REVIEW

A review on the beneficial aspects of food processing

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The manuscript reviews beneficial aspects of food processing with main focus on cooking/heat treatment, including other food-processing techniques (*e.g.* fermentation). Benefits of thermal processing include inactivation of food-borne pathogens, natural toxins or other detrimental constituents, prolongation of shelf-life, improved digestibility and bioavailability of nutrients, improved palatability, taste, texture and flavour and enhanced functional properties, including augmented antioxidants and other defense reactivity or increased antimicrobial effectiveness. Thermal processing can bring some unintentional undesired consequences, such as losses of certain nutrients, formation of toxic compounds (acrylamide, furan or acrolein), or of compounds with negative effects on flavour perception, texture or colour. Heat treatment of foods needs to be optimized in order to promote beneficial effects and to counteract, to the best possible, undesired effects. This may be achieved more effectively/sustainably by consistent fine-tuning of technological processes rather than within ordinary household cooking conditions. The most important identified points for further study are information on processed foods to be considered in epidemiological work, databases should be built to estimate the intake of compounds from processed foods, translation of *in-vitro* results to *in-vivo* relevance for human health should be worked on, thermal and non-thermal processes should be optimized by application of kinetic principles.

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1 Introduction

1.1 Reasons for the study

In a previous study, an Expert Group of the ILSI Europe Process-Related Compounds Task Force focussed on risk-benefit considerations of food processing and published a paper: 'Risk benefit considerations of mitigation measures on acrylamide content of foods – a case study on potatoes cereals and coffee' [1]. This article serves as a useful tool for risk assessors and risk managers to help in science-based

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Abbreviations: ACE, angiotensin I-converting enzyme; **CGA,** chlorogenic acid; **CI,** confidence interval; **CML,** carboxymethyl-lysine; **CVD,** cardiovascular disease; **DM,** diabetes mellitus; **GST,** glutathione *S*-transferase; **MRPs,** maillard reaction products; **RS,** resistant starch; **TAC,** total antioxidant capacity

decision making, and it can be used as a basis for education and communication to food processors and consumers.

In contrast to the adverse effects of processing and cooking, the beneficial effects of processing received little attention to date, but it is an important component in any risk-benefit debate on the thermal or heat treatment of foods. The previous study focussed on the risks rather than the benefits of (thermal) food processing. Consequently, the ILSI Europe Process-Related Compounds and Natural Toxins Task Force held a brainstorming meeting and it was decided to undertake a study on the beneficial aspects of thermal food processing.

Thus, the ILSI Europe task force commissioned an independent Expert Group to undertake this study to identify the beneficial changes to food that occur during thermal treatment of foods, to ensure the safety and quality of food while ensuring consumer acceptability as occurs during industrial food processing and during household cooking. The objective of this activity is to review the beneficial aspects of cooking and food processing. Beneficial should be understood as desirable in relation to health, to food safety, to attractiveness of the food, to shelf-life and to sustainability. This requires a holistic approach and aims at a comprehensive description of beneficial aspects of cooking and beneficial compounds involved. It will address the current knowledge of compounds that may be formed during the cooking process that may benefit to health. Gaps in research will be identified. It is interesting to note that most of the information related to health aspects of heat-processed foods is on products such as coffee, cocoa, barley in which the Maillard reaction plays an important role.

Food safety and palatability issues require the use of thermal processes for modulation of food raw materials during food processing at industrial and household levels. Thermal treatment of foodstuffs induces several biological, physical and chemical modifications, leading to sensory, nutritional and textural changes. In general, the thermal treatment of foods results in enhanced food safety and quality.

Although some undesirable reactions are known to occur, leading to loss of nutritional value and the formation of potentially mutagenic and carcinogenic molecules, this review will focus on the beneficial changes that occur during processing of food. These include enhancement of nutritional quality, release of bioactive and generation of beneficial components, as well as destruction of negative activity (anti-nutritional substances) that occur as a result of heat treatments of food at household and industrial levels.

1.2 Anthropological aspects – historic aspects of cooking and development of food processing

Foods have been heat-treated for many centuries, since our ancestors learned, by trial and error, to master fire for cooking purposes approx. 700 000 years ago, to modify and preserve the organoleptic and nutritional properties of foodstuffs. It is widely accepted by today's anthropology that the invention and continuous development of thermal food treatment had a substantial, if not the major impact on phenotypical, intellectual, societal and economic development of mankind [2]. Since the late 19th century, the focus began to change from home cooking to more industrialized processes with initially an emphasis on preservation and later on (1920–1930s) safety (microbial safety) and quality issues, especially nutritional quality issues after the Second World War.

Meanwhile new technologies became available, such as steam and irradiation treatments to the use of microwaves. This has revolutionized the way home cooking is nowadays done, with the availability and use of semi-finished processed foods in everyday cooking. With the availability of modern technology, it becomes possible to pay more attention to gastronomic issues of foods. It is estimated that 80–90% of foods used in home cooking are semi-processed and therefore it makes sense to consider the beneficial effects of processing on safety and quality of food [3].

Food-processing methods started with heating in bottles and cans, as developed by Appert and later on Pasteur to modern sterilization techniques, such as ultra high temperature and other non-thermal techniques, as will be discussed in the next section. The fact that less time is spent at home cooking as a result of food processing has resulted in a change in lifestyle and has also affected the health status of the population (longer life expectancy) as a result of the availability of consistently high quality, safe and nutritious food. Thermal processing may lead to the formation of compounds in food that warrant a critical review in terms of their impact on health.

1.3 Processing of foods – at household and industrial levels

The application of heat during household cooking of foodstuffs encompasses a variety of processes, such as boiling, frying, steaming, baking, stewing and roasting, in traditional and microwave and steam ovens. Industrial thermal treatment of foodstuffs includes many of the processes also listed for household cooking. In addition, heat has been used in traditional transformation processes other than cooking, such as toasting, kilning, coffee roasting, drying processes, canning, pasteurization and related technology (ultra high temperature treatment) smoking and extrusion cooking. It is important to note that these processes can be controlled much better on industrial scale than on household level.

The quality of food, from the nutritional, microbial safety point of view and sensory aspects depends on a range of variables from farm to fork, including the quality of the raw material, processing techniques, packaging and cooking.

The main purpose of industrial food processing is to provide safe and high-quality food as demanded by the consumer [4, 5].

2 Factors in processing that impact food

The most important beneficial aspects can be summarized as follows:

(i) Food safety (pathogens): The main benefit of food processing is inactivation of food-borne pathogens, as is normally required by Food Safety Legislation.
(ii) Food safety (other aspects): inactivation of natural toxins and enzymes, prolongation of shelf-life.
(iii) Nutritional value: improved digestibility, bioavailability of nutrients.
(iv) Sensory quality: taste, texture and flavour.
(v) Functional health benefits: *e.g.* probiotics, prebiotics, Maillard reaction products (MRPs), flavonoids, other food constituents and their reaction products.
(vi) Convenience: availability of ready-to-eat and semi-prepared foods, *e.g.* microwavable frozen meals.
(vii) Cost: economy of scale.
(viii) Diversity: independence from the seasonal availability of foods, and introduction of global food supply chain.
(ix) Quality of life: improved because less time required for food supply and preparation.

The focus of this study is on the first five benefits using the example of foods identified at the beginning of this section.

The first and foremost beneficial effect of food processing is the destruction of unwanted compounds and micro-organisms. The minimal heat treatment in this respect is pasteurization: this comprises a time–temperature combination that inactivates pathogenic bacteria such as *Mycobacterium tuberculosis*, *Salmonellae* species, *Staphylococcus aureus*, among others. Provided that the food is properly packed (*i.e.* no recontamination after processing) and stored refrigerated, pasteurized food is generally safe from a microbiological point of view. A more intense heat treatment is sterilization in which not only vegetative cells but also spores are inactivated. If properly packed and if recontamination is prevented, such foods are completely safe from a microbiological point of view and can be kept indefinitely. Another beneficial effect is the inactivation of anti-nutritional factors such as protease inhibitors (*e.g.* trypsin inhibitors in soy) and other natural toxins. However, the quality of sterilized foods will diminish over time because of chemical changes taking place in the food. The second effect is the prolongation of shelf-life, which is generally determined by microbial changes, biochemical changes, chemical and physical changes. Although microbial and enzymatic changes may be stopped by heat inactivation, chemical and physical changes continue to occur in foods, whether they are sterilized or not. This is the reason why sterilized foods can still have a limited shelf-life because chemical changes such as the Maillard reaction may lead to undesired flavour compounds and discolouration. A third beneficial effect of cooking is enhanced digestibility of food and bioavailability of nutrients. For example, denatured proteins are generally more digestible than proteins that are not denatured, because digestive enzymes can more easily hydrolyse unfolded molecules than folded ones. Also, gelatinization of starch makes possible its hydrolysis by amylase enzymes. Destruction of cell walls in plant materials may improve the bioavailability of compounds such as carotenoids and polyphenols. A fourth effect is the formation of desired compounds such as flavour compounds, antioxidants and colouring agents and a prime example of this is the Maillard reaction, which leads to the formation of desired flavour and colour changes in many foods. The fifth effect is related to specific effects of processing on health-promoting compounds.

These five effects illustrate the positive aspects of food processing and some of them will be elaborated below. However, processing can also damage food quality, leading to undesired consequences, such as:

(i) losses of certain (essential) nutrients due to chemical reactions (*e.g.* vitamin C, available lysine),
(ii) formation of undesired compounds, *e.g.* acrylamide, acrolein chloropropanediols and –esters, heterocyclic amines, *etc.*,
(iii) in some cases, formation of compounds that have a negative effect on flavour perception (for instance, sulphur compounds formed during heating of milk [6]),
(iv) loss of texture, discolouration, *etc.*

Hence, processes need to be optimized such that the desired beneficial effects are promoted and the undesired effects are counteracted as much as possible. This section discusses some technological means to realize such optimization.

2.1 Optimization through reaction kinetics

Quality changes of food, be it heat-processed or not, are due to changes at the chemical, biochemical, physical and microbial levels. For example, the nutritional value of a product may change because of oxidation of a vitamin (chemical reaction), the colour of a food may change because of enzymatic conversion of polyphenols (biochemical reaction), phase changes may occur in food (physical reaction) and foods may spoil because of bacteria or moulds (microbial reaction). If we want to steer such reactions in a desired way, there are two things we need to know: (i) understanding of the reaction taking place and (ii) the

speed at which the reaction takes place. Foods are unstable from a thermodynamic point of view, which basically means that spoilage cannot be prevented in principle. However, what can be influenced is the speed at which spoilage takes place. We need at least two relations, one is to express the change in a quality attribute as a function of time, and the other is about the dependence of that change on conditions such as temperature and pressure. If we apply heat, temperature dependence needs to be known, if high-pressure technology is used, the pressure dependence of a reaction has to be known, if we apply electric fields, the dependence on electric field strength, *etc*. This is the domain of kinetics. van Boekel has addressed this topic extensively and we refer to this reference for further details [7].

2.2 Types of processes

2.2.1 Thermal processes

Traditionally, thermal processes have been used extensively in food technology. Only fairly recently have other technologies emerged (see below). Thermal processes can be classified according to the intensity of heat treatment: pasteurization (in the range 70–80°C, sterilization (in the range 110–120°C), and ultra-high-temperature treatment (in the range 140–160°C). The reason why thermal treatment is effective is that the speed of reactions is increased with temperature. Chemical reactions always increase with temperature; biochemical and microbial reactions also increase with temperature, but above a certain temperature enzymes and microorganisms become inactivated. Physical reactions and radical reactions are usually not so temperature dependent.

Various reactions of importance for food quality have different temperature sensitivity, and this we can exploit to our benefit (Fig. 1). Such a plot can be constructed using the kinetic equations given in, for instance, [7]. Note that this is not an Arrhenius plot because the *x*-axis does not reflect the inverse of absolute temperature. The important process variables here are time and temperature, and food technologists can manipulate these variables to the foods benefit, *i.e.* producing foods that have the highest quality possible.

Such plots show at a glance which time–temperature combinations lead to a desired effect, and they can be constructed with the aid of reaction kinetics. Basically, such a concept can be applied to any quality change that is considered of importance, but the parameters need to be derived from experiments.

Just one example shows the possibilities of this approach; it is on reduction of acrylamide while keeping the quality attribute of a desired brown colour, taken from the work of De Vleeschouwer *et al.* [8] on a potato-based model system. It shows the time at which a level of 0.2 mM acrylamide (as an example of an undesired compound) is reached in the system and the time to obtain a colour level that corresponds to a melanoidin level of 10 mM (melanoidins are the brown pigments and are usually desired compounds) (Fig. 2).

Figure 2 shows that acrylamide formation is slightly more temperature dependent than colour formation. This implies that reducing temperature will reduce acrylamide formation more than colour formation. Hence, by heating at a lower temperature and a longer time, acrylamide formation can be reduced while maintaining the same colour. This is, thus, one of the ways to mitigate acrylamide formation. In passing, we note that although many articles have been published on acrylamide formation, it is not so

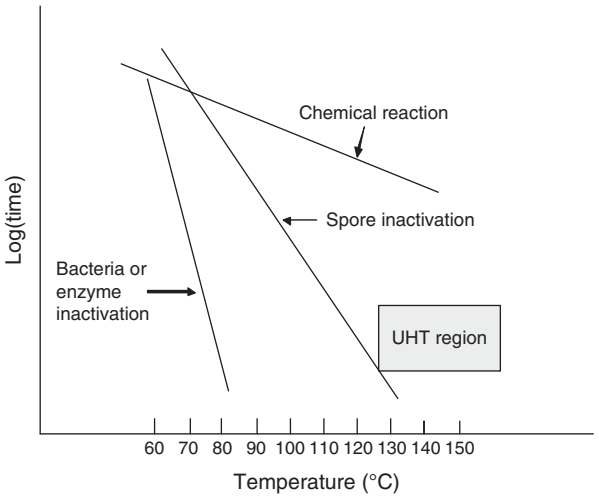

Figure 1. Schematic picture showing how the time to achieve a certain effect depends on the temperature of a chemical reaction and microbial/biochemical inactivation (after van Boekel, [7]).

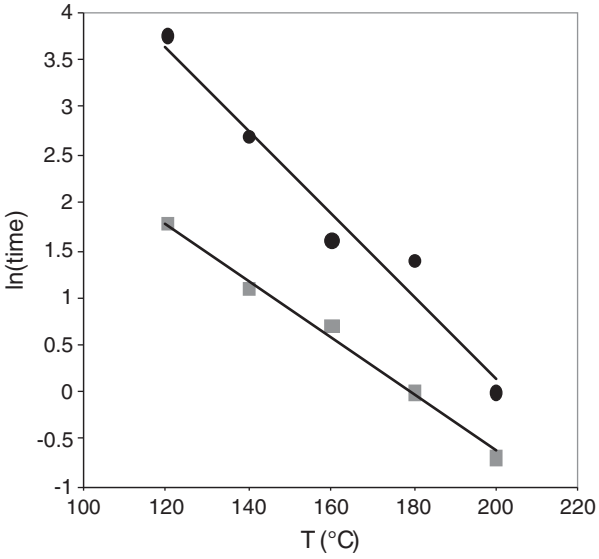

Figure 2. Temperature-time plot showing the formation of 0.2 mM acrylamide (■) and 10 mM melanoidins (●) in heated potato-based asparagine-glucose model system (extracted from Figure 4 in De Vleeschouwer *et al.*, 2008).

easy to find reliable kinetic data that can be used to optimize processing.

Such knowledge is now actively applied in the food industry to optimize quality of food, something that is impossible to do in home cooking. An important aspect of this is to also consider process design. Applying a heat treatment can be done in various ways, by indirect heat exchange but also in some cases by direct heat exchange by injecting steam into a liquid product. The speed of heat transfer is then important and aspects such as residence time distributions and temperature distributions come into play. Obviously, it should be avoided that some parts of the food receive a very intense treatment, whereas other parts are under-processed. Hence, equipment design (including hygienic design) is an important aspect to take into consideration.

After processing, it is of utmost importance to use the right packaging material to retain the highest quality level during storage. In addition, modern techniques such as modified atmosphere and controlled atmosphere packaging offer excellent opportunities to extend shelf-life, and thereby enhance the beneficial effects of processing.

2.3 Flavour and texture

Flavour and texture are very important desirable, key quality attributes. However, these attributes are not easily translated into measurable parameters that can be controlled in a technological way. Sensory studies are needed for this and outcomes are not easily translated into industrial scale processes and technologies. Although many such processes can be conducted at laboratory and even at pilot plant scale, upscaling to industrial scale remains a major technological challenge. Qualitatively, however, it is clear that processing has large, usually desired, effects on flavour and texture.

Flavour compounds are formed predominantly in the Maillard reaction, *e.g.* [9] as a result of heat processing, but also other reactions contribute [10]. Another reaction of great importance to food quality is oxidation; this is, however, not a desired reaction as it leads to formation of undesired flavour compounds [10], sometimes also called off-flavours.

Texture is certainly influenced by processing. Basically, a desired texture arises from (i) gel formation caused by biopolymers, *i.e.* proteins and polysaccharides, (ii) from closely packed systems and (iii) from cellular materials [11, 12]. A term used in this respect is soft solids. Gels can be formed because of heat processing, but also because of pH changes and changes in ionic strength and as a result of enzymatic action; examples include many dairy products and meat products, jams and gellies. Closely packed systems are, for instance, vegetable purees and concentrated emulsions; these are obviously processed foods. Cellular materials comprise fruits and vegetables, and processing has large effects on their behaviour because processing usually disrupts cell walls to some extent. Texture is the result of many complex interactions of components in the food matrix and processing offers the opportunity to influence these interactions in a desired way [12]. The topic is too vast to discuss in much detail here; we refer to a recent review [13].

2.4 Non-thermal processes

Some of the non-thermal-processing techniques are still in their infancy and in an exploratory phase of investigation. The ones we consider here are those that have commercial application in the food industry, namely high-pressure, pulsed electric fields, membrane processing, dehydration and freezing. Table 1 summarizes advantages and disadvantages of these non-thermal processes in comparison to thermal processes. It should be noted that high pressure alone does not inactivate spores, but a combination of mild heat treatment under pressure may be effective (which makes it partly a heat treatment again); research is ongoing (*e.g.* [14, 15]). Pressure-dependent freezing point depression of water to $-20^{\circ}C$ at 200 MPa and the formation of different ice crystal types under pressure leads to unique high pressure–low temperature applications [16, 17].

Pressure induces protein denaturation and individual starch granules, which can also, – pressure and temperature dependent – lead to gelatinization [16]. It should be noted that pressure-induced gels differ in physico-chemical properties from heat-induced ones, thus allowing *via* T and p combinations to create a wide variety of different gel structures [18]. In addition, pressure supported protein–polysaccharide interactions have led to the creation of new food textures [16]. Compared with thermal processing, less work has been done for non-thermal processes in terms of inactivation kinetics and reaction mechanisms of nutrients, toxins, allergens, microbes and viruses. More data are needed on mechanisms of spore inactivation and on enzyme inactivation. Shelf-life studies of non-thermally treated products are desirable.

2.5 Fermentation processes

Fermentation is a non-thermal process that can be exploited to produce beneficial effects in foods. It is a process by which foods and drinks undergo chemical changes caused by enzymes produced from bacteria, microorganisms or yeasts, and is one of the oldest known food preservation techniques. The process involves the action of desirable microorganisms or their enzymes on food ingredients causing biochemical changes, leading to significant modification of the food product. During fermentation, the carbohydrate energy source in food, such as lactose in milk is converted to lactic acid, as occurs during production of yogurt and cheese from milk, and pickles from fruits and vegetables. Yeasts (typically of the species *Saccharomyces*) convert glucose to ethanol and carbon dioxide during bread

Table 1. Advantages and disadvantages of non-thermal processes in comparison to thermal processes

Process	Advantage	Disadvantage	Conditions	Comments	Reference
Thermal processes	Safety Quality Nutritional value largely maintained Inactivation of anti-nutritional factors achieved (*e.g.* trypsin inhibitors) and some allergens Inactivation of enzymes Sensory attractiveness Ease of use for industry and house hold processing In general, cost effective	Formation of undesired compounds (*e.g.* acrylamide) Loss of freshness and related sensory attributes	Pasteurization: 60–70°C Sterilization: 110–150°C	Inactivation of cells Inactivation of spores Formation of desired flavour and texture (*e.g.* Maillard reaction)	[7, 201]
High pressure	Safety No formation of undesired compounds Nutritional value largely maintained Retention of freshness Physical modification	Desirable flavour compounds generated by heat are not formed	Pasteurization: 600 MPa, ambient temperature (batch process) Sterilization: 600 MPa, 121.1°C rapid, homogenous heating and cooling due to adiabatic heating (batch process)	Inactivation of vegetative micro-organisms and spores Possible inactivation of enzymes, viruses and prions (p,T dependent) Treatment of packaged food Cold storage (4°C) or storage at ambient temperature	[16, 17], [18]
Pulsed electric field	Safety No formation of undesired Compounds Gentle processing, retention of freshness Cell disintegration Improvements of mass transfer processes Physical modification	Spores are not inactivated Desirable flavour compounds generated by heat are not formed Inactivation of anti-nutritional factors not achieved No inactivation of enzymes	Cell disintegration: 1–5 kV/cm 1–10 kJ/kg Non-thermal pasteurization: 25–40 kV/cm 50–200 kJ/kg Short processing times Continuous operation	Intensity-dependent occurrence of electrochemical and thermal side effects Refrigerated storage of products required	[202, 203] [204]
Membrane processing	Safety No formation of undesired compounds	Desirable flavour compounds generated by heat are not formed Inactivation of anti-nutritional factors not achieved		Only applicable for liquids Expensive for complete products Added value for separation of ingredients *e.g.*, lactoferrin)	[205]
Dehydration processes	Preservation Reduced reaction rates No formation of undesired compounds.	No inactivation of microbes and anti-nutritional factors Formation of heat-generated substances	Sublimation drying (freeze drying) −10 to −40°C. 0.01–1 mbar Evaporation drying (40–90°C) Convection drying Contact drying Radiation drying Osmotic drying (<50°C, solute concentration 30–70%)	Room temperature storage Suitable for a wide range of products	[206] [207]

Table 1. Continued

Process	Advantage	Disadvantage	Conditions	Comments	Reference
Freezing	Preservation Reduced reaction rates No formation of undesired compounds Retention of freshness and nutrients (*e.g.* vitamins)	No inactivation of pathogens, and anti-nutritional factors No inactivation of enzymes Loss of structure	Cold air freezing. −30 to −50°C Contact freezing: approx. −40°C Cryogenic freezers: −40 to 196°C	Applicable to almost all foods, retention of microbial viability *e.g.* probiotics Industrial freezing can be precisely controlled, frozen storage at −20°C	[208]

making, and in the production of alcoholic beverages. Moulds are also used in certain fermentation, such as production of blue cheese and soy sauces. One of the achievements of the past 50 years is that fermentation processes are now well-controlled and can be tailored to produce desired compounds, based on the selection of starter bacteria and understanding the fermentation process and thus manipulation of conditions [19].

The benefits of fermentation are shelf-life extension, enrichment of the nutritive value of the food, enhancing the taste and digestibility of milk and indeed improved food safety *e.g.* by low pH and elimination of antinutrients during the fermentation process.

2.6 Beneficial compounds from fermentation

During fermentation, a range of secondary metabolites are produced, some of which have been associated with health-promoting properties, most notably B vitamins and bioactive peptides released from food proteins through microbial action. Peptides with inhibitory properties against angiotensin I-converting enzyme (ACE) are known to have an anti-hypertensive effect and have been isolated from enzymatic digests of various food proteins, whereas other properties ascribed to bioactive peptides include antimicrobial, opiate, cholesterol-lowering, immuno-stimulatory and mineral-utilizing properties (for review, see [20]). It is thus not surprising that the consumption of fermented foods has long been associated with good health. Indeed, dating back to 76 A.D., the Roman historian Plinio advocated the use of fermented milks for treating gastrointestinal infections, whereas the French pediatrician Tissier proposed in the early 1900s that bifidobacteria may be effective in preventing infection in infants.

2.7 Probiotics

Furthermore, around this time, Elie Metchnikoff found that the consumption of fermented milks could reverse putrefactive effects of the gut micro flora, leading to the development of the probiotic concept. Probiotics are defined as ''live microorganisms which when administered in adequate amounts confer a health benefit on the host'' (ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf) [21]. There is accumulating clinical evidence that probiotic bacteria positively affect certain human health conditions, including overcoming lactose intolerance, preventing various diarrheal illnesses, allergic disorders, bacterial vaginosis and urinary tract infections, respiratory infections, dental caries, necrotizing enterocolitis and certain aspects of inflammatory bowel disease [22]. Although specific numbers are not mentioned in the definition, high levels of viable microorganisms are recommended in probiotic foods for efficacy [23], given that many of the clinical studies use daily doses in excess of 10^9 cfu/day.

For successful delivery in foods, probiotics must survive food processing and storage during product manufacture, maturation and shelf-life. Appropriate processes must be applied to guarantee viability of the bacteria at the end of the shelf-life of the product. Probiotic strains are generally of the genera *Lactobacillus* and *Bifidobacterium*, and to a lesser extent *Pediococcus*, *Propionibacterium*, *Enterococcus*, *Bacillus*, *Streptococcus* and *Saccharomyces*. Freeze-dried powders and frozen concentrates of probiotic *Lactobacillus* and *Bifidobacterium* spp. have been developed, whereas spray drying has been applied to the dehydration of a limited number of probiotic cultures including lactobacilli and bifidobacteria (for review, see [24]).

During freeze drying, cells are first frozen at −196°C and then dried by sublimation under high vacuum and while good probiotic survival rates are typically achieved in freeze-dried powders, inactivation does occur, and is mainly associated with the freezing step. The lipid fraction of the cell membrane is known to be a primary target area for damage during drying, where lipid peroxidation may occur while the secondary structures of RNA and DNA destabilize, resulting in reduced efficacy of DNA replication, transcription and translation. Consequently, approaches that minimize damage to these cellular components during the desiccation of probiotics will deliver optimum results (for review, see [25]).

For successful spray drying of probiotic cultures, selection of the particular probiotic strain and the outlet temperature of the spray drier are critical factors. The process results in exposure of the live bacteria to heat stress, leading to viability losses. The cytoplasmic membrane is among the most susceptible sites in bacterial cells to the stresses associated with spray drying, while the cell wall, DNA and RNA are also affected, leading to loss of metabolic activity. Therefore, in order to minimize damage during spray drying of probiotic cultures, approaches such as optimization of the drying technology, selection of suitable drying matrix for encapsulation and manipulating probiotic bacteria by classical (microbiological) approaches have proved effective. For example, studies have shown that probiotic performance can be improved *via* the addition to the media of thermoprotectants (such as skim milk powder, whey protein, trehalose, betaine, adonitol, *etc.*) prior to drying (for review, see [24, 25]). The generation of live probiotic cultures in dried format thus presents challenges in terms of retaining probiotic functionality during powder manufacture and storage. Both freeze drying and spray drying can be used for manufacture of probiotic powders on a large scale; however, both approaches expose the cultures to extreme environmental conditions. Methods of production of dried probiotic powders should be such that viability is maintained in the dried powders following manufacture, and storage to ensure that an adequate number of bacteria can be delivered in the final product.

Consequently, the processing conditions relating to the development of foods containing probiotics in sufficient numbers in a stable live form that can withstand the product shelf-life need to be overcome. These requirements pose a significant challenge from a technological standpoint, since many probiotic bacteria, being of intestinal origin, are sensitive to stresses such as heat, oxygen and acid exposure. Manipulation of the processing conditions and use of processing aids may lead to improved delivery of viable probiotics in foods.

2.8 Food additives

Beneficial aspects of food processing can be reached in many ways. One of these ways is by adding ingredients. Food additives have a bad reputation among consumers. Nevertheless, they are intentionally used to improve food quality. It comprises the use of flavouring agents, thickening agents, emulsifiers and foam stabilizers. They are generally used to improve the stability of processed foods. We discuss here the use of enzymes, preserving agents and vitamins in somewhat more detail.

2.8.1 Enzymes as processing aids

The basic mechanism behind the action of enzymes is that they act as catalysts and thereby speed up chemical reactions enormously without the need for temperature increase. Enzymes can act on the major nutrients in foods, carbohydrates, proteins, fats, but also on other compounds such as phenols, chlorophyll. A detailed overview of the use of enzymes in food technology is given by Parkin [26]. A typical recent new example is the use of asparaginase to remove one of the precursors of acrylamide during processing of potatoes and doughs. The effect of enzymes is to improve either the properties of a food, or to help in processing. For example, in the production of fruit and vegetable juices, pectolytic enzymes can be used to break down pectin in cell walls, thus facilitating and improving yields in the extraction process.

2.8.2 Preserving agents

Preserving agents obviously help to preserve food, the beneficial effect being that spoilage of foods is postponed. In most cases, these agents limit or prevent the growth of micro-organisms. This concerns agents such as benzoic acid, sorbic acid, lactic acid and acetic acid. Part of their action is to lower the pH such that micro-organisms cannot grow, but there are also specific effects on micro-organisms, for instance, they may damage the membrane of micro-organisms. Other preserving agents, such as sulphite, interfere with chemical reactions, or they inhibit enzymes. Salts such as NaCl, nitrite and nitrate can be added as preserving agents. A recent overview on food additives can be found in Lindsay [27].

2.8.3 Vitamins, minerals and antioxidants

For nutritional reasons, one can add micro-nutrients to foods. For instance, folic acid to bread, iodine to bread and calcium to drinks. Such foods are nowadays classified as functional foods, the beneficial effect being that specific needs can be fulfilled, such as folic acid for pregnant women, extra calcium to combat osteoporosis. The prime concern here is that in order to be really beneficial, the added compounds need to retain their activity in the food up until the moment they are consumed. Folic acid may be subjected to chemical reaction, added salts may react. An example of the latter is the following. If a product like soy milk is fortified with a calcium salt, it strongly depends on which salt is actually used. Some calcium salts may crystallize, so if, for instance, calcium carbonate would be used, it will precipitate in the milk, form a sediment layer (a sort of cement) and is thus not adsorbed. Hence, although the product does contain added calcium, it is of no use. In cow's milk, such crystallization does not happen because of the association of calcium phosphate with casein micelles. Such micelles are not present in soy milk. To prevent such things from happening, a thorough knowledge of the behaviour of the added compound in conjunction with interactions in the food matrix is needed.

Antioxidants are used to prevent oxidation of compounds that are prone to reaction with oxygen, such as vitamins and unsaturated fatty acids. Oxidation is typically a problem for stored processed foods and the use of antioxidants, in combination with suitable packaging materials is clearly beneficial.

3 Effects of processing on bioactive compounds in food

The previous section described how the use of specific technologies can result in beneficial effects. This section deals with the chemical changes, the modification and formation of compounds considered beneficial for human health in terms of digestibility, bioavailability and antioxidant activity.

In the past, much attention was paid to compounds formed by heat processing that have potential adverse effects for human health (*e.g.* acrylamide, furan, 3-monochloropropane-1,2-diol (3-MCPD) and trans fatty acids), shedding a rather negative light on processed foods. These compounds will not be detailed here, the scope of this article being the potential health beneficial effects, in line with other recent efforts to balance potential adverse against beneficial effects (risk-benefit assessment) (http://europe.ilsi.org/activities/ecprojects/BRAFO/) [1, 28].

New compounds are formed upon processing, or existing components are transformed that may express biologically beneficial activities. Table 2 summarizes examples of such compounds linked to the processes used. In addition, Table 3 summarizes the effects of processing on the formation/destruction of compounds with potentially adverse effects.

3.1 Starch, dietary fibre, protein and allergens

Processing strongly affects the digestibility of macronutrients. Cooking starchy foods (legumes, cereals and potatoes) results in starch gelatinization and increases digestibility of starch, which has been very beneficial for mankind because it allowed to markedly enhance the caloric intake. Paradoxycally, in the present era of abundancy where people take in too many calories, research is now focused on resistant starch (RS) which is slowly hydrolysed by amylase and reaches the lower gut where it is fermented by gut microbiota. The differing rates of absorption between RS and digestible starch influence their differential metabolic responses. RS intake decreases postprandial glycemic and insulinemic responses, lowers plasma cholesterol and triglyceride concentrations, improves whole body insulin sensitivity, increases satiety and reduces fat storage [29].

Processing parameters such as time/temperature of cooking, water availability and storage conditions can be used to modulate the amount and the nature of RS. This opens up many opportunities for future developments, especially for tailor-made starch derivatives with multiple modifications and with the desired functional and nutritional properties [30].

Despite common beliefs, dietary fibre structure can also be significantly affected by processing. Dietary fibre is defined as non-digestible carbohydrates and lignin that are intrinsic and intact in edible parts of plants. A definition for Dietary Fiber was adopted in June 2009 by the CAC based on the recommendation for endorsement of the CODEX Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in November 2008. The definition listed three categories of carbohydrate polymers which are not hydrolyzed by the endogenous enzymes in the small intestine of humans [31]:

(i) Edible carbohydrate polymers naturally occurring in the food as consumed,
(ii) Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities and
(iii) Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Dietary fibre has traditionally been categorized according to its solubility. Soluble fibres include gums, pectins and inulin, whereas cellulose and fibre in wheat bran are two examples of

insoluble fibres. Although the distinction between soluble and insoluble fibre was thought to determine the physiological effect of fibre, other properties may be of more importance. Two of these include viscosity and fermentability. Heat treatment in wet conditions usually determines the hydrolysis of the fibre (the more severe the treatment the higher the hydrolysis) (for review, see Nyman [32]).

Processing can modify the solubility of the fibre by reducing its molecular weight, enzymatically [33, 34] or mechanically, for example, during extrusion by applying different shear forces [35, 36].

Also for proteins, a number of factors affect their digestion and limit their absorption. Some proteins are resistant to proteolytic enzymes and pass through the small intestine relatively intact. The protein digestion rate influences the amount of protein that is absorbed in the small intestine. Processing has a double contrasting effect on protein digestibility that has been summarized by Friedman [37, 38].

(i) Denaturation can facilitate proteolysis, thus increasing digestibility. This is particularly the case for some vegetable proteins, which are poorly accessible in uncooked material.
(ii) Processing results in the formation of protein aggregates that are less digestible than the unprocessed protein. This is particularly the case for animal proteins and for systems heated in the presence of carbohydrates (formation of crosslinked proteins).

3.1.1 Allergens

Allergens are proteins and therefore subject to denaturation upon processing steps such as heating and high pressure. Whether denaturation (and/or subsequent reactions) leads to changes of the epitopes (the region on a protein recognized by an antibody) that influence potential allergenicity strongly depends on the molecular properties of the allergen concerned [39]. An important point to note is that some allergens are able to refold from the denatured state [40] when the cause of denaturation is removed (*e.g.* upon cooling), but it remains to be investigated whether these refolded proteins are still able to provoke allergic reactions.

Food processing may increase or decrease the intrinsic allergenicity of a protein, but current data do not facilitate the identification of specific variables that could be used to reliably determine how processing will influence protein allergenicity [41].

The allergens that occur in fruits and that are related to birch pollen allergies (the PR10 protein family, [42]) have molecular similarities and are in general not very heat stable. Their denaturation temperature is somewhere between 45 and 80°C [40]. For instance, the allergen from apple, Mal d1, is quite heat labile. Thus, people allergic to apples are generally able to eat processed apples as in apple cake or apple sauce. The allergen from soya, Gly m4, seems to be more heat stable and has been found to cause allergies when present in processed soy products [43]. Also the major allergen in peanuts, Ara h1, is very heat stable [44]. The allergen from Brazil nut, Ber e1, is so heat stable that it does not denature during common food processing [45].

Processing may also lead to enhancement of allergenic activity. This is documented for instance, for allergens subjected to the Maillard reaction. The phenomenon has been demonstrated quite a while ago already for β-lactoglobulin, the major allergen in milk [46]. Since the ability of sugars to cause glycation differs considerably, this implies that the extent of increased allergenicity depends on the type of sugar. Also, temperature has a strong effect on the Maillard reaction, and hence it depends on the intensity of heating how strong this allergenicity increase will be. It has been shown that Maillard-modified peanut allergens bind more effectively to IgE and are at the same time also more resistant to gastric hydrolysis [47, 48]. Hence, roasting of peanuts could lead to an increased allergic reaction, but it is not completely sure whether an increased IgE binding is causing an increase in allergenicity. Supposedly, high-pressure processing will not lead to an increase in allergenicity if there is no additional heat treatment. Depending on the extent of denaturation and the ability to refold after the treatment, allergenicity may actually decrease, as shown for celery allergens [49].

Table 2. Effects of processing on the presence/absence (formation/destruction) of compounds to which beneficial effects have been attributed

Compounds	Foods	Process	Effect	Reference
Antioxidant MRPs	Different foods	Thermal treatment	Increase amount	[101]
Catechins	Cocoa	Alkalinization	Decrease	[209]
Proanthocyanidins	Cocoa	Fermentation	Increase amount	[210]
Carotenoids	Tomato	Heating to dissociate carotenoid protein	Increase amount	[59, 60]
Glucosinolates	Broccoli	Steam heating	Increase amount	[60, 82]
Glucosinolates	Broccoli	Frying, boiling	Decrease amount	[60]
Flavonoids	Different vegetables	Heating	Increase amount	[60, 89, 90–94]
Anthocyanins	Fruits juice	High pressure	Avoid degradation	[211]
Organosulphur compounds	Garlic onions	Thermal treatment	Decrease amount	[212]
Ascorbic acid	Fruits and vegetables	Thermal treatment	Decrease amount	[56]

Table 3. Effects of processing on the presence/absence (formation/destruction) of potentially harmful compounds

Compounds	Food product	Process	Effect	Reference
3-MCPD	Soy sauce	Acid hydrolysis, heating	Increase	[213, 214]
Diterpenes	Coffee	Filtration	Decrease	[215]
Trans fatty acid	Spreads	Transesterification	Decrease	[216, 217]
Trans fatty acid	Spreads	Hydrogenation	Increase	[218]
Furan	Coffee, baby foods	Stirring	Decrease	[219]
Acrylamide	Potato, cereals, coffee	Frying, roasting at low water activity	Increase	[37]
Polyaromatic hydrocarbons	Meat	Barbecueing, grilling	Increase	[167]
Heterocyclic amines	Meat	Roasting, frying	Increase	[167]

A very effective way of reducing or removing allergenicity is enzymatic hydrolysis, destroying the responsible epitopes. By this way, hypo-allergenic foods (especially milk products) can be developed. The downside of this treatment is, of course, that the properties of the resulting food are drastically different from the original food. The treatment notably affects texture and taste, as well as appearance. The resistance to enzymatic hydrolysis is, incidentally, also of importance for the occurrence of allergic reactions upon digestion [50].

Other treatments such as γ-irradiation, pulsed electric fields and acoustic shocks, are not expected to have much effect on allergens, because they do not affect protein conformation. A possible exception is reported for γ-irradiation, which affected the allergenic potential [51, 52], depending on the dose. This may be due to chemical changes occurring in the proteins, perhaps *via* free radical reactions. Molecular separation techniques such as ultrafiltration could, in principle, be used to remove allergens, but in actual practice this will not be easy. Such an operation will change the properties of a food, and not all food matrices lend themselves to such an operation.

A completely different approach is to develop and select plant varieties *via* breeding that are containing a reduced content of major allergens. This has been investigated for wheat in relation to celiac disease [53] and for apples [54]. In combination with food-processing operations, such an approach can lead to foods that can be eaten by people who would normally be allergic for such foods.

Recently, it has been found that the molecular behaviour of recombinant allergens can be quite different from allergens isolated from their natural environment [55]. This is of importance, because many studies are actually performed with recombinant allergens and it is thus not straightforward to translate such results to foods.

3.2 Secondary plant metabolites

Secondary plant metabolites comprise compounds such as carotenoids, polyphenols and glucosinolates. They have received much attention as non-nutrients in the last decade because of their putative role of health-promoting capacity in relation to consumption of fruits and vegetables. There is quite some discussion whether or not it is beneficial to eat fruits and vegetables raw or processed. There seems to be a common understanding among the public that fresh fruits and vegetables are to be preferred over processed ones. The question is whether this image is supported by scientific research. Indeed, processing does have an effect. Maceration, heating and various separation steps can result in oxidation, thermal degradation, leaching and other events that lead to lower levels of food components in processed food compared with fresh food [56]. However, below we present results that show that there are also positive beneficial effects of processing.

3.2.1 Carotenoids

Carotenoids appear generally to be affected by processing such that bioavailability is improved, especially when destructive factors (*e.g.* oxygen, light and heat) are kept to a minimum [57]. Carotenoids are hydrophobic compounds present in plant leaves in chloroplasts as lipoprotein complexes and, in the case of carrot root and tomato fruit, in chromoplasts where they are deposited in crystalline form [58]. Heat treatment is able to disrupt the molecular linkages between carotenoids and proteins. For instance, it has been reported that boiling of fresh broccoli promotes the release of β-carotene from the matrix due to denaturation of carotenoproteins, leading to better extractability and higher concentrations in cooked samples [59, 60]. Similarly, boiling of fresh carrots resulted in a slight increase of 14% of β-carotene content compared with the raw ones [60].

The role of heating for carotenoid bioavailability has been reported in human dietary intervention studies. In a human cross-over study, the daily consumption of processed carrots and spinach over a 4-wk period produced a plasma β-carotene response that averaged three times that associated with the consumption of the same amount of β-carotene from these vegetables in the raw form [61]. This finding was recently confirmed in a human intervention study, demonstrating that the rate of β-carotene absorbed from a single portion of cooked carrots is significantly higher (two-tailed *t*-test) than the rate values obtained by ileostomy volunteers

eating raw carrot meals ($p = 0.048$). The absorption of an oral dose of 15 mg β-carotene provided in a raw carrot meal was 41.4 ± 7.4%, whereas absorption of β-carotene from a cooked carrot meal reached 65.1 ± 7.4% [62]. Similarly, the bioavailability of lycopene, of which the major dietary sources are tomato and tomato products, has been shown to be much higher from processed tomato products (*e.g.* tomato paste) as compared with fresh tomato in human dietary intervention studies both after acute and after chronic consumption [63, 64]. However, different results were obtained after the administration of a test meal containing fresh or domestic cooked cherry tomatoes in human [65]. Among the reasons discussed by the authors to explain the lack of significant changes in carotenoid plasma concentrations in volunteers, the short cooking period (only 15 min) was mentioned.

The influence of food matrix on carotenoid bioavailability is not the same among carotenoids. Indeed, the relative bioavailability of lutein from spinach is greater than that of β-carotene (*i.e.* 67 and 14%, respectively) and less affected by the food matrix [66]. This emerged from a human dietary intervention study where differently processed spinach products were investigated. It was observed that the bioavailability of lutein from spinach was higher than that of β-carotene and that enzymatic disruption of the matrix (cell-wall structure) enhanced the bioavailability of β-carotene from whole leaf and minced spinach, but had no effect on lutein bioavailability [66]. The release of lutein into an aqueous environment is probably higher than that of β-carotene because of its lower lipophilicity compared with β-carotene [67].

In addition to light and the presence of triplet sensitizers (*e.g.* chlorophyll), heat treatment also promotes the isomerization of carotenoids in foods, from trans to cis isomeric forms, and the degree of isomerization is directly correlated with the intensity and duration of heat processing [61]. Fresh sweet potatoes, carrots and tomatoes contain negligible quantities of *cis*-β-carotene. However, after canning, the proportion in these vegetables was found to be substantially increased [68]. Usually, cis-isomers are less stable and have lower melting points than their all-trans counterparts, due to a decreased tendency to crystallization [69]. Moreover, 13-*cis*-β-carotene and 9-*cis*-β-carotene possess lower relative provitamin A capacity than all-*trans*-β-carotene (53 and 38%, respectively). Apart from reduced provitamin A activities, trans-cis isomerization also affects bioavailability and antioxidant capacity of carotenoids. Several human studies indicate that in the case of β-carotene, the all-trans-isomer is absorbed preferentially to the cis-isomers [69].

Experimental results indicate that, in contrast to β-carotene, lycopene remained relatively resistant to heat-induced geometrical conversion during typical food processing of tomatoes and related products [70]. Nevertheless, serum and tissue lycopene consist of between 50 and 90% cis-lycopene isomers. This observation has led to the hypothesis that cis-isomers of lycopene are produced during food processing, cooking or digestion and are more bioavailable [71]. The improved absorption of lycopene cis-isomers is hypothesized to result from greater solubility in mixed micelles, a lower tendency to aggregate and the shorter length allowing the molecules to ''fit'' into micelles with greater ease [72]. This hypothesis has been recently confirmed in a human dietary intervention study in which a significant increase in lycopene absorption was observed when tomato sauce was enriched in cis-isomers by heat treatment [73]. The additional heat treatment of the tomato matrix, enhancing availability of lycopene to digestive and absorptive processes, could also have contributed to this result. However, the results further suggest that preferred absorption of cis-lycopene isomers alone cannot explain the dramatically increased proportion of lycopene cis-isomers in biological samples upon heating, an observation that probably reflects an additional *in vivo* isomerization from all-trans- to cis-isomers. The authors conclude that, based on these findings, tomato-based food products could be manipulated by temperature processing to favour the formation of specific isomer patterns to improve lycopene bioavailability and possibly health benefits.

In addition, the key role of heat treatments in the carotenoid bioavailability emerges indirectly from a recent cross-sectional study in which vitamin A and carotenoids status and related food sources were evaluated in raw food diet adherents in Germany [74]. Fruit and vegetable consumption by raw food diet adherents was higher (approximately 1800 g/day, mainly fruits) than the average consumption of the general population in the USA (391 g/day) [75] or the recommended fruit and vegetable consumption (400–800 g/day) [76]. The raw food diet adherents showed normal vitamin A status and achieved favourable plasma β-carotene concentrations as recommended for chronic disease prevention. However, despite the high intake of plant foods, they showed low-plasma lycopene levels, demonstrating that processing is an important factor in determining lycopene plasma concentration.

3.2.2 Glucosinolates

Glucosinolates occur in Brassica vegetables and these compounds are considered to be health promoting because of the isothiocyanates derived from them [77]. The latter are potent inducers of Nrf2, a transcription factor involved in the cell protection against oxidative stress. Isothiocyanates need to be cleaved from their glucosinolate precursors to exert activity [77–79]. This is mediated by the plant-derived enzyme myrosinase that is sequestered in myrosin cells in the intact plant. Only upon injury of the plant during harvesting, food preparation and mastication, the myrosinase comes into contact with the glucosinolates to liberate the active compounds. Verkerk and Dekker [77] give an extensive review of the fate of glucosinolates in the food chain, including the effect of industrial as well as home cooking processing. Glucosinolates themselves are affected

by heat [80], as well as the enzyme myrosinase. Myrosinase is inactivated by heating but is also present in gut microbiota [81]. Thus, for glucosinolates to be converted/cleaved into active isothiocyanates the plant cells need to be damaged to liberate myrosinase. Some studies demonstrated that among cooking methods steaming entails an increase of glucosinolates present in broccoli probably due to the disintegration of plant tissue upon heat that favours the release of these compounds from the cell walls [60, 82]. On the contrary, glucosinolates, which are water-soluble compounds, are usually lost during conventional cooking methods (*e.g.* boiling) because of leaching into surrounding water. In that respect, microwaving may be a better alternative because of the absence of cooking water [77]. Dekker *et al.* [83] and Dekker and Verkerk [84] have presented mathematical models to predict the fate of glucosinolates over the food chain, including processing. They also discuss the consequences of a food chain approach for epidemiological studies on the role of phytochemicals.

3.2.3 Polyphenols

Among phytochemicals present in plant foods, polyphenols are a huge group of secondary metabolites of plants and are generally involved in defence against oxidative stress, ultraviolet radiation or aggression by pathogens. These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made among the phenolic acids, flavonoids, stilbenes and lignans. In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids [85] and they are generally dissolved in vacuoles and the apoplast of plant cells [56]. Polyphenols are highly reactive compounds and good substrates for various enzymes, including polyphenoloxidases, peroxidases, glycosidases and esterases. They undergo numerous enzymatic and chemical reactions during post-harvest food storage and processing. Although the occurrence of such reactions and their roles in the development or degradation of food quality is well documented, the structures of the resulting products are still poorly understood and few studies are available on the effects of thermal treatment on these compounds as well as on their bioavailability in cooked foods.

The consequences of food processing on the fate of polyphenols may differ greatly in relation to their concentration, chemical structure, oxidation state, localization in the cell, possible interactions with other food components and type of thermal processing applied. Food processing may be responsible for a decrease, increase or minor changes in content and in functionality of polyphenols. Zhang and Hamauzu [86] observed a loss of phenolic compounds for boiled and microwaved broccoli, and similar results were reported by Sahlin *et al.* [87] for fried tomatoes and by Ismail *et al.* [88] for blanched and boiled spinach. The cooking of vegetables, causing softening and breaking of cell-wall components with subsequent release of the molecules, determines the extent of leaching of water-soluble polyphenols into the surrounding water during water-cooking methods (*e.g.* boiling) or may destroy polyphenols by high temperature as in the case of frying. However, different cooking methods may differently affect the content of polyphenols. Applying three domestic cooking methods (*i.e.* boiling, steaming and frying), it has recently been demonstrated that for carrots, zucchinis and broccoli, there was a significant decrease in phenolic acids and flavonoids in boiled and fried vegetables, whereas steaming preserves polyphenols [60]. Similar results were obtained on four vegetables belonging to the cruciferous vegetable family cooked by steaming, microwaving and boiling: steamed vegetables showed the highest total phenolic value, following by boiled and microwaved ones [89].

On the contrary, the content and the functionality of some phenolic compounds might also increase during heating. Some studies described the influence of thermal treatments on the conversion of caffeoylquinic acids into other isomers. Recently, Takenaka *et al.* [90] found an increase of 3-caffeoylquinic, 4-caffeoylquinic, 3,4-di-caffeoylquinic and 4,5-di-caffeoylquinic acids on boiled sweet potatoes due to the isomerization of 5-caffeoylquinic and 3,5-di-caffeoylquinic acids, the major phenolic compounds of this vegetable. The authors hypothesized that heat is one of the factors causing the isomerization. When the plant is heated, the cell and organelles can sustain damage, causing phenolic compounds and polyphenoloxidase to meet and react, and parts of the phenolic compounds are isomerized. Thus, the behaviour of phenolic compounds during cooking of the sweet potato is influenced by heat; they can react with the enzyme until the enzyme is inactivated by temperature. A significant increase (ranging from 66 to 94%, depending on the cooking method applied) in total caffeoylquinic acids was also observed in artichoke after boiling, steaming and frying procedures [91]. This enhancement of polyphenolic content was the result of both isomerization and hydrolysis events, leading to a substantial re-distribution of phenolic acid concentrations. This behaviour determined a consistent increase of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids, particularly in steamed and fried samples, and of 3,5- and 4,5-di-*O*-caffeoylquinic acids, the concentration of which is very low in the raw product and strongly enhanced during processing. Accordingly, Slanina *et al.* [92] observed an increased caffeoylquinic acids isomer content in the leaves of *Cynara cardunculus* L. (artichoke thistle) caused by the intramolecular trans-esterification of 5-*O*-caffeoylquinic and 1,5-di-*O*-caffeoylquinic acids promoted by the high temperatures. Higher polyphenol content in blanched artichokes compared with raw samples was also reported [93, 94].

Beside isomerization and intramolecular trans-esterification, chlorogenic acid (CGA) can undergo hydrolysis during

heating, resulting in a consequent increase in caffeic acid. This effect has recently been observed during domestic heating (*e.g.* steaming and frying) of carrots in which a significant increase in caffeic acid content was reported [60]. As far as polyphenol bioavailability is concerned, it is well known that polyphenols are poorly adsorbed, highly metabolized or rapidly eliminated [85]. Whether heat processing can affect the bioavailability of polyphenols is not yet known. To our knowledge, the only study that explored the effect of processing on the polyphenolic bioavailability is that of Bugianesi *et al.* [65], who carried out a human cross-over study to evaluate the effect of domestic cooking on the bioavailability of antioxidants (*i.e.* carotenoids and polyphenols) after the administration of a test meal containing cherry tomatoes. The results demonstrated that plasma concentrations of naringenin and CGA increased significantly with respect to baseline ($p < 0.05$) after administration of cooked cherry tomatoes, but not after administration of raw cherry tomatoes. Naringenin is trapped in the cutin matrix of the membrane of the ripe fruit where it strongly interacts with insoluble polyesters that are constituents of tomato fibre. As stated by the authors, the mechanical and heat treatments may provide the energy necessary to break the interactions, improving naringenin bioaccessibility *in vivo*. In a recent study of the same group [95], an indirect role of domestic cooking on the bioavailability of artichoke polyphenols was demonstrated. Indeed, in steamed artichoke heads, the content of mono- and di-caffeoylquinic acids was higher than in the uncooked vegetable. The consumption of cooked artichoke in humans resulted in a significant increase in plasma levels of hydrocinnamic acids (*i.e.* CGA, caffeic, ferulic, dihydrocaffeic and dihydroferulic acids), demonstrating for the first time the bioavailability of metabolites of these acids.

3.2.4 Total antioxidant activity

Along with changes in the content of bioactive compounds, some authors showed that various heat treatments are able to increase the total antioxidant capacity (TAC) of vegetables. The antioxidant activity of a compound can either be a radical scavenging activity based on single electron transfer or be a hydrogen abstraction reaction, or based on the induction of cell-signalling pathways that lead to the induction of Nrf-2. The TAC is an index that describes the ability of antioxidants present in food to scavenge preformed free radicals. The TAC depends on the synergistic and redox interactions between the different molecules present in the food [96]. Obviously, different cooking methods have different effects on TAC. In a recent study exploring the effect of domestic cooking methods on TAC of 17 vegetables, boiling generally resulted in positive TAC changes, a general negative effect was observed with pan-fried vegetables, whereas deep frying produced an increase in TAC of potato, artichoke and aubergine, but a TAC reduction of mushroom and onion [97].

Miglio *et al.* [60] reported an overall increase in TAC values of all cooked vegetables (*i.e.* carrots, zucchinis and broccoli). In particular, in the case of carrots and zucchinis, frying resulted in the highest TAC increase, followed by boiling and steaming, whereas steamed broccoli showed the highest TAC values compared with vegetable cooked by other methods. Similarly, steaming led to an increase in antioxidant capacity of four vegetables belonging to the cruciferous vegetable family: the effect was modest for cabbage (13%), but in cauliflower, broccoli and choy-sum the TAC values more than doubled after 5 min of steaming, compared with the uncooked vegetable [89]. The positive effect of steaming was observed also in the case of artichoke; indeed, this cooking method resulted in the highest TAC increase of artichoke compared with the other two methods (*i.e.* boiling and frying) [91]. In two studies, Dewanto *et al.* [98, 99] demonstrated that thermal processing was able to enhance the nutritional value of tomatoes, by increasing the bioaccessible lycopene content and the TAC values, and of sweet corn by 44%, despite the loss of vitamin C. Accordingly, Turkmen *et al.* [100] stated that moderate heat treatment might be considered a useful tool in improving health properties of some vegetables since they observed an increase in TAC of pepper, green beans, broccoli and spinach during cooking procedures (*i.e.* boiling, steaming and microwaving).

The observed increase in TAC values in cooked vegetables, extremely high in some cases [91], cannot be merely attributed to a release of antioxidant compounds from the plant matrix. The trans-cis isomerization of carotenoids, the intramolecular trans-esterification of polyphenols as well as their polymerization, leading to the formation of compounds which are not yet identified but probably showing very high antioxidant activity, promoted by high temperatures cannot be ruled out. Moreover, the antioxidant properties of polyphenols may change with respect to their oxidation state. In fact, although enzymatic and chemical oxidations of polyphenols are generally responsible for a decrease in their antioxidant properties, recent findings have suggested that partially oxidized polyphenols can exhibit higher antioxidant activity than the corresponding non-oxidized forms [101]. This was attributed to the increased ability of partially oxidized polyphenols to donate a hydrogen atom from the aromatic hydroxyl group to a free unpaired electron. Moreover, during heating, the increases of TAC values might be due to the formation of MRPs that possess antioxidant activity (see the following section).

3.3 Bioactive effects of processed foods due to the MRPs

The Maillard reaction is the prime example of a processing-related reaction with large consequences. Many reviews on the Maillard reaction are available and the chemistry will not be discussed here. Rather, we focus on metabolic effects.

The first metabolic transit data of fructoselysine as an Amadori compound in human volunteers have been reported by Erbersdobler *et al*. [102], showing that urinary excretion of orally administered fructoselysine is about 3% and fecal excretion about 1%. These data confirmed a hypothesis made 16 years before [103], when the intestinal microflora was thought to degrade Amadori products. This hypothesis was finally proven by Wiame *et al*. [104] who discovered the enzyme fructoseamine-6-kinase, which enables *Eschericha coli* and other species to metabolize Amadori products.

In the past 20 years, a few more studies focused on the metabolism of food-derived MRPs (for review, see [105–108]), all demonstrating an absorption rate of low-molecular-weight MRPs of up to 30%, depending on the structure and the administered dose. Bergmann *et al*. [106] used positron emission tomography to monitor biodistribution and elimination of radiofluorinated carboxymethyllysine (CML), a compound formed in the Mailllard reaction. The authors found that the compound was rapidly absorbed, transported into circulation and rapidly excreted through the kidneys.

For high molecular weight melanoidins, [107, 108] reported a urinary excretion rate of a 10 000 Da fraction of a ^{14}C-labeled, at 100°C heated casein/glucose mixture of 4.3%, as opposed to a 27% urinary excretion of the administered dose for the low-molecular-weight fraction of less than 10 000 Da. Taken together, there is considerable evidence that low- and even high-molecular MPRs are being absorbed at least to some extent, albeit metabolic transit data for an MRP structure showing an antioxidant activity *in vivo* are still lacking. For heat-treated foods, however, numerous intervention trials in animals and humans have been performed aiming at increasing the antioxidant capacity in plasma or tissues.

Numerous publications report on the antioxidant capacity of MRPs. In most cases, either MRPs prepared by heating reducing sugars with amino acids or proteins, or heat-treated foods, such as *e.g.* coffee, were tested for their antioxidant activity in cell-free systems (*in situ*), in cell-based assays (*in vitro*), or in animal-feeding studies or human intervention trials (*in vivo*). Also, a few *ex vivo* experiments where blood, namely LDL isolated thereof, from healthy volunteers was treated with MRPs are reported.

Results from cell culture experiments in which heat-treated foods, food-derived MRPs or heated MRP model mixtures of reducing sugars and amino acids/proteins or isolated compounds thereof are tested for their antioxidant activity cannot always be transferred to in *vivo* situations, especially when pharmacokinetic data are missing. Cell-free *in situ* tests might have some merit as indicators for the food's oxidative stability and shelf-life.

The formation of MRPs with antioxidant activities in model reactions between reducing sugars and amino acids *in situ* has been demonstrated in [109] who analysed model browning reactions between the reducing sugars glucose or fructose and cysteine or glutathione. The radical scavenging activity measured as Trolox equivalents increased after heat treatment, although model mixtures heated for 4 h 20 min showed higher Trolox values than those heated for 14 h and no correlation to browning was found. For the glucose/cysteine model mixture, the antioxidant activity was also demonstrated in a bacterial-based assay in hydrogen peroxide exposed salmonella. In this experiment, treatment with the glucose/cysteine mixture heated at 103°C for 14 h prevented hydrogen peroxide-induced mutations. This result was demonstrated only without metabolic activation. When, in the same experimental setting, salmonella were pre-incubated with a metabolic activation system using liver microsomes, treatment of hydrogen peroxide exposed salmonella with model mixtures heated for shorter times such as 4 h 20 min resulted in increased mutations, indicating a pro-oxidative effect. These results clearly demonstrate an antioxidant effect of MRPs formed from the reaction of glucose and cysteine *in situ*. The fact that metabolic activation by liver microsomes *ex vivo* abolished this effect indicates that an antioxidant activity measured *in situ* and, depending on the reaction conditions, also *in vitro* might not be transferable to *in vivo* situations.

Dittrich *et al*. [110] analyzed MRPs from model reactions for their capacity to prevent copper-induced oxidation of LDLs isolated from human volunteers. MRPs were generated by heating glucose with glycine, lysine or arginine. All of the heated mixtures were more or less equally effective as ascorbic acid in preventing LDL oxidation. Two individual amino reductone compounds, supposed to be formed by the Maillard reaction from glucose, were also tested for their antioxidant capacity and were found to be less effective than ascorbic acid at equimolar concentrations. However, the question whether these two compounds were the active principles in the heated glucose/amino acid mixtures was not answered since the formation of these two compounds was not shown.

The most recent evidence that food-derived MRPs elicit antioxidative properties in human has been reported by Dittrich *et al*. [111]. The authors administered diets poor and rich in MRPs to eight healthy volunteers in a weekly turn for 3 wk. The diet rich in MRPs contained dark beer, bread crust and roasted coffee and led to a statistically significant increased oxidative stability by 35.5% of isolated LDL against copper-induced oxidation. Although the experimental design of this study does not allow to draw conclusions about the active principles, the fact that administration of severely heat treated foods *versus* mildly heat treated foods resulted in an increased oxidative stability of LDL strongly supports the hypothesis that dietary MRPs exhibit antioxidant properties *in vivo*.

Coffee is one of the most commonly studied foods for the formation of MRPs and their biological effects, probably due to the intense heat treatment of the raw coffee beans during roasting and its widespread consumption. Given the fact that most of the naturally present phenolic antioxidants are degraded during roasting, the overall antioxidant activity of

roasted coffee is mainly attributed to high-molecular-weight MRPs, *i.e.* the melanoidins [112, 113]. Anese and Nicoli [114] studied the influence of some technological variables on the changes of the antioxidant capacity of ready-to-drink coffee brews and demonstrated a higher redox potential for brews prepared from dark roasted *versus* medium and light roasted coffee beans. When coffee beans are roasted with the addition of sugar (so-called torrefacto roasting), the antioxidant activity is even higher than that of conventionally roasted coffees, probably due to increased formation of MRPs [115].

Next to the roasting conditions, Lopez-Galilea *et al.* [115] demonstrated that also the brewing procedure, by applying different temperatures, hydrostatic pressures and using different ratios of coffee powder to water, has an impact on the antioxidant activity of the coffee brews: Espresso coffees showed the highest antioxidant activity, measured by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, over mocha, plunger (french press) and filtered coffee.

When coffee brews were administered to human volunteers, the TAC of the plasma increased shortly after consumption. Natella *et al.* [116] reported a statistically significant 7% increase in the antioxidant capacity of plasma samples taken from healthy volunteers 2 h after intake of 200 mL regular coffee beverage. These results are in accordance with those reported by Esposito *et al.* [117], demonstrating an increase of the main water-soluble antioxidant in the plasma, glutathione, after administration of five cups of regular coffee *per* day to healthy volunteers for 1 wk.

CGA undergoes numerous transformations *in vivo*, resulting in the formation of derivatives that might be not as effective as the parent compound [118]. Trigonelline is degraded into *N*-methylpyridinium during roasting [119], leading to relatively low concentrations in the coffee beverage. Interestingly, strong antioxidant effects of *N*-methylpyridinium and a lyophilized de-caffeinated coffee brew have been described by Somoza *et al.* [120] following a 10-day administration to rats. In both experimental groups, plasma TAC and also α-tocopherol concentrations were significantly increased compared with non-treated controls. Next to *N*-methylpyridinium, there might be other coffee-derived antioxidants yet not identified, *e.g.* melanoidins [121]. Although melanoidins comprise high-molecular-weight compounds, unlikely to be absorbed to exert antioxidant activities *in vivo*, these compounds might be degraded upon intestinal digestion, resulting in absorbable structures with antioxidant effects. Rufian-Henares and Morales [122] reported that low-molecular-weight compounds released by gastrointestinal digestion from a coffee melanoidin fraction with a molecular weight >10 kDa exerted high antioxidant activities, even higher than those compounds bound to melanoidins by ionic interaction. Thus, gastrointestinal digestion is able to modify coffee melanoidins to some extent, either by modifying/releasing the ionic interaction with melanoidins and/or by the generation of new structures from the melanoidin skeleton after enzymatic treatment.

Coffee surrogates are often made of barley that also contains MRPs after roasting. Papetti *et al.* [123] compared effects of fractionated water extracts prepared from roasted and natural barley for their antioxidant activity by means of the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay and the inhibition of linoleic acid peroxidation in hepatic microsomes of rats. The highest antioxidant activity was attributed to a MW fraction between 1000 and 2000 kDa of roasted barley. Based on the elemental composition, the authors hypothesized that the active compound was a polysaccharide-containing melanoidin.

Beer is also rich in MRPs and melanoidins due to the malting and brewing process. The antioxidant properties of beer were reported to correlate both with the level of total polyphenols and with the content of melanoidins. Rivero *et al.* [124] studied the antioxidant capacity of blond, dark and alcohol-free lager beers, showing that the oxidative damage of calf thymus DNA resulting in the formation of 8-OH-dG after induction by copper/ascorbic acid was prevented by co-incubation with the various beers, of which the darkest beer was most effective. When malt (5% w/w) was fed to rats for 15 days, plasma contents of tocopherol increased by 14%, whereas the levels of thiobarbituric acid reactive substances were decreased [125]. In this study, the antioxidant effect of malt was demonstrated *in vivo* for the first time. Further investigations by Somoza *et al.* revealed that one of the key antioxidants in malt is pronyl-lysine, earlier identified as the most effective antioxidant formed in bread crust by means of cell culture experiments and an animal feeding trial [126]. Main systemic effects of dietary malt, bread crust and pronyl-BSA were demonstrated to be enhanced antioxidant capacity and increased expression of chemopreventive enzymes.

Cocoa, like coffee, is thermally processed and contains MRPs as well as numerous bioactive flavonoids, such as epicatechin. A recent study on potential beneficial effects of cocoa melanoidins could not identify significant antioxidant, antimutagenic or pro-mutagenic activity [127]. No effects, neither pro- nor antioxidant, were observed with water-soluble fractions of green and roasted cocoa in Salmonella strains TA100 and TA98, with or without metabolic activation using liver microsomes. A weak indication for a pro-oxidative effect in Salmonella strain TA102 was observed with the 5–10 kDa MW fraction of roasted cocoa at the highest concentration tested (10%), the only sample that reached statistical significance. The highest antioxidant scavenging activity was found in the 10–30 kDa fraction of roasted cocoa. All other MW fractions (<5, 5–10, 10–30, >30 kDa) prepared from non-roasted and roasted cocoa showed similar curves for growth inhibition of bacterial strains (*E.coli*, *Enterobacter cloacae*, *Bifidobacteriaum* Bb-12 and B 7.1), with some inhibition at the highest concentration tested (100 μg/mL). In contrast to these results from *in vitro* experiments, Jalil *et al.* [128] performed an animal study on the effects of cocoa extract which was given to obese-diabetic rats in a dose of 600 mg/kg body weight for 4 wk. The results indicated that plasma contents of 8-isoprostane as oxidative stress biomarker were significantly

($p<0.05$) reduced, whereas the catalytic activity of the antioxidant enzyme superoxide dismutase was significantly enhanced after cocoa supplementation. These results are in line with those reported by Ramiro-Puig *et al.* [129] who also showed an increased superoxide dismutase activity and antioxidant capacity in plasma and tissues following natural cocoa supplementation (4 or 10% food intake) in weaned rats for 3 wk. However, whether the *in vivo* antioxidant effects of cocoa are caused by flavonoids or MRPs has to be identified in future studies.

4 Evaluation of potential health benefits

4.1 General considerations concerning epidemiological studies

Depending on the type of epidemiological study (cross-sectional or prospective cohort studies, randomized controlled trials), different issues need to be taken into consideration. A great concern and reason for conflicting outcomes of epidemiological studies in humans is related to confounding factors. For instance, coffee drinking is part of an individual's lifestyle and can be directly linked to other lifestyle factors that may influence the outcome, such as alcohol intake, smoking, stress, sedentary lifestyle/lack of physical activity and dietary habits. Heavy coffee drinking is also associated with lower socioeconomic status. On the other hand, people may have switched to decaffeinated coffee or avoid coffee because of the perception that caffeinated coffee is part of an unhealthy lifestyle [130–132]. Misclassification of food intake can occur depending on the methods used for assessment, variability in portion size but also due to the variability of food components depending on the type of processing and preparation.

Polymorphisms in the metabolism of food constituents may play an important role, as well as dietary habits or medication that potentially influence the activity of enzymes involved in the metabolism of food components (or *vice versa*). Reverse causation is another factor to be taken into account. For instance, an association may be identified between low coffee intake and increased blood pressure. Other than a causal relationship (low coffee intake causes high blood pressure) it is likely that a person who knows about its high blood pressure or another health problem avoids or reduces coffee intake [130–132].

Epidemiological studies available to date mostly deal with specific foods, nutrients, micronutrients or even contaminants present in the diet and their association with human health (*e.g.* whole grain, red meat and aflatoxin). Little information is available on how people cook foods before eating. Therefore, specific information on process-related changes and their effects on human health is rarely addressed in food intake assessments, mainly because these changes are difficult to quantify. No information on processing is generally available on some food items, for instance breakfast cereals, potatoes and bread, epidemiological evidence for which will not be discussed in this review.

In the following sections, we discuss the epidemiological evidence related to coffee, cocoa, beer and tomato, for which some information related to processing is available, as shown in the previous section. It is known that nutritional quality of such food items is strongly affected by processing. The roasting process of coffee and cocoa strongly affects which compounds are going into the cup: in fact, many compounds are formed, modified, destroyed or remain unchanged upon roasting [133]. Dark-coloured beers (*e.g.* dark and stout) are produced from dark-coloured malts that have been kilned for a longer period and at a higher temperature than lager/pilsner malt. In dark-coloured beers, the kilning process of malt produces compounds that make them different from the lager beers.

As already discussed in the previous section, the bioavailability of protective compounds in tomato is strongly affected by processing. In the majority of epidemiological studies on health effect of tomato, relative risks for the combined intake of raw and cooked tomato products are reported. However, in few of them information about processing is included in the analysis. The effect of uncooked tomato should be described separately from that of cooked tomato, making tomato a perfect case for considering the effect of processing on chronic disease risks.

4.2 Coffee

Epidemiological studies addressing the health effects of coffee were originally initiated due to the presumed adverse effects. Coffee consumption was associated with increased risk factors for cardiovascular disease (CVD) including increased blood pressure, blood cholesterol and plasma homocysteine. There is some evidence that risk factors such as serum cholesterol (total and LDL cholesterol) can be increased by coffee consumption. The effect is strongly depending on the type of brew, being associated with boiled, non-filtered coffee that contains significant amounts of the coffee diterpenes cafestol and kahweol [134, 135]. The increase of blood homocysteine through coffee intake has also been discussed as a risk factor for coronary heart disease; however, epidemiological study results are inconclusive [132, 136].

However, more and more recent epidemiological studies did not confirm some of the presumed health risks, on the contrary, some seem to indicate even beneficial effects [137]. There is good epidemiological evidence that regular intake of coffee may decrease the risk for type 2 diabetes [132, 138]. Most epidemiological studies on the association between high coffee intake and various diseases such as coronary heart disease (arrhythmia, stroke and infarction), cancer or bone health revealed conflicting results, often depending on the type of study used (case control or prospective cohort studies), with the majority showing no association after correction for various confounding factors like smoking [131, 136, 137].

Recommendations to limit coffee intake are maintained today, for special risk groups, like pregnant women or women of childbearing age, children and older adults, whereas for normal subjects, mounting evidence indicate possible beneficial effects of coffee consumption.

4.3 Bioactive compounds in coffee that may affect human health

Caffeine is a natural alkaloid found in coffee beans, tea leaves, cocoa beans and other plants. Of these, coffee has the highest content, with varying amounts in the final beverage depending on the type of preparation of the coffee brew. For instance, brewed coffee was reported to contain 72–130 mg of caffeine in one cup in the US, whereas the amount in espresso was lower ranging from 58 to 76 mg in one cup [139].

The diterpenes cafestol and kahweol are reported to be responsible for the increase in serum total and LDL cholesterol levels. Observational studies have indicated that the type of preparation of the brew may influence the concentration of the diterpenes in coffee. An association between coffee intake and increased serum cholesterol levels was most evident in Scandinavian countries, where coffee was brewed preferably by boiling, whereas an association was less evident in countries where filtered coffee was preferred. The diterpenes, which are present in coffee oil, are extracted to the brew, but were found to be trapped when filtering through paper filters [131].

CGAs are a family of esters between quinic acid and *trans*-cinnamic acid (caffeic acid), which are an important group of dietary polyphenols. Coffee is a rich source of these dietary antioxidants. Roasting affects the CGA composition and also generates some derivatives with potential beneficial activity [140]. Coffee is the most important source of CGAs in the human diet. Part of the CGAs is absorbed in the human gastrointestinal tract and metabolized in the body and part of it reaches the colon, where CGAs are metabolized by the gastrointestinal microflora. The antioxidant capacities of CGAs are well established *in vitro*; however, due to their extensive metabolism, the biological and antioxidant effects in humans at usual dietary intakes remain to be established [141].

4.4 Potential beneficial effects of coffee consumption

4.4.1 Type 2 diabetes mellitus

Several epidemiological studies have found an inverse association between coffee consumption and type 2 diabetes. The association seems to be consistent between different populations and after adjustment for various confounders. Furthermore, a dose dependency was established with a greater reduction of the risk with higher consumption across gender. Consumption of four or more cups of coffee was generally associated with a significantly reduced risk, with more variable results for lower levels of consumption. There are indications that this holds true also for decaffeinated coffee, but not for tea, indicating that components other than caffeine are responsible for the effect. These associations seem to be supported by experimental studies in animals and humans relating coffee consumption to beneficial effects on insulin sensitivity, glucose homeostasis and the blood glucose response. On the contrary, in short-term metabolic studies, caffeine intake was shown to increase insulin sensitivity and to exaggerate the blood glucose response to glucose loads, which was hypothesized to be related to the antagonistic effects of caffeine on the adenosine receptor. Some beneficial effects are discussed of decaffeinated over caffeinated coffee, regarding postprandial glucose response, fasting plasma insulin concentrations and insulin sensitivity. However, based on the available evidence, long-term consumption of caffeinated coffee appears to decrease rather than increase the risk for type 2 diabetes [132].

4.4.2 Prevention of Parkinson's disease

Both case control and prospective cohort studies have found inverse associations between coffee and caffeine intake and Parkinson's disease in men. In women, the association was initially not evident, but it turned out that an association was found in women not taking postmenopausal hormone replacement therapy, whereas an association was absent in women undergoing this therapy. The association was also present with caffeine intake from sources other than coffee. One study even found an increased risk for Parkinson's disease from high coffee intake (>6 cups) in women under hormone replacement therapy. As a possible mechanism, the co-localization in the brain of adenosine receptors that are restricted to the striatum, the target receptors of caffeine and the target of the dopaminergic neurons that degenerate in Parkinson's disease is discussed. Animal studies suggested that blocking of adenosine receptors reduced chemically induced dopaminergic neurotoxicity in an animal model of Parkinson's disease [131].

4.4.3 Cancer

The strongest epidemiological evidence relating coffee and reducing risk of cancer pertains to colorectal and liver cancers [142].

4.4.3.1 Colorectal cancer

An inverse association between the coffee consumption and the risk of colorectal cancer has been found in several case–control studies, but an association was not

consistent in prospective cohort studies. A recent meta-analysis, including 12 prospective cohort studies from the US, Europe and Japan showed no significant effect of coffee consumption on colorectal cancer risk. However, an indication of an inverse association was found in women (consuming >4 cups/day) that was slightly stronger when controlled for the confounding factors smoking and alcohol, and in studies with shorter follow-up time [143].

4.4.3.2 Hepatic injury, cirrhosis and hepatocellular carcinoma

Chronic liver inflammation can result in cirrhosis, and progressive formation of fibrotic scar tissue may eventually result in hepatocellular carcinoma. The most common causes for cirrhosis in developing countries are alcohol abuse and viral hepatitis B and C infection. A number of cross-sectional studies have found an inverse association between coffee intake and serum γ-glutamyl-transferase, a marker for hepatic injury and alcohol intake. An inverse association was also identified for alanine aminotransferase, a marker for hepatic injury. However, a reversed causality was put forward to explain this effect; since people experiencing adverse effects after drinking coffee due to liver injury might abstain from drinking coffee (caffeine metabolism is inhibited in cirrhotic liver resulting in adverse effects). In one study, the association persisted when correcting for this possibility. The risk of liver cirrhosis was consistently inversely correlated with the consumption of two or more cups of coffee in several case control and prospective cohort studies. This was also observed for hepatocellular carcinoma and for heavy alcohol drinkers [144], whereas the strongest inverse association was found in people infected with hepatitis C virus. The mechanism by which coffee prevents hepatocarcinoma is not well established, but the induction of phase II liver detoxification enzymes and increased glutathione levels by the coffee diterpenes cafestol and kahweol is discussed [131].

4.4.3.3 Stroke

A recent study reports an inverse association between coffee consumption and stroke in women [145]. After adjustment for factors such as age, smoking, menopausal status, hormone replacement therapy, physical activity, alcohol intake, aspirin use and dietary factors, the association persisted. This was also the case for further adjustment for high blood pressure, hypercholesterolemia and type 2 diabetes. Decaffeinated coffee intake also showed a trend towards lower stroke risk.

4.5 Processed barley

Processed barley is employed to prepare coffee substitutes and to produce beers. Up to date, epidemiological and interventional studies have not addressed the health effects of barley coffee, whereas more information is available on beer consumption. Unfortunately, most of the studies on beer did not consider the type of beer (lager, sprout, dark *etc.*). As a consequence data, interpretation was mainly addressed on the effect of alcohol and in some cases discussion was focused on the polyphenols moiety mainly to explain the differences observed between wine and beer.

After wine intake was suggested as a possible explanation for the lower than expected CHD mortality rates in France (the French Paradox), many studies have dealt with the question of whether different alcoholic beverages are equivalent in their ability to protect against CHD or whether a specific beverage might offer a greater protection. Evidence obtained from a meta-analysis of 26 observational studies [146] indicates an average significant reduction of 32 and 22% of overall vascular risk associated with drinking wine and beer, respectively.

Regarding the effect of beer in human intervention studies, controversial results have been obtained. Imhof *et al.* [147] evaluated the effect of different alcoholic beverages (red wine, beer and ethanol and the same beverages de-alcoholized) on *ex vivo* migration of monocytes, which represents a crucial step in early atherosclerosis, in a human open randomized intervention study over 3 wk. Results demonstrated a statistically significant inhibitory effect after ethanol and de-alcoholized red wine consumption, but no effect for consumption of beer, de-alcoholized beer and red wine. However, in a randomized controlled crossover trial performed recently by the same group [148], an increase of adiponectin concentration was observed among women after consuming red wine (29.8%, $p<0.05$), whereas this was different among men after ethanol solution (17.4%, $p<0.05$) and consuming beer (16.1%, $p<0.05$). The authors concluded that moderate amounts of ethanol-containing beverages increased adiponectin concentrations, but sex-specific effects might depend on the type of beverage consumed. An influence of gender on the effect of beer consumption was reported also by a Spanish group. In several human intervention longitudinal studies carried out by these researchers, moderate consumption of beer during a month was associated, especially in women, with favourable changes on the blood lipid profile (*i.e.* increase of HDL-cholesterol, erythrocytes, haematocrit and mean corpuscular volume) [149], on parameters describing the non-specific immunity (*i.e.* increase of white blood cell counts and phagocytic and oxidative burst activity) [150] and on the immune function (*i.e.* increase of $CD3^+$ cells only in women, increase of IgG, IgM and IgA concentrations, IL-2, IL-4, IL-10 and IFN-γ cytokine production in both gender) [150].

Components produced during the kilning process at higher temperatures may be responsible for the higher *in vitro* anti-mutagenic activity of dark-coloured beer against a variety of mutagens, including heterocyclic amines, than that of pilsner-type beer [151]. However, single-cell gel

electrophoresis assay (comet assay) revealed that oral ingestion of pilsner-type and stout beers for 1 wk significantly inhibited DNA damage in the liver cells of male mice exposed to 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline, a heterocyclic amine. This demonstrates that anti-mutagenic effects of two beers *in vivo* were approximately the same, although the anti-mutagenic effect of the dark-coloured beers was stronger than that of pilsner-type beers in the *in vitro* experiments. This inconsistency may be caused by the difference in bioavailability of active components from both types of beer, as stated by the authors [151]. Similarly, at the high dose (1/2-diluted beer) both lager and dark beer significantly inhibited atherosclerosis compared with a control of 2% alcohol when these beverages were provided for 10 w to cholesterol-fed hamsters, an animal model of atherosclerosis [152]. Moreover, lager significantly decreased cholesterol and triglycerides, and both beers acted as *in vivo* antioxidants by decreasing the oxidizability of LDLs.

4.6 Tomatoes

In the past years, a large body of epidemiological studies has explored the relationship between the intake of tomato and tomato-based products on cancer risk. Based on the most extensive review of the epidemiological literature published by Giovannucci [153] 10 years ago, the intake of tomatoes and tomato-based products was consistently associated with a lower risk of a variety of cancers. Evidence was strongest for cancers of the lung, stomach and prostate gland and is suggestive for cancers of the cervix, breast, oral cavity, pancreas, colorectum and esophagus. More recently, the US Food and Drug Administration (FDA) received a petition for qualified health claims regarding tomatoes and the risk reduction of some forms of cancer [154]. Based on FDA's review of the strength of the total body of publicly available scientific evidence for the consumption of tomatoes or tomato-based food and reduced risk of cancer, the agency found that there was very limited evidence to support an association between tomato consumption and reduced risks of prostate, ovarian, gastric and pancreatic cancers. The reasons for such judgement, in disagreement with the observational evidence mentioned above, are due to: (i) mixed results found in case–control, case–cohort and ecologic studies evaluated in the case of prostate cancer; (ii) very limited credible evidence for other cancer sites. Moreover, the FDA found no credible evidence for an association between tomato consumption and a reduced risk of lung, colorectal, breast, cervical or endometrial cancer.

The majority of studies reported relative risks for the combined intake of raw and cooked tomato products. Only a few studies reported relative risks for raw tomatoes and cooked tomato products separately, suggesting stronger protective effects of cooked tomatoes than for raw tomatoes. Bosetti *et al.* [155] conducted a case–control study in Greece with 320 cases of prostate cancer and 246 controls. The intake of both raw and cooked tomatoes was inversely associated with prostate cancer risk, but the relationship for cooked tomatoes was stronger than for raw ones (odds ratios for the lowest *versus* the highest tertile of intake = 1.91, 95% confidence interval (CI) = 1.20–3.04 for cooked tomato and = 1.55, 95% CI = 1.00–2.52 for raw tomato). In a prospective cohort study, Giovannucci *et al.* [156] followed 47 365 men for approximately 12 years, during which 2481 cases of prostate cancer were identified. Among all the tomato sources measured, consuming one or more than one serving of tomato sauce *per* week was associated with a statistically significant decreased incidence of prostate cancer (RR = 0.80, 95% CI = 0.70–0.91). Tomato sauce intake was evaluated with the use of a 131 items food–frequency questionnaire that was administered at the beginning of the study and at 4-year intervals thereafter. These results confirmed an earlier reported association between tomato sauce and a reduced risk of prostate cancer in the same cohort [157]. A protective effect of tomato sauce was also observed in a case–control study in which the association between tomato and tomato-based food intakes and the risk of ovarian cancer among 549 case patients and 516 control subjects from the United States was explored [158]. This study found that those who ate tomato sauce two or more times *per* week had a statistically significantly lower risk of ovarian cancer (OR = 0.60, 95% CI = 0.37–0.99), whereas no association was found between intake of raw tomato and the risk of ovarian cancer (OR = 0.88, 95% CI = 0.50–1.54).

Less explored is the relationship between the risks of CVD and type 2 diabetes mellitus (DM) and the consumption of tomato and tomato-based products. In a cohort of middle-aged and older women from the United States, the RR of CVD for those consuming tomatoes or tomato juice suggested no clear association with either CVD or important vascular events [159]. Conversely, comparing women consuming ≥ 2 servings/wk *versus* no tomato sauce, the age- and treatment-adjusted RR of total CVD was 0.66 (p for linear trend = 0.008). Different results were obtained in the same cohort when the intake of tomato and tomato-based products was explored in relationship with the risk of DM [160]. On average, women consumed a total of 4.33 ± 3.22 servings of tomato-based food products *per* week, of which 2.18 were from tomatoes, 1.14 from tomato sauce, 0.57 from pizza and 0.44 from tomato juice. Women who consumed greater amounts of tomato-based food products had neither a significantly decreased nor a increased risk of type 2 DM and the associations for individual tomato-based food products were similar to the results for the combination of all tomato products.

The stronger inverse associations seen for tomato sauce compared with other sources of tomatoes are probably linked to, as argued by Giovannucci [161], the higher bioavailability of lycopene after heat treatment and the additional presence of oil, which facilitates its absorption into the intestinal mucosal cell. Although tomato juice is high in

lycopene, the absorbed amount will vary dramatically, depending on how it is processed and whether it is consumed alone or with a meal containing lipids.

5 Mechanistic evidence for benefits related to compounds formed during food thermal processing

The studies on the biological effects of compounds specifically formed during processing were traditionally focused on the potential harmful effects, as they are considered somehow artificial. However, the evidence of the beneficial effects exerted by some processed foods, such as coffee, promoted the studies aimed at investigating the mechanisms and the compounds responsible for the beneficial effects. Some small molecular weight compounds such as pronyl-lysine and *N*-methylpiridinium, as well as some quinide compounds derived from CGAs have been identified. Recently, the structures of two classes of food melanoidins such as those present in coffee [113, 162–165] and in bread crust [126] were elucidated allowing to shed some light on the possible biological activities of these compounds. Table 4 summarizes an overview. In the following section, the available evidence is grouped according to the biological effects.

5.1 Effects on liver detoxification enzymes

5.1.1 Functioning of the liver detoxification system

To understand the effect of compounds on the liver detoxification system, it is useful to have an overview of the functioning of this complex system. Detoxification enzymes generally function to minimize the potential of damage from xenobiotics living organisms are exposed to, such as pharmaceuticals, environmental or food components. Many of these compounds show little relationship to previously encountered compounds or metabolites and most of them are of non-polar structures, which do not allow urinary excretion. In order to prevent xenobiotics from being accumulated in tissues, a complex system of detoxifying enzymes has evolved to transform these compounds into polar, water-soluble structures that can be easily excreted through the urine.

Literature suggests considerable evidence for an association between impaired detoxification and the risk for various diseases, such as some types of cancer [166–168], Parkinson's disease [169] or chronic immune dysfunction syndrome [170]. On the other hand, the intake of fruits and vegetables containing compounds that induce detoxification enzymes, such as antioxidants, has been shown to lower the risk for, *e.g.* colorectal cancer [171].

The biotransformation of xenobiotics occurs in two phases: functionalization, which uses oxygen to form a reactive site, and conjugation, which results in addition of a water-soluble group to the reactive site. These two steps, functionalization and conjugation, are termed Phase I and Phase II detoxification, respectively. The result is the biotransformation of a lipophilic compound into a water-soluble compound to be excreted in urine (Fig. 3).

The Phase I detoxification system, composed mainly of the cytochrome P450 supergene family of enzymes, is generally the first enzymatic defense against xenobiotic compounds. Most pharmaceuticals are metabolized through Phase I biotransformation. In a typical Phase I reaction, a cytochrome P450 enzyme uses oxygen and, as a cofactor, NADH, to add a reactive group, such as a hydroxyl radical. As a consequence of this step in detoxification, sometimes highly reactive molecules, which may be more toxic than the parent molecule, are produced. If these reactive molecules are not further metabolized by Phase II conjugation, they may cause damage to proteins, RNA and DNA within the cell. Several types of conjugation reactions are present in the body, including glucuronidation, sulfation, and glutathione and amino acid conjugation. These reactions require conjugating compounds such as glucuronic acid, sulfate, glycine, glutamine, taurine, ornithine or glutathione. Antioxidants exert health beneficial effects by scavenging reactive Phase I intermediates (see previous section) or by inducing Phase II enzymes.

A Phase III detoxification system is currently under discussion, referring to transmembrane transporter/antiporter activity (*p*-glycoprotein or multi-drug resistance proteins). Such membrane transporter activity is an important factor in the first pass metabolism of pharmaceuticals and other xenobiotics. The antiporter is an energy-dependent efflux pump, which pumps xenobiotics out of a cell, thereby decreasing the intracellular concentration of xenobiotics [172]. Since antiporter activity in the intestine appears to be co-regulated with intestinal Phase I Cyp3A4 enzyme [173], antiporter proteins may support and promote detoxification. Possibly, their function of transporting non-metabolized xenobiotics out of the cell and back into the intestinal lumen may allow more opportunities for Phase I activity to metabolize the xenobiotic before it is taken into circulation. However, Phase I and Phase II enzyme proteins are highly expressed in the liver and the kidneys, whereas enterocytes show some but not highest expression levels.

5.1.2 Modulation of liver detoxification enzymes by thermally treated foods

Heat treated-foods and even MRP structures formed therein have been demonstrated to modulate Phase I and Phase II enzymes in animal-feeding trials. Kitts *et al.* [174] first reported decreased Phase I aryl hydrocarbon hydroxylase activity in small intestinal enterocytes of mice which were fed an experimental diet containing 2% MRPs for 10 wk. MRPs were prepared by heating an equimolar mixture of

Table 4. Beneficial effect of melanoidins and food components rich in food melanoidins

Compounds	Study design	Measure	Outcome	Reference
Bread crust	Animal	Liver enzyme activity	Enhanced antioxidant capacity increases chemopreventive enzymes	[126]
Bread crust	*In vitro* Gut model	Growth of bacteria species	Prebiotic activity	[220]
Bread crust	*In vitro* tests	ACE and MIC	Anti-ACE activity antibacterial activity	[186]
Fractionated melanoidins Roasted malt	Cultured cells (Caco2)	Enzyme activity	Activation CCR and the GST	[221]
Coffee Silverskin	*In vitro* Gut model	Growth of bacteria species	Prebiotic activity	[222]
Various coffee melanoidins	*In vitro* system	Prevention of dental diseases	Inhibition of adhesion Binding of Streptococcus mutans to tooth enamel	[223]
Coffee melanoidins, beer			ACE inhibitory	[122]
Coffee melanoidins	*In vitro* Gut model	Growth of bacteria species	Prebiotic activity	[188]
Coffee melanoidins	*In vitro* Gut model	Growth of bacteria species	Prebiotic activity	[162]
Coffee melanoidins	*In vitro* Gut model	Growth of bacteria species	Prebiotic activity	[163]

glucose and L-lysine at 121°C for 4 h. Pintauro and Lucchina [175] administered a heat-treated protein, egg albumin, to rats for 10 wk and also demonstrated a decreased activity of the Phase I enzyme aminopyrine *N*-demethylase in small intestinal enterocytes, whereas hepatic Phase I benzo[alpha]pyrene hydroxylase activity was significantly increased. Although both feeding trials from Kitts *et al.* [174] and Pintauro and Luccina [175] clearly indicated an effect of MRPs on detoxifying enzymes, the active principles were still unknown. Wenzel *et al.* [176] administered heat treated casein that was selectively fortified with the MRP N^{ε}-CML. After a feeding period of 10 days, Phase II glutathione *S*-transferase (GST) activity in rat colonic enterocytes and kidneys increased by 64 and 86%, respectively, compared with control animals on a standard diet containing equivalent amounts of non-heated casein. However, the heat-treated casein did not only contain CML and other MRPs might have contributed to this Phase II enhancing effect. When CML as a purified, non-protein-linked compound was tested for its GST-modulating activity in Caco-2 cells, a statistically significant decrease of 10% was observed after the cell's treatment with 0.5 μM CML for 48 h [105]. Thus, CML is unlikely to be a potent inducer of Phase II GST activity. In order to clarify whether the Phase I/II modulating activity is more affected by high- or by low-molecular-weight compounds, Faist *et al.* [105] tested various melanoidin fractions of different molecular weight prepared from hot water extracts of roasted caraffa malt in Caco-2 cell cultures. The low-molecular-weight fraction of $<10\,000$ Da was most effective in activating Phase I NADPH-cytochrome *c* reductase and Phase II GST activity (+122 and +33% *versus* control, respectively). The majority of the mid-molecular-weight compounds tested showed an activating effect on Phase I NADPH-cytochrome *c* reductase and an inhibitory effect on GST activity. These effects were most pronounced for compounds of up to 70 000 and $>200\,000$ Da, but less distinct for fractions of an average molecular weight of 100 000 Da.

Lindenmeier *et al.* [125] first identified an MRP structure with strong Phase I and Phase II modulating activities that was formed in bread crust upon heat treatment. In accordance with the well accepted fact of antioxidants being potent inducers of Phase II enzymes through cellular binding to the "antioxidant responsive element", this compound was also characterized as the key antioxidant in bread crust. Briefly, application of an *in situ* antioxidant assay to solvent fractions isolated from bread crust revealed the highest antioxidative potential for dark brown, ethanol soluble compounds. Both bread crust and, in particular, the intensely brown, ethanolic crust fraction induced a significantly elevated Phase II GST activity and a decreased Phase I NADPH-cytochrome *c* reductase activity. Antioxidant screening of Maillard-type model mixtures, followed by structure determination revealed a pyrrolinone reductonyl-lysine, abbreviated as pronyl-lysine, of which high concentrations were quantified in the bread crust (62.2 mg/kg), whereas low concentrations were analyzed in the crumb (8.0 mg/kg). Exposing Caco-2 cells for 48 h to either synthetically pronylated albumin or to purified pronyl-glycine significantly increased Phase II GST activity by 12 or 34%, respectively, thus demonstrating for the first time that "pronylated" proteins as part of bread crust melanoidins act as monofunctional inducers of GST, serving as a functional parameter of an antioxidant, chemopreventive activity *in vitro*.

Next to bread crust and malt, coffee brews were also studied for their effects on Phase II enzymes [125]. In this study, *N*-methylpyridinium iodide was identified as the key compound modulating Phase II GST. *In vivo* effects of a decaffeinated coffee beverage and *N*-methylpyridinium iodide

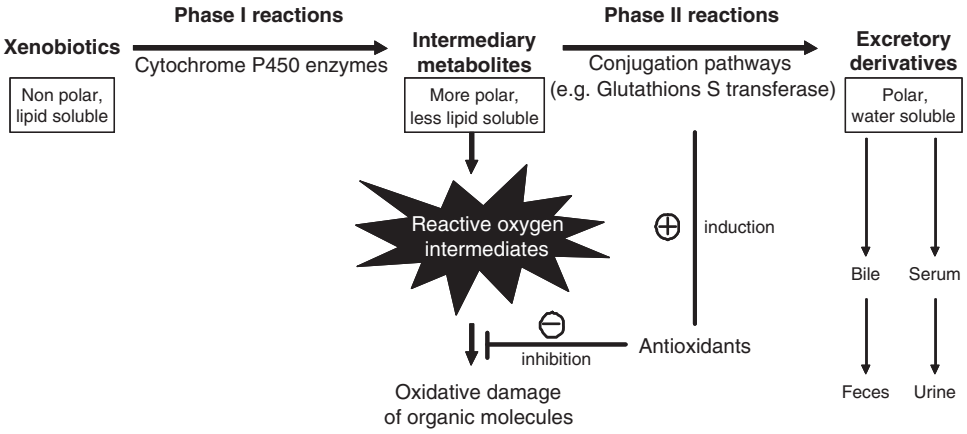

Figure 3. Liver detoxification pathways and their interaction with antioxidants.

were tested in a 15-day animal trial on rats. As a result, feeding of 4.5% coffee beverage resulted in an increase of Phase II GST and UDP-glucuronyl-transferase activity by 24 and 40%, respectively, compared with animals fed the control diet. Animals on the *N*-methylpyridinium diet showed an increase in liver Phase II UDP-glucuronyl-transferase of 65% compared with controls. The mechanism by which *N*-methylpyridinium ions induce Phase II enzymes was recently identified by Böttler *et al.* [177] who showed that this compound effectively induced the gene transcription and translocation of Nrf2, a major transcription factor leading to the expression of antioxidant and Phase II enzymes, such as GST. These results are in line with findings reported by Cavin *et al.* [178] who demonstrated an induction of Nrf2-mediated cellular defence and alteration of detoxifying enzyme activities as mechanisms of chemoprotective effects of coffee in the liver of rats. However, controlled intervention trials are still needed to verify the contribution of *N*-methylpyridium ions and potential other health beneficial compounds in coffee to the reduced risk for diseases such as various types of cancer which are associated with an endogenous load of reactive oxygen species and decreased activities of detoxification enzymes.

5.2 Experimental and mechanistic studies on anticancer activity

The potential anticarcinogenic activity of food compounds is usually assessed by inhibitory activity on the growth of human tumor cells, mutagenicity tests as well as by investigating the effect on DNA oxidation and on the mitogen-activated protein kinase cascade. Traditionally, compounds formed upon thermal treatment were mainly considered for their potential carcinogenic activity (*i.e.* acrylamide or heterocyclic amines). However, there are some *in vitro* studies performed with the chemically characterized Maillard-type chromophores formed under mild heating conditions. These compounds were shown to be potent inhibitors of the growth of human tumor cells in the low micromolar range, causing tumor cell cycle arrest and apoptosis induction. This effect is due to their ability to suppress the induction of the mitogen-activated protein kinase cascade, one of the major signalling pathways in the regulation of cell growth [179]. Antimutagenic properties of MRP or melanoidin mixtures have been noted by Kim *et al.* [180] and were attributed to the inhibition of mutagen absorption or to the inhibition of mutagen activation [181].

Oxidative stress and subsequent DNA damage can be considered as potential biomarkers to investigate the mechanisms of the potential anticancer activity of whole foods. As far as coffee and liver cancer is concerned, Bichler *et al.* [182] reported a protection of human lymphocytes against DNA damage induced. Blood samples were taken from human volunteers who consumed 600 mL of regular coffee *per* day for 5 days and lymphocytes were cultivated in the presence of H_2O_2 or Trp-P-2 assessing DNA damage by the comet assay (single-cell gel electrophoresis). DNA damage was significantly reduced after coffee consumption, as compared with the induced damage before the intervention. Analysis of the cytosolic enzymes of the lymphocytes after coffee consumption showed an increased activity of superoxide dismutase (+38%), whereas glutathione peroxidase remained unchanged.

Similar results were obtained in a human intervention study showing protection against DNA damage induced by the polycyclic aromatic hydrocarbon BPDE (anti-benzo-[a]pyrene-dihydrodiol-epoxide) in isolated peripheral lymphocytes after coffee consumption [183]. Experiments comparing paper filtered *versus* non-filtered coffee furthermore indicated that this effect cannot be ascribed to the well-known bioactive coffee diterpenes cafestol and kahweol.

5.3 Antimicrobial and antibacterial activity

The first study showing the inhibitory mechanism of antibacterial MRPs was run by Einarsson [184] using the Salmonella mutagenic test system. They showed that the

MRPs tested had no mutagenic effect and that their antibacterial effect was primarily due to the interaction between MRPs and iron, resulting in reduced oxygen uptake. Recently, Rufian-Henares and de la Cueva [185] tested the ability of coffee melanoidins against the growth of different pathogenic bacteria. They found that at low concentrations melanoidins exerted a bacteriostatic activity mediated by iron chelation from the culture medium, whereas at high concentrations they exerted a bactericidal activity by removing Mg^{2+} cations from the outer membrane, promoting the disruption of the cell membrane and allowing the release of intracellular molecules. Also different molecular weight fractions isolated from roasted cocoa have been tested for their antibacterial effects [127]. All fractions reduced the growth of pathogenic bacteria; however, the authors highlighted that the growth of Bifidobacteria was also inhibited.

Hiramoto *et al.* [186] determined the inhibitory activity of a variety of melanoidins on urease-gastric mucin adhesion. Extracellular urease proteins located on the surface of *Helicobacter pylori* are gastric mucin-targeted adhesins, which play an important role in infection and colonization to the host. In addition, they have determined the anti-colonization effect of melanoidins prepared by heating casein and lactose, in an animal model and in human subjects infected with this bacterium. They concluded that melanoidins might be an alternative to antibiotic-based therapy to prevent *H. pylori* growth that combines safety, ease of administration and efficacy.

The high-molecular-weight material obtained by an enzymatic extraction procedure from bread crust inhibited the growth of *Escherichia coli* ATCC 25922 and *Staphlococcus aureus* ATCC 25923 [187]. Data demonstrated that the more severe the thermal treatment the higher the antibacterial activity, suggesting that melanoidins play an important role.

5.4 Prebiotic activity

Prebiotics are non-digestible food ingredients that stimulate the growth or activity of bacteria in the digestive system, which are beneficial to the health of the body. Usually, this term refers to soluble dietary fibre (fructo-oligosaccharides and inulin) that is able to increase the concentration of lactobacillus and bifidobacteria. Actually also food melanoidins, as part of food indigestible material that reach the lower gut and can be metabolized by the gut microflora, should be considered as potential prebiotic material. This concept was first investigated by Ames *et al.* [188]. They used glycated BSA as a test substance and no prebiotic activity was found. Borrelli *et al.* [112] demonstrated that the high-molecular-weight fraction enzymatically extracted from bread crust increased the growth of Bifidobacteria and a similar effect could be observed using the coffee silverskin, a by-product of coffee roasting very rich in coffee melanoidins [113].

Recently, a series of articles [162, 189, 190] showed that the high-molecular-weight fraction from coffee brew contains three fractions that can be distinguished into galactomannans, arabinogalactans and melanoidins. The chemical characteristics of these fractions depended not only on the roasting conditions, but also on the coffee preparation procedure. They demonstrated that coffee melanoidins behave as a soluble dietary fibre since they are fermented by the gut microflora. High amounts of acetate and propionate were produced after microbial degradation of high-molecular-weight components from coffee.

It can be hypothesized that polysaccharide-rich melanoidins such as those present in coffee are preferentially metabolized by *Bifidobacteria*, whereas protein-rich melanoidins, as obtained by protein glucose mixtures or milk-like systems, are good substrates for protein metabolizing bacteria predominantly present in the descending tract of the colon.

5.5 Anti-ACE activity

Two articles have addressed the possibility that MRPs influence the activity of angiotensin-I converting enzyme. This enzyme, whose activity plays a key role in the increase of blood pressure, is inhibited by various food-derived peptides, which are candidates for the management of blood pressure. Del Castillo *et al.* [187] showed that the natural ACE inhibitory activity of wheat gluten was reduced by thermal treatment as a consequence of the reaction with sugars. On the other hand, Rufian-Henares and Morales [122] found that melanoidins isolated from beer and coffee were able to inhibit ACE and that the efficacy of coffee melanoidins increases with roasting degree.

5.6 Effect on type 2 diabetes

Results reported by Natella *et al.* [116], Esposito *et al.* [117], Bichler *et al.* [182] and Steinkellner *et al.* [183] clearly show that drinking of coffee enhances the antioxidant capacity of human plasma and isolated cells thereof. Subclinical inflammation, imparting enhanced oxidative stress to the organism, has been considered to be a risk factor for type 2 DM and its complications. However, it is still not clear whether and to what extent antioxidative effectiveness might contribute to the evidence that moderate coffee consumption (three to four cups *per* day) is associated with decreased risks for certain diseases, including DM type 2 [191, 192–194], colon cancer [195], liver cirrhosis [144], Alzheimer's disease [196] and Parkinson's disease [197].

Although for all of these diseases, the endogenous load of reactive oxygen species is increased compared with healthy subjects, controlled intervention trials to prove this hypothesis are mostly lacking. Very recently, results of a human trial published by van Dijk *et al.* [198] demonstrated that the

intake of two major coffee components, CGA (1 g) and trigonelline (500 mg) significantly reduced glucose and insulin concentrations in the plasma of healthy volunteers 15 min, following an oral glucose tolerance test compared with placebo. Since reactive oxygen species are hypothesized to play a pivotal role in the progression of DM [199], the reduced risk for this disease following regular coffee consumption might be at least in part caused by its contents of CGA and trigonelline, although these two compounds might not be the only or most effective antioxidants ingested with a moderate coffee consumption of two to three cups *per* day *in vivo*.

Further evidence for beneficial effects on type-2 diabetes also comes from a Canadian group focusing on the role of CGA derivatives [140]. Roasting transforms CGA into quinides that can alter blood glucose levels acting through gut peptides (glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1). Shearer *et al.* [200] demonstrated that 3,4-diferuloyl-1,5-quinide increases whole-body glucose disposal independently of skeletal muscle and that decaffeinated coffee has a beneficial effect on glucose management in rats [200].

6 Concluding remarks

Consumer perception of (mainly industrial) processing is rather negative, probably due to the large attention to formation of undesired compounds, but clearly processing also leads to the formation of compounds with beneficial properties. Moreover, industrial processing can be controlled and optimized much better through the application of kinetic principles than household cooking. It appears from this review that many individual compounds as well as compounds from model reactions or whole foods have been analysed *in vitro* for health beneficial characteristics. The major problem is that it is as yet not clear if these effects can be directly interpreted as health beneficial for the consumer, because metabolism by the gastrointestinal microflora, bioavailability or degradation by human metabolism are of great influence. Epidemiological data indicative of positive effects for consumers are merely available on whole foods but information on if and how these foods are processed is usually not available. It is also fair to conclude that precise mechanisms of action of individual compounds responsible for the observed effects are less well understood.

A clear understanding of the mechanisms underlying the biological effect is essential for obtaining the intended beneficial effect in the food product. If such knowledge becomes available, new technologies may provide new opportunities to deliver health, quality and safety in food systems.

For the few compounds that have been isolated up to now from processed foods, the mechanisms identified seem to support the epidemiological data available for each specific food. Such individual compounds are known to be formed during the thermal processing of the whole foods.

The translation of consumer perceptions (particularly flavour, texture and the presence of health promoting components) into manageable industrial scale technologies is a major challenge for the food industry and it is a limitation of the state of the current science underpinning modern food-processing technology. Systematic studies are required to provide a balanced optimization for the thermal processes that are accepted and widely used by the food industry in terms of food safety, providing acceptable risk and the desired benefits that are satisfactory to both to the consumers and to the food safety risk managers (*i.e.* nutritional and organoleptic quality, release of bioactive or functional compounds formed during food proccessing). A systematic application of kinetics to optimize thermal processes to deliver benefits while ensuring safety needs to be pursued by food technologists and food safety scientists with urgent view to commercial implementation.

Non-thermal processes may provide less risk and equal or even better benefits than thermal processes but are still in their infancy and in an exploratory phase of investigation, especially those that have potential commercial applications in the food industry. Current non-thermal processes can, in general, not yet offer acceptable safety alternatives compared with thermal processes but studies are on the way, and combinations of mild heat treatment and non-thermal processes may give new opportunities. Also here, a systematic application of kinetics is needed. Data on non-thermal processes in terms of inactivation kinetics and reaction mechanisms of nutrients, toxins, allergens, microbes and viruses are required and shelf-life studies of non-thermally treated products are desirable. To date, unfortunately, there seems to be little progress in the development of industrial application of technologies for non-thermal processes. In a similar manner, non-thermal processes such as fermentation can generate metabolites, giving food unique health benefits when these processes are implemented in a controlled way. However, new strains and substrates for release of beneficial compounds need to be developed and the performance of starter strains needs to be enhanced during the process.

There are rather strong indications in the scientific literature for the effects of individual compounds that are formed, and epidemiological data do indicate positive beneficial effects of whole foods, but the evidence is not always conclusive. The mechanisms or the compounds responsible for effects are less well understood. Areas for further investigations include bioavailability and biotransformation of phytochemicals in general and translation of *in vitro* antioxidant capacity to beneficial health effects in humans.

However, there is a lack of information about the food processing in epidemiological studies and epidemiological studies should be designed for addressing this purpose. Further study is required to find or synthesize pure standard compounds to enable the conduct of more accurate mechanistic studies and to further identify other bioactive or functional compounds, thus providing stronger evidence of the beneficial effects of food processing.

A better understanding of the relevance of *in vitro* results for human health benefits is needed, with due consideration of intake of these compounds from foods. Databases and fit for purpose food intake surveys to estimate the levels of intake of these compounds are also required if the beneficial effects of compounds derived from thermal food processing are to be clearly established.

In conclusion, the following gaps are identified

(i) Lack of information about how foods are processed that are considered in epidemiological studies.
(ii) Translation of antioxidant capacity *in vitro* to health beneficial effects in humans.
(iii) Effects of processing on protein digestibility, notably the effects on allergens.
(iv) Effects of non-thermal processes on phytochemicals, melanoidins and allergens.
(v) Relevance of the *in vitro* results for human benefit considering the reasonable intake of these compounds from foods.
(vi) Lack of databases to estimate the level of intake of compounds from processed foods.

It is the authors' intention and hope that this review helps in designing future study in this exciting area.

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