Food Microstructure Affects the Bioavailability of Several Nutrients

J. PARADA AND J.M. AGUILERA

ABSTRACT: There is an increased interest in the role that some nutrients may play in preventing or ameliorating the effect of major diseases (for example, some types of cancer, cardiovascular diseases, eye disorders, among others). In this respect, the bioavailability or the proportion of an ingested nutrient that is made available (that is, delivered to the bloodstream) for its intended mode of action is more relevant than the total amount present in the original food. Disruption of the natural matrix or the microstructure created during processing may influence the release, transformation, and subsequent absorption of some nutrients in the digestive tract. Alternatively, extracts of bioactive molecules (for example, nutraceuticals) and beneficial microorganisms may be protected during their transit in the digestive system to the absorption sites by encapsulation in designed matrices. This review summarizes relevant *in vivo* and *in vitro* methods used to assess the bioavailability of some nutrients (mostly phytochemicals), types of microstructural changes imparted by processing and during food ingestion that are relevant in matrix-nutrient interactions, and their effect on the bioavailability of selected nutrients.

Keywords: allergenicity, antioxidants, bioavailability, encapsulation, food matrix, *in vitro* methods, microstructure, phytochemicals, probiotics, processing

Introduction

Health and well-being of consumers are major drivers of the modern food industry. The large scientific evidence linking important diseases such as cardiovascular diseases (CVD) and some types of cancers with diet is well documented. All these diet-related problems are likely to change eating habits, processing technologies, and products. Many beneficial and detrimental health effects of specific nutrients present in foods are well documented. Although the total amount of a nutrient may be obtained from composition tables, its "availability" for absorption in the gut is in many cases quite uncertain or varies for the same food depending on processing conditions, presence of other components, and so on. The fraction of an ingested nutrient that can be used by the organism is obviously of major importance and several factors influence its availability: the chemical state of the nutrient, its release from the food matrix, possible interactions with other food components, presence of suppressors or cofactors, formation of stable compounds that are slowly metabolized, and so on. However, recent scientific data appear to demonstrate that in the case of certain nutrients the state of the matrix of natural foods or the microstructure of processed foods may favor or hinder their nutritional response *in vivo*. The objective of this hypothesis paper is to present some available evidence that food microstructure may affect the final uptake of nutrients in the gut, hence, their presence in the blood plasma. Although reported food matrix effects span from proteins and polysaccharides (for example, starch) to minerals, this article deals mostly with phytochemicals.

MS 20060594 Submitted 2/11/2006, Accepted 13/12/2006. *Authors are with Dept. of Chemical and Bioprocess Engineering, Pontificia Univ. Católica de Chile, P.O. Box 306, Santiago, Chile. Direct inquiries to author Aguilera (E-mail: jmaguile@ing.puc.cl).*

Food Composition Compared with Nutrient Bioavailability

Databases of food composition

A typical food composition database (FCD) provides values for the amount of energy, protein, fat, vitamins, minerals, and some other specific nutrients present in a food item. The values are normally determined by standard chemical analyses (for example, "Official Methods") or sometimes derived, in the case of complex foods, from the nutrient composition of ingredients (Schakela and others 1997). FCDs have been methodically compiled over the years in many countries and provide information about the nutrients contained in most consumed foods (Ottley 2005; Pakkala and others 2006; Similä and others 2006). There are over 150 food composition tables and electronic databases already in existence worldwide. In the internet, there is an Intl. Directory of Food Composition Tables, which lists FCDs from different regions and countries (http://www.fao.org/infoods/directory_en.stm).

FCDs are generally used to assess the nutrient content of diets and to derive nutrition guidelines. They are also often utilized in food policy recommendations and in nutrition monitoring. Criticism of the actual merit of FCDs to accomplish the previous tasks abounds, starting with the appropriateness of the analytical methods used to determine nutrient concentration. For example, Gregory and others (2005) state that values of folate content in some FCDs are determined by obsolete microbiological assays having many drawbacks. Another shortcoming is the limited detail of the reported data, for example, inadequate descriptions of the foods and samples analyzed, omission to cite the number of samples analyzed, and lack of statistical parameters of data variability. Food composition tables for most foods and nutrients give estimations of the mean concentration, but rarely estimates of the variance of those values (Stewart 1997). Moreover, there is a limited availability of data for some bioactive components that are known to play important physiological roles

doi: 10.1111/j.1750-3841.2007.00274.x

(Pennington 2002). One effort to overcome some of these shortcomings is the *EuroFIR* initiative aimed at creating a Pan-European Food Information Resource by linking databases and allowing effective management, updating, extending, and comparability of food composition data (Ottley 2005). Notwithstanding the previous limitations, the real problem is that information contained in FCDs is related to the amount of nutrients present in foods prior to ingestion but gives no clue of the actual amount that becomes available for physiological activity after absorption in the gut.

Nutrient bioavailability

The FDA has defined *bioavailability* as the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action (Shi and Le Maguer 2000). This definition also applies to active substances (nutrients) present in foods. However, even today nutrient bioavailability is an important but often nebulous concept associated with the efficiency of absorption and metabolic utilization of an ingested nutrient (Gregory and others 2005). Another term that is commonly used is *bioaccessibility*, which is defined as the amount of an ingested nutrient that is available for absorption in the gut after digestion (Hedrén and others 2002). Thus, it is not equivalent to speak of bioavailability or bioaccessibility. If the amount of recovered nutrient after digestion is of relevance then the term to use is bioaccessibility. On the other hand, bioavailability of nutrients is usually measured in the blood plasma of humans (*in vivo* assay) so factors such as the individual variability, physiological state, dose, and presence of other meal components come into play (Faulks and Southon 2005). These authors established that although all of a nutrient is potentially bioaccessible, in reality almost no nutrient is totally converted during digestion into a potentially absorbable form. In almost every case, bioaccessibility and bioavailability of a nutrient are governed by the physical properties of the food matrix, which affect the efficiency of the physical, enzymatic, and chemical digestion processes (Boyer and Liu 2004). Table 1 summarizes commonly used definitions pertaining to the utilization of an ingested nutrient.

Microstructure

Definition

Through evolution, humans have received nutrients in the form of palatable foods that are easily recognized by the sensations experienced during mastication. Foods produced by nature are generally organized hierarchically from molecules into assemblies and organelles that are later compartmentalized into cells and tissues. "Natural" food structures may be classified into 4 broad categories: (1) fibrous structures assembled hierarchically from macromolecules into tissues for specific functionality (for example, muscles) and held together at different levels by specific interfacial interactions; (2) fleshy materials from plants that are hierarchal composites of hydrated cells that exhibit turgor pressure and are bonded together at the cell walls (for example, tubers, fruits, and vegetables); (3) encapsulated embryos of plants that contain a dispersion of starch, protein, and lipids assembled into discrete packets (for example, in grains and pulses); and (4) a unique complex fluid called milk, intended for nutrition of the young mammal containing several nutrients in a state of dispersion.

Most fruits, vegetables, meat, fish, grains, and tubers are eaten around the world with minor processing; thus their edible microstructure has been largely imparted by nature. Processed foods (for example, baked and confectionery products, dried pasta, processed meats, and so on), on the other hand, are multicomponent structured matrices where the individual components (proteins, fats, carbohydrates, and so on) have been reassembled as colloidal dispersions, emulsions, amorphous or crystalline phases, or gel networks by heating and/or cooling and the application of shear. By food microstructure we understand the spatial arrangement of identifiable elements in a food and their interactions at levels below 100 μm (Aguilera and Stanley 1999). Typical microstructural elements in foods are cell walls, starch granules, proteins, water and oil droplets, fat crystals, gas bubbles, and so on. The concept of a "food matrix" points to the fact that nutrients are contained into a larger continuous medium that may be of cellular origin (in fruits and vegetables) or a microstructure produced by processing, where they may interact at different length scales with the components and structures of the medium. For example, nutrients may be found as individual molecules bound to plant organelles (for example, carotenoids in carrots) or entrapped in a complex macromolecular matrix of swollen starch granules and protein (for example, isoflavones in baked products). Table 2 shows the most common locations of several nutrients in the tissue of plant materials. As pointed out by Cuvelier and others (2000) the molecular structure of a nutrient is the smallest structural level relevant for its biological role and activity (for example, *cis* or *trans* conformation, number of hydroxyl groups or chelating sites in phenolic antioxidants). Methods to probe into the food microstructure or the state of the food matrix at different length- and time-scales are reviewed elsewhere (Kaláb and others 1995; Aguilera and Stanley 1999). The most common microscopy techniques to study the food microstructure are light microscopy in its many versions, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and, recently, the confocal laser microscope and atomic probe microscopes. These techniques are now being routinely used by food scientists to characterize food microstructures and food matrices, providing qualitative information about their physical state (Aguilera 2006).

Relation between microstructure and nutritional properties

As stated before, nutrients are often located in natural cellular compartments or within assemblies produced during processing. In either case, they need to be released during digestion so they can be absorbed in the gut. Ellis and others (2004) studied the role of plant cell walls in the bioaccessibility of lipids in almond seeds (where almond seeds and fecal samples were examined by microscopy to identify cell walls and intracellular lipids).

Table 1—Commonly used definitions pertaining to the utilization of an ingested nutrient

Term	Definition
Bioavailability	The fraction of ingested nutrient that is available for utilization in normal physiologic functions and for storage.
Bioconversion	Fraction of bioavailable nutrient that is converted into the active form.
Bioefficacy	Fraction of ingested nutrient that has a nutritional effect
Bioaccessibility	Fraction that is released from food matrix and is available for intestinal absorption (typically based on *in vitro* procedures)
Bioequivalence	Absence of a significant difference in the rate and extent to which 2 active ingredients become available at the site of action, when administrated at the same molar dose under similar conditions.

They successfully identified intact almond tissue in fecal material collected from healthy subjects fed an almond-rich diet. The main structures of almond tissue were found to be preserved even after chewing and digestion. In particular, cell walls that remained intact hindered the release of intracellular lipid.

Even if complete disruption of cellular structure is effected, full absorption of a particular nutrient is not ensured and may depend on the presence and interactions with other food components. For example, while Brown and others (1997) indicated that food matrix components such as fiber could decrease carotenoid absorption, Rondini and others (2004) found good bioavailability of ferulic acid in the presence of bran. Although not covered in this review, there is extensive evidence that the food matrix is also of considerable influence in the bioavailability of some minerals (Brouns and Vermeer 2000; Moretti and others 2006). Even though work still needs to be done in characterizing food microstructure, there is ample evidence that the physical state of the matrix plays a key role in the release, mass transfer, accessibility, and biochemical stability of many food components (Aguilera 2005).

The 1st physical transformation of food matrices during eating occurs in the mouth, and mastication is considered the initial step in the digestion of foods. Mastication consists of grinding the food into small pieces and impregnating these pieces with saliva to form a swallowable bolus. Decreasing the particle size enlarges the surface area available for the attack by digestive enzymes, thus increasing the overall digestion efficiency and the gastrointestinal absorption of nutrients (Kulp and others 2003; Suzuki and others 2005).

Methods to Determine Bioavailability/Bioaccessibility

Types of experimental procedures

Methods for determining bioavailability and/or bioaccessibility of nutrients involve human (*in vivo*) or simulated experiments performed in a laboratory (*in vitro*). In vivo methods provide direct data of bioavailability and have been used for a great variety of nutrients and foods. Usually a response is measured after consumption of a pure nutrient (natural or synthetic) by living beings, either humans (most common) or animals, and compared to an equivalent nutrient dose found in a food source (Yeum and Russell 2002). Ethical restrictions and abiding to severe protocols when humans and/or animals are used in biological research are severely limiting this type of studies (van het Hof and others 2000a, 2000b). Most commonly, *in vivo* bioavailability studies imply the consumption of certain dose of a nutrient and following changes of its concentration (measured by standard analytical procedures) in the blood plasma compared with time (for example, postprandial period, or the time after the meal). Three parameters are derived from the kinetics: the area under curve (AUC), the maximal plasma concentration (C_{max}), and the time to reach C_{max}, t_{max}. AUC is a measure of the absorption intensity, whereas C_{max} and t_{max} give an idea of the rate of absorption (Heacock and others 2004; Manach and others 2005). Another method to assess bioavailability is to measure the plasma concentration of a nutrient through an extended period (day, week) of constant consumption of a specific food (van het Hof and others 1999; Richelle and others 2002). Relevant parameters in this case are C_{sat} (value at which the concentration remains constant in the time) and the t_{sat} (time at which C_{sat} is attained). The main drawbacks of *in vivo* data are the variability in physiological state of individuals and the possible interaction of the nutrient with other components in the diet.

In vitro methods are being extensively used at present since they are rapid, safe, and do not have the ethical restrictions of *in vivo* methods. *In vitro* methods either simulate the digestion and absorption processes (for bioavailability) or only the digestion process (for bioaccessibility) and the response measured is the concentration of a nutrient in some kind of final extract. The digestion process is simulated under controlled conditions using commercial digestive enzymes (for example, pepsin, pancreatin, and so on) while the final absorption process is commonly assessed using Caco-2 cells cultures. "Caco-2 cells" is the short name of polarized human colon carcinoma cells line (Verwei and others 2005). Figure 1 shows the

Table 2—Some key nutrients in plant foods and their location within the tissue

Nutrient	Organelle(s) or tissue	Main plants foods of occurrence	Reference
Lycopene	As carotenoid-protein complexes in chloroplasts or in crystalline form inside chromoplasts	Tomato, watermelon, guava, rosehips, papaya, sweet potato, cantaloupe	Sommerburg and others (1998); Shi and Le Maguer (2000)
β-carotene	As carotenoid-protein complexes in chloroplasts or in crystalline form inside chromoplasts	Carrot, peach, cantaloupe, nectarine, honeydew, mangos, apricot, pumpkin, spinach, broccoli, apple, tomato, green pepper, red seedless grapes	Sommerburg and others (1998); Krinsky and Johnson (2005)
α-carotene	As carotenoid-protein complexes in chloroplasts or in crystalline form inside chromoplasts	Carrot, yellow squash, red pepper, lettuce, celery (stalks, leaves), tomato, peach, orange, pepper, apple, butternut squash, grapes, green beans, yellow pepper	Sommerburg and others (1998)
Lutein (and zeaxanthin)	As carotenoid-protein complexes in chloroplasts or in crystalline form inside chromoplasts	Corn, kiwi, squash, pumpkin, spinach, peppers, yellow squash, cucumber, pea, red grape, orange, honeydew, celery (stalks, leaves), green grapes, Brussels sprouts, scallions, green beans, broccoli, apple, mango, green lettuce, tomato, peach, nectarine, carrot	Sommerburg and others (1998); Krinsky and Johnson (2005)
Polyphenols (phenolic compounds)	As conjugates with proteins and polysaccharides	Apple, banana, blackberry, blueberry, cherry, cranberry, guava, litchi, mango, peach, papaya, persimmon, pineapple, plums, prunes (pitted), raisins, rambutan, raspberry, red grape, starfruit, strawberry, broccoli, Brussels sprouts, cabbage, carrot, cucumber, mint, spinach, tomato, onion, wines	Balasundram and others (2006)
Ferulic acid	Bound to arabinosyl chains of plant cell walls	Cereals as corn, wheat, oats, and rice	Rondini and others (2004)
Folate	Covalently bound to macromolecules such as proteins	Leafy green vegetables such as spinach, green peas, broccoli, Brussels sprouts, green beans, and citrus fruits	Brouwer and others (1999)

basic elements in a digestion Caco-2 cell assay, where conditions of time, pH, temperature, and so on, depend on the compound to be analyzed and the type of food matrix. The amount of a nutrient present in the extract (after the Caco-2 cell step) is assumed as the final amount bioavailable (Glahn and others 1998). Several protocols following the scheme of Figure 1 have been adapted to assess the bioavailability of specific nutrients. A major problem to be resolved is that nutrients in foods may be transformed from the gut into metabolites (for example, by colonic bacteria) that are the active form in which they are absorbed (thus "bioavailable").

Gastrointestinal models

Methods that simulate under laboratory conditions the gastrointestinal digestion process are known as gastrointestinal models (GIMs). They are used as a suitable alternative to *in vivo* assays to determine bioavailability, in spite of their limitations regarding the significance of data generated. GIMs try to reproduce the physiological conditions in the mouth, stomach, and small intestine during mastication, digestion, and absorption. In general, GIMs fall into 2 broad categories: *static models*, where the products of digestion remain largely immobile and do not mimic physical processes such as shear, mixing, hydration, and so on, whereas *dynamic models* try to include physical and mechanical processes and temporal changes in luminal conditions to mimic conditions *in vivo*. The latter are particularly useful where the physical condition of the digesta (mixture of ingested food particles and fluids released during digestion) changes over time (for example, particle size and viscosity) and take into account some temporal effects not considered otherwise (mixing, diffusion, formation of colloidal phases, and so on).

GIMs have been used to study the release of phenolic compounds from the matrix of orange juice, strawberries, and strawberry jam by Gil-Izquierdo and others (2002). Bermúdez-Soto and others (2007) investigated the effects of an *in vitro* gastric and pancreatic digestion on the stability and composition of the major polyphenols in chokeberry juice. Their GIM consisted of an initial pepsin-HCl digestion (to simulate gastric digestion), followed by a pancreatin digestion with bile salts (to simulate conditions in the small intestine), and ended with a dialysis step (to simulate the absorption process). Krul and others (2001) used a sort of dynamic GIM to determine potential antimutagenic activity of extracts of black tea and green tea. Their apparatus consisted of water-jacketed glass tubes internally lined with flexible walls, which simulated peristaltic movements. Hoebler and others (2002) designed an *in vitro* digestion system that mimicked the physical and chemical processes in the mouth and stomach to understand the kinetics of digestion of carbohydrates and proteins in bread. To simulate the oral phase digestion, bread was minced and subjected to amylase digestion, measuring the release of oligosaccharides. During the gastric phase the mean particle size of bread pieces decreased progressively. Nagah and Seal (2005) studied the release of antioxidants from a total of 41 of wholegrain foods with a GIM having enzymatic and fermentation steps. Unveiling some of the limitations of *in vitro* testing, the 3 methods used to determine antioxidant activity (the ferric reducing antioxidant capacity, Trolox equivalent antioxidant capacity, and the oxygen radical absorption capacity method) gave different trends, and the range of values was dependent on the type of food. Chu and Beauchemin (2004) described a method to assess the maximum bioaccessibility of nutrients released from foods into artificial gastrointestinal fluids (saliva, gastric juice, and intestinal juice) using flow injection and coupled plasma mass spectrometry. Recently, Haro-Vicente and others (2006) used a GIM to measure available iron from different fortificants (ferrous sulfate, micronized dispersible ferric pyrophosphate, and ferrous bis-glycinate) in citric fruit juices. For flavonoids, Boyer and others (2005) recommended a procedure based on enzymatic digestion with pepsin + pancreatin/bile + lactase, before exposing the extract to the Caco-2 cells culture. Chitchumroonchokchai and others (2004) studied lutein bioavailability simulating the gastric and small intestinal phases of digestion, followed by a Caco-2 cell assay. Similarly, Garrett and others (1999, 2000) concluded that the *in vitro* digestion/Caco-2 cell culture procedure was a rapid and cost-effective model for screening the bioavailability of carotenoids from plant foods (fresh spinach, fresh carrots, and tomato paste). A dynamic gastrointestinal model consisting of gastric and small-intestinal compartments and Caco-2 cell culture (absorption step) was used by Verwei and others (2005) and results predicted well the bioavailability of folate from milk products in humans. GIMs have also been used to prescreen mycotoxins/adsorbent combinations as an alternative or complement to animal experiments (Avantaggiato and others 2003). At least 1 commercial GIM is now available in the market: TNO's gastrointestinal model, widely used in pharmacological and food testing for human and animal trials (TNO 2006).

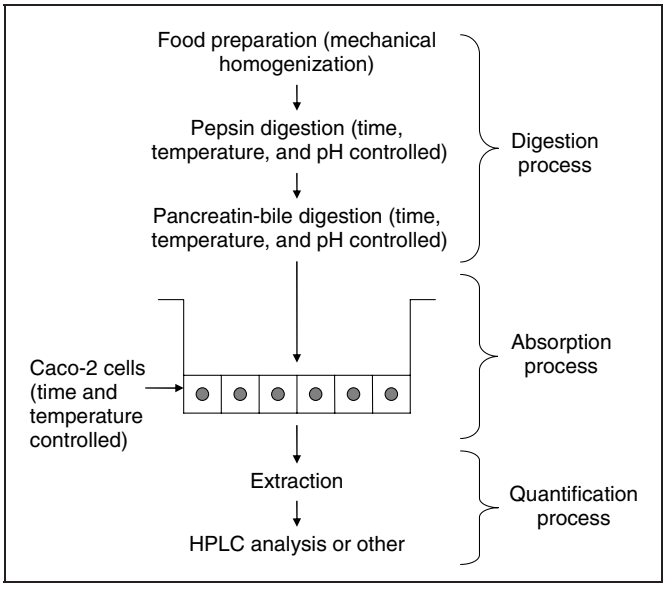

Figure 1—Diagram of an *in vitro* method to determine bioavailability involving a digestion/absorption step using a Caco-2 cell culture

Nutrient Bioavailability from Plant Foods

Importance of nutrient bioavailability and processing

The bioavailability of nutrients and bioactive compounds present in plant products (fruits and vegetables) is presently an extremely important area of food and nutrition research. The objective of these studies is to determine the real contribution of actual foods as sources of specific nutrients (for example, antioxidants) and to establish processing conditions that maximize the health benefits. Although several photochemicals are known to be important in promoting human health, only those with evidence that microstructure affects their bioavailability will be reviewed here. For example, food microstructure seems to be quite relevant in the bioavailability of several antioxidants.

Food processing such as grinding, fermentation, and/or mild heating may improve bioavailability, most likely as a result of disruption of the cell walls of plant tissues, dissociation the nutrient-matrix complexes, or transformation into more active molecular structures.

The scheme in Figure 2 depicts the case where the total amount of a nutrient may decrease in the food chain due to chemical degradation during storage and physical losses in processing, while at the same time the bioavailability may increase by the aforementioned processes.

Carotenoids

Carotenoids are a family of fat-soluble plant pigments that provide red and orange colors to fruits and vegetables. Their function is to absorb light in photosynthesis, protecting plants against photosenzitation. Dietary carotenoids are considered to be beneficial in the prevention of a variety of diseases, including certain cancers and eye disorders. The 5 principal carotenoids found in human plasma as a result of ingestion of plants are α-carotene and β-carotene, cryptoxanthin, lutein, and lycopene, but over 600 carotenoids have been identified to date. Carotenoids present in a wide variety of plants are partially concentrated in chromoplasts or chloroplasts in different ways. The extent of release from the food matrix is highly variable depending on whether carotenoids are noncovalently bound to protein or fiber, dissolved in oil (as in corn and palm oil), or in crystalline form (carrots), making their optimal absorption difficult to achieve (Deming and Erdman 1999; Yeum and Russell 2002; Zaripheh and Erdman 2002). In general, the bioavailability of carotenoids has been estimated to vary from 10% in raw, uncooked vegetables to 50% in oil and commercial products (Deming and Erdman 1999). Even when extracted from the food matrix, the bioavailability of carotenoids may be very low as revealed by *in vivo* studies of capsanthin and capsorubin from paprika oleoresin (Perez-Galvez and others 2003). Since carotenoids are hydrophobic their absorption depends not only on the release from the food matrix but also on the subsequent solubilization by bile acids and digestive enzymes, culminating in their incorporation into micelles. For this reason dietary lipids have been considered to be important cofactors for carotenoid biovailability, particularly in carotenoid-rich fruits that are low in lipids.

In general, the release of carotenoids from plant foods occurs only when the cells in the food matrix are disrupted, as is usually the case during food preparation, processing, and/or mastication, but not during digestion, at least in the ileum of humans (van het Hof and others 1999, 2000a, 2000b; Edwards and others 2002; Faulks and Southon 2005). In addition Zhou and others (1996) suggested that the food matrix, probably pectin-like fibers, and the crystalline form of carotenoids in carrot chromoplasts were the primary factors that reduced the relative bioavailability of carotenoids from carrot juice (so-called "incomplete release"). Serrano and others (2005) concluded that the proportion of β-carotene (and lutein) released by the sole action of digestive enzymes from spinach, chaya (*Cnidoscolus aconitifolius*), and macuy (*Solanum americanum*) ranged from 22% to 77%. Following release from the food matrix, the major limiting factor governing the extent of absorption of carotenoids is their solubilization in digesta (Faulks and Southon 2005). It is well known that cooking can increase the extractability of β-carotene from the plant matrix, thereby improving its bioavailability. Daily consumption of processed carrots and spinach by women over a 4-wk period produced an increase in plasma β-carotene concentration that averaged 3 times that associated with consumption of the same amount of β-carotene from the raw vegetables (Rock and others 1998). Livny and others (2003) concluded that significantly more of the β-carotene was absorbed from cooked and pureed carrots (65.1% $\pm$ 7.4%) than from the raw vegetable (41.4% $\pm$ 7.4%). One conclusion is that providing cooked and pureed vegetables rather than raw vegetables would appear to be a better approach to providing bioavailable β-carotene from carotenoid-rich foods, which may be applicable for populations who rely on these foods to meet vitamin A requirements.

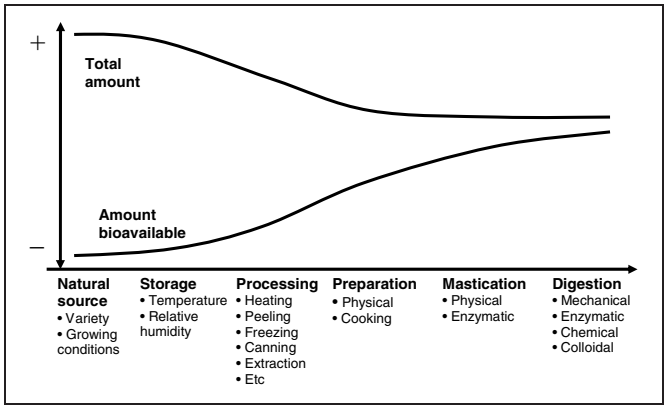

Figure 2—Example of a food where the total amount of a nutrient diminishes with time, but the amount bioavailable increases due to suppression of food matrix effects

Lycopene

Lycopene, a carotenoid responsible for the distinctive red color of ripe tomatoes, is usually located within cell membranes and its release is determinant on the bioavailability. Epidemiological studies have suggested that consumption of lycopene may protect against CVD and reduce the risk of several types of cancer, most notably those of the prostate, breast, lung, and digestive tract (Omoni and Aluko 2005).

Food processing like cooking or heating may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and the tissue matrix, thus making it more accessible. Moreover, *cis*-isomerization is enhanced in free lycopene with the advantage that *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons. Shi and Le Maguer (2000) and Re and others (2001) concluded that the matrix may contribute to the stability of all *trans*-forms of lycopene in tomatoes, thus preventing the isomeric equilibrium from occurring. During digestion the prevailing food matrix is further disrupted and lycopene may be incorporated into micelles prior to absorption. It is plausible that once this disruption occurs, further isomerization of *trans*-lycopene may occur. The practical result is that lycopene is 2.5 times more bioavailable in humans when present in tomato paste than in fresh tomatoes (Omoni and Aluko 2005).

Xanthophylls

Xanthophylls (for example, lutein, zeaxanthin, capsanthin, canthaxanthin, astaxanthin, echionine, and β-cryptoxanthin) are the typical yellow pigments of leaves. These are oxygenated carotenoids that are synthesized within the plastids. The xanthophylls lutein and zeaxanthin accumulate in the eye lens and macular region of the retina and have been implicated in helping to protect the eye against oxidative damage and cataracts. For optimal absorption of xanthophylls, they must be released from their food matrix and then transferred to lipid micelles in the small intestine. This requires the presence of dietary fat in the small intestine, which stimulates the gallbladder to release bile acids (that is, emulsifiers). Zaripheh and Erdman (2002) concluded that major factors limiting the bioavailability of xanthophylls included the molecular structure, the interactions of xanthophylls with other nutrients (mainly dietary fat), and the physical disposition of xanthophylls in the food matrix. Efforts to determine lutein directly within the food matrix using photoacoustic spectroscopy are under way (Bicanic and others 2005).

Folates

Folate and folic acid are forms of B vitamin that are necessary for the production and maintenance of new cells, especially important during periods of rapid cell division and growth, such as throughout infancy and pregnancy. Inadequate folate intake has been associated with development of birth defects (Takimoto and Tamura 2006); esophageal, gastric, and pancreatic cancers (Larsson and others 2006); and brain disorders such as depression (Abou-Saleh and Coppen 2006). Leafy green vegetables such as spinach and turnip greens, dry beans and peas, and some other fruits and vegetables are rich natural sources of folate (Rychlik and others 2003). In nature, folates are covalently bound to macromolecules. Results obtained by Verwei and others (2003, 2005) showed that folate-binding proteins decrease the bioavailability of folate (especially pteroylmonoglutamic acid [PGA], which is synthetic folic acid) in fortified milk products, which was confirmed in human studies. The food matrix itself and its individual components can also influence folate bioavailability by entrapment, thereby hindering diffusion to the absorptive surface during digestion (van het Hof and others 1999; McNulty and Pentieva 2004). Also simple entrapment in the food bolus during digestion is a possible mechanism for incomplete absorption of food folates. In the absence of food matrix effects, the various folates are absorbed effectively and to an approximately equivalent extent (Gregory and others 2005).

Polyphenols

Polyphenols represent a wide variety of compounds belonging to several classes, for example, hydroxybenzoic and hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes, and lignans (Manach and others 2005). Phenolic compounds (or polyphenols) are secondary metabolites of the pentosephosphate, shikimate, and phenylpropanoid pathways in plants. These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants (Balasundram and others 2006). Polyphenols are abundant micronutrients in our diet and alleged to play several roles in the prevention of degenerative diseases, acting as anti-allergenic, antiatherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, and vasodilatory agents. Derivatives of phenolic acids account for about one-third of the total intake of polyphenols in our diet and flavonoids account for the remaining two-thirds. They have been associated with the health benefits derived from consuming high levels of fruits, vegetables, and wine, mainly as antioxidants (Balasundram and others 2006).

Reported bioavailability of polyphenols is highly variable depending on their structure and conjugation (for example, to sugars): $< 0.1\%$ for most anthocyanins (colored flavonoids in berries and red wine), 1% to 5% for quercetin (red wine, apples, and onions), 10% to 30% for flavanones (citrus) and flavanols (red wine, tea, and cocoa), and 30% to 50% for isoflavones (soya products) and gallic acid (red wine, tea, and various fruits) (Scalbert and Williamson 2000). A major part of the polyphenols ingested (75% to 99%) is not found in urine. This implies they have either not been absorbed through the gut barrier, absorbed and excreted in the bile, or metabolized by the colonic microflora or our own tissues. Polyphenols are highly sensitive to the mild alkaline conditions in the small intestine and a good proportion of these compounds can be transformed before absorption (Bermúdez-Soto and others 2007). This has been shown in a recent *in vitro* study using a digestion Caco-2 cell model by Laurent and others (2007), who obtained evidence that only pancreatic digestion plays a determining role in the bioavailability of phenolic compounds from grape seed extracts. Salivary and gastric digestion had no effect on polyphenol stability, because interactions between proteins (for example, digestive juice proteins and/or brush border cell proteins or enzymes) and phenolic compounds occurred mainly during the intestinal step and decreased their bioavailability. As a consequence, the most abundant polyphenols in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach and others 2005). The bioavailability of phenolic compounds can be also affected by differences in concentration within plant tissues, variations in cell wall structure, location of glycosides in cells, and binding of phenolic compounds within the food matrix (Balasundram and others 2006). Methods of culinary preparation have also a marked effect on the polyphenol content of foods. For example, simple peeling of fruit and vegetables can eliminate a significant portion of polyphenols because these substances are often present in higher concentrations in the skin than in the pulp. Interestingly, Manach and others (2004) commented that the effect of the food matrix on the bioavailability of polyphenols had not been examined in much detail. In a later review by these authors, an extensive variability in the bioavailability and bioefficacy of polyphenols in humans was observed with up to 10-fold variation in the C_{max} values for most phenolic compounds. Among several factors that could explain this variability were the food matrix and the background diet (Manach and others 2005). Milbury and others (2002) also concluded that the information on the bioavailability of different flavonoid groups is limited. These authors suggested that anthocyanins appear to be absorbed in their unchanged glycosylated forms by humans and provided measurements of the pharmacokinetic parameters of dietary anthocyanins absorption. In their review, Aherne and O'Brien (2002) concluded that flavonol content in processed foods (canned, glass jars, frozen) from onion, kale, apple, bean, and so on can be significantly lower (approximately 50%) than levels found in fresh products. However, processing of food such as tomatoes may increase flavonol availability (free form flavonol) due to hydrolysis and extraction from food matrix (Stewart and others 2000). The accumulation of quercetin or release of the aglycone form in processed foods can occur during digestion as a consequence of enzymatic hydrolysis of quercetin that has become conjugated during pasteurization and fermentation. Simonetti and others (2005) have shown that flavonoid glucosides such as rutin were absorbed from tomato puree even at low amounts of intake, suggesting that this food was probably a good vehicle for these polyphenolic compounds.

Polyphenols from wine, in particular resveratrol, anthocyanins, catechins, and quercetin, have attracted a great deal of attention. Tannins (complex polyphenols) in the grape berry are located in specialized tissues of the skin and seed, and because of their differential extraction from these matrices during pressing and fermentation (especially in red wines) their presence in wine may not necessarily reflect their relative abundance in the fruit at harvest. This is important when invoking health-related benefits from wines. However, most of the major solutes present in the grape berry at harvest contribute to wine composition. This is the case of resveratrol where higher concentrations are found in red grapes rather than in white varieties, and in red wines (fermented with the skins) rather than in white wines (King and others 2006). Tannins are tightly bound to cellulose and hemicellulose in the cell walls of fresh grapes, but not to pectin (Adams 2006). It was found that only a fraction of the tannin was extracted during winemaking and some of the nonextracted tannin was tightly bound to the insoluble matrix of the grape berry. The capacity of the insoluble matrix to capture tannin can amount to more than 22% of the tannin present in the fruit. This result indicates that tannin binding to the insoluble matrix of grape berries

may be an important factor in the ability to extract tannin from fruit during fermentation (Hazak and others 2005). In addition new polyphenols may be formed during processing (van de Wiel and others 2001). Once extracted, the absorption of quercetin, catechin, and resveratrol in humans was almost equivalent in white wine, grape juice, and vegetable juice.

Berries that accumulate large quantities of anthocyanins, pigments associated with the red and blue colors of plant organs (fruits, flowers, and leaves), have been proposed to have important health-related benefits apart from their antioxidant activity. Anthocyanins are composed of 6 anthocyanidin aglycones linked to sugar groups. However, the bioavailability of anthocyanins is very low and their metabolism is still not fully understood (Wu and others 2002). Felgines and others (2003) suggested that anthocyanins in fresh strawberries were glucuro- and sulfo-conjugated in humans and that their absorption was probably affected by the food matrix. On the other hand, in a study by Mazza and others (2002) the absorption of anthocyanins in humans was investigated after the consumption of a high-fat meal with a freeze-dried blueberry powder containing 25 individual anthocyanins. Nineteen of the 25 anthocyanins were detected in blood serum and their presence was directly correlated with an increase in serum antioxidant capacity. These results appear to indicate that anthocyanins can be absorbed in their intact glycosylated and possibly acylated forms in human subjects. *In vitro*, the exposure to differences in pH, oxygen, and heating combines to greatly reduce raspberry (extracts) anthocyanin availability to the serum fraction, but codigestion with common foodstuffs (such as bread, breakfast cereal, ice cream, and cooked minced beef) may help protect the labile anthocyanins and certainly does not markedly decrease the level of serum bioavailability polyphenols. Results suggests that polyphenols transiently bind to food matrices during digestion, which protects the more labile anthocyanins from degradation; however, the details about the components involved in the process require further attention (McDougall and others 2005). Based on the limited knowledge available on absorption and metabolic fate of phytochemicals found in berries and conflicting results of bioavailabilty studies, Beattie and others (2005) in their comprehensive review have recommended that "... it would be unwise to ascribe additional health promoting benefits from berries beyond those recognized for fruits and vegetables in general."

Isoflavones (subclass of polyphenols) are phenolic compounds strikingly similar in chemical structure to mammalian female estrogens and occur naturally in plants, predominantly in soybeans, and thus are known as "phytoestrogens." They are currently heralded as offering potential alternative therapies for a range of hormone-dependent conditions, including some cancers, menopausal symptoms, cardiovascular disease, and osteoporosis (Setchell and Cassidy 1999; Birt and others 2001). Isoflavones occur in different chemical forms: aglycones, ß-glucosides, manolyl-, and acetyl-glucosides. Although in soy foods the predominant form is as glucosides, the concentration and composition vary according to the part of the seed where the isoflavones are found (seed coat, cotyledon, and axis). Food processing can alter the ratio of glucosides and fermentation processes may result in an increase in the levels of aglycones in commercial soy products (Setchell and Cassidy 1999; de Pascual-Teresa and others 2006). In addition, isoflavone glycosides are not absorbed intact across the enterocytes of healthy adults, but require the hydrolysis of the sugar moiety by intestinal ß-glucosidases (Setchell and others 2002).

There is little information about the effect of the food matrix (and its changes along the digestion process) on the bioavailability of isoflavones. Low recovery of isoflavones after *in vitro* digestion (bioaccessibility) was reported from cookies (22.0% ± 14.1%) compared to fruit juice (90.0% ± 12.7%) and chocolate bars (99.5% ± 0.7%) (de Pascual-Teresa and others 2006). These results were related to the complexity of the sugar/starch/protein matrix in cookies, which may have hindered the extraction of isoflavones. However, these findings were not replicated in a human study (bioavailability), unveiling the difficulties in extrapolating results from *in vitro* experimentation to humans. Apparently, these differences could be explained because during *in vivo* digestion the gut microflora degrades isoflavones, in particular, daidzein. Additionally, interactions between released isoflavones and proteins are more likely to happen *in vivo* than *in vitro* (as is the case, in general, for all polyphenols) (Cassidy and others 2006).

Various extents of release, partitioning, and stability of the isoflavones occur at different stages of digestion. Sanz and Luyten (2006) using an *in vitro* method studied custard desserts made with starch or carboxymethylcellulose (CMC) and enriched with a soy germ extract as source of isoflavones. Incubation under simulated mouth conditions did not affect the amount and partitioning of isoflavones (aqueous/fat phase). A lower recovery and different partitioning were found after the stomach incubation, which was associated with the low pH, whereas after the intestine incubation, a higher recovery and an effect on partitioning were found. Regarding the matrix effect, custards containing starch released a significantly higher amount of isoflavones than those made with CMC, probably due to the higher enzymatic resistance of the latter. Finally, the presence of fat significantly increased the bioaccessibility of the aglycone forms, especially of genistein. The complexity in interpreting experimental results is also confirmed in other studies; for example, it has been observed that fractional absorption of isoflavones (as genistein) is influenced by the matrix and chemical composition of the food, and by gender (Birt and others 2001; Hendrich 2002; Faughnan and others 2004). Thus, isoflavones in supplements are likely to be absorbed at a faster rate compared with those ingested within a food matrix (Rowland and others 2003).

Other sources of antioxidant activity

The overall intake of several natural antioxidants present in foods has been associated with lower incidence of various aging diseases (Roberts 2005). It has already been mentioned that different methods to determine "antioxidant activity" *in vitro* not only give conflicting results among them but also do not necessarily correlate with results *in vivo*. Dekker and others (1999) concluded that attempts to relate the total antioxidant activity determined by standard tests to that *in vivo* ignored that sometimes interactions and matrix effects may lead to a lower activity than expected. For some products (for example, black tea) a good match exists between the predicted and measured antioxidant activity while for other products (for example, apple juice) up to an 80% reduction in activity has been observed. These results emphasize again that predicting health benefits based only on compositional data of nutrients does not take into account possible matrix effects and results in an overprediction of the *in vivo* activity. In a previously cited study by Nagah and Seal (2005) on the antioxidant activity of wholegrain foods (cooked and uncooked), it was suggested that cooking may destroy soluble antioxidants but not those bound within the food matrix. Cocoa is rich in polyphenols, particularly catechins (flavan-3-ols) and procyanidins. There are indications that cocoa flavanols have the ability to act as *in vivo* antioxidants and may be associated with reduced risk for vascular disease. A sharp decrease in flavanols occurs during fermentation and drying of cocoa beans but not during roasting (Wollgast and Anklam 2000). In a study published in *Nature*, researchers reported that absorption of chocolate flavonoids into the bloodstream was 69% less in milk chocolate compared to absorption from dark chocolate alone,

insinuating that these compounds interacted with milk proteins during manufacturing or in the gut (Serafini and others 2003). However, Keen and others (2005) suggested that differences in flavonol activity and its bioavailability between dark and milk chocolate were the result of the food matrix altering the kinetics of absorption rather than due to flavanol–milk protein interactions. These contradictory explanations are not uncommon in the scientific literature on nutrient bioavailability and matrix effects and stress the urgent need for further research.

Other compounds

Due to space limitations only a few examples of the effect of the food microstructure on the bioavailability of other compounds are mentioned. The absorption efficiency of highly lipophilic food microconstituents—that include the fat-soluble vitamins (A, E, D, and K) and phytochemicals with potential health benefits (the carotenoids and phytosterols)—depends on factors such as the presence of fat and the type of food matrix (Borel 2003). The food matrix has been found relevant in the absorption of other vitamins as well. Three decades ago Yasumoto and others (1977) reported that pyridoxine in rice bran and wheat germ was associated with the starch fraction and needed to be released from the food matrix to perform as vitamin B_6. Ekanayake and Nelson (1986) proposed an enzymatic digestion method of the food matrix to release and determine biologically available vitamin B_6 in processed foods. Jeanes and others (2004) demonstrated that both the amount of fat and the food matrix influenced vitamin E absorption. Höller and others (2006) emphasized the importance of the food matrix in determining the total amount of biotin in feed, food, tablets, and premixes. Kulp and others (2003) concluded that the bioaccessibility of each heterocyclic aromatic amine (HA) depended upon the doneness of meat, although it is generally assumed that all HAs in the meat matrix are equally bioavailable. Sulforaphane (SF) is considered to be the major anticarcinogenic component in broccoli. Keck and others (2003) experimenting with rats concluded that although neither endogenous nor bacterial hydrolysis of the precursor glucoraphanin gives as good a yield of SF as the exogenous white mustard myrosinase, the broccoli matrix greatly enhances SF uptake compared to absorption of purified SF. Selected examples of plant nutrients, their respective matrix, and the measured indices related to increased bioavailability (or bioaccessibility) are shown in Table 3.

Nutrients in Designed Matrices for Delivery

Man-made matrices can improve the stability of purified nutrients during storage, increase the effectiveness at the absorption site, and ensure optimal dosage. Thus, encapsulation or microencapsulation of bioactive substances, nutrients, and beneficial microorganisms (probiotics) is becoming a widely used technology in the pharmaceutical and food industries. Among microencapsulation techniques are spray-drying, spray-chilling, extrusion, coacervation, liposomes, cocrystallization, and freeze-drying (Gouin 2004). Sanz and Luyten (2006) commented that the selection of the food matrix into which the bioactive ingredient is incorporated was crucial to deliver an effective dose in the human body. To be bioavailable, the ingredient should be released from the food matrix, dissolved in the appropriate phase of digesta (usually aqueous phase), and subsequently absorbed in the human body.

Structuring matrices for nutrient delivery is a subject of enormous interest nowadays, and several matrix materials are under study. A recent article by Chen and others (2006b) describes the potential role of food proteins as materials in nutraceutical delivery systems for bioactive compounds (for example, vitamins, probiotics, bioactive peptides, and antioxidants) in the form of hydrogels and micro- or nano-particles. Embedding lycopene into whey protein matrices enhanced its bioavailability to that equal to tomato paste, the most valuable food source of bioavailable lycopene for humans (Richelle and others 2002). Hydrocolloids are potential capsule materials and have been widely used as matrices for delivery. Yoo and others (2006) found that β-tocopherol encapsulated in sodium alginate microcapsules was largely protected when exposed to simulated gastric fluid and subsequently largely released in a simulated intestinal fluid. A recent study reports that over 80% of the original lycopene could be embedded in the walls of gelatine/sucrose microcapsules by spray drying, although no information was provided as far as the activity and release (Shu and others 2006). Protein hydrogels are attractive carriers for controlled release of bioactive molecules when weak interactions occur with the polymeric as is the case of flavor compounds in gelatin gels (Boland and others 2004). Remondetto and others (2004), using protein hydrogels, showed that different iron release profiles could be obtained depending on the microstructure of the gels in which the mineral was entrapped. This result is of relevance since extrusion encapsulation of micronized dispersible ferric pyrophosphate in rice meal has proven to result in a low bioavailabilty (Moretti and others 2006).

Probiotics are defined as "live microbial food supplements (mainly lactic acid bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium*) that benefit the health of consumers beyond inherent general nutrition by maintaining or improving their intestinal microbial balance" (Guarnen and Schaafsma 1998). In general, these bacteria exhibit a low ability to survive the harsh conditions in the gastrointestinal tract and need to be protected to preserve their activity, and several food matrices and encapsulation techniques have been successfully used for this purpose (Krasaekoopt and others 2003a; Chen and others 2006a). Dairy products are obvious carriers of probiotics; thus, yogurt, fermented milks, and cheeses containing probiotics are well established in the market (Doleyres and Lacroix 2005). The tolerance of dairy *Propionibacteria* to immobilization in several food matrices was studied by Leverrier and others (2005), who showed that some matrices can significantly improve protection of bacterial cells from stress injury (*in vitro* tolerance to digestive stresses). Capela and others (2006) concluded that microencapsulation with alginate improved the viability of probiotic organisms in freeze-dried yogurt stored for 6 mo at 4 and 21 °C. Similarly, Krasaekoopt and others (2006) concluded that the survival of encapsulated probiotic bacteria in chitosan-coated alginate beads was higher than that of free cells by approximately 1 log cycle over a period of 4 wk at 4 °C. The problem is that processing conditions to protect microorganisms seem to be specific for each genus and even for each particular strain. Picot and Lacroix (2004) reported that the viable counts of *Bifidobacterium breve* R070 cells entrapped in whey protein microcapsules were significantly higher than those of free cells after 28 d in yogurt stored at 4 °C (+2.6 log cycles), and after sequential exposure to simulated gastric and intestinal juices (+2.7 log cycles). However, no protective effect of encapsulation was observed with the strain *Bifidobacterium longum* R023.

The use of vegetable matrices (for example, fruit pieces, spent grains after extraction) as carriers of nutrients is a novel concept in the design of functional foods that opens new product categories and commercial opportunities. Vacuum and/or atmospheric impregnation allows introduction of controlled quantities of a nutrient solution into the porous structure of fruits and vegetables (Fito and others 2001). Alzamora and others (2005) described the main aspects of the kinetics of matrix fortification via solution impregnation, the stability of some active compounds (probiotics and minerals), interactions between calcium and the cell structure, and the mechanical properties of impregnated fruit and vegetable tissues.

Table 3 – Effect of the state of the matrix on measured bioavailability/bioaccessibility of selected nutrients

Nutrient	Food (Matrix)	Processing	Bioavailability, bioaccessibility	Method and units	Reference
β-carotene	Green vegetable leaves	Raw	3 to 6	Plasma response ("relative carotenoid bioavailability"); percent	van het Hof and others (2000b)
	Carrot	Raw	19 to 34		
		Carrot juice	70% higher than raw		
	Spinach	Whole leaf	5.1	Plasma response ("relative carotenoid bioavailability"); percent	Castenmiller and others (1999), van het Hof and others (2000), Castenmiller and others (1999)
		Minced leaf	6.4		
		Liquefied leaf	9.5		
	Tomato	Minimal heat treatment	7.53 $\pm$ 8.8	Postprandial TRL response; nmol$*$h/L	van het Hof and others (2000a)
		Extensive heat treatment	20.5 $\pm$ 8.8		
		Homogenization, none	2.51 $\pm$ 6.5		
		Homogenization, mild	16.6 $\pm$ 6.5		
		Homogenization, severe	25 $\pm$ 6.5		
	Carrot	Boiled-mashed (home preparation)	0.9 $\pm$ 0.6	Mass of β-carotene absorbed intact (*in vivo*); percent	Edwards and others (2002)
		Puree (industrial processing)	2.4 $\pm$ 0.8		
Trans-β-carotene	Carrot	Raw carrot	41.4 $\pm$ 7.4	Trans-β-carotene in the ileal effluent; percent	Livny and others (2003)
		Cooked puree	65.1 $\pm$ 7.4		
α-carotene	Carrot	Boiled-mashed (home preparation)	1.2 $\pm$ 0.5	Mass of β-carotene absorbed intact (*in vivo*); percent	Edwards and others (2002)
		Puree (industrial processing)	3.5 $\pm$ 0.9		
Lutein	Spinach	Whole leaf	45	Plasma response ("relative carotenoid bioavailability"); percent	Castenmiller and others (1999)
		Minced leaf	52		
		Liquefied leaf	55		
		Whole leaf spinach	0.33	Plasma levels after 4d consumption; μmol/L	van het Hof and others (1999)
		Chopped spinach	0.37		
Lycopene	Tomato	No homogenization	54.9 $\pm$ 11	Postprandial TRL response; nmol$*$h/L	van het Hof and others (2000a)
		Mild homogenization	72.2 $\pm$ 11		
		Severe homogenization	88.7 $\pm$ 11		
		Minimal heat treatment	59 $\pm$ 16.6		
		Extensive heat treatment	84.9 $\pm$ 16.6		
Folic acid	Spinach	Whole leaf spinach	23.4	Plasma levels after 4d consumption; nmol/L	van het Hof and others (1999)
		Chopped spinach	25.7		
α-tocopherol	Fresh broccoli	Before cooking	0.32 $\pm$ 0.05	HPLC-method; mg/100g	Bernhardt and Schlich (2006)
		Boiling	1.54 $\pm$ 0.16		
		Stewing	1.61 $\pm$ 0.07		
		Steaming	1.58 $\pm$ 0.16		
		Pressure steaming	1.70 $\pm$ 0.08		
Antioxidant activity	Tomato	No homogenization	63.9 $\pm$ 16.5	Postprandial TRL response; μmol$*$h/L	van het Hof and others (2000a)
		Mild homogenization	82.6 $\pm$ 16.5		
		Severe homogenization	118 $\pm$ 16.5		
		Minimal heat treatment	78.6 $\pm$ 15.4		
		Extensive heat treatment	97.9 $\pm$ 15.4		
Total antioxidant	Whole-wheat spaghetti	Raw	7.38 $\pm$ 0.097	FRAP antioxidant content (*in vitro* procedure); μM ferrous ion equivalents	Nagah and Seal (2005)
		Cooked	8.07 $\pm$ 0.500		
	Corn spaghetti	Raw	5.00 $\pm$ 0.588		
		Cooked	6.01 $\pm$ 0.317		
Heterocyclic amines (HA)	Chicken meat	Large particle size ($>$3 mm^2)	100	HA in the supernatant (*in vitro* system); digestibility ratio	Kulp and others (2003)
		Small particle size ($<$3 mm^2)	126 to 188		

Anino and others (2006) studied the potential of fresh apple pieces as a matrix for calcium incorporation by 2 different impregnation techniques (under vacuum and at atmospheric pressure). Impregnation treatments promoted a significant increase in calcium concentration, especially during a long-term atmospheric process, with the amount of Ca^{2+} incorporated in pieces enough to satisfy about 23% to 62% of the recommended daily intake. Preliminary results of sensory characteristics of the final product have shown that, in spite of the softening observed, textural characteristics of infused apple pieces appeared to be acceptable.

Food Matrix and Allergenicity

Because accumulating evidence that the activity of food allergens depends on the microstructure of foods, this topic will be briefly reviewed here. Allergenicity is an extreme case where a food component elicits a negative immunological reaction in the body. In the United Kingdom approximately 1% to 2% of adults and 5% to 7% of children are thought to suffer some kind of IgE-mediated food allergies (IFST 2005). In order to produce sensitization, allergens have to be released from the food matrix, survive digestion, and be absorbed through the gut epithelial barrier in an immunological active form (van Wijk and others 2005). The action of food processing on the food matrix may affect the stability and release of allergens, as well as destroy or induce "neo-epitopes" in food proteins (Maleki 2004; Mills and others 2006). For example, it has been reported that texturization neutralizes the action of a major allergen present in soy protein (Franck and others 2002).

Unlike their common role as nutrients, some proteins, in particular glycoproteins present in a number of foods, may be major food allergens. Certain proteins may not exert their allergenicity unless they are released from protein body organelles in which they are naturally present. Digestion, hydration, interactions with other proteins, and other matrix effects may contribute to the ability of a protein to reach the sites of immune action in the gastrointestinal mucosa and thus exert their potential allergenicity. Moreover, in the case of peanut extracts the food matrix itself activates immune cells, thus eliciting immune responses to peanut protein (Teuber 2002; van Wijk and others 2005). It has been suggested that allergens from tree nuts and peanuts may become protected during digestion by the fat released from the cells. Formation of colloidal structures in the gut and their effect on the action of allergens or the bioavailability of nutrients is a subject that requires further research.

Conclusions

A major subject in food technology in the 1970s was to assess the effect of processing on nutrient losses (Harris 1975). Nowadays the issue is nutrient bioavailability, or how much of an ingested nutrient is effectively absorbed and appears in the blood plasma. As repeatedly noted in this review, evidence is accumulating that food microstructure (or the food matrix) plays a major role in the release and bioavailability of several nutrients and allergenic substances. This has enormous consequences in assessing the nutritional role and real impact of many foods and prepared nutraceuticals in the prevention and therapy of some chronic human diseases.

Some nutrients found in plants are protected in nature against degradation inside cells, whether attached to membranes, occluded inside cell organelles, or bound to cell walls, but this natural protection lowers bioavailability. Thermal and physical processing, mastication, and to limited extent digestion break down the cell walls, making the release of nutrients from the food matrix easier and rendering them available for absorption in the intestine. However, even if released during processing and digestion, nutrients may possibly interact with other food components in the gut by binding to macromolecules, forming chemical complexes and colloidal structures that reduce or improve their bioavailability, a subject that needs urgent research. Alternatively, food microstructure can be manipulated to our advantage by protecting nutrient extracts and beneficial microorganisms during storage and through transit in the stomach inside man-made matrices. Figure 3 summarizes some possible mechanisms related to microstructure that influence the bioavailability of nutrients in the gut.

There is still conflicting information regarding the effects of the food matrix on the bioavailability of some nutrients that needs to be urgently resolved. Some of the future directions of research to clarify this issue include: (1) Improve *in vitro* methods for the reliable determination of bioavailability of genuine *in vivo* metabolites (that may be different from the forms found in ingested foods), as they are rapid, cheap, and circumvent ethical issues related to the use humans or animals; (2) In vivo assays will continue to be used as confirmatory tests to validate results of laboratory studies and

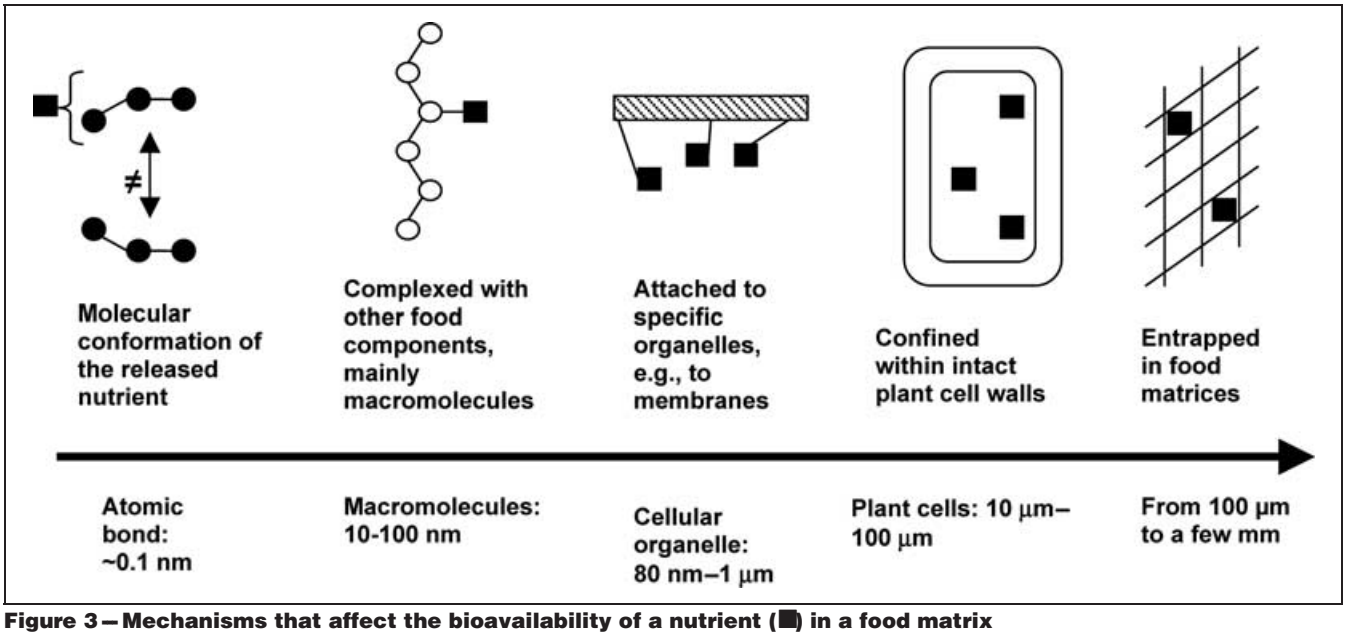

Figure 3—Mechanisms that affect the bioavailability of a nutrient (■) in a food matrix

to analyze some physiological conditions hard to reproduce experimentally; (3) More specific analytical and microscopy techniques should be used to study the location, release, and complexing of nutrients within an evolving food matrix and in model systems; (4) Experiments assessing the effect of processing variables (for example, temperature, shear) need to be carefully controlled so that data can be scaled up into actual industrial applications; and (5) The subject of interactions between bioactive molecules, macromolecules, and fat in the gut should be actively examined since new microstructures (for example, at the colloidal level) may be assembled, thus affecting the bioavailability. Similarly, the role of colonic bacteria in bioavailability also needs to be determined.

Acknowledgments

This research has been funded by the Marcel Loncin Award of the Institute of Food Technologists to JMA and a CONICYT doctoral fellowship to JP.

References

Abou-Saleh MT, Coppen A. 2006. Folic acid and the treatment of depression. J Psychosom Res 61:285–7.
Adams DO. 2006. Phenolics and ripening in grape berries. Am J Enol Viticult 57:249–56.
Aguilera JM. 2005. Why food microstructure? J Food Eng 67:3–11.
Aguilera JM. 2006. Food product engineering: building the right structures. J Sci Food Agr 86:1147–55.
Aguilera JM, Stanley DW. 1999. Microstructural principles of food processing and engineering. 2nd ed. Gaitherburg, Md.: Aspen Publishers Inc.
Aherne SA, O'Brien NM. 2002. Dietary flavonols: chemistry, food content, and metabolism. Nutrition 18:75–81.
Alzamora SM, Salvatori D, Tapia MS, López-Malo A, Welti-Chanes J, Fito P. 2005. Novel functional foods from vegetable matrices impregnated with biologically active compounds. J Food Eng 67:205–14.
Anino SV, Salvatori DM, Alzamora SM. 2006. Changes in calcium level and mechanical properties of apple tissue due to impregnation with calcium salts. Food Res Int 39:154–64.
Avantaggiato G, Havenaar R, Visconti A. 2003. Assessing the zearalenone-binding activity of adsorbent materials during passage through a dynamic *in vitro* gastrointestinal model. Food Chem Toxicol 41:1283–90.
Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203.
Beattie J, Crozier A, Duthie GG. 2005. Potential health benefit of berries. Curr Nutr Food Sci 1:71–86.
Bermúdez-Soto MJ, Tomás-Barberán FA, García-Conesa MT. 2007. Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion. Food Chem, 102:865–7040.
Bernhardt S, Schlich E. 2006. Impact of different cooking methods on food quality: retention of lipophilic vitamins in fresh and frozen vegetables. J Food Eng 77:327–33.
Bicanic D, Luterotti S, Becucci M, Fogliano V, Versloot P. 2005. Photoacoustic measurement of lutein in biological matrix. J Phys IV (France) 125:825–8
Birt DF, Hendrich S, Wang WQ. 2001. Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol Therapeut 90:157–77.
Boland AB, Buhr K, Giannouli P, van Ruth SM. 2004. Influence of gelatin, starch, pectin and artificial saliva on the release of 11 flavour compounds from model gel systems. Food Chem 86:401–11.
Borel P. 2003. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). Clin Chem Lab Med 41:979–94.
Boyer J, Liu RH. 2004. Apple phytochemicals and their health benefits. Nutr J 3:5.
Boyer J, Brown D, Liu RH. 2005. *In vitro* digestion and lactase treatment influence uptake of quercetin and quercetin glucoside by the Caco-2 cell monolayer. Nutr J 4:1.
Brouns F, Vermeer C. 2000. Functional food ingredients for reducing the risks of osteoporosis. Trends Food Sci Tech 11:22–33.
Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CM, Duran M, van het Hof KH, Eskes TK, Hautvast JG, Steegers-Theunissen RP. 1999. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. J Nutr 129:1135–9.
Brown ED, Blakely SR, Babu U, Grundel E, Mitchell GV. 1997. Vegetable concentrates interact with canthaxanthin to affect carotenoid bioavailability and superoxide dismutase activity but not immune response in rats. Nutr Res 17:989–98.
Capela P, Hay TKC, Shah NP. 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. Food Res Int 39:203–11.
Cassidy A, Brown JE, Hawdon A, Faughnan MS, King LJ, Millward J, Zimmer-Nechemias L, Wolfe B, Setchell KD. 2006. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. J Nutr 136:45–51.
Castenmiller J, West CE, Linssen JP, van et Hof KH, Voragen AG. 1999. The food matrix of spinach is a limiting factor in determining the bioavailability of ß-carotene and to a lesser extent of lutein in humans. J Nutr 129:349–55.
Chen K, Chen M, Lin C. 2006a. Optimal combination of the encapsulating materials for probiotic microcapsules and its experimental verification (R1). J Food Eng 76:313–20.
Chen L, Remondetto GE, Subirade M. 2006b. Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Tech 17:272–83.
Chitchumroonchokchai C, Schwartz SJ, Failla ML. 2004. Assessment of lutein bioavailability from meals and a supplement using simulated digestion and Caco-2 human intestinal cells. J Nutr 134:2280–6.
Chu M, Beauchemin D. 2004. Simple method to assess the maximum bio-accessibility of elements from food using flow injection and inductively coupled plasma mass spectrometry. J Anal Atom Spectrom 19:1213–6.
Cuvelier M, Bondet V, Berset C. 2000. Behavior of phenolic antioxidants in a partitioned medium: structure-activity relationship. J Am Oil Chem Soc 77:819–23.
Dekker M, Verkerk R, van der Sluis AA, Khokhar S, Jongen WM. 1999. Analysing the antioxidant activity of food products: processing and matrix effects. Toxicol In Vitro 13:797–9.
Deming DM, Erdmann JW. 1999. Mammalian carotenoid absorption and metabolism. Pure Appl Chem 71:2213–33.
de Pascual-Teresa S, Hallund J, Talbot D, Schroot J, Williams CM, Bugel S, Cassidy A. 2006. Absorption of isoflavones in humans: effects of food matrix and processing. J Nutr Biochem 17:257–64.
Doleyres Y, Lacroix C. 2005. Technologies with free and immobilized cells for probiotic bifidobacteria production and protection. Int Dairy J 15:973–88.
Edwards AJ, Nguyen CH, You C, Swanson JE, Emenhiser C, Parker RS. 2002. α- and ß-carotene from a commercial carrot puree are more bioavailable to humans than from boiled-mashed carrots, as determined using an extrinsic stable isotope reference method. J Nutr 132:159–67.
Ekanayake A, Nelson PE. 1986. An *in vitro* method for estimating biologically available vitamin B6 in processed foods. Brit J Nutr 55:235–44.
Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, Jenkins DJ. 2004. Role of cell walls in the bioaccessibility of lipids in almond seeds. Am J Clin Nutr 80:604–13.
Faughnan MS, Hawdon A, Ah-Singh E, Brown J, Millward DJ, Cassidy A. 2004. Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. Brit J Nutr 91:567–74.
Faulks RM, Southon S. 2005. Challenges to understanding and measuring carotenoid bioavailability. Biochim Biophys Acta 1740:95–100.
Felgines C, Talavéra S, Gonthier M, Texier O, Scalbert A, Lamaison J, Rémésy C. 2003. Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. J Nutr 133:1296–301.
Fito P, Chiralt A, Betoret N, Gras M, Cháfer M, Martinez-Monzó J, Andrés A, Vidal D. 2001. Vacuum impregnation and osmotic dehydration in matrix engineering. Application in functional fresh food development. J Food Eng 49:175–83.
Franck P, Vautrin DM, Dousset B, Kanny G, Nabet P, Guenard-Bilbaut L, Parisot L. 2002. The allergenicity of soybean-based products is modified by food technologies. Int Arch Allergy Imm 128:212–9.
Garrett DA, Failla ML, Sarama RJ. 1999. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. J Agr Food Chem 47:4301–9.
Garrett DA, Failla ML, Sarama RJ. 2000. Estimation of carotenoid bioavailability from fresh stir-fried vegetables using an *in vitro* digestion Caco-2 cell culture model. J Nutr Biochem 11:574–80.
Gil-Izquierdo A, Zafrilla P, Tomás-Barberán FA. 2002. An *in vitro* method to simulate phenolic compound release from the food matrix in the gastrointestinal tract. Eur Food Res Technol 214:155–9.
Glahn RP, Lee OA, Yeung A, Goldman MI, Miller DD. 1998. Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an *in vitro* digestion/Caco-2 cell culture model. J Nutr 128:1555–61.
Gouin S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Tech 15:330–47.
Gregory JF, Quinlivan EP, Davis SR. 2005. Integrated the issues of folate bioavailability, intake and metabolism in the era of fortification. Trends Food Sci Tech 16:229–40.
Guarnen F, Schaafsma GJ. 1998. Probiotics. Int J Food Microbiol 39:237–8.
Haro-Vicente JF, Martínez-Graciá C, Ros G. 2006. Optimisation of *in vitro* measurement of available iron from different fortificants in citric fruit juices. Food Chem 98:639–48.
Harris RS. 1975. Nutritional evaluation of food processing. 2nd ed. Westport Conn.: Avi Publishing Co.
Hazak JC, Harbertson JF, Lin CH, Ro BH, Adams DO. 2005. The phenolic components of grape berries in relation to wine composition. Acta Hortic 689:189–96.
Heacock PM, Hertzler SR, Wolf B. 2004. The glycemic, insulinemic, and breath hydrogen responses in humans to a food starch esterified by 1-octenyl succinic anhydride. Nutr Res 24:581–92.
Hedrén E, Mulokozi G, Svanberg U. 2002. *In vitro* accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. Int J Food Sci Nutr 53:445–53.
Hendrich S. 2002. Bioavailability of isoflavones. J Chromatogr B 777:203–10.
Hoebler C, Lecannu G, Belleville C, Devaux MF, Popineau Y, Barry JL. 2002. Development of an *in vitro* system simulating bucco-gastric digestion to assess the physical and chemical changes of food. Int J Food Sci Nutr 53:389–402.
Höller U, Wachter F, Wehrli C, Fizet C. 2006. Quantification of biotin in feed, food, tablets, and premixes using HPLC-MS(MS. J Chromatogr B 831:8–16.
IFST 2005. Available from: http://www.ifst.org/uploadedfiles/cms/store/ATTACHMENTS/allergy.pdf. Accessed Oct 16, 2006.
Jeanes YM, Hall WL, Ellard S, Lee E, Lodge JK. 2004. The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix. Brit J Nutr 92: 575–9.
Kaláb M, Allan-Wojtas P, Miller SS. 1995. Microscopy and other imaging techniques in food structure analysis. Trends Food Sci Tech 6:177–86.
Keck A, Qiao Q, Jeffery EH. 2003. Food matrix effects on bioactivity of broccoli-derived sulforaphane in liver and colon of F344 rats. J Agr Food Chem 51:3320–7.
Keen CL, Holt RR, Oteiza PI, Fraga CG, Schmitz HH. 2005. Cocoa antioxidants and cardiovascular health. Am J Clin Nutr 81:298S–303S.

King RE, Bomser JA, Min DB. 2006. Bioactivity of resveratrol. Compr Rev Food Sci Food Safety 5:65–70.
Krasaekoopt W, Bhandari B, Deeth H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13:3–13.
Krasaekoopt W, Bhandari B, Deeth HC. 2006. Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT- and conventionally treated milk during storage. Food Sci Technol-Leb 39:177–83.
Krinsky NI, Johnson EJ. 2005. Carotenoid actions and their relation to health and disease. Mol Aspects Med 26:459–516.
Krul C, Luiten-Schuite A, Tenfelde A, van Ommen B, Verhagen H, Havenaar R. 2001. Antimutagenic activity of green tea and black tea extracts studied in a dynamic *in vitro* gastrointestinal model. Mutat Res 474:71–85.
Kulp KS, Fortson SL, Knize MG, Felton JS. 2003. An *in vitro* model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. Food Chem Toxicol 41:1701–10.
Larsson SC, Giovannucci E, Wolk A. 2006. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology 131: 1271–83.
Laurent C, Besançon P, Caporiccio B. 2007. Flavonoids from a grape seed extract interact with digestive secretions and intestinal cells as assessed in an *in vitro* digestion/Caco-2 cell culture model. Food Chem 100:1704–12.
Leverrier P, Fremont Y, Rouault A. 2005. *In vitro* tolerance to digestive stresses of propionibacteria: influence of food matrices. Food Microbiol 22:11–8.
Livny O, Reifen R, Levy I, Madar Z, Faulks R, Southon S, Schwartz B. 2003. ß-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. Eur J Nutr 42:338–45.
Maleki SJ. 2004. Food processing: effects on allergenicity. Curr Opin Allergy Clin Immunol 4:241–5.
Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. 2004. Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–47.
Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 81:230S– 42S.
Mazza G, Kay CD, Cottrell T, Holub BJ. 2002. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. J Agr Food Chem 50:7731–7.
McDougall GJ, Dobson P, Smith P, Blake A, Stewart D. 2005. Assessing potential bioavailability of raspberry anthocyanins using an *in vitro* digestion system. J Agr Food Chem 53:5896–904.
McNulty H, Pentieva K. 2004. Folate bioavailability. Proc Nutr Soc 63:529–536.
Milbury PE, Cao G, Prior RL, Blumberg J. 2002. Bioavailability of elderberry anthocyanins. Mech Ageing Dev 123:997–1006.
Mills C, Sancho AI, Moreno J. 2006. The effects of food processing on allergens. In: Mills C, Wichers H, Hoffmann-Sommergruber K, editors. Managing allergens in food. Cambridge, U.K.: Woodhead Publ. Ltd.. p 120–40.
Moretti D, Zimmermann MB, Wegmüller R, Walczyk T, Zeder C, Hurrell RF. 2006. Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr 83:632–8.
Nagah AM, Seal CJ. 2005. *In vitro* procedure to predict apparent antioxidant release from wholegrain foods measured using three different analytical methods. J Sci Food Agr 85:1177–85.
Omoni AO, Aluko RE. 2005. The anti-carcinogenic and anti-atherogenic effects of lycopene: a review. Trends Food Sci Tech 16:344–50.
Ottley C. 2005. Food composition databases. Trends Food Sci Tech 16:507–9.
Pakkala H, Reinivuo H, Ovaskainen ML. 2006. Food composition on the world wide web: a user-centred perspective. J Food Compos Anal 19:231–40.
Pennington JA. 2002. Food composition databases for bioactive food components. J Food Compos Anal 15:419–34.
Perez-Galvez A, Martin HD, Sies H, Stahl W. 2003. Incorporation of carotenoids from paprika oleoresin into human chylomicrons. Brit J Nutr 89:787–93.
Picot A, Lacroix C. 2004. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int Dairy J 14:505–15.
Re R, Fraser PD, Long M, Bramley PM, Rice-Evans C. 2001. Isomerization of lycopene in the gastric milieu. Biochem Bioph Res Co 281:576–81.
Remondetto GE, Beyssac E, Subirade M. 2004. Iron availability from whey protein hydrogels: an *in vitro* study. J Agr Food Chem 52:8137–43.
Richelle M, Bortlik K, Liardet S, Hager C, Lambelet P, Baur M, Applegate LA, Offord EA. 2002. A food-based formulation provides lycopene with the same bioavailability to humans as that from tomato paste. J Nutr 132:404–8.
Roberts DC. 2005. Nutrition and metabolism: antioxidants, the food matrix and methodological considerations. Curr Opin Lipidol 16:111–3.
Rock CL, Lovalvo JL, Emenhiser C, Ruffin MT, Flatt SW, Schwartz SJ. 1998. Bioavailability of ß-carotene is lower in raw than in processed carrots and spinach in women. J Nutr 128:913–6.
Rondini L, Peyrat-Maillard M, Marsset-Baglieri A, Fromentin G, Durand P, Tomeä D, Prost M, Berset C. 2004. Bound ferulic acid from bran is more bioavailable than the free compound in rat. J Agr Food Chem 52:4338–43.
Rowland I, Faughnan M, Hoey L, Wähälä K, Williamson G, Cassidy A. 2003. Bioavailability of phyto-estrogens. Brit J Nutr 89:S45–S58.
Rychlik M, Netzel M, Pfannebecker I, Frank T, Bitsch I. 2003. Application of stable isotope dilution assays based on liquid chromatography-tandem mass spectrometry for the assessment of folate bioavailability. J Chromatogr B 792:167–76.
Sanz T, Luyten H. 2006. Release, partitioning and stability of isoflavones from enriched custards during mouth, stomach and intestine *in vitro* simulations. Food Hydrocoll 20:892–900.
Scalbert A, Williamson G. 2000. Dietary intake and bioavailability of polyphenols. J Nutr 130:2073S–85S.
Schakel SF, Buzzard M, Gebhardt SE. 1997. Procedures for estimating nutrient values for food composition databases. J Food Compos Anal 10:102–14.
Serafini M, Bugianesi R, Maiani G, Valtuena S, de Santis S, Crozier A. 2003. Plasma antioxidants from chocolate. Nature 424:1013.
Serrano J, Goñi I, Saura-Calixto F. 2005. Determination of ß-carotene and lutein available from green leafy vegetables by an *in vitro* digestion and colonic fermentation method. J Agr Food Chem 53:2936–40.
Setchell KD, Cassidy A. 1999. Dietary isoflavones: biological effects and relevance to human health. J Nutr 129:758–67.
Setchell KD, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, Kirschner AS, Heubi JE. 2002. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr 76:447–53.
Shi J, Le Maguer M. 2000. Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit Rev Biotechnol 20:293–334.
Shu B, Yu W, Zhao Y, Liu X. 2006. Study on microencapsulation of lycopene by spray-drying. J Food Eng 76:664–9.
Similä M, Ovaskainen ML, Virtanen MJ, Valsta LM. 2006. Nutrient content patterns of Finnish foods in a food composition database. J Food Compos Anal 19:217–24.
Simonetti P, Gardana C, Riso P, Mauri P, Pietta P, Porrini M. 2005. Glycosylated flavonoids from tomato puree are bioavailable in humans. Nutr Res 25:717–26.
Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Brit J Ophthalmol 82:907–10.
Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Lean ME, Crozier A. 2000. Occurrence of flavonols in tomatoes and tomato-based products. J Agr Food Chem 48:2663–9.
Stewart KK. 1997. What are the variances of food composition data? J Food Compos Anal 10:89.
Suzuki H, Fukushima M, Okamoto S, Takahashi O, Shimbo T, Kurose T, Yamada Y, Inagaki N, Seino Y, Fukui T. 2005. Effects of thorough mastication on postprandial plasma glucose concentrations in nonobese Japanese subjects. Metabolism 54:1593–9.
Takimoto H, Tamura T. 2006. Increasing trend of spina bifida and decreasing birth weight in relation to declining body-mass index of young women in Japan. Med Hypotheses 67:1023–6.
Teuber SS. 2002. Hypothesis: the protein body effect and other aspects of food matrix effects. Ann Ny Acad Sci 964:111–6.
TNO. 2006. Available from: http://www.tno.nl/kwaliteit_van_leven/producten_en_diensten/food_and_nutrition/health_promotion/tnos_gastro_intestinal_mo/KvL%20voeding%20042e_TIM.pdf. Accessed October 27, 2006.
van de Wiel A, van Golde PH, Hart HC. 2001. Blessings of the grape. Eur J Intern Med 12:484–9.
van het Hof KH, Tijburg LB, Pietrzik K, Weststrate JA. 1999. Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. Brit J Nutr 82:203–12.
van het Hof KH, de Boer BC, Tijburg LB, Lucius BR, Zijp I, West CE, Hautvast JG, Weststrate JA. 2000a. Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. J Nutr 130:1189–96.
van het Hof KH, West CE, Weststrate JA, Hautvast JG. 2000b. Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503–6.
van Wijk F, Nierkens S, Hassing I, Feijen M, Koppelman SJ, de Jong GA, Pieters R, Knippels LM. 2005. The effect of the food matrix on *in vitro* immune responses to purified peanut allergens. Toxicol Sci 86:333–41.
Verwei M, Arkbage K, Havenaar R, van den Berg H, Witthöft C, Schaafsma G. 2003. Folic acid and 5-methyltetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic *in vitro* gastrointestinal model. J Nutr 133:2377–83.
Verwei M, Arkbåge K, Groten JP, Witthöft C, vandenBerg H, Havenaar R. 2005. The effect of folate-binding proteins on bioavailability of folate from milk products. Trends Food Sci Tech 16:307–10.
Wollgast J, Anklam E. 2000. Polyphenols in chocolate: is there a contribution to human health? Food Res Intl 33:449–59.
Wu X, Cao G, Prior RL. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. J Nutr 132:1865–71.
Yasumoto K, Tsuji H, Iwami K, Mitsuda H. 1977. Isolation from rice bran of a bound form of vitamin B6 and its identification of 5'-0-(ß-D-glucopyranosyl) pyridoxine. Agr Biol Chem Tokyo 41:1061–7.
Yeum K, Russell RM. 2002. Carotenoid bioavailability and bioconversion. Annu Rev Nutr 22:483–504.
Yoo S, Song Y, Chang P, Lee HG. 2006. Microencapsulation of a-tocopherol using sodium alginate and its controlled release properties. Int J Biol Macromol 38:25–30.
Zaripheh S, Erdman JW. 2002. Factors that influence the bioavailability of xanthophylls. J Nutr 132:531S–4S.
Zhou JR, Gugger ET, Erdman JW. 1996. The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta- and alpha-carotene in the ferret model. J Am Coll Nutr 15:84–91.