

# Plant Metabolites and Nutritional Quality of Vegetables

N. HOUNSOME, B. HOUNSOME, D. TOMOS, AND G. EDWARDS-JONES

**ABSTRACT:** Vegetables are an important part of the human diet and a major source of biologically active substances such as vitamins, dietary fiber, antioxidants, and cholesterol-lowering compounds. Despite a large amount of information on this topic, the nutritional quality of vegetables has not been defined. Historically, the value of many plant nutrients and health-promoting compounds was discovered by trial and error. By the turn of the century, the application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites. Approximately 50000 metabolites have been elucidated in plants, and it is predicted that the final number will exceed 200000. Most of them have unknown function. Metabolites such as carbohydrates, organic and amino acids, vitamins, hormones, flavonoids, phenolics, and glucosinolates are essential for plant growth, development, stress adaptation, and defense. Besides the importance for the plant itself, such metabolites determine the nutritional quality of food, color, taste, smell, antioxidative, anticarcinogenic, antihypertension, anti-inflammatory, antimicrobial, immunostimulating, and cholesterol-lowering properties. This review is focused on major plant metabolites that characterize the nutritional quality of vegetables, and methods of their analysis.

**Keywords:** analysis, nutritional quality, plant metabolites, vegetables

## Introduction

Vegetables contain most, if not all, of the essential components of human nutrition. Nutrients have traditionally been viewed as food components that either cannot be synthesized in the body (for example, vitamin C) or whose synthesis requires a specific factor that may in certain circumstances be absent or inadequate (for example, some amino acids, fatty acids, and vitamins). However, there is now recognition that many other compounds of plant food, such as dietary fiber, flavonoids, sterols, phenolic acids, and glucosinolates, are associated with lower disease risk. This has been widely reported, sometimes erroneously, by the popular press. Nevertheless, a large number of phytochemicals capable of antioxidant, antimutagenic, cytotoxic, antifungal, and antiviral activities have been identified in broccoli, cauliflower, Brussels sprouts, turnips, kale, mustard, asparagus, spinach, lettuces, and endives (Prior and Cao 2000; Goldberg 2003). These phytochemicals have been linked to many positive effects on human health, including coronary heart diseases, diabetes, high blood pressure, cataracts, degenerative diseases, and obesity (Liu and others 2000; Djoussé and others 2004). The links between fruit and vegetable consumption and protection against cancers of stomach, esophagus, lung, pharynx, endometrium, pancreas, and colon have also been extensively reported (Temple and Gladwin 2003; Hung and others 2004). The realization of the importance of plant products to the human diet has led to the five-a-day campaign in the United Kingdom to persuade people to eat at least 5 portions of fruit and vegetables ev-

ery day. This campaign is driven by the nutritional importance of plant foods in terms of essential nutrients, trace elements, and fiber (WHO 2002, 2003).

Nutrient composition of vegetables is very complex and difficult to assess. Levels of plant metabolites are strongly affected by genetic and environmental factors as well as transportation and storage conditions. Growth factors such as light, temperature, humidity, type of soil, application of fertilizers, damage caused by microorganisms and insects, stress induced by UV radiation, heavy metals, and pesticides all alter metabolite composition of plants (Orcutt and Nilsen 2000). Before vegetables appear on a supermarket shelf they have been handled by plant growers, transporters, packagers, storehouse operators, distributors, and/or processors. The chemical and physical changes that occur in vegetables during these stages can lead to loss of potentially beneficial components (MacEvilly and Peltola 2003).

Over the past decade, significant amounts of information have been accumulated on identification, biochemical characterization, localization, and health benefits of plant metabolites. This review represents an attempt to summarize the information about metabolites, which determine the nutritional value of vegetables, their occurrence in plants, role in the human diet, and advances in their analysis.

## Primary Metabolites

The diverse variety of organic compounds in vegetables represents the product of primary and secondary plant metabolism. Primary metabolites such as carbohydrates, amino acids, fatty acids, and organic acids are involved in growth and development, respiration and photosynthesis, and hormone and protein synthesis. Primary metabolites are found across all species within broad phylogenetic groups, and are produced using the same (or nearly the same) biochemical pathways. Secondary metabolites such as flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates determine the color of vegetables, protect

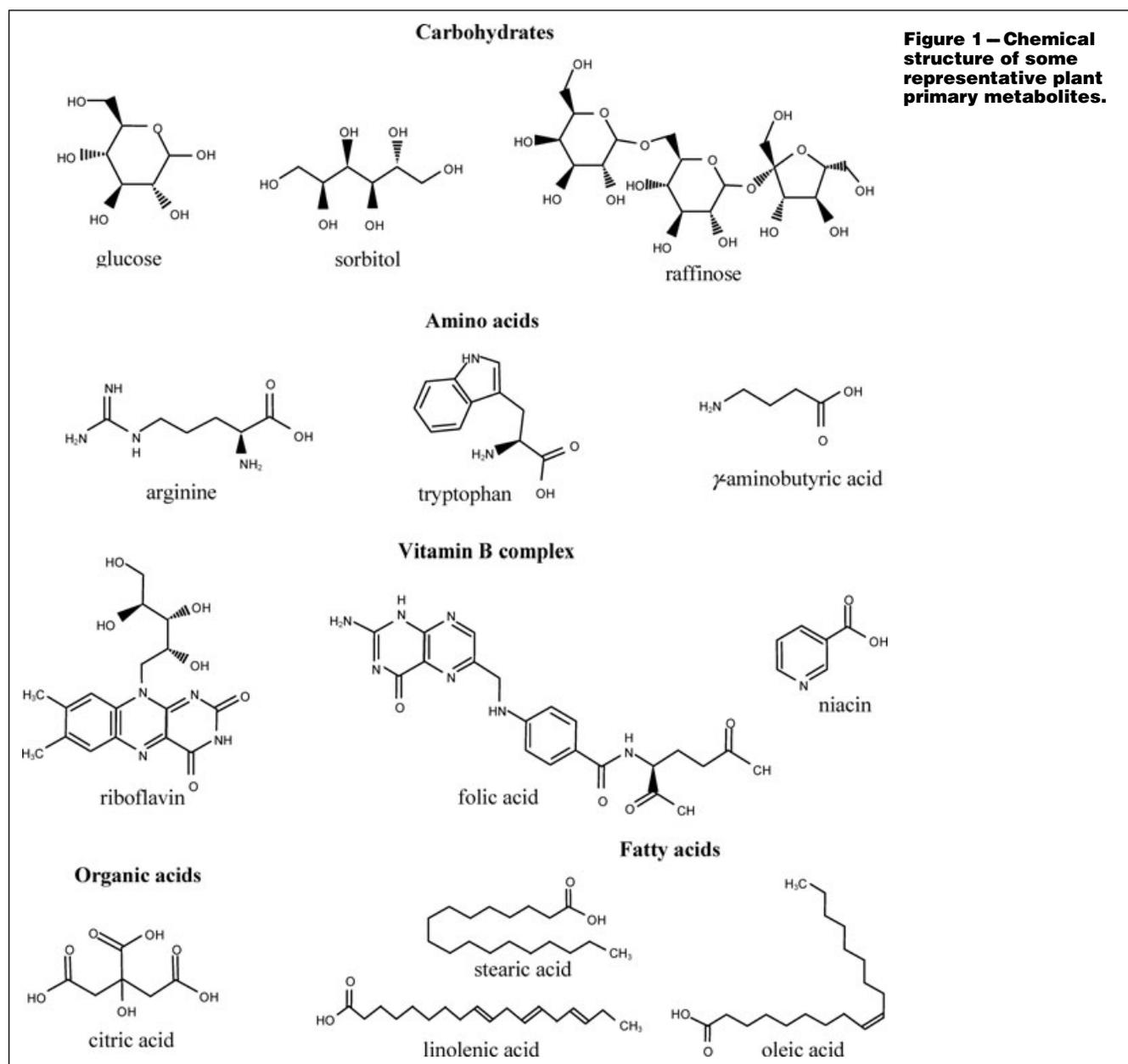
MS20070712 Submitted 9/17/2007, Accepted 1/26/2008. Authors N. Hounsome and Edwards-Jones are with College of Natural Sciences, School of the Environment and Natural Resources, Bangor Univ., Deiniol Rd., Bangor, LL57 2UW, Wales, U.K. Author B. Hounsome is with College of Health and Behavioural Sciences, Inst. of Medical and Social Care Research, Bangor Univ., Dean St. Building, Bangor, LL57 1UT, Wales, U.K. Author Tomos is with College of Natural Sciences, School of Biological Sciences, Bangor Univ., Memorial Building, Deiniol Rd., Bangor, LL57 2UW, Wales, U.K. Direct inquiries to author N. Hounsome (E-mail: afs20e@bangor.ac.uk).

plants against herbivores and microorganisms, attract pollinators and seed-dispersing animals, and act as signal molecules under stress conditions (Seiger 1998; Crozier and others 2006). The chemical structure of representative primary metabolites is shown in Figure 1. Information about major primary metabolites and their content in vegetables is summarized in Table 1.

### Carbohydrates

Carbohydrates in vegetables occur as sugar monosaccharides (glucose, fructose, arabinose, galactose, rhamnose), disaccharides (sucrose, maltose, trehalose), sugar alcohols (sorbitol, mannitol, xylitol), oligosaccharides (raffinose, stachyose, fructooligosaccharides), and polysaccharides (starch, cellulose, hemicellulose, pectins). Monosaccharides, sucrose, and polysaccharides are present in all vegetables. Raffinose and stachyose are found in beans, peas, and lentils (Kuo and others 1988; Obendorf and others 1998; Frias and others 1999; Peterbauer and others 2001). Fructooligosaccharides (fructans) are accumulated in

asparagus, peas, beans, leeks, onions, and chicory (Shiomi 1992; Shiomi and others 2005). Mannitol is present in celery, carrot, and parsley, and xylitol is found in maize corn and strawberries (Mäkinen and Söderling 1980; Lewis 1984; Stoop and others 1996). In terms of their physiological or nutritional role, carbohydrates are often classified as available and unavailable. Available carbohydrates are those that are hydrolyzed by enzymes of the human gastrointestinal system to monosaccharides such as sucrose and digestible starch. Monosaccharides require no digestion and can be absorbed directly into the blood stream. Unavailable carbohydrates (sugar alcohols, many oligosaccharides, and nonstarch polysaccharides) are not hydrolyzed by endogenous human enzymes. They can be fermented by microorganisms in the large intestine to varying extents and then absorbed (Asp 1996). Fructooligosaccharides and nonstarch polysaccharides are important components of dietary fiber (discussed subsequently). Available carbohydrates are the most accessible source of energy for the human body and an adequate supply of carbohydrates in the diet



**Figure 1 – Chemical structure of some representative plant primary metabolites.**

**Table 1 – Primary plant metabolites and their content in vegetables.**

Compounds	Vegetables	Concentrations	References
<b>Carbohydrates</b>			
Glucose	Cabbage	1.4 to 2.06 g/100 g FW	Lee and others (1970)
	Onion	1.76 to 2.34	
	Pumpkin	1.54 to 1.84	
	Tomato	0.88 to 1.25	
Fructose	Asparagus	1.2 to 1.4 g/100 g FW	Lee and others (1970)
	Broccoli	0.52 to 0.87	
	Cabbage	1.14 to 1.74	
	Cucumber	0.82 to 0.97	
Sucrose	Brussels sprouts	0.6 to 0.9	Lee and others (1970)
	Red beet	5.58 to 6.64 g/100 g FW	
	Cabbage	0.02 to 0.5	
	Broccoli	0.36 to 0.5	
Raffinose	Carrot	3.68 to 4.54	Lee and others (1970)
	Sweet corn	2.62 to 4.0	
	Broccoli	0.1 to 0.16 g/100 g FW	
	Cabbage	0.06 to 0.1	
Stachyose	Cauliflower	0.02 to 0.06	Lee and others (1970)
	Red beet	0.04 g/100 g FW	
	Broccoli	0.18 to 0.22	
	Cabbage	0.06	
Mannitol	Leeks	0.56	Wang and Eys (1981)
	Onion	0.24 to 1.16	
	Artichoke	184 mg/100 g DW (2)	
	Asparagus	170	
Xylitol	Endive	334	Wang and Eys (1981)
	Onion	47.5	
	Pumpkin	200	
	Carrot	86.5 mg/100 g DW	
Sorbitol	Cauliflower	300	Wang and Eys (1981)
	Lettuce	131	
	Spinach	107	
	Red beet	77 mg/100 g DW	
Total dietary fiber	Cabbage	23.24 g/100 g DW	Anderson and Bridges (1988)
	Carrot	23.76	
	Lettuce	21.02	
	Potato	9.48	
Nonstarch polysaccharides	Tomato	13.13	Anderson and Bridges (1988)
	Cabbage	22.41 g/100 g DW	
	Carrot	22.75	
	Lettuce	19.0	
Lignin	Potato	8.58	Anderson and Bridges (1988)
	Tomato	11.44	
	Cabbage	0.83 g/100 g DW	
	Carrot	1.01	
<b>Amino acids</b>	Lettuce	2.02	Anderson and Bridges (1988)
	Potato	0.9	
	Tomato	1.69	
Arginine	Broccoli	242 mg/100 g FW	(FAO)
	Cabbage	135	
	Carrot	50	
	Cucumber	61	
Histidine	Peas	575	(FAO)
	Spinach	139	
	Brussels sprouts	108 mg/100 g FW	
	Cauliflower	54	
Isoleucine	Endive	31	(FAO)
	Lettuce	21	
	Onion	14	
	Peas	140	
Leucine	Brussels sprouts	230 mg/100 g FW	(FAO)
	Carrot	33	
	Lettuce	50	
	Peas	273	
	Spinach	106	(FAO)
	Tomato	20	
	Broccoli	201 mg/100 g FW	
	Cabbage	86	
	Onion	37	(FAO)
	Peas	457	

Continued on next page

**Table 1 – Continued.**

Compounds	Vegetables	Concentrations	References		
<b>Amino acids</b>					
Lysine	Spinach	208	(FAO)		
	Tomato	30			
	Brussels sprouts	252 mg/100 g FW			
	Cauliflower	160			
	Cucumber	35			
	Carrot	44			
	Peas	479			
Methionine	Endive	50	(FAO)		
	Broccoli	44 mg/100 g FW			
	Cucumber	8			
	Endive	22			
	Peas	61			
	Onion	16			
	Tomato	7			
Phenylalanine	Brussels sprouts	172 mg/100 g FW	(FAO)		
	Cabbage	49			
	Carrot	31			
	Cucumber	19			
	Endive	78			
	Peas	289			
	Tryptophan	Brussels sprouts		58 mg/100 g FW	(FAO)
Valine	Cauliflower	39	(FAO)		
	Cucumber	6			
	Onion	20			
	Cauliflower	156 mg/100 g FW			
	Cabbage	68			
	Onion	30			
	Peas	311			
<b>Vitamin B complex</b>	Spinach	133	(FAO)		
	Tomato	24			
	Thiamine	Broccoli		0.15 mg/100 g FW	(FAO)
		Cucumber		0.04	
		Garlic		0.32	
		Leeks		1.46	
		Spinach		0.16	
Brussels sprouts		0.12 mg/100 g FW			
Riboflavin		Lettuce	0.15	(FAO)	
Niacin	Onion	0.17	(FAO)		
	Spinach	0.19			
	Tomato	0.05			
	Broccoli	0.8 mg/100 g FW			
	Cabbage	0.5			
	Endive	0.5			
	Leeks	1.7			
Pantothenic acid	Peas	2.4	(USDA)		
	Sweet pepper	0.9			
	Broccoli	0.593 mg/100 g FW			
	Cabbage	0.211			
	Endive	0.9			
	Sweet pepper	0.319			
	Spinach	0.07			
Pyridoxine	Tomato	0.17	(USDA)		
	Broccoli	0.175 mg/100 g FW			
	Cauliflower	0.222			
	Carrot	0.11			
	Cucumber	0.05			
	Spinach	0.2			
	Folic acid	Broccoli		62.5 mg/100 g FW	(USDA)
Celery		35.8			
Endive		142			
Spinach		190			
Tomato		15			
<b>Organic acids</b>					
Ascorbic acid		Cabbage	58 mg/100 g FW	(FAO)	
	Cauliflower	79			
	Onion	30			
	Sweet pepper	146			
	Spinach	56			
	Tomato	20			

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Table 1 – Continued.

Compounds	Vegetables	Concentrations	References
<b>Organic acids</b> Oxalic acid	Broccoli	0.19 g/100 g FW	(USDA)
	Brussels sprouts	0.36	
	Cabbage	0.10	
	Lettuce	0.33	
	Onion	0.05	
Aconitic acid	Maize	2.3 $\mu$ mol/g FW	Nelson and Rinne (1977)
	Soybean	26.8	
<b>Fatty acids</b> Saturated	Broccoli	0.038 g/100 g FW	(USDA)
	Cauliflower	0.032	
	Carrot	0.041	
	Endive	0.048	
	Sweet pepper	0.07	
	Tomato	0.046	
Monounsaturated	Broccoli	0.011 g/100 g FW	(USDA)
	Celery	0.03	
	Cucumber	0.003	
	Onion	0.023	
	Spinach	0.01	
Polyunsaturated	Broccoli	0.0375 g/100 g FW	(USDA)
	Carrot	0.117	
	Lettuce	0.08	
	Sweet pepper	0.156	
	Spinach	0.17	
	Tomato	0.135	

FW = fresh weight, DW = dry weight, FAO = Food and Agriculture Organization of the United Nations Database, USDA = U.S. Dept. of Agriculture National Nutrient Database.

spares protein from being broken down for energy. There are no specific sugar requirements for humans since all monosaccharides can be synthesized by the body. Sugars are involved in control of blood glucose and insulin metabolism, intestinal microflora activity, and food fermentation. Monosaccharides bound to protein and lipid molecules (glycoproteins and glycolipids) are involved in cell signaling. Nonenzymatic binding of sugars to proteins, called glycation, produces advanced glycation end products implicated in many age-related chronic diseases such as type 2 diabetes, cardiovascular diseases, Alzheimer's disease, cancer, and peripheral neuropathy (Foster-Powell and others 2002; Krajčovičová-Kudláčková and others 2002).

Plant components described as dietary fiber typically include nonstarch polysaccharides, resistant oligosaccharides, lignin, and associated substances such as resistant starch, waxes, cutin, and suberin (De Vries 2003). All these materials pass through the gastrointestinal tract as bulk fiber, undergoing modification and digestion by colon microorganisms (Blaut 2002). Substances produced by intestinal bacteria may be absorbed into the body. Some products such as vitamin K, biotin, and fatty acids may be beneficial. Other substances such as alcohols, lactic acid, and formate, as well as hydrogen gas produced by colon fermentation, may be undesirable (Flamm and others 2001; Tungland and Meyer 2002; McGarr and others 2005). The consumption of high dietary fiber foods has been found to reduce symptoms of chronic constipation, diverticular disease, and some types of colitis (Stollman and Raskin 2004). It has been suggested that diets with low fiber may increase the risk of developing colon cancer, cardiovascular diseases, and obesity (Marlett 2001; McGarr and others 2005; Slavin 2005). Some researchers believe that dietary fiber improves the ability of diabetics to process blood sugar (Willett and others 2002). Increasing fiber consumption in a diet has been a difficult challenge, as high-fiber containing vegetables do not always have appealing taste properties (Tungland and Meyer 2002). Therefore, despite its positive in-

fluence on health, the intake of dietary fiber remains low in many populations worldwide.

### Amino acids

Amino acids play a role as intermediates in plant and animal metabolism, and join together to form proteins. Proteins provide structural material for the human body and function as enzymes, hormones, and antibodies. Dietary proteins are the major source of amino acids. Most proteins are broken down by enzymes into amino acids and absorbed from the small intestine. Humans can synthesize a range of amino acids, including alanine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine. Nine amino acids, called essential, must come from the diet, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, and valine. The amino acids arginine, methionine, and phenylalanine are considered essential for reasons not directly related to lack of metabolic pathway, but because the rate of their synthesis is insufficient to meet the needs of the body (Spallholz and others 1999). Histidine is considered an essential amino acid in children. Vegetables contain all essential amino acids, but some may be in lower proportions than are required for humans (Young and Pellett 1994). High levels of arginine are found in asparagus, Brussels sprouts, watercress, and potatoes; histidine in broccoli, Brussels sprouts, and cauliflower; phenylalanine in beets, carrots, parsley, spinach, and tomatoes; and methionine in cabbage, cauliflower, radish, kale, and watercress (FAO 1970). Over 250 nonprotein amino acids (for example, homoarginine, carnitine, citrulline, taurine,  $\alpha$ -aminobutyric acid,  $\gamma$ -aminobutyric acid) have been identified in plants.  $\gamma$ -Aminobutyric acid (GABA), present in beans, spinach, potatoes, and kale, is an inhibitory neurotransmitter in human central nervous system and in the retina (Oh and others 2003). Carnitine, found in peas, potatoes, and zucchini, is involved in lipid metabolism in heart and skeletal muscle (Demarquoy and

others 2004). Taurine, identified in maize, lentils, and peanuts, is involved in detoxification and membrane stabilization in human cells (Stapleton and others 1997). Some nonprotein amino acids (for example,  $\beta$ -cyanoalanine and canavanine found in beans) are reported to be toxic for humans due to inhibition of protein synthesis and immune system (Bell 2003). Besides the importance for human metabolism, free amino acids contribute to the taste of vegetables. Glycine and alanine are sweet, valine and leucine are bitter, and aspartic acid and glutamate have sour and “savory” (umami in Japanese) tastes (Solms 1969).

### Vitamin B complex

The vitamin B complex of vegetables includes the water-soluble vitamins thiamine (B1), riboflavin (B2), nicotinic acid (B3, niacin), pantothenic acid (B5), pyridoxine (B6), biotin (B7), and folic acid (B9) (Bender 2003). In plants, these compounds are vital cofactors for enzymes, involved in photosynthesis (riboflavin), respiration (thiamine, riboflavin, biotin,) synthesis of organic and amino acids (thiamine, folic acid), and regulation of cell division and flowering (niacin) (Heldt 2005). In humans, B vitamins are involved in tissue respiration and carbohydrate, fatty acid, and amino acid metabolism. Vitamin B deficiency can cause polyneuritis (thiamine), cheilosis, angular stomatitis and dermatitis (riboflavin), pellagra, diarrhea, dermatitis and dementia (nicotinic acid), seborrhea, glossitis, peripheral neuropathies and microcytic anemia (pyridoxine), nausea, dermatitis (pantothenic acid, biotin), and anemia (folic acid) (Combs 1998). Green leafy vegetables such as asparagus, spinach, Brussels sprouts, cauliflower, turnip, and lettuce are good sources of B vitamins (USDA 2005).

### Organic acids

Organic acids are a group of organic compounds containing carboxylic groups. In solution organic acids release protons, which determine their “acidic taste.” Plants contain citric, acetic, malic, oxalic, succinic, fumaric, quinic, tartaric, malonic, shikimic, aconitic, ascorbic, and other organic acids (Heldt 2005). The predominant acids are malic and citric. Succinic, fumaric, and quinic acids are widespread. Tartaric acid was found in carrots, lettuce, endives, chicory, and celery (Ruhl and Herrmann 1985). Green vegetables generally contain low concentrations of organic acids. Nevertheless, they play important roles as flavor enhancers and natural antimicrobial agents. Organic acids give the vegetables tartness, and affect flavor by acting on the perception of sweetness (Fisher and Scott 1997). The sugar/acid ratio is often used to give a technological characterization of vegetable ripeness (Bartz and Brecht 2002). For example, the value of about 7.5 is usually accepted as a beneficial sugar/acid ratio in tomatoes, although values in the range of 3.3 to 21.7 have been reported (Kmieciak and Lisiewska 2000). Organic acids influence the color of vegetables since many plant pigments are natural pH indicators (Davies 2004). For example, some anthocyanins, found in red cabbages and lettuces, change color from red to blue as pH increases. Ascorbic acid, known as vitamin C, is an organic acid with strong antioxidant properties. Vegetables rich in ascorbic acid include spinach, spring onions, cress, cabbage, broccoli, cauliflower, sweet peppers, peas, and beans (USDA 2005). Vitamin C is involved in the synthesis of neurotransmitters, steroid hormones, and collagen, in the conversion of cholesterol to bile acids, and in the absorption of iron and calcium. It assists in the healing of wounds and burns, in the prevention of blood clotting and bruising, and in strengthening the walls of the capillaries (Combs 1998). Because vitamin C is a strong biological antioxidant, it is also linked to the prevention of degenerative diseases such as cataracts, certain cancers, and cardiovascular disorders (reviewed

by Carr and Frei 1999). The content of ascorbic acid in vegetables is affected by growth conditions and the application of nitrogen fertilizers (Mozafar 1993).

### Fatty acids

Fatty acids consist of a hydrophilic carboxylate group attached to a long hydrocarbon chain. Fatty acids are major components of fats. They provide the human body with energy and structural material for cell membranes and organ padding. Fatty acids are involved in the absorption of certain vitamins, blood clotting, and the immune response (Nettleton 1995; Shahidi and Miraliakbari 2005). Some of them are chemical precursors to a number of hormones (Yehuda and others 1999). Fatty acid classification is based on the number of double bonds. Saturated fatty acids (such as capric, myristic, palmitic, stearic acids) do not contain double bonds. Unsaturated acids with 1 double bond are called monounsaturated (oleic, palmitoleic), and those with two or more double bonds are polyunsaturated (docosahexaenoic acid, eicosapentaenoic acid,  $\alpha$ -linolenic acid, arachidonic acid) (Gurr and others 2002). Two unsaturated fatty acids that cannot be made in the body (linoleic acid and  $\alpha$ -linolenic acid) must be provided by diet and are known as essential fatty acids (Innis 1991).  $\alpha$ -linolenic acid can be found in soybeans and in most vegetable oils, including corn, sunflower, and safflower oil (Connor 1999). Linolenic acid is present in soybeans, wheat germ, and pumpkin seeds (Jakab and others 2002). Green vegetables such as Chinese cabbage, Brussels sprouts, watercress, and parsley are known to contain a relatively high proportion of polyunsaturated fatty acids, primarily in the form of  $\alpha$ -linolenic acid (Pereira and others 2001). The consumption of monounsaturated fatty acids has been shown to reduce cholesterol levels and have a beneficial effect on some of the traditional risk factors for cardiovascular disease and type 2 diabetes (Simopoulos 1999; Connor 2000).

### Secondary Metabolites

Plants produce a diverse assortment of organic compounds that do not participate directly in growth and development. These substances, traditionally called secondary metabolites, are often differentially distributed among taxonomic groups within the plant kingdom. Their functions, many of which remain unknown, are being elucidated with increasing frequency. According to the nomenclature adopted by the British Nutrition Foundation, plant secondary metabolites can be divided into 4 major groups: phenolic and polyphenolic compounds (about 8000 compounds), terpenoids (about 25000 compounds), alkaloids (about 12000 compounds), and sulfur-containing compounds (Goldberg 2003). The chemical structure of representative primary metabolites is shown in Figure 2. Information about secondary metabolites and their content in vegetables is summarized in Table 2.

### Phenolic and polyphenolic compounds

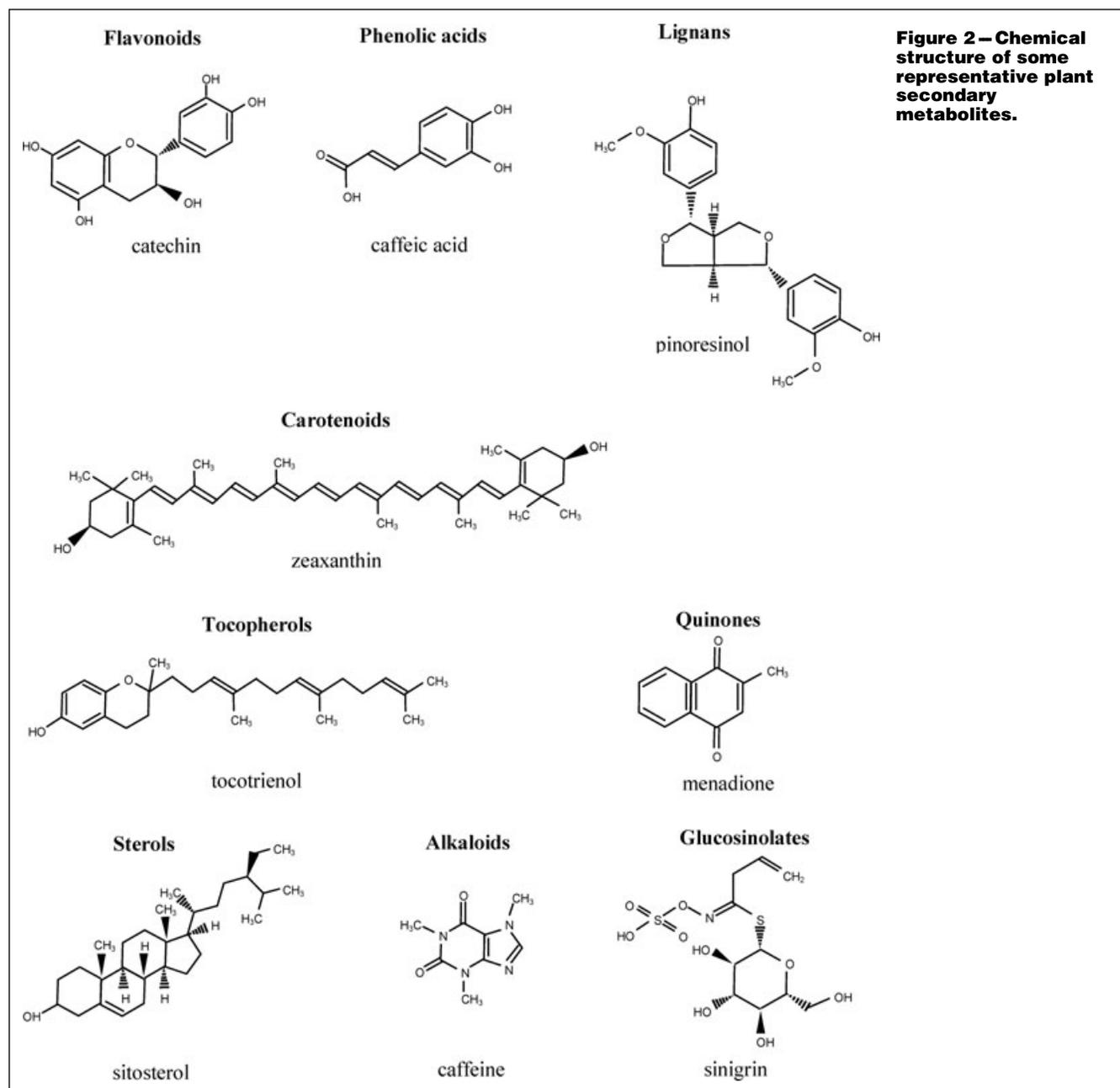
Phenolic and polyphenolic compounds, characterized by an aromatic or phenolic ring structure, include flavonoids, phenolic acids, and lignans. Phenolic compounds are feeding deterrents for many insects and other animals; high concentrations of phenolic compounds are often associated with increased resistance to fungal plant pathogens (Nicholson and Hammerschmidt 1992). Some phenolics determine the color and smell of plants, attracting pollinators. Phenolics play a role in cold acclimation and protection against UV radiation. In plant cells, most phenolic compounds are coupled to sugars to reduce their endogenous toxicity. External stimuli such as microbial infection, ultraviolet radiation,

temperature, and chemical stressors induce their synthesis (Parr and Bolwell 2000).

Flavonoids, the most numerous phenolic compound group, include flavonols (quercetin, kaempferol, and isorhamnetin) present in onions, leeks, endives, and broccoli; flavones (apigenin, luteolin, and chrysoeriol) present in parsley, thyme, and celery; anthocyanidins (cyanidin, delphinidin, and malvidin) present in red cabbage, radish, and red lettuces; chalcones and dihydrochalcones present in tomatoes; and isoflavones present in soy (Hollman and Katan 1999; Yao and others 2004). Many flavonoids such as anthocyanidins, chalcones, and flavones are plant pigments that determine the color of vegetables (Hou and others 2004). Dietary flavonoids possess antiviral, anti-inflammatory, antihistamine, and antioxidant properties. They have been reported to inhibit lipid peroxidation, to scavenge free radicals, to chelate iron and copper ions (which can catalyze production of free radicals), and to modulate cell signaling pathways (Heim and others 2002; Rice-Evans

and Packer 2003). Production of peroxides and free radicals, which damage lipids, proteins, and DNA, has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases (Parkinson's and Alzheimer's). Flavonoids protect low-density lipoprotein cholesterol from being oxidized, preventing the formation of atherosclerotic plaques in the arterial wall. They stimulate enzymes involved in detoxification of cancerogenic substances and inhibit inflammation associated with local production of free radicals (reviewed by Hollman and Katan 1999; McCord 2000). Most flavonoids have a bitter or astringent taste or a bitter taste with sweet aftertaste (Drewnowski and Gomez-Carneros 2000).

Phenolic acids are chemical compounds with at least 1 aromatic ring bearing one or more hydroxyl groups. Phenolic acids, such as gallic and caffeic acid, are found in lettuce and pac choi; vanillic and cinnamic acid in onions, parsley, and spinach; coumaric acid in tomatoes, carrots, and garlic (Rice-Evans and Packer 2003;



**Figure 2 – Chemical structure of some representative plant secondary metabolites.**

**Table 2 – Secondary plant metabolites and their content in vegetables.**

Compounds	Vegetables	Concentrations	References
<b>Flavonoids</b>			
Quercetin	Broccoli	3.12 mg/100 g FW	(USDA)
	Cabbage	0.01	
	Endive	7.71	
	Lettuce	1.95	
	Onion	13.27	
	Tomato	0.57	
Apigenin	Cabbage	0.01 mg/100 g FW	(USDA)
	Celery	4.61	
	Lettuce	0.01	
Luteolin	Cauliflower	0.08 mg/100 g FW	(USDA)
	Celery	1.31	
	Spinach	1.11	
	Sweet pepper	0.63	
Myricetin	Lettuce	mg/100 g FW	(USDA)
	Spinach	0.01	
	Green lettuce	0.3 mg/100 g FW	
Cyanidin	Red lettuce	13.7	Harnly and others (2006)
<b>Phenolic acids</b>			
Chlorogenic acid	Beans	0.29 mg/100 g FW	Mattila and Hellström (2007)
	Carrot	10	
	Cauliflower	0.14	
	Lettuce	0.42 to 23	
	Soya beans	2.0	
	Tomato	0.86	
	Carrot	0.1 mg/100 g FW	
Caffeic acid	Turnip	0.42 mg/100 g FW	
Ferulic acid	Cabbage	0.21 mg/100 g FW	
	Cauliflower	0.31	
<i>p</i> -Coumaric acid	Soya beans	0.5 mg/100 g FW	
	Cauliflower	0.15 mg/100 g FW	
Vanillic acid	Turnip	1.4	
Sinapic acid	Beans	0.26 mg/100 g FW	
	Carrot	0.46	
Protocatechuic acid			
<b>Lignans</b>			
Lariciresinol	Broccoli	972 mg/100 g FW	Milder and others (2005)
	Cauliflower	124	
	Kale	599	
	Lettuce	5	
	Onion	19	
	Sweet pepper	164	
	Broccoli	315 mg/100 g FW	
	Cabbage	568	
	Kale	1691	
	Endive	9	
Pinoresinol	Leeks	3	
	Sweet pepper	1	
	Broccoli	38 mg/100 g FW	
	Brussels sprout	34	
	Leeks	38	
Secoisolariciresinol	Carrot	93	
	Lettuce	8	
	Tomato	2	
	Kale	12 mg/100 g FW	
Matairesinol			
<b>Carotenoids</b>			
$\alpha$ -Carotene	Broccoli	1 mg/100 g FW	(USDA)
	Carrot	4.6	
	Peas	19	
	Sweet pepper	59	
	Tomato	112	
$\beta$ -Carotene	Broccoli	779 mg/100 g FW	
	Brussels sprout	450	
	Carrot	8.8	
	Peas	485	
	Tomato	393	
$\beta$ -Cryptoxanthin	Sweet pepper	2.205 mg/100 g FW	
	Carrot	23 mg/100 g FW	
	Celery	3	
	Kale	173	
	Lettuce	187	
Zeaxanthin	Spinach	331	
	Tomato	3.025 mg/100 g FW	

Continued on next page

Table 2 – Continued.

Compounds	Vegetables	Concentrations	References		
<b>Tocopherols and tocotrienols</b>					
$\alpha$ -Tocopherol	Broccoli	1.44 mg/100 g FW	Chun and others (2006)		
	Cabbage	0.21			
	Carrot	0.86			
	Celery	0.26			
	Onions	0.04			
	Spinach	1.96			
	Tomato	0.53			
$\beta$ -Tocopherol	Carrots	0.01 mg/100 g FW			
	Lettuce	0.01			
	Cucumber	0.01			
$\gamma$ -Tocopherol	Broccoli	0.31 mg/100 g FW			
	Cauliflower	0.20			
	Lettuce	0.11 to 0.74			
	Spinach	0.21			
$\alpha$ -T3	Tomato	0.14			
	Cabbage	0.04 mg/100 g FW			
	Cauliflower	0.06			
$\gamma$ -T3	Onions	0.12			
	Corn	0.21 mg/100 g FW			
<b>Quinones</b> Phylloquinone	Peas	0.05	Damon and others (2005)		
	Broccoli	102 $\mu$ g/100 g FW			
	Carrot	8.3			
	Celery	29			
	Cucumber	16.4			
	Lettuce	24.1 to 127			
	Onion	0.2			
<b>Sterols</b> Campesterol	Sweet pepper	4.9 to 21.4	Normén and others (1999)		
	Broccoli	6.9 mg/100 g FW			
	Brussels sprouts	8.0			
	Carrot	2.2			
	Cauliflower	9.5			
	Onion	0.82			
	Tomato	0.28			
	Campestanol	Broccoli		0.10 mg/100 g FW	
		Kale		0.07	
		Leeks		0.09	
Stigmasterol	Tomato	0.05			
	Broccoli	1.1 mg/100 g FW			
	Brussels sprouts	0.38			
	Carrot	2.8			
$\beta$ -Sitosterol	Cauliflower	3.7			
	Celery	7.0			
	Tomato	1.7			
	Broccoli	31 mg/100 g FW			
	Brussels sprouts	34			
	Cauliflower	26			
	Celery	7.3			
$\beta$ -Sitostanol	Kale	7.4			
	Tomato	2.4			
	Broccoli	0.08 mg/100 g FW			
	Carrot	0.08			
	Cauliflower	0.06			
<b>Alkaloids</b> $\alpha$ -Tomatine	Celery	0.13	Kozukue and others (2004)		
	Tomato	0.23			
	Tomato	521 to 795 $\mu$ g/g FW			
	Dehydrotomatine	41.6 to 68.0 $\mu$ g/g FW			
	$\alpha$ -Solanine	0.01 to 0.43 mg/kg FW			
	$\alpha$ -Chaconine	0.7 to 1.93 mg/kg FW			
Lactucin	Potato	178 to 245 mg/kg FW	Peters and others (1997)		
	Chicory	112 to 143 mg/kg FW			
<b>Glucosinolates</b> Glucobriferin	Chicory		Song and Thornalley (2007)		
	Broccoli	17.1 $\mu$ mol/100 g FW			
	Brussels sprouts	1.5			
	Cauliflower	1.34			
	Green cabbage	3.88			

Continued on next page

Table 2 – Continued.

Compounds	Vegetables	Concentrations	References
Glucosinolates	Broccoli	29.4 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	0.55	
	Cauliflower	0.31	
	Green cabbage	0.35	
Glucoalyssin	Broccoli	3.86 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	0.33	
Sinigrin	Broccoli	1.40 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	8.56	
	Cauliflower	5.28	
	Green cabbage	5.09	
Gluconapin	Broccoli	2.87 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	2.77	
	Cauliflower	3.36	
	Green cabbage	0.38	
Progoitrin	Broccoli	3.33 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	2.41	
	Cauliflower	0.45	
	Green cabbage	0.62	
Gluconasturtiin	Broccoli	4.44 $\mu\text{mol}/100\text{ g FW}$	
	Brussels sprouts	1.06	
	Cauliflower	2.79	

FW = fresh weight, USDA = U.S. Dept. of Agriculture National Nutrient Database.

Crozier and others 2006). In plants, these compounds fulfill antipathogen, antiherbivore, and allelopathic roles (Nicholson and Hammerschmidt 1992; Chou 1999). Salicylic acid plays an important role in cell signaling under stress conditions (Klessig and Malamy 1994). Dietary phenolic acids such as benzoic, hydrobenzoic, vanillic, and caffeic were reported to have antimicrobial and antifungal action, probably due to enzyme inhibition by the oxidized compounds (Cowan 1999). Hydroxycinnamic acid derivatives such as caffeic, chlorogenic, sinapic, ferulic, and *p*-coumaric acid possess strong antioxidant activity due to inhibition of lipid oxidation and scavenging reactive oxygen species (Sroka and Cisowski 2003; Cheng and others 2007). Some phenolics like syringic acid may contribute to the bitter and astringent taste of vegetables (Drewnowski and Gomez-Carneros 2000).

Lignans are diphenolic compounds involved in the synthesis of lignin, a hydrophobic component of plant cell wall. Lignans are present at high concentrations in broccoli, kale, Brussels sprouts, beans, and garlic (Milder and others 2005). Lignans play a role in the defense of plants against insects, acting as regulators of insect feeding and development (molting) (Harmatha and Dinan 2003). Some plant lignans such as secoisolariciresinol, matairesinol, lariciresinol, and pinoresinol can be converted into the “enterolignans” by the intestinal microflora, and absorbed into the human body (Heinonen and others 2001). In humans, lignans possess several biological activities, such as antioxidant and (anti)estrogenic properties, and may thus reduce the risk of certain cancers as well as cardiovascular diseases (Adlercreutz and Mazur 1997; Arts and Hollman 2005). It was postulated that, compared to the semivegetarian diet in many Asian countries, the Western diet may alter hormone production, metabolism, or action at the cellular level, increasing incidences of breast, colorectal, and prostate cancer. Reduced cancer risks were associated with higher urinary lignan excretion (Webb and McCullough 2005).

## Terpenoids

Terpenoids are a large family of chemical compounds derived by repetitive fusion of branched 5-carbon isoprene units. Terpenoids have diverse functional roles in plants as structural com-

ponents of membranes (sterols), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastoquinone), and hormones (gibberelins, abscisic acid) (Seiger 1998). Major dietary terpenoids include carotenoids, tocopherols and tocotrienols, quinones, and sterols.

Plant carotenoids ( $\alpha$ -carotenes,  $\beta$ -carotenes, xanthophylls, lycopene) are orange, yellow, and red lipid-soluble pigments. They are found in all green leafy vegetables (where their color is masked by chlorophyll), as well as in carrots, tomatoes, pumpkin, and sweet potatoes (Granado and others 1992; Crozier and others 2006). In plants, carotenoids protect photosynthetic tissues against photooxidative damage and are precursors of phytohormone abscisic acid, which modulates developmental and stress processes (Demmig-Adams and Adams 1996; Taylor and others 2000). Carotenoids with provitamin A activity are essential components of the human diet. Vitamin A is involved in hormone synthesis, regulation of cell growth and differentiation, and immune responses (Combs 1998; Bender 2003). It can be produced within the body from certain carotenoids, notably,  $\beta$ -carotene (present in carrots, spinach, and sweet potatoes) and  $\alpha$ -carotene (found in carrots, pumpkin, and red and yellow peppers) (Bureau and Bushway 1986). Lack of carotenoids in human diet can lead to xerophthalmia (night blindness) and premature death. Carotenoid-rich diets are correlated with a significant reduction in the risk for certain forms of cancer, coronary heart disease, and some degenerative diseases such as coronary heart diseases and cataracts. Carotenoids play an important role by acting as biological antioxidants, protecting cells and tissues from oxidative damage (Combs 1998; Bender 2003).

Tocopherols and tocotrienols, known as vitamin E, are found in kale, broccoli, Brussels sprouts, cabbage, cauliflower, lettuce, asparagus, spinach, sweet potatoes, tomatoes, and turnip (Pironen and others 1986; Eitenmiller and Lee 2004). In plants, tocopherols protect chloroplast membranes from photooxidation to provide an optimal environment for the photosynthetic machinery (Munné-Bosch and Alegre 2002). In humans, vitamin E is present in all cell membranes and plasma lipoproteins (especially in red blood cells). As the major lipid-soluble chain-breaking antioxidants in humans,

vitamin E protects DNA, low-density lipoproteins, and polyunsaturated fatty acids from free radical-induced oxidation. It plays a role in stabilizing the structure of membranes, hemoglobin biosynthesis, and modulation of immune response (Brigelius-Flohe and Traber 1999).

Quinones possess aromatic rings with 2 ketone substitutions. Phylloquinone, known as vitamin K1, is found in lettuce, spinach, asparagus, cabbage, kale, lettuce, cauliflower, and broccoli (Bolton-Smith and others 2000). In plants, phylloquinone is involved in electron transport during photosynthesis and in the generation of the active oxygen species observed as a reaction to pathogen attack or stress (Lochner and others 2003). In humans, vitamin K1 plays a role in the control of blood clotting, bone formation, and repair. A deficiency of this vitamin results in hemorrhagic disease in newborn babies as well as postoperative bleeding, hematuria, muscle hematomas, and intercranial hemorrhages in adults (Combs 1998; Bender 2003). Menadione, known as vitamin K3, was shown to possess cytotoxic activity and inhibit growth of human tumors (Taper and others 2004). Quinones are highly reactive, and are responsible for the browning reaction in cut or injured vegetables (Cowan 1999).

Plant sterols (for example,  $\beta$ -sitosterol, campesterol, brassicasterol, and stigmasterol) are found in high amounts in soy, broccoli, Brussels sprouts, cauliflower, and spinach (Piironen and others 2003). In plant membranes sterols regulate the fluidity and the permeability of phospholipid bilayers (Hartmann 1998). Certain sterols are precursors of plant hormones brassinosteroids, involved in embryonic development, cell division, plant growth, and fertility (Clouse and Sasse 1998). Upon UV irradiation of human skin, these sterols give rise to calciferol (vitamin D2), involved in the absorption of calcium and bone growth. Sterols are essential for synthesis of prostaglandins and leukotrienes, important components of the immune system. Due to their structural similarity to cholesterol, plant sterols inhibit cholesterol absorption. In addition to their cholesterol-lowering effect, plant sterols may possess anticancer, antiatherosclerosis, anti-inflammation, and antioxidant activities (Awad and Fink 2000; Ostlund 2002; Dutta 2003).

## Alkaloids

Alkaloids are a group of basic nitrogen-containing compounds, mainly derived from amino acids. Alkaloids have traditionally been of great interest because of their pronounced physiological and medicinal properties (for example, caffeine, nicotine, morphine, atropine, quinine). Alkaloids are usually classified by their common molecular precursors, such as pyridine (coniine, nicotine), tropane (atropine, cocaine), isoquinone (morphine, codeine), purine (caffeine), and steroids (solanine) (Facchini 2001). The importance of alkaloids in plant metabolism is under debate. Most alkaloids are very toxic and, therefore, have the potential function in the chemical defense against herbivores and microorganisms (Wittstock and Gershenzon 2002). It has also been suggested that these compounds serve as protectants against damage by UV light (Jansen and others 1998). Plant alkaloids like berberine, palmatine, and mahanine are reported to have antimicrobial and cytotoxic activities (Facchini 2001). Some vegetable alkaloids can be toxic for humans. Unripe tomatoes and potatoes exposed to light contain 2 major alkaloid fractions, solanine and chaconine (Jadhav and others 1981). Solanine is a cholinesterase inhibitor and can cause neurological and gastrointestinal symptoms, including depression of the activity of the central nervous system (Dalvi and Bowie 1983). However, it has not been proven that consumption of these vegetables would be toxic unless they comprised an excessively high proportion of the diet. Most alkaloids (for example, lactucin and lactucopicrin,

present in lettuces and chicory) have a bitter and acrid taste (Van Beek and others 1990).

Plant glucoalkaloids solanine, tomatine, and chaconine are called saponins since these compounds have surfactant properties and form foam in aqueous solutions (Hostettmann and Marston 2005). Saponins are found in peas, beans, tomatoes, spinach, asparagus, onions, garlic, and potatoes (Sparg and others 2004). Saponins defend plants against attack by microorganisms and herbivores due to their insecticidal and molluscicidal activity, and have allelopathic effects on many weeds (Haralampidis and others 2001). Dietary saponins cause a reduction of blood cholesterol, inhibit growth of cancer cells, and stimulate the immune system (Francis and others 2002). Saponins contribute to the bitter and acrid taste of vegetables (Drewnowski and Gomez-Carneros 2000). Sweet saponins, like periandrin V, however, are 100 times sweeter than sucrose (Kinghorn and others 1998). Some saponins, such as sapotoxin, can be toxic for humans. They cause irritation of membranes of the respiratory and digestive tract, and increase the membrane permeability of red blood cells and urticaria (skin rash) (Francis and others 2002).

**Sulfur-containing compounds.** Glucosinolates are a large functional group of sulfur-containing amino acid derivatives, containing a group derived from glucose. The glucosinolates progoitrin and sinigrin are found in white and red cabbage, Brussels sprouts, and cauliflower; glucoiberin and glucoraphenin in broccoli, red radish, and daikon; and sinigrin and gluconasturtiin in mustard and horseradish (Fahey and others 2001; Johnson 2002). Substances formed from glucosinolates act as natural pesticides and as a defense against herbivores. Upon tissue disruption, glucosinolates are rapidly hydrolyzed by the enzyme myrosinase, which cleaves off the glucose group. The remaining molecule quickly converts to thiocyanate, isothiocyanate, or nitrile, which acts as an allelochemical, protecting plants against herbivores, pests, and pathogens (Bennett and Wallsgrave 1994; Bones and Rossiter 1996). Some glucosinolates are important precursors for flavor compounds. Isothiocyanates such as allylisothiocyanate and benzylisothiocyanate, commonly termed "mustard oils," have pungent or lachrymatory taste, and acrid smell (Drewnowski and Gomez-Carneros 2000). Other glucosinolates are unwanted as their breakdown products have undesirable sensory or physiological characteristics. Sinigrin and its degradation product have a bitter taste. Progoitrin and glucanapoleiferin are tasteless, but their hydrolytic products are very bitter (Van Doorn and others 1998). Overconsumption of glucosinolate-rich food can disrupt synthesis of thyroid hormone and cause inflammation of the mucous membranes of the stomach (Fenwick and Heaney 1983). However, these cases are rare. Certain glucosinolates (glucoraphenin, glucobrassicin, glucotropaeolin) and their breakdown products have been linked to a reduction in the prevalence of certain types of cancer (Das and others 2000). The anticarcinogenic effect of glucosinolates is explained by the activation of enzymes involved in the detoxification of carcinogens, inhibition of enzymes modifying steroid hormone metabolism, and protection against oxidative damage (Johnson 2002).

As discussed previously, a diverse variety of plant metabolites, beneficial to human health, determines the nutritional quality of vegetables. However, the nutritional value of food cannot be readily estimated by consumers, as it requires technical expertise. Therefore, nutritional information, provided to the consumer, will influence the acceptability of the product. The next section reflects on the definition of vegetable quality and developments in analytical technologies.

### Metabolite Analysis in Vegetables

Public concern and legislation are demanding the provision of quality fruit and vegetables that have reached a satisfactory state of ripeness and exhibit their true organoleptic characteristics (UNECE 2007). Quality is a combination of characteristics, attributes, and properties that have significance for product acceptability and determine the decision to repurchase the product (Schröder 2003). The various components of quality can be classified into 2 groups, namely, external and internal parameters. External parameters include size, shape, color, and external defects. Internal parameters include flavor, texture, nutrient compounds, and internal defects, for example, cavities, water core, frost damage, decay (Shewfelt and Brückner 2000). Various techniques such as electrical and mechanical impedance measurements, visible, and near-infrared spectroscopy, soft X-ray imaging, acoustic (sonic and ultrasonic), chlorophyll fluorescence, and nuclear magnetic resonance imaging have been applied to estimate size, shape, color, texture, internal defects, freshness, and maturity of vegetables (Abbott 1999; Butz and others 2005).

Nutritional quality of vegetables can be analyzed using destructive and nondestructive approaches. Most standardized methods of food chemical analyses approved by European Commission (Buchgraber and Karaali 2005) are destructive, and based on liquid chromatography, thin layer chromatography, gas chromatography, and spectrophotometry. Analytical procedures are carried out by sampling a limited number of vegetables and sending them for laboratory testing. Destructive methods reach the highest accuracy with high detection limits, but are expensive, time-consuming, and require specially trained personnel. Nondestructive methods are faster and economical. They allow the testing of a large number of vegetables without damaging them, and therefore can be used for their grading and sorting (reviewed by Jha and Matsuoka 2000; Butz and others 2005). Instruments for nondestructive analysis used commercially, or which are still under research, are based on spectroscopic, electrochemical, and nuclear magnetic resonance techniques. These devices, however, detect only a limited number of chemical compounds, and are usually less informative and less pre-

cise compared to destructive methods. The information about non-destructive and destructive techniques, applied for vegetable nutrients analysis, is summarized in Table 3.

### Nondestructive analytical techniques

Most sensors for nondestructive analysis of organic compounds are based on detection of molecule interaction with electromagnetic waves. These sensors use visible (400 to 700 nm), ultraviolet (10 to 400 nm), and infrared (700 to 30000 nm) waves, microwaves (1 to 10 cm), X-rays (100 pm to 1 nm), and high frequency electromagnetic waves (magnetic resonance, MHz to GHz). Each class may be further subdivided according to the wave range: near infrared (700 to 2500 nm), mid infrared (2500 to 30000 nm), and so on. Sensors may also be classified according to their principle of signal detection, such as absorbance, transmittance, or reflectance. Techniques operating in the near-infrared and visible region, such as near-infrared transmittance and time-resolved diffuse reflectance spectroscopy, have been used to determine sugar, oil, and fiber content in vegetables (Sato and others 1995; Katayama and others 1996; Schulz and others 1998; Garrido and others 2001).

Near-infrared spectroscopy (NIR spectroscopy) deals with the irradiation of objects with 700 to 25000 nm waves, and the subsequent analysis of their absorbance/transmittance spectrum. The concentrations of components are calculated from the radiation intensity by using previously developed calibration equations. Application of NIR spectroscopy is, however, limited to small fruits and vegetables with a thin peel. This method has found its application in industry for sorting/grading small fruits and vegetables according to their sweetness (Butz and others 2005). Nondestructive NIR spectroscopy has been used to determine beta-carotene and lycopene content in processed tomatoes and tomato products (Pedro and Ferreira 2005).

Raman spectroscopy provides information about the wavelength and intensity of inelastically scattered light from molecules. Raman scattering is a relatively weak optical effect that requires lasers for efficient excitation. In many biomaterials, the fluorescence induced by this incident light is much more intense than

**Table 3—Techniques applied for metabolite analysis in vegetables.**

Methods	Metabolites	References
<b>Nondestructive techniques</b>		
NIR spectroscopy	Sugars, carotenoids	Matsunaga and others (2004), Pedro and Ferreira (2005)
Raman spectroscopy	Fatty acids, carotenoids	Bhosale and others (2004), Schulz and others (2005)
NMR spectroscopy	Fatty acids, organic acids, carbohydrates	Ni and Eads (1993), Ishida and others (1994), Chen and others (1996)
E-nose	Fatty acids	Gan and others (2005)
<b>Destructive techniques</b>		
LC	Flavonoids, carotenoids, polysaccharides, organic acids, tocopherols, phenolics	Hertog and others (1992), Müller (1997), Villanueva-Suárez and others (2003), Marconi and others (2007), Singh and others (2007)
GC	Flavonoids, glucosinolates, phenolic acids, polysaccharides,	Bilyk and others (1984), Shaw and others (1989), Robbins (2003), Villanueva-Suárez and others (2003)
SFC and SFE	Carotenoids, tocopherols, fatty acids	Schmitz and others (1989), Señoráns and others (1999), Ollanketo and others (2001)
CE and CEC	Tocopherols, carotenoids	Aturki and others (2005), Herrero-Martinez and others (2006)
IR spectroscopy	Glucosinolates, polysaccharides, hemicelluloses	Velasco and Becker (1998), Kačuráková and others (2000)
MAE	Fatty acids, saponins, phenolics,	Lucchesi and others (2004), Kerem and others (2005), Japón-Luján and others (2006)
MS	Carotenoids, sterols, phenolics, glucosinolates, alkaloids, flavonoids, oligosaccharides	Khachik and others (1992), Dutta and Appelqvist (1996), Price and Rhodes (1997), Tolrá and others (2000), Cherkaoui and others (2001), Boue and others (2003), Latovikov and Chmelik (2006)
Biosensors	Organic acids, phenolics, glucosinolates	Maines and others (2000), Mello and others (2003), Wu and others (2005)

the Raman scattered light. This characteristic can make the Raman signals difficult to measure. The optimal wavelength region for Raman excitation for rapid tissue analysis is between 780 and 850 nm. Excitation in this region minimizes the fluorescence emission to an acceptable level and allows the use of sensitive CCD cameras, which capture high signal to noise spectra in short time exposures (a few seconds). This method has great potential for nondestructive biochemical analysis of pigments and fatty acids in plant foods (Schrader and others 1999; Hanlon and others 2000). Raman spectroscopy was applied for the detection of carotenoids in vegetables (Bhosale and others 2004; Baranska and others 2006).

Nuclear magnetic resonance spectroscopy (NMR), or magnetic resonance (MR), so called because of the negative connotations associated with the word "nuclear" in the late 1970s, is based on interactions between atomic nuclei and recording perturbations in their resonance frequencies in response to an electromagnetic field. Unlike near-infrared spectroscopy, NMR does not require a complicated calibration procedure and its operation is straightforward. Numerous studies have demonstrated that proton magnetic resonance ( $^1\text{H-MR}$ ) can be applied to the measurement of quality and composition of fruits and vegetables (Clark and others 1997; Jha and Matsuoka 2000; Butz and others 2005).  $^1\text{H-NMR}$  sensor was used to determine oil content in avocados moving on a conveyor belt (Chen and others 1996).  $^1\text{H-NMR}$  measurements were conducted to assess water, oil, carbohydrates, and organic acid content in fruits and vegetables (Ni and Eads 1993; Ishida and others 1994).

Electronic nose (e-nose) is a technique to analyze odors and odorless volatile compounds. The device consists of an array of chemical gas sensors with a broad and partly overlapping selectivity and a pattern recognition electronic system with multivariate statistical data processing tools. Chemical gas sensors are based on different signal detection principles, such as heat generation, conductivity, electrical polarization, electrochemical activity, optical, dielectric, and magnetic properties. Chemical interaction between volatile compounds and the gas sensors produces electrical signals that are interpreted by multivariate pattern recognition techniques. The data processing utilizes feature extraction and pattern recognition routines such as principal component analysis (PCA), partial least squares (PLS), functional discriminant analysis (FDA), cluster analysis, fuzzy logics, or artificial neural network (ANN). The signals generated by a gas sensor array provide qualitative and quantitative information about the chemical composition of the gas sample (Gardner and Bartlett 1999; Deisingh and others 2004; Scott and others 2006). E-noses can be applied to assess freshness/spoilage of vegetables, to detect taints and off-odors, and to control processing and packaging procedures (Deisingh and others 2004). Several studies have been conducted with e-nose to monitor changes in the aroma profile during storage of apples, to assess the postharvest quality of peaches, pears, bananas, and nectarines, and to detect spoilage in potatoes (Llobet and others 1999; De Lacy Costello and others 2000; Brezmes and others 2001, 2005). Most of these trials, however, represent preliminary feasibility studies. Much effort has to be put into solving problems with sensor stability and lifetime, calibration, and standardization of gas array instruments.

### Destructive analytical techniques

Most techniques for destructive chemical analysis require mechanical destruction of plant material followed by extraction and separation of analyzed compounds.

Liquid chromatography (LC) is the technique most frequently used for analyses of organic compounds in foods (Nollet 2000). It allows the separation of chemical components from a mixture, based on a variety of chemical interactions between the substance

being analyzed and the chromatography column. Modern high-performance liquid chromatography (HPLC) has many applications, including the separation, purification, identification, and quantification of various compounds. The HPLC system consists of several elements: columns, detector, and solvent delivery system. The most common HPLC detectors are the UV and refractive index detector. Several HPLC methods such as reversed phase chromatography, size exclusion chromatography, and ion exchange chromatography have been applied to analyze the chemical composition of vegetables (Mínguez-Mosquera and Hornero-Méndez 1993; Cole and Cousin 1994; Van Waes and others 1998). The majority of HPLC techniques employ reversed-phase separation mode. It involves a nonpolar stationary phase and a polar mobile phase. The retention time is, therefore, longer for molecules, which are more nonpolar in nature, allowing polar molecules to elute more readily. Reversed-phase HPLC is applied to analyze sugar, amino acids, organic acids, fatty acids, carotenoids, tocopherols, flavonoids, phenolic acids, saponins, and nonstarch polysaccharides in vegetables (Nollet 2000). Using this technique, carotenoids lycopene, lutein, beta-carotene, neoxanthin, violaxanthin, and luteoxanthin have been identified in tomato, carrot, red peppers, kale, spinach, lettuce, and parsley (Müller 1997). Nonstarch polysaccharides arabinose, mannose, galactose, and rhamnose have been measured in radish, leek, celery, asparagus, broccoli, tomato, and green pepper (Villanueva-Suárez and others 2003). Using liquid chromatography, potentially anticarcinogenic flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin have been quantified in onions, kale, broccoli, beans, endives, leek, and turnip (Hertog and others 1992).

Gas chromatography (GC) is the major technique for the detection of volatile compounds in plants. In gas chromatography, the mobile phase is a carrier gas (usually an inert gas such as helium or nitrogen), and the stationary phase is a microscopic layer of liquid on an inert solid support, inside glass, or metal tubing, called a column. The most common GC detectors are the flame ionization detector and the thermal conductivity detector. This method is suitable for the analysis of mixtures of relatively low molecular mass compounds (< 800 Da) such as hydrocarbons, fragrances, and essential oils. Chemical derivatization (for example, trimethylsilylation) can often be employed to increase the volatility of compounds containing polar functional groups (for example,  $-\text{OH}$ ,  $-\text{COOH}$ ,  $-\text{NH}_2$ ), thereby extending the range of potential analytes to such compounds as steroids, organic acids, amino acids, and small peptides (Adams and others 1999; Lehotay and Hajšlová 2001). Gas chromatography was applied to analyze flavonols (Bilyk and others 1984), glucosinolates (Shaw and others 1989), sterols (Normén and others 1999), phenolic acids (Robbins 2003), polysaccharides (Villanueva-Suárez and others 2003), and aroma compounds (Jeleń and others 2000) in vegetables and their products.

Supercritical fluid chromatography (SFC) and extraction (SFE) is a hybrid of gas and liquid chromatography. Supercritical fluids are produced by heating a liquid above its critical temperature or compressing a gas above its critical pressure. Supercritical fluids (such as xenon, ethane, and carbon dioxide) possess unique properties that are different from those of either gases or liquids under standard conditions (Chester and others 1994; Williams and others 2002). Carbon dioxide is the most commonly used supercritical fluid because of its low critical temperature (31 °C), inertness, low toxicity, and reactivity. Supercritical fluid chromatography permits the separation and determination of compounds that are not conveniently handled by gas or liquid chromatography, for example, nonvolatile or thermally labile. Compared with GC, SFC offers high resolution at much lower temperatures. Compared with HPLC, SFC

provides rapid separations without the use of organic solvents. SFC has been applied to the separation of vegetable carotenoids and carotenoid isomers (Schmitz and others 1989), and the isolation of vegetable lipids, tocopherols, and carotenoids (Señoráns and others 1999; Lesellier and Tchaplá 2000; Ollanketo and others 2001). Due to lack of toxicity, the method has been extended to supercritical fluid extraction (SFE) of plant nonpolar substances such as essential oils, flavor and fragrance compounds, lipids, carotenes, and alkaloids (Jarvis and Morgan 1997).

Capillary electrophoresis (CE) and capillary electrochromatography (CEC) are separation techniques that use narrow-bore fused-silica capillaries to separate a complex array of large and small molecules. High electric field strengths are used to separate molecules based on differences in charge, size, and hydrophobicity. Sample introduction is accomplished by immersing the end of the capillary in a sample vial and applying pressure, vacuum, or voltage (Altria 1999). Capillary electrochromatography is a hybrid method that couples the high separation efficiency of CE with HPLC and uses an electric field rather than hydraulic pressure to propel the mobile phase through a packed bed (Altria and others 1997). Capillary electrochromatography was applied to analyze carotenoids in carrot, tomato, and spinach (Herrero-Martínez and others 2006) and tocopherols in vegetable oils (Aturki and others 2005).

Infrared spectroscopy (IR spectroscopy) is a subset of techniques that deal with the infrared part of the electromagnetic spectrum (750 nm to 1 mm). Radiation in this region can be utilized in organic structure determination by making use of the fact that it is absorbed by interatomic bonds in organic compounds such as C–O, –NO<sub>2</sub>, C=N, C–F, and so on. There are a number of ways in which the IR technique may be implemented for analyses of chemical compounds: transmission IR spectroscopy, diffuse reflectance IR spectroscopy, reflection–absorption IR spectroscopy, and multiple internal reflection spectroscopy (Smith 1996). Fourier transform infrared spectroscopy (FTIR) is the technique most frequently used for identification of organic compounds present in complex biological samples. It can be utilized to quantify some components of an unknown mixture. The term Fourier transform refers to a fairly recent development in the manner in which the data are collected and converted from an interference pattern to a spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Smith 1996). Infrared spectroscopy was applied for analyses of plant pectic polysaccharides and hemicelluloses (Kačuráková and others 2000), quantification of glucosinolates in Brassica species (Velasco and Becker 1998), classification of vegetable oils (Dahlberg and others 1997), and metabolic fingerprinting of salt-stressed tomatoes (Johnson and others 2003).

The microwave-assisted extraction (MAE) and its patented variation microwave-assisted process (MAP) are high-speed methods used to selectively extract target compounds from various raw materials (Paré and others 1994; Eskilsson and Björklund 2000). The technology uses a microwave applicator as the energy source during solvent extraction, leading to direct extraction capability and reduced solvent levels as compared to conventional extraction methods. The raw material from which the desired compound has to be removed is immersed in a solvent. The solvent is selected on the basis of its ability to dissolve the sought compound(s) and its transparency to microwaves. This source material is pumped into the process vessel where it is subjected to microwave irradiation. A target compound is then selectively heated by adjusting various MAP parameters such as microwave power, exposure time, and flow rate. The temperature difference between the solvent and the target compound forces the compound into the solvent. The resulting substrate is then filtered off and the filtrate is processed conven-

tionally to remove the target compound. Microwave-assisted extraction has been applied to isolate saponins (Kerem and others 2005), phenolic compounds (Japón-Luján and others 2006), and essential oils from plant tissues (Lucchesi and others 2004).

Separation methods, described in this section, are often used as tandem techniques with mass spectrometric analyses.

Mass spectrometry (MS) is the most powerful analytical technique used to identify chemical compounds by measuring mass-to-charge ratio ( $m/z$ ) of their ions. This is achieved by ionizing the sample, separating ions of differing masses, and recording their relative abundance by measuring intensities of ion flux. The most frequently used ionization techniques for organic molecules are electron impact ionization (EI), chemical ionization (CI), electrospray ionization (ESI), plasma desorption ionization (PDI), matrix-assisted laser desorption/ionization (MALDI), and fast atom bombardment ionization (FAB) (Herbert and Johnstone 2003).

Electron impact ionization is widely used in mass spectrometry for relatively volatile samples that are insensitive to heat and have relatively low molecular weight. It utilizes 70 eV electrons for the ionization of volatile compounds. This relatively harsh ionization technique can produce molecular ions as well as fragment ions.

Chemical ionization is a softer ionization technique for volatile compounds. The method is based on proton transfer from a reagent gas ion (typically isobutane, methane, or ammonia) present in great excess relative to the sample of interest. For both ionization methods, the molecular weight range is 50 to 800 Da.

Electrospray ionization can be used for small and large molecular weight biopolymers (for example, peptides, proteins, carbohydrates, and DNA fragments) and lipids. The solvated sample is passed through a needle held at a high potential of 3 to 10 kV. Typically this leads to formation of protonated molecules with little or no fragmentation.

Plasma desorption ionization utilizes fission fragments from <sup>252</sup>Californium for ionization. The sample is applied to a nitrocellulose matrix, either by droplet or electrospraying. The sample ions, which are formed, are accelerated into a time-of-flight mass spectrometer for mass analysis. The useful mass range is up to  $m/z$  5000.

Matrix-assisted laser desorption/ionization analysis is used to determine the molecular weight of peptides, proteins, polysaccharides, and other compounds of biological origin. This technique typically utilizes a nitrogen laser at 337 nm as the ionization source. The sample is mixed with a matrix and allowed to dry prior to insertion into the mass spectrometer. This method can be applied to molecules up to  $m/z$  300000.

Fast atom bombardment is another “soft” ionization method, used to analyze sensitive, nonvolatile compounds. It involves the bombardment of a solid spot of the analyte/matrix mixture at the end of a sample probe by a fast atom beam (for example, neutral inert gas, usually argon or xenon). The detected mass range is about 300 to 6000 Da.

The most common mass detectors include magnetic sector analyzer, time-of-flight, quadrupole, quadrupole ion trap, and ion cyclotron resonance (McLucky and Wells 2001). Magnetic sector uses an electric and/or magnetic field to affect the path and/or velocity of the charged particles. The ions enter a magnetic or electric field that bends the ion paths depending on their mass-to-charge ratios. Time of flight analyzer uses an electric field to accelerate the ions through the same potential, and then measures the time they take to reach the detector. Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize ions passing through a radio frequency field. Fourier transform ion cyclotron resonance analyzer detects the image current produced by ions cyclotroning in the presence of a magnetic field. Orbitrap is the

most recently introduced mass analyzer (Hu and others 2005). In the Orbitrap, ions are electrostatically trapped in an orbit around a central, spindle-shaped electrode. The electrode confines the ions so that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates, which is recorded by the instrument.

Mass spectrometry, along or in combination with chromatographic separation techniques, has been applied to analyze carotenoids in tomatoes, Brussels sprouts, and kale (Khachik and others 1992), oligosaccharides in Jerusalem artichoke and red onion (Latovikov and Chmelík 2006), flavonoids in soybean and kale (Boue and others 2003; Zhang and others 2003), phenolic compounds in onions (Price and Rhodes 1997), glucosinolates in Brassica species (Tolrà and others 2000), alkaloids in Solanaceae (Cherkaoui and others 2001), and sterols in vegetable oils (Dutta and Appelqvist 1996).

Biosensors are analytical tools incorporating biotic elements, such as immunochemical, enzymatic, nonenzymatic receptors, and DNA recognition sensors. In biosensors, biological components are immobilized at the transducer surface. Biosensors can be classified as electrochemical, optical, thermal, piezoelectric, and surface plasmon resonance sensors according to their transducing mechanism. The main advantage of these techniques is their ability to determine analytes in complex matrices and to identify biological compounds that are difficult to determine by other analytical procedures (for example, nonpolar molecules). The main limitations of biosensors are the narrow range of detecting compounds and their short lifetime. Biosensors have been applied to environmental monitoring, food control, agricultural, clinical, and pharmaceutical research. Sensors suitable for food analysis can detect phenolic compounds, alkanes, hormones, surfactants, antibiotics, and bacterial toxins (O'Kennedy and others 2005). A biosensor based on a myrosinase and glucose oxidase bienzyme system was developed to detect glucosinolates in cabbage, rape, mustard, and caixin (Wu and others 2005). A biosensor based on horseradish peroxidase, immobilized onto silica-titanium matrix, was applied to measure polyphenol compounds in vegetable extracts (Mello and others 2003). A pyruvate biosensor was constructed using pyruvate oxidase, immobilized on meldolas blue electrodes, to determine pungency in onions (Abayomi and others 2006). A polyphenol oxidase sensor incorporated into conducting polymer matrices was tested to determine phenolic content in red wines (Yildiz and others 2005). A biosensor with citrate lyase and oxaloacetate decarboxylase enzymes in a flow injection analysis system has been constructed for the determination of citrate concentration in fruit juices (Maines and others 2000). Despite the large amount of research literature on biosensors, most of commercially available devices are used for medical diagnostics.

### Conclusions

Plant foods contain almost all nutrient compounds essential for human nutrition as well as a large number of health-promoting chemical substances. The number of metabolites present in the plant kingdom is estimated to exceed 200000 (Pichersky and Gang 2000; Fiehn 2002). All these compounds are involved in primary and secondary plant metabolism. They play important role in plant growth and development, defense against insects and other plant-feeding animals, resistance to plant pathogens, and stress responses induced by UV radiation, heavy metals, and pesticides. Development of strategies to improve the nutritional quality of plant foods requires understanding of the biochemical and molecular mechanisms of metabolite synthesis in plants as well development

of analytical approaches for metabolite identification. Over the past decade, significant efforts have been made to enhance the production of plant metabolites through traditional breeding and application of genomics technologies (DellaPenna 2001; Capell and Christou 2004; Morandini and others 2005). Successful examples are increases in the amount of oleic acid in soybean (Kinney 1996), increased levels of flavonoids and carotenoids in tomato (Fraser and others 2002; Mehta and others 2002), and increases in starch and amino acid content in potato (Regierer and others 2002). However, the introduction of high phytochemical-containing vegetables requires an extensive metabolite control, because background levels of other metabolites are largely unknown.

Plant metabolomics, or large-scale phytochemical analysis, is a new research discipline, which aims to develop a comprehensive approach to metabolite detection and identification. Mass spectrometry, nuclear magnetic resonance, and infrared spectrometry are the most common metabolomics platforms. Statistical tools such as hierarchical cluster analysis and principal component analysis are often used to compare large metabolite data sets. Metabolite profiling and metabolite fingerprinting are fast growing technologies for phenotyping and diagnostic analyses of plants (Krishnan and others 2004; Schauer and Fernie 2006). They allow identification of the most important compounds (or groups of compounds) underlying differences between genotypes or phenotypes. Using this approach, the metabolic profiles of potato, tomato, and lettuce were characterized (Roessner and others 2001; Garratt and others 2005; Schauer and others 2005). Metabolite fingerprinting has been applied to compare equivalence of conventional and transgenic tomatoes and peas (Noteborn and others 2000; Charlton and others 2004). Due to recent developments in analytical and computational methods we are now approaching comprehensive high-throughput analysis of plant metabolites.

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