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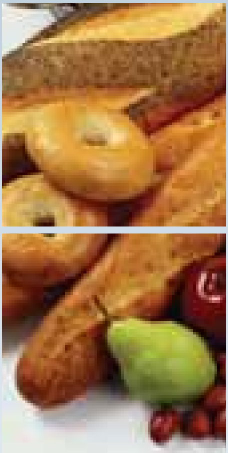
Synthesis Report No 4:

Plant Foods and Health: Focus on Plant Bioactives

By Anna Denny and
Prof. Judith Buttriss
British Nutrition Foundation



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Contents

1. Introduction

It has been recognised for some time that people consuming diets rich in plant foods (*e.g.* fruits and vegetables and wholegrain cereals) are at reduced risk of developing chronic diseases. These include cardiovascular disease (Dauchet *et al.* 2006), cancer (WHO 2002) and other chronic conditions such as age-related eye conditions (van Leeuwen *et al.* 2003; Hogg & Chakravarthy 2004) and lung disorders *e.g.* obstructive pulmonary disease (Denny *et al.* 2003).

The relationship between plant food intake and health has been the focal point of much scientific investigation in recent years to try to identify the specific plant components that convey health benefits. Initially much of this research focused upon the 'antioxidant' nutrients (*e.g.* vitamins C, E and ß-carotene) and their ability to prevent free-radical induced tissue damage but, as yet, no clear causal link between low plasma levels of antioxidant nutrients and disease occurrence has been established (see Stanner *et al.* 2003), possibly due to the design limitations of scientific studies used to investigate this relationship.

More recently, the search for specific plant components that convey health benefits has widened to encompass the vast range of 'non-nutritive' compounds present in plant foods, and their potential to improve health. Evidence is growing that such plant constituents, belonging to the group termed **"Bioactive compounds"**, may help to promote optimal health and to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke and perhaps Alzheimer's disease.

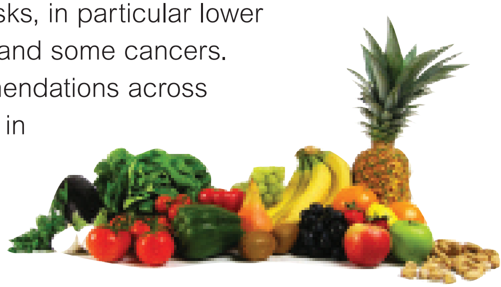
Quality and comprehensive composition data on bioactives in edible plants and plant-based foods are vital to underpin epidemiological research on plant foods and health. **EuroFIR**, short for **European Food Information Resource**, is an EU funded 'Network of Excellence' project that aims to develop an integrated, comprehensive and validated databank or *food information resource* which will provide a single, authoritative source of food composition data in Europe for nutrients and newly emerging bioactive compounds with potential health benefits. A unique feature of the EuroFIR BASIS Bioactives database is that, in addition to the inclusion of critically assessed composition data on the bioactives present in edible plants and plant-based foods, critically assessed data on the biological effects of these bioactives are also being compiled.

This Synthesis Report:

- explores the evidence linking plant food intake with health;
- identifies plant components that may convey health benefits, including potentially bioactive compounds;
- introduces the main classes of plant bioactives with potential health benefits;
- introduces work underway within EuroFIR to provide quality and comprehensive information on the levels and biological effects of bioactive compounds with anticipated health benefits in plant and plant-based foods commonly consumed in Europe.

2. Plant foods and health

It is known that diets rich in plant-derived foods are generally associated with lower disease risks, in particular lower rates of cardiovascular disease and some cancers. Because of this, dietary recommendations across Europe recommend an increase in consumption of plant foods, including wholegrain cereals and fruits and vegetables.



2.1 What counts as a plant food?

Plant-derived foods can be categorised in many ways, the most common of which use the terms ‘fruit’ and ‘vegetable’ in a culinary, rather than botanical, sense. For the purposes of this report, plant–derived foods are categorised according to the following model (Table 1).

Table 1: Categorisation of plant-derived foods and drinks (modified from BNF 2003)

Group	Examples
Fruits	
Fruits from trees	Apples, pears, plums, apricots, peaches, cherries, citrus fruit, dates, mango, papaya, fig, kiwi
Other fruits	Strawberries, raspberries, blackberries, cranberries, pineapple, red and black currants, melons, grapes, bananas
Vegetables	
Root crops	Carrots, turnips, swedes, parsnips; potato, sweet potato, yam*
Cabbage group	Cabbages, broccoli, Brussels sprouts
Onion group	Onions, leeks, garlic
Salad vegetables	Lettuce, celery
Fruit crops	Tomatoes, sweet peppers, chilli peppers, aubergine, cucumbers, squashes
Other	Sprouted seeds, sea vegetables
Cereals (grains)	Wheat, barley, maize (corn), millet, oats, rice, rye
Tree nuts & seeds	Walnuts, cashews, almonds, chestnuts, pecans, brazils, hazelnuts, pistachio, pine kernels, sesame seeds
Pulses (legumes)	Soya beans, kidney beans, butter beans, chick peas, lentils, peanuts
Beverages	Tea, coffee, cocoa, wine, beer
Edible oils	Sunflower oil, maize (corn) oil, rapeseed oil, linseed oil, olive oil, soya oil, peanut oil
Herbs, spices	Sage, rosemary, thyme, ginger, pepper, cumin
Other	Chocolate

* Potato, sweet potato and yam are classified as starchy foods, rather than vegetables, in many food guidance models.

2.2 Constituents of plant-derived foods

Plant-derived foods include rich sources of a number of nutrients that may have a beneficial effect on health, for example carbohydrates, vitamin C, folate, dietary fibre, ß-carotene and vitamin K.

In economically developed countries, diets abundant in a variety of plant foods, such as brightly coloured fruits and vegetables, are associated with reduced risk of developing chronic disease, and with general health and wellbeing. Before focussing on the evidence linking plant food intake and health, it can be helpful to consider the diverse range of nutrients and other potentially bioactive compounds found in plant-derived foods and drinks that may convey such health benefits (Table 2).

Table 2: Nutrients and other constituents found in plant-derived foods and drinks (adapted from BNF 2003)

Plant constituent	Important sources (if eaten regularly)
Minerals	
Calcium	Pulses, green vegetables, dried fruit and nuts
Chromium	Wholegrains, and to a lesser extent legumes and nuts
Copper	Cereals, vegetables
Iodine	Sea vegetables *e.g.* kelp, beer
Iron	Vegetables, pulses; to a lesser extent potatoes and dried fruit
Magnesium	Cereals (especially wholegrain), green vegetables, nuts & seeds
Manganese	Tea is a major source. Other sources include wholegrain cereals, vegetables, nuts and seeds
Molybdenum	Vegetables and cereals. Also present in pulses
Potassium	Particularly abundant in vegetables, potatoes and fruit. It is also found in cereals, nuts and seeds

Table 2 continued: Nutrients and other constituents found in plant-derived foods and drinks (adapted from BNF 2003)

Plant constituent	Important sources (if eaten regularly)
Selenium	Cereals
Zinc	Cereals (especially wholegrain varieties), lentils, nuts, sweetcorn and rice
Vitamins	
Folate	Green leafy vegetables (especially sprouts & spinach), green beans & peas, potatoes; fruit, especially oranges
Niacin	Potatoes
Thiamin	All cereals, potatoes. Also present in vegetables
Vitamin B6	Potatoes
Vitamin C	Richest sources are citrus fruit, kiwi fruit and soft fruits *e.g.* blackcurrants, strawberries. Other sources include green vegetables, peppers and potatoes (especially new potatoes)
Vitamin E	Vegetable oils, wholegrain cereals, vegetables (especially dark green leafy types), fruit, cereals
Vitamin K	Green leafy vegetables. Also in other vegetables, fruit, vegetable oils and cereals
Other nutrients	
Dietary fibre	All cereals (especially wholegrain varieties), vegetables, fruit, pulses, nuts

Table 2 continued: Nutrients and other constituents found in plant-derived foods and drinks (adapted from BNF 2003)

Plant constituent	Important sources (if eaten regularly)
Fatty acids	
MUFA	Olive oil and rapeseed oil. Also present in other seed and nut oils
PUFA (n-6)	Rich sources are sunflower, safflower and corn oils. Also present in other seed and nut oils
PUFA (n-3)	There are no plant sources of the long chain n-3 fatty acids (EPA & DHA) associated with heart health, but the essential fatty acid ß-linolenic acid is present in large amounts in linseed (flax) oil, grapeseed and rapeseed oils, walnut oil and walnuts. It is also present in green leafy vegetables, soya beans (and soya oil) and hazelnuts
Other plant constituents	
Carotenoids	
α-carotene	Carrots, squash, oranges, tangerines; other sources include passion fruit & kumquats
ß-carotene	Orange vegetables (*e.g.* carrots), green leafy vegetables (*e.g.* spinach), tomato products; other sources include apricots, guava, mangoes, orange melons, passion fruit
Lycopene	Tomato
ß-cryptozanthin	Oranges
Lutein and zeaxanthin	Green leafy vegetables
Flavonoids, *e.g.* flavonols, flavan-3-ols, flavones, flavanones, anthocyanidins	Tea, red wine, onions and apples are rich sources of this large group of compounds. Cocoa and hence dark chocolate primarily provide flavan-3-ols. Sources of specific types of compounds include grapes, berries and cherries (flavonols, anthocyanidins); parsley, thyme and celery (flavones) and citrus fruit (flavanones)

Table 2 continued: Nutrients and other constituents found in plant-derived foods and drinks (adapted from BNF 2003)

Plant constituent	Important sources (if eaten regularly)
Glucosinolates	Brassica vegetables *e.g.* Brussels sprouts, cabbage, broccoli
Phytoestrogens (isoflavones, lignans)	Soya, other pulses, seeds *e.g.* linseed, grains, nuts
Sterols	Naturally present in vegetable oils *e.g.* soya oil. Also present in cereals, nuts and vegetables
Sulphur-containing compounds	Onions, leeks, garlic, chives (also see glucosinolates)
Terpenoids	Herbs & spices *e.g.* mint, sage, coriander, rosemary, ginger

2.3 What is the evidence linking plant food intake and human health?

Evidence that consumption of plant-derived foods is protective of human health has been gathered from a substantial number of large epidemiological (population-based) studies.

To date, many of these studies have focused upon constituents in fruits and vegetables but, more recently, compounds found in other plant-based foods such as tea, cocoa, cereals, wine and herbs have been investigated for their potentially beneficial effects.

2.3.1 Cardiovascular disease

Cardiovascular disease (CVD, which includes heart disease and stroke) is a major cause of death and ill health in Europe. Nearly half of all deaths in Europe (49%) are caused by CVD and, although CVD incidence and mortality are falling in most Northern, Southern and Western European countries, incidence and mortality rates are falling more slowly in Central and Eastern European countries (Petersen *et al.* 2005).

Important risk factors for CVD include obesity, high blood cholesterol level, high blood pressure and type 2 diabetes. Eating a healthy diet[1], being physically active, not smoking and not drinking alcohol excessively can all help to reduce a person's risk of CVD (BNF 2005).

A number of studies have looked at the relationship between the consumption of whole plant foods (*e.g.* fruit and vegetables, nuts, pulses, wholegrains) and CVD risk, and between plant-derived food components (*e.g.* dietary fibre) and CVD risk.

2.3.1.1 Fruit and vegetables and CVD risk

Trend data demonstrating an inverse relationship between declining fruit and vegetable consumption and increasing rates of coronary heart disease (CHD) across Europe can be tracked back to the 1970s, when Armstrong and Doll (1975) reported that CHD mortality rates were higher in areas of the UK where consumption of fruit and vegetables was lowest.

In more recent years, growing interest in preventing CVD using plant-based foods has generated numerous epidemiological studies (Hu *et al.* 2002) and,

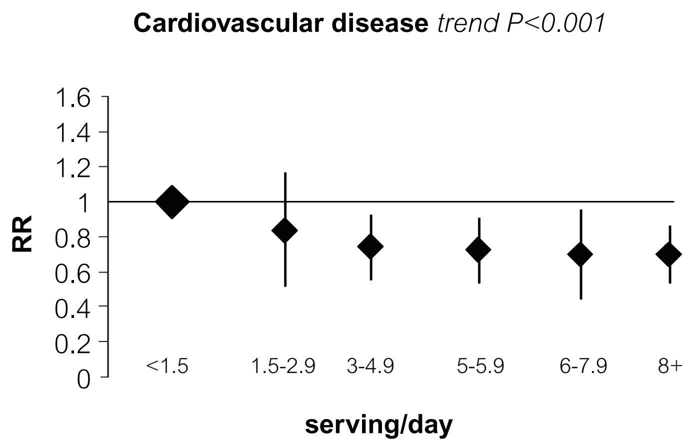
1 A healthy diet is a diet based on breads, potatoes and other cereals and is rich in fruits and vegetables. A healthy diet will include moderate amounts of milk and dairy products, meat, fish or meat/milk alternatives, and limited amounts of foods containing fat or sugar.

in the last five years, a number of large long-term prospective cohort studies investigating the relationship between fruit and vegetable intake and CVD risk have been published. The following section summarises some of this work.

The relationship between fruit and vegetable intake and CVD incidence in the American *Nurses' Health Study* and the *Health Professionals' Follow-up Study* prospective cohorts has been investigated by Hung *et al.* (2004).

Almost 72,000 female participants in the *Nurses' Health Study* and 37,725 male participants in the *Health Professionals' Follow-up Study* completed baseline food frequency questionnaires, in 1984 and 1986 respectively, and were followed up for incidence of CVD or death until 1998. Total fruit and vegetable intake was inversely associated with risk of CVD. For example, risk of CVD was reduced by 28% among men and women eating at least five servings of fruit and vegetables daily, compared with participants eating fewer than 1.5 servings daily (relative risk of 0.72 for an intake of 5-5.9 servings of fruit and vegetables a day compared with fewer than 1.5 servings) (Figure 1) (Hung *et al.* 2004).

Figure 1: Multivariable-adjusted relative risks and 95% confidence intervals of total fruit and vegetable consumption for cardiovascular disease using <1.5 servings per day as reference. Source: Hung *et al.* 2004



Of the food groups analysed, green leafy vegetable intake showed the strongest inverse association with CVD. For each additional serving of green leafy vegetables consumed a day, risk of CVD fell by 11% [RR 0.89 (95% CI = 0.83 to 0.96) for an increment of one serving per day of green leafy vegetables].

2.3.1.2 Fruit and vegetables and risk of coronary heart disease and stroke

In a second analysis of data from the American *Nurses' Health Study* and the *Health Professionals' Follow-up Study* including 2190 incident cases of CHD and 570 cases of ischaemic stroke (Joshipura *et al.* 1999, 2001), Hu (2003) identified RRs of CHD associated with different categories of fruit and vegetables (Table 3).

Table 3: Multivariate relative risks (RRs) of coronary heart disease and stroke based on a comparison of the highest and the lowest quintiles of fruit and vegetable intakes in the pooled analyses of the Nurses' Health Study and the Health Professionals' Follow-up Study

	RR (95% Confidence Intervals)[2]	
Food	**Coronary heart disease**	**Stroke**
All fruit	0.80 (0.69, 0.92)	0.69 (0.52, 0.91)
All vegetables	0.82 (0.71, 0.94)	0.90 (0.68, 1.18)
Total citrus fruit	0.88 (0.77, 1.00)	0.72 (0.47, 1.11)
Citrus fruit juice	1.06 (0.85, 1.32)	0.65 (0.51, 0.84)
Cruciferous vegetables	0.86 (0.75, 0.99)	0.71 (0.55, 0.93)
Green leafy vegetables	0.72 (0.63, 0.83)	0.76 (0.58, 0.99)
Vitamin C rich fruit and vegetables	0.91 (0.79, 1.04)	0.68 (0.52, 0.89)
Legumes	1.06 (0.91, 1.24)	1.03 (0.77, 1.39)
Potatoes (including French fries)	1.15 (0.78, 1.70)	1.18 (0.90, 1.54)

RRs adjusted for standard cardiovascular disease risk factors
Source: Am.J.Clin.Nutr. (2003; **78** (3): 544S-551S), American Society for Nutrition.

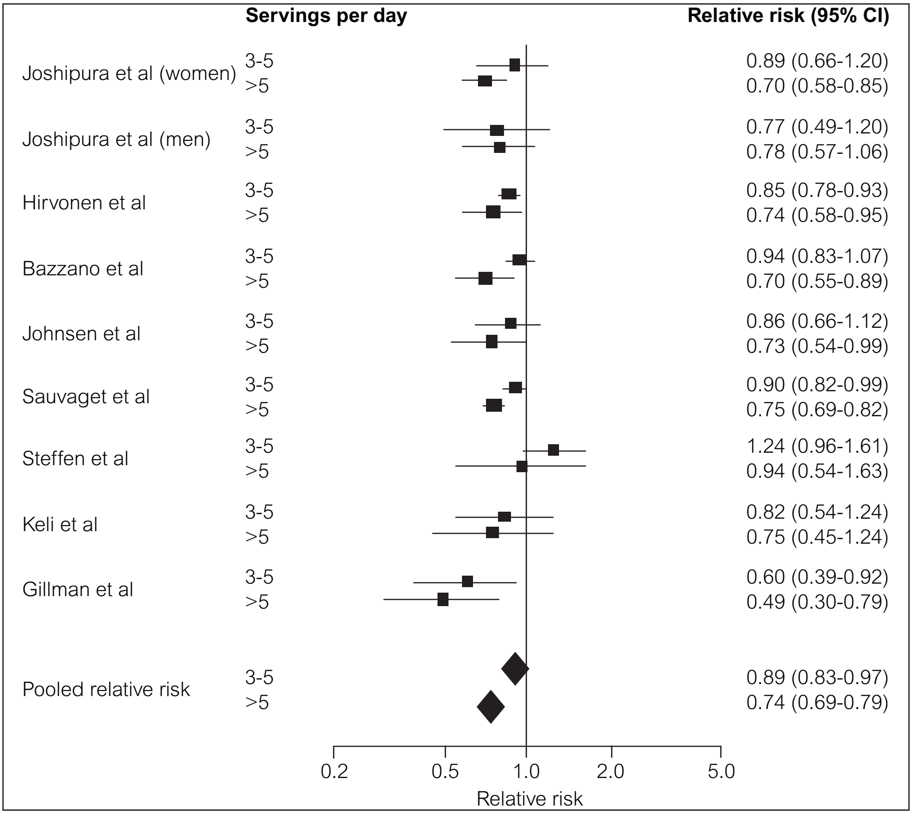
2 If the confidence intervals span 1.0 the association is not significant

Stronger support for an association with CHD than stroke was evident for vegetables (RR=0.82, so an 18% reduction in risk). Among individual items, green leafy vegetables contributed most to the protective effects for CHD, whilst cruciferous vegetables and vitamin C rich citrus fruit and juices contributed most to the protective effects for stroke (*e.g.* a statistically significant 35% reduction in risk of stroke for those in the highest quintile of citrus fruit juice intake). A particular role for citrus fruit in reducing CHD risk has also been reported in the *PRIME* study of subjects in France and Northern Ireland (Dauchet *et al.* 2004).

Dauchet *et al.* (2006) assessed the strength of the relationship between fruit and vegetable consumption and risk of CHD in a meta-analysis of 9 observational cohort studies (comprising 221,080 individuals and adjusted for major confounding factors). Pooled RRs were calculated for each additional portion of fruit and/or vegetables consumed per day. The risk of CHD was decreased by 4% [RR 0.96 (95% CI = 0.93–0.99)] for each additional portion per day of fruit and vegetable intake and by 7% [RR 0.93 (95% CI = 0.89–0.96)] for fruit intake. The authors note that, due to publication bias (the tendency for investigators, reviewers and editors to submit or accept manuscripts for publication based on the direction or strength of the study findings (Dickersin 1990)), the reported pooled RRs may be overestimated.

He *et al.* (2006) have recently assessed the relationship between fruit and vegetable intake and incidence of stroke in a meta-analysis of nine independent cohort studies. The studies were adjusted for major confounding factors and included 257,551 individuals with an average follow-up of 13 years. The authors reported an 11% reduction in risk of stroke in those consuming 3-5 servings of fruit and vegetables per day compared to less than 3 servings, and a reduction in risk of 26% in those consuming more than 5 servings (Figure 2). Subgroup analyses showed that fruit and vegetables had a significant protective effect on both ischaemic and haemorrhagic stroke.

Figure 2: Risk of stroke for 3-5 and 5+ servings of fruit and vegetables per day compared with less than 3 servings[3]



Size of the squares is proportional to weight of the studies in the meta-analysis

Figure reprinted from The Lancet, Vol. 367, He FJ, Nowson CA, MacGregor GA, Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. P. 320-326, Copyright (2007) with permission from Elsevier.

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3 Squares to the left of the line indicate a reduced risk. The reduction in risk is statistically significant if the confidence limits (indicated by the horizontal lines) do not cross the vertical line.

One of the most important risk factors for stroke is high blood pressure (hypertension). The beneficial effects of increased intake of fruit and vegetables on blood pressure have been demonstrated in several clinical trials (Appel *et al.* 1997; John *et al.* 2002). As fruit and vegetables contain a variety of nutrients and non-nutrients that may be protective against CVD, it is almost impossible to pinpoint the exact nutrients or other constituents that are responsible for these protective effects.

The most widely quoted intervention study to change subjects' dietary patterns as a whole, and thereby demonstrate an effect of fruits and vegetables on blood pressure, is the *DASH* trial (Appel *et al.*, 1997). This study showed that a diet rich in fruits and vegetables, low in fat and incorporating low-fat dairy products, without changes in salt or weight loss, could lower blood pressure. Other trials have broadly supported the findings from *DASH*, showing that moving dietary patterns towards a more plant-based food intake is associated with lower blood pressure.

Alongside hypertension, raised serum total cholesterol is an important risk factor for CVD (particularly for CHD) and the beneficial effects of fruits and vegetables on CVD risk are mainly mediated through an effect on LDL-cholesterol level.

In a cross sectional study, Djousse *et al.* (2004) found that subjects in the groups with highest fruit and vegetable intakes had LDL concentrations that were 6-7% lower than those in the groups with the lowest intakes. This effect on LDL-cholesterol is likely to be at least partially attributable to the fibre content of fruit and vegetables; propionic acid produced by fermentation of fibre in the large bowel is believed to help reduce blood cholesterol (Lunn & Buttriss 2007).

However, results of other studies have been inconsistent. For example, a similar response (a 7.3% reduction) was seen in the *Indian Diet Heart Study*, a 12 week intervention study (Singh *et al.* 1992), but no significant effect was reported in the 8-week *DASH* trial (Obarzarnek *et al.* 2001).

2.3.1.3 Wholegrains and CVD risk

Evidence is mounting, especially in the USA where oats are the major whole grain, that there are considerable health benefits to be gained from consuming cereals in the form of the whole grain (see Lunn & Buttriss 2007). For instance, consuming the intact grain has been associated with reduced risk of coronary heart disease, ischaemic stroke and type 2 diabetes (Smith *et al.* 2003; Seal 2006; Seal *et al.* 2006). Wholegrains contain a number of components that may contribute to a reduced risk of heart disease, such as vitamin E and dietary fibre. They also contain resistant starch and oligosaccharides that are fermented by intestinal bacteria to short chain fatty acids that may help reduce blood cholesterol, as well as plant sterols that have cholesterol lowering effects (when consumed in sufficient quantities) (see Seal *et al.* 2006).

2.3.1.4 Nuts and CVD risk

There is a substantial body of evidence suggesting that frequent consumption of nuts is associated with lower risk of cardiovascular disease (see Aisbitt 2007). Nuts are a good source of dietary fibre, essential fatty acids and protein. They also provide vitamins including folate, niacin, vitamins E and B_6 and minerals, such as iron, magnesium, zinc, selenium, phosphorus and potassium. In addition, they are a source of plant bioactives that may have important health benefits. Although they are high in fat and thus energy, the fatty acids are predominantly unsaturated.

A number of large-scale epidemiological studies have found that individuals who frequently consume nuts have a lower risk of heart disease than those who do not. The reduction in risk ranges from 25-55% (Kris-Etherton *et al.* 2001). There have also been a number of intervention trials where the effects of including nuts in the diet on cardiovascular risk factors, particularly blood cholesterol levels, have been investigated. Overall, these studies have shown a beneficial effect of nut-containing diets on cholesterol levels. However, it is unclear whether this is mediated by the high levels of unsaturated fatty acids in the nuts or by another component of the nuts. Some, but not all, of the studies that compared the nut diets with other nut-free diets that were high in unsaturated fatty acids, found that the nut diets had a greater effect on blood cholesterol levels. In addition, when the expected changes in CHD were calculated according to the fat profile of the nuts, the actual changes in CHD risk reported in epidemiological studies were greater than expected, suggesting that nuts have effects beyond their contribution of unsaturated fatty acids to the diet (Kris-Etherton *et al.* 2001).

The additional components of nuts that may contribute to a reduction in CHD risk include fibre and the bioactive compounds they contain. Among the many bioactive compounds found in nuts are phytosterols, flavonoids and other phenolic compounds. Although these have potential health benefits, it has not yet been established whether any of these are responsible for the apparent reduction in CHD risk from nut consumption. In addition, data on the composition of nuts (with regard to bioactive compounds) is incomplete, and so more research is needed before it is possible to attribute a beneficial effect to any of the bioactive compounds in nuts (Kris-Etherton *et al.* 2001). EuroFIR is contributing to this work (see Section 4.1.1).

2.3.1.5 Conclusions for cardiovascular disease

Plant-based diets are associated with lower risk of cardiovascular disease (*e.g.* CHD and stroke). Fruit and vegetable consumption is generally associated with other health-promoting behaviours, *e.g.* taking more exercise and not smoking, as well as higher consumption of wholegrain cereals and lower consumption of high-fat animal-derived products. Even when such factors are taken into account, however, an association between high fruit and vegetable intake, and reduced risk of CVD, remains (although adjusting for these factors often weakens the strength of the association). Whilst the specific patterns of plant components responsible for this association remain to be established, there is evidence of a greater effect at higher levels of fruit and vegetable consumption, in line with current recommendations to increase fruit and vegetable consumption to a minimum of 400g of fruits and vegetables per day (WHO 2002).

2.3.2 Cancer

Cancer remains a leading cause of death in Europe. Each year there are 2.7 million new cases and 1.7 million deaths from the disease (Ferlay *et al.* 2007). In recent years, age-standardised cancer incidence has generally been declining in most European countries, with the notable exception of lung cancer in women, which is continuing to increase and reflects trends in cigarette smoking.

It has long been recognised that diet and lifestyle factors can influence the risk of cancer, with associations with some forms of cancer being stronger than with others. It has been estimated that approximately 30% of cancers could be prevented by dietary means in European countries (Key *et al.* 2002). Excess body weight and physical inactivity are estimated to account for approximately 20-30% of the incidence rate of those cancers commonly found in developed countries (Key *et al.* 2004 a).

2.3.2.1 Fruit and vegetables and cancer risk

The World Cancer Research Fund (WCRF)/American Institute of Cancer Research (AICR) (World Cancer Research Fund 1997) and the UK COMA Working Group on The Nutritional Aspects of Cancer (Department of Health

1998) reviewed the evidence on the effect of diet on cancer risk about 10 years ago. Both panels reached similar conclusions on the strength of the evidence for an effect of consuming fruit and vegetables on risk of cancer at a number of sites. These are summarised in Table 4 below:

Table 4: Conclusions of the WCRF-AICR and COMA reviews on the strength of the evidence for a protective effect of fruit and vegetable consumption on risk of cancer at a number of sites

Cancer site	World Cancer Research Fund/American Institute of Cancer Research, 1997	Committee on Medical Aspects of Food and Nutrition Policy, UK, 1998
Mouth and pharynx	Convincing	Weakly consistent for fruit, inconsistent for vegetables
Larynx	Probable	Moderately consistent, limited data
Oesophagus	Convincing	Strongly consistent
Lung	Convincing, particularly for green vegetables & carrots	Moderately consistent for fruit, weakly consistent for vegetables
Stomach	Convincing, particularly for raw vegetables, allium vegetables, and citrus fruit	Moderately consistent
Pancreas	Probable	Strongly consistent, limited data
Liver	Possible for vegetables, not fruit	Not included in the review
Colon & rectum	Convincing for vegetables, limited and inconsistent data for fruit	Moderately consistent for vegetables, inconsistent and limited data for fruit
Breast	Probable, in particular for green vegetables	Moderately consistent for vegetables, weakly consistent for fruit

Cancer site	World Cancer Research Fund/ American Institute of Cancer Research, 1997	Committee on Medical Aspects of Food and Nutrition Policy, UK, 1998
Ovary	Possible	Insufficient data
Endometrium	Possible	Insufficient data
Cervix	Possible	Strongly consistent, limited data
Prostate	Possible for vegetables, inconsistent for fruit	Moderately consistent, especially raw and salad type for vegetables
Kidney	Possible for vegetables, limited evidence for fruit	Not included in the review
Bladder	Probable	Moderately consistent, limited data

(From Riboli and Norat 2003)

Overall, although the evidence was not conclusive for a protective effect, in many cases there was a trend towards increased fruit and vegetable consumption being associated with reduced cancer risk at many sites.

A more recent review of the evidence, in the form of a meta-analysis, was conducted by Riboli and Norat in 2003. By the time of this review, more cohort studies had been published, and there was therefore less reliance on evidence from case-control studies. Findings from the review suggested that the evidence for a protective effect of fruit and vegetable consumption on cancer risk was by then weaker than it had appeared at the time of the WCRF and COMA reviews. A summary of the study's findings is shown in Table 5. As can be seen, in the cohort studies, which are generally thought to provide more robust evidence than the case-control approach, the relationship is generally not significant with the exception of fruit with lung and bladder cancers.

Table 5: Summary analysis of the meta-analyses on fruit and vegetables and the risk of some cancers in case control and cohort studies

	Vegetables		**Fruit**	
	Case Control	Cohort	Case Control	Cohort
Mouth & pharynx	NS	?	↓	?
Larynx	NS	?	↓	?
Oesophagus	↓	?	↓	?
Breast	↓	NS	NS	NS
Lung	↓	NS	↓	↓
Bladder	NS	NS	↓	↓
Stomach	↓	NS	↓	NS
Colorectum	↓	NS	↓	NS

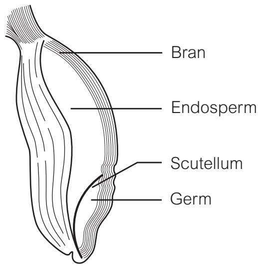
↓ significant protective effect; NS non significant protective effect; ? inconclusive

Source: Am.J.Clin.Nutr. (2003; **78** (3): 559S-569S), American Society for Nutrition.

A second major analysis of the global data, by WCRF/AICR, is to be published in November 2007.

The *European Prospective Investigation into Nutrition and Cancer* (EPIC), a cohort study, has been collating data on dietary patterns and cancer incidence in 9 European countries since 1992, some of which relates to fruit and vegetable intake. Some recent findings for fruits and vegetables include a non-significant decreased risk between fruit and vegetable intake and risk of prostate cancer (Key *et al.* 2004 b), between fruit and vegetable intake and risk of breast cancer (van Gils *et al.* 2005) and between vegetable intake and risk of lung cancer (Miller *et al.* 2004). One large study within EPIC has shown that plasma ascorbic acid (vitamin C) concentrations are inversely related to cancer mortality (*i.e.* higher the level, lower the risk) in men but not in women (Khaw *et al.* 2001).

2.3.2.2 Wholegrains and cancer risk



According to the 1997 WCRF/AICR review (World Cancer Research Fund 1997), diets rich in wholegrain cereals possibly decrease the risk of stomach cancer (based on evidence from six case control studies), but insufficient evidence to support an inverse association with colon cancer was found. A more recent US prospective study (McCullough *et al.* 2001) supports a modest role for plant foods (including wholegrain cereals) in reducing the risk of fatal stomach cancer. Levi *et al.* (2000) have reported protective effects of wholegrains on risk for cancers of the oral cavity, pharynx, oesophagus and the larynx.

In the *Iowa Women's Health Study*, Jacobs *et al.* (1999) reported a reduction in cancer risk for all cancers studied, among participants who had a high consumption of wholegrains. Similar findings were reported among consumers of wholegrain bread in the in the *Norwegian County Study* (Jacobs *et al.* 2001), although the food frequency questionnaire used in the study contained little information on fruit or vegetable intake, which may have confounded the association. A review by Smith *et al.* (2003) provides evidence of an inverse association between wholegrain intake and several cancers. The authors of the review note that whilst the support for a dose-response relationship between wholegrain intake and cancer is only modest, confounding with other dietary and lifestyle factors does not appear to explain the relationship between wholegrains and cancer risk.

2.3.2.3 Conclusions for cancer

Overall, there is some epidemiological evidence that consumption of fruit and vegetables is inversely related to cancer risk, but it now appears that the benefits of fruits and vegetables may have been overstated in relation to some cancers. However, these findings do not rule out a benefit of specific plant foods; one possible explanation for the lack of association with cancer risk is that the category ‘fruit and vegetables’ is too broad to capture any effects exerted by specific nutrients or bioactive compounds found only in subclasses of fruits and/or vegetables.

2.4 The antioxidant hypothesis

There are several plausible reasons why there may be an association between fruit and vegetable consumption and reduced risk of chronic disease, apart from a possible influence on the relationship of other associated factors, such as tobacco use and physical inactivity among those with low fruit and vegetable intake (known as confounding) (Lampe 1999).

A popular explanation among scientists, and more recently in the media, has been that food components with antioxidant properties (including vitamins C and E, selenium and ß-carotene) present in these foods may prevent some of the processes involved in the development of cancer (*e.g.* by protecting DNA from oxidative damage) and in the development of cardiovascular disease (*e.g.* by inhibiting oxidative damage to LDL-cholesterol).

Normal oxidative metabolism in the human body produces large quantities of potentially damaging toxins (‘free radicals’ or ‘pro-oxidants’) that can cause damage to cells by disrupting their normal repair mechanisms. This damage is known as oxidative stress. The delicate balance between pro- and antioxidants in the cell determines the degree of oxidative stress to the cell, and the balance between pro- and antioxidants has been implicated in the development of many chronic diseases, including heart disease, diabetes, cancer and the ageing process (Jackson 2003).

2.4.1 Antioxidant nutrients and CVD

The first hint of an inverse association between antioxidant nutrient intake and CVD risk arose from research indicating that inhabitants of European countries with high rates of heart disease had lower blood plasma levels of ß-carotene, vitamin E and, to a lesser extent, vitamin C (see Khaw *et al.* 2001). These epidemiological data were supported by data from case-control and cohort studies (see Stanner *et al.* 2003) and similar observations were reported for cancer (Department of Health 1998; Loira *et al.* 2000; Khaw *et al.* 2001).

It was assumed that antioxidant nutrients were responsible for the association and this hypothesis was tested in a series of intervention trials using antioxidant supplements. It came as a considerable surprise to researchers to find that not only did these supplements not result in reduction of CVD and cancer risk, but in some cases taking supplements actually increased risk (Stephens *et al.* 1996; Stanner *et al.* 2003; Virtamo *et al.* 2003).

In conclusion, despite evidence from observational studies suggesting an association between low intakes or plasma levels of antioxidant nutrients and incidence of CVD, single antioxidants or mixtures of antioxidants given in supplement form have not been found to be effective in preventing CVD.

It remains unclear which components of fruits and vegetables are responsible for their apparent protective effect and the scope of work in this area is now broadening to include other plant components with anticipated health promoting effects, such as flavonoids (see Section 3).

2.4.2 Antioxidant nutrients and cancer

The findings of intervention trials, which have the potential to identify causative relationships, have been summarised by Stanner *et al.* (2003).

Beta-carotene supplements seem to offer no protection against cancer and among smokers may actually increase risk (Virtamo *et al.* 2003). There is no published evidence from randomised controlled trials to support a role for high-dose vitamin C in cancer prevention, perhaps because dietary supply has been adequate in the subjects studied. There is also little evidence for a beneficial effect of vitamin E supplementation. However, there is some evidence to suggest that an adequate supply of selenium is important for cancer prevention, based on an association between increased risk of cancer at several sites in subjects with low baseline plasma selenium status in a selenium supplementation trial (see Rayman 2005).

2.4.3 Conclusions on antioxidant nutrients

Despite considerable research effort it remains unclear as to whether antioxidants in fruits and vegetables are responsible for the apparent protective effect of these foods against CVD and some cancers.

It is quite plausible that mechanisms other than those involving antioxidants may be responsible for some of the protective effects observed, and evidence is accumulating to support other mechanisms.

The following are commonly proposed mechanisms by which plant components may protect against chronic disease:

- Detoxification of cancer-causing agents (by the activation of phase I and II detoxification enzyme systems)
- Causing cancer cells to die (apoptosis/suppression of mitosis)
- Influencing cell-to-cell communication (effects on cell signalling cascades)
- Modification of the hormonal profile (*e.g.* steroid hormone levels)
- Modification of the lipid profile

- Protection against DNA damage that results in abnormal gene expression (See Mathers 2006)
- Increased repair of DNA damage (See Mathers 2006)
- Stimulation of the immune system
- Anti-inflammatory effects
- Reducing serum cholesterol
- Antimicrobial activity.

Much of the current focus of research is on the effects of plant bioactives on cell-signalling cascades. For example, recent findings from research into the pathogenesis of chronic neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, demonstrate a role for specific flavonoids in interacting selectively within signalling cascades that regulate neuronal survival following exposure to oxidative stress (Spencer, *in press*). This is potentially significant, as we now know that numerous dietary flavonoids can cross the blood brain barrier and thus have the potential to act within the central nervous system. Accumulating evidence now suggests that flavonoids might exert effects on neuronal cells independently of classical antioxidant capacity through selective actions at different points within a number of cell signalling cascades.

3. Plant Bioactives

3.1 What are plant bioactives and where are they found?

Bioactive plant food constituents ("plant bioactives") have been defined in the EuroFIR Project as inherent non-nutritive constituents in food plants with anticipated health promoting/beneficial effects, and/or toxic effects, when ingested (Gry *et al.* EuroFIR 6th SMB Meeting, January 2007, Rome; *Gry et al.* 2007). Plant bioactives are commonly found in plant foods such as fruits, vegetables and grains and, although not classified as essential nutrients in the traditional sense (as is the case for protein, fat, vitamins and minerals), there is increasing evidence that, as dietary constituents, at least some of them may have a role in promoting health.

Thousands of plant bioactives have been identified (BNF 2003), many of which are involved in plants' metabolic processes, and in the interaction of the plant with its surrounding environment. The specific functions of plant bioactives are diverse, but they may include:

- attracting pollinating insects and other seed dispersing insects
- protecting plants from being attacked by insects or being eaten by herbivores
- protecting plants from microbial infection
- protecting plants from UV light.

Plant bioactives are found in a vast range of foods consumed as part of the human diet. They may be consumed via the leaves, stems, roots, tubers, buds, fruits, seeds and flowers of whole plants, or consumed in plant-derived foods and drinks such as chocolate, tea, coffee and fermented foods such as wine and bread (BNF 2003).

In plants, bioactives tend to be present in groups. It is very rare for the edible part of a plant to contain just one type of plant bioactive, but some bioactives are found in only a very limited range of plant species (BNF 2003), or in only one or few families (*e.g.* glucosinolates in cruciferous vegetables).

3.2 What are the major classes of plant bioactives?

Plant bioactives with anticipated health benefits can be classified into different major groups:

- flavonoids and other phenolic compounds
- carotenoids
- plant sterols
- glucosinolates found in brassicas (*e.g.* sprouts, broccoli, cabbage)
- other sulphur containing compounds.

Within most of the groups of bioactives exists a number of sub-categories. For example, anthocyanins, isoflavones, flavanones, flavones, flavan-3-ols and flavonols are sub-classes of flavonoids. The following sections explore three of the major classes of bioactives and review some of the evidence for the proposed health effects of plant bioactives.

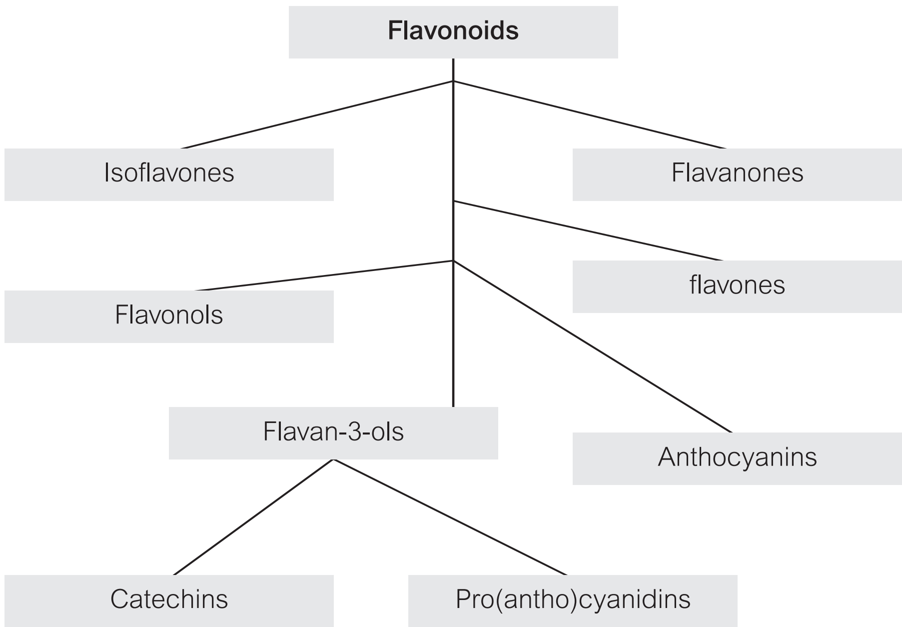
3.2.1 The flavonoids and other phenolic compounds

3.2.1.1 Flavonoids

Flavonoids are a large group of ‘phenolic’ bioactives that are found in a range of plant derived foods, mainly in the skin of fruit such as grapes, and in the epidermis of leaves such as tea leaves (BNF 2003).

As Figure 3 shows, the flavonoids category encompasses a range of bioactives including: flavan-3-ols (sometimes called flavanols), anthocyanins, flavonols, flavones, isoflavones and flavanones. Flavonoids have a polyphenolic structure (see Figure 4).

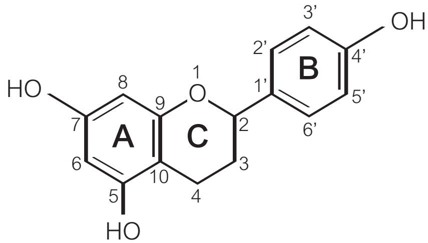
Figure 3: Categories of flavonoids



Flavonoids are the most numerous of the phenolics and are involved in diverse processes in the plant, such as UV protection, pigmentation, stimulation of nitrogen fixing nodules and disease resistance (Pierpoint 2000).

The basic structure for most of the flavonoids is shown in Figure 4. The flavonoid skeleton can have numerous components attached to it, for example hydroxyl groups, or sugars that cause the flavonoid to form a 'glycoside'.

Figure 4: General features of a flavonoid skeleton



Source: Adapted from BNF 2003

The majority of dietary flavonoids are absorbed in some form from the intestine after consumption from food and beverages, and can pass through the gut wall into the blood stream. In order for the absorption of flavonoids from the small intestine to occur, the sugars (glycosides) that are attached to the flavonoid skeleton must first be removed. This process is controlled by the action of enzymes manufactured in the small intestine (*e.g.* mammalian ß-glucosidase), resulting in the release of the flavonoid skeleton (the aglycone) from its sugar.

Scientists have found that it is the type of sugar attached to the flavonoid skeleton that determines the site and the extent of absorption of glycosylated flavonoids (Donovan *et al.* 2006). The location at which the sugar is attached to the flavonoid skeleton affects the mechanism by which glycosylated flavonoids are absorbed (Donovan *et al.* 2006).

Once the sugars have been removed from the flavonoid skeleton for absorption, flavonoids are further metabolised in the gut and subsequently in the liver and kidneys to produce a vast number of modified flavonoids, known as flavonoid **secondary metabolites**. This process involves further conjugation of the flavonoid – for example glucuronate, sulphate or methyl groups may be joined to the flavonoid. In the intestine this process is controlled by enzymes produced by the gut bacteria, and so the gut bacteria play an important role in the metabolism of plant bioactives.

Flavonoid secondary metabolites can be detected in blood and urine following the consumption of flavonoid-containing foods and beverages, but only very small quantities of non-conjugated flavonoids in their original form are to be found in blood and urine (Kroon *et al.* 2004). It is therefore clear that flavonoid secondary metabolites enter the circulation, and mounting evidence suggests that it is these secondary metabolites, rather than the native flavonoids found in food, that exert biological effects in the body.

Flavonols are the most widespread of the flavonoids and are found throughout the plant kingdom. Quercetin, kaempferol and myricetin are all examples of flavonols. Flavonols are found in onions, kale, broccoli, apples, cherries and berries, tea and red wine. These compounds have potential anti-mutagenic, anti-carcinogenic and anti-hypertensive effects.

Isoflavones are found almost exclusively in leguminous plants such as soybean. Genistein and daidzein found in soya, and the coumestan 'coumestrol', are three of the isoflavones that are recognized to have an effect on mammalian health. Due to their structural similarities with the mammalian hormone *oestradiol*, isoflavones have an affinity for the oestrogen receptor ß, which means that these compounds can exert weak pro- and anti-oestrogenic effects. Potentially these effects could have either positive or negative repercussions on the health of mammals. For example, consumption of genistein, daidzein and coumestrol by animals grazing on leguminous plants has been found to adversely affect their reproduction. For these reasons, genistein and daidzein are also known as phytoestrogens (**lignans**, found in grains and linseed for example, also have weak oestrogenic properties).

Flavan-3-ols (flavanols) are a subclass of flavonoids and are found in tea, apples, apricots and cherries, for example. The flavan-3-ols category includes **catechins** and the larger **proanthocyanidins**, which are formed from catechin units. Red wines contain flavan-3-ols that originate from the seeds of black grapes (BNF 2003) and dark chocolate is a rich source of flavan-3-ols derived from roasted cocoa seeds (Gu *et al.* 2004). Green tea contains high levels of flavan-3-ols in the form of catechins *e.g.* epicatechin, epigallocatechin and epigallocatechin gallate (Hollman *et al.* 1997).

Flavones (*e.g.* apigenin and luteolin) are structurally very similar to flavonols but, unlike the flavonols, they are not widely distributed throughout the plant kingdom. Flavones are found in parsley, thyme and celery, for example.

Anthocyanidins are principally found in plant foods as their glycosides (*i.e.* attached to sugar molecules), **anthocyanins**. Anthocyanins are widely dispersed throughout the plant kingdom, giving characteristic red, blue and purple colours to flowers and berry fruits such as blackcurrants, sweet cherries and blueberries. Other foods and drinks containing anthocyanins include wine, raisins and berry juices.

Flavanones are dietary components that are particularly abundant in citrus fruits. Hesperetin, for example, is commonly found in citrus peel and naringenin imparts intensely bitter flavours to grapefruit peel (BNF 2003).

3.2.1.2 Other phenolics compounds

In addition to the flavonoids, a number of other phenolic bioactives are found in foods. These include simple **phenolic acids** (*e.g.* gallic acid), the **hydroxycinnamates** (*e.g.* caffeic acid from coffee and ferulic acid from cereal brans), and the **stilbenes** (*e.g.* resveratrol found in red wine). Vanillin, capsaicins (such as capsaicin in chilli peppers) and zingerone in ginger are also examples of phenolics.

Phenolic acids are also known as hydroxybenzoates. The most important phenolic acid is gallic acid, which serves as the basis for many of the **tannins** that are used in the leather-making (tanning) industry. Plant-derived tannins play an important role in giving foods such as tea and red wine their distinctive flavours – tannins bind to salivary proteins in the mouth producing a characteristic mild astringent taste (BNF 2003). Tannins are also able to bind to some proteins in the gut and this process can have a negative impact on the absorption of non-haem iron from our diets. Whilst it appears that mammals that regularly consume tannin-rich plant material (*e.g.* rabbits) adapt somewhat to remove tannins from their digestive systems (BNF 2003), it may be prudent for people who are at risk of iron-deficiency to avoid drinking tea with meals.

Salicylic acid is also a phenolic acid. The manufactured compound *acetyl salicylic acid* (aspirin) has well-defined health effects, protecting against CVD (Dalen 2006) and possibly some intestinal cancers (Hoensch & Kirch 2005). It is possible that food salicylates may also demonstrate such effects, although levels found in foods are generally low and therefore may not have any biological significance (Paterson *et al.* 2006).

3.2.2 Carotenoids

Carotenoids belong to the group of bioactives known as the terpenoids, one of the most diverse classes of natural products, comprising over 30,000 different compounds including flavours, fragrances, insect attractants and antibiotics.

Carotenoids are structurally diverse compounds that are classified as tetraterpenes. In plants, carotenoids work alongside chlorophyll in photosynthesis, extending the range of light that can be absorbed by the photosynthetic pigments, and giving plant foods that contain carotenoids their distinctive red or orange colours (*e.g.* lycopene in red tomatoes, lutein in yellow peppers and α- and ß-carotene in orange carrots).

Important dietary sources of carotenoids in Europe are carrots, tomatoes, peas, spinach, citrus fruit and carrots (O'Neill *et al.* 2001). Average total carotenoid intakes in European adults vary from 9.5mg per day in Spain, to 14.4mg/d in the UK and 16.1mg/d in France (O'Neill *et al.* 2001).

3.2.3 Plant Sterols

Plant sterols are also terpenoids and some have been shown to reduce plasma cholesterol levels when incorporated into foods such as spreads, in the form of plant sterol and stanol esters. Plant sterols and stanols have a similar structure to dietary cholesterol but are not absorbed by the human gastrointestinal tract, and so have the ability to inhibit the absorption of cholesterol from the diet and facilitate its elimination from the body. Evidence from clinical trials shows that plant sterols and stanols can have a beneficial effect on blood lipid profiles by significantly reducing total and LDL-cholesterol.

In a review of the use of sterol- and stanol-enriched margarines, Law (2000) concluded that the consumption of around 2g/day of plant sterol in margarine is associated with a substantial (~10%) reduction in LDL-cholesterol. Although stanols and sterols are found naturally in a variety of foods, such as nuts, seeds, grains and avocados, high quantities of these foods would need to be eaten regularly in order to achieve the intakes of sterols required to have a beneficial effect on blood cholesterol levels. 'Functional' products such as mini-drinks containing a 'whole serving' of plant sterols, or 2-3 servings a day of products such as spreads and cream cheeses with added plant sterols, are therefore an effective way of ensuring sufficient intake to lower cholesterol.

3.2.4 Glucosinolates

Cruciferous vegetables, such as sprouts, broccoli, cabbage and watercress contain the plant bioactives **glucosinolates**. The importance of sulphur-containing bioactives as flavour compounds is well known. When cruciferous vegetables are chopped, crushed or chewed, the mechanical action breaks down the walls of the plant food cells, causing the glucosinolates contained within the cells to come into contact with the enzyme 'myrosinase', which is contained within a different area of the plant cell (Mithen 2006). The action of myrosinase on glucosinolates causes their conversion to **isothiocyanates** and/or other products, which provide the characteristic hot and pungent flavours of many of our cruciferous salad crops (*e.g.* watercress, rocket, salad cress) and condiments (*e.g.* mustard, horseradish, wasabi) (Mithen 2006).

The amount of isothiocyanates to which we are exposed when we eat cruciferous vegetables is dependent upon five factors:

- the amount of glucosinolates present in the plant food itself (which is dependent upon plant genetic and environmental factors)
- the structure of the glucosinolates
- the extent to which glucosinolates are converted to isothiocyanates within the plant, by the plant enzyme myrosinase (which is dependent on preparation and cooking methods)
- the extent to which any remaining glucosinolates are converted to isothiocyanates within the intestines (by gut bacteria)
- the extent to which the isothiocyanates are absorbed and metabolised by the individual (which is dependent upon human genetic factors).

Cooking methods have an important influence on the extent to which glucosinolates are converted to isothiocyanates by the plant enzyme myrosinase, because prolonged exposure to heat can destroy myrosinase. Thus when cruciferous vegetables are chopped up and eaten raw, a relatively large proportion of the glucosinolates may be converted to isothiocyanates before reaching the gut, owing to the chopping action causing the myrosinase to come into contact with the glucosinolates, and the action of the enzyme on the glucosinolates. More prolonged cooking destroys the myrosinase enzyme, meaning that the glucosinolates (which are not destroyed by cooking) are not converted to isothiocyanates until they reach the intestines. The best option for preparing cruciferous vegetables may be to steam or microwave them for two minutes, as this does not destroy the myrosinase and also causes the breakdown of another enzyme (known as ESP) that can sometimes hamper the conversion of glucosinolates to isothiocyanates when vegetables are eaten completely raw (Mithen 2006).

At least part of the potential health promoting effect of isothiocyanates on the body seems to be due to their metabolism in the colon by gut bacteria. Once the biologically active isothiocyanates have been released into the small intestine, they are available for interactions with the colonic epithelial cells and for uptake into the circulation via the colonic mucosa.

A paradox exists in that whilst studies are suggesting possible health effects of isothiocyanates, it is the glucosinolates that give sprouts and other brassicas their characteristic taste, which is not universally popular. Any attempts to increase the glucosinolate content for health purposes, via plant breeding, needs to take this paradox into account.

3.2.5 Other sulphur containing compounds

Onions and garlic, which are Allium species, have been an important part of the human diet for thousands of years. Sulphur containing compounds (S-alkyl cysteine sulphoxides) are found in all varieties, along with flavonols such as quercetin. It has been suggested that the sulphur containing compounds **allicin** and **ajoene** are responsible for claimed health benefits of garlic but there is limited evidence to substantiate this view.

3.3 Why are bioactives of interest to human health?

Despite substantial evidence from epidemiological studies that a diet rich in plant foods (particularly fruits and vegetables) is protective against chronic disease (see Section 2.3), a causal link between low plasma levels of antioxidant nutrients and disease incidence, or between antioxidant supplementation and disease prevention, is yet to be established (Stanner *et al.* 2003). This failure to demonstrate an effect for antioxidant nutrients has led researchers to look at non-nutrient plant bioactives with antioxidant properties, such as flavonoids, in the search for a protective mechanism in chronic disease.

The following sections review some of the evidence for the proposed effects on health of the different classes of plant bioactives, and the evidence for the purported health benefits of some foods and beverages known to be rich sources of these bioactives.

3.3.1 What is the evidence that flavonoids are protective?

3.3.1.1 Cardiovascular Disease

Several studies over the last decade have investigated associations between flavonoids and coronary heart disease. For example an inverse association between flavonoid intake and coronary mortality was observed in an ecological study of middle aged men from 16 different cohorts of the *Seven Countries Study* (Hertog *et al.* 1995). In a review of prospective epidemiological studies, Hollman and Katan (1999) found that intake of flavonols and flavones was inversely associated with subsequent coronary heart disease in most but not all studies (Hollman & Katan, 1999).

The findings for the association between flavonoid intake and coronary heart disease have not always been consistent, however, and, as with cancer (see Section 2.3.2.1), the stronger findings are often from cohort studies. For example, in a prospective study of over 38,000 women followed-up for a mean period of 6.9 years, no significant trend was identified across quintiles of flavonoid intake, categorised using a food frequency questionnaire that assesses total flavonoids and components such as flavonols and flavones (Sesso *et al.* 2003). A cross sectional analysis using data from the *SU.VI.MAX* study in France suggested that women with a high intake of flavonoids were at lower risk, and men at higher risk, of cardiovascular disease (Mennen *et al.* 2004), which is difficult to interpret.

Other studies have focused on flavonoid rich foods or on specific classes of flavonoids. Sesso *et al.* reported non-significant inverse associations for cardiovascular disease risk and tea consumption (4 or more cups per day), and cardiovascular disease risk and consumption of broccoli and apples (Sesso *et al.* 2003), although some scientists have criticised the way in which the authors interpreted this study (Donovan 2004). In contrast, others have reported a stronger association (Arts *et al.* 2001).

There has also been interest in flavonols present in cocoa, red wine and tea in the context of a possible beneficial effect on blood vessel lining (endothelial function) and hence cardiovascular health. To date there have been some clinical trials (in healthy volunteers and in subjects at risk of CVD) that have been suggestive of a beneficial effect on endothelial function but they have typically been short term.

A case control study in Italy looked at the impact of intake of specific flavonoids on risk of heart attack and reported a reduced risk with high intakes of anthocyanins, which are abundant in berries (Tavani *et al.* 2006). Another study in Greece reported an inverse association between CHD risk and flavan-3-ols, which are largely found in tea and wine; they reported that an increase in intake of about 24mg/day corresponded to a 24% reduction in risk (Lagiou *et al.* 2004).

Whilst these findings are promising, longer term studies and mechanistic investigations are needed to establish whether these short term effects on endothelial function translate into long term cardiovascular health benefits. There is also ongoing work focussing on other potential mechanisms via which flavonoids might exert a beneficial effect on cardiovascular health, including potential anti-inflammatory effects (Selmi *et al.* 2006) and effects on cell signalling cascades.

3.3.1.2 Cancer

Several studies have investigated associations with flavonoids but, as for CVD, findings have been inconsistent. In an analysis of data from the *Seven Countries Study* after 25 years of follow-up, no association was found between the intake of five major flavonoids and mortality from lung cancer, colorectal cancer or stomach cancer (Hertog *et al.* 1995). Similarly, no association between flavonoid intake and lung cancer was found in the *Zutphen Elderly Study* (Arts *et al.* 2001).

In countries where habitual intakes of isoflavones are high compared to Western countries (*e.g.* Japan and China), observational studies have suggested that the lower rates of some cancers (particularly breast and prostate) in these countries may be related to increased isoflavone intakes (Bingham *et al.* 1998). However, a report by the UK Committee on Toxicity found that, although plausible mechanisms for a protective effect had been tested *in vitro*, there was not enough evidence to confirm a causal association *in vivo*, as data from intervention trials in humans are inconsistent (COT 2003).

3.3.1.3 Menopausal Symptoms

There has been speculation that the oestrogenic properties of isoflavones may help to alleviate menopausal symptoms such as hot flushes, night sweats, irritability, joint pains and irregular or heavy periods, as well as poor concentration. Much of the research conducted relates to hot flushes. These have been found to be less common in Japan and China compared to Western countries and it has been suggested that consumption of isoflavones might be a contributing factor. However, it is unclear whether a lifetime exposure to higher levels of isoflavones is necessary in order to see an effect (Cassidy 2003). A recent critical review of the health effects of soya phytoestrogens in post-menopausal women concluded that there was limited evidence that soya protein isolates or soya foods were effective in reducing menopausal symptoms, but soyabean isoflavone extracts may be effective in reducing hot flushes (Cassidy *et al.* 2006). Studies on the effects of soya phytoestrogens on bone and heart health in post-menopausal women remain inconclusive at present.

3.3.2 What is the evidence that carotenoids are protective

Most epidemiological research on the protective effects of carotenoids has focused on a number of individual antioxidants studied in relative isolation (see Section 2.4.1 and 2.4.2).

Whilst observational studies have shown an inverse association between dietary intake of carotenoids and risk of cancers such as lung and stomach cancer, results from intervention studies using supplements have failed to back up these findings. It is likely therefore that there is a complex interaction between the many components found in plant foods, and the rest of the diet, which may have an integrated effect on cancer risk.

3.3.3 What is the evidence that glucosinolates are protective?

The most promising evidence for a protective effect of plant bioactives on human health comes from studies of glucosinolates. Epidemiological evidence associating high intakes of brassicas with reduced risk of cancer at a number of sites is relatively strong (van Poppel *et al.* 1999), although human intervention studies to corroborate these findings are hampered by the absence of good biomarkers of cancer. The evidence is most consistent for lung, stomach, colon and rectal cancers. Feeding extracts of cruciferous and *Allium* vegetables to rats and mice treated with an agent to cause colon cancer has provided further evidence of the protective effect of glucosinolates (Lynn *et al.* 2006).

3.3.4 Conclusions on the protective effects of plant bioactives

Whilst there is a growing body of research on the putative protective effects of plant bioactives on human health, the role of specific foods, and indeed specific bioactives, remains to be established. Much of the evidence for the protective effects of plant bioactives is drawn from *in vitro* or animal experiments, which are often performed with doses much higher than those to which we are normally exposed via the diet. Before the health benefits of these substances can be established with certainty, more work is needed to establish the effect *in vivo* in humans and to demonstrate the effects of these compounds when consumed in normal amounts as part of normal diets.

One major difficulty with such studies is accurately assessing actual intakes of plant bioactives, given the sheer numbers of bioactives that have been identified, the variations in concentration from plant to plant, the major differences in bioavailability of these bioactives, and the limitations of current food composition databases. The following section summarises work being undertaken by the EuroFIR project to provide comprehensive, high quality information on the levels of bioactives contained in plant foods commonly consumed in Europe.

4. EuroFIR and the EuroFIR BASIS Database

Knowledge of the distribution, intake, absorption and metabolism of plant bioactives is essential in order to fully evaluate their potential benefit to human health. There is currently, however, a lack of information available to researchers on the content and concentrations of plant bioactives present in foods commonly consumed in Europe.

The emerging evidence for a protective effect of plant bioactives against diseases has also generated great interest from the food and nutritional supplement industry regarding the development of bioactive-rich products (Scalbert 2005). The need for data on the content and concentrations of plant bioactives in foods consumed in Europe is therefore becoming increasingly important to researchers and the food industry alike.

In order to address this deficit of information, work is currently being undertaken by researchers working within **EuroFIR** (short for European Food Information Resource), an EU funded ‘Network of Excellence’, to build an internet-based database of critically assessed data on the levels and biological effects of bioactives with anticipated health benefits contained in plant-based foods commonly consumed in Europe. The database, called **‘EuroFIR BASIS’ BioActive Substances in Food Information System**, will help researchers assess exposure of European populations to plant bioactives and will provide an overview of the extent to which the possible health effects of these plant bioactives have been studied, and how thorough this evaluation has been.

4.1 The EuroFIR project

EuroFIR is a five-year project funded by the European Commission through the EU Sixth Framework Programme on Food Quality and Safety. The main objective of *EuroFIR* is to develop an integrated, comprehensive and validated databank or *food information resource* that will provide a single, authoritative source of food composition data in Europe for nutrients and newly emerging bioactive compounds with potential health benefits. This objective provides an essential foundation for all food and health research in Europe and underpins nutrition labelling and potential health claims.

EuroFIR is a partnership involving 48 universities, research institutes and small-to-medium sized enterprises (SMEs) from 25 European countries. The main aims of the project include:

- strengthening the scientific and technological excellence of food databank systems and tools in Europe
- training a new generation of European scientists in the development, management and application of food databank systems
- communicating with all user and stakeholder groups to develop food databank systems for the benefit of European food and nutrition research
- disseminating and exploiting new scientific and technological knowledge to create a sustainable resource
- identifying and providing new information on missing data for nutrients and bioactive compounds for all food groups, including traditional and ethnic foods.

For further information on the EuroFIR project, see the EuroFIR website: www.eurofir.net

4.1.1 The Bioactives work package

EuroFIR's work to build an internet-based database of critically assessed data on the levels and biological effects of bioactives contained in edible plants and plant-based foods commonly consumed in Europe is being managed by the EuroFIR Workpackage Leader Dr Jørn Gry (National Food Institute, Technical University of Denmark) and co-led by Dr Mairead Kiely (University College Cork, Ireland) and by Dr Paul Kroon (Institute of Food Research, UK). There are 17 other partners involved in the Bioactives work package, from 13 different countries.

Currently, in Europe, databases of individual bioactive compounds exist but there is no integrated system that encompasses several bioactive compound classes. Three previous EU 5th Framework-funded projects, NETTOX, BASIS and VENUS, developed databases of information on bioactive compounds. The overall objective of the EuroFIR Bioactives work package is to develop an integrated database system that enables users to retrieve information on the levels and biological effects of a comprehensive range of the bioactives found in food plants and plant-based foods commonly consumed in Europe. The database system is designed to conform to the standards and specifications outlined for the overall EuroFIR food composition databank. Furthermore, the plant list published from the NETTOX project (Gry *et al.* 1997) has already been updated to include additional plant parts consumed in Europe. The list, now known as the **EuroFIR NETTOX plant list**, has recently been published by EuroFIR (see www.eurofir.net).

The scientific work of the Bioactives work package is focused around the activities of three task groups with specific objectives:

i. Compositional Evaluators Group: to establish and populate a web-based database on critically assessed composition data on bioactive constituents in plant-based foods (The composition part of the EuroFIR BASIS Database).

ii. Biological Effects Group: to extend the web-based database system to allow the inclusion of critically assessed data on the biological effects of bioactive constituents (The biological effects part of the EuroFIR BASIS Database).

iii. Plant List Group: to update the plant and plant part lists to include major European food plants in 2007 and to produce new lists covering edible plants such as exotic fruits and processed plant-derived foods (The plants part of the EuroFIR BASIS Database).

In its entirety, the EuroFIR BASIS Database will provide comprehensive coverage of both prioritised plant foods in Europe and bioactive compound classes, thereby facilitating calculations of exposure to bioactive compounds such as flavonols, phenolic acids, phytosterols, carotenoids, isoflavones and lignans. Of the 328 edible plants in the database, the Plant List Group has to date focussed on 108 of those most commonly consumed in Europe. The Compositional Evaluators Group have searched the literature and collected over 600 papers on bioactive composition, of these over 150 references have been critically evaluated producing more than 6500 individual data points. The Biological Effects Group has critically evaluated over two hundred papers providing *in vitro* and *in vivo* data on the beneficial biological effects of bioactive compounds, and has incorporated them into the database. Together these papers provide in excess of 550 biological effects inputs. Such a compilation of critically assessed biological effects data in the database will help users to establish to what extent reliable studies for a specific compound are available.

5. Conclusions

Epidemiological evidence shows the benefits of consuming a diet that is rich in plant foods. It remains unclear which components of plant foods are responsible for this apparent protective effect on health but, recently, the scope of work in this area has broadened beyond micronutrients to include plant bioactive compounds with anticipated beneficial effects. A vast array of bioactive compounds that may have protective effects against chronic disease has been identified, but the role of specific compounds remains to be established. To date, much of the evidence in this field comes from *in vitro* or animal studies, the findings of which require corroboration in human intervention studies using normal dietary amounts of plant bioactives given as part of normal diets.

High quality, comprehensive food composition data for plant foods commonly consumed in Europe is vital to underpin epidemiological research on plant foods and health. EuroFIR aims to develop an integrated, comprehensive and validated databank, or *food information resource*, that will provide a single, authoritative source of food composition data in Europe for nutrients and bioactive compounds with potential health benefits. Findings from the EuroFIR project and an update on developments can be found on the EuroFIR website www.eurofir.net.

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