Hydroxycinnamic acids as natural antioxidants

by Leonardo Setti, Craig Faulds and Silvia Giuliani

Functional foods containing a high amount of antioxidant compounds are of great interest for the food and pharmaceutical industries like foods for the health care. In fact the lifelong production of free radicals through the human biochemical functions have to be balanced by a series of biomolecules named as antioxidants. Recently, a group of dietary phenolic compounds with antioxidant activity have received increasing interest, these being derivatives of cinnamic acid; e.g. caffeic acid, chlorogenic acid, and ferulic acid.

The human diet contains an array of different compounds that possess antioxidant activities based on their structural properties and their ability to scavenge reactive oxygen species (ROS). ROS affect the development and the existence of an organism in the presence of O_2, even under physiological conditions. These radicals are responsible for oxidative damage to biological macromolecules such as DNA, carbohydrates and proteins. Components in our diet, antioxidants, help decrease the risk of oxidative damage caused by oxygen radicals. The most prominent representatives of dietary antioxidants are ascorbate (vitamin C), tocopherols (vitamin E), carotenoids and flavonoids. Apart from vitamin C, each of these antioxidants consists of a number of structurally different compounds that might occur in the human diet through synergistic effects. These effects are difficult to assess.

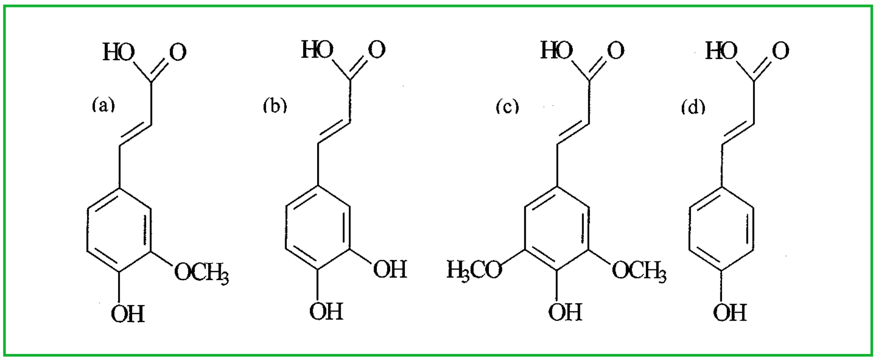

Figure 1 - Structure of hydroxycinnamic acids. (a) Ferulic, (b) p-coumaric, (c) sinapic and (d) caffeic acids are all based on the phenylpropionic acid skeleton of cinnamic acid

The term vitamin E is a generic description for all tocols and tocotrienol derivatives that exhibit the biological activity of α–tocopherol. These compounds are highly lipophilic, and operative in membranes or lipoproteins. Their most important antioxidant function appears to be the inhibition of lipid peroxidation, scavenging lipid peroxyl radicals yielding lipid hydroperoxides and a tocopheroxyl radical. The richest sources of vitamin E in the diet are vegetable oils (soyabean, maize, cottonseed, and sunflower seed), and products made from these oils such as margarine and mayonnaise. Furthermore, wheat germ, nuts, and some green leafy vegetables contribute considerable amounts to the vitamin E supply. Like vitamin E, carotenoids belong to the group of lipophilic antioxidants present in lipoproteins such as LDL and HDL. It has been shown that they are consumed when isolated LDL is exposed to the process of lipid peroxidation. A variety of structurally different carotenoids are present in fruits and vegetables. Some of the major sources are carrots (α-carotene and β-carotene), tomatoes (lycopene), citrus fruit (β-cryptoxanthin), spinach (lutein), and maize (zeaxanthin). Flavonoids are a large group of polyphenolic antioxidants that occur in several fruits, vegetables, and beverages such as tea, wine and beer mainly as *o*-glycosides. They are efficient antioxidants capable of scavenging radical species (peroxyl radicals, hydroxyl radical, $O_2^{\cdot}$) forming a phenoxy radical. The term flavonoids summarizes a number of structurally different subgroups including flavanols (catechin and epicatechin), flavonols (quercetin, myricetin and kaempherol), flavanones (naringenin and taxifolin), flavones (apigenin and hesperidin), isoflavones (genestein) and anthocyanidins (cyanidin and malvidin). In addition to the flavonoids, a number of other phenolic compounds of potential interest occur in foods. Olive oil contains a number of phenolic substances, such as tyrosol, which is object of important studies for its contribute to the antioxidant content of diets rich in olive oil [1].

Recently, another group of dietary phenolic compounds with antioxidant activity have received increasing interest, these being derivatives of cinnamic acid; e.g. caffeic acid, chlorogenic

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Table 1 - Examples of functional food innovation

Food	*Functional benefit*
Live fermented milks and yoghurts with probiotic cultures	Improve digestive functioning and gut health
Margarine, yoghurt, cheese spreads	Plant stereols and stanols reduce cholesterol and lower the risk of heart disease
Eggs rich in omega-3 essential fatty acids	3 – 4 Eggs a week would provide the same amount of n-3 fatty acids as recommended to help reduce the risk of heart disease
Breakfast cereals	Added folic acid may help reduce the risk of babies being born with spina bifida
Bread, Muesli style bars	Added isoflavones may help reduce the risk of breast and prostate cancers, heart disease and osteoporosis
Potatoes rich in selenium	Potential protective role in preventing carcinogenesis and other chronic diseases

acid, and ferulic acid. A number of other dietary constituents are involved in the antioxidant defence system either by direct action as antioxidants or by effects related to the induction of detoxifying enzymes. Enzymes such as glutathione peroxidase and superoxide dismutase, which require a dietary supply of Se, and of Cu and Zn, respectively, contribute to the overall oxidative defence mechanism.

Enhancement of the dietary intake of these antioxidants may be beneficial, however the absorption and transport processes of these compounds are quite complex. Several factors influencing antioxidants bioavailability from food, such as co-ingestion of fat or fibre, cooking or food processing, should be investigated.

To the extent that certain foods confer additional, and sometimes unexpected, health benefits, they are, according to today's terminology, recognised as "functional foods", and are of increasing scientific interest. "Functional foods" are "health-enhancing" foods containing biologically active ingredients or food components. The functional activity is based on a demonstrable positive interaction between a food component and some physiological, cellular and biochemical functions. A beneficial action is based on the prevention or the reduction of the risk of diseases. Functional foods beneficially affect one or more target functions in the body when they are consumed as part of a normal food pattern (Table 1). A strategy in food technology is represented by the implementation of specific compounds into the food in order to confer particular functional characteristics. A typical example is potatoes enriched with selenium, which are produced through specific selenium-based foliar fertilisers in order to increase the up-take of this compounds by the plant in growing conditions [2]. However, the most useful strategy is to supplement the human diet by using equilibrated supplements.

Biological activities of hydroxycinnamic acids

The class of compounds known as the hydroxycinnamic acids are found both covalently attached to the plant cell wall polysaccharides and as soluble forms in the cytoplasm. The free form ocurs very rarely in plants. Hydroxycinnamic acids and their derivatives all stem from cinnamic acid, and are present as four basic molecules: *p*-coumaric, caffeic, ferulic and sinapic acids (Figure 1). The diversity of hydroxycinnamic acids and their resultant nutraceutical properties derives from the nature of the bonds and that of the molecule(s) involved. In addition, the presence of a double bond in the lateral chain of the hydroxycinnamic acids leads to the possible existence of two isomeric forms: *cis* (Z) and *trans* (E). Native compounds are mainly of the *trans* form, although interconversion of the two forms *in situ* may lead to certain physiological responses. They are synthesised from either *L*-phenylalanine or *L*-tyrosine as apart of the lignin precursor pathway. Almost all the biosynthetic enzymes from phenylalanine and tyrosine have been identified in the plant chloroplast, but the cytosolic fraction may also have a split pathway for flavonoids biosynthesis [3]. In general, ferulic acid is uniformly distributed across the plant cell wall, and is particularly abundant in epidermis, xylem vessels, bundle sheaths and sclerenchyma. Dietary hydroxycinnamic acids consist predominantly of ferulic and caffeic acid. Total dietary intake has not been determined, but estimates put up to 2 g of chlorogenic acid (a caffeic acid-quinate conjugate) per day for heavy coffee drinkers [4]. Cereals are another good source of dietary hydroxycinnamates, especially ferulic acid (Table 2).

Hydroxycinnamic acids exhibit antioxidant properties, as measured *in vitro*. All have higher potential than the standard vitamin E analogue used in these assays [5]. Ferulic and caffeic acid are the most potent of phenolic compounds in exhibiting a cytoprotective effect against LDL oxidation in cultured endothelial cells [6]. Wheat bran has been reported as having the ability to scavenge free radicals, especially nitrites, a reactive ion which upon reaction with secondary amines and amides under acidic conditions (such as in the stomach) form *N*-nitroso compounds, many of which are carcinogenic. Ferulic acid has been implicated as being a strong contributor to this activity, as after alkali treatment, scavenging activity is transferred from the insoluble bran to the soluble fraction [7]. The lignin fraction, which can scavenge nitrite on its own, did not have as great affect as ferulic acid. Hydroxycinnamic acids have *in vitro* activities in the induction of leukocyte apoptosis, suppression of acute inflammation in response to viral infection possibly through inhibition of viral multiplication by binding to the membrane glycoprotein of such viruses as HIV and influenza [8]. They have been shown to arrest the growth of a broad range of human tumour cells and induce the natural immune response in chemo- and radiotherapy during treatment for carcinomas. Wheat bran has been associated with reduced risk of colorectal cancers, and the protective effect is greatest for the insoluble fibres and the degree of fermentation [9]. As ferulic acid was only partially released at a slow rate in a human gut model, another role could be in the scavenging of potential carcinogens in the colon or by changing the gut microbiota composition and gut physiology, or both [10]. Cereal derived oils, such as rice bran or corn bran oil, contain phytosterols, many of which are conjugated with ferulic acid. These phytosterols have been reported to block gastric secretions stimulated by histamine and insulin, and thus reduce stress-induced gastric ulceration, possibly by increasing brain norepinephrine content, suppressing the central nervous system and acting in a

Table 2 - Main dietary sources of hydroxycinnamic acids and derivatives (adapted from Clifford, 2000)*

Cinnamates	*Main dietary source*	*Levels (mg/kg)*
Ferulic acid	Coffee; citrus juice; cereal brans; sugar beet fibre	700-3,000
Caffeic acid	Coffee; apples; cider; blueberries	12,500-37,500
Sinapic acid	Broccoli; kale; citrus juice; rice	>100
p-Coumaric acid	Spinach; cereal brans; sugar beet fibre	
Caffeoylquinic acids (chlorogenic)	Coffee; blueberries; apples; cider	25,000-75,000
Feruloylquinic acids	Coffee	
p-Coumaroylquinic acids	Sweet cherries	
Rosmarinic acid	Herbs	~10
Tartaric conjugates	Spinach; lettuce; grapes; wine	50-350
Malic conjugates	Lettuce; spinach; legumes	>30
Cell wall conjugates	Spinach; cereal brans; sugar beet fibre	5-30

* M.N Clifford,. *Journal of the Science of Food and Agriculture,* 2000, **80,**1033

similar manner to a minor tranquillizer [11]. These phytosterols also significantly reduce plasma total cholesterol and cholesterol absorption, while having no effect on cholesterol biosynthesis [12]. Ferulic acid is an ingredient of many traditional Chinese herbal medicines, which have been clinically shown to be better than control drugs in reducing platelet aggregation and thrombus formation, and that sodium ferulate may be as effective as aspirin in the treatment of thromboembolic disease [13].
Ferulic acid has been shown to be beneficial to sperm viability and motility in both fertile and infertile individuals, and may be an active ingredient in potential cures for asthenozoospermic infertility [14]. Inhibition of uterus contraction was observed with ferulic acid together with tetramethylpyrazine (TMP), with ferulic acid acting on the oxytocin-receptor system in the uterus [15]. This may be due to the inhibition of prostaglandin biosynthesis.
Although the health promoting benefits of hydroxycinnamic acids have been publicized on the basis of their antioxidant status, it must be remembered that the gastrointestinal tract is primarily an anaerobic environment, and measured biological effects cannot be due to these compounds, once ingested, acting as antioxidants. Consideration must also be taken of the potential biological effects of metabolites of hydroxycinnamates formed through microbial transformation.

Feruloyl esterases

Hydroxycinnamates require de-esterification to have an effect, either in a biological system or in an industrial process. This can be achieved either through alkali-extraction or by the action of specific esterases. While alkali hydrolysis is the most effective mean to release high quantities of phenolic acids, there is the problem of neutralisation, especially on a large scale. A ‘greener’ approach is the use of specific feruloyl esterases. These enzymes have been mostly studied from microbial sources, but they are also present in plants [16]. At the time of writing, there have been over 30 feruloyl esterases identified and most have been purified, 10 protein and nucleotide sequences and no structure. These esterases, which appear to be serine esterases, contain a putative conserved catalytic triad comprising serine, histidine and a carboxylic acid group, whose function is to increase the nucleophilicity of the serine residue. Some feruloyl esterases are capable of releasing ferulic acid from insoluble residues by themselves, but most require the presence of a main-chain polysaccharide-acting glycosidase, such as xylanase or arabinanase. For those esterases acting in combination, a synergistic interaction occurs where the rate of activity of the main-chain degrading enzyme is also increased. However, there has been a reported case of antisynergy involving feruloyl esterases, where the action of one type of enzyme inhibits or restricts the action of a second, and this is important in industrial processing of plant cell wall material, if, by adding enzymes at the wrong stage, the overall process becomes less efficient.
Most, but not all of the feruloyl esterases detected to date are inducible and are secreted into the culture medium. This makes their utilisation in industrial processes much easier. With an increasing number of sequences available, the use of recombinant feruloyl esterases will become more prevalent, either as monocomponent systems or as enriched enzymic preparations. Recently, the first heterologously expressed feruloyl esterase was obtained using the yeast *Pichia pastoris*, at yields of up to 300 mg per litre [17]. Putative health-promoting bacterial species have recently been shown to have feruloyl esterase activity and are involved in the release of hydroxycinnamic acids in the human colon [18]. Given that the gut flora has a major role in human nutrition and health, some of the beneficial effects of phenolic acids may be ascribed to the microflora involved in metabolism.

Industrial applications of hydroxycinnamic acids and feruloyl esterases

Flavour - Microbial transformations of ferulic acid by bacteria and fungi have concentrated on the identification of metabolic pathways and routes of degradation. It is interesting to discover novel systems for the formation of phenolic-based flavourings and aromas. Microorganisms able to produce vanillin, the world’s principle flavouring compound, in excess of 6 g per litre from ferulic acid have now been isolated [19].
Medicinal and pharmaceutical - As described in the section above on biological properties, the hydroxycinnamates have many potential applications in the medical and health industries. Certain acids, such as ferulate and caffeate, are essential ingredients of Oriental herbal medicines. Processes exist by which polysaccharide gels are formed by peroxidase-mediated oxidation of the ferulic acid groups to form a cross-linked matrix [20]. This gelation process has a wide potential in products ranging from wound dressings to food ingredients, including

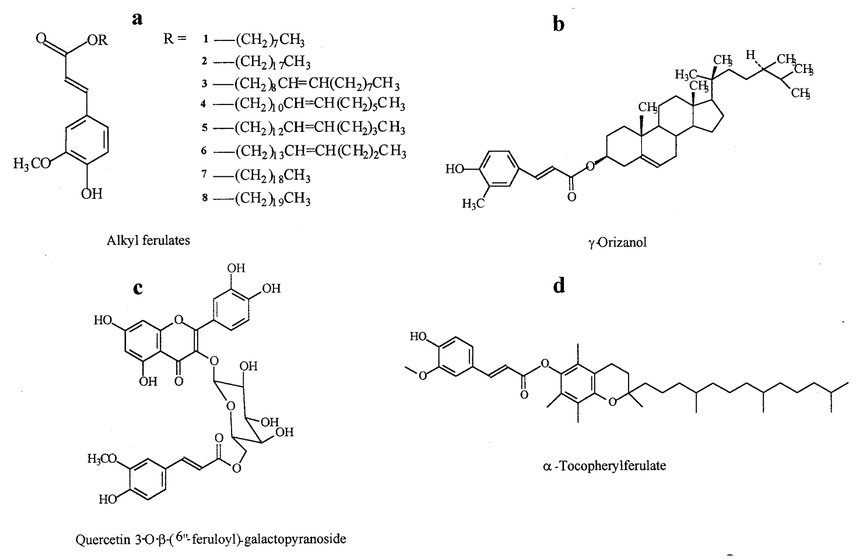

Figure 2 - Structure of ferulyc acid esters having an industrial interest: a) Ferulate alkyl esters; b) Ferulate phytosterol esters; c) Flavonol glucoside ferulate esters; d) α-Tocopheryl ferulate

improvements in bread dough technologies. Ferulic acid is being marketed as a commercial supplement for body builders in the form of phytosterols, where it is believed to stimulate growth and hormonal secretion in human, although there is no published research to substantiate this claim. In cattle, there were claims for weight or muscle gain and anabolic or growth-promoting properties. The use of ferulic acid in the formulation of an ophthalmological solution for the prevention of cataracts in humans by UV damage has been proposed, making use of the photoprotective properties of ferulate [21]. A similar approach for topical sunscreen application has been discussed above under biological properties.

Animal feeds - Agro-industrial wastes, such as wheat bran (baking and milling), barley spent grain (brewing), sugar beet pulp (sugar production), olive pulp and waste water (olive oil manufacturing) and fruit pulp (purée and juice production), are all rich sources of hydroxycinnamic acids, whose normal fate is to be either used as animal feed or stored until disposal. Some material can be too rich in phenolics so that digestibility problems occur. Ruminants and poultry are fed on cereal-based diets and performance can be affected due to the inability to breakdown polysaccharides. Enzymic supplementation or processing can reduce some of these problems such as reduce viscosity of the arabinoxylans. Addition of esterases to such enzymatic preparations can help such digestion, or by extracting esterase-released phenolic acids prior to being used as animal feeds, these high added-value phenolics products have the potential to be used in processes as described above. Further understanding of animal and human intestinal microbiology, physiology and biochemistry could allow stimulation of health promoting, feruloyl esterase-producing species, or the design of prebiotic and probiotic (functional) foods.

Food preservatives - Certain phenolic acids are indigestible and even toxic to many soil and other microorganisms which come into contact with living, decaying or processed plant material, or may bind with various compounds to reduce hydrolytic activity [22]. Phenolics have long been used as food preservatives to inhibit microbial growth [23]. Stead has shown that hydroxycinnamic acids and their soluble esters inhibited growth of wine spoilage bacteria and yeast, but only when used at high levels (500 mg/litre and above). Lower levels stimulated growth. A 1 mM concentration also inhibited growth of some ruminal bacteria [24].

Synthesis of cinnamic acid derivatives by feruloyl esterase as a possible future development

Recently, Giuliani *et al.* [25] have shown the possible use of feruloyl esterase from *Aspergillus niger* in the synthesis of pentyl ferulate in water-in-oil microemulsion. The specificity as well as the selectivity of this enzyme for the phenolic group represents an important characteristic for the synthesis of new active products in food, health and cosmetic field. One factor limiting the use of esterases-catalysed reactions on an industrial scale is the cost of the enzyme, which could be cheaper by reusing the enzyme for several reactions through various techniques of immobilization.

Ferulic acid esters of saturated or unsaturated higher alcohols

Due to a high degree of conjugated unsaturation, ferulic acid is a strong UV absorber. By absorbing UV radiation, phenoxy radicals are formed leading to *cis-trans* isomerization. This in turn inhibits other free radical reactions, aiding in the antioxidative potential of the hydroxycinnamates. At high concentrations, ferulic acid may protect light-sensitive compounds against oxidative damage by attenuating the amount of UV radiation impinging on the soluble material, such as in seeds, leaves, etc. Ferulic acid can impart photoprotection to several layers of cells below the level immediately exposed to UV radiation. Ferulic and caffeic acids penetrated the main barrier (the stratum corneum) against endogenous substance penetration of skin samples, and thus protect liposomes from damage by UV peroxidation and nitric oxide [26]. This property is the basis behind the use of cinnamic acid derivatives as the most widely used UV-absorbing chemicals typically incorporated (2-15%) into topical sunscreen formulations. Four and seventeen derivatives are already approved in the United States and in Europe, respectively. Most common cinnamic acids and short-chain esters are water soluble, limiting their usefulness as waterproof sunscreens. To overcome this problem, cinnamic acid derivatives have been designed with long-chain hydrocarbons (Figure 2a). Several lipases were tested to determine their activity toward feruloyl compounds using two main approaches: direct esterification of ferulic acid with aliphatic alcohol or trans-esterification of ferulic acid with triolein [27]. Transesterification reactions could lead to new sunscreen products from natural sources, with a higher acceptability in the cosmetic field as well as interesting application in vegetable oils. The use of feruloyl esterase can improve the efficiency in the production of alkyl hydroxycinnamates, especially for the construction of ferulic acid esters with unsaturated long-chain alcoholic residues (C18-24) possessing a double bond at unusual position.

Many alkyl hydroxycinnamates have been reported to have antimicrobial activity against yeast, *Aspergillus niger* and *Penicillium* sp. as well as against *Pythium* sp. where the efficiency is comparable to that of the commercial fungicide iprobenfos (Kitazin P) [28].

Ferulate phytosterol esters

γ–Oryzanol is an important fraction, along with tocotrienols and other unsaponifiable, of rice bran oil, and has been demonstrated to exhibit hypocholesterolemic and antiatherogenic properties as well as having antioxidant functionality (Figure 2b) [29]. Generally, organic solvents such as *n*-hexane, isopropanol and ethyl acetate are used for extracting lipid and γ-oryzanol from rice bran. Ten components of γ-oryzanol were identified and three of these, cyclo artenyl ferulate, 24-methylenecycloartenyl ferulate, and campesteryl ferulate, were the major components. The separation and the purification of the individual components is then a challenge, however they become difficult because of the high costs and the high fractionation time. We have some evidence that the synthesis of γ-oryzanol is possible when using feruloyl esterase through selective esterification reactions of ferulic acid and sterols in organic solvent.

Ferulic acid derivatives with glucosides and amino acids

A so-called "tertiary function" exists in food expected to affect the disease process by modulating the immune, endocrine, nerve, circulatory and digestive systems. Functional foods can be designed and constructed based on a variety of synthetic substances or natural products with tertiary functions. The amount of bioactive compounds naturally occurring in food is generally too small, so that it is necessary to separate them.

Flavonol glucoside ferulate esters (Figure 2c) exhibit potent inhibitory effects on superoxide production by leukocytes, which play an important role in aging, carcinogenesis, and certain neurological disorders of human beings in addition to the host-defensive mechanism of inflammation [30]. These compounds are extracted from some natural sources such as the aerial parts of *Persicaria lapathifolia* [31] or fresh broccoli florets [32].

Kayahara *et al.* [33] described the synthesis of twenty-two kinds of ferulic acid-amino acid derivatives, which exhibited both tyrosinase-inhibitory and superoxide-scavenging activities, and also demonstrated strong UV absorption potential. These compounds may then serve in functional skin care products for whitening, aging prevention and photoprotective functions. Ferulic acid derivatives were obtained both introducing a *C*-terminal protected amino acid into the carboxyl group by the dicyclohexyl carbodiimide/1-hydroxybenzotriazole and introducing a free amino acid into the carboxyl group by the *N,N'*-disuccinimidyl carbonate.

DL-α-tocopheryl ferulate

Scavenging as well as inhibitors of the active oxygen radicals are studied as whitening agents to reduce or cure pigmented molecules or prevent new melanogenesis by UV irradiation. α-Tocopheryl ferulate is the product of the esterification reaction between α-tocopherol and ferulic acid (Figure 2d). It efficiently inhibits melanin formation compared with other whitening agents such as arbutin, kojic acid, magnesium *L*-ascorbyl 2-phosphate (VC-PMG) and tranexamic acid [34].

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