Hydroxycinnamic acids as natural antioxidants

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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 O₂, 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.

The term vitamin E is a generic description for all tocots 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, O₂⁻) forming a phenoxy radical. The term flavonoids summarizes a number of structurally different subgroups including flavanols (catechin and epicatechin), flavonols (quercetin, myricetin and kaempferol), flavones (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...
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 uptake 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 occurs 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 an effect 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 phytoestrogens, many of which are conjugated with ferulic acid. These phytoestrogens 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
similar manner to a minor tranquilizer [11]. These phytosterols also significantly reduce plasma total cholesterol and choles-
terol absorption, while having no effect on cholesterol biosyn-
thesis [12]. Ferulic acid is an ingredient of many traditional Chi-
nese 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 infer-
ty [14]. Inhibition of uterus contraction was observed with fer-
ulic 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, act-
ing as antioxidants. Consideration must also be taken of the
potential biological effects of metabolites of hydroxycinna-
mates 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 ac-
tion of specific esterases. While alkali hydrolysis is the most ef-
fecitive 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 se-
quences and no structure. These esterases, which appear to be
serine esterases, contain a putative conserved catalytic tri-
ad 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 fer-
ulic acid from insoluble residues by themselves, but most re-
quire the presence of a main-chain polysaccharide-acting gly-
cosidase, such as xylanase or arabinanase. For those esteras-
es 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, ei-
ther as monocomponent systems or as enriched enzymic
preparations. Recently, the first heterologously expressed feru-
loyl 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 hydroxycinna-
matic 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 mi-
croflora 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 discov-
er novel systems for the formation of phenolic-based flavour-
ings 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 indus-
tries. Certain acids, such as ferulate and caffeate, are essential
ingredients of Oriental herbal medicines. Processes exist by
which polysaccharides gels are formed by peroxidase-mediated
oxidation of the ferulic acid groups to form a cross-linked ma-
trix [20]. This gelation process has a wide potential in products
ranging from wound dressings to food ingredients, including

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

<table>
<thead>
<tr>
<th>Cinnamates</th>
<th>Main dietary source</th>
<th>Levels (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid</td>
<td>Coffee; citrus juice; cereal brans; sugar beet fibre</td>
<td>700-3,000</td>
</tr>
<tr>
<td>3-Caffeoylquinic acid</td>
<td>Coffee; blueberries; apples; cider</td>
<td>12,500-37,500</td>
</tr>
<tr>
<td>3,4-Dimethoxysinapic acid</td>
<td>Broccoli; kale; citrus juice; rice</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3-Methoxysinapic acid</td>
<td>Lettuce; spinach</td>
<td>25,000-75,000</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>Lettuce; legumes</td>
<td>12,500-37,500</td>
</tr>
<tr>
<td>4-Methoxysinapic acid</td>
<td>Spinach; cereal brans; sugar beet fibre</td>
<td>5-30</td>
</tr>
<tr>
<td>H.-Coumaroylquinic acid</td>
<td>Coffee</td>
<td>5-30</td>
</tr>
<tr>
<td>3-Caffeoylquinic acid</td>
<td>Coffee</td>
<td>75,000-200,000</td>
</tr>
<tr>
<td>3,4-Dimethoxysinapic acid</td>
<td>Lettuce; broccoli; grapes; wine</td>
<td>50-350</td>
</tr>
<tr>
<td>3-Methoxysinapic acid</td>
<td>Lettuce; spinach</td>
<td>&gt;90</td>
</tr>
<tr>
<td>4-Methoxysinapic acid</td>
<td>Spinach; cereal brans; sugar beet fibre</td>
<td>5-30</td>
</tr>
</tbody>
</table>

* M.N. Clifford, *Journal of the Science of Food and Agriculture*, 2000, 80, 1033
improvements in bread dough technologies. Ferulic acid is being marketed as a commercial supplement for body builders in the form of phytoestrols, 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 (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 water-soluble 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 transesterification 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.

Figure 2 - Structure of feruloy acid esters having an industrial interest: a) Ferulate alkyl esters; b) Ferulate phytosterol esters; c) Flavonol glucoside ferulate esters; d) α-Tocopheryl ferulate
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 (Kitaizin P) [28].

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 molecules 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. 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.

References