Review

Reactions of Plant Phenolics with Food Proteins and Enzymes under Special Consideration of Covalent Bonds

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Secondary plant metabolites are important native food components, which are becoming more and more interesting due to their physiological effects on human beings. One of the largest groups of these compounds is represented by plant phenols. This review summarizes the structure, classification and distribution of the phenolic compounds in plant foods, their chemistry and signification with regard to food processing and -storage as well as their physiological effects. This work focuses mainly on such reactions of the phenolic substances with proteins and enzymes that lead to covalent bonds. The derivatives formed have been characterized in terms of changes in their physicochemical and structural properties. The effect on the proteolytic in vitro digestion has been also illustrated. Further aspects reported include the influence on enzyme activity and -kinetic parameters. The different aspects of the nutritional-physiological consequences of such reactions in food and body, especially considering their significance to food science and technology are discussed.

Keywords: phenolic acids, flavonoids, food protein/enzyme derivatization, physicochemical characterisation, structure, in-vitro proteolytic degradation, biological activity

1. Phenols as natural substances of plant foods

1.1 Introduction

Phenolic compounds as food components represent, with more than 6000 identified substances, the largest group of secondary metabolites in plant foods. They are characterized by a large range of structures and functions, but generally possessing an aromatic ring bearing one or more hydroxy substituents (Robards et al., 1999). They are usually found in plants bound to sugars as glycosides (Hollman & Arts, 2000). Plant phenols are present in many foodstuffs as colour imparting ingredients (e.g. anthocyanins in red wines, red cabbage, eggplant, strawberry, blueberry, raspberry, plum, cherry etc.) (Robards et al., 1999; Clifford, 2000a; Hollman & Arts, 2000). Interest in these compounds is related to their dual role as substrates for oxidative browning reactions and as antioxidants, underlining their impact on organoleptic and nutritional qualities of fruits and vegetables, their role in plant growth and metabolism and, more recently, their demonstrated physiological activity in humans (Friedman, 1996; Robards et al., 1999; Hollman & Arts, 2000). In both roles, the key process is oxidation (Robards et al., 1999). The oxidative browning is probably restricted to foods and is invariably detrimental although, in some instances (e.g. tea, cacao, dates, currants, sultanas), it is intentional and essential to the character of the product (Friedman, 1996; Robards et al., 1999). The action of phenolics as antioxidants is viewed as beneficial in both foods (e.g. galls as supplementary food ingredient) and the body where phenolics are oxidized in preference to other food constituents or cellular components and tissues (Vinson et al., 1995; Hertog et al., 1997; Meyer et al., 1998; Robards et al., 1999; Yoshida et al., 1999; Miura et al., 2001).

1.2 Structure, classification, distribution of phenols in plant foods, intake and their physiological effects

With regard to their structure, the major groups of phenolic compounds are distinguished by a number of constitutive carbon atoms in conjunction with the basic phenolic skeleton. The food relevant groupings include firstly, the hydroxybenzoic acid derivatives (HBAs) with a general structure C₆-C₃ (e.g. salicylic and gallic acids) as illustrated in Fig. 1. Variations in their basic structure being hydroxylations and methoxylation of the aromatic ring. As a general rule HBAs are present as conjugates (Tomás-Barberán & Clifford, 2000a), although they can also be detected as free acids in some fruits (e.g. gallic acid in persimmons), after being released during fruit and vegetable maturation or more importantly during food processing. The main source of gallic acid is tea, especially green tea, where it occurs as (-)-epigallocatechin-3-gallate (EGCG) and (-)-epigallocatechin. The consumption of EGCG was estimated for Japan to be as high as 1 g per day (Authors-group, 1999). A detailed account on the specific occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods has also been reported (Herrmann, 1989; Authors-group, 1999; Clifford, 1999; Robards et al., 1999; Clifford, 2000b; Tomás-Barberán & Clifford, 2000a).

The second group is that of those phenolic compounds having the general formula C₆-C₃ (hydroxycinnamic acid derivatives, Fig. 2), representing a series of trans-phenyl-3-propenoic acids differing in their ring substitution. These compounds are widely distributed as conjugates in plant material including many foods and beverages (Herrmann, 1989; Clifford, 1999; Clifford, 2000b). The commonest (Fig. 2) is caffeic acid (3,4-dihydroxycinnamic acid); ferulic (3-methoxy, 4-hydroxy), sinapic (3,5-dimethoxy, 4-hydroxy) and p-coumaric (4-hydroxy) also are wide-
spread (Clifford, 2000b). The best-known conjugate is 5-caf-feoylquinic acid (IUPAC numbering, Fig. 2), commonly referred to as chlorogenic acid (CGA) (Clifford, 2000b). Accurate data on population-wide intakes of phenolic compounds are not available. Recent studies show, that the human dietary intake of chlorogenic acids and other cinnamates ranges from 25 mg up to 1 g/day depending upon the dietary constitution (Clifford, 1999). The content of chlorogenic acid is relatively high with 6–10% in green coffee beans, and a cup of coffee may contain up to 675 mg (Clifford, 1999).

The third and the largest group are represented by the flavonoids, with more than 4000 compounds characterized (Ader et al., 2000). They are also the most widespread and diverse, built upon a C₆-C₃-C₆ flavone skeleton (Fig. 3), in which the three-carbon bridge between the phenyl groups is commonly cyclised with oxygen (Robards et al., 1999). Several sub-classes of flavonoid are differentiated on the degree of unsaturation and degree of oxidation of the three-carbon segment (e.g. flavones, isoflavones, flavonols, flavanones, catechin, anthocyanins etc.). Within the various classes, further differentiation is possible, based on the number and nature of substituent groups attached to the rings (Robards et al., 1999). The main dietary sources of flavonoids include fruits, vegetables as well as tea, and detailed accounts of their content have been reviewed recently (Kühnau, 1976; Cook & Samman, 1996; Böhm et al., 1998; Robards et al., 1999; Hollman & Arts, 2000; Miean & Mohamed, 2001). The estimated total intake of flavonoids in the USA was 1 g/person/day (expressed as glycosides) (Kühnau, 1976). This seems to be an overestimation, as recent reviews indicate a daily intake of only 6–60 mg/person/day (Böhm et al., 1998; Hollman & Arts, 2000).

The largest number of flavonoids is represented by the sub-group of flavonols (Fig. 4), usually found in plants bound to sugars as O-glycosides (Robards et al., 1999). The most important representatives of flavonols are kaempferol, quercetin and myricetin. Aglycones of flavonols do not occur in fresh plants but may be present as a result of food processing. Sugars are predominantly bound to the flavonoid nucleus via a β-glycosidic

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**Table 1.** The structure of hydroxybenzoic acid derivatives with a general structure C₆-C₁.

<table>
<thead>
<tr>
<th>Hydroxybenzoic Acid</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Para-hydroxybenzoic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Protocatechatic acid</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

**Table 2.** The structure of hydroxycinnamic acid derivatives with a general formula C₆-C₃.

<table>
<thead>
<tr>
<th>Hydroxycinnamic Acid</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Para-coumaric acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>OH</td>
<td>OCH₃</td>
<td>OCH₃</td>
</tr>
</tbody>
</table>

**Fig. 1.** The structure of hydroxybenzoic acid derivatives with a general structure C₆-C₁.

**Fig. 2.** The structure of hydroxycinnamic acid derivatives with a general formula C₆-C₃.

**Fig. 3.** The structure of the Flavonoids with a C₆-C₃-C₆ flavone skeleton.

**Fig. 4.** The structure of the sub-group of flavonols.
bond. Sugar molecules can bind to various positions in the parent flavonoid, although there is a preference for the C_2-position. 179 different glycosides of quercetin alone have been described in nature (Hollman & Arts, 2000), rutin (quercetin-3-O-rhamnosyl-glucoside) being one of the commonest. A further physiological-interesting sub-group of the flavonoids is that of isoflavones (Fig. 5), representing the hormone-like bisphenolic phytoestrogens of dietary origin (Mazur et al., 1998; Rice-Evans, 1996; Meyer et al., 1998; Robards et al., 1999; Yoshida et al., 1999; Miura et al., 2001; Hodek et al., 2002). Most of these physiological effects are based on either in vitro models, cell/tissue culture studies, animal experiments or epidemiological data. The results thus obtained are generally projected to show if a strong association or correlation of activity in humans is possible or not, since data on basis of human investigations are relatively rare.

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
</tr>
</thead>
<tbody>
<tr>
<td>daidzein</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>genistein</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>glycitein</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>OCH_3</td>
</tr>
<tr>
<td>prunetin</td>
<td>OH</td>
<td>OH</td>
<td>OCH_3</td>
<td>H</td>
</tr>
<tr>
<td>formononetin</td>
<td>OCH_3</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>biochanin A</td>
<td>OCH_3</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

Fig. 5. The structure of the sub-group of isoflavones.

1.3 Chemistry and signification regarding to food processing and -storage

Enzymatic browning is the most known reaction of plant foods. Browning reactions can be in some instances (e.g. tea, cacao, dates, currants, sultanas), intentional and essential to the character (e.g. coloring and taste) of the product (Friedman, 1996; Robards et al., 1999). The action of phenolics as antioxidants is perhaps the most beneficial effect in both foods and the body (Cook & Samman, 1996; Rice-Evans et al., 1996; Meyer et al., 1998; Robards et al., 1999; Yoshida et al., 1999; Miura et al., 2001; Hodek et al., 2002). In order to function as an antioxidant, the phenolic compound must be capable of forming a resonance stabilized phenoxy radical, which in turn can react with other available radicals (e.g. peroxyl radical), consequently deactivating them (Robards et al., 1999; Pedrielli et al., 2001). However, the reactivity of the phenoxy radicals should remain so low, such that the radical formation e.g. by H-subtraction from unsaturated fatty acids is not possible. A further beneficial property of phenolic compounds in food processing and -storage is their antimicrobial ability (Scalbert, 1991; Cook & Samman, 1996).

The presence of polyphenols in foods and beverages (e.g. sorghum, millet, fruit, cocoa, coffee, tea, beer, and wine) also produces a sensation of astringency, which is perceived as a diffuse feeling of extreme dryness and roughness that is not confined to a particular region of the mouth or tongue, thus effecting the taste of foodstuffs (Baxter et al., 1997). Proteins and polyphenolic compounds can combine to form soluble complexes; these can grow to colloidal size, leading to visible haze formation and as a result limiting the shelf life of products the consumer expects to be clear including beer, wine, fruit juices, coffee an tea (Siebert et al., 1996; Baxter et al., 1997; Siebert, 1999). The same phenomenon can contribute to the fouling of process equipment surfaces with deposits that are difficult to remove by in-place cleaning (Siebert et al., 1996). Polyphenol-oxidase-catalyzed oxidation of polyphenol compounds in fruits and vegetables to quinones, which then polymerize to dark melanin pigments of unknown structure causing discoloring of foodstuffs, e.g., formation of brown or black spots from chlorogenic acid in bananas, pears, lettuce, and potatoes and the browning of fruit juices (Friedman, 1996). Enzyme-catalyzed browning reactions of amino acids and proteins with oxidized plant phenols may cause deterioration of food during storage and processing leading to a loss in nutritional quality, which is especially serious in underprivileged countries (Friedman, 1996). Plant phenols may also display many other possible detrimental effects, including inhibition of iron absorp-
tion and irreversible complexation of dietary proteins (Mehansho et al., 1987; Baxter et al., 1997). The loss of nutritional quality is also attributed to the destruction of essential amino acids and a decrease in digestibility and inhibition of proteolytic and glycolytic enzymes (Friedman, 1996). The production of antinutritional and toxic compounds may further reduce the nutritional value and possibly the safety of foods (Friedman, 1996).

With regard to the chemical reactivity of phenolic compounds, it is now generally accepted that two reaction steps are involved (Nicolas et al., 1994). The first reaction step consists of the hydroxylation of monophenols into o-diphenols and the second, the oxidation of the o-diphenols into o-quinones. These two reactions consume oxygen and are catalyzed by the enzymes monophenolase (E.C. 1.14.18.1, cresolase activity) and o-diphenolase (E.C. 1.10.3.1, catecholase activity) (Nicolas et al., 1994). Similarly the laccases (E.C. 1.10.3.2) oxidize o-diphenols as well as p-diphenols forming their corresponding quinones. The quinones resulting as the primary products of enzymatic oxidation represent a species of highly reactive substances (Fig. 6) which normally react further with other quinones to produce dark melanin pigments (Nicolas et al., 1994; Friedman, 1996; Robards et al., 1999). The quinone, being a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such as lysine, methionine, cysteine and tryptophan moieties in a protein chain (see also chapter 1.3) (Pierpoint, 1969a,b; Hurrell & Finot, 1984; Hurrell & Finot, 1984; Matheis & Whitaker, 1984a,b; Machholz & Lewerenz, 1989; Nicolas et al., 1994). Upon further oxidation of this addition product to form its quinone, a second addition may occur, which leads to formation of cross-linked protein polymers (Matheis & Whitaker, 1984a,b; Machholz & Lewerenz, 1989). In most of these reports, theoretical aspects of covalent linkages between proteins and phenolic compounds with corresponding reaction schemes are discussed. Few results showing such reactions of o-quinone with proteins are reported (Pierpoint, 1969a,b; Vithayathil & Murthy, 1972; Hurrell et al., 1982; Hurrell & Finot, 1984; Muralidhara & Prakash, 1995).

Contrary to the above-mentioned non-covalent type of interactions, our main interest was to focus our studies on covalent linkages between plant phenols and proteins/enzymes. Recently, it was possible for the authors of this review to derivatize proteins/enzymes with phenols and related compounds in absence of polyphenoloxidases. The derivatives were characterized, to demonstrate the changes induced in their physicochemical and structural properties and as well as in the biological activities of the enzymes. The food proteins and enzymes tested by us included myoglobin, lysozyme, bovine serum albumin, whey proteins, soy proteins, α-amylase, bromelain, chymotrypsin and trypsin. A series of simple phenolic and related compounds (quinic-, furfural-, caffeic-, chlorogenic-, and gallic acid; rescorcinol, pyrocatechol, hydroquinone representing m-, o-, p-dihydroxyphenols and finally p-quinone) were applied and the results reported in detail (Kroll et al., 2000; Rawel et al., 2000a,b; Kroll et al., 2001; Kroll & Rawel, 2001; Rawel et al., 2001a,b; Rohn et al., 2001; Rawel et al., 2002a,b; Rohn et al., 2002a,b). The studies were further extended to include the reaction of proteins with flavonoids (flavone, apigenin, kaempferol, quercetin and myricetin; C_{6}-C_{3}-C_{6} structure) (Kroll et al., 2002; Rawel et al., 2002a; Rawel et al., 2003). Some of the characteristic changes in proteins and enzymes derivatized with these phenolic compounds are discussed with help of recent and partly unpublished data in the following sections.

2. Reactions of phenolics and related substances with food proteins

Most of the literature sources deal with non-covalent type of interactions between phenolic compounds and proteins. There is only a very limited, especially experimental data on the covalent bonds between these two reactants. Principally, five potential types of interactions of phenolics and proteins can be proposed: hydrogen bonding, π-bonding, and hydrophobic, ionic, and covalent linkages (Hagerman, 1992; Bianco et al., 1997). The main mechanisms of protein polyphenol interaction at moderate pH are thought to be governed by π-bonding (Bianco et al., 1997). Phenols may also be oxidized with ease in an alkaline solution to its corresponding quinone (Hurrell & Finot, 1984). The quinone, being a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such as lysine, methionine, cysteine and tryptophan moieties in a protein chain (see also chapter 1.3) (Pierpoint, 1969a,b; Hurrell & Finot, 1984; Hurrell & Finot, 1984; Matheis & Whitaker, 1984a,b; Machholz & Lewerenz, 1989; Nicolas et al., 1994). Upon further oxidation of this addition product to form its quinone, a second addition may occur, which leads to formation of cross-linked protein polymers (Matheis & Whitaker, 1984a,b; Machholz & Lewerenz, 1989). In most of these reports, theoretical aspects of covalent linkages between proteins and phenolic compounds with corresponding reaction schemes are discussed. Few results showing such reactions of o-quinone with proteins are reported (Pierpoint, 1969a,b; Vithayathil & Murthy, 1972; Hurrell et al., 1982; Hurrell & Finot, 1984; Muralidhara & Prakash, 1995).

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2.1 Reactions at the reactive side chains of proteins: aminosulphhydral groups and tryptophan

The derivatization of the proteins was conducted under relatively mild conditions (pH 5–9, room temperature, different concentrations) as found during the technological food processing. The specific details can be referred to in our publications listed above.

The amount of free amino groups was determined using trinitrobenzenesulphonic acid (TNBS), which binds to the nucleophilic nitrogen of the ε-amino side chains of lysine. The reaction of the
phenolic compounds with the free amino groups of the proteins results in the consequent binding and blocking of this reaction site for TNBS. This leads to a corresponding decrease in the amount of the free amino groups in the myoglobin derivatives of flavonoids as illustrated in Fig. 7. Since the amount of free amino groups was determined in presence of 1% SDS (a well known agent, which destroys non-covalent protein interactions), we can assume, that the derivatization has occurred through covalent binding. The reaction of lysine side chains of myoglobin with simple phenolic and related compounds (quinic-, ferulic-, caffeic-, chlorogenic-, and gallic acid, $m$, $o$, $p$-dihydroxyphenols and $p$-quinone) has already been reported (Kroll et al., 2000; Kroll & Rawel, 2001). The basic requirement for such a reaction is the formation of a corresponding quinone, this condition being fulfilled by caffeic-, chlorogenic-, and gallic acid, $o$- and $p$-dihydroxyphenols. Ferulic acid and $m$-dihydroxyphenol cannot be oxidized to their corresponding quinone derivative, but were found to react with the proteins applied (Rawel et al., 2000b).

One possible explanation can be given by considering the formation of a radical, which in turn can attack a nucleophilic reaction partner. The possible reaction intermediates from ferulic acid and $m$-dihydroxyphenol have been postulated (Kroll & Rawel, 2001; Rawel et al., 2001b). Quinic acid as a simple cyclic compound also reacts with the free amino groups only to a small, but significant level (Rawel et al., 2000b). The mechanisms of this reaction are not yet clear.

From the flavonoids tested, flavone (having no hydroxy substituents) and apigenin (hydroxy substituents on ring A and 4’ of ring B) showed no significant reactivity towards the free amino groups, whereas kaempferol, quercetin and myricetin did undergo a reaction (Fig. 7). This would mean for quercetin and myricetin that primarily the hydroxy substituents on the ring B and C (positions 3, 3’, 4’, 5’) of the flavonoids invoke a strong reaction with lysine side chains in proteins. A possible route for the reaction of kaempferol with proteins can be explained by means of enol-keto tautomerization across the 2–3 bond (Fig. 8). Therefore, the occurrence of the catechol moiety, i.e. the two adjacent (ortho) aromatic hydroxyl groups in quercetin and myricetin (C3’ and C4’, ring B) and the subsequent autoxidation (alkaline pH 9, presence of oxygen during the derivatisation) of these to reactive and redox active $o$-quinones appears to be a prerequisite condition, to generate electrophilic species capable of undergoing such nucleophilic addition.

A further possibility of reaction of oxidized phenolic compounds is at the heterocyclic $N$-atom of tryptophan as already discussed (Matheis & Whitaker, 1984a; Machholz & Lewerenz, 1989). The quenching of the fluorescence intensity as an indicator of tryptophan changes was studied after activation at 295 nm and measurement of the emission between 300–360 nm. The results did also show a decrease of tryptophan content in dependence of the added reactant as documented in Fig. 9 for the applied flavonoids. A similar observation was also made with simple phenolic and related compounds as reported earlier (Kroll et al., 2000; Rawel et al., 2000a,b; Kroll & Rawel, 2001; Rawel et al., 2001a,b). Further, from the flavonoids (Fig. 9), even flavone and apigenin were found to react. Kaempferol, quercetin as

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**Fig. 7.** Changes in the amount of the free amino groups in the myoglobin derivatives of flavonoids (1.07 mmol phenol/g protein). Code: 1=unmodified (control) myoglobin; 2=flavone derivative; 3=apigenin derivative; 4=kaempferol derivative; 5=quercetin derivative; 6=myricetin derivative.

**Fig. 9.** The change of tryptophan content of myoglobin in dependence of the added reactant (1.07 mmol phenol/g protein). Code: 1=unmodified (control) myoglobin; 2=flavone derivative; 3=apigenin derivative; 4=kaempferol derivative; 5=quercetin derivative; 6=myricetin derivative.
well as myricetin were more reactive (Fig. 9). The mechanisms of the interactions of flavone with the tryptophan groups are not yet clear and that of apigenin are likely to be similar to that with ferulic acid over the formation of a radical as mentioned above. Since the tryptophan fluorescence was measured in presence of 8 M urea (destroying of non-covalent protein interactions), it can be assumed that covalent binding has occurred. These trends in the decrease of tryptophan were also confirmed by performing alkali hydrolysis of the derivatives, followed by consequent amino acid analysis (unpublished data).

Besides the above mentioned influence on lysine and tryptophan concentration of proteins, the content of the thiol groups can also be decreased by undergoing similar reactions with oxidized phenolic compounds as postulated and documented (Rawel et al., 2002b; Kroll et al., 2002; Rawel et al., 2002a,b). As recently reported, o-quinone trapping method by glutathione appeared to be an accurate method for quantification of o-quinone metabolites (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001; Awad et al., 2002a,b). In these studies, the method proved useful for analysing the nature and biochemical behavior of quercetin o-quinone, and characterization of its p-quinone methide isomers (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001; Awad et al., 2002a,b). According to these authors, for quercetin o-quinone and p-quinone methide III, glutathione adducts can be formed only at C2’, C5’, and C6’, whereas for p-quinone methides I and II, adduct formation at C2’, C5’, C6’, C6, and C8 is possible (Boersma et al., 2000; Awad et al., 2001).

Translating this information to our case and consequently in accordance to the observed decrease in the nucleophilic protein side chains, similar covalent adduct formation at the proposed C atoms for the flavonoids is possible.

This estimation of the reactivity of the protein side chains is incomplete, since it has been theoretically and experimentally observed that further amino acid residues like proline, methionine, histidine and tyrosine also have the potential to react with the phenolic compounds (Matheis & Whitaker, 1984a; Machholz & Lewerenz, 1989; Rawel et al., 2000b).

On the other hand, the methods of characterization and identification of plant phenolics show that their spectrophotometric absorption properties can be applied to estimate the amount of the phenolic compound covalently bound to the proteins. But consideration should be given to the fact that the extinction coefficient and absorption maximum wavelength can change in an adducted species. Hydroxycinnamic acids (like chlorogenic acid) and flavonoids (like quercetin) exhibit absorption bands at 305–330 nm and 320–385 nm respectively (Robards et al., 1999).

This absorption characteristics were successfully applied to estimate the amount of the individual chlorogenic acid and quercetin covalently bound (Rawel et al., 2002b; Rawel et al., 2003).

2.2 Changes in solubility induced by derivatization

Solubility is a prime requisite for a functional ingredient protein and is critically necessary for products such as beverages. For example, whey proteins are nitrogenous fraction remaining soluble in the supernatant at pH 4.6 after precipitation of casein. Thus, the loss in solubility at this pH is commonly used to assess the extent of protein denaturation (Kinsella & Whitehead, 1989).

Therefore, it was possible to estimate the degree of denaturation in whey proteins caused by their reaction with the simple phenolic and related compounds (Rawel et al., 2001a) and recently with flavonoids (Rawel et al., 2003). The complexation of myoglobin with oxidized flavonoids is demonstrated by the pH dependent solubility profile, whereby the unmodified control myoglobin had a good solubility in buffer at the investigated pH range (Fig. 10). The reaction of myoglobin with the derivatizing flavonoids is accompanied by a corresponding blocking of the hydrophilic amino acid side chains, causing a parallel change of its solubility (Fig. 10). Flavone caused hardly any influence on the solubility behavior, where as apigenin, kaempferol, quercetin as well as myricetin produced significant changes (Fig. 10). These changes in solubility reflect the respective changes in the amount of charged groups as documented for free available amino groups in Fig. 7, having a good correlation with the structure of the flavonoids. Certainly, with pH near 4, phenols are also known to complex with proline-containing proteins by non-covalent associations that lead to reduced solubility (Siebert, 1999). Myoglobin has about 2.5 mol% proline (GenBank Protein sequences, Accession No. 2506462 for myoglobin from horse). Under these conditions a complexation of phenols with proline would be expected, whereby phenolic compounds are known to be much stronger in binding to proline-rich proteins than other configurations (Siebert & Lynn, 1998; Siebert, 1999). From the nutritional and technological-functional points of view, such decrease in solubility may adversely affect not only protein extraction from raw materials with a relative high content of phenolic compounds (such as sorghum, sunflower seeds, rapeseed), but also the digestion properties as shown below.

2.2.3 Changes in hydrophilic/hydrophobic character of the protein derivatives

The surface activity of proteins is important in a number of functional applications, e.g. foams and emulsions. The number and disposition of apolar groups contribute to the effective hydrophobicity of proteins by promoting a greater affinity of the protein e.g. for an oil phase. The covalent attachment of the phenolic compound to proteins causes on one hand the blocking of the hydrophilic groups like amino and thiol groups as discussed above, but on the other hand there is also an increase in the amount of apolar groups (benzene ring) and polar groups (hydroxyl and carboxyl groups as in the case of the phenolic acids)
being introduced. Simultaneously, the solubility behaviour indicates denaturation, which means structural and conformational changes. All these changes affect the hydrophilic/hydrophobic character of the derivatized proteins, which can be illustrated by RP-HPLC. This is documented in a rise of the retention times of the main peak e.g. illustrated for myoglobin flavonoid derivatives in Fig. 11. The peak formation may be adversely affected, indicating possible denaturation and molecular interactions (Fig. 11). Contrary to these results, an increase in the hydrophilic character (using RP-HPLC) of the derivatives of whey proteins with phenolic compounds was also observed (Rawel et al., 2001a; Rawel et al., 2003). To summarize, it appears that the effect of the treatment of proteins with phenolic compounds on their hydrophilic/hydrophobic character seems to be governed by different factors, some of which like pH, phenol concentration, protein and/or phenol configuration have been documented (Kroll et al., 2000; Rawel et al., 2000a,b; Kroll et al., 2001; Kroll & Rawel, 2001; Rawel et al., 2001a,b; Rohn et al., 2001; Rawel et al., 2002a,b; Rawel et al., 2003). On the other hand, to optimize the biological and technological utilization of proteins, especially of low-quality proteins in underdeveloped countries, a better understanding is needed of the various interrelated parameters that influence their nutritional value and functional properties. As noted above, the hydrophobic-hydrophilic properties and solubility behavior have also been modified by reactions of proteins with phenolic compounds, which in turn may influence the functional properties (emulsion-, foam properties) of the derivatives.

2.4 Characterization of the protein-phenol-derivatives with electrophoretical methods

As a result of the covalent linkages of the phenolic compounds with the proteins and in accordance with the observed changes in the pH dependent solubility of the derivatives (Fig. 10), there is a change in the net charge of the protein molecules, which is reflected in the corresponding change of the isoelectric point of the derivatives. Exemplary, the changes obtained by the reaction of selected flavonoids with myoglobin is depicted in the Fig. 12. With the increasing number of hydroxyl groups and their positions in the reacting flavonoids and their corresponding higher reactivity as witnessed by the blocking of free amino groups (Fig. 7) and tryptophan (Fig. 9), a shift in the isoelectric points of the myoglobin fractions can be documented (Fig. 12). Generally we can summarize following: With higher degrees of derivatization, lower isoelectrical points for proteins can be observed i.e. the proteins become more acidic in nature. This observation is consistent in all our investigations regarding interactions of proteins with phenolic compounds.

The estimated data obtained for the total number of amino acid residues blocked in proteins (BSA, whey proteins) was generally higher as compared to the amount of phenolic compound bound (chlorogenic acid, quercetin) (Rawel et al., 2002b; Rawel et al., 2003) underlining the fact that some protein groups must be involved in intra- and intermolecular cross-linking. The later can be confirmed by SDS-PAGE analysis as documented in Fig. 13 for myoglobin-flavonoid derivatives. Again flavon and apigenin did not show any change in the electrophoretical behavior, whereas kaempferol, quercetin as well as myricetin induced strong polymerization. These high molecular complexes produced, were not spliced by use of SDS and mercaptoethanol. The latter reagent is generally used to split disulfide bonds during sample preparation. This is a further indication, that the bonds formed between the molecules must be of covalent nature. The general picture emerging from these studies is that the oxidation of fla-
The formation and dimerization of glutathione adducts originating from glutathione conjugation with quercetin were observed and identified by 1H NMR (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001; Awad et al., 2002a,b). According to these studies, the adduct formation at following sites-C2′, C5′, C6′, C6, and C8 of quercetin is possible.

2.5 Characterization of the protein-phenol-derivatives with mass spectrometry

The confirmation of the covalent addition of the phenolic compounds to the monomer protein molecules was obtained by applying the analytical methods of mass spectrometry like MALDI-TOF-MS and SELDI-TOF-MS. Exemplary, the results obtained for myoglobin with simple phenolic and related compounds (quinic-, ferulic-, caffeic-, chlorogenic-, and gallic acid, m-, o-, p- dihydroxyphenols and p-quinone) document this covalent conjugation with quercetin were observed and identified by 1H NMR (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001; Awad et al., 2002a,b). According to these studies, the adduct formation at following sites-C2′, C5′, C6′, C6, and C8 of quercetin is possible.

Fig. 13. SDS-PAGE of the myoglobin-flavonoid derivatives (1.07 mmol phenol/g protein). Code: 1=unmodified (control) myoglobin; 2=flavone derivative; 3=apigenin derivative; 4=kaempferol derivative; 5=quercetin derivative; 6=myricetin derivative.

2.6 Influence of the reaction parameters and conditions

The pre-requisite condition for the reaction of phenolic compounds with proteins is the presence of oxygen, in order to produce a reactive and redox active quinone or a semi-quinone radical, an electrophilic species capable of undergoing nucleophilic addition at the reactive side chains of the proteins. It is clear that the reaction conditions like time period, temperature, pH, oxygenation, concentration of the phenolic compounds as well as the position and the amount of hydroxyl groups present, may greatly influence the extent of their covalent attachment to proteins. The presence or absence of enzymes (e.g. tyrosinase) can also be important with regard to the oxidation products formed and consequently the reaction products produced with proteins (Hurrell et al., 1982; Hurrell & Finot, 1984). Some interesting aspects of reactions of caffeic- and chlorogenic acids with casein, showing the influence of pH, reaction time, temperature and presence or absence of enzymes (tyrosinase) have been reported (Hurrell et al., 1982; Hurrell & Finot, 1984). The influence of pH was also documented for reaction of chlorogenic acid with lysozyme and α-amylase, where even at low pH values (pH 4–5) derivatization occurred and consequent change of their activity was witnessed (Rawel et al., 2002b; Rohn et al., 2002b). With regard to flavonoids, recent results showed that, the adduct formation of quercetin quinone or quercetin quinone methide was found to be pH dependent (Awad et al., 2002a). A shift in adduct formation was noted from the C ring at pH 3.5, to the A ring at pH 7.0 and finally to the B ring at pH 9.5. This indicates a significant influence of the pH on the regioselectivity of glutathione conjugation to quercetin quinone/quinone methide (Awad et al., 2002a). The concentration of the phenolic compounds present during the derivatization also showed considerable effect, the degree of derivatization increasing with their rising concentration as documented for myoglobin and phenolic acids in (Kroll et al., 2000) and for α-amylase activity in (Rohn et al., 2002b). The mass spectra of the myoglobin derivatives with chlorogenic acid and p-quinone illustrating such changes are shown in Fig. 14. The reaction of myoglobin with chlorogenic acid (352.3 Da) delivered the highest molecular mass of 18025 Da, which in turn could account for addition of at least 3 molecules of chlorogeno-quinone to one myoglobin molecule (Fig. 14). In case of p-quinone (108.1 Da), a high molecular weight of 18117 Da was derived, which accounts for a corresponding addition of ca.11 molecules of p-quinone (Kroll & Rawel, 2001).

Fig. 14. MALDI-TOF-MS of myoglobin-phenol derivatives (0.42 mmol phenol/g protein). Code: 1=unmodified (control) myoglobin; 2=chlorogenic acid derivative; 3=p-quinone derivative.
The different configurations of hydroxyl groups, i.e. their amount and their position in the phenolic compounds, also determine the degree of protein derivatization (Kroll & Rawel, 2001; Rawel et al., 2001a,b; Rawel et al., 2002a; Rawel et al., 2003). The dependence of the reactivity on the different configurations of hydroxyl groups in flavonoids is also demonstrated by studies using the glutathione trapping method followed by HPLC, NMR, MALDI-TOF, and LC/MS analysis to identify the different glutathionyl adducts formed (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001).

3. Characterization of structural changes in protein-phenol-derivatives

The structural changes were studied using circular dichroism, differential scanning calorimetry, intrinsic fluorescence, and binding of anilinonaphthalenesulfonic acid. These results have been published recently (Rawel et al., 2002a,b; Rawel et al., 2003). A far-UV-CD study was conducted e.g. to evaluate the influence of derivatization by chlorogenic acid on the secondary structure of BSA (Rawel et al., 2002b). The results showed that there was a significant perturbation of the secondary structure with increasing degree of derivatization, causing a loss of the \( \alpha \)-helix and a parallel increase in the content of unordered structure elements (Rawel et al., 2002b). The derivatisation of \( \beta \)-lactoglobulin with quercetin and rutin (quercetin-3-O-rhamnosylglucoside) also caused a decrease in \( \alpha \)-helix (esp. the regular \( \alpha \)-helix) with a parallel increase in the remaining structure confirming the destructuring effect (Rawel et al., 2003). The perturbation of the secondary structure became more significant after derivatisation with rutin leading to an increased amount of unordered structure elements (Rawel et al., 2003). The reason being its bulkiness resulting from the attached rhamnosylglucoside at C3. Investigations on thermodynamics of interactions of caffeic acid and quinic acid with multisubunit proteins from sunflower seeds also suggested a marginal increase in \( \beta \)-sheet content, while \( \alpha \)-helix and \( \beta \)-turn contents were marginally decreased (Suryaprapaksh et al., 2000). Interaction of chlorogenic acid with human serum albumin was also shown to cause a decrease in \( \alpha \)-helix structure with other structures unaltered (Muralidhara & Prakash, 1995). These results are in agreement with our observations for BSA and \( \beta \)-lactoglobulin.

The interactions of flavonoids (apigenin, kaempferol and quercetin) with soy glycynin on the other side produced an increase in \( \alpha \)-helix and \( \beta \)-strand (Rawel et al., 2002a). A similar observation was also made during experiments concerning interactions of a globular protein (arachin) exposed to simple polyphenols (catechol and pyrogallol) and was attributed to the number of the introduced hydroxyl groups (Neucere et al., 1978). The interaction was thought to be of the hydrogen bond type, either through interpeptide chains or encompassing the phenols themselves (Neucere et al., 1978). Derivatization with myricetin causes a decrease in \( \alpha \)-helix, with a parallel increase in \( \beta \)-strand and -turn. In summary, CD studies and estimation of changes in secondary structures clearly demonstrates a perturbation, that is dependent of phenolic compounds and proteins applied.

The influence of the derivatization on the tertiary structure of soy glycynin and \( \beta \)-lactoglobulin were evaluated by means of a near-UV-CD study (Rawel et al., 2002a; Rawel et al., 2003). The CD of proteins in the near UV (310–240 nm) derives from transitions in the prosthetic groups e.g. tryptophan. The variation of CD in this region can be used to monitor changes in conformation and local environment. Chlorogenic acid caused a decrease in the molecular ellipticity compared to the unmodified soy glycynin, where as caffeic- and gallic acid an increase. Conversely, flavonoids-flavone and apigenin caused hardly any change, kaempferol only a slight, and quercetin as well as myricetin a significant increase in molecular ellipticity compared to the control sample (Rawel et al., 2002a). An increase in molecular ellipticity was also obtained by the derivatization of \( \beta \)-lactoglobulin with quercetin and rutin (Rawel et al., 2003). These changes in spectra highlight some conformational changes induced by the derivatization, affecting the tertiary structure and perturbing the initial conformation of the protein. Chemical modification (acylation and sulfoxidation) also produces a similar destructuring of proteins (Gerbanowski et al., 1999).

An intrinsic fluorescence study was also performed e.g. to evaluate changes in tertiary structure caused by reaction of BSA with chlorogenic acid (Rawel et al., 2002b). The maximum emission decreased in intensity as a function of the level of BSA modification by the covalent attachment of the chlorogenic acid and a red shift of the maximum emission was observed (Rawel et al., 2002b). The interaction of the aromatic ring of caffeic acid with aromatic residues such as tyrosine and tryptophan of the protein may be responsible for quenching of fluorescence intensity based on tryptophan fluorescence experiments (Suryaprapaksh et al., 2000). Such interactions can be confirmed as shown by the progressive decrease in the amount of free tryptophan (Suryaprapaksh et al., 2000; Rawel et al., 2002b). From the progressive quenching and the red shift observed in the maximum fluorescence emission of derivatized BSA, it can be deduced that conformational changes induced by the modification lead to a further exposure of tryptophan residues to the polar solvent (Jackman & Yada, 1989; Gerbanowski et al., 1999). Similar observations were also recorded for the reaction of \( \beta \)-lactoglobulin with quercetin and rutin (Rawel et al., 2003).

Differential scanning calorimetry was applied to assess the response of BSA-chlorogenic acid derivatives from a thermal stability perspective (Rawel et al., 2002b). The thermal stability of BSA, as reflected by denaturation temperature \( (T_d) \), decreased significantly as a function of the level of derivatization (Rawel et al., 2002b). Generally the enthalpy of denaturation \( (\Delta H) \) of derivatized BSA was also lowered (Rawel et al., 2002b). This decrease was not continuous making conformational changes apparent. The end-product of such a denaturation is generally a partly random coiled protein; this result is caused primarily by the disruption of non-covalent interactions (Ismond et al., 1985). The increase in the random coiled or unordered structure has been documented by CD analysis as described above (Rawel et al., 2002b).

The influence on surface hydrophobicity of BSA molecules by covalent linkage of chlorogenic acid was documented by binding of hydrophobic fluorescence probe, 1-anilino-8-naphthalensulfonate (ANS) (Rawel et al., 2002b). ANS is widely used to monitor conformational changes in proteins and to characterize surface exposure of hydrophobic sites (Hayakawa & Nakai, 1985; Ismond et al., 1985; Ismond et al., 1988; Gerbanowski et al., 1999). The surface hydrophobicity decreased with increasing degree of derivatization of BSA as shown by the low initial
slopes \( (S_\text{H}, \text{hydrophobicity index}) \), calculated from the corresponding fluorescence intensity vs. protein concentration plots (Rawel et al., 2002b). This means that the surface of BSA becomes hydrophilic. This also explains the better solubility of the BSA-chlorogenic acid derivatives as compared to e.g. myoglobin-phenol derivatives, which exhibited increased hydrophobicity (Kroll et al., 2000; Kroll & Rawel, 2001). According to Hayakawa and Nakai (1985), the decrease in solubility correlates generally well with an increase in surface hydrophobicity as measured by the hydrophobic fluorescence probe ANS. The surface hydrophobicity was also decreased when \( \beta \)-lactoglobulin was derivatized by quercetin and rutin respectively (Rawel et al., 2003). The observed decrease in the surface hydrophobicity can be explained as combined effect of a conformational change of the protein that increased the exposure of some additional hydrophilic regions which were previously buried, the covalent blocking of exposed hydrophobic residues like tryptophan and that the phenolic compounds bound, introduced new hydrophilic components (hydroxyl- and carboxyl groups).

In summary, CD studies and estimation of changes in secondary structures clearly demonstrates that the reaction of proteins with phenolic compounds leads to changes in \( \alpha \)-helix. The intrinsic fluorescence, DSC measurements and ANS binding experiments further indicate significant changes in the conformation of protein derivatized with the phenolic compounds.

4. Effect of protein derivatization on the proteolytic in vitro digestion

The enzymatic hydrolysis of derivatized food proteins as tested by trypsin, chymotrypsin, pepsin and pancreatin were differently influenced. Exemplary, an increase in concentration of phenolic substance present during derivatization caused the proteolytic digestion to become negatively influenced as reported for myoglobin derivatives (Kroll et al., 2000). Similarly, an alkaline pH lead to a higher derivatization and the proteolytic digestion (e.g. peptic hydrolysis of lysozyme derivatives with chlorogenic acid) was correspondingly poor (Rawel et al., 2000a). Quinic acid (a non aromatic substance) reacts only to small extent with protein, where as starting from resorcinol, ferulic acid, chlorogenic-, caffeic acid, pyrocatechol, hydroquinone, \( p \)-quinone and gallic acid a corresponding stronger reactivity and a corresponding stronger negative effect on proteolytic digestion was observed. The effect of derivatization of myoglobin and whey proteins with some of these phenolic compounds on all of the four proteolytic digestions tested, were negatively effected (Kroll et al., 2000; Kroll & Rawel, 2001; Rawel et al., 2001a). The results also showed that lysozyme derivatization with these plant phenols leads generally to a better digestion by trypsin, chymotrypsin and pancreatin, whereas the peptic digestion is not favoured (Rawel et al., 2001b). With regard to the derivatization of soy glycinin with selected flavonoids, general observations were that the tryptic, chymotryptic and pancreatic hydrolysis were promoted (except that flavone had little or no effect), whereas the peptic digestion remained more or less unaffected (Rawel et al., 2002a). A similar observation was also made, when digesting myoglobin derivatized with the these flavonoids, except here even the peptic digestion was also promoted. Exemplary, the chymotryptic digestion is presented in Fig. 15.

In conclusion, the proteolytic degradation of food proteins derivatized with phenolic compounds was effected by the pH milieu and the concentration of the phenolic substance present during the derivatization as well as from the phenol and protein configuration.

5. Reactions of phenolics and related substances with enzymes

The plant phenolc substances react with enzymes influencing their physicochemical and structural properties as described above and consequently their in vitro enzymatic activity was also adversely affected (Rawel et al., 2000b; Rawel et al., 2001b; Rohn et al., 2001; Rohn et al., 2002a,b). The proteolytic digestion of food proteins with \( \alpha \)-chymotrypsin derivatized by phenolic compounds was adversely affected as reported in Rohn et al. (2001). This decrease depended on the reactivity of the phenolic and related substances tested as well as on the kind of substrate applied (Rohn et al., 2001). Reactions of phenolic compounds with other enzymes (trypsin, lysozyme, \( \alpha \)-amylase) showed similar results with regard to changes in physicochemical properties and the effect on their activities (Rohn et al., 2002b). The evaluation of the lytic activity against gram-positive bacteria (Micrococcus lysodeikicus) showed that the lytic degradation of their cell walls by lysozyme derivatives of different phenolic compounds was significantly reduced due to the covalent attachment of the phenolic substances (Rawel et al., 2001b; Rohn et al., 2002b). The soy trypsin inhibitor activity against tryptic digestion declined as tested for the two different substrates (N-\( \beta \)-benzyol-arginin derivative; 4

Fig. 15. Chymotryptic digestion of the myoglobin-flavonoid derivatives (1.07 mmol phenol/g protein; Enzyme:Substrate ratio-E : S =1:60). Code: 1=□ unmmodified (control) myoglobin; 2=△ flavone derivative; 3= ● apigenin derivative; 4= ▲ kaempferol derivative; 5= ○ quercetin derivative; 6= ◇ myricetin derivative.
lase-potato starch; trypsin-BAEE (N\textsubscript{\textalpha{}}-benzoyl-arg-ethyl ester); α-chymotrypsin-N-suc-ala-ala-pro-phe-p-nitroanilide; brome-
lain-N-CBZ-LNPE (N\textsubscript{\textalpha{}}-carbobenzoxy-lys-p-nitrophenyl ester); lysozyme-cells from Micrococcus lysodeikticus; soy trypsin
inhibitor-N-benzoyl-arg-nitroanilide. Detailed experimental condi-
tions are reported elsewhere (Rawel et al., 2001b; Rohn et al.,
2001; Rohn et al., 2002b; Rawel et al., 2002a).

The kinetics of the enzymatic reactions showed that the hy-
drolysis of selected food proteins becomes slower and the affinity
of the enzyme to these substrates declined as measured by
Michaelis-Menten constant and maximum velocity of the enzy-
matic reaction (Rohn et al., 2003). This enzyme inhibition de-
pended on the reactivity of the phenolic and related substances
tested as well as on the degree of the derivatization. Further, in-
fluence of the enzyme-substrate ratio was also demonstrated. The
effects of the derivatization are more pronounced with increasing
concentration of the substrates.

6. Conclusions: Nutritional-physiological consequences of
such reactions

These interactions of flavonoids with myoglobin confirm our
former results with other proteins/enzymes, showing that the per-
quisite condition for a phenol to form covalent bonds with pro-
teins lies in its capability to form corresponding quinones or
semi-quinone radicals. The later being in position to undergo
nucleophilic addition at the amino side chains of the proteins.
The reaction proceeds in a further stage, where polymerization
takes place.

The nutritional value of food proteins is governed by amino
acid composition, ratios of essential amino acids, susceptibility
to hydrolysis during digestion, source, and the effects of processing.
To optimize the biological utilization of proteins, especially of
low-quality proteins in underdeveloped countries, a better under-
standing is needed of the various interrelated parameters that
influence their nutritional value. Enzyme-catalyzed brown-
ing reactions of amino acid and proteins with oxidized plantphe-
nols may cause deterioration of food during storage and process-
ing leading to a loss in nutritional quality, which is especially
serious in underprivileged countries. Some complex polyphenols
such as tannins are present in many cereal foods, e.g., grain sor-
gghum, the latter being widely used in many African countries. A
similar problem also encountered in the utilization of proteins
from sunflower, safflower, cotton seed and alfalfa leaf is that they
contain high levels of phenolic compounds (Hurrell et al., 1982;
Hurrell & Finot, 1984; Matheis & Whitaker, 1984a). Further,
alkaline processing of proteins may also be applied in technolog-
tical treatments of foods and feeds by solubilization and purifi-
caction, to destroy toxic contaminants, to obtain functional prop-
ties, including the formation of textured vegetable protein fibers
(Hurrell et al., 1982). These conditions would promote reactions
of proteins with secondary plant metabolites as shown above
inducing changes in their physico-chemical properties. The enzy-
matic digestion of the food protein derivatives by trypsin, chy-
mutrypsin, pepsin and pancreatin is adversely effected. And,
finally the biological function e.g. enzyme activity could also be
negatively influenced.

Further, after intake of food materials containing phenolic
compounds and after their corresponding absorption, these free
ligands may interact with many transport proteins and enzymes
as shown by the inhibition of human spleen tyrosine kinases
(Lazarro et al., 1995). Further, phenols have also been attributed
with positive properties like having antimutagenic, antimicrobial
and anticancerogen effects as well as being antioxidants—a
possible mechanism by which dietary components protect the
body from free radicals and reactive oxygen species (Robards
et al., 1999). This is biologically relevant especially with respect to
possible toxicological risks of these supposed beneficial antioxid-
ants, apparently able to be converted to electrophilic pro-oxi-
dants (Awad et al., 2000; Boersma et al., 2000; Awad et al.,
2001; Awad et al., 2002a,b). The p-quinone methide of quercetin
may prove to be an important toxic metabolite involved in the
cytotoxicity and pro-oxidant effects of quercetin (Boersma et al.,
2000). Further, this is of utmost importance, in connection with
the recent trends of introducing more and more supplementary
foods on the commercial level with the intention of marketing
the positive (antioxidative!) effects of flavonoids. These supple-
mentary foods contain high concentrations of flavonoids, which
are physiologically disquieting, especially since depending on
the concentration and hydroxyl substituent pattern, they can also act as pro-oxidants (Awad et al., 2000; Boersma et al., 2000; Awad et al., 2001; Awad et al., 2002a,b). Recently, the quercetin adduct formation with glutathione was indicated to be reversible, leading to possibilities for release and further electrophilic reactions of the quercetin quinone methide at cellular sites different from those of its generation (Boersma et al., 2000).

The different aspects of consumption of protein-phenol derivatives and their consequent proteolytic digestion have been adequately commented by Friedman (Friedman, 1997) as follows: “the nutritional and toxicological significance of consuming such protein derivatives is largely unknown.”

References


