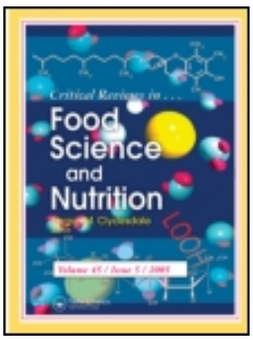

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Polyphenol Conjugates and Human Health: A Perspective Review

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In recent years, antioxidants have gained great importance because of their potential use in food, pharmaceutical, and cosmetic industries. This interest is rooted in the cumulative evidence connecting active oxygen and free radicals with numerous human degenerative disorders, such as cardiovascular diseases, cancer, aging, and atherosclerosis. Polyphenols are the major class of antioxidant able to reduce the oxidative damages of lipids, proteins, enzymes, carbohydrates, and DNA in living cells and tissues. Among the realm of polyphenol compounds, polyphenol conjugates have been proposed as innovative materials which, by combining the advantageous properties of both the components, can increase the efficiency of antioxidants and their range of application in nutritional and biomedical fields. This work is an overview of the different class of polyphenol conjugates, which will be analyzed in terms of nutritional and biological properties, showing how these bio-conjugates will positively affect the human health.

Keywords Polyphenols, bio-conjugates, biological properties, polymers, nanomaterials

INTRODUCTION

Antioxidants are a class of compounds able to protect living tissue by inhibiting the activation of oxygen to highly reactive products or by quenching oxidants before they can react with biological environments (Sies, 1997; Venkat Ratnam et al., 2006). Although not many antioxidants are listed in pharmacopoeias, extensive researches are being globally carried out on these agents for their potential use in food, pharmaceutical, biomedical, and cosmetic industries (Halliwell and Gutteridge, 1994; Azzi et al., 2004) by virtue of the evidence proving the link between the oxidative stress and the development of human diseases, such as cancer, premature aging, atherosclerosis, and prostaglandin-mediated inflammatory processes (Muller et al., 2007; Stephens et al., 2009).

Antioxidants can be classified into two major groups: enzymatic and nonenzymatic antioxidants, some of which, e.g., enzymes, low molecular weight molecules, and enzyme cofactors, are endogenously produced (Halliwell, 2011). The nonenzymatic antioxidants, a class of compounds including the dietary antioxidants, can be classified into various sub-classes, such as polyphenols, vitamins, carotenoids, and organosulfural compounds (Cai et al., 2004).

Polyphenols represent the largest class of natural antioxidants, basically consisting of phenolic acids and flavonoids, with a high chemical variety in relation to the number of aromatic rings and to the number and the position of phenolic groups (Bravo, 1998). Their antioxidant activity is mainly due to the ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal ions.

Several interesting research works devoted their attention to the understanding of the biochemical mechanism at the basis of the different biological activities of polyphenols (Frei and Higdon, 2003), which were evaluated by considering the polyphenols as both nutraceutical supplement and/or therapeutic agents to undergo intravenous administration.

The effects of polyphenols on cardiac functionality were well elucidated in a review of Gresele et al. (2011). In this work, by using a model of myocardial ischemia, it was assessed that different polyphenols, including resveratrol, tyrosol, and hydroxytyrosol, displayed cardioprotective properties, as a consequence of their ability to improve the post-ischemic ventricular performance by reducing myocardial infarction size, cardiomyocyte apoptosis, and peroxide formation.

As regards resveratrol, it was found to be able to stimulate endothelial production of nitric oxide, reduce oxidative stress,

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inhibit vascular inflammation (effective dose of 1.00–10.0 μM in human coronary artery), and prevent platelet aggregation (4 mg/kg/day; Li et al., 2012). In animal models of cardiovascular disease, it was demonstrated that resveratrol protects the heart from ischemia–reperfusion injury, reduces blood pressure and cardiac hypertrophy (effective dose of 50–100 μM) in hypertensive animals, prevents the endothelial dysfunction, and slows the progression of atherosclerosis (Bhatt et al., 2011; Rimbaud et al., 2011).

The effect of polyphenols on myocardial function was also the objective of the work of Aneja et al. (2004), where they extensively analyzed the beneficial effect of epigallocatechin-3-gallate (EGCG), the most prominent catechin in green tea extract (GTE), on myocardial ischemia reperfusion injury. EGCG, at a concentration of 10 mg/kg/hour after intravenous administration, acts by inhibiting the biological pathways (nuclear factor-kappaB–NFkB and activator protein-1–AP1) involved in the myocardial injury.

GTE was also found to reduce the risk of coronary artery disease as a consequence of an antiinflammatory effect related to the modulation of the cardiac mRNA of genes involved in insulin and lipid metabolism and inflammation (Qin et al., 2010).

In recent years, a large amount of experimental and some clinical data have been accumulated regarding the effects of flavonoids on the endothelium under physiological and pathological conditions (Perez-Vizcaino et al., 2006). Kleemann et al. (2011) focused their attention on the antiinflammatory and antiatherogenic effects of quercetin (Q). Authors showed that Q and related flavonoids exert direct (endothelium-independent) vasodilator effects in isolated arteries by virtue of a molecular mechanism involving multiple actions on protein kinases. Interestingly, Q and its metabolites are more potent in coronary arteries and in resistance than in conductance vessels.

In addition to heart damage, polyphenols also show protective effect on liver. The biochemical data of a study of Vitaglione et al. (2010) showed that the treatment of high-calorie solid diet (HFD)-fed rats with polyphenols found in coffee leads to a reduction of fat and collagen deposition, systemic lipid peroxidation, and proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interferon-γ (IFN-γ). Moreover, it was shown that coffee's polyphenols by their ability to stimulate, through Nrf2/EpRE activity, antioxidant response element-dependent genes encoding for antioxidant proteins, and phase II detoxifying enzymes increase antiinflammatory molecules (IL-4 and IL-10) in liver tissue and the glutathione (GSH), which can play a role in the prevention of liver carcinogenesis.

The administration of GTE also prevents liver injury after hemorrhage/resuscitation (Relja et al., 2011), in which the release of pro-inflammatory cytokine IL-6, neutrophil infiltration, and thus the activation of NFkB, is promoted.

Shen et al. (2011) successfully utilized a model of lipopolysaccharide (LPS) administration to female rats to investigate the impact of GTE supplementation in chronic inflammation-induced deterioration of bone microarchitecture. The results of histomorphometric analyses showed the GTE potent effect in preserving bone mass and microarchitecture by attenuating chronic inflammation-induced LPS.

Polyphenols also showed beneficial effects on neuronal cells, and this aspect was the main topic of several interesting research and review works in the last decade (Simonyi et al., 2005). In brain injury, such as that arising from trauma, epileptic seizures, neurodegenerative diseases, and cerebral ischemia, either necrosis or apoptosis of the neurons (Lu and Mattson, 2001) can be observed, as a consequence of the activation of N-methyl-D-aspartate (NMDA), AMPA, kainate, and metabotropic glutamate receptors (Ciani et al., 1996). Oxidative stress is involved in the whole of these effects (Abe et al., 1988), not only by a direct neuronal damage, but also by the increase of the blood–brain barrier (BBB) permeability, allowing the extravasation of high molecular weight materials like the protein albumin (Nelson et al., 1992). This phenomenon results in edema formation, an important acute clinical complication and a major factor contributing to a poor outcome after an ischemic insult. EGCG and GTE were found to reduce neuronal damage after transient global ischemia (Lee et al., 2000), β-amyloid protein-induced neurotoxicity (Choi et al., 2001), and AMPA-induced calcium influx (Bae et al., 2002). Lee et al. (2000) demonstrated that EGCG, when administered at the dose of 25 or 50 mg/kg after intravenous administration, reduced hippocampal neuronal damage in a dose-dependent manner in gerbils after transient global ischemia induced by the occlusion of both common carotid arteries. The same research groups (Lee et al., 2004) found that a treatment with EGCG reduced cell death after exposure to the glutamate receptor agonist NMDA, AMPA, or kainate in primary hippocampal cell cultures. Shah et al. (2010) hypothesized and verified that epicatechin (EC) provides neuroprotection against brain injury induced by transient middle cerebral artery occlusion (MCAO) or N-methyl-D-aspartate (NMDA), and that the protection would occur through activation of the enzyme heme oxygenase 1 (HO1)/transcriptional factor Nrf2 pathway. The same mechanism of neuroprotection was found in Ginkgo biloba extract (Saleem et al., 2008; Shah et al., 2011). A study of Shin et al. (2006) demonstrated the robust neuroprotective effects of a systemic administration of the naturally occurring amentoflavone against the neonatal brain from hypoxic-ischemic injury. The authors showed that amentoflavone blocks the caspase-dependent apoptosis and caspase-independent excitotoxic/necrotic cell death as well as the inflammatory activation of microglia.

GTE was also demonstrated to be potentially used as a low-cost dietary support to combat *Helicobacter pylori*-linked gastric diseases without affecting the beneficial intestinal bacteria (Ankolekar et al., 2011). The most accepted regime for the eradication of *H. pylori* infection currently includes a triple therapy, which combines the antibiotic clarithromycin and amoxicillin with a proton pump inhibitor such as omeprazole. However, this therapy can produce side effects and it can fail to eliminate infection in 10–30% of patients (Cavallaro et al.,

2006). In a recent work (Romero et al., 2007), it was demonstrated that the dialdehydic form of decarboxymethyl ligstroside (Ty-EDA), a phenolic component of olive oil, is able to kill *H. pylori* at a concentration much lower than any other polyphenols from food sources. The bacteria were sensitive to more than 100 μg/mL of tea catechins, 12–25 μg/mL of resveratrol, 12 μg/mL of flavonoids from medicinal plants, 20–100 μg/mL of essential oils, and 1.3 μg/mL of Ty-EDA.

Polyphenols extracted from Annurca apple showed chemopreventive properties in colorectal cancer cells by reducing the polyp number in the range 35–42% (Fini et al., 2011). Fujiki et al. (2003) characterized the anticancer properties of geraniin and corilagin, polyphenol of traditional Japanese herbal medicines, and they found that these molecules act by inhibiting TNF-α with IC_{50} values of 43 μM for geraniin, 76 μM for corilagin, and 26 μM for EGCG. In the same work, authors proved the synergistic effects of EGCG with sulindac or tamoxifen on cancer preventive activity in PC-9 cells, and cancer prevention of intestinal tumor development in multiple intestinal neoplasia (Min) mice by co-treatment using EGCG with sulindac (Suganuma et al., 2001).

To complete the overview on the biological activity of polyphenols, the skin care effects should be cited, because many topical and dietary antioxidants were demonstrated to alleviate the oxidative skin damage, retard skin aging (Cecchi et al., 2011), and prevent the UVB-induced keratinocyte death. In the last case, the actual mechanism includes the inhibition of UVB-induced intracellular hydrogen peroxide production, lipid peroxidation, and c-jun-NH_2 terminal kinase activation (Huang et al., 2010). More interestingly, polyphenol have been found to be effective against melanoma, as shown by Ellis et al. (2011). Authors demonstrated that EGCG inhibits melanoma cell growth at physiological doses (0.1–1 μM) with the mechanism involving inflammation down-regulation, decreased IL-1β secretion, and decreased NFkB activities.

All the antioxidant and biological properties of polyphenols are related not only to the structure of phenolic compounds, but also to their solubility properties that greatly influence their application fields (Tsao, 2010). Hydrophilic antioxidants, indeed, could prevent the oxidation of bulk oil, while their hydrophobic counterpart effectively retards lipid oxidation in oil-in-water emulsion (Maqsood and Benjakul, 2010). Several research groups have focused their attention to the chemical modification of polyphenols to influence the solubility properties. Recently, by-products from wood industry, in particular sawdust from sawmills, have been proposed as a potential source of tannins, cheap antioxidants for food industry (Poaty et al., 2012). Being water-soluble because of their structure containing many OH groups, tannins show, however, a limited solubility in lipid environment such as the cell membrane, and thus they do not act as effective antioxidizing agents in the lipid bilayer. To improve this defect, chemical reactions, e.g., esterification with a fatty acid or alcohol, have been proposed, which improves their lipophilicity, while preserving the antioxidant properties (Jin and Yoshioka, 2005). The esterification of polyphenols to modify their hydrophilic properties is one of the several different chemical modifications of these antioxidant compounds; in the present review, a specific modification of the basic skeleton of polyphenols, such as their conjugation with biomaterials, will be explored. We will give an overview of the most important polyphenol conjugates, with specific attention to the naturally occurring derivatives as well as to the most innovative synthetic materials.

NATURAL POLYMERS–POLYPHENOL CONJUGATES IN PLANTS

Polyphenol conjugates are commonly extracted from different plants. Several scientists have devoted their activity to the isolation and to the physic-chemical and biological characterization of these compounds (Table 1).

Polyphenol–polysaccharide conjugates were isolated into plant extracts from 17 Poland traditional medicinal plants, belonging to *Asteraceae* and *Rosaceae* families, by Pawlaczyk et al. (2009). These authors investigated the role of these polyphenolic–polysaccharide conjugates in the defense against oxidative stress induced by peroxynitrite anion in human platelets (Pawlaczyk et al., 2011). Blood platelets are involved in inflammatory responses because they can deliver various biologically active compounds like serotonin, histamine, and platelet activation factor. Moreover, $NO^{\bullet}$ and $O2^{-\bullet}$, reactive nitrogen and oxygen species, are generated in platelets. The reaction of $O2^{-\bullet}$ and $NO^{\bullet}$ leads to production of peroxynitrite ($ONOO^{-}$), which can alter the mechanism of platelet activation and their hemostatic function, inducing the oxidation of platelet thiols, as well as the carbonylation and nitration of platelet and plasma proteins (Saluk-Juszczak et al., 2007). The whole of these modifications can alter the platelet response, and may be an important factor involved in cardiovascular diseases and in inflammatory conditions. Authors confirmed the suitability of polyphenol–polysaccharide conjugates extracted from *C. Canadensis* in reducing the nitrative and oxidative damage induced by $ONOO^{-}$ in blood platelets.

Harish Nayaka et al. (2010) determined the phenolic acid composition in swallow root (*Decalepis hamiltonii*) free phenolic acid (SRFP), swallow root conjugated phenolic acid (SRCP), and swallow root insoluble-bound phenolic acid (SRIBP) extracts. Moreover, it was demonstrated that these components possess cytoprotection activity on NIH 3T3 fibroblast cells because they showed radical scavenging ability and protection against DNA damage induced by hydroxyl radical. In the performed experiments, the cytoprotection was evaluated after oxidation of NIH 3T3 cells induced by *tert*-butyl hydroperoxide, while the effect on DNA damage was determined by means of electrophoresis measurements on DNA subjected to oxidation by Fenton's reagent (0.3 mM hydrogen peroxide, 0.5 μM ascorbic acid, and 0.8 mΜ ferric chloride). All the tested phenolic

Table 1 Summary of the most relevant natural polyphenols and polymer–polyphenol conjugates found in plants and their biological activity

Polyphenol	Source	Effect
Epigallocatechin-3-gallate	Green tea	- Antioxidant properties - Anticancer effects - Cardioprotective effect - Prevention of liver injury - Prevention of deterioration of bone microarchitecture - Neuroprotection - Antiulcer properties
Resveratrol	Red wine	- Protection of endothelial functions - Reduction of oxidative stress - Inhibition of vascular inflammation - Prevention of platelet aggregation - Reduction of blood pressure - Reduction of cardiac hypertrophy - Antiatherogenic effect - Cardioprotective effect
Quercetin	Red wine	- Protection of endothelial functions - Antiinflammatory effect - Antiatherogenic effect
Tyrosol-Hydroxytyrosol	Grapes–olives	- Cardioprotective effect
Epicatechin	Green tea	- Neuroprotection
Myricetin	Grapes	- Keratinocytes protection
Procyanidin	Parkia biglobosa	- Protection of endothelial functions
Oligomeric Procyanidins	Larix gmelinii bark	- Antioxidant properties
Proanthocyanidins	Persimmon leaf	- Antihypertensive effect
Polyphenol-polysaccharide conjugates	Asteraceae and Rosaceae	- Antiinflammatory effect
Polyphenol-polysaccharide conjugates	C. Canadensis	- Protection of blood platelets function
Hydroxybenzoic and cinnamic acid derivatives	Swallow root	- Antioxidant properties - Fibroblast protection - Protection against DNA damage
Semimyrtucommulone and Myrtucommulone A	Myrtle	- Inhibition of oxidative degradation of cholesterol and LDL - Inhibition of linoleic acid peroxidation
Amentoflavone	Selaginella tamariscina	- Neuroprotection on neonatal brain
Decarboxymethyl ligstroside	Olive oil	- Antiulcer properties
Geraniin and corilagin	Japanese medical plants	- Anticancer properties
Insoluble components of foods	Cereals, vegetables, and oat flour	- Antioxidant properties in gastro-intestinal tract

acid extracts showed a dose-dependent protection between 0.03 and 0.15 μg/mL concentration: SRCP showed the best efficiency. In particular, at equal concentration of 0.12 μg/mL, SRCP showed 87% protection, while SRFP and SRIBP showed 47% and 65%, respectively. Finally, it was verified that these phenolic acid extracts contained both hydroxybenzoic and cinnamic acid derivatives as antioxidant molecules to different extent.

Oligomeric non-prenylated acylphloroglucinols named semimyrtucommulone and myrtucommulone A, isolated from myrtle, represent a particular kind of natural polyphenol conjugates (Rosa et al., 2008). These compounds showed potent protective effect in simplified models of oxidative degradation of cholesterol and LDL, which play a crucial role in the development of cardiovascular diseases. Specifically, myrtucommulone A exerted a complete inhibition of cholesterol degradation (after 2 hours) at an amount of 10 nmol, while for semimyrtucommulone, the 100% protection was recorded at 20 nmol. Reducing the reaction time to 1 hour, these values become 5 nmol for both the acylphloroglucinols. In contrast to cholesterol assay, semimyrtucommulone was much more powerful in protecting linoleic acid autoxidation and $FeCl_3$-mediated oxidation.

In a work of Serpen et al. (2007), authors analyzed the healthy effect of the insoluble matters in many food samples, such as cereals, vegetables, and oat flour, proving that antioxidant functional groups, such as phenolic acids, bound to the insoluble components of foods, may quench free radicals by a surface reaction phenomenon. To confirm that the antioxidant activity of these insoluble food components was related to the presence of fiber-bound antioxidant compounds, authors evaluate the reduction of the antioxidant activity by means of alkaline hydrolysis, and as a result, a 90% reduction of the antioxidant activity was recorded. Furthermore, authors elucidated that the insoluble matters of food remain in the gastrointestinal tract for a long time and may help in quenching the soluble radicals those are continuously produced in the intestinal tract and could be involved in the etiology of colon cancer (Babbs, 1990).

NATURAL POLYMERS AS SUBSTRATE FOR THE SYNTHESIS OF INNOVATIVE POLYPHENOL CONJUGATES

In literature, several researchers report on the design and synthesis of bio-conjugates obtained by the covalent linkage of polyphenols to natural polymers, like proteins and polysaccharides, which could be applied in food, biomedical, and "health-care" industry (Table 1). These materials are very interesting from an application point of view, because both the components are already widely used and studied. Proteins and polysaccharides, indeed, are common constituting materials of food packaging and are often used as food preservative and prebiotics. The main limitation in the practical employment of polyphenols as preservatives in food industry is their low stability when exposed to high temperature, light, etc. The conjugation of antioxidants to biomacromolecules allows overcoming this drawback, conferring a high value to both the components: the antioxidant molecule, when linked to a macromolecule, becomes more stable, while the biopolymer acquires the properties of the linked antioxidant. Several different polysaccharides and proteins have been used as starting materials for the preparation of innovative antioxidant bioconjugates and an overview of the most significative are reported below.

By virtue of its special biocompatible and antimicrobial activity, chitosan is one of the most studied polysaccharides, and it is applied in different fields, such as wound healing and food packaging (Maeda et al., 1992; Chae et al., 2004). The synthesis of chitosan–polyphenol conjugates has been proposed by employing different synthetic approaches. Tyrosinase has been used to graft flavonoids onto chitosan macromolecules as a nontoxic attractive alternative, environmental unfriendly, and nonspecific chemical approach (Sousa et al., 2009). With the grafting of the flavonoid onto chitosan fibers, the antioxidant properties were enhanced when compared with the unmodified fibers. An enhancement of the antibacterial activity was also recorded by virtues of damages to the bacterial membrane. Increased bacterial membrane permeability and the dissipation of the membrane potential were observed. The flavonoid-functionalized chitosan is more effective against Gram-positive than Gram-negative bacteria, and a possible explanation is the composition of the Gram-negative cell membrane, constituted of LPS, lipoprotein, and phospholipids, acting as a potential barrier for foreign molecules with high molecular weight (Xie et al., 2002).

Catechin– and gallic acid–chitosan conjugates, retaining the antioxidant properties of the linked polyphenol, were obtained by free radical grafting procedure (Curcio et al., 2009). This synthetic approach allowed us to work in ecofriendly conditions, because the use of organic solvents and the generation of toxic by-products were completely avoided; to initiate the grafting reaction, a water-soluble redox initiator system, composed of ascorbic acid and hydrogen peroxide, was employed. A possible mechanism of antioxidants insertion onto chitosan involves the interaction between the hydroxyl radicals, generated by the redox pair initiator system, and the H-atoms of α-methylene (CH_2), the hydroxyl groups (OH) on the hydroxymethylene group, or the amino group. At those sites, the insertion of the antioxidant molecules can subsequently occur.

In another work, chitosan/(3-chloropropyl)trimethoxysilane hybrid scaffolds (CTS/CPTMS) were functionalized with caffeic acid by a grafting approach (Shiu et al., 2010). The conjugation of caffeic acid to CTS/CPTMS hybrid scaffold increased the compressive strength of the material and enhanced its scavenging activity, anti-cancer property, and antibacterial activity against *Staphylococcus aureus*. In culturing human osteosarcoma UMR-106 cells, the caffeic acid grafted scaffolds showed inhibitions on cell growth, alkaline phosphatase activity, and cell attachment of UMR-106 cells.

In an article of 2010 (Pasanphan et al., 2010), electron paramagnetic resonance (EPR) was employed to demonstrate a wide range of antioxidant activity for a chitosan–gallic acid conjugate synthesized in the presence of 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide as coupling agent. Even if the functionalization degree was only 15%, high antioxidant ability was recorded, as highlighted by the EC_{50} values (0.021 mg/mL for $^{\bullet}R$ and 0.066 mg/mL for $^{\bullet}OH$), when compared with other chitosan derivatives, such as sulfated chitosan ($EC_{50} = 0.1$ mg/mL for $^{\bullet}R$ and 3.269 mg/mL for $^{\bullet}OH$).

Another interesting work reports on the preparation of nanoparticles with both antioxidant and antibacterial properties by grafting eugenol and carvacrol (two components of essential oils) on chitosan nanoparticles (Chen et al., 2009). Eugenol and carvacrol were first formylated, and then the grafting of these oils to chitosan nanoparticles was carried out via the Schiff base reaction, obtaining carvacrol-grafted chitosan nanoparticles (CHCA NPs) and eugenol-grafted chitosan nanoparticles (CHEU NPs). The scavenging activity of CHCA NPs and CHEU NPs conjugates was evaluated by performing diphenylpicrylhydrazyl test (DPPH), with an EC_{50} value for CHEU NPs and CHCA NPs of 2.6 and >4.0 mg/mL, respectively. Moreover, antibacterial assays, carried out with *Escherichia coli* (a representative gram-negative bacterium) and *Staphylococcus aureus* (a representative gram-positive bacterium), demonstrated an antibacterial activity equivalent to or higher than that of the unmodified chitosan nanoparticles. In the assays with *Escherichia coli*, the minimum inhibition concentrations (MICs) recorded were 0.5–1 mg/mL for CHCA NPs and 0.25–0.5 mg/mL for CHEU NPs, while MICs of 0.5–1 mg/mL for CHCA NPs and 0.5 mg/mL for CHEU NPs were obtained in the *Staphylococcus aureus* case. Finally, cytotoxicity assays using 3T3 mouse fibroblast showed that the cytotoxicity of CHCA NPs and CHEU NPs conjugates was significant lower than those of the pure essential oils, and the recorded IC_{50} values were both around 1 mg/mL.

Besides chitosan, other polysaccharides have been exploited for the synthesis of antioxidant conjugates. In a work of 2012 (Cirillo et al., 2012), starch-quercetin conjugate (ST-Q) was

synthesized by free radical grafting procedure using, as reported before for chitosan, ascorbic acid and hydrogen peroxide as free radical initiators. Stability tests, performed by UV-irradiation, demonstrated that the insertion of Q in starch structure increased the stability of macromolecule, as confirmed by a degradation value of 9% compared with 24% for native starch. Similarly, the conjugate was found to be stable against α-amylase degradation, with a 44% lower degradation value compared with that obtained when native starch is used. The antioxidant tests confirmed high antioxidant ability as showed by the EC_{50} values of 2.52 mg/mL for DPPH, 1.15 mg/mL for peroxynitrite, and 4.31 mg/mL for lipid peroxidation assay. Results also show an enhanced ability of starch conjugate in preventing gallic acid (used as a model drug) from degradation. The amount of preserved drug by the antioxidant macromolecular system was almost 95% in all the environments tested, while native starch was less effective, with preserved gallic acid of about 30% under light stress, 43% under thermal treatment, and 52% under oxidative stress. Finally, authors evaluated the enzymatic activity of the conjugate against acetylcholine esterase (EC_{50} of 12.1 mg/mL) and tyrosinase (EC_{50} of 15.7 mg/mL) to prove the potential beneficial effects of ST-Q on human health. The same synthetic procedure was employed to obtain the covalent conjugation of catechin to alginate and inulin, linear polysaccharides widely used in food and beverage areas (Spizzirri et al., 2010). The characterization of the conjugates showed that the antioxidant properties of catechin are completely retained after the conjugation process. In a subsequent work, inulin–catechin conjugate was further engineered by adding the thermo-responsive functionality in the polymer backbone. As thermo-responsive element, N-isopropylacrylamide (NIPAAm) was chosen because of its peculiar properties: NIPAAm omo-polymer, poly (NIPAAm), undergoes a sharp coil-globule transition and phase separation at a temperature of 30–32°C (low critical solution temperature–LCST; Spizzirri et al., 2011). The covalent insertion of such stimuli-responsive moieties in the polymer backbone allows modulating the antioxidant properties of the biomaterial in relation to the temperature variation around the LCST, tailoring the polymeric materials to specific applications. In order to obtain this material, one pot reaction, consisting of direct polymerization of inulin with catechin (CA), NIPAAm, and N,N-ethylenebisacrylamide (EBA) (used as cross-linking agent), was performed, and hydrogels with controllable antioxidant properties depending of the external temperature were obtained. In a different work, a dextran–catechin conjugate obtained by radical grafting was proposed as anticancer agent in the treatment of anticancer drug against pancreatic ductal adenocarcinoma (Vittorio et al., 2012). The MTT assays confirmed significant cytotoxicity against cancer cells (IC_{50} of 1.197 mg/mL for MIA PaCa-2 and 1.032 mg/mL for PL45 cells) with lower effects on Human Pancreatic Neuroendocrine Cells (HPNE) non-neoplastic cells (IC_{50} of 838.33 mg/mL), indicating its potential for reducing systemic cytotoxicity on normal tissues commonly associated with standard chemotherapy treatments.

In other works, the functionalization of carboxylic group with dicyclohexylcarbodiimide was used as synthetic strategy to couple ferulic acid and α-tocopherol to microcrystalline cellulose (Trombino et al., 2008), and ferulic acid to dextran (Cassano et al., 2009). These systems were found to be effective in inhibiting the free radical damage in rat liver microsomal membranes in the range of 0.5–6 mg/mL.

When considering the protein materials, only few examples of protein-based antioxidant conjugates can be find in literature. An interesting study was published by Valenta et al. (1998). In this work, lysozyme was modified by the covalent attachment of caffeic acid and cinnamic acid, in order to generate preservatives, which exhibits a broad antimicrobial spectrum against Gram-positive as well as Gram-negative bacteria. Condensation reaction mediated by a water-soluble carbodiimide was employed as synthetic strategy to obtain the constitution of amide bindings between the carboxyl groups of ligands and primary amino groups of the enzyme. It was found that the conjugates were characterized by a wider antimicrobial spectrum compared with nonmodified lysozyme and the results show a concentration of 0.2% of the lysozyme–caffeic acid/lysozyme mixture (1+1) as the minimum required to exert a bacteriostatic effect for Gram-negative as well as for Gram-positive bacteria.

Gelatin, a degradation product of collagen, extensively used in food, adhesives, and pharmaceutical fields, is another key protein modified with antioxidant moieties through different synthetic routes. This protein is characterized by a low level of immunogenicity and cytotoxicity, is easy to modify at the level of amino acids, and has a good biodegradability (Maddux et al., 2011). Chung et al. (2003a) proposed the use of a laccase-catalyzed oxidation to covalently conjugate catechin to gelatin through the lysine residues. As a result, the gelatin–catechin conjugate was more capable of inhibiting oxidation of LDL than unconjugated catechin. At a concentration of 70 μM, the conjugate reduces the fluorescence due to the oxidative marker to 45%, while this value was 60% for free flavonoid.

The addition of antioxidant molecules on gelatin side chains was also obtained by radical reaction of gelatin with gallic acid and catechin. The hydrogen peroxide redox pair was used as initiator system (Spizzirri et al., 2009). As reported in the case of polysaccharides, the hydroxyl radicals generated by the interaction among redox pair components attack the sensible residues in the side chains of protein, producing radical species on the aminoacidic structure. After that, antioxidant molecules were added to the reaction mixture, obtaining the formation of antioxidant–gelatin covalent bonds. The conjugates were characterized by good scavenging and antioxidant activities: the inhibition (%) of DPPH, $^{\bullet}$OH radical, and lipid peroxidation by selected amounts of antioxidant conjugate were all in the range of 66–98%, with an interference of pure gelatin of 28–43%. An extensive biological characterization of the gelatin–gallic acid conjugate was

performed in view of a possible biomedical application of the proposed macromolecular systems (Cirillo et al., 2010). First, the scavenging properties toward peroxynitrite anion were tested showing that the EC_{50} value of gallic acid does not significantly change after the conjugation process. Second, the functionality of the conjugate was explored in terms of beneficial effects on human health by testing its activity on selected enzymes. Specifically, acetyl cholinesterase and α-amylase were tested to investigate putative beneficial effects of the synthesized biopolymer in the treatment of Alzheimer disease and diabetes, with recorded EC_{50} values of 7.1 and 9.8 mg/mL, respectively. Furthermore, the anticancer activity of the bioconjugate was determined in prostate carcinoma and renal carcinoma cell lines by comparing its effect on cancer cell viability with pure gelatin. The results showed that the conjugate is able to reduce the viability of all the tested cancer cell lines with a considerable higher efficiency than the pure carrier.

A particular class of antioxidant conjugates is known as antioxidant peptides (Park et al., 2005). These materials could be prepared through enzymatic hydrolysis from various natural proteins (Kim et al., 2001; Sakanaka et al., 2004; Mendis et al., 2005; Hsu et al., 2009), synthetic peptide libraries (Saito et al., 2003), or chemical modification of preformed peptides. In a study of 2009 (Kwak et al., 2009), authors focused their attention on the enhancement of the antioxidant activity of hydroxycinnamic acids through conjugation with antioxidative peptides. They concluded that Caffeic Acid-b-Ala-His-NH_2 was an ideal antioxidant in both hydrophilic and hydrophobic environments. In a subsequent study (Seo et al., 2010), the same authors focused their attention on histidine residue and constructed histidine dipeptide libraries in order to find a better antioxidant. Experimental data showed that the conjugation of caffeic acid to histidine-containing dipeptides enhanced its antioxidant activity because of the radical trapping ability of imidazole in histidine. Caffeic Acid-Pro-His-NH_2 was found to be a powerful ideal antioxidant in both hydrophilic and lipophilic systems, probably because the structure of proline enabled histidine to stabilize the hydroxyls of the polyphenol, leading to the enhanced antioxidative activities. Furthermore, proline gives a critical effect on the enhanced antioxidant activity (Kwak et al., 2012).

In a work of Ouberai et al. (2009), an interesting class of polyphenol–peptide conjugate was synthesized, and their applicability in the treatment of Alzheimer disease was proposed by virtue of its ability to inhibit the aggregation of β-amyloid peptides into toxic aggregates (Murphy, 2002), and the curcumin-polypeptide conjugates were found to be the most effective ones (Findeis et al., 1999).

SYNTHETIC POLYPHENOL CONJUGATES

The high antioxidant properties of conjugated polyphenols, as well as their enhanced chemical and physical properties, have led the scientific community to the development of synthetic materials able to couple the biological functionalities of polyphenols with the chemical properties of polymeric materials (Table 2).

As an example on this concept, the preparation of a synthetic derivative of curcumin (Simoni et al., 2010) should be cited. Despite its broad effects on the biological functions of cells, the potential use of this polyphenol as a therapeutic agent is severely affected by its low water solubility, poor in vivo bioavailability, and rapid metabolism. In different works, its structure has been widely modified to improve the pharmacokinetic profile, focusing mainly on changes in the β-diketone structure and aryl substitution pattern of the molecule (Padhye et al., 2010). Simoni et al. (2010), considering the ability of polyamine to specifically convey a bioactive functionality into mitochondria by virtue of electrostatic forces (Casero and Woster, 2009), developed polyamine derivatives of the curcumin analog, 3,5-dibenzylidenepiperidin-4-one (DBP). The results demonstrated that the proposed bio-conjugate retained all the antioxidant activities of DBP, which are accompanied by a significant loss of cytotoxicity.

In a work of Li et al. (2011a), resveratrol was coupled through a hydrolysable covalent bond with the carboxylic acid groups in porous poly-ε-caprolactone surface grafted with acrylic acid. Authors demonstrated that the conjugation of the polyphenol results in preserved resveratrol antioxidant properties, and in an increased DNA synthesis and alkaline phosphatase activity in osteoblasts, and prevent femoral bone loss in ovariectomized rats. Grafting procedures were also exploited for the direct co-polymerization of ferulic acid and methacrylic acid (Puoci et al., 2008). Both soluble and microparticulate systems were prepared by this innovative technique (Parisi et al., 2010) and the resulting functional polymers were found to be suitable for application as food preservatives and antimicrobial agents as a consequence of their ability to inhibit the free radical formation and the *S. Aureus* proliferation, respectively (Iemma et al., 2010). Methacrylic acid was also conjugated with quercetin (Puoci et al., 2012) and the obtained macromolecular system was found to be effective as anticancer agent against HeLa cancer cells, as a result of a higher stability of the flavonoid.

With the aim to improve the physiological and pharmacokinetic properties of polyphenols, enzyme-catalyzed conjugation reactions were developed (Gogoi et al., 2010). The biomimetic process using oxidative enzymes is of great interest for the "in situ" production of biopolymers and for further applications onto fabrics, satisfying an environmental friendly concept by replacing chemical processes. Laccase-catalyzed enzymatic synthesis of catechin conjugate with poly(allylamine) has been reported in literature (Chung et al., 2003b), leading to materials with improved physiological properties in comparison with unconjugated catechin. Similarly, a work of 2011 highlights the ability of laccase to catalyze the conjugation of flavonoids (quercetin and catechin) to proteins (α-casein and BSA; Kim and Cavaco-Paulo, 2012). The results showed that quercetin conjugates presented much higher antioxidant activity compared with protein–catechin conjugates (2–3 times higher).

Table 2 Summary of the most recent natural and synthetic polymers modified by conjugation with polyphenols and their biological activity

Polyphenol	Matrix	Effect
Flavonoids	Chitosan	- Antioxidant properties - Antimicrobial activity
Catechin–gallic acid	Chitosan	- Antioxidant properties
Caffeic acid	Chitosan	- Antioxidant properties - Antimicrobial activity - Anticancer effects
Eugenol and carvacrol	Chitosan	- Antioxidant properties - Antimicrobial activity
Quercetin	Starch	- Antioxidant properties - Antiinflammatory effects
Catechin	Dextran	- Anticancer effects
Catechin	Alginate–inulin	- Antioxidant properties
Catechin	Inulin	- Thermo-responsive antioxidant
Ferulic acid–α-tocopherol	Cellulose	- Antioxidant properties
Ferulic acid	Dextran	- Antioxidant properties
Caffeic acid–cinnamic acid	Lysozyme	- Antimicrobial activity
Catechin	Gelatin	- Antioxidant properties - Inhibition of LDL oxidation
Gallic acid	Gelatin	- Antioxidant properties - Antiinflammatory effects - Anticancer effects - Neuroprotection - Antiiperglycemic effects
Hydroxycinnamic acids	Antioxidant peptides	- Antioxidant properties
Caffeic acid	Antioxidant peptides	- Antioxidant properties
Curcumin	Peptides	- Inhibition of β-amyloid fibril formation
Curcumin	Polyamine	- Neuroprotection
Catechin	Poly(allylamine)	- Antioxidant properties
Catechin–quercetin	Bovine serum albumin–α-casein	- Antioxidant properties
Catechin	Poly(ε-lysine)	- Antioxidant properties - Inhibition of collagenase-hyaluronidase
Salicylic derivatives	Poly(melamine)	- Antimicrobial activity
Resveratrol	poly-ε-caprolactone-*co*-acrylic acid	- Antioxidant properties - Bone protection
Ferulic acid	Poly(methacrylic acid)	- Antioxidant properties - Antimicrobial activity
Quercetin	Poly(methacrylic acid)	- Anticancer effects
Quercetin	β-cyclodextrin-Polyvinylpyrrolidone–pluronic	- Antioxidant properties
Cinnamic acid	Epoxidized soybean oil	- Antioxidant properties
Catechin	Oligomeric silsesquioxane	- Antioxidant properties
Curcumin	Polyethylene glycol	- Antioxidant properties
C. Platycladi extract	Polysaccharides	- Antimicrobial activity
Gallic acid	Carbon nanotubes	- Antioxidant properties - Antiinflammatory effects
Catechin	Omopolymer	- Antioxidant properties
Epigallocatechin-3-gallate	Omopolymer	- Antioxidant properties
Quercetin	Omopolymer	- Antioxidant properties
Rutin	Omopolymer	- Protection of Endothelial functions
Catechin	Omopolymer	- Inhibition of LDL oxidation - Inhibition of xanthine oxidase
Trolox	Omopolymer	- Antioxidant properties

Similarly, a conjugate of catechin and poly(ε-lysine) was proposed as inhibitory agent against the disease related to enzymes such as collagenase, hyaluronidase, and xanthine oxidase (Ihara et al., 2004).

In different works, the conjugation of polyphenol was obtained in terms of synthesis of oligo-derivative of the antioxidant. This was the cases of trolox (Wattamwar et al., 2011), quercetin (Kakran et al., 2011), catechin (Kurisawa et al., 2003a; Mita et al., 2003), and EGCG (Kurisawa et al., 2004). In the first case, poly (trolox ester) nanoparticles were evaluated for their ability to either inhibit or induce cellular oxidative stress in a dose-dependent fashion. This polymer delivery form possessed a unique ability to suppress protein oxidation, a feature not seen in the free drug form, emphasizing the

advantage of the delivery/dosage formulation on the regulating cellular response.

In the case of quercetin, the dissolution rate of the poorly water-soluble antioxidant drug was enhanced by fabricating its nanoparticles, complexes, and solid dispersions with β-cyclodextrin, polyvinylpyrrolidone, and pluronic F127 using evaporative precipitation of nanosuspension. Similarly, a biocompatible route for the direct synthesis of water-soluble polyquercetin was proposed by using horseradish peroxidase as catalyst (Bruno et al., 2010). The resulting polymer was envisioned in order to be more thermally stable suitable for the highly regulated food industry and/or drug industry since the starting materials, solvents, and catalyst used are biocompatible.

Regarding EGCG, the polymer obtained by its laccase-catalyzed oxidative coupling showed much higher superoxide anion scavenging activity than the EGCG monomer (Kurisawa et al., 2004).

Catechin was polymerized by using both laccase and horseradish peroxidase as catalysts, and the obtained poly(catechin) was found to greatly scavenged superoxide anion in a concentration-dependent manner, and almost completely scavenged at 200 μM, while the inhibition activity of monomeric catechin starts at 300 μM (Kurisawa et al., 2003a).

Laccase was also employed for the synthesis of polymeric derivatives of rutin (Kurisawa et al., 2003b), with the resulting poly(rutin) able to enhance the cell viability in the endothelium with higher protection effects against the oxidative damage in comparison to that of the rutin monomer.

Uyama (2007) made a detailed overview of polyphenol oligo-derivative of catechin. He focused the attention on poly (catechin)s condensed through acetaldehyde, showing that this synthetic approach is one of the most efficient synthetic approaches to improve the physiological properties of catechin. The first tested effect was the inhibitory activities against LDL peroxidation, and it was found that catechin-acetaldehyde polycondensate showed greater activity compared to monomeric catechin and that the activity was dependent on the catechin unit concentration. The activity on xanthine oxidase was then evaluated for *p*-hydroxybenzaldehyde polycondensate; an inhibition effect higher than that recorded for monomeric catechin (which did not exhibit inhibition activity to a concentration up of 300 μM) was demonstrated. Moreover, an increasing inhibition effect as the concentration of the repeating catechin unit enhanced was also verified. According to the author evaluation, this may be due to the interactions (hydrogen bonds and electrostatic interactions) of a phenol group in the side chain of *p*-hydroxybenzaldehyde polycondensate and xanthine oxidase.

In Esen and Kusefoglu (2003), with the aim to obtain rigid and load-bearing thermosetting polymers from renewable resources, epoxidized soybean oil was reacted with cinnamic acid using triphenyl phosphine as a catalyst, obtaining cinnamate esters of epoxidized soybean oil (ESOCA). The linkage of a cinnamate ester to epoxidized triglyceride gives entry to inherently photopolymerizable derivatives of plant oils, which were then homopolymerized or copolymerized by free radical initiation. Depending on the amount and nature of the comonomer used (styrene, vinyl acetate, and methyl methacrylate), structurally rigid copolymers have been obtained.

Finally, an emerging research field is the conjugation of natural occurring polyphenols within nanomaterials (Li et al., 2011b).

In recent works, a bio-conjugate of polyhedral oligomeric silsesquioxane and catechin, synthesized via horseradish peroxidase catalysis, has been proposed as a starting substrate to construct nanocomposites with precise control of nano-architecture and properties (Ihara et al., 2005).

The conjugation of curcumin with poly(ethylene glycol) assisted magnetic iron oxide nanoparticles was achieved through a sonication mediated route (Konwarh et al., 2010), and, as a result, a synergic effect in free radical scavenging potency of the phytocompound in conjugated state was detected.

In a more recent work, the synthesis of Ag nanoparticles by reducing $AgNO_3$ with the *C. Platycladi* extract was investigated and the antibacterial activities of the nanoparticles were evaluated. Authors found that the biomolecules in the extract, such as reducing sugars, flavonoids, saccharides, and proteins, played a dual role as reducing and protecting agents and that the reducing sugars and flavonoids were mainly responsible for the bioreduction of silver ions (Huang et al., 2011).

As reported for natural polymers, the conjugation of polyphenol compounds to nanomaterials was also performed by grafting procedure. In particular, the grafting of gallic acid onto CNTs was obtained by the radical insertion of gallic acid on the CNT's shell (Cirillo et al., 2011). Specific tests were performed to prove that the GA-CNT conjugate retains all the biological properties of the free polyphenol. In particular, antioxidant and enzymatic tests (DPPH and hydroxyl radical scavenging activity, inhibition of linoleic acid peroxidation, total antioxidant activity, AChE inhibition) imply the reducing power, the scavenging effect, and the AChE inhibitory activity of the conjugate. Importantly, the HET-CAM test confirmed the biocompatibility of the bio-conjugate, indicating that it is nonirritant and well tolerated within the biological environment.

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