Antioxidant and prooxidant actions of plant extracts and plant-derived compounds

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Summary

Free radical oxidation of the lipid components in foods by the chain reaction of lipid peroxidation is a major strategic problem for food manufacturers. For this reason, lipid containing foods are stabilised during processing by addition of food-grade antioxidants. There is a growing interest in the use of plant extracts and plant-derived antioxidant compounds (such as those from rosemary) in the preservation of food materials and, in their propensity to serve as antioxidant prophylactic agents. Before advocating in vivo applications, it is critical to first establish the antioxidant and prooxidant profile(s) of the extracts and compounds in vitro.

Introduction

Lipids are important reserve storage materials which play important roles in metabolism within cells by providing the source of energy. Within the food matrix, the free radical reaction of peroxidation contributes to food deterioration often as a consequence of a number of products which affect overall food quality, taste, smell and nutritional value.

The extent to which oxidation of fatty acids and their esters occurs in foods depends on the chemical structure of the fatty acid, the water activity of the food matrix, the nature of food processing and the temperature at which the foods are stored and/or cooked, and the constituent antioxidants. Free radical oxidation in foods and in living organisms may be considered from the standpoint of four distinctive interfacial groups: bulk food lipids (eg oils), dispersed food lipids (eg membranes and emulsions - salad dressing), dispersed lipids in living organisms (membranes and organelles) and free radical reactions in watery fluids in organisms (eg cytoplasm, plasma) (reviewed in 1-5).

The pro-oxidant and antioxidant considerations may have different implications for each of the groups, eg it may be totally irrelevant for the bulk food lipids but highly relevant for the dispersed food lipids and biological fluids in organisms (3). It is also perfectly possible for an antioxidant to protect in one system but to fail to protect, or even sometimes to cause damage, in others. Antioxidant inhibitors of lipid peroxidation may not protect other targets (such as DNA and protein) against damage, and sometimes can even aggravate such damage (see below). This may not matter much in the food matrix, since damage to DNA and proteins, unless very excessive, will not alter the taste or texture of food (6). However, it is of the greatest importance in vivo. For example, butylated hydroxyanisole (BHA) is a powerful inhibitor of lipid peroxidation, and yet huge dietary doses of it can induce cancer of the rat fore-stomach, and it has been suggested that oxidative DNA damage could be involved (7).

The interrelationship between free radicals and oxidants collectively referred to as reactive oxygen species (ROS) and antioxidants in humans is very complex indeed. A compound might exert antioxidant actions in foods by inhibiting lipid peroxidation or in vivo by inhibiting generation of ROS, by directly scavenging free radicals and/or by raising the levels of endogenous antioxidant defences (eg by up-regulating expression of the genes encoding the antioxidant enzymes superoxide dismutase, catalase or glutathione peroxidase). There is an interest in the use of natural antioxidants, such as those from rosemary extracts, in the preservation of food materials (8-11).

Herbor 025 (rosemary extract fortified with carnosol and carnosic acid) and spice cocktail provencal (an aromatic blend of rosemary, thyme, oregano and sage fortified with carnosic acid, carnosol, thymol and carvacrol) (Nestlé Research Center, Lausanne, Switzerland), are classic examples of such new generation food-grade plant extract antioxidants. Plant extracts that have also been proposed to contain antioxidant capabilities include cocoa shells, oats, tea, olives, garlic, ginger, red onion skin, grapes, apple cuticle, wheat gliadin, korum rind, licorice, nutmeg, clove, oregano, thyme, mustard leaf seed, chia seed, peanut seed coat, birch bark, carob pod, tempeli, yam, mango, vanilla, and mangostum. Flavonoids and other polyphenols found in some of these extracts are widely discussed as potential antioxidant prophylactics (12-14).

Antioxidant versus prooxidant actions: Basic considerations

Simple experiments (3) have been developed to facilitate assessment of antioxidant ability and to test for possible prooxidant effects using different molecular targets—DNA, proteins, lipids membranes or oil systems.

This 'screening' approach can be used to rule out direct antioxidant activity in vivo: a compound that is poorly effective in vitro will not be any better in vivo. It can also alert one to the possibility of damaging effects. In theory, β-carotene has remarkable antioxidant chemistry but this has been difficult to demonstrate in a beneficial manner in biological systems (11-16). During in vitro testing, it is essential to examine the action of a compound over a concentration range that is relevant to its intended use. Scavenging of peroxynitrite ($ONOO^-$) by antioxidants based on assays involving tyrosine nitration and inactivation of α1-antiproteinase is suggested as a useful research tool providing additional valuable information on the antioxidant profile of the biomolecules. This is illustrated with the action of ergothioneine, a natural antioxidant (17).

Assessment of potential antioxidant and prooxidant actions

Antioxidants may act at different levels in the oxidative sequence involving lipids. They may for example, act by decreasing localised oxygen concentration, preventing first-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ions in forms that will not generate the lipid peroxidation initiating species, decomposing peroxides by converting them to non radical products such as alcohols, and chain-breaking whereby intermediate radicals such as peroxyl and alkoxyl radicals are scavenged to prevent continued hydrogen abstraction. The extent to which oxidation of fatty acids and their esters occurs depends on the chemical structure of the fatty acid (1, 2, 4, 8).

The antioxidant activities of plant extracts and antioxidants can be determined with a Rancimat apparatus by measuring the induction period of oils or fats containing the anti-oxidant, using the Automated Swift Test. The principle of this test is to bubble air through heated oil and to monitor continuously the conductivity of water in which the effluent gas is trapped. The tests for which data are given below were performed with 5 g fat and 0.05-1% concentration of antioxidant mixture at an air flow rate of 20 litres/hr and a temperature of 110°C. The antioxidant index was calculated as:

$$\text{Antioxidant index} = \frac{\text{Induction period of fat with extract or antioxidant}}{\text{Induction period of fat alone}}$$

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Extract	Chicken fat	Lard	Soya oil	Sunflower oil
Herbor 025 0.05% w/w	1.6	3.6	1.3	1.2
Spice cocktail 1% w/w	8.3	9.1	1.8	2.0
Rosemary 1% w/w	12.6	11.4	2.1	2.3
Sage 1% w/w	8.4	8.5	1.8	1.8
Thyme 1% w/w	5.7	4.8	1.2	1.3
Oregano 1% w/w	3.4	2.9	1.3	1.3
Ginger 1% w/w	2.4	2.9	1.1	1.1
Turmeric 1% w/w	1.8	1.6	1.1	1.1
Cayenne pepper 1% w/w	1.2	1.1	1.0	1.1

Other measures of antioxidant action

Measurements of lipid peroxidation using rat liver or cardiac microsomes, ox-brain phospholipid liposomes, arachidonic acid, and other lipid model systems (eg emulsified oil systems) should be the next line of tests to establish the potential antioxidant action of the dietary antioxidant compounds (9, 14, 18-21). Antioxidant index based on the ability to scavenge peroxyl radicals (22) and alkyl peroxyl radicals (23) also provide worthwhile data. The spectrophotometric technique, total antioxidant activity (TAA) or the Trolox equivalent antioxidant activity (TEAC) method (24), involves the generation of the long-lived specific radical cation chromophore of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) by controlled chemical oxidation. The $ABTS^{\cdot+}$ radical cation has absorption maxima in the near-infrared region at 645, 734 and 815 nm. The TEAC reflects the ability of hydrogen- or electron-donating antioxidants to scavenge the $ABTS^{\cdot+}$ radical cation compared with that of Trolox C. The antioxidant suppresses the A734 to an extent and on a time scale dependent on the antioxidant activity.

Brand-Williams et al (19) discussed a method involving use of the free radical 2,2 diphenyl-1-picryl-hydrazyl (DPPH.). Antioxidant compounds are allowed to react with the radical in a methanol solution. The reduction of DPPH. is followed by monitoring the decrease in its absorbance at a characteristic wavelength during the reaction. The DPPH. radical absorbs at 515 nm, but upon reduction by an antioxidant (AH):

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$$

or a radical species ($R^{\cdot}$):

$$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH\text{-}R,$$

this absorption disappears.

Antioxidants that protect lipids against free radical damage may actually accelerate damage to other molecules such as DNA, carbohydrates, and proteins under certain conditions. This points to the need to examine suggested antioxidant activ-ity or the activity of components with a proposed 'anti-oxidant cocktail' using assays involving DNA, proteins and carbo-hydrates.

The deoxyribose assay

The deoxyribose assay allows determination of rate constants of reactions with $OH^{\cdot}$ radicals, assessment of abilities to exert prooxidant action, and assessment of abilities to chelate metal iron. Studies using the deoxyribose assay can provide useful information on the likelihood that molecules could chelate iron ions in a way that prevents them from catalysing $OH^{\cdot}$ formation. Thus when iron is added to the assay mixture as ferric chloride instead of as ferric-EDTA, some

of the Fe^{3+} ions bind to deoxyribose, and damage to the sugar becomes site-specific such that the $OH^{\cdot}$ formed by bound iron ions immediately attacks the deoxyribose (25). The ability of a substance to inhibit deoxyribose degradation under these reaction conditions is a measure of its ability to interfere with site-specific Fenton chemistry (25, 26).

When ascorbate is omitted from the deoxyribose reaction mixture, the ability of added compounds to reduce the Fe^{3+}-EDTA complex can be tested. This gives an index of prooxidant action, allowing assessment of antioxidant molecules in non-lipid systems. The Fe^{3+}-EDTA complex has a tested propensity to be reduced by the prooxidant (if, indeed, it is able to do so). It follows that the redox potentials of other metal complexes (which may be physiologically relevant) would vary.

This simple idea led to the proposal to use assays involving DNA damage to specifically test for the abilities of dietary antioxidants to exert prooxidant actions, different from their intended abilities to minimize oxidation of lipids. The tests involve measurement of DNA damage in the presence of bleomycin-iron and copper-1,10-phenanthroline complexes. They do have unique features (3).

Measurement of prooxidant action by use of the bleomycin-iron dependent DNA damage:

The bleomycin-iron(III) complex by itself is inactive in inducing damage in DNA. Oxygen and a reducing agent or hydrogen peroxide are required for the damage to DNA to occur. DNA cleavage by bleomycin releases some free bases and base propenals in amounts that are stoichiometric with strand cleavage. When heated with thiobarbituric acid (TBA) at low pH, base propenals rapidly decompose to give malondi-aldehyde (MDA) which combines with TBA to form a pink adduct. A positive test is obtained when the compound is able to reduce bleomycin-Fe^{3+}-DNA complex to the more active bleomycin-Fe^{2+}-DNA complex (in the presence of oxygen) in the absence of added ascorbate in the reaction mixture resulting in DNA damage (3, 14).

Measurement of prooxidant action by the copper-phenanthroline dependent DNA oxidation

The original copper-phenanthroline assay was developed to measure copper-ions in biological fluids, but has been adapted as a method for assessing the prooxidant action of food additives and/or nutrient components (3, 14). Hydrogen peroxide is implicated in the mechanism of the DNA damage by the copper-phenanthroline system. Hydroxyl radicals are involved in the damage to DNA caused by the copper-phenanthroline system. Unlike the bleomycin-iron mediated damage to DNA, damage in the copper-phenanthroline system is confined mainly to the DNA bases. The small amount of DNA sugar damage is what the copper-phenanthroline assay measures. When a reducing agent is omitted from the reaction mixture, no damage to deoxysugar in DNA occurs in this system. Increasing the concent-rations of the reducing agents such as ascorbate and/or mercapto-ethanol lead to increased deoxysugar damage. This is in agreement with the view that the nuclease activity of copper-phenanthroline complex is potentiated by thiols, a superoxide generating system, xanthine-xanthine oxidase and NADH in the presence of hydrogen peroxide (27).

Concluding remarks

Circumventing potential prooxidant action could contribute to increased protective ability of dietary antioxidants towards susceptible substrates. For example, proteins can protect DNA against the prooxidant actions of some flavonoids and polyphenolic compounds in vitro (14).

Table 2 (data abstracted from reference 14) compares the rate constants of the reactions of various plant-derived antioxidants with the reactive trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$) and contrasts them with the respective values for Trolox C and propyl gallate.

Current research directed towards the understanding the role of free radicals, plant extracts, plant-derived antioxidants in foods and in nutrition and in human health is being complemented with the development and validation of biological markers with which scientists could begin to delineate the efficacy of dietary antioxidants (28).

Table 2 Reactions of 'natural' antioxidants in assays involving ROS

Dietary components	Lipid oxidation	Rate constants with CCl_3O_2 ($M^{-1}s^{-1}$)	Dietary sources
vanillic acid	antioxidant	3.97×10^6	vanilla
vanillin	antioxidant	9.54×10^7	vanilla
thymol	antioxidant	3.82×10^6	oil of thyme
carvacrol	antioxidant	3.92×105	oil of thyme
6-gingerol	antioxidant	4.67×10^6	ginger oil
zingerone	weak antioxidant	5.63×10^6	ginger root
ferulic acid	antioxidant	7.5×10^6	Chia, Mexican plant
+catechin	antioxidant	6.1×10^6	green tea
±catechin	antioxidant	6.1×10^6	green tea
-Epicatechin	antioxidant	7.3×10^6	green tea
hydroxytyrosol	antioxidant	8.37×10^6	olive
carnosic acid	antioxidant	2.7×10^7	rosemary
carnosol	antioxidant	1.3×10^6	rosemary
vitamin E	antioxidant	4.89×10^8	synthetic, plant-derived
vitamin C	prooxidant but often considered as an antioxidant	1.3×10^7	synthetic, plant-derived
trolox C	antioxidant	2.23×10^7	vitamin E analogue
propyl gallate	antioxidant	1.67×10^7	synthetic
quercetin	antioxidant	3.0×10^7	flavonoid
morin	antioxidant	3.01×10^7	flavonoid
chrysin	antioxidant	9.86×10^7	flavonoid
fisetin	antioxidant	4.09×10^7	flavonoid

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