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Review

We have been actively involved in the isolation and characterization of endogenous plant antioxidants that are believed to inhibit lipid peroxidation and offer protection against oxidative damage to membrane functions. Antioxidants have been isolated from conventional food sources, such as tea (green and black), sesame and wild rice, and also from other plant sources, such as rice hulls, and crude plant drugs. Data on new types of water-soluble and lipid-soluble plant antioxidants are provided, and the biological activity and functionality of these antioxidants are discussed.

It is well known that humans, as they grow older, become less active, have an increased probability of illness, and generally experience a loss of optimum function of all physiological systems. Evolutionary processes have engendered the survival of individuals of all living species until the time that they are able to reproduce and pass on their genetic codes to their offspring. At sexual maturity, an age-dependent process sets in, progressing gradually, and leading eventually to the death of an individual, if death has not occurred from another means before then¹.

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The contribution of plant food antioxidants to human health

Narasimhan Ramarathnam, Toshihiko Osawa, Hiroto Ochi and Shunro Kawakishi

Oxygen metabolism and free-radical formation in biological systems

Oxygen, a vital component for the survival of the human species, is present in the atmosphere as a stable triplet biradical (3O_2), in the ground state. Once inside the human body, it can be transformed, by a four-electron reduction process, to water, producing a superoxide radical ($O_2^{\cdot-}$), a hydroxyl radical ($\cdot OH$) and hydrogen peroxide (H_2O_2) as the reactive intermediates (Fig. 1). Singlet oxygen (1O_2) is formed from the excited state of various sensitizers such as chlorophyll, acridine and other pigments. Among the major cellular and extracellular components, proteins, enzymes, lipids, DNA and RNA form the primary targets for these reactive oxygen species. However, oxidation of the unsaturated fatty acid components of cell membranes is the oxidative event that occurs most frequently inside the human body².

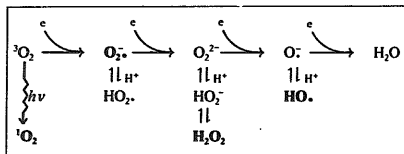


Fig. 1

Scheme to illustrate the formation of reactive oxygen species (shown in bold type) from the stable triplet oxygen, ${}^3\text{O}_2$.

Lipid oxidation has both positive and negative effects. At low levels, the peroxidation products of lipids are responsible for the desirable aroma of fried foods³, and some of the characteristic flavor properties of cooked meats of different species⁴, roasted nuts⁵, and so on. On the other hand, lipid peroxidation not only poses problems in the development of rancidity in processed foods⁶, but also causes serious damage to the human body⁷. The excess production of reactive oxygen species, particularly hydroxyl radicals, can easily initiate lipid oxidation in the cell membrane, resulting in the formation of lipid peroxides. Many researchers have shown that lipid peroxides and reactive oxygen species are involved in the development of a variety of diseases, including cancer, and also accelerate aging. Several secondary products of lipid oxidation, especially malondialdehyde and 4-hydroxynonenal, are themselves reactive; they have been shown to react with biological components such as proteins, amino acids and DNA⁸. Malondialdehyde, which has been shown to be formed both enzymatically and non-enzymatically, has been implicated in aging, mutagenesis and carcinogenesis⁹.

The metabolic processes that involve complex oxidation-reduction reactions are governed collectively by enzymes, hormones, trace elements, and so on. Most living organisms possess extremely efficient defense and protective systems in those cells that are essential for defending the organism against oxidative stress induced by reactive oxygen species. However, the capability of such protective systems gradually decreases with age, resulting in disturbances in the normal redox equilibrium that is established in healthy systems. Such disturbances lead to the malfunctioning of the vital organs, the gradual loss of immunity to diseases that tend to be contracted with aging, and eventually death. The endogenous antioxidants distributed in and around living cells, which regulate the various oxidation-reduction reactions, are therefore seen as a potential class of determinants of longevity¹⁰. Thus, in order to replenish the age-induced loss in the capability of endogenous antioxidant defense mechanisms, there is a need to identify new phytochemicals that could be made readily available by the regular intake of conventional foods.

Impact of life style on human health

Smoking is one of the major causes proposed for cancer around the globe, and has been implicated in

over 30% of the cases. Improper diet among subjects, especially in industrialized nations, is another major factor in 35% of cancer and degenerative diseases associated with aging. On the contrary, inflammation due to chronic infection, especially in under-developed nations, has been cited as the cause in over 30% of the cases, whereas occupational hazards (2%) and environmental factors (1%) are the other minor causes¹¹. Thus, if all the external factors could be controlled and kept within reasonable limits, cancer and degenerative diseases associated with aging could be adequately regulated by the intake of a healthy diet.

In 1945, the average life span of Japanese people was 50 years for men and 53 years for women. Recently published figures indicate that women in Japan can expect to live for 81.4 years and men for 75.6 years¹². Many factors such as improvements in public sanitation, the introduction of new antibiotics, and awareness of eating habits and health through proper education have been suggested to be responsible for the increase in the average life span in Japan. However, of these factors, a combination of the best of Western food and of traditional Japanese food is currently the focus of attention for improving the health of old people in Japan.

Recent publications indicate that there is much evidence that plant antioxidants play an important role in biological systems *in vitro* as agents for antioxidative defense. Antioxidant compounds have already been found in numerous plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves and leaf wax, barks and roots, spices, herbs and crude plant drugs. The isolation and identification of several plant antioxidants from leaf wax, rice and sesame seeds have been undertaken by us. With this concept of food and health in mind, this article will emphasize the contribution of plant food antioxidants to human health, in general. The biological activity and functionality of some of the components will also be discussed.

Green tea consumption and implications on human health

The custom of drinking green tea in Japan originated from China in ~800 AD. Green tea has been valued from ancient times as a powerful and miraculous medicine, vital for the maintenance of health. In recent years, research on green tea has progressed to such an extent that this belief has almost been proved to be true. Epidemiological studies have shown that the death rate from cancer is surprisingly low in the Shizuoka Prefecture of Japan, a major green-tea-producing region¹³. Animal studies, using mice, have also proved the beneficial effects of components of green tea, mainly catechin, in both inhibiting the initiation of cancer and suppressing the growth of tumor cells¹⁴.

Green tea also has beneficial effects on the regulation of blood cholesterol levels. A rapid increase in the levels of low-density lipoprotein (LDL) was observed in the blood plasma of rats fed with a fat-rich diet, whereas rats fed the same diet supplemented with 1% catechin, the main antioxidant in green tea, showed

remarkably reduced levels of LDL cholesterol. However, the catechin supplement did not have any impact on the levels of high-density lipoprotein (HDL), an essential cholesterol component¹⁵. Green tea catechin is also believed to regulate blood pressure, and can help to lower blood sugar levels. Extracts of green tea or green tea catechin, when fed to diabetic mice at a dose of 1%, reduced blood sugar levels by 20–35%. Tolbutamide, a synthetic blood-glucose-lowering drug, lowered blood sugar levels by only 15%, when administered orally¹⁶.

The membranes of red blood cells are known to contain high levels of polyunsaturated lipids, and are therefore very susceptible to oxidation. We have evaluated the antioxidative activity of tea polyphenols by a simple and convenient *in vitro* antioxidative assay system that uses rabbit erythrocyte (red blood cell) membrane 'ghosts'. [The rabbit erythrocyte membrane ghost system was prepared by first washing commercial rabbit blood with isotonic buffer solution several times and then subjecting it to centrifugation. The resulting cells were lysed in phosphate buffer (10 mM) and then ultra-centrifuged.] Erythrocyte ghosts prepared from both human and rabbit red blood cell membranes showed almost the same extent of lipid peroxidation, induced by *t*-butylhydroperoxide¹⁷. Thus, the evaluation of the *in vitro* antioxidative activity of the tea polyphenols was carried out using the rabbit erythrocyte membrane ghost system. Of the four tea catechins evaluated (Fig. 2), (-)-epicatechin gallate and (-)-epigallocatechin gallate produced the strongest protection against lipid peroxidation, and were also found to be more active than the standard antioxidants α -tocopherol and propyl gallate².

On the applications front, we have compared the antioxidant properties of green tea collected from different tea-producing regions in Japan. Samples were procured at various times during the tea-producing season, such as during early, mid- and late spring and early summer. Aqueous extraction of the tea samples was carried out under controlled conditions, so as to give the extract the optimum aroma, taste and color characteristics. The extracts were tested for their antioxidant activities, as determined by the suppression of the oxidation of deoxyribose induced by hydroxyl radicals and also of the autoxidation of linoleic acid. The superoxide radical and hydroxyl radical scavenging properties of the tea extracts were also determined by the use of electron spin resonance (ESR) spectrometry. Although differences in the antioxidant and radical-scavenging properties of the extracts could not be detected between samples collected from different regions of production, seasonal differences had a pronounced effect on their activities.

Tea leaves collected between late spring and early summer were found to be extremely effective in scavenging superoxide radicals (Table 1). The autoxidation of linoleic acid and the oxidation of deoxyribose induced by hydroxyl radicals were also found to be suppressed more effectively by extracts from leaves procured in late spring. However, the extracts obtained from these samples had a strong astringent taste, and also lacked the desirable fresh green tea aroma. These

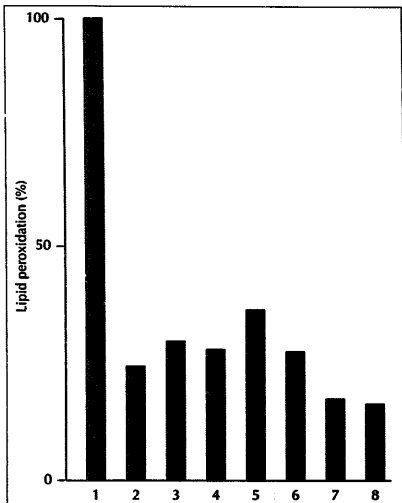


Fig. 2
Antioxidative activity of green tea polyphenols (final concentration: 100 μ M), compared with a control sample and α -tocopherol, and determined by the rabbit erythrocyte ghost system. (1) Control; (2) α -tocopherol; (3) (+)-catechin; (4) (-)-epicatechin; (5) (+)-gallocatechin; (6) (-)-epigallocatechin; (7) (-)-epicatechin gallate; and (8) (-)-epigallocatechin gallate. (Data taken from Ref. 2.)

characteristics have been carefully studied at the Japan Institute for the Control of Aging, the problems resolved, and an instant type of 'antioxidant green tea' has been developed (marketed by Nikken Foods, Fukuroi City, Japan). This product, which has good antioxidant and organoleptic properties, has been commercially available since the beginning of 1993.

Polyphenol-type antioxidants in black tea

Black tea is richer than green tea in theaflavins (Fig. 3), which are dimers of catechins formed by enzymatic oxidation during the manufacture of black tea. In the rabbit erythrocyte ghost system, theaflavin (TF1) was shown to have stronger antioxidative activities than α -tocopherol; however, in a rat liver microsomal system, it was found to be less potent than α -tocopherol. [The rat liver microsomal system was prepared using fresh liver removed from sacrificed Wistar rats. The liver was first homogenized thoroughly, and then the microsomes were prepared using a differential centrifugation method.] Theaflavins were also shown to inhibit the cleavage of calf thymus DNA induced by H_2O_2

Table 1. Superoxide radical scavenging activity (SOSA) values of extracts from green tea leaves harvested at different times of the year*

| Sample | SOSA value (SOSA units/g) ^b |
|---------------------|--|
| Early spring | 145 000 ± 20 000 |
| Mid-spring | 170 000 ± 5000 |
| Late spring | 190 000 ± 5000 |
| Early summer | 230 000 ± 30 000 |
| (Vitamin C control) | (170 000 ± 5000) |

*N. Ramaratnam *et al.*, unpublished

^b3500 SOSA units correspond to the scavenging activity of 1 mg of pure superoxide dismutase enzyme

in the presence of horse heart cytochrome *c*. Hence, theaflavins are expected to be effective in protecting cellular DNA against oxidative damage. Of the theaflavins tested, theaflavin digallate (TF3), which has two gallic acid moieties, exhibited the strongest antioxidative

activity in the rabbit erythrocyte ghost system. Based on a detailed investigation of structure–activity relationships, we concluded that the gallic acid moiety is probably important for the antioxidative and antimutagenic activities of theaflavins¹⁸. The protective roles of other water-soluble antioxidants on mutagenicity and DNA damaging activity induced by reactive oxygen species and lipid peroxidation products are currently being investigated. In addition, attempts are being made to develop anti-oxidant-rich health drinks that contain extracts from green and black tea in combination with other natural extracts.

Antioxidative components found in rice seeds

Rice (*Oryza sativa*) is the principal cereal food in Asia, and the staple food for nearly half of the world's population. We have recently undertaken a comparative investigation of the germination potential of rice seeds from the subspecies *japonica* and *indica* during long-term storage. We have observed that the *indica* rice seeds, on average, retained their ability to germinate when stored at 30°C, even after one year, whereas cultivars of the *japonica* subspecies showed a gradual decline in their germination potential after storage for six months at 30°C¹⁹. We hypothesized that the differences in the

storage stability of *japonica* and *indica* rice seeds could have been due to the differences in the effectiveness of the defense systems in the rice hull, which until now was believed to offer only physical protection to the rice grain. Model experiments were undertaken to induce accelerated aging by initiating lipid peroxidation at 30°C, using hydroxyl radicals generated during the γ -irradiation of the seeds. As shown in Table 2, the seeds showed wide differences in their germination potential and thiobarbituric acid (TBA) values depending on whether or not their hulls were intact during irradiation²⁰. It was concluded that the hull fraction of long-life (*indica*) rice seeds must contain some unique antioxidative constituents²¹. Similar results were obtained when accelerated aging was induced in rice seeds stored with and without intact hulls at a temperature of 60°C²².

A large-scale extraction, isolation and purification process was used to identify the active components as flavonoid substances. One of the active components, when tested by the *in vitro* thiocyanate method, was found to be as active as α -tocopherol, and was identified as isovitexin, a C-glycosyl-flavonoid²¹. A study to determine the mechanism by which these hull antioxidants offer protection is currently being undertaken²³. Also, food

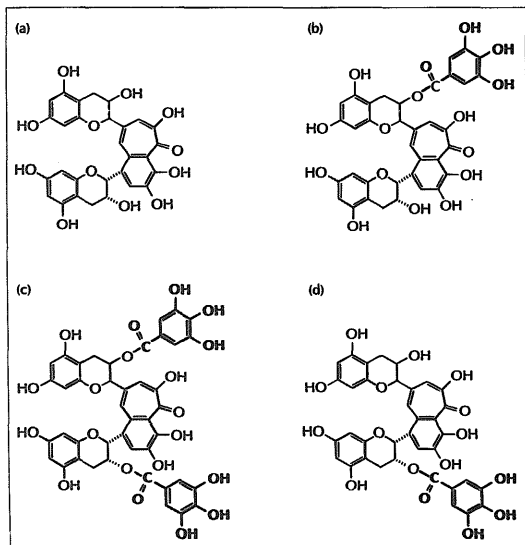


Fig. 3

Structures of black tea polyphenols: (a), theaflavin (TF1); (b), theaflavin monogallate A (TF2A); (c), theaflavin digallate (TF3); and (d), theaflavin monogallate B (TF2B) (bold type indicates the gallic acid moieties).

applications of rice hull antioxidants are being explored. In the cosmetic industry, attempts are being made to use rice hull antioxidants in the formulation of body lotions and creams used mainly for the protection of the skin against the adverse effects of aging.

Recently, we have also isolated and identified antioxidative pigments from black rice. Black rice seeds have the ability to maintain their viability even after long-term storage, retaining their viability longer than the regular white rice seeds from the same species. Large-scale isolation and purification of the antioxidative pigments led to the identification of cyanidine-3-O- β -D-glucopyranoside²⁴, which was found to possess strong antioxidative activity when tested using the *in vitro* thiocyanate method. Cyanidine-3-O- β -D-glucopyranoside was also isolated and identified as the antioxidative pigment in black and red beans²⁵; thus, studies of the relationship between the variety and levels of these antioxidative pigments in cereal crops and beans are now in progress.

Natural antioxidants in other cereal crops

We have also succeeded in isolating the antioxidative components present in young barley leaves; 2'-O-glucosylisovitexin was identified as a unique antioxidative compound²⁶, the activity of which was determined by the *in vitro* TBA assay method. Peroxidation of methyl linoleate was induced by the hydroxyl radical, generated in a Fenton reaction system (Fig. 4). When 2'-O-glucosylisovitexin was added to this system, methyl linoleate peroxidation was drastically suppressed, proving that 2'-O-glucosylisovitexin possesses strong antioxidant activity²⁷. Also, it has been demonstrated that 2'-O-glucosylisovitexin protects squalene against peroxidation induced by irradiation with UVB²⁸. The potential applications of this antioxidant in value-added processed foods are being investigated.

Wild rice (*Zizania aquatica*) is another cereal crop whose applications in the food industry have shown a rapid increase; for example, it is being used in the development of ready-to-eat soups, breakfast cereal mixes, baking mixes and gourmet salad preparations. Unlike regular rice, wild rice belongs to the genus *Zizania*, comprising a grass-like family of plants. Though traditionally grown by native Indians in shallow lakes and streams in northern Minnesota and northern Wisconsin in the USA, and in southern Ontario and Manitoba in Canada, wild rice is now cultivated as a regular crop, chiefly in Minnesota. Its composition resembles that of oats, low in fat (less than 1%) and high in protein (12.5–15%).

The application of wild rice as an extender in the preparation of processed meat products has been well investigated²⁹. Sensory evaluations indicated that beef patties supplemented with wild rice had higher sensory

Table 2. Effect of γ -irradiation on the germination potential and thiobarbituric acid (TBA) values of rice (*Oryza sativa*) seeds, irradiated at different doses with and without intact hulls^{a,b}

| Cultivars | Germination (%) at Radiation dose (kGy): | | | TBA value (OD ₅₃₅) at Radiation dose (kGy): | | |
|------------------------|---|---------|-------|--|-------------|-------------|
| | 0 | 10 | 15 | 0 | 10 | 15 |
| <i>Subsp. indica</i> | | | | | | |
| Katakutara | 100 (100) | 54 (28) | 0 (0) | 0.1 (0.1) | 0.11 (0.11) | 0.12 (0.13) |
| Century Patna | 100 (99) | 52 (26) | 0 (0) | 0.1 (0.1) | 0.12 (0.13) | 0.13 (0.15) |
| <i>Subsp. japonica</i> | | | | | | |
| Koshihikari | 100 (100) | 42 (22) | 0 (0) | 0.1 (0.1) | 0.13 (0.14) | 0.14 (0.18) |
| Kusabue | 99 (99) | 35 (20) | 0 (0) | 0.1 (0.1) | 0.14 (0.16) | 0.15 (0.17) |

^a The figures in parentheses refer to values for rice seeds irradiated without intact hulls

^b Data taken from Ref. 20

OD₅₃₅, Optical density at 535 nm

scores than control patties. Also, the processed product made with wild rice possessed lower cholesterol levels, and a low TBARS value (level of thiobarbituric acid-reactive substances; a measure of the extent of lipid oxidation). These observations indicated that wild rice might contain inherent antioxidants. As an extension of their work, Wu *et al.* investigated the chemical nature of the antioxidant system in wild rice, and attributed the antioxidant activity of wild rice to phytic acid³⁰.

Recently, we have begun investigating the antioxidant components of wild rice. Ethanolic extracts of wild rice were fractionated by column chromatography, followed by purification on high-performance liquid chromatography (HPLC). Instrumental analyses have revealed the presence of other novel antioxidants with stronger antioxidant activities than phytic acid, as determined by the *in vitro* TBA assay method. The structures of three of the active components have been determined. These three active components were 3,4,5-trimethoxybenzoic acid, a phenolic glycoside with 3,4,5-trimethoxycinnamate and *p*-hydroxy acetophenone as the aglycone moieties, and a flavonoid glycoside with 3,4,5-trimethoxycinnamate and luteolin as the aglycone moieties³¹. Preliminary investigations have also shown the presence of other biofactors in wild rice that may have promising applications in the development of an anti-diabetic diet (i.e. a diet rich in functional ingredients that have been shown, in an *in vitro* assay, to reduce the formation of advanced glycation end products, which are found at very high levels in blood samples from diabetic patients). At present, we are actively involved in the application of wild rice in various processed health-food products in combination with traditional Japanese flavoring ingredients.

Antioxidative components in spices, herbs and plant drugs

Herbs and crude drugs prepared from plant materials are traditionally used in many Asian countries, and their pharmacological effects have been extensively studied.

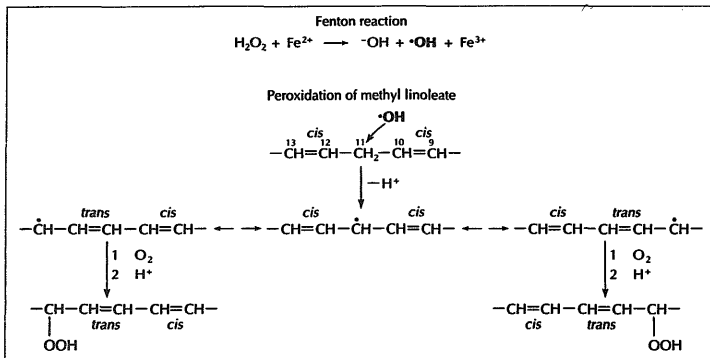


Fig. 4

Peroxidation of methyl linoleate, induced by a hydroxyl radical generated in a Fenton reaction system.

However, only a few reports are available on the antioxidative components of herbs and crude drugs. In view of this, we have screened 32 different types of herbs and crude drugs obtained from Japanese markets and drug stores in Taipei, Taiwan. The levels of tocopherol derivatives in these drug extracts have been quantified using HPLC, and it was concluded that *Osebeckia chinensis* did not possess any tocopherols. However, all of the other herbs and crude drugs screened were found to contain tocopherols as the antioxidative components. These results prompted us to isolate and identify the active principles in the extracts of *O. chinensis*³².

The whole of the *O. chinensis* plant has long been used as a traditional medicine in Taiwan, Japan and China. The dried leaf, stalk and stem of the plant are normally extracted in hot water, and administered as a quick remedy to alleviate body pain, fever and cases of severe influenza. Large-scale purification has resulted in the isolation and identification of many different types of antioxidative components. Most of the antioxidative activity of the extracts of *O. chinensis* is believed to be due to tannin-type antioxidants, in particular casuarinin (Fig. 5). These isolated tannins contain an ellagic acid moiety in their structures, and have almost the same antioxidant activity as ellagic acid itself³³.

Sesame seeds and oils have been used traditionally in Japan, China, Korea and other Asian countries for many years. Sesame oil, in particular roasted sesame oil, is widely used in Chinese and Japanese cooking, and has strong antioxidant activity. Sesaminol, an active component of sesame oil, is unique among antioxidants because it has a superior heat stability³⁴. Also, sesaminol has been shown to effectively suppress the degradation of tocopherols in model systems using corn oil³⁵. The antioxidant activity of sesaminol was evaluated using several *in vitro* lipid peroxidation systems, in particular

the rabbit erythrocyte membrane ghost and rat liver microsomal systems; sesaminol was found to effectively inhibit lipid peroxidation induced by *t*-butylhydroperoxide in the microsomes and the erythrocyte ghost membranes.

The protective role of sesaminol against oxidative damage has also been reported in studies using cultured human diploid fibroblasts at various *in vitro* ages³⁶. Yamashita *et al.*³⁷ recently reported that sesaminol inhibited *in vivo* oxidative damage induced by carbon tetrachloride in senescent-accelerated mice. More recently, in an *in vivo* system, we have found that rats fed a diet supplemented with sesaminol at a dose of 10 mg/100 g of body weight suffered less DNA damage due to oxidative stress induced by carbon tetrachloride than the control rats. The levels of TBARS in blood plasma and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine were measured. Although the detailed examination remains incomplete, we have found that sesaminol inhibits both lipid peroxidation and the excretion of 8-OHdG in urine, as determined by a monoclonal antibody enzyme-linked immunosorbent assay (ELISA) assay method³⁸. The assay system, which is very accurate, fast and convenient, was developed by the Japan Institute for the Control of Aging as one of several assay procedures to measure the degree of oxidative stress and *in vivo* oxidative damage.

Sesame seeds also contain a large quantity of water-soluble lignan glucosides³⁹. These glucosides were found to act as strong water-soluble antioxidants as well as being precursors of lipid-soluble antioxidant lignans. A diet comprising sesame from which all lipid-soluble antioxidants, including sesaminol and tocopherol, were removed by hexane extraction was fed to rats for two months. This diet, which therefore contained only water-soluble antioxidants, inhibited both lipid peroxidation in blood plasma and the excretion of 8-OHdG in

urine, as determined by the monoclonal antibody ELISA assay method³⁸. The application of the antioxidants found in sesame and sesame oil for the development of novel health foods is being actively pursued.

Influence of phenolic components in red wine on human health

Polyphenol oligomers, namely procyanidines, are known to be widely distributed in the plant kingdom. They have been successfully applied in the treatment of several vascular disorders in humans because of their positive biochemical effects on blood vessels⁴⁰. Procyanidines are also effective free-radical scavengers and inhibitors of oxidative enzymes such as xanthine oxidase⁴¹, as determined by measuring the extent of conversion of hypoxanthine to uric acid. The vasorelaxing activity of wine and other grape products including grape juices and grape skin extracts has been well documented⁴². The effect is believed to be induced by quercetin and tannic acid, compounds known to be present in grape skin.

Phytochemicals, especially those in wine products, may also play a critical role in reducing mortality from coronary heart diseases in certain sectors of the French population. A comparative study initiated by the World Health Organization has shown marked differences in mortality and morbidity from coronary heart disease between countries, in particular between French and US populations^{43,44}. Despite having a similar dietary intake of saturated fatty acids, and comparable plasma cholesterol levels, the French subjects were shown to be less susceptible to coronary heart disease than the US subjects.

Using multivariate analyses, it was postulated that the consumption of wine was the only dietary factor responsible for this discrepancy, commonly referred to as the 'French paradox'⁴⁴. An extended investigation demonstrated that the non-alcoholic portion of red wine, particularly the phenolic substances, inhibited the oxidation of human LDL *in vitro*^{45,46}. The assay was carried out by measuring the levels of hexanal and conjugated dienes formed by a Cu²⁺-catalysed oxidation of freshly prepared human LDL.

In view of all these observations, it appears that the consumption of red wine, in moderate amounts, may have a long-term health benefit. Quite often, healthy centenarians in Japan, when interviewed about the secrets of their longevity, strongly advocate drinking moderate amounts of sake, the traditional Japanese rice wine, believing it to be one of the probable reasons for their long life. Work is currently being carried out to characterize the non-alcoholic phenolic antioxidants in rice wine. Steps are also being taken to investigate other non-alcoholic fermentation products like soy sauce, and fermented soybean products such as natto and miso, which have long been used traditionally in Japan.

Antioxidative defense system in seaweeds

Seaweeds are another foodstuff that has been traditionally used in the Japanese diet for centuries. The lipid content of seaweeds is low, and is rich in eicosa-

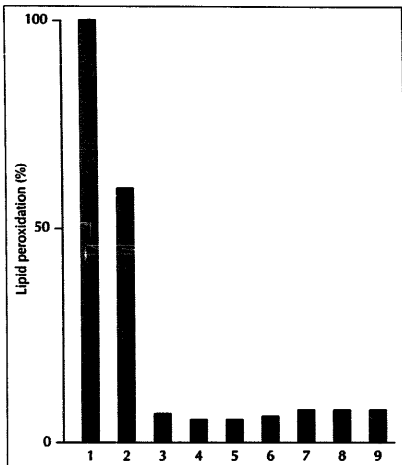


Fig. 5

Antioxidative activities of tannins (final concentration: 25 μ M) isolated from *Osebeckia chinensis*, compared with a control sample and α -tocopherol, and determined by the rabbit erythrocyte ghost system. (1) Control; (2) α -tocopherol; (3) ellagic acid; (4) casuarinin; (5) casuarin; (6) punicacone A; (7) degalloyl-punicacone A; (8) 2,3-[(5)-4,4',5,5',6,6'-hexahydroxydiphenoyl]-D-glucopyranoside; and (9) 4,6-[(5)-4,4',5,5',6,6'-hexahydroxydiphenoyl]-D-glucopyranoside. (Data taken from Ref. 33.)

pentaenoic acid, which has been shown to be effective in lowering blood cholesterol levels in rats⁴⁷. Despite their high content of polyunsaturated fatty acids, it is well known that seaweeds are stable against oxidation during storage. Recently, we undertook an investigation to characterize the chemical nature of the defense system present in seaweeds. Extracts of brown algae were found to contain strong hydroxyl-radical scavengers, as determined by the ESR spectrometry method⁴⁸. Some of the extracts also displayed a strong potential for scavenging superoxide radicals. Studies are in progress to identify the key antioxidant components in seaweeds. In addition, attempts are being made to utilize seaweed extracts in the preparation of unconventional soups, seasonings and salad dressings, both for oriental food applications and to meet the needs of the Western palate.

Conclusions

Lipid peroxidation *in vivo* has been indicated to be the primary cause of many of the cardiovascular diseases such as atherosclerosis, and also in cancer and aging. Consumers, especially in the Western world, were

advised in the 1980s to reduce their fat intake and to increase their intake of food sources that would provide more crude fiber. The current emphasis is on drastically reducing the intake of red meat, and increasing the intake of fresh vegetables and fruits. Without doubt, consumers all over the globe are becoming more health conscious, and are prepared to educate themselves about nutrition.

Scientists in the fields of aging, cancer, diabetes, cardiovascular diseases and many of the diseases related to aging are becoming aware of the value of preventive therapies. Terms like functional foods, designer foods, therapeutic foods, nutraceuticals and the like are already being used by the media. However, the information that is currently available is not sufficient to address all of the unsolved health problems. There is certainly a strong need to investigate plant products in a more systematic way. Effects that have been observed at the test-tube level need to be interpreted carefully, and documented with more *in vivo* evidence. Traditional foods that have been used for several generations are likely to be the subjects of in-depth research in the near future. New types of plant antioxidants and novel biofactors will be developed, and it is very likely that the increased consumption of plant foods will become the trend of the next decade.

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