Oxygen, free radicals, antioxidants and food - some views from the supply side

B.A. Goodman & J.R. Hillman

Introduction to free radicals and antioxidants

Free radicals are chemical species (excluding transition metal and rare earth ions) with unpaired electrons. They are often, but not always, highly reactive, and in biological systems usually have only a transient existence. The reasons for free-radical reactivity lie in the electronic structure of atoms and molecules; the structure of dioxygen, O_2, and the products of its reactions are of particular importance.

All aerobic systems utilise metabolic processes, which are based on free-radical reactions, but some of the products of such reactions can be dangerous, if not properly controlled. Many chemical species are involved in the control of oxidative chemical processes in all biological systems, and these are often referred to as *antioxidants*. The words *antioxidants* and *free-radical scavengers* are often used interchangeably, but antioxidants are not necessarily free-radical scavengers, and free-radical scavengers are not necessarily antioxidants. For example, an extremely important antioxidation reaction is the control of hydrogen peroxide, H_2O_2, which is a precursor of the highly reactive hydroxyl radical, ·OH, but H_2O_2 is not itself a free radical.

Although biological processes are often complex, they are usually highly specific, and the roles played by different molecules possessing, for example, antioxidative properties, can be extremely varied. The general expressions 'antioxidant' and 'free-radical scavenger' are not, therefore, particularly helpful in discussions of health and nutrition. At the present time, it would be better if both were avoided as far as possible, and instead, to concentrate on descriptions of specific chemical reactions, if our understanding of this extremely important area of science is to develop.

Background to the electronic structure of oxygen

The energy states for an electron in an atom are characterised by four quantum numbers (referred to in order of decreasing magnitude as *n*, *l*, *m*, and *s*), which correspond to the electronic shell, orbital shape, orbital direction and electron spin. The principal quantum number, *n*, has integer values, 1,2, 3, *etc.*; *l* can have values n-1, n-2, …0; *m* can have values 0, ±1, ±2, … ±l; and *s* has values of ±1/2 (called m_s). It is also conventional to use letters instead of numbers to describe *l*, and directional coordinates to describe *m*. Thus,

l =	0	1	2	3	4
	s	*p*	*d*	*f*	*g*

so that in terms of *n* and *l*, electronic orbitals are referred to as *1s*, *2s*, *2p*, *3s*, *3p*, *3d*, *etc*. In *s*-orbitals, *m* = 0, and the radical distribution of electron density is spherical. There are three distinct *p*-orbitals (*m* = 0, ±1), with identical radial, but different directional characteristics. The *p*-orbitals are generally depicted as p_x, p_y, and p_z, which represent complex combinations of the quantum descriptions. Similarly, there are five *d*-orbitals (*m* = 0, ±1, ±2), which are depicted as d_{xy}, d_{xz}, d_{yz}, $d_{x^2-y^2}$, d_{z^2} (Fig. 1). Each of these orbitals may contain two electrons, differing in their values of m_s, usually just referred to as 'spin'.

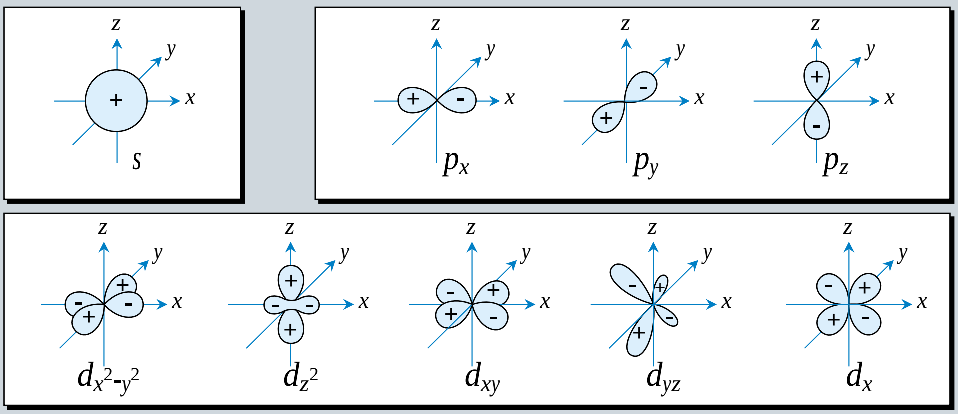

Figure 1 Diagrammatic representation of the radial distribution of electrons in s, p, and d atomic orbitals.

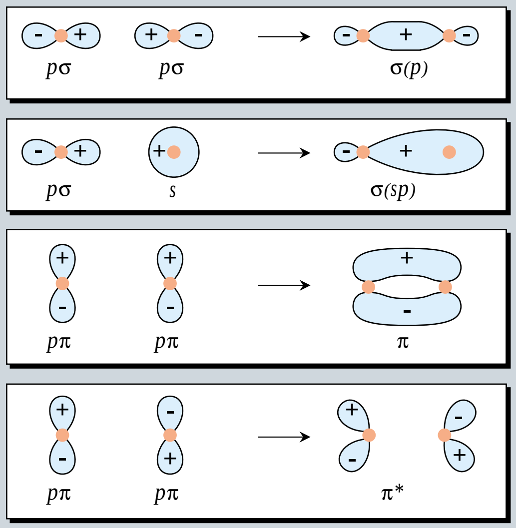

Figure 2 The formation of some simple 2-centred molecular orbitals by combinations of atomic orbitals.

There are a number of rules, which govern the distribution of electrons within atoms. The most important state that (i) no two electrons can have an identical set of quantum numbers, and (ii) the electronic orbitals in multielectron atoms (or ions) are filled in order of decreasing stability. Thus, for example, a nitrogen atom with seven electrons has the electronic configuration $1s^2\ 2s^2\ 2p^3$. The *1s*- and *2s*-orbitals are completely filled, whereas only three of the six *2p* states are occupied. The occupancy of these orbitals is determined by Hund's rules, which state that electrons

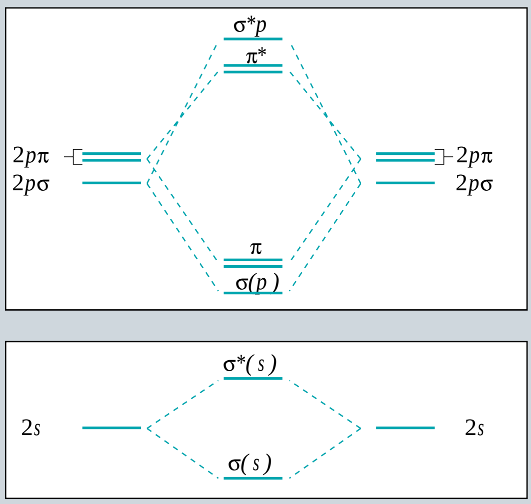

Figure 3 Schematic energy level diagram for molecular orbitals formed from two identical atoms of the 1st row of the Periodic Table (ignoring the core 1s electrons).

with the same *n* and *l* values occupy orbitals with different values of *m* and, as far as possible, have the same values of m_s (*i.e.* their spins are not paired). Similarly, the oxygen atom has the electronic configuration $1s^2\ 2s^2\ 2p^4$ with one *p*-orbital containing a pair of electrons and the others each with a single electron with the same spin. Isolated atoms are not generally stable (except for the inert gases helium, neon, argon, *etc*., which have filled electron shells) and they react to form molecules with more stable electronic structures. Molecular orbitals are formed by combining atomic orbitals on two or more atoms to produce a new set of orbitals (Fig. 2), and a typical molecular orbital energy level diagram for two identical atoms of the 1st row of the Periodic Table is shown in Figure 3.

The population of molecular orbitals is governed by the same rules as those for atomic orbitals. Thus, in the N_2 molecule the orbitals are completely filled up to and including the π-orbitals; all electrons are, therefore, 'paired'. The two additional electrons in O_2 are located in the π*-orbitals. Their combined spin, *S* (Σm_s), can be either 1 or 0 depending on whether the individual spin vectors have the same or opposite sign. It is common to refer to the spin states by their multiplicities, *2S+1*, so the two states for O_2 are $^3O_2$ and $^1O_2$ (triplet or singlet oxygen). Hund's rules then make $^3O_2$ the more stable state. Thus, molecular O_2 is a free radical with two unpaired electrons, a property which is the key to understanding the free-radical chemistry of oxidation processes.

The involvement of free radicals in oxidation processes

The complete reduction of molecular oxygen (*i.e.* oxidation of substrates) involves the transfer of four electrons *per* oxygen molecule to generate the oxide, O^{2-} ion, which has the stable $1s^2 2s^2 2p^6$ electronic configuration:

$$O_2 + 4e^- \rightarrow 2O^{2-}$$

However, intermediate levels of reduction can (and often do) occur. The one-electron reduction of O_2 produces the superoxide free radical anion, $O_2^{\cdot -}$, which contains three electrons (2 paired, 1 unpaired) in the π*-orbitals in Figure 3. Further reduction produces the peroxide ion, O_2^{2-}, in which the π*-orbitals are fully occupied (*i.e.* there are no unpaired electrons).

Peroxides are broken down in the presence of oxidisable transition metal ions, and the hydroxyl free radical (·OH) is generated (the Fenton reaction):

$$\text{e.g. } Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

The ·OH radical is highly reactive and is able to abstract a hydrogen atom (·H) from a wide range of organic compounds, generating a carbon-centred free radical in the process:

$$R\text{-}H + {\cdot}OH \rightarrow R{\cdot} + H_2O$$

These reactions are of particular importance in biology and in the stability of foods, because of their roles in the decomposition of unsaturated lipid molecules. There is, however, a wide range of other reactions in foods that involve free-radical processes, some probably detrimental, some beneficial, and others benign as far as quality perceptions are concerned. Some of these are discussed later in this article, but generalisation is not possible with the current status of our knowledge.

Food quality – what are the most important criteria?

The relative importance of the various food quality criteria differs in different parts of the world. In the UK, in common with other developed countries, however, *safety* is perceived as of overwhelming importance. In addition, microbial contamination causes greater concern than chemical components, although at the current time, the prion agents for transmissible spongiform encephalopathies (TSEs) represent a major problem. However, the public has little ability to use safety as a criterion in food selection, and has to place a huge element of trust in the food producers and suppliers. The recently established Food Standards Agency has a crucial role in ensuring that this trust is justified.

Sensory properties, summarised as appearance, texture, aroma and taste, along with cost and responses to marketing activities, are the main selection criteria that are used for food purchases. With fresh fruit and vegetables, sensory properties, along with relative cost, are the over-riding factors determining choice. In contrast, marketing activities are probably the main factor determining the choice of prepared products, although sensory properties, especially taste, are factors, which determine the extent of any further purchases of a product.

Where then do *nutritional properties* feature as criteria? Except where they are used in marketing tactics, they would appear to be seldom a factor in consumer choice at the present time. A major reason for this is a lack of relevant data upon which the public can make informed choices. Although processed and many prepackaged food products contain typical analytical values for a number of their components, it is not clear, to most people at least, how these should be used in formulating a healthy diet. In the case of fresh products, the impracticability of performing sample analyses at the point of sale invariably means that the public has no information on their chemical compositions. Even at a research level, for most plant foods there is a dearth of analytical data on how the composition of different varieties of particular plant products *vary with environmental conditions during growth and different post-harvest storage regimes.*

For certain types of food product, selection may be based on medicinal properties, and the production of functional foods is anticipated to be a major growth area in the future. There is also growing evidence that specific food products that are already widely available can provide protection against certain ailments (*e.g.* coffee can provide protection against allergic reactions). Other products, purchased because of physiological effects that are produced when they are consumed, may have side effects that can be either beneficial or detrimental. For example, small quantities of alcohol consumed on a regular basis may have beneficial effects on the cardiovascular system, although excessive consumption is demonstrably detrimental to health.

Oxidation processes in foods – impact on quality

Oxidative processes generally lead to a depletion in the levels of antioxidant molecules in stored foods. This is particularly important for fresh fruit and vegetables, which represent the major sources of vitamins in many diets. In Scotland, the decline in ascorbic acid (Vitamin C) contents of potatoes during storage is of particular significance, since for many people this represents the major source of this essential vitamin. Post-harvest storage conditions may, therefore, have a key role in micronutritional quality.

Levels of micronutrient molecules, such as the antioxidant vitamins, in fresh plant materials can be affected greatly by the plant being subjected to biotic or abiotic stress processes. An example is shown in Figure 4 of the decline in ascorbic acid content of leaf tissue from plants as a result of prolonged high humidity conditions. Similar effects have been seen for other stress processes, including the apparently healthy regions of plant tissue subjected to biotic damage. *Oxidative reactions caused by stress processes can, therefore, have a marked effect on the composition of food products.*

In foods in general, it is now generally accepted that consumption of saturated fats is linked to heart disease, whereas unsaturated lipid molecules are thought to have beneficial effects on the consumer.

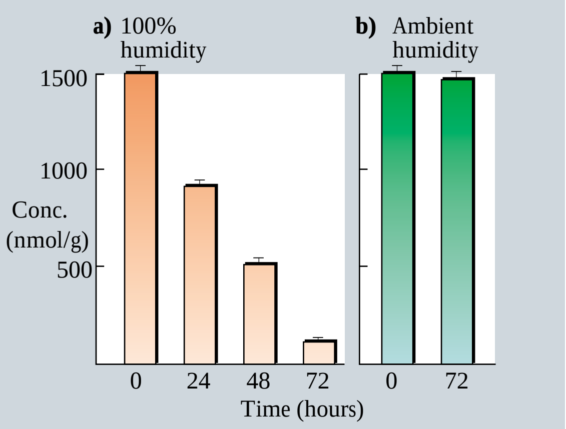

Figure 4 Variation with time of the ascorbic acid contents of *Phaseolus vulgaris* leaves from glasshouse grown plants (a) subjected to 100% humidity in incubation boxes, and (b) control samples at normal humidity levels.

Unsaturated lipids, however, have much lower stability with respect to oxidative processes than their saturated counterparts. The carbon-centred free radicals (L·), derived from reaction of ·OH with lipid molecules, react with oxygen to form lipid peroxy radicals:

$$L\cdot + O_2 \rightarrow LOO\cdot$$

Lipid peroxy radicals initiate a chain reaction with other lipid molecules:

$$LOO\cdot + LH \rightarrow LOOH + L\cdot$$

Decomposition of lipid hydroperoxides occurs through heating or catalysis by transition metal ions:

$$LOOH + M^{n+} \rightarrow LO\cdot + M^{(n+1)+} + OH^-$$
$$LOOH + M^{(n+1)+} \rightarrow LOO\cdot + M^{n+} + H^+$$

The lipid peroxidation process can be terminated by the reaction of two radicals with one another:

$$L\cdot + L\cdot \rightarrow L\text{-}L$$
$$LOO. + L\cdot \rightarrow LOOL$$
$$LOO\cdot + LOO\cdot \rightarrow LOOL + {}^1O_2$$

${}^1O_2$ as described above is the unstable form of O_2 and highly reactive. It readily reacts with other lipid molecules, for example, to produce lipid peroxides, which continue the reaction described above.

In addition to the reaction sequence described above, autoxidation of lipids results in the formation of off-flavours and other undesirable chemical compounds. The lipid peroxy radicals can also undergo intramolecular rearrangement to produce endoperoxides. These are then transformed into endoperoxy hydroperoxides after further rearrangement and reaction with oxygen. Finally, the endoperoxy hydroperoxides decompose in the presence of metal ions to produce malondialdehyde and other low-molecular-mass fragments. Aldehydes and ketones generally have distinct sensory properties, and the unattractive sensory properties of some of these lipid peroxidation products make them sensitive markers of oxidation processes. Addition of antioxidant molecules to food products can delay these lipid decomposition processes, thus increasing the shelf-lives of the products. Improvements in product quality at the point of consumption may not necessarily follow, however, especially if the extended lives of the products are spent in storage.

Oxidation reactions in foods are not limited to the lipid components; reactions involving proteins, carbohydrates and nucleic acids are also extremely important. One of these is the Maillard or non-enzymatic browning reaction, which involves the condensation between reducing sugars and the free amino groups of proteins. The Maillard reaction is ubiquitous in nature, but is particularly important during the processing and storage of foods. It is responsible for changes in flavour, colour and nutritive value during thermal processing, such as during roasting of meat, baking of bread, or brewing of coffee. The initial stage of the Maillard reaction involves the formation of a Schiff's base (aldamine) between amino and carbonyl groups (Fig. 5), which may rearrange to an Amadori compound (1-amino-1-deoxy-2-ketose). Amadori compounds may then react further by several pathways, including enolization, dehydration, aldol condensation and oxidative degradation. Many of the

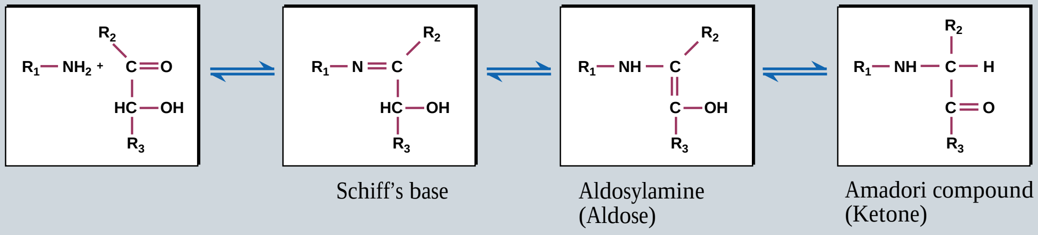

Figure 5 Chemical reactions involved in the initial stages of the Maillard reaction.

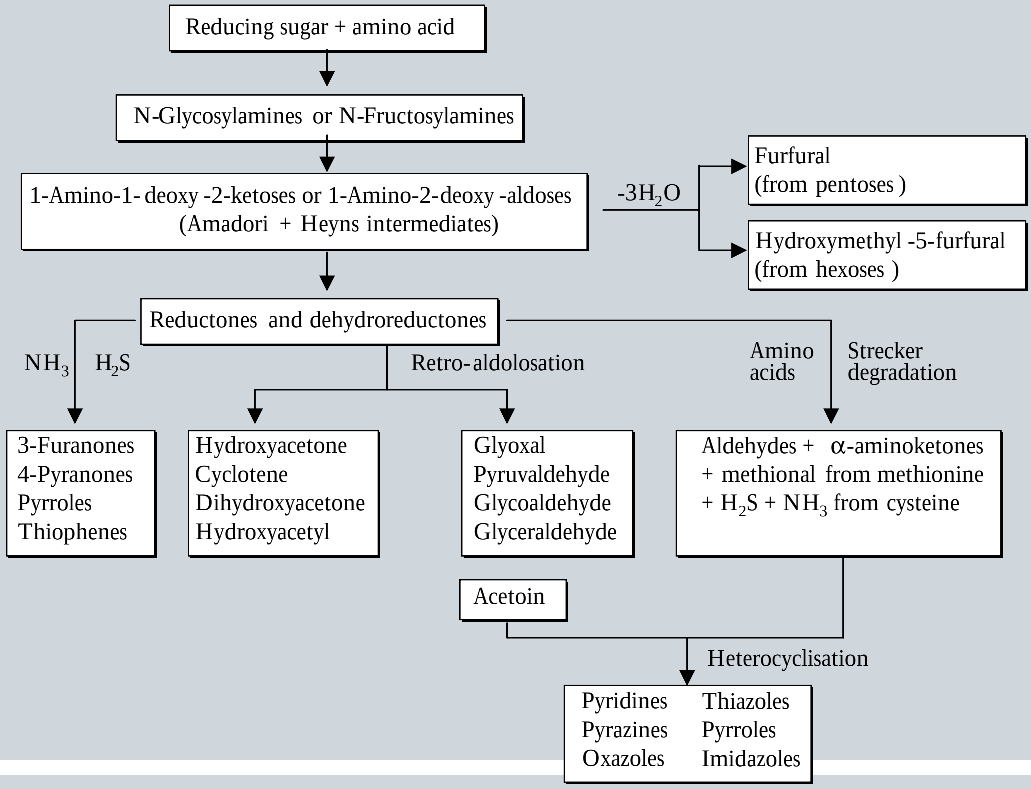

Figure 6 Reactions schemes for the formation of aroma compounds in foods as a result of Maillard reactions.

low-molecular-mass products of Maillard reactions have important sensory properties and biological activities, and various types of reaction, which lead to the formation of aroma compounds in food products, are summarised in Figure 6. The final stages of the Maillard reaction are complex and involve the condensation of many low-molecular-mass compounds into high-molecular-mass polymers, known as melanoidins, which have considerable variability in their compositions and structures.

Oxidation processes in food are, therefore, by no means always detrimental to quality. Many Maillard reaction products behave as strong antioxidants, and, as can be seen with the examples in the previous paragraph, some are also responsible for desirable sensory properties. In this respect, it is often the temperature of the reaction, which is important, and the 'best' products may be obtained by heating at the 'right' temperature for the 'right' period of time. Usually, we do not understand the chemistry, and the statements in the previous sentence are derived from generations of empirical measurements. *Many food preparation activities are still perceived as arts rather than science, and there is a need to develop our understanding of these important processes.*

Oxygen and biological systems – living with free-radical processes

For most organisms, free radicals are an inevitable consequence of living in an aerobic environment, and many fundamental metabolic processes proceed *via* free-radical mechanisms. In photosynthesis and respiration, for example, a series of free radicals are involved in the basic biochemical reaction schemes. Despite such fundamental processes, the crucial role played by free radicals in biological processes is often ignored, and free radicals are frequently seen in terms of their chemical reactivity and ability to bring about molecular change. In the context of health and nutrition, such change is often equated with being detrimental, despite its necessity in evolution and adaption to continually changing environments. One of the keys to survival of any organism, however, is the prevention (or minimisation) of free-radical damage to itself, whilst utilising free radicals for its own benefit (for providing energy and inflicting damage upon competitor organisms).

In order to avoid the damaging consequences of uncontrolled free-radical reactions, all aerobic organisms have evolved a vast array of protective mechanisms, which operate under normal conditions. Many

Generic name	Specific compounds
Vitamin A	Retinol Retinal Retinoic acid
Vitamin B_1	Thiamine
Vitamin B_2	Riboflavin
Vitamin B_6	Pyridoxine Pyridoxal Pyridoxamine
Vitamin B_{12}	Cobalamin derivatives
Vitamin C	L-ascorbic acid
Vitamin D	Cholecalciferol
Vitamin E	α-,β-,γ-,δ-tocopherol α-,β-,γ-,δ-tocotrienol
Vitamin K	Phylloquinone and derivatives
Biotin	Biotin
Folic acid	Tetrahydrofolic acid and derivatives
Niacin	Nicotinic acid

Table 1 Major vitamin molecules.

of these are enzymatic, and free-radical neutralisation is accompanied by a change in oxidation state of one or more transition metal ions in the enzyme. In addition, there are a number of organic molecules, which can play important roles in controlling free-radical processes in biological systems. The best known of these molecules are the vitamins (Table 1), but there are other molecules, many uncharacterised, which are believed to possess beneficial antioxidant properties. *There is now considerable interest in identifying and testing the efficacy of such molecules in protection against a number of causes of premature death, including cardiovascular disease and cancers.*

Free radicals, antioxidants and health: are deteriorative processes inevitable?

Disease states may result either from attack by competitor organisms (pathogens), or from the deterioration or breakdown in the functioning of essential components of the body. Free-radical-induced damage has been widely implicated in disease processes, but this should not be seen as surprising, since any interference with normal metabolic processes would be expected to have implications for free-radical reactions. Physical damage or pathogen attack both invoke a free-radical response in both plants and mammals. Also, the presence of elevated free radical levels in tumours may be primarily a reflection of a higher metabolic turnover in malignant tissue rather than being associated with the source of the disease. There is, therefore, an important problem of distinguishing between 'cause' and 'effect', and this represents a major difficulty in understanding free-radical aspects of the chemistry of disease processes.

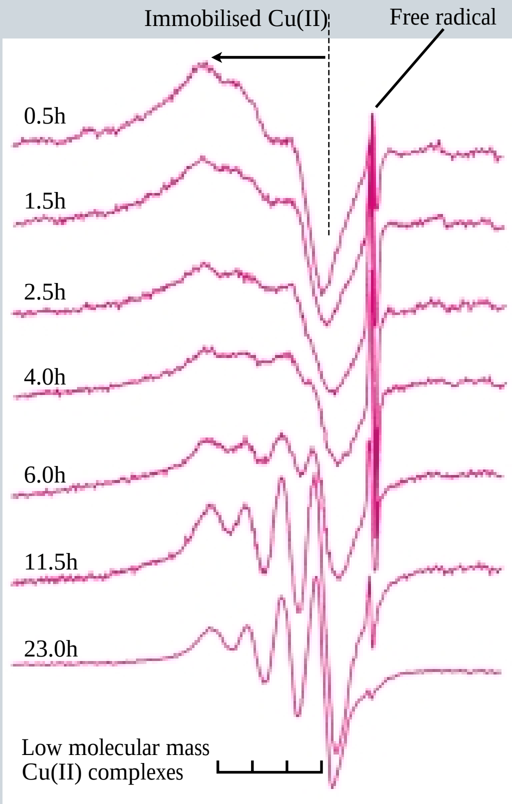

Figure 7 Electron paramagnetic resonance (EPR) spectra of wheat roots at various periods following a short exposure (30 minutes) to toxic levels of Cu(II) (10^{-5}M $CuSO_4$). Note: The free radical signal increased in intensity initially as the plant attempted to respond to the challenge, then decreased to virtually zero as it failed and died.

In contrast, the free-radical theory of ageing holds the premise that deteriorative ageing processes are the result of cummulative free-radical damage. Hence, *anything, which can slow down this free-radical damage, should have a beneficial effect on the ageing process.* A good example here is the maintenance of fluidity and structural integrity of cell membranes. Lipid-soluble antioxidants, such as tocopherols, play an important role in protecting the membrane lipids, and this fact is now widely recognised in the formulations of anti-ageing creams. Although the effectiveness of such preparations may still be a matter of debate, the protection of cells against antioxidative damage is a major reason why the various antioxidant vitamins are essential dietary components.

The oxidative free radicals involved in ageing processes are short-lived, and it appears that they are chemically identical to those involved in normal metabolic processes. *Contrary to popular opinion, however, there is not a build-up of free-radical activity* (or even levels of stable free radicals) *as a result of ageing*. In fact, just the opposite occurs, and senescing tissues show decreased levels of free radicals as a result of slower metabolic turnover. Death actually results in a virtual cessation of free-radical production (see Fig. 7).

In addition to the long-recognised vitamins, many other molecules found in foodstuffs have antioxidant properties. In recent years, there has been a great deal of effort to determine the extent to which they may be bioavailable, and to identify whether or not there is a positive health role for them. Although the vast majority of chemical species present in all foodstuffs is unknown at the molecular level, a current strategy is to explore in detail the properties of those molecules that can be isolated relatively easily. A positive response in test systems produces an identification of (potentially) 'desirable molecules', which can be used to produce 'healthier' food products. Since most of such products are of plant origin, many plant breeders aim to produce new varieties in order to satisfy projected consumer demand for foods enriched in these 'healthy molecules'. Caution is required in this approach, with respect to antioxidants especially, because it is often not possible to monitor the knock-on effects of changes in selected biochemical pathways. As a consequence of their need to survive in an environment of solar radiation and a vast range of other abiotic stresses, plants produce naturally a plethora of antioxidant molecules in their various organs. Enhancement of the levels of production of one type of molecule, therefore, carries the possibility of depression in the production of another, which performs a similar function in the plant, but which might be a more effective dietary component for humans and livestock. *Development of appropriate analytical techniques for large-scale metabolic profiling, should in the future allow a much higher fraction of the metabolic products to be characterised, and consequently to result in valuable improvements in our understanding of the functions of a much wider range of food components.*

The antioxidant argument, as commonly presented, is also a gross oversimplification of the complex range of reactions in which such molecules are involved. Health properties of foods cannot realistically be equated to their overall antioxidative capacities, since if that were the case, healthier food could be produced simply by addition of cheap synthetic chemical antioxidants, such as butylated hydroxytoluene (BHT). Unfortunately, the answer to improving health is not that simple. *Life is complex, and anyone looking for simple answers, is likely to get the wrong ones.*

The crucial role of plants in health and nutrition

The importance of plants in human nutrition was established at the beginnings of civilisation and, for most of human history, plants have represented the principal means for the control of disease as well as the provision of nutrients for healthy living. Several specific molecules have been shown to possess beneficial properties for maintenance of good health or treatment of disease, but, in most cases, the nature of the association between food and health is of a long-standing empirical nature. Nevertheless, around 30% of current world-wide sales of therapeutic agents are derived from plants or micro-organisms, and some 60% of the drugs currently used in cancer treatment are of natural origin. In Asia, reports of medicinal properties of plants go back 3,000 years, and formulations based on plants still account for a considerable fraction of traditional Asian and African medicines. There is also a growing awareness in Western countries of medicinal properties associated with specific plants or plant-derived products. In addition to the frequent reports of beneficial health properties associated with red wine, tea, and coffee, there have been many other reports concerning medicinal properties of individual plant products. Recent examples include claims that broccoli and apples help to prevent cancer, cranberries provide an effective treatment of urinary infections, tomatoes reduce blood clotting, black pepper prevents hair greying, and kiwi fruit are good laxatives, to name just a few.

There is a current tendency to attempt to explain beneficial health effects of foods in terms of protection provided by particular chemical components (*e.g.* resveratrol in red wine), even though the chemical composition of much of the product remains unidentified. This is indeed an important concept, because current legislation requires detailed information on the identities and quantities of any additives to foods, but very little is known about the precise chemical nature of most of the natural components. For example, consider the case of coffee. This is probably the most extensively characterised of any foodstuff and has been the subject of many hundreds (if not thousands) of man-years of investigation. There are estimates of between 4,000 and 15,000 for the number of chemical components in the beverage; around 1,000 of these have been identified, less than 20 have been subjected to basic toxicological evaluation, and only one (caffeine) has had its physiological effects thoroughly investigated. There is, therefore, a high level of ignorance about the natural chemical make-up of even the most common foods, and their *acceptability for consumption is based primarily on historical evidence of safety*. Consequently, *advancing our understanding of food composition would appear to be a priority area for investigation with modern technology*, *if the nutritional quality is to be developed as a food selection criterion*.

In humans, some antioxidant molecules are synthesised within the body, but many are derived from food. Plants, because of their abilities to synthesise large quantities of antioxidant molecules, have a special place in human nutrition. However, many of the chemical species in our bodies are not components of the foods we eat. Some are generated through reactions between different food components and others during digestive processes. Research, in which single food components are tested for their contributions to health, should consider the point that their chemical reactions could be quite different when presented to the body in a whole foodstuff. Even the consideration of individual foodstuffs is still an inappropriate over-simplification to understanding human nutrition. *It is unrealistic simply to present foods as 'healthy', or 'unhealthy', although there are clearly healthy and unhealthy diets (lifestyles).*

Of particular significance are the simplistic dietary claims which prey on public concerns about their own mortality, especially where emotive issues such as cancer and heart disease are concerned. The beneficial role of antioxidants in the diet as a result of their free-radical scavenging activities is an example of an over-simplified message, and much greater effort should be directed to understanding the relevant chemistry of foods and their transformations during digestion.

To what extent do we need improved food quality, or just better information?

There is now considerable interest in the production of plants with enhanced nutritional quality as a mechanism for improving the overall health of the population. This is currently a shared objective of the agricultural and food-processing industries, and major research efforts are being devoted to the breeding of plants (using both conventional and molecular techniques) to produce nutritionally enhanced products. There have been some notable successes as, for example, in the production of Vitamin A-enriched rice, which is starting to make an impact in China and SE Asia, but in the developed countries, there are currently more problems relating to over-consumption of food than to malnutrition. In this respect, Scotland is no exception with some 60% of the population estimated to be overweight or obese.

There is now more choice of foods than at any time in history, and in every supermarket there is a wider variety of food products than could have been imagined by our grandparents. How then do we transfer these opportunities to the dinner table? The answer has to be through knowledge, experience and education. At present, the public does not know the identities of most of the natural chemical components of foods, and clearly has little or no knowledge of their behaviour in the digestive system. Even when attention can be focused on one or more molecules with known nutritional properties, there is usually limited understanding of the extent to which they affect other (frequently uncharacterised) species. *We now have biological techniques, which allow us to make rapid changes in the composition of our foods, but substantial investments are required in analytical techniques to monitor fully the consequences of these changes.* Although it is relatively easy to screen out potentially toxic products, the subtleties in the differences in composition of most novel and conventional foods, means that thorough evaluation of the effects on humans is still only possible through the observation of generations of consumers. *There is, therefore, a desperate need for the development of good models for the prediction of the consequences of dietary changes, especially with respect to antioxidant molecules, if we are to reap the benefits from recent advances in biological techniques.* Fortunately, with the continuing advances in computer power, the technology to achieve such sophisticated models is already on the horizon.

Can health effects of food be considered in isolation, or should diet be based on lifestyle?

It is now a priority in many developed countries to advance understanding of the role of diet in the maintenance of health. This should, however, be seen as a long-term programme, and it must be recognised that there can be no quick fix. Inappropriate diets in childhood have been linked to diseases of middle age and, because of maternal inherited traits, it may take generations to develop a scientific understanding of the principles our forebears derived by trial and error. This is all made more difficult by living in an age of constant change – new foodstuffs, new methods of processing, pre-prepared meals, *etc*. *The development of new chemical- and bio-assay systems should be a great asset in advancing our knowledge of novel foods, but it is of crucial importance that these analytical approaches are*

Country	Life expectancy at birth (years)		Probability of dying between 15 and 59 years	
	Males	Females	Males	Females
UK	74.7	79.7	11.1%	6.7%
Ireland	73.3	78.3	11.6%	6.7%
France	74.9	83.6	14.6%	5.9%
Germany	73.7	80.1	13.6%	6.7%
Italy	75.4	82.1	10.9%	5.1%
Spain	75.3	82.1	12.9%	5.4%
Sweden	77.1	81.9	8.9%	6.0%
USA	73.8	79.7	14.8%	8.5%
Japan	77.6	84.3	9.5%	4.8%
Australia	76.8	82.2	9.4%	5.3%
China	68.1	71.3	17.0%	12.5%
India	59.6	61.2	27.5%	21.7%
South Africa	47.3	49.7	60.1%	53.3%

Table 2 Selected life expectancy data from the WHO World Health Report 2000.

developed in parallel with the food technology, if a fuller understanding of the relationships between diet and health is to be achieved.

In recent years, much attention has been given to the 'French paradox', and dietary differences between Mediterranean and North European countries have been invoked to explain longer life expectancies in France compared to the UK. There have also been claims that consumption of red wine is the explanation for this effect. It is interesting then to examine closer recently published data by the World Health Organisation (Table 2). Firstly, there is a relatively small difference between life expectancies for males in France and the UK, and both are similar to those of other major countries in Europe. Furthermore, the probability of premature adult death (i.e. between 15 and 59 years) for French males is one of the highest in Europe. In contrast, the life expectancy of the French female is the longest in Europe. *If it really exists, the 'French paradox' is, therefore, a gender-related issue*. It is completely inappropriate to just consider diet to explain life expectancy data, and to ignore environmental contributions to health, such as the climate, housing conditions, and working practices; nor should genetics be discounted. Hopefully, the next few years will bring huge advances in our knowledge of some of the subtleties in the relationships between genetics and health.

It is also unreasonable to assume that every individual has the same dietary requirements. The diet of an office worker will not be adequate for a professional athlete, and an athlete's diet will probably not be beneficial to the office worker. For many years we havehad information on the energy requirements for different occupations, but, although various governments produce recommended daily allowances for a number of vitamins, detailed information on a wider range of micronutrient requirements is not currently available. *Increases in understanding how diet could be adjusted in the context of lifestyle in order to achieve optimum health effects should be regarded as a priority in nutritional research.*

What is the case for nutritionally enriched foods and dietary supplementation?

There is now a considerable demand in the developed countries for food supplements, despite the fact that our supermarkets now have year-round stocks of fresh produce from all over the world. The need for dietary supplementation, therefore, has never been less than it is at the present time, but does it still convey a real benefit? Unfortunately, except for a few special cases where individuals are subjected to severe stress conditions, the answers are largely unknown. Nutritionally enriched foods are beneficial to people in areas of the world where only a limited range of foodstuffs is available, but there is debate as to what fraction of the population of the UK could benefit from such products.

It can be argued that the increased use of synthetic antioxidant food preservatives in the latter half of the 20th century has been responsible for general improvements in the health of people in developed countries. Also, the longer active life-spans in the USA compared to the UK is sometimes attributed to nutritional supplementation, although there is no significant difference in actual life-spans. There are, however, many other lifestyle differences between these countries, and at the present time it is not possible to confirm or refute such claims. Indeed, public attitudes to physical activity might be a major factor, not to mention the availability of facilities, and in some parts of the country, a more amenable climate. Nevertheless, the perceived importance to health of various antioxidant molecules has led to the development of an industry devoted to the production of dietary supplements, the objective being provision of the means to avoid nutritional deficiencies.

The cost of routine dietary supplementation is not insignificant, and this raises the question as to when it is valuable, especially since nutritional requirements are dependent on a number of lifestyle factors. Also, there is a question of the extent to which conventional foods can be used to alleviate the need for dietary supplementation. The problem is identifying what sort of improvement is desirable and how this can be achieved. Apart from the vitamin molecules, the present state of our knowledge on many dietary supplements is limited, and detailed chemical compositions of popular supplements, such as for example ginseng or *Ginkgo biloba,* are largely unknown. Without such information, it is not possible to begin a programme of identifying active ingredients (if activity is associated with individual molecules) or of developing an understanding of the factors which influence the qualities of different samples of these products.

Meanwhile, the European Commission is concerned about claims relating to the effectiveness of food supplements. It is relatively straightforward to determine toxicological effects by following the methods used for screening pharmaceuticals, but it is much more difficult to establish the efficacy of various formulations. The approach used to establish the essential requirement of vitamins, by testing their abilities to remove adverse symptoms from subjects suffering from severe deficiencies, is largely irrelevant to the populations in developed countries. As stated above, there is an urgent need for the development of good micro-nutritional models in order to remove some of the evangelical approaches from research in diet and health.

Natural versus added antioxidants in the diet

Popular current opinion is that the key to beneficial nutritional properties of plants is the antioxidants, which are produced in large quantities in order to aid survival in an aerobic environment. The logic behind this thinking is that humans need protection against the detrimental effects of oxidative stress reactions, and that plants have evolved mechanisms for synthesising the appropriate chemicals to enable them to survive in an environment of acute oxidative stress. There is then the thought that what works for plants, should also work for humans, although it neglects the production of toxic plant components. Generalisation is, therefore, potentially dangerous, and the safety of all plants has to be considered on an individual basis. Fortunately, for most food plants this has been done on an empirical basis over thousands of years.

There is good epidemiological evidence that a diet rich in fruit and vegetables provides health benefits over one that is devoid of fresh plant products. The role of fish, especially oily or uncooked fish, should also be considered in a healthy diet, since Japan, with its high levels of fish consumption, has the highest life expectancy of any developed country. Specific health claims have been made for several individual products (see above), whereas others are thought to make an overall contribution to health. Such statements should, however, be treated with caution, since an over-reliance on a limited range of food products can be seriously detrimental (*e.g.* deaths from excessive carrot consumption). It has been recognised recently that excessive consumption of vitamin molecules can be detrimental to health, and it is likely that excessive consumption of any type of food product is undesirable.

Because of its importance, the validity of the link between antioxidant molecules in the diet and human health should be carefully scrutinised. There is a strong current view that this link is an established fact, but is such confidence really justified? The basis for the concept lies in the essential role of vitamins, but the current status of other ‘antioxidant’ molecules in foods still remains in the ‘non-proven’ category.

The acceptability of chemical food additives has changed appreciably since the 1980s, when there was concern about the presence of synthetic preservatives in foodstuffs and their supposed adverse implications for health. These molecules were antioxidants and were (and still are) used to inhibit oxidative degradation processes. Positive benefits were not generally recognised then, and even now there seems to be a conception that the natural products are in some way superior. Sometimes, of course, that may be the case, but it can not be taken as a generality, since many of the most toxic compounds known to man are of a natural origin. Also, humans do not live in biological isolation, but in an environment in which there is a constant competition with other biological organisms. We may be the dominant mammalian species on this planet, but we have by no means developed control of all micro-organisms. Our battle against them involves the use of toxins, which are more lethal to them than to ourselves, *i.e.* in the right circumstance toxic materials are beneficial, whereas nutritious materials can be detrimental, because they could help the organisms with which we are in conflict. *A full understanding of the roles of various foodstuffs is, therefore, an extremely complex issue, and at the present time there is a need to keep an open mind about the health implications of individual food components.*

Techniques to measure free radicals, antioxidants and free radical scavengers

Free radicals can be detected directly by electron paramagnetic resonance (EPR) spectroscopy (Fig. 8), a technique that is designed to study specifically molecules with unpaired electrons. The same technique, therefore, is able to characterise the paramagnetic transition metal centres in some antioxidant enzymes. As mentioned in earlier paragraphs, many free radicals have extremely short half-lives and, except where high steady-state levels are generated, cannot easily be studied directly in biological samples. There are, however, various practical procedures that allow EPR to be used for the study of unstable radicals. One of the most useful of these is 'spin trapping', where a molecule, known as a spin trap, is added to a reaction medium and reacts specifically with unstable radicals to produce adducts, which are themselves radicals, but with greater stability. In many cases, the spectral parameters of these radical adducts allow identification of the original radical. Other techniques for the study of unstable radicals include stabilisation by rapid quenching to low temperature, or generation within the spectrometer by irradiation or mixing the reactants which produce the radicals in the spectrometer using a flow system.

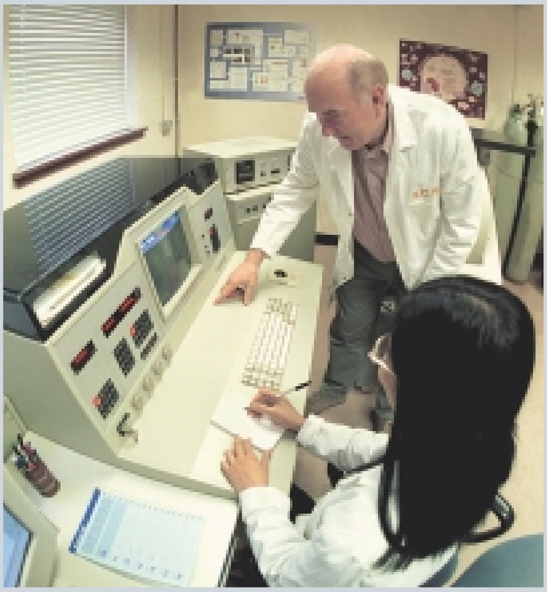

Figure 8. Electron paramagnetic resonance spectrometer.

There are various approaches available for the study and characterisation of antioxidant molecules. Accredited chemical analytical methods are available for the routine determination of the known vitamin molecules, and assay kits have been produced for measurement of activity of various enzyme preparations. Measurements of uncharacterised antioxidant molecules are more difficult and a range of assays has been produced with the objective of assessing the overall antioxidant capacity of a sample. In reality, such assays measure the ability to carry out a specific reaction and are far removed from any assessment of the contribution to health of a particular food product. Nevertheless, they have some value in screening exercises. In recent years, there have been rapid developments in chromatographic techniques for molecular separation. These have been paralleled by advances in techniques for molecular characterisation, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR). Coupling these techniques should in the near future lead to rapid progress in identifying a new generation of molecules that are able to perform specific antioxidant reactions in foodstuffs, such as the inhibition of lipid autoxidation.

Different approaches are required to determine free radical scavengers, depending on whether they are known molecules, or uncharacterised mixtures. In the first case, a standard analytical chemistry approach can be used, but dealing with the latter is more difficult. As mentioned at the beginning of this article, the expression 'free radical scavenger' is not particularly helpful in the context of food and health. Some radicals, such as $^{\cdot}OH$, are highly reactive and unspecific in their reaction. With these radicals, there is no such thing as a specific scavenger, although the nature of the reaction products can be definitive for a particular radical (spin trapping is a specialised example). Other radicals are much less reactive, and may be scavenged specifically. In such a situation, however, *different free radical scavengers are required for reaction with different types of radical*. The enzyme superoxide dismutase (SOD) is an example of a specific scavenger for the $O_2^{\cdot -}$ radical.

The future – what are the nutritional priorities for the 21st Century?

The framework for the human genome has now been established and, within the next few years, a good knowledge of the natural variations in individuals will likely be obtained. Identification of the functions of the various genes will then follow, thus opening up the possibility for routine treatment of the adverse consequences of many genetic weaknesses – perhaps identified through screening at birth. The pharmaceutical industry offers a route to the treatment of the genetic basis of diseases, but economic factors are likely to limit its availability. In a practical sense, there is a need to learn how to adjust lifestyles to counter genetic susceptibilities. Diet and nutrition should, therefore, represent the foundation upon which it will be possible to devise ‘recipes’ for each individual that will provide the optimum dietary conditions for a healthy life. This might well include functional foods as a practical link between basic diets and conventional medicine.

In order to achieve these aims, huge advances need to be made in the characterisation of our foodstuffs and identification of a much wider range of food components than is presently available. This will need to be supplemented by data on the physiological effects, not just as individual molecules, but of food ingredients in combination with one another, in the digestive system. Simultaneously, information needs to be generated on the natural variation in the levels of physiologically active molecules, and the extent to which these can be controlled in the food production pathways. This type of *analytical chemical knowledge will provide the scientific basis from which it should be possible to generate progressive improvements in the nutritional qualities of our foodstuffs, and hopefully to produce a general extension in the period of high-quality life for individuals.*

Conclusions

Understanding the health implications of diet and other lifestyle factors offers the most practical approach for a long-term increase in the overall health of populations. At the present time, there is good information on the functions of a relatively small number of essential molecules in the diet, but a paucity of knowledge of the chemical composition and physiological effects of many of the components of our foods. Generation of this knowledge is only a first step, however, since for it to be effective, the public must both understand and accept the information. This latter may yet prove to be the most formidable obstacle, given the current willingness of many people to risk health damage by consuming substances known to have long-term adverse effects.

There is also the question of the extent to which it is reasonable to expect individuals to monitor their daily intake of a wide range of nutrients. Particular attention may be beneficial when bodies are physiologically stressed (*e.g.* after major illness, professional athletes, *etc.*) and recovery rates may be enhanced by the consumption of certain types of molecule. In such situations there may be positive advantages in using dietary supplements. Alternatively, the stress generated by worrying too much about details of a diet may negate potentially beneficial effects for some individuals. As a society, a long-term aim should be to use education to encourage individuals to adopt healthy lifestyles naturally, without thought.

There is an argument that changes in food compositions are currently happening too quickly for non-clinical effects to be detected, but this is a trend that is likely to continue for the foreseeable future. Potential problems must be seen and addressed in the context of a rapidly changing world. There is no such thing as a risk-free change, or even existence, and at the present time, we have to place a large degree of trust in risk/benefit analyses. It is imperative, then, that such exercises are conducted diligently, and independent of short-term profit motive. However, there will inevitably be mistakes (just as there have always been), and all reasonable steps must be taken to ensure that they are minimised, including the encouragement of open and informed debate on on-going science and its implications. Nevertheless, *no change is not a no-risk scenario*. The dynamic nature of the world means that increasingly more innovative solutions are required to address a rapidly evolving set of problems. In order to achieve this, it is vital that all relevant branches of science are developed simultaneously.

The final take-home message is that the science underlying the links between food, diet and health is exceedingly complex and demands greater investment, if our understanding is to be increased to a level that will produce tangible benefits in terms of human health. Such an investment at the present time will represent a valuable legacy for future generations.