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Carbohydrate polymers in food preservation: an integrated view of the Maillard reaction with special reference to discoveries of preserved foods in *Sphagnum*-dominated peat bogs

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Abstract

Well-preserved human bodies more than 2000 years old have been found in peat bogs derived mainly from *sphagnum* mosses. Preservation is correlated with the occurrence of α-keto-carboxylate groups in a glycuronoglycan (‘sphagnan’) that comprises ~60% of the holocellulose in the hyaline cell walls of the mosses [Painter (1991b). *Carbohydr. Polym*., *15*, 123–142]. There is now renewed interest in other biodegradable materials that have been found preserved in peat, including carcasses of domestic animals, loaves of bread, dried fruits, berries, and kegs of butter or cheese up to 1800 years old. This review attempts to correlate these examples of fortuitous preservation in peat with other, more familiar methods of food preservation that depend in the first instance upon the condensation of highly reactive carbonyl compounds with primary amino-groups or ammonia. The Maillard reaction inhibits microbial growth by sequestering ammonia, aminoacids and peptides, while the brown, polymeric end-products (‘melanoidins’) inhibit by cross-linking polypeptide chains and sequestering essential, multivalent metal cations. These reactions could find broader or entirely new applications in food preservation. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Maillard reaction; Carbohydrate polymers; *Sphagnum*-dominated peat bogs

1. Introduction

1.1. The ‘carbonyls’ of wood smoke

Exposure to the volatile, organic components of wood smoke has been used for millennia to preserve fish, meat and hides. For example, leather artefacts found on the 5300 year-old body of the ‘Ice Man’ (near Innsbruck, in September 1991) are believed to have been tanned by smoke (Spindler, 1994). The practice probably evolved concomitantly with cooking over open wood fires.

Typical wood-smoke condensates contain 0.5–2.0% of a phenolic fraction which includes cresols and xylenols with significant bactericidal potencies (‘phenol coefficients’ $>$ 4). Their contribution to preservation is, however, probably small compared to that of the much larger ‘carbonyl’ fraction ($>$ 30% of the total condensate). This fraction is a mixture of at least 133 different aldehydes and ketones, including potent cellular toxins such as formaldehyde and acrolein (Maga, 1988). Its size (~30%) may be underestimated owing to polymerization catalysed by the carboxylic acids that are also present in wood smoke.

Most of these carbonyl compounds, and certainly all that are essential for preservation, can be obtained by pyrolysis of pure cellulose or hemicelluloses alone. This kind of preservation is, therefore, a significant part of the technology of carbohydrate polymers (Shafizadeh, 1984).

1.2. Mechanism of preservation by carbonyl compounds

Formaldehyde, acrolein and other toxic carbonyl compounds kill microbial cells by penetrating the cell wall and inactivating the enzymes on the cytoplasmic membrane and in the cytoplasm. They do this by condensing with the free, primary amino-groups in the polypeptide chains. These occur mostly in the side-chains of basic amino-acids, but also in the *N*-terminal amino-acid and the amido-groups of Asn and Gln. In some cases, these amino-groups are an essential part of the enzymes’ active centres, while in others they help to bind the substrate by hydrogen-bonding. In all cases, however, Schiff-base formation changes the charge-distribution in the polypeptide chain, and this can eliminate or distort its catalytically active conformation.

Schiff-base formation is generally acid–base catalysed,

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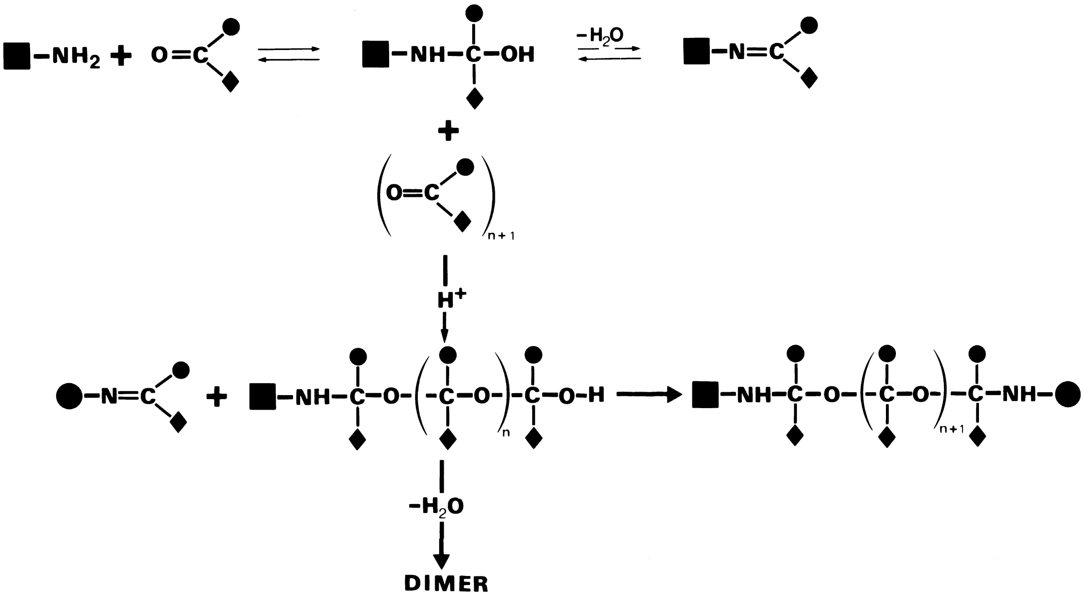

Fig. 1. Schematic representation of possibilities for the acid-catalysed cross-linking of proteins by a compound containing a single carbonyl group.

and occurs spontaneously and reversibly under neutral conditions, but under mildly acidic conditions (brought about by the large carboxylic-acid fraction of wood smoke), a specific acid-catalysed polymerization occurs in which the Schiff base reacts with additional molecules of the carbonyl compound. The polymeric chains that are built on to the polypeptide in this way are essentially polyaldals or polyketals, at least when the carbonyl compounds are relatively simple alkanals or alkanones. Some of the growing side-chains inevitably join to form cross-linkages (Fig. 1). This cross-linking is the basis of 'aldehyde tanning' (Gustavson, 1956).

The reactivity of any carbonyl group in the initial condensation with a primary amino-group increases when there is an electron-withdrawing group close by in the molecule. Hence, an α-keto-carboxylic acid group is especially reactive at pH < 5, that is, when the carboxyl groups are incompletely ionized. Pyridoxal phosphate is an example of a highly reactive carbonyl compound in which the electron-withdrawing group is a pyridinium cation.

In contrast, primary amines are activated by electron-releasing groups; hence the ε-amino group in lysine and the guanido-group in arginine are especially reactive with carbonyl compounds (Gustavson, 1956).

1.3. Passive inhibition of microbial growth by nutrient deprivation

Even when a carbonyl compound cannot penetrate the wall of a microbial cell, it can still inhibit growth by interfering with the uptake of nutrients. It can do this in at least three different ways:

Type A inhibition entails reaction with a nutrient of low molecular weight, leading to a decrease in its effective concentration in the growth medium. A carbonyl compound can be said to 'sequester' an amino-acid or ammonia by condensing with it. Many carbonyl compounds, including α-keto-carboxylic acids, enediols, 3-hydroxyketones and 1,3-diones can also form stable chelate complexes with essential, multivalent metal cations.

Type B inhibition can arise in situations where saprogenic bacteria or moulds utilize a polymeric substrate such as a protein or a glycan. To do this, they must secrete exocellular proteases or glycanases that break down the polymer into small fragments which can be taken up by the cells. Carbonyl compounds can inhibit such depolymerisation either by inactivating these enzymes in the ways described earlier, or by immobilising them in insoluble particles or a three-dimensional polymeric network which physically isolates them from their polymeric substrates.

Type C inhibition also refers to polymeric substrates, but involves direct chemical modification of the substrate itself, so that it becomes less accessible or susceptible to the microbial depolymerases. Leather production by the tanning of the collagen fibres in hide is a typical example. Aldehydes, including especially formaldehyde, acrolein and glutaraldele (also found in wood smoke) are extensively used in industrial tanning (Gustavson, 1956; Nayudamma, 1975). Fish skins also contain collagen, and their leathery texture after smoking illustrates that exposure to the carbonyl compounds in wood smoke is essentially a form of aldehyde tanning.

1.4. Loss of nutritional value with Type C inhibition

The word 'tanning' is usually reserved for collagen, but the myosin fibres in muscular tissue are derivatized and cross-linked by wood-smoke carbonyls in the same way. Obviously, this treatment has to be restricted to a thin, surface layer because the fish or meat would otherwise become as resistant to the enzymes in the human digestive tract as to the microbial ones. The degree of preservation is rationally expressed as an observed extension to the ‘lag’ phase in the colonisation of the smoked surface by a test micro-organism (Maga, 1988).

Normally, the objective is to increase ‘shelf life’ at 4°C by 2–3 months. Salting and smoking work synergistically in prolonging shelf life. Provided that the fish or meat is lightly salted first (which is normal in Western countries, but less usual in Japan), the degree of smoking required to give the desired, additional protection probably causes no more than a 10–15% loss in nutritional quality, expressed as lysine (Maga, 1988; Opskvedt, 1988).

1.5. Similarity of smoking to a Maillard reaction

When fish is smoked for too long by mistake, the familiar, light brown colour of the smoked muscle gradually darkens to that of black coffee or molasses. It is firmly established that colour development involves the free amino-groups in the muscle proteins, and that it is strongly correlated with smoke’s content of certain, specific aldehydes. The latter include glycolic aldehyde, methyl glyoxal and furfural (Ruiter, 1979).

The carbonyls in wood smoke are dominated by furfural and its derivatives. Samples of freshly generated smoke from six different kinds of wood contained 64–72% of 2-*C*-formyl-furan (furfural), 2-*C*-formyl-5-*C*-methyl-furan and 2-*C*-acetyl-furan (Fujimaki et al., 1974). These compounds are also found (together with 2-*C*-formyl-5-hydroxymethyl-furan) among the pyrolysis products of pure cellulose (Shafizadeh, 1984), which must therefore be the main wood component from which they originate.

Maga (1988) argues strongly that there is little point in distinguishing between this colour reaction of wood smoke with proteins and a conventional Maillard reaction, carried out in homogeneous, aqueous solution. In both cases, the carbonyls are formed by dehydration of carbohydrates, and the only significant difference is that in smoking the site of their generation is more widely separated in space and time from that of their reaction. Maga could have added that the dominant carbonyls in a conventional Maillard reaction are also furfural derivatives (Olssen et al., 1978).

2. Natural herbs and spices

2.1. Hops

The bitter taste imparted to beer by hops is widely appreciated, but this is not the only reason for their use. The dried flowers are added to the boiling wort and subsequently filtered off. The soluble extract helps to clarify the beer by promoting the coagulation of soluble protein, and after cooling it selectively inhibits the growth of Gram-positive bacteria while having no effect upon the added yeast. Bacteria that produce lactic acid, especially *Lactobacillus* and *Pediococcus* spp., are particularly troublesome.

Hops contain many different carbonyl compounds. The most important ones, which make up about a third of the hot-water extract and 5–15% of the original hops, are called ‘α-acids’. They comprise three closely similar compounds that differ only in the identity of one side-chain. One of them, ‘humulone’ (Fig. 2, no. 1), has well-documented bactericidal properties (Simpson and Smith, 1992). During boiling, it is partially isomerized to isohumulone and oxidised by dissolved oxygen to humulinone (Fig. 2, no. 2).

It is usually implied that all of these carbonyl compounds have either bactericidal or bacteriostatic activities. Moreover, they all contain enolic hydroxyl groups with a pK_a of ~ 3, and their antibacterial potencies decrease with increasing pH. There is, therefore, a positive correlation between biological activity and carbonyl reactivity (Fernandez and Simpson, 1995).

2.2. Indian curry spices

‘Curry’ is a collective term for mixtures of spices from many different herbs. The composition of these mixtures varies widely, but almost all contain coriander, and hence also the essential oils coriandrin (2.3), coriandrone A (2.4) and coriandrone B (2.5). Turmeric is usually also included, and hence also curcumin (2.6) and curcuminone (2.7). Root ginger is also very popular. Its main component is gingerol (2.8), with smaller amounts of gingerdiones (2.9). Sage contains salvone (2.10), and the bitter taste of saffron is owing to picrocrocin (2.11). Celery roots contain sedanonic acid (2.12), and D-carvone (2.13) occurs in both caraway and dill. *p*-Isopopylbenzaldehyde is found in cumin, cinnamaldehyde in cinnamon, and vanillin in vanilla beans. A longer list could be prepared with little effort. About 80% of the essential oils found in curry spices are carbonyl compounds. Most of them have antimicrobial properties and will prolong the ‘lag’ phase in new microbial growth after the sterilization effected by cooking. This is especially important in countries with hot climates (Billing and Sherman, 1998).

Whereas most of the carbonyls in wood smoke are soluble in water and readily polymerised by the carboxylic acids that are also present in abundance, those that occur in curry spices are only very sparingly soluble in water and are used at about pH 5. They do condense readily with the free amino-groups in food proteins, a fact well illustrated by the yellow colour imparted to proteins by turmeric, but there is no evidence that they cross-link myosin fibres.

Curry spices are seldom refined before use. They consist simply of seeds, roots, nuts, barks or leaves that have been dried in the sun or a current of air, and then coarsely ground or chopped. The essential oils exist in these lignocellulosic or pectinous tissues, enclosed in special cells, glands or ducts which are only partly ruptured by the grinding or chopping (Parry, 1969).

The low solubility of these essential oils in water, together with their partial occlusion in particles of insoluble vegetable tissue under the conditions of use, suggests that

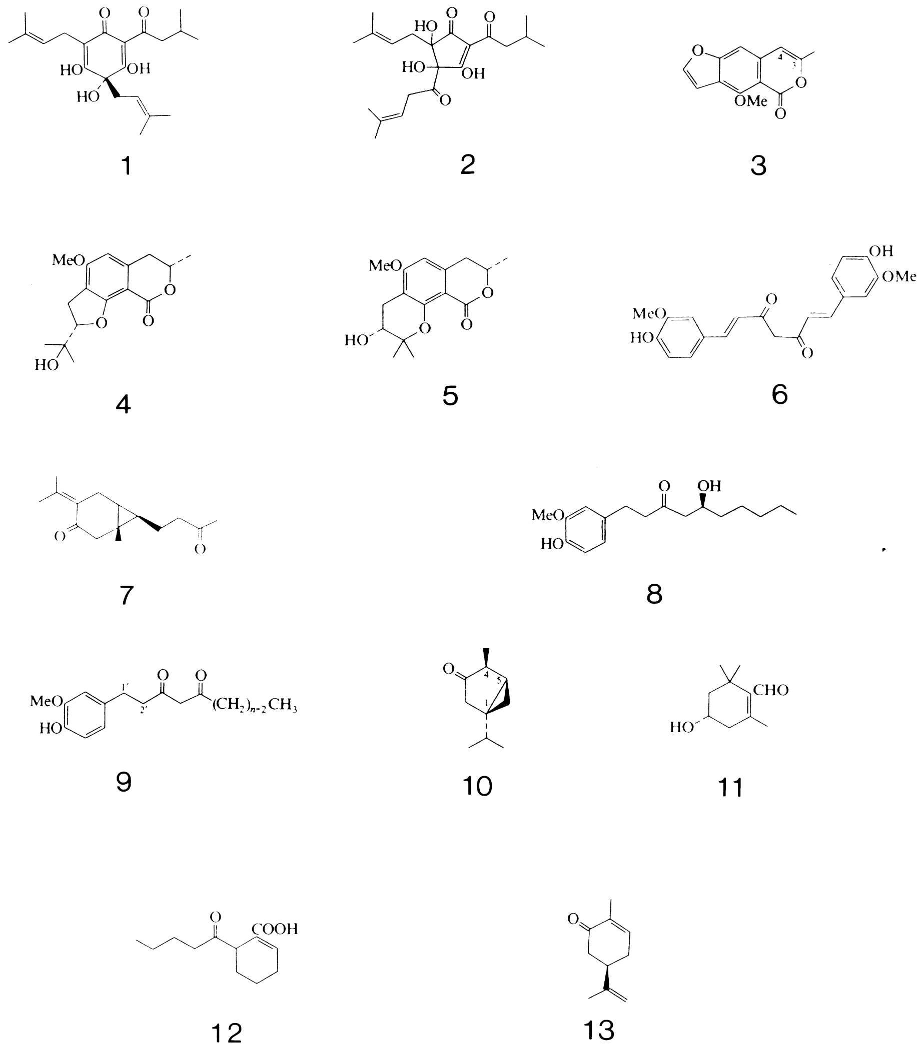

Fig. 2. Carbonyl compounds in the essential oils of herbs and spices that have a documented or putative preservative effect upon foods and beverages.

their preservative action depends more upon Type B inhibition and less upon Type C inhibition than is the case with wood smoke. A consideration of the likely protective function of these occlusions in the living plants seems to predict this. If confirmed, it would imply that any loss of nutritional quality in the preserved protein would be smaller than is the case with smoking.

3. Artificial spices

3.1. Soya sauce

On proceeding eastwards from India through southeastern Asia and then northwards to China and Japan, the traveller finds that curries become progressively milder, and that they are served less often, while soya sauce gradually replaces them. Indeed, soya sauce is usually used as a substitute for crystalline salt because of its high salt content, in any and every savoury dish.

According to a traditional recipe, soya beans are first boiled with water and then mashed with roasted wheat kernels. Salt is added, and the mash is kept in a barrel for 1–3 years. The salt prevents the growth of undesirable micro-organisms, but permits a slow and selective fermentation by salt-tolerant moulds, yeasts and bacteria. Moulds, notably *Aspergillus sojae* and *A. oryzae*, begin the fermentation by secreting proteases that hydrolyse the soya protein and α-amylases that hydrolyse the wheat starch. The liberated amino-acids and reducing sugars then support the development of a more complex microbial flora, but they also react spontaneously together in a typical Maillard reaction, with production of a dark-brown polymer. Modern technology has shortened the brewing time to about 6 months (Sasaki and Nunomura, 1993), but its slowness suggests that some of the reaction products may have antimicrobial activities.

3.2. Brewing

The Maillard-reaction products are also important in brewing beer. The colour of the beer, ranging from pale yellow in some lagers to dark brown in stouts, is developed by heating or roasting a part of the malted barley before it is mashed with water. The duration and temperature of this treatment determine how dark the beer will be. The melanoidins produced during this process contribute more than colour to the final brew, however. They also contribute ‘body’ to the flavour of the beer, inhibit microbial spoilage, and increase the shelf life of bottled beers by suppressing photochemical spoilage (Fernandez and Simpson, 1995; Øvre, 1996, personal communication).

In former times hops were not universally used in brewing as they are today. In mediaeval England, for example, ‘mild’ was a very dark, full-bodied ale, totally without bitterness. It probably did not ‘keep’ so well as a ‘hopped’ beer (‘bitter’), and it is partly for this reason that modern ‘mild’ is lightly hopped. There is, however, a clear awareness in the industry today that the melanoidins improve microbiological stability. For example, certain strains of lactic-acid producing bacteria have developed resistance towards the iso-α-acids in hops, but they cause problems mainly with the lighter, lager-type beers (Fernandez and Simpson, 1995; Hammond, 1996, personal communication). Moreover, certain strains of yeast are unsuitable for brewing dark beers, because their growth is inhibited by the melanoidins.

In Europe, new EEC legislation now permits the use of burnt sugar (caramel, E 150) to darken beer. Since the caramel can be added after the fermentation, the use of a wider range of yeast strains is now possible, but the contribution of the true melanoidins to flavour and stability may have been lost.

3.3. Baking

Bread crusts are seldom thought of as ‘spices’, but their contribution to the flavour of bread is universally appreciated. There is also a popular acceptance, rooted in custom but seldom verbalized, that it is safe to handle unwrapped, whole loaves without tongs or gloves, but not sliced bread or sandwiches. This implies a belief that bread crusts, and perhaps especially their brown, melanoidin pigments, have an antimicrobial property (Schulz and Drews, 1957).

4. The Maillard reaction products

4.1. Structural features

The end-product of the Maillard reaction is a dark-brown polyampholyte described by Maillard himself as a ‘humic substance’ (Maillard, 1916, 1917). Even when it is formed from a single sugar (e.g. D-glucose) and only one amino-acid (e.g. glycine), its structure must be extremely complex. This can be inferred from the many different intermediates

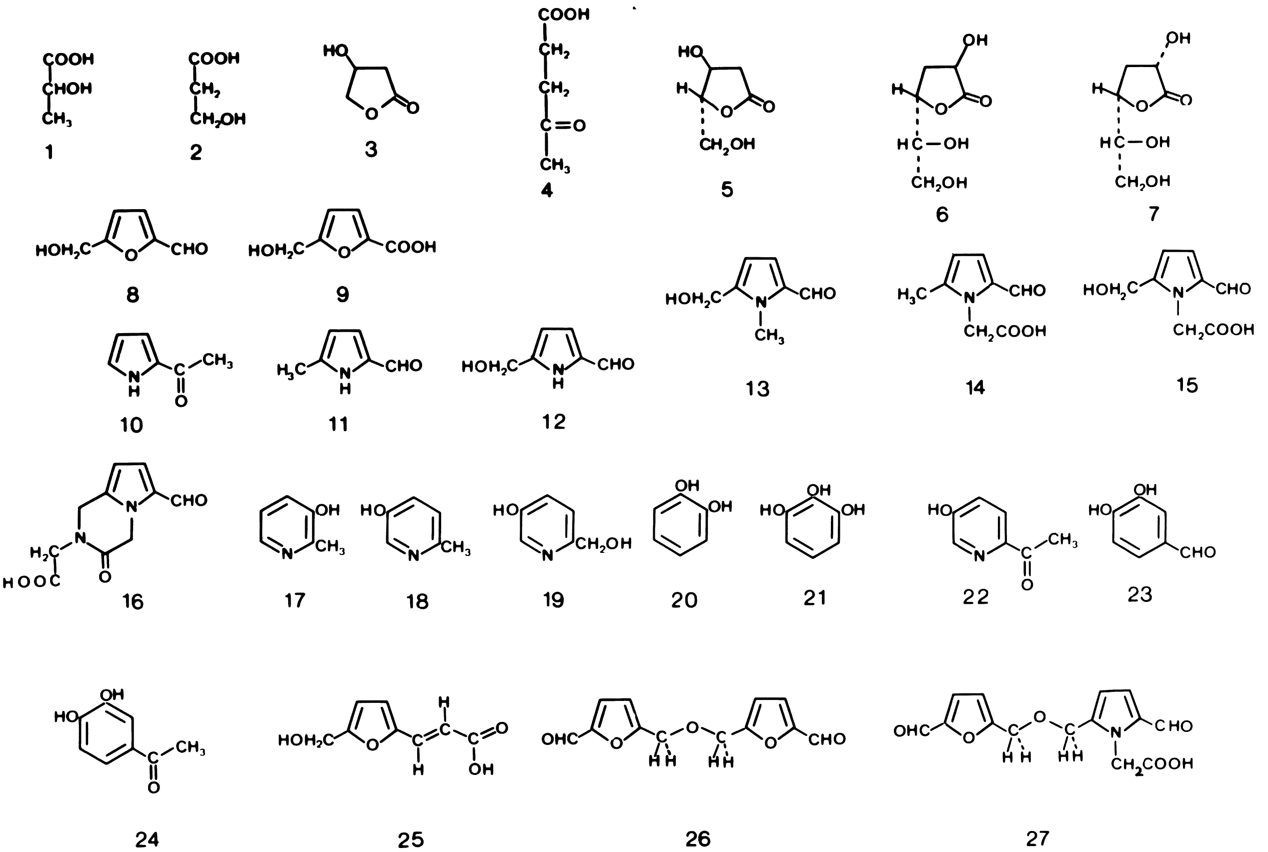

Fig. 3. Compounds identified as intermediates in the Maillard reaction between D-glucose and glycine under mildly acidic conditions (Olssen et al., 1978).

that are formed first in the reaction, and which then polymerize (Fig. 3). Olssen et al. (1978) identified, inter alia, 27 intermediates, of which 18 were carbonyl compounds. The intermediate fraction was dominated by furfural derivitives (3.8, 3.26 and 3.27), and one of these, 2-*C*-formyl-5-hydroxymethyl-furan (3.8), alone accounted for more than half of the total intermediate fraction.

The eight derivatives of 2-*C*-formyl-pyrrole (3.10, 3.11, 3.12, 3.13, 3.14, 3.15, 3.16 and 3.27) are especially noteworthy because their endocyclic nitrogen atom, when protonated, becomes strongly electron-attracting. Under mildly acidic conditions, therefore, these intermediates would resemble pyridoxal phosphate in reacting rapidly with primary amino-groups. The fact that they made up only a minor part of the total intermediate fraction is consistent with this expectation, because they should polymerize rapidly instead of accumulating in the reaction mixture.

The reactivity of D-glucose as a carbonyl compound in aqueous solution is strongly suppressed by cyclic-hemiacetal formation, and perhaps the most significant feature of the Maillard reaction is that it dehydrates sugars at pH 5 with the formation of carbonyl compounds that are much more highly reactive than the sugars themselves. It, therefore, achieves almost the same results as pyrolysis (smoking) with the expenditure of much less energy.

It is noteworthy that polyphenols (3.20, 3.21, 3.23 and 3.24) were also produced in the Maillard reaction of glucose with glycine, though their 'phenol coefficients' are low (~0.5). With amino-acids such as alanine, valine, leucine or isoleucine, it is more likely that antibacterial compounds resembling *p*-cresol, thymol or carvacrol would be produced.

4.2. Antibacterial activity

Much of the earlier work in this area (reviewed by Einarsson, 1987) is unsatisfying because no effort was made to monitor the Maillard reaction or to fractionate its products before testing them for antibacterial activity. Some of the crude reaction mixtures that have been tested, especially those obtained after short heating periods, would have contained large amounts of unreacted sugars and amino-acids, and this would have increased the concentrations of nutrients in the test cultures relative to those in the controls. This may explain some anomalies, but the bulk of the evidence strongly supports the traditional wisdom outlined in the section on artificial spices.

Einarsson et al. (1983) used colour development (expressed as optical density at 450 nm) to monitor the reaction, and dialysis to separate the polymeric end-product from any residual reactants and intermediates of low molecular weight. A product obtained by boiling an aqueous mixture of arginine and xylose for 20 h at an initial pH of 7 totally prevented growth of twenty different test organisms at concentrations ranging from 0.04% to 0.8%. The organisms included different strains of *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Lactobacillus helveticus*, *L. plantarum*, *Proteus stuarti*, *P. vulgaris*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. fragi*, *Salmonella montevideo*, *S. senftenberg*, *Staphylococcus aureus* and *Streptococcus faecalis*.

The mechanism of the inhibition was also investigated. It was found that the Maillard reaction products inhibited the uptake of glucose, serine and oxygen, and that they decreased the solubility of ferric ions, which were essential for oxygen uptake. The inhibitory effect could be reversed by adding more iron, and the polymeric fraction inhibited more effectively than the intermediate one, evidently because it precipitated out the iron more effectively. This was, therefore, a case of Type A inhibition. The authors concluded that it consisted primarily in the sequestration of ferric ions, which led to reduced metabolic activity in general and, hence, to reduced uptake of the other nutrients investigated (Einarsson et al., 1988).

In mechanistic studies of this kind, it is important to recognize the significance of experimental design. It is logical to expect that the reactive carbonyl compounds in the intermediate fraction (Fig. 3) would sequester the amino-acids in the culture medium, because this would simply represent an extension of the Maillard reaction already initiated. It would, however, only be possible to observe an inhibitory effect upon growth if the latter were taking place under conditions of nitrogen limitation. Likewise, Type B and C inhibition could only be demonstrated if the nitrogen source were exclusively polymeric.

5. *Sphagnum* mosses and peat

5.1. Use of wet peat as a coolant

Sphagnum mosses and relatively young peat (<1000 years old) absorb up to 20–25 times their own weight of water, and their porous surfaces facilitate rapid evaporation. For thousands of years, unglazed clay pots and dishes, saturated with water, have been used to cool milk, wine, butter and cheese. It is, therefore, not surprising that subterranean 'larders', excavated in peat bogs, have been used to keep foods cool over a similar period of time. This custom is still practised on a small scale today, for example by people on camping trips and by peat-cutters who have found it a convenient way to keep their lunches cool until they are ready to consume them. Anecdotes dating from the recent past back to the 14th century recount that people on angling excursions buried their 'catches' in peat until they were ready to collect them (Riddervold, 1990; Celius, 1996).

In the course of centuries, it is inevitable that people have occasionally forgotten food items that they had buried in peat, or possibly the locations of their larders. Moreover, little effort seems to have been made to enclose their butter or cheese in watertight vessels, so that in time they would have come into direct contact with bog water. Hundreds of crudely constructed, wooden kegs, many of them containing

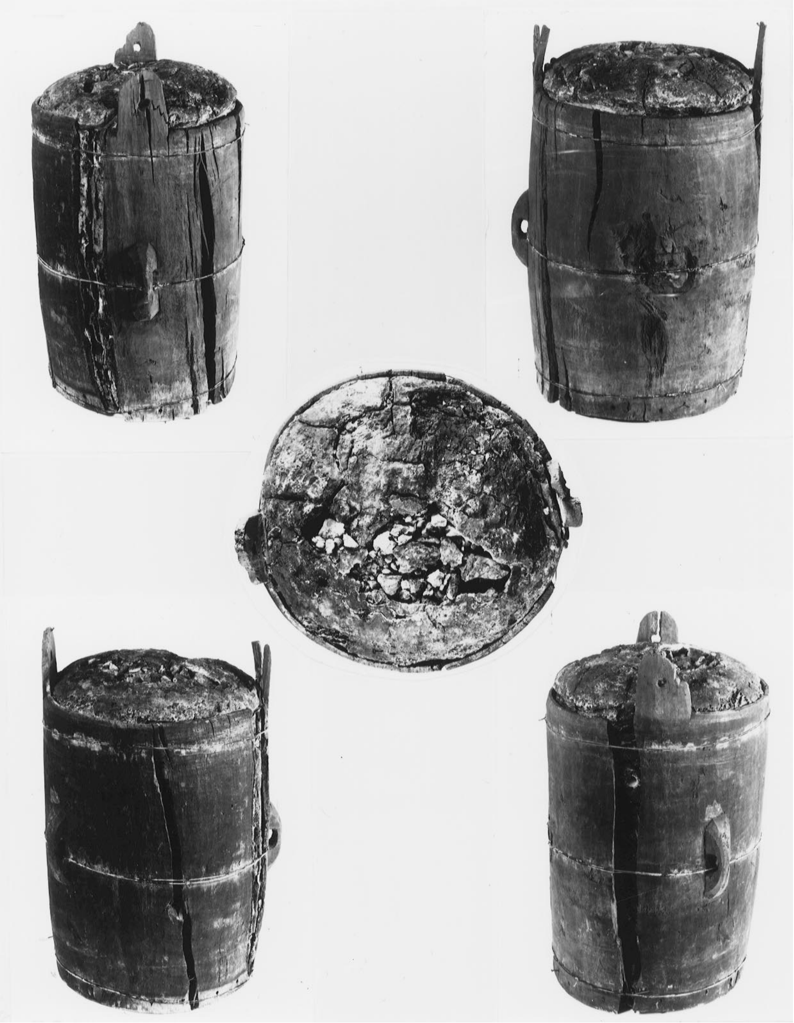

Fig. 4. Bog butter, 1800 years old, found in a peat bog in Morvern, Argyll, Scotland. Reproduced by permission of The National Museums of Scotland. The keg is constructed from a hollowed-out birch or alder trunk (Earwood, 1993).

ancient butter or cheese, have been found in Irish and Scottish peat bogs (Earwood, 1993). Lumps of butter or cheese have also been found wrapped in cloth (usually linen) or in skin (usually a bladder) or simply deposited in a wickerwork basket (Raftery, 1942; Rynne, 1989; O'Floinn, 1997, personal communication).

The emphasis on dairy products that spoil quickly in warm weather suggests that people buried them simply to keep them cool for relatively short periods during the summer months. They could scarcely have known or expected that their butter or cheese would keep for up to 1800 years (Fig. 4). Biochemical analyses on eight Scottish samples of 'bog butter' have shown, however, that they were sufficiently modified by anaerobic bacterial activity that it was hard to tell whether they were originally butter, cheese, or some other kind of animal fat (Thornton et al., 1970).

5.2. Peat bogs as garbage dumps

Peat bogs are important as havens for wildlife, but until they are drained they have little practical value except as garbage dumps. Most of those that have preservative properties are produced by the growth of *Sphagnum* in water that has collected in basins in impervious rocks. The water typically has a very low content of nutrients or other solutes, including especially calcium ions (Clymo and Hayward, 1982).

In the first stage of peat formation, the moss grows over the surface of the lake from the sides. As successive generations die, they sink beneath the surface, gradually increasing the thickness of the floating raft of vegetable matter until the whole basin is filled with peat containing at least 96% of water. During this stage, which may take 2000 years or more, depending upon the depth of the lake, anything thrown into the bog will sink and come into immediate contact with soluble, brown products of decay of the dead mosses on the surface (Moore and Bellamy, 1974).

In the second stage of bog development, the living moss on the surface grows up above the level of the groundwater, while the layers of dead moss underneath become steadily compressed.

From this point onwards, the peat becomes 'ombrogenous' (rain-fed). Ombrogenous peat is especially noted for its preservative properties. It can grow up well above the level of the surrounding terrain, engulfing and preserving whole villages that had originally been built by the lakeside.

Between about 4000 BC and AD 1000, a number of communities chose to live in houses built of wood and wattle beside lakes and wetlands (bogs and marshes), or over them on wooden piles. The remains of these villages, preserved in peat, have been discovered throughout Western Europe from Italy in the south to Denmark and Sweden in the north, and eastwards as far as Poland (Coles and Coles, 1989). Predictably, the 'lake dwellers' deposited their domestic debris in the lake waters, or dumped them on the edge of the wetlands. Fire was used for heating and cooking, and the buildings occasionally caught fire and burned down. This explains the discovery of many food materials that had been charred by fire. They may have been deliberately dumped because they were no longer palatable, or they may have fallen into the bog-water when the building in which they were stored caught fire and collapsed.

The charred foodstuffs included grains of wheat and barley, whole loaves of bread, apples and pears that had been halved and dried for storage, sloes and berries of many kinds, nuts, water-chestnuts and acorns. Domestic animals, including cows, goats, pigs and sheep, must have been housed in these settlements, because their faeces were, predictably, also thrown into the bog and preserved there in recognizable form. This is confirmed in part by the discovery of numerous, whole carcasses of pigs, 'frozen' in attitudes which suggested that they had died by drowning. The recent discovery of the well-preserved head of an ox, 2500 years old, in Solway Moss, near Gretna, Scotland, should also be noted. A fascinating and meticulously documented account of these and many other examples of the preservation of biodegradable materials in peat is provided by Coles and Coles (1989).

5.3. *Sphagnum mosses as scavengers of atmospheric ammonia*

Sphagnum mosses emerged as a recognizably distinct group from other bryophytes about 280–300 million years ago. Like other bryophytes they have no roots, and like most mosses they have leaves that are only one cell thick and able to absorb nutrients directly from the ambient water. Once *Sphagnum* has grown above the influence of groundwater it becomes entirely dependent upon rainwater and the atmosphere for nutrients, and its exceptional biological success is owing largely to the efficiency with which it extracts them.

About 90% of the volume of a *Sphagnum* leaf is made up of long, empty, thin-walled ('hyaline') cells with circular or elliptical openings ('pores') through which rainwater enters and leaves the leaf under gravity (Fig. 5). The water passes through tubes supported on the inside by hoops with transverse struts. The walls between the hoops are flexible, which permits them, within limits, to expand or contract proportionately with the water supply. The diameter of the pores on the concave surfaces of the leaves are such that surface

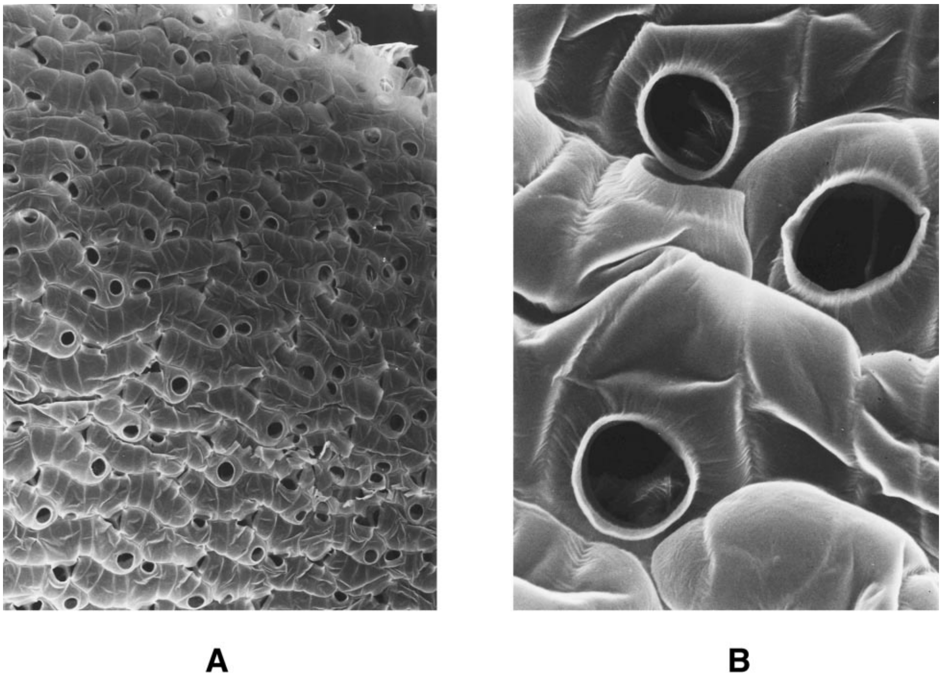

Fig. 5. Scanning electron micrograph of a leaf of *Sphagnum palustre* from which the lignin has been removed by a mild chlorite bleach. The average diameter of the circular openings (pores) is 13 μm. As rainwater flows under gravity through the tubes formed by these empty hyaline cells, it passes over the surfaces of the smaller, chlorophyllose cells inside the leaf, which absorb the traces of ammonia and nutrient salts that it contains (S.E.M.: Sissel Herzberg).

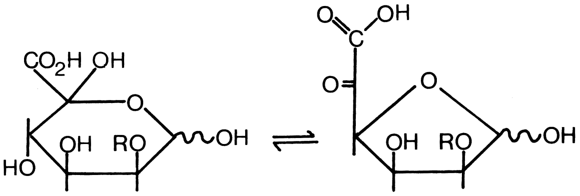

Fig. 6. 5-Keto-D-mannuronic acid (5 KMA) in its hemiketal (pyranose) ring form and its highly-reactive keto- (furanose) ring form. In sphagnan, both forms are glycosidically linked through positions 1 and 2, and some of the pyranose units are additionally linked through position 5 (Painter, 1983a, 1998; Andresen et al., 1987).

tension prevents the cells from discharging their water until more is supplied (Clymo and Hayward, 1982).

The nitrogen extracted from the rainwater is mainly in the form of ammonia washed out from the atmosphere or absorbed directly as gas. It originates mainly from the decay of organic matter outside the bog area, but forest fires, the combustion of fossil fuels by man, and the activities of farmers also contribute significantly. Other contributors to the total input of ammonia, though numerous, are believed to be relatively minor (Mattson and Koutler-Andersson, 1955).

That part of the ‘extraction plant’ that binds ammonia is, not unexpectedly, a highly reactive carbonyl compound. In the roots of higher plants, that compound is α-ketoglutaric acid. It is first reductively aminated to give glutamic acid, which is then amidated to give glutamine (Woodall et al., 1996). In contrast, in the hyaline cell-walls of *Sphagnum*, the α-ketocarboxylic acid groups are built into the structure of a polysaccharide, sphagnan, that comprises about 60% of the total holocellulose. They occur in residues of D-*lyxo*-5-hexosulofuranuronic acid (Fig. 6). This sugar is referred to trivially as 5-keto-D-mannuronic acid (5 KMA). Its pyranose ring-form (Fig. 6), which has two anomeric centres, is also present in the holocellulose. It is involved, in part, in cross-linking sphagnan with cellulose and a xyloglucomannan resembling the ‘amyloids’ of higher plants (Painter, 1983a, 1991a, b, 1995a, 1998; Andresen et al., 1987).

It is natural to enquire how this helps to increase the efficiency of ammonia uptake. Any answer will have to take account of the fact that the hyaline cells have no cytoplasmic contents. They are dead cells that function as a water reservoir while the living cells, which make up only 10% of the volume of the leaf, extract nutrients from the water and do all the photosynthesis. A plant that was able to conduct photosynthesis only while it was raining could hardly compete with a vascular plant that was able to absorb nutrients through its roots continuously. Hence, the 5 KMA units that line the walls of the hyaline cells are likely to have a storage function. Schiff-base formation is reversible in the first instance, and the 5 KMA units may help to stabilize the concentration of free ammonia in the stored rainwater, so that the living cells can continue to consume it during periods of dry weather.

5.4. Type A, B and C inhibition in Sphagnum-dominated peat bogs

In the living moss that carpets the surface of the bog, sphagnan is covalently cross-linked to other cell-wall glycans and is an integral part of the structure of the holocellulose. As such, it is completely insoluble in water. The moss therefore functions as a Type A inhibitor of microbial growth, simply by removing ammonia from the rainwater that filters through its leaves (Fig. 5). Any amino-acids or other primary amines would also be removed. Enzymes can also be bound through their free amino-groups, and hence the living moss should also be a Type B inhibitor.

These ideas have been fully confirmed in the laboratory by studying the immobilization of ammonia, amino-acids, basic dyestuffs, and model globular proteins and enzymes on *Sphagnum* holocellulose (Painter, 1998). In general, the enzymes lost their activity considerably faster when bound to the holocellulose than when kept in aqueous solution in the same buffer and at the same temperature. Hence, they were not only removed physically from solution, but also deactivated. The immobilization was reversible in the first instance, but it was followed by a visible ‘browning’ reaction, after which the amine could not be removed by washing at any pH.

When the moss dies, sphagnan with its highly reactive 5 KMA units is slowly released in soluble form into the bog water. This process takes place continuously over a period of 2000–3000 years (Mattson and Koutler-Andersson, 1955). The degradation is chemical rather than microbiological. It consists in a kind of selective, acid-catalysed autohydrolysis. It can be simulated convincingly in the laboratory by elevating the temperature to increase the rate of reaction (Painter, 1991b). Simultaneously with the autohydrolysis, the unstable 5 KMA residues lose carbon dioxide and the elements of water, to form a brown, acidic polymer, still containing most of the other sugar residues in sphagnan, but also a dark brown chromophore (Painter, 1983b, 1991b).

This process of spontaneous ‘humification’ takes place even in the absence of amino-acids or other amines, but it goes much faster when amines are present. Hence, humic-acid formation in the presence or absence of amines may be compared with a true Maillard reaction vis-à-vis caramelization (Painter, 1983b, 1991b). In the natural situation, amines are always present. The soluble ones (ammonia, amino-acids and globular proteins) react with the soluble form of sphagnan to produce the familiar brown polymer (‘aquatic humus’) that gives bog water its colour. The insoluble ones include the fibrous proteins in any animal tissue that finds its way into the bog. The collagen in hide, cartilage and tendons is converted into leather; the myosin in muscle is cross-linked as on the surface of smoked fish or meat; and the keratin in horns, hooves, nails, claws, wool, feathers, hair and fur becomes more resistant to biodegradation. They all acquire the characteristic colour of melanoidin

(Painter, 1991a, 1995a). Sphagnan in its soluble form is therefore primarily a Type C inhibitor.

5.5. Special significance of Type A and B inhibition

From the point of view of food preservation, Type A and B inhibition are especially interesting because they do not depend upon derivatization of food proteins and do not, therefore, reduce their nutritional value. It is easy to assess the significance of these mechanisms in *sphagnum* peat simply by examining the well-preserved remnants of higher plants that it contains. This activity constitutes an area of active research in palaeobotany (Grosse-Brauckmann, 1963; Godwin, 1981; Barber, 1986). Cotton grass (*Eriophorum*) is a special case (because it contains silica), but the leaves of dicots, including especially heather (*Calluna*), the cranberry (*Vaccinium*), alder (*Alnus*), willow (*Salix*) and birch (*Betula*) are easily identifiable in peat up to at least 2000 years old. They must have been preserved by the *Sphagnum* in the peat, because they decay rapidly when composted alone. On the other hand, they could not have been tanned or derivatized by water-soluble sphagnan, because their morphology is determined by polysaccharides (cellulose and hemicelluloses) rather than by fibrous proteins. Similar conclusions are indicated by the preserved garbage from the lake villages. Cereal grains, loaves of bread, and dried fruits and berries are composed mainly of polysaccharides and could not be preserved in recognizable form by derivatizing their protein components alone.

5.6. Humic acids as Type A inhibitors

Since sphagnan is only slowly released in soluble form after the moss dies, the part that remains in the insoluble fragments of *Sphagnum* leaves can continue to sequester ammonia and amino-acids, and to immobilize and deactivate the exocellular enzymes of putrefactive bacteria up until the peat is at least 2000 years old. At the same time, humification reactions occur within the insoluble leaf fragments. Indeed, a high proportion of the humic acids in peat are never released in soluble form, and they become an increasingly significant part of the insoluble residue as the peat ages (Mattson and Koutler-Andersson, 1955).

Peat humic acids must be regarded as a ‘family’ of glycoconjugates containing a continuously variable proportion of unmodified sphagnan vis-à-vis a dark brown, nitrogen-containing chromophore. The latter can be isolated by hydrolysing away the carbohydrate moiety with mineral acid, and is found to be insoluble in water at the pH of the bog (pH 3.2–5.5). Hence, the essential difference between aquatic humus and the humic acids that are insoluble at the pH of the bog water is that the former contains more unmodified sphagnan, covalently linked to the chromophore (Painter, 1983b; Smidsrød and Painter, 1984).

There is little merit in distinguishing between this kind of humification and a Maillard reaction, or between the chromophore and a melanoidin. Both reactions entail decarboxylation and dehydration, an increase in carbon content and equivalent weight, a decrease in solubility, and similar spectral changes. Both products are polymeric and contain firmly bound nitrogen which cannot be hydrolysed away with concentrated mineral acids (Olssen et al., 1978; Painter, 1983b, 1991b).

Another important similarity is that the polymeric products show greatly enhanced affinities and altered selectivities for multivalent metal cations compared to those of either reactant. The previously cited work of Einarsson et al. (1983, 1987) demonstrated this for a classical Maillard reaction between D-xylose and L-arginine. Similar effects were noted when sphagnan was compared with a sample of aquatic humus isolated from natural peat-bog water (Smidsrød and Painter, 1984).

Pure, unmodified sphagnan showed only a modest selectivity for Ca^{2+} compared with Mg^{2+}. Expressed as a ‘selectivity coefficient’ at 50% saturation with Ca^{2+}, this was $((K^{Ca}_{Mg})_{50} = 1.33$. The corresponding value for aquatic humus was 27.4. Likewise, $(K^{Cu}_{Mg})_{50}$ was 30 for sphagnan, but 1000 for aquatic humus. Some selectivities were even inverted; for example, Zn^{2+} was bound more firmly than Ca^{2+} by sphagnan $\{(K^{Zn}_{Ca})_{50} = 2.3\}$, but less so by the aquatic humus $\{(K^{Zn}_{Ca})_{50} = 0.4\}$. Considerable efforts were made to find out whether the living moss contained some other component that had become incorporated into the aquatic humus and which could explain these differences. Samples of the whole moss and of its chlorite holocellulose, however, showed essentially the same cation-bindinq selectivities as sphagnan (Smidsrød and Painter, 1984). Moreover, the total cation-exchange capacity of the whole moss could be accounted for entirely as that of the holocellulose (Painter and Sørensen, 1978).

The correct explanation was finally revealed by measuring selectivity coefficients for similar pairs of cations on samples of sphagnan that had been ‘humified’ in the laboratory by heating aqueous solutions either alone (Painter, 1983b) or with selected amino-acids (Painter, 1991b; Holsen and Painter, unpublished results). The results were decisive: increasing degree of humification was accompanied by marked changes in selectivity, and especially an increase in the affinity for Ca^{2+}, Cu^{2+} and Pb^{2+} relative to Mg^{2+}.

Other cations have not so far been investigated in the author’s laboratory, but cation-binding by humic acids in general has been intensively investigated because of its relevance for soil fertility (Schnitzer, 1978) and waterworks technology (Gjessing, 1976). High affinities are reported for Co^{2+}, Fe^{3+}, Al^{3+}, Mn^{2+}, and $Mo^{2 \rightarrow 4+}$. These are prominent among the essential trace elements that micro-organisms need for growth, and humic acids should accordingly be effective Type A inhibitors whenever their concentration in the culture medium exceeds the total supply of essential, multivalent metal cations. This has been confirmed in the laboratory (Painter, 1991b).

Whereas there is a massive excess of metal-complexing capacity in an unpolluted *Sphagnum*-dominated peat bog, this is not the case in most ordinary soils. There is also unlikely to be an excess of chelatory capacity in the human intestine after a meal cooked with curry or soya sauce, a slice of bread, or a glass of dark beer taken as part of an adequate diet. It should be emphasized that humic acids do not kill bacteria, and when the supply of essential trace elements exceeds their capacity to sequester them, the bacteria grow quite normally. When the cations of toxic metals such as cadmium or lead are present, a little humic acid can even stimulate growth by sequestering them selectively (Prakash et al., 1973; Steinberg and Muenster, 1985). Low concentrations of humic acids can also stimulate growth by acting as a carrier for iron (Foster et al., 1991). It would, therefore, be more accurate to describe humic acids and melanoidins as ‘regulators’ of microbial growth than to categorize them simply as ‘inhibitors’.

5.7. Origin of the sequestering properties of melanoidins

The emergent cation-binding affinities have a profound significance for food preservation and human nutrition, and an effort must be made to understand them. It is instructive to look again at the Maillard reaction intermediates in Fig. 3, and to note how many of them contain constellations of functional groups that could in theory bind metal cations differently or more strongly than those in either of the two reactants (D-glucose and glycine). Whereas glycine could form a bidentate complex only, 3.9 could form a terdentate one, 3.15 and 3.27 a quadridentate one, and 3.16 possibly a quinquedentate one (Chaberek and Martell, 1959). Similar constellations are almost certainly present in the polymeric end-products of the Maillard reaction, and the polymers could well include additional ones, such as α,α'-dipyridyl structures or even tetrapyrrole (porphine) rings.

These for intramolecular co-operativity in cation-binding are supplemented by those for intermolecular co-operativity. With a polyanion such as alginate (Smidsrød and Haug, 1972), and especially with a polyampholyte such as a proteoglycan (Painter, 1995b, 1996), such effects can arise in the cross-linking of different chains by chelate-bridge formation. They can enhance cation-binding affinities by up to two orders of magnitude compared to a system in which cross-linking is prevented by the immobilization of isolated molecules in a gel or on a support of inert material.

6. Discussion

Formaldehyde is perhaps the most powerful known preservative, but it kills all cells indiscriminately, and even a trace amount, applied superficially as in smoking, downgrades the nutritional value of protein to some extent. In contrast, a hypothetical polymer chain carrying numerous, pendant carbonyl groups would probably not poison any cells, but only inhibit their uptake of amino-nitrogen and inactivate any enzymes that they secrete into the medium. If used in the form of absorbent but insoluble particles, it could not modify fibrous food proteins chemically, except perhaps very superficially in regions of close contact between the two insoluble surfaces.

Nature has provided a prototype of such a polymer in the holocellulose of the hyaline cell walls of *sphagnum* mosses, while dedicated archaeologists have provided a painstakingly documented account of its performance so far. It should be emphasized that very little of this evidence is anecdotal, and much of it can be freely inspected in museums all over Europe.

It is true, of course, that peat does not always preserve biodegradable materials that find their way into it. Its capacity to absorb ammonia, amines and multivalent metal cations is not unlimited, and peat that has been polluted by industrial waste or run-off from agricultural soil or pastureland is unlikely to preserve anything, though it could still participate in cation-exchange processes.

Two different, chemical steps are involved in the preservation process, corresponding to different stages in a Maillard reaction. Their relative contributions to the total preservative effect depend upon the age (and hence the depth) of the peat immediately surrounding the material that is to be preserved. In the living moss and in young peat, ammonia, amino-acids and proteins, including any exocellular enzymes secreted by saprogenic microorganisms, are bound and immobilized on the hyaline cell-walls and their fragments in the peat. Cations, including multivalent metal cations, are also bound by these materials, though the multivalent ones are subsequently bound more firmly by humic acids.

As the peat ages, a humification reaction takes place spontaneously within the insoluble cell-wall fragments. It consists in a transformation of the complex formed between the bound amines and the sphagnan chains in the holocellulose. This reaction shows all the general characteristics of a classical Maillard reaction, and the dark-brown chromophore that is produced as an end-product is essentially a kind of melanoidin, like the brown pigment in molasses, dark beers, soya sauce, and European condiments such as Worcester Sauce and Marmite. The cation-binding affinities of this melanoidin are radically different from those of the starting-materials in the living moss, and some of them are greatly enhanced. This means that a new, altered or enhanced kind of Type A antibacterial activity develops as peat ages.

During the aging process, some free sphagnan is liberated in water-soluble form, and it condenses with any free, primary amino-groups in the fibrous proteins of animal remains that have found their way into the bog. Such complexes undergo a similar, spontaneous transformation during which the fibrous proteins are tanned by cross-linking and acquire the characteristic, dark-brown colour of melanoidin.

With this formidable arsenal of antimicrobial activities, it may be supposed that peat is sterile, and this was actually believed to be true for many years until Waksman (1930) showed otherwise. The very existence of peat is testimony enough that the intensity of microbial activity in the anoxic region (which extends from about 40 cm below the surface downwards) must be very low. Although extreme variations are reported (some of which are almost certainly owing to pollution), it is perhaps possible to estimate that the number of anaerobic bacterial cells per gram of wet peat is, on average, a millionth of the number in heap of rotting grass or a septic tank.

Bacteria are known which could, in principle, escape the rigors imposed by the affinity of peat for ammonia, primary amines and multivalent metal cations. Among the most likely candidates are some *Clostridium* species which can fix atmospheric nitrogen anaerobically; and facultatively anaerobic *Pseudomonas* species which can utilize nitrate and nitrite (originating from thunderstorms, for example) as a nitrogen source. Some bacteria are also known to produce 'bio-chelators' that surpass even peat humic acids in their affinity for certain multivalent metal cations (Painter, 1995b, 1996). Organic carbon may be supplied by the reducing sugars that are believed to be slowly released during the spontaneous autohydrolysis of *Sphagnum* holocellulose (Painter, 1991b).

It is obviously inadvisable to bring peat into direct contact with susceptible foods such as fish or meat unless it has first been sterilized. Peat that has been polluted, for example by run-off from mining operations, may also contain the cations of toxic or radioactive heavy metals. These could be removed, if necessary, by an acid wash. There is, however, no serious argument against using purified peat for food preservation.

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