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Maillard Reaction

Chemistry and Consequences in Food Properties

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Contents

Introduction

Maillard reaction is a non-enzymatic browning reaction, caused by the condensation of an amino group and a reducing compound. It is a complex network of diverse reactions involving mixtures of compounds with varying initial concentrations. The Maillard reaction is divided in four steps: 1) the formation of an N glucosamine from sugar amino condensation; 2) rearrangement of the glycosamine via Amadori mechanism; 3) degradation and fragmentation of the Amadori product and amino acid degradation; and 4) the condensation and polymerization of aldol, aldehyde and amino compounds forming melanoidins.

This reaction occurs in many different fields, and the analysis under any particular set of conditions represents a continuous challenge for scientists. Great numbers of both desirable and undesirable changes occur in raw materials and foods as a result of the Maillard reaction. In food, the condensation is commonly between the amino acids (both free and peptide-bound), and reducing sugars. During heat treatment like frying, roasting and baking the reaction produces different flavours and colours, most of them were desirable. Roasting of cereals is an example of processes in which the reaction brings about a compromise between positive and negative effects.

The Maillard reaction was first studied with respect to humus formation and only later became concerned with color and flavour formation in food. The significance of the reaction in nutritional physiology and food safety was also began to be realized. Recently, the Maillard reaction has been the junction of food and biomedical sciences. Maillard products are now known to contribute to the natural and normal aging of tissue proteins and other biomolecules. It is also implicated in the pathologic processes of a range of age-related chronic diseases, including arthritis, arteriosclerosis, diabetes, and neurodegenerative diseases. The role of this reaction in diabetes and its complications has become one of the major focuses of research, giving a better understanding of oxidative stress, lipid peroxidation, and hyperlipidemia in diabetic complications (Baynes et al., 2005).

In this paper the discussion will be focus on three main points: the Maillard reaction in food science, the chemistry of the reaction, and the properties of the food that are affected and become a product of the Maillard reaction.

History

The Maillard reaction was first described by Louis-Camille Maillard in 1912 when he presented a paper to the French Academy. The concise paper described some recent experiments where he had made a very simple observation: softly heating sugars and amino acids in water lead the development of a yellow brown color. Maillard described the original protocol in only 77 lines, including the title. He outlined the chemical reaction between amino acids and sugars, providing details on the mechanism of the reaction, which were later confirmed (Fayle and Gerrard, 2002; Finot, 2005).

Maillard knew biology was saturated with sugars and amino acids and, therefore, he imagined the implications of this reaction for human physiology and pathology, plant physiology, geology, agronomy, and analytical chemistry. Furthermore, he also utilized the terms of chemical pathology and mentioned pathological disorders in relation to diabetes. Moreover, the consequences or Maillard chemistry are indeed extensive, and cut across many disciplines, the most notable being food science and medicine. In other words, there cannot be many other fields in which the same fundamental chemical knowledge can be so broadly applied. This particular reaction has attracted the interest of a range of researchers from organic chemists to food scientists to medics (Fayle and Gerrard, 2002).

The reaction described by Maillard was ignored for 24 years. In 1941, Weast and MacKinney (cited by Finot, 2005), from the University of California at Berkeley, published a paper on the nonenzymatic darkening of fruits and fruit products, in contrast to enzymatic browning. This was the first time that the name of Maillard appeared again since the paper was published in 1916. In 1942 Dood et al. (cited by Finot, 2005) discovered the influence of moisture on browning of dried whey and skim milk, but the name Maillard was not mentioned. His name is after all cited in 1946 in Nature by Henry et al. (cited by Finot, 2005) to explain the multiple modifications of skim milk upon storage, but with no reference to his publications. In 1950–51 Patron (cited by Finot, 2005), gave some credit to Maillard and his reaction when, on the basis of his experience with the browning of fruits, proposed to extend the consequences of the Maillard reaction to other foods (milk powder, egg powder, canned fish, protein hydrolysates, and bread). He stated that the Maillard reaction was responsible for the development not only of off-flavours but also the production of aromas such as those in beer, caramel, bread, maple sugar,

fried potatoes, and cheese (Finot, 2005). Two years later, Hodge publish a famous review on the chemistry of browning reactions. In fact, the scheme of the Maillard reaction that he proposed in that publication is still valid today.

More than 25 original Maillard products have been identified and structurally characterized in tissue proteins during the last 25 years, and new products continue to be described at the International Symposium on the Maillard Reaction. Since 1979, this meeting has gradually shifted from interest in glycation to advanced glycation, from studies on the effects of Maillard products on protein structure and function to studies on the interplay between the Maillard reaction and regulatory biology – oxidative stress, apoptosis, and the mechanism of glucotoxicity and lipotoxicity. In addition, the attention has extended from the original interest in aging and diabetes to the much broader scope of research today on human health and disease – the influence of dietary and endogenous Maillard product on renal and vascular function and on chronic neurodegenerative disease (Baynes *et al*, 2005).

To provide an historical appreciation of research on the Maillard reaction, the following list is a short overview of the program contents of previous "International Symposium on the Maillard Reaction" meetings:

- **1st, 1979, Sweden** - A critical review of nutritional aspects of the Maillard reaction, followed by presentations addressing Chemical, Physiological and Technological Aspects of the reaction. Workshops on Caramelization, Separation and Fractionation, Toxicology, Water Activity, and Volatile Products.
- **2nd, 1982, USA** - An historical overview on the Maillard reaction, followed by presentations on: Chemistry of the Maillard reaction; Flavours, Tastes and Odours; Food Technology; Nutrition; Maillard reactions *in vivo*; and Toxicology.
- **3rd, 1985, Japan** - Presentations in sessions devoted to Food Chemistry, Food Technology, and Biological (mostly nutritional and toxicological) Aspects of the Maillard reaction. Besides, workshops devoted to: Low Molecular Weight Products, AminoCarbonyl reactions of protein *in vitro* and *in vivo*; and Melanoidins. The Maillard reaction under physiological conditions *in vitro* or the growing evidence for the role of the Maillard reaction in diabetes and aging.

- **4th, 1989, Switzerland** - A total of 59 presentations divided into sessions on Chemistry, Food Science, Nutrition, Toxicology, and In Vivo Reactions. Sixteen papers dealt with the Maillard reaction in living systems, including one paper on the use of organic germanium compounds and aminoguanidine to inhibit the Maillard reaction.
- **5th, 1993, USA** - Research presentations in the areas of Chemistry, Food, and Health. This meeting was the first at which presentation were equally drawn from research in the food and biomedical sciences. Approximately half of the presentations dealt with physiological aspects of the Maillard reaction, including the first papers on AGE receptors in biological systems.
- **6th, 1997, England** - Research presentations on Reaction Mechanisms, Food Technology, Kinetics and Analytical Chemistry, Flavour Chemistry, Toxicology and Antioxidants, and Health and Disease. At the 5th and subsequent International Maillard Symposia, the presentations were approximately equally derived from laboratories in the food and biomedical sciences, with a substantial number of presentations being interdisciplinary in nature.
- **7th, 2001, Japan** - The beginning of the postgenomic era, and the Maillard reaction is one of the most important post-translational modifications of proteins in life science. The program was divided equally into oral presentations in the food and biomedical sciences. The earlier covered the reaction mechanism, kinetics and analytical aspects of the Maillard reaction, food technology, flavour chemistry, ecology and antioxidants, whereas the latter covered *in vivo* aspects of the Maillard reaction affecting human health and diseases, which included oxidative stress, glycation and cell biology, pharmacological prevention of glycation, new horizons in glycation research, and glycation and diseases.
- **8th, 2004, USA** – Discussion about the formation of an International Maillard Society, an organization designed to foster research on the Maillard reaction and to provide a formal structure for sponsoring future symposia in this expanding area of research.

Maillard Reaction in Food

Since food as been cooked, the Maillard reaction has played an important role in improving the appearance and taste of foods. Describing the analysis of Maillard reactions in

food is a challenge since the products of the reaction are numerous, changing rapidly and are often chemically undefined. In addition, defining the problem is often a project in itself, because one has to identify precisely what is being looked for without any clue how to measure it (Martins et al., 2001; Fayle and Gerrard, 2002).

Several changes in the properties of food have been attributed to the Maillard reaction including: changes in color (particularly browning); production of aroma and flavour compounds; production of bioactive compounds (beneficial and toxic); loss of nutritional quality (especially proteins) and changes in texture. These changes occur in traditional process such as roasting (coffee), baking (breads), toasting (cereals) and cooking (meat). Food processing conditions can include high temperature and pressure, which result in the formation of many Maillard reactions products that can vary in concentration depending on the different reactions (Martins et al., 2001; Fayle and Gerrard, 2002; Miller and Gerrard, 2005).

Heating is a process used in the food industry to obtain safe products with a prolonged shelf life. It is also used to improve the sensory properties of food; however, it may as well cause changes that decrease food quality. Many desirable and undesirable effects of heating are due to Maillard reaction (Brands and van Boekel, 2001).

High pressure is also used as an aid to preserve food or as a means of making products with different quality attributes. Ames (1998) demonstrated that the effects of pressure varied according to the pH of the system. In all systems, pressure may modify the chemical reactions taking place, accordingly influencing the rate of color development.

As a result the Maillard reaction is very difficult to control. Various factors involved in food processing influence it and they can be considered as food processing variables. The kinetic approach tends to present a complementing view of this mechanism, because it considers the rate determining steps of the reaction, and this provides control points (Martins et al., 2001).

As mentioned at the beginning, the Maillard reaction is one of the non enzymatic browning reactions. A non-enzymatic browning reaction is a chemical process that produces a brown color in foods without the activity of enzymes. Browning is caused by the formation of unsaturated, coloured polymers with different composition (Hodge, 1953). Three different non enzymatic browning reactions are recognized in food technology:

1) Carbonyl-amino reactions, which includes the reactions of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides and proteins. The sugar interacts with the amino acid, producing a variety of odours and flavours. The Maillard reaction is the basis of the flavouring industry, since the type of amino acid involved determines the resulting flavour.

2) Caramelization, which occurs when polyhydroxycarbonyl compounds (sugars polyhydroxycarboxylic acids) are heated to relatively high temperatures in the absence of amino compounds, a complex process in which sugar reaction products condense and form brown macromolecules. This type of browning characteristically requires more energy to get started than the carbonyl-amino reactions. This reaction is preceded by sugar isomerisation and sugar degradation reactions. Monosaccharides in aqueous alkaline medium undergo both reversible and irreversible transformations. The reversible reactions included ionization, mutarotation and enolization. As the process occurs, volatile chemicals are released producing the characteristic caramel flavour. Neither carbonyl-amino nor caramelization reactions are dependant upon the presence of oxygen to produce browning. In this type of reaction, the rate of color formation is markedly increased with higher pH (Hodge, 1953; Brands and van Boekel, 2001).

3) Oxidative reactions, in which ascorbic acid and polyphenols, are converted into di or polycabonyl compounds. This reaction may or may not be enzyme catalyzed. Zamora and Hidalgo, 2005, propose that lipid oxidation pathway affected the Maillard reaction pathway and vice versa. Both mechanisms have common intermediates and polymerization mechanisms, seeming to produce similar products with a nonenzymatic point of view. As a result, it is recommended to consider both reactions simultaneously in order to have a better understand of the products of Maillard reaction in the presence of lipids.

Finot (2005) reported several different reasons for studying the chemistry of the Maillard reaction:

- In food science, to evaluate the influence of reaction parameters (pH, Tº, time, sugar reactivity, concentration of the reagents, water activity, glass transition temperature) on the evolution of the reaction and on changes in food quality.

- In nutrition, to quantify the loss of bioavailability of essential amino acids; to understand the metabolism of the reaction products and to define the physiological affects of the ingested Maillard reaction products.

- In the food industry, concern with the occurrence of this reaction in process and to understand the phenomena and to optimize the processes and conditions of food preparation in order to preserve the nutritional, safety, and organoleptic qualities of foods.

Most of the information on the chemical mechanism of the Maillard reaction has been based in the analysis of model systems, in which chemical reactions mimic the chemistry of food stuff and cells. To know what happens inside the food, we need methods to measure the concentrations of Maillard products amongst a huge number of potentially interfering substances. In addition, the situation is more complicated by the propensity of Maillard products to interconvert during the course of the purification or measurement (Fayle and Gerrard, 2002).

The methodology presently used for analysis of the Maillard reaction, is a combination of extraction methods like: Gas Chromatography (GC), Liquid Chromatography (LC or HPLC), Capillary Electrophoresis; with detection methods like: Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR). The most common combination is GC-MS, because of high reproducibility, standardized technique, and high separation efficiencies, which allows separation of a mixtures into individual components, identifying components as well as providing valuable structural information. In the last ten years NMR studies have begun to yield not only unambiguous structural information about previously unidentified reaction products, but also insights in mechanism by which these products may have been formed. Most studies involve isolation of the compound of interest, followed by analysis by one dimensional and/or two dimensional NMR methods. The majority of research has so far employed proton ^{1}H, or carbon ^{13}C NMR of molecules in solution. There are also other studies with ^{31}P, ^{19}F, ^{15}N and the latest ^{17}O NMR, used to understand the mechanism of sugar fragmentation (Fayle and Gerrard, 2002; Robert et al., 2005; Zamora and Hidalgo, 2005).

Fluorescent compounds have been recognized as important early markers of the reaction in food products since 1942. However, the recent advances in the characterization of fluorophores development have been in the biological and biomedical areas. Application of fluorescence

measurement is considered a potential tool for dealing with key problems of food deterioration through early detection of markers or indexes of the damaged biomolecules (Matiacevich et al., 2005).

Other techniques from the biochemical sciences have also begun to find application in the Maillard field. In particular, immunochemistry has begun to allow the detection of specific molecules of interest within a complex mixture. This method obviates the need to separate Maillard reaction products from food, and has a considerable potential in monitoring specific molecules of interest that may be formed during food processing (Fayle and Gerrard, 2002).

Chemical Mechanism of Maillard reaction

Previous studies revealed seven different types of reactions which are known to occur during browning in sugar-amine systems. Hodge (1953) classified these reactions in three stages of development according with the production of coloured products. The initial stage result in colourless compounds; therefore the reactions of sugar-amine condensation and Amadori rearrangement do not produce colour absorption in near-ultraviolet. In the intermediate stage, the reactions of sugar dehydration, sugar fragmentation and amino acid degradation occur. The compounds formed in these reactions absorb in the ultraviolet (UV), eg hydroxymetilfurfural (HMF), but do not absorb in the visible region of the spectra. The final stage is highly coloured, as it produces a very strong absorption in the UV region with the tail of the curve progressively entering into the blue region of the visible spectrum. In this stage, the reactions of aldol condensation, aldehyde-amine polymerization and the formation of heterocyclic nitrogen compounds are occurring (Hodge, 1953; MacDougall and Granov, 1998).

The scheme developed by Hodge in 1953 is shown in **Figure 1**. This scheme represents the types of reactions and products which have been found in model systems. Although this scheme was developed in 1953 it is still valid today, but new schemes, new intermediates products and the influence of the pH in the reaction is still in discussion (Yaylayan, 1997; Ames, 1998; Martins et al., 2001; Matiacevich et al., 2005).

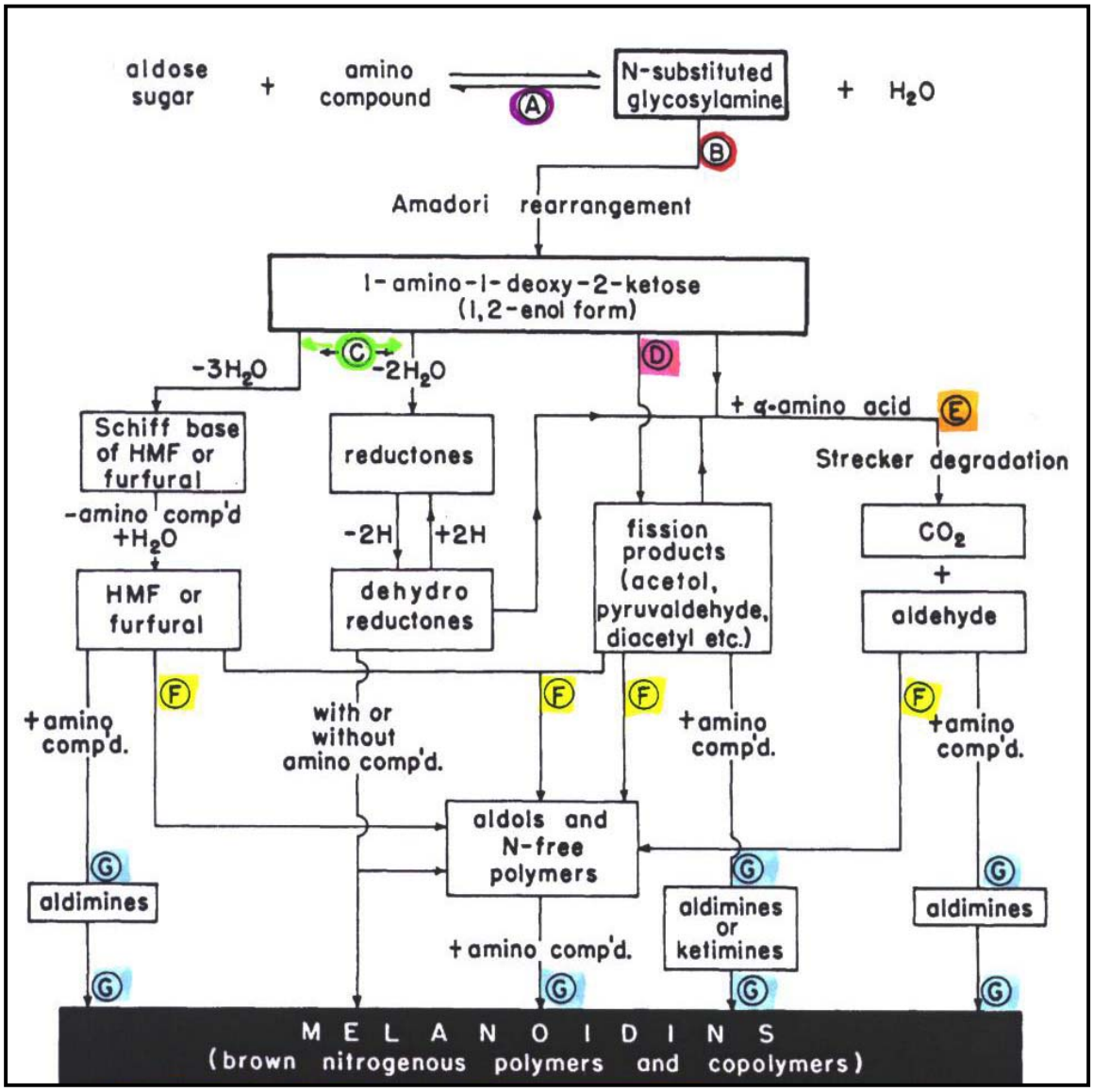


Figure 1 – Maillard reaction (Hodge, 1953)

The first reaction in **Figure 1** is the sugar amine condensation (A). In a series of reversible reactions, the carbonyl moiety of the sugar molecule forms a Schiff base with an amine, typically an amino acid or lysine residue of protein, **Figure 2**. This is the result of a nucleophilic attack by the amino group of the amino acid on the electrophilic carbonyl of sugar. Color formation in aqueous reducing sugar/amino acid mixtures is directly proportional to the percentage of reducing sugar in the aldehyde form (Hodge, 1953; Kramhöller et al., 1993; Mlotkiewicz, 1998; Maritn et al., 2001; Fayle and Gerrard, 2002).

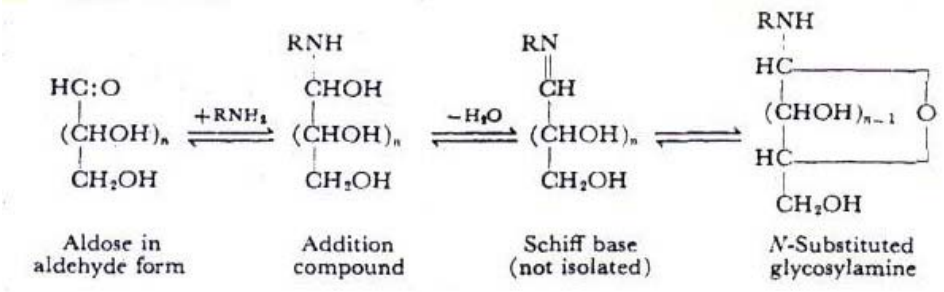


Figure 2 – sugar amine condensation (Hodge, 1953)

Hodge (1953) concluded that reducing sugars and amines condense in an equimolar ratio in browning reactions. The initial reaction is reversible, but irreversible reactions soon follow. The accepted mechanism for sugar amine condensation involves opening of the ring form of the sugar, addition of the amine to the carbonyl group, and subsequent elimination of a molecule of water to form the N-substituted glycosylamine. As you see in **Figure 2**, one mole of water was liberated for each mole of sugar combined with the protein.

The second reaction is the Amadori rearrangement, which is a key reaction for browning in aldose amine, and ketose amine systems, (Reaction B in **Figure1**).

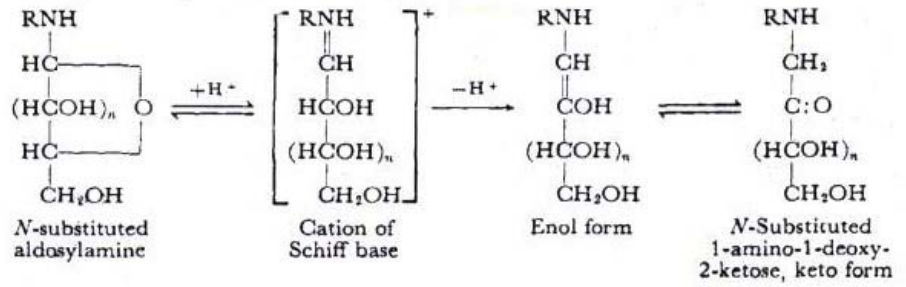


Figure 3 – The Amadori rearrangement (Hodge, 1953)

The Amadori rearrangement is the isomerisation of the N-substituted aldosylamines to 1-amino-1-deoxy-2-ketoses **(Figure 3)**. The reaction is acid catalyzed. Studies review by Hodge in 1953, showed that autoisomerization is a general reaction of N-substituted aldosylamines on standing in moist atmospheres, since most unsubstituted sugars hydroxyls show decomposition with browning to some extent on storage. N glycosyl derivatives of proteins should react similarly. Although the Amadori rearrangement products are more stable than the original glycosylamines in moist, acidic atmospheres they are still heat-labile, even in the dry state. On heating, they undergo dehydration and fission and yielding colorless reductones as well as brown

fluorescent substances (Hodge, 1953; Martins et al., 2001). The reversibility of the Amadori product into its parent sugar and amino compound has been reported under different conditions: physiological, pyrolysis and relevant to food processing. However, the mechanism of reversion is still not fully understood nor is it quantitatively of relevance in the Maillard reaction (Martins and van Boekel, 2005).

The products of this rearrangement undergo browning decomposition alone in aqueous solutions at alkaline pH with the rate of browning considerably enhanced in the presence of amino acids. From the Amadori rearrangement product, none of the aldose is recoverable after acid hydrolysis, although about 50% of the amine moiety is released (Hodge, 1953; Fayle and Gerrard, 2002).

In the ketose amino system, ketoses such as fructose, react with amines to form aminoaldoses and this is called the Heyns reaction. The intermediates of this reaction are imines. Aminoaldoses are not very stable and readily react forming the Amadori compound (Ledl and Schleicher, 1990; Brands and van Boekel, 2001). The N-glycosylamine can also degrade to fission products via free radicals without the formation of Amadori or Heyns rearrangement products (Mlotkiewicz, 1998). The apparently simple reaction between a sugar and amino group leads to an enormous variety of products in varying yields. Each compound it self reactive and will participate in a lot of reactions, depending on the conditions that each molecule encounters (Fayle and Gerrard, 2002).

As was mentioned before, the initial stage of browning (reactions A and B) cannot be detected by spectropohotometric measurements in the ultraviolet; however, before visible browning has begun, a strong absorption appears to announce the beginning of the intermediate stage. The initial strong absorption occurs at the furfural region, for the reaction of reducing sugars with most amino acids. The conjugated unsaturation indicated by the spectra could develop either by sugar dehydration alone or by sugar fission and dehydration of the (recombined) fragments.

The third reaction is the dehydration of the sugar moiety or Amadori product (Reaction C in **Figure 1**). This reaction is the beginning of the second stage, in which the flavours compounds are formed. Depending of the pH of the system there are two sugar dehydration reactions: one in acidic systems, at pH 7 or below, undergoes mainly 1,2-enolization with the formation of furfural or HMF; the another reaction in anhydrous systems (pH>7), in which the degradation of the

Amadori compound is though 2,3-enolization, where reductones and a variety of fission products are formed, **Figure 4** (Hodge, 1953; Martins et al., 2001; Mottram, 2002).

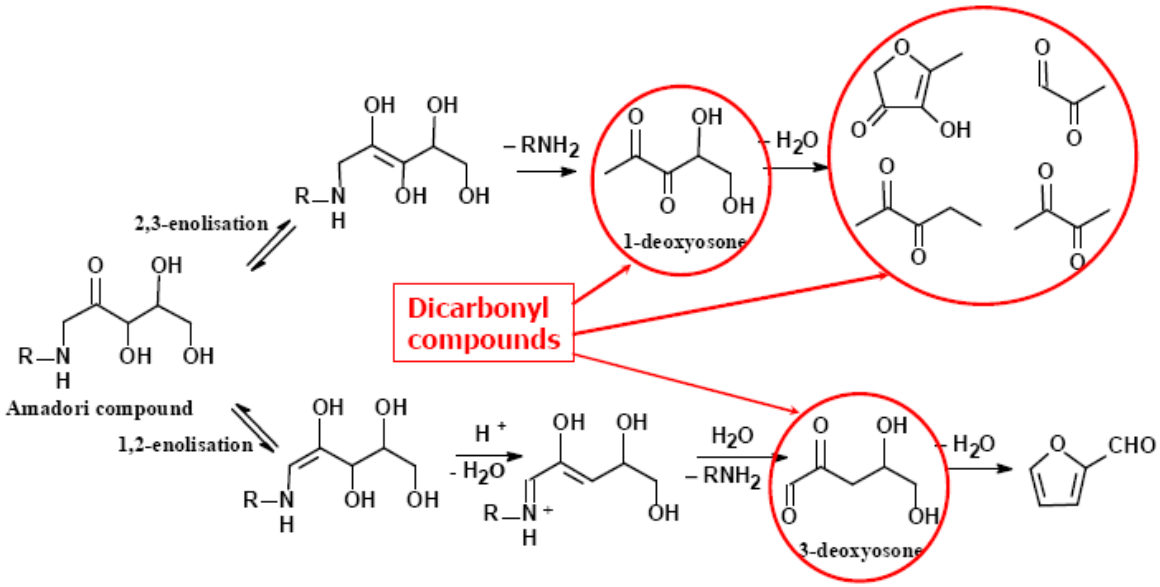


Figure 4 – Dehydration of sugar moiety (Mottram, 2002).

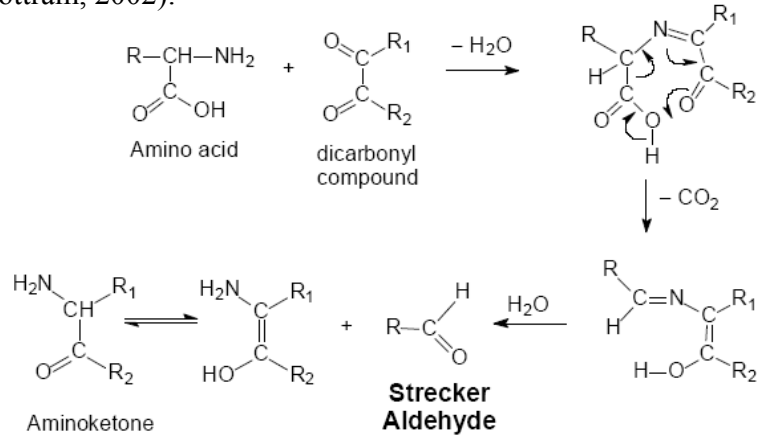
The mechanism involved in the formation of furfural is initiated with the loss of one molecule of water resulting in the production of the α-enolic-α,β-unsaturated aldehyde, which would exist in equilibrium with 3-deoxyosone.

Chichester et al. (cited by Hodge, 1953), demonstrated chromatographically the formation of the HMF in glucose glycine browning reactions under Maillard conditions. In addition, other studies demonstrated the accelerating effect of amino acids in sugar dehydration. The amine is hydrolyzed from the Schiff base of HMF after the dehydration of the sugar moiety. The deoxyaminoketose per se is not hydrolyzed. After rearrangement to the easily dehydrated deoxyaminoketose or tautomeric 1,2-enolic structure (reaction B), the loss of three molecules of water (reaction C) occurs readily (acid catalyzed) to form the Schiff base (but only in part) by hydrolysis (Hodge, 1953; Martins et al., 2001; Fayle and Gerrard, 2002). The presence of amines in the dry state or in non aqueous solvents results in the formation of reductones. Ascorbic Acid is the best known reductone, and most of the studies have involved this single endiol (Hodge, 1953).

The fourth reaction of Maillard scheme is the fragmentation of sugar moiety (reaction D in **Figure 1**). The accepted mechanism for sugar fragmentation is dealdolization, the reverse of aldol condensation. As amines or amine salts are known to catalyze aldol condensation they will also catalyze the reverse reaction, if the reaction is truly reversible. Studies found that the dealdolization is subject to amine catalysis, but not to general base catalysis. Fragments produced for sugar fission vary considerably in their potential for browning. Some which retain the α-hydroxycarbonyl grouping will undergo browning alone in aqueous solutions, and in the presence of amino compounds, the browning is greatly accelerated. The most highly reactive compounds formed are glycolaldehyde, glyceraldehyde, pyruvaldehyde, acetol, dihydroxyacetone, acetoin, and diacetyl. Formic acid has not been shown to be a source of browning, and formaldehyde is not only inactive, but is actually an inhibitor for furfural, caramelization, and carbonyl-amino types of browning (Hodge, 1953; Martins et al., 2001).

The formation of acetic acid by sugar degradation has been know for several decades, but most recent data indicate that acetic acid is formed in high levels form free and bound protein Amadori compounds. Hodge postulated that 1-deoxy-2,3-diuluoses was a possible precursor of acetic acid via retroaldolization of 1-deoxy-2,3-diuluoses followed by sugar rearrangement of the glycolaldehyde. Recent kinetics studies and labelling experiments confirmed that 1-deoxy-2,3-diuluoses is a efficient precursor of acetic acid; however, the via is direct degradation of 1-deoxy-2,3-diuluoses by α-dicarbonyl cleavage (Brands and van Boekel, 2001; Davidek et al., 2005).

The last reaction that is included in the second stage is the Strecker degradation of amino acid moiety (reaction E in **Figure 1**). In this reaction, the α-amino acids are degraded to aldehydes containing one carbon less than the amino acids, with the liberation of carbon dioxide. The reaction is (Mottram, 2002):



The transformation VIII to IX, **Figure 5** (part of the Reaction D), would give the α-dicarbonyl structure necessary for the Strecker degradation. The fission products of VIII, such as pyruvaldehyde and diacetyl, as well as reductones dehydrogenated by dismutation, are other possible sources of the required dicarbonyl compounds.

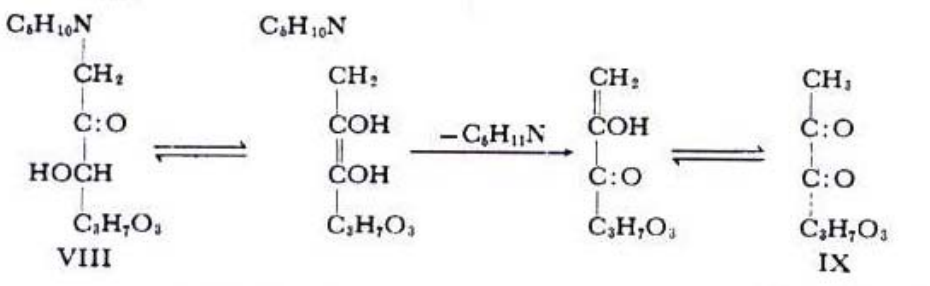


Figure 5 – Par of the reaction D, VIII -1-deoxy-1 –piperidinofructose IX- α-dicarbonyl

The aldehydes formed by the Strecker degradation are a source of browning. They could condense with themselves, with sugar fragments, with furfurals and other dehydration products, or with aldimines and ketimines to form brown pigments. Hodge (1953) concluded that the Strecker degradation is a nonessential but possibly important reaction leading to melanoidin formation. Mlotkiewicz (1998) considered this reaction to be one of the most important pathways in which amino acids react with carbonyls to generate a wealth of reactive intermediates.

The final stage of the Maillard reaction is the production of the intermediates polymers and the unsaturated, fluorescent and colored polymers. The reactions that are occurring in this stage are: the aldol condensation (reaction F in **Figure 1**) as well as the aldehyde-amine polymerization. The result is the formation of heterocyclic nitrogen compounds (reaction G in **Figure 1**) such as pyrroles, imidazoles, pyridines, and pyrazines, since amine catalysts are present, and aldehydes can be generated by sugar dehydration (reaction C), by sugar fission (reaction D), and by the Strecker degradation (reaction E). It is evident that aldol condensation (F) is a highly probable reaction for melanoidin formation. In this stage, a range of reactions takes place, including cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations, and further condensations, which ultimately lead to the formation of brown nitrogenous polymers and co-polymers known as melanoidins. The melanoidins which are produced from acetaldehyde and amino compounds are not acidic, contrary to the case with sugar-amine melanoidins (Hodge, 1953; Martins et al., 2001).

The molecular weights of the predominant fractions obtained in the Maillard reaction, as measured by exclusion chromatography, begin about 7000Da or even higher. Melanoidins can have molecular weights of up to 100 kDa. Nevertheless it has not yet proved possible to isolate homogeneous high molecular weight these Maillard products, and little is know about the structure of the melanoidins. Besides ^{1}H ^{13}C-NMR (nuclear magnetic resonance) spectra of melanoidins isolated by different research groups differed substantially, and melanoidins with signals similar of Amadori compounds contrast with those that exhibit signals in the olefin, aromatic and carbonyl regions. Additional studies with ^{15}N labelled amino acids showed that melanoidins obtained exhibit signals in pyrrole, indole, and amide region of ^{15}N-NMR spectrum (Miller and Gerrard, 2005; Zamora and Hidalgo, 2005).

Different theories exist on the structure of melanoidins. One theory indicates that high molecular weight colour structures are formed by polymerization of low molecular weight intermediates such as pyrrole derivatives. However, other studies have recently found that heating an aqueous solution of glucose in the presence of amino acids produces coloured compounds exhibiting exclusively low molecular weights <3 kDa. The fact that polymers with molecular weights >3 kDa were not detected indicates that the formation of melanoidins by polymerization of low molecular weight intermediates is not very likely to occur in a food system. As a result, Hofmann (1998a) proposed that melanoidins might be generated by a cross-linking reaction between low molecular weight coloured Maillard reaction products and high molecular weight non-coloured proteins. This hypothesis was confirmed by it self in an experiment in which an aqueous solution of glucose was heated in the presence of α-casein. Fractionation of the brown solution revealed that the predominant part of the coloured compounds consists of coloured casein pentamers or even higher oligomers exhibiting molecular weights >100 kDa. These coloured proteinoligomerization products showed characteristics typical for food melanoidins (Hofmann, 1998a)

The browning reaction can also be inhibited with various factors including; low pH, temperature, water content, removal of reactants as well as carbonyl reagents, such as cyanide, Dimedon, hydroxylamine, hydrazines, mercaptans, bisulfite and sulphur dioxide (Hodge, 1953; Ames, 1998).

Food Properties under Maillard reaction

As mentioned before, the Maillard reaction affects a whole host of sensory properties of food during storage and processing. Many of the properties that make food attractive are highly subjective qualities. The relevant effects of the Maillard reaction in food are: color, aroma and flavour, bioactivity, nutrition and texture. The controlling factors of the Maillard reaction products include: nature of the reactants (the composition of the raw material), the temperature and time combination used during heating and storage, pH and water content of food. In addition, other controlling factors could include the presence of oxygen and metals, and the presences of any reactions inhibitors, such as sulphur dioxide are other controlling factors (Ames, 1998). The profile of reactions products can also be strongly influenced by pH.

Water is produced during Maillard reaction, thus the reaction occurs less readily in foods with a high water activity values (a_w). At low a_w, the mobility of reactants is limited, despite their presence at increased concentrations. In practice, the Maillard reaction occurs most rapidly at intermediate a_w values (0.5-0.8) and, therefore, a_w is of most significance to the reaction in dried or intermediate-moisture foods. However, a_w values for maximum browning are affected by other components of the system: humectants, such as glycerol, can lower a_w value for maximum browning (Bell et al., 1998).

The rest of the paper will now discuss those food properties that are affected by the Maillard reaction and that are most relevant to the food industry. Properties include colour, aroma and flavour, bioactivity, nutrition and texture

Color

Color is often the first sensory quality by which foods are judged, and it may also provide an indication of chemical changes. Food colors are the result of variety of factors, endogenous and exogenous to the food. The exogenous factors consist of things like packaging films, display lights, and processing, while endogenous factors involve pigments within the food, added colors, and physical characteristics that affect glossiness and haze (Zamora and Hidalgo, 2005).

Most of the natural food pigments can be divided into four groups: tetrapyrrol compounds (chlorophylls, hemes, and bilins), isoprenoid derivatives (carotenoids), benzopyran derivatives

(anthocyanins and flavonoids) and betanidin derivatives (betalains). In addition to natural pigments, the color of foods may be modified by a series of reactions, including the Maillard reaction (Zamora and Hidalgo, 2005).

The Maillard reaction is commonly associated with color formation. Often, browning is an integral part of the food processing of interest, and "brownness" is the quality to which the consumer responds, and thus a property that must be measured. In many instances, Maillard browning is highly desirable as part of food processing. Without Maillard chemistry we would not have a dark bread crust or golden brown roast turkey and cakes and pastries would be pale an anemic. In other cases, Maillard browning can be detrimental to product quality, for example when if consumers find the browned product unappealing, such us browning of dry milk or the color of dehydrated products (Fayle and Gerrard, 2002).

Color compounds can be divided in two classes: low molecular weight (LMW) color compounds which comprise two to four linked rings (<1kDa) or the high molecular weight melanoidins (>100kDa). Color development increases with increasing temperature, time of heating, pH and by intermediate moisture content (a_w = 0.3-0.7). Generally, browning occurs slowly in dry systems at low temperatures and is relatively slow in high moisture foods. Color generation is enhanced at pH>7 (Mlotkiewicz, 1998; Coghe et al., 2006). Hashiba (cited by Matrins et al., 2001) concluded, by comparing different sugars with one single amino acid, that browning was directly proportional to the reducing power of the sugar and the amounts of glycine consumed. The compounds produced in the Maillard reaction under alkaline conditions exhibit distinct characteristics in both the UV and visible spectral regions with different absorption values during their development (MacDougall and Granov, 1998).

Lately, many non-volatile colour Maillard reaction products including melanoidins and their precursors have been identified through independent synthesis of the isolated products. Acetylfomoin has been characterized as a chemical switch directing the formation of different color compounds depending on the presence of either primary or secondary amino acids. This information is very important for two reasons: allows for understanding of the parameters that control Maillard reaction and it gives some clues about the presence of other key intermediates that form during the reaction and act as chemical switches able to control production of different chromophores and also of aromas and, perhaps, of carcinogens. For the identification of such key chemicals, Yaylayan et al. (2005) developed Py-GC/MS (Pyrolisis Gas Chromatography/Mass

Spectroscopy) based methodologies that allow for the analysis of the volatiles and nonvolatiles under dry or wet conditions while performing the reactions in the presence of air or helium. Their suggested that the net outcome of the Maillard reaction depends on the balance of four key precursor moieties: α-dicarbonyl (1), α-hydroxy-carbonyl (2), α-amino-carbonyl (3), and 1-amino acid-2-carbonyl (4) **(Figure 6)**. This balance can be manipulated or easily disrupted through initiation of redox reactions that are affected by the amount of dissolved oxygen, and by the amount and timing of the release of reducing species produced by the reaction (reductones), disproportionate, and dehydration reactions as well as the concentration of metal ions.

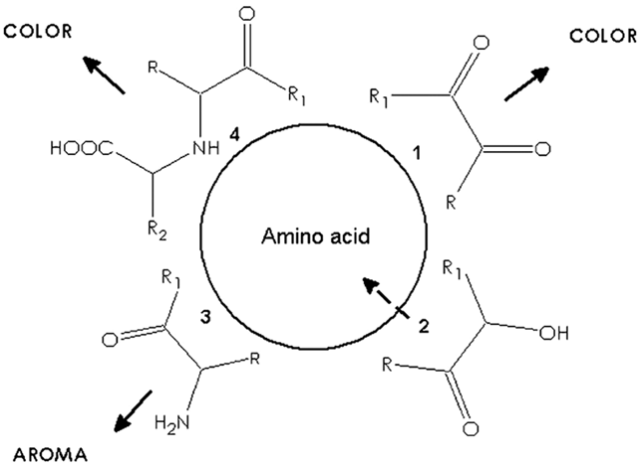


Figure 6 – Key precursors moieties: α-dicarbonyl (1), α-hydroxy-carbonyl (2), α-amino-carbonyl (3), and 1-amino acid-2-carbonyl (4) (Yaylayan et al., 2005).

Even though there are no studies yet on the relative ability of the structures represented in Figure 6 the effects of aroma and browning, Hofmann (1998b) investigated the role of the different α-dicarbonyl and α-hydroxycarbonyl intermediates (structures 1 and 2 in Figure 6) as browning precursors. He calculated relative browning activity of various carbohydrate precursors such as glyoxal, glycolaldehyde, pyruvaldehyde, and hydroxyacetone in the presence of alanine, when refluxed in phosphate buffer (pH 7.0) for 15 min. The results indicated that in addition to the nature of the chemical moieties involved as key precursors, their carbon chain lengths could also play a decisive role in promoting aldol condensations that lead to formation of furanoid species and hence induced browning through an ionic pathway (Hoffman, 1998b; Yaylayan et al., 2005).

Browning of a whole food can be monitored by visual inspection and comparison to standard samples, or by image analysis. Food extracts and model systems, which can form transparent, non turbid solutions, can be monitored as they brown by following the absorbance at a chosen wavelength (typically 420-460nm). This technique will give a reasonable indication of reaction rates and or the extent of the reaction, but cannot provide sufficient information to define

the qualities of the colour. For characterization of the molecular changes that accompany browning, or to follow the kinetics of specific Maillard events, simple photometric detection is limiting (MacDougall and Granov, 1998; Martins et al., 2001; Fayle and Gerrard, 2002).

One example of color variation under Maillard conditions is in the process of malt roasting. Depending on the thermal treatment applied during the kilning or rusting, a wide collection of dark malts can be produced ranging in color from pale yellow through to amber to brown to nearly black (caramel and roasted malts). The rate of color formation depends on time-temperature profiles. Chromophores of LMW (<10kDa) are the predominant colorants in paler malts, while melanoidins (>100kDa) prevails in roasted malt. In addition, capillary electrophoresis revealed that most malt colorants are negatively charged over a broad of pH range, and this may cause the low pH values in dark wort. The degree of non enzymatic browning is a key factor in malt quality, as it not only involves the formation of colour but also the generation of antioxidants and flavour active volatiles (Coghe et al., 2004a; Coghe et al., 2006). Another important factor on color development is the degree of malt modification, in particular the degree of protein modification. The lower degree of protein modification cause lower degree of modification of the malt and, as a result, less degradation of cell walls and later decomposition of the starch. On the other hand, a high degree of protein modification causes many degradation products, for example amino acids, into the malt. The numerous amino acids formed create Maillard products during the kilning with the already formed sugars. These Maillard products do not only increase the color of the beer, but also their transformation products lead to a reduction of the flavour stability of the beer. In fact, depending of the kind of beer to be produced the necessarily of the generation of Maillard products. In the case of pale beer, during malt production the generation of Maillard products need to avoid, and in the case of dark beer, dark malt is important to produce a lot of theses compounds.

Aroma and Flavour

Hofmann (2005) defined flavour as the sensory impression that results from the simultaneous sensing by humans of chemicals odour and taste. This can be triggered by chemical compounds already present in raw food material or generated during food processing from specific precursors such as reducing carbohydrates and amino acids.

In addition Maillard chemistry is known to generate more than 2500 different flavour compounds. Distinctive flavours are formed in many foods and beverages for example, chocolate, coffee and bakery products. In fact, the aroma of most foods that are subjected to baking, roasting and grilling will contain Maillard products. Almost invariably, hundreds of volatile compounds have been isolated form each food studied (Fayle and Gerrard, 2002; Jousse et al., 2002).

A number of factors in food influence the generation of flavours by the Maillard reaction, and they can be separated into two categories. A) The nature of the sugars and amino acids, as well as the pH at which the reaction is conducted, greatly influence the nature of the volatiles formed and, the flavour of the final product. B) The reaction temperature and time, and the water activity of the system, mainly influence the kinetics of the reaction, while leaving the nature of the volatiles broadly unchanged (Jousse et al., 2002). One example of this is the studied of Pripis-Nicolau et al. (2000) which showns the importance of the genesis of aromatic products from carbonyl compounds even under difficult conditions of low pH and low temperature. In the studied the products of reactions between α-dicarbonyl compounds and/or α-hydroxyketones compounds and amino acids presents in wine. They found that formation of odours products or strong smelling additives resulting form the Maillard and Stecker reaction in a primarily aqueous medium at low temperature and low pH (3.5) of the wine.

Food flavour can be investigated by sensory evaluation or by instrumental methods. Although the sensory evaluation is subjective, it is widely accepted because current detectors do not exhibit the same sensitivity and selectivity as the human olfactory system (Coghe et al., 2004b). The food industry has invested great effort in trying to create synthetic flavours and aromas by reconstituting combinations of these compounds, so called “aromagrams”, during processing. As mention above, measurement of aroma and flavour is a subjective process, and relating flavour to molecular structure is a very challenging field. Considerable progress has been made using the concept of “odour activity”, which is a parameter relating to the threshold concentration at which a compound must be present before its odour is detectable (Fayle and Gerrard, 2002).

In comparison to aroma-active volatiles, relatively little attention has been paid to tongue responses induced by nonvolatiles that are not present in the foods per se, but are generated during food processing by Maillard-type reactions from carbohydrates and amino acids. One reason for that lack of information is that most studies focused primarily on the quantitatively

predominating compounds, rather than selecting the target compounds to be identified with regard to taste activity. Hofmann (2005) developed a taste dilution analysis called TDA to bridge the gap between pure structural chemistry and human taste perception. This method offers the possibility of screening and characterizing the key compound formed during thermal food processing.

Continuing with the example mentioned in color properties, as mentioned before dark specialty malts can be defined as all malts kilned at higher temperatures than pilsner malt or roasted in a roasting drum. The most important properties of dark malts, color, flavour and antioxidative activity, result from the Maillard reaction. An unlimited number of colors and flavours can be achieved by the use of different dark speciality malts for brewing, but also by the variation of the dark malt level or by the combination of different malt types. Dark malts may also improve the foam stability and the mouthfeel of beer, presumably by the presence of melanoidins. Furthermore, due to a higher level of antioxidants, beers brewed with dark malts normally have a better flavours stability and a longer shelf life than pale beers (Coghe et al., 2006). The compounds that most contribute to the flavour of dark caramel malts and roasted malt are: oxygen heterocyclic components such as pyrones, furans, and furanones predominate while nitrogen containing heterocycles such us pyrazine, pyradines and pyrroles. Coghe et al., 2004b demonstrate that Maillard compounds increased with increasing wort color. However, not all Maillard reaction compounds evolve in the same way. The Strecker aldehyde 3-methylbutanal had a maximal level in wort produced with caramel malt. The aldol condensation product of two 3-methylbutanal molecules increased steadily with the color of the wort. In contrast, furfural and 5-methylfurfural were mainly observed in the darkest wort samples. Hexanal was the only compound that clearly decreasing with wort color.

Bioactivity

Measuring beneficial effects of Maillard products, such as antioxidants, is not a simple process, since some sort of bioassays is required before we can confidently predict and real effect. Once potentially useful compounds, or mixtures of compounds, are identified, they can be assessed for efficacy in food. Beyond that, a bioassay using a cell line, or an animal trial, typically with mice, might be devised to test the efficacy of compounds *in vivo* (Fayle and Gerrard, 2002).

Malt is one example where beneficial bioactive products, antioxidants, are produced by the Maillard reaction. In malt, heating induces the formation of antioxidants. The existence of at least two types of Maillard reaction related antioxidants has been postulated by Coghe et al. (2006): redox indicator reducing antioxidants and radical scavenging antioxidants. Antioxidants in beer brewed from dark malts can lead to longer shelf life and better flavour stability of the beer. With regard to flavour, it was demonstrated that it was not the darkest roasted malts, but rather the caramel malts that contained the most volatile Maillard reaction products.

In the case of toxic compounds produced by the Maillard reaction measurements in the literature are many and varied. They are also subject to the same sort of constraints dicussed earlier. Amongst the raft of commonly accepted toxicity tests there are thought to be most relevant to human health is the Ames test (Fayle and Gerrard, 2002). Some examples of toxicity are explained in junction with the nutritional loss in the next point.

Nutrition

The nutritional quality of foods may be damaged by the Maillard reaction during processing. This can occur for two main reasons. Firstly, aggregation of proteins on heating is known to decrease protein digestibility and this is exacerbated by Maillard reactions. Secondly, the Maillard reaction often leads to the loss of amino acids, especially lysine which if of great nutrition concern because it is commonly the limiting nutrient in the overall nutritional value of a food. This is of particular concern in the intensive livestock industry, where the precise nutritive value of a feed is of paramount importance for animal performance (Fayle and Gerrard, 2002). In many foods, the α-amino groups of the lysine residues of proteins are the most important source of reactive amino groups. As a result of blockage in the Amadori product, these lysine residues are no longer available for digestion and consequently the nutritive value decreases (Brands and van Boekel, 2001). One example of this is the work by Barneveld et al. (cited by Fayle and Gerrard, 2002) has focussed on the effect of heat on amino acid availability in feed for growing pigs. Comparisons of lysine digestibility, lysine availability and lysine utilization showed that heating the feed and thence increasing the rate of Maillard reaction, renders lysine in a form that is chemically measurable and efficiently absorbed by the pig, ineffectively utilised. This study wants to emphases the difficulty in predicting nutritional value from simple chemical measurement.

A nutritionally satisfactory food consisting of starch, proteins and lipids, together with other necessary minor components as required. This basic food would contain very few functional groups, and would undoubtedly have poor taste. The kind of food we wish to have daily usually contains larger amounts of low molecular compounds such as amino acids, sugars and fatty acids. Not only do these components make food taste good on their own but they are also sources, via the Maillard reaction when the food is cooked, processed or stored properly, of desirable flavours, as well as appetizing colors and smells (Fujimaki et al., 1986).

The formation of beneficial compounds during the Maillard reaction has been found and is gaining a lot of attention. The Maillard reaction products contain antioxidant, antiallergenic, antimicrobial and cytotoxic properties. It has been demonstrated that this products can contribute greatly to the shelf life of heat treated foods. In addition, studies in vitro shows that the Maillard reaction products may offer health promoting activity as they can act as reducing agents, metal chelators and radical scavengers (Silván et al., 2006).

One of the most obvious negative consequences of the Maillard reaction in food is the loss of nutritive value of proteins involved. There is also the possibility of loss of quality and a possible decrease of food safety. In previous studies loss of nutrition was attributed to decrease of digestibility, destruction and/or biological inactivation of amino acids (lysine, tryptophan), inhibition of proteolytic and glycolitic enzymes, and interaction with metal ions (Martins et al., 2001).

Moreover, this loss of nutritional value has been associated with the formation of mutagenic compounds. Some of these are dicarbonyl compounds, methylglyoxal (highest mutagenic activity), diacetyl and glyoxal; however no quantitative correlation with carcinogenic properties was found (Martins et al., 2001). Studies about the presence of acrylamide in a range of fried and oven cooked foods have caused concern because this compound has been classified as possible carcinogenic in humans. Mottram et al., (2002) showed how acrylamide can be generated from food components during heat treatment as a result of the Maillard reaction. They found that the amino acid in potatoes and cereals, asparagine, was a crucial member of the pathway in the production of acrylamide.

The most toxic effect of the Maillard reaction is evidently the result of the mutagens and carcinogens formed by cooking meat and fish. Multiple heterocyclic amines derived from the Maillard reaction in the presence of creatinine have been identified. They belong to the classes of

imidazo-quinolines and imidazo-quinoxalines. Others, derived from the heating of tryptophan, glutamic acid, or phenylalanine, were also identified. Very sensitive detection methods have been developed, particularly by the food industry. The food industry has remained very concerned with the problem and have modified the process conditions of meat extracts and meat flavours accordingly, to avoid formation of toxins (Finot, 2005).

However, the physiological effects of Maillard reaction products are not only negative. The desmutagenic effect has been reported in the Maillard reaction. Flavones and flavonoids are one example of this effect where they act like inhibitors of the heterocyclic amine type mutagens (Martins et al., 2001). Antimutagenic effects have been shown in some Maillard products that to some extent counterbalance the negative properties of Maillard products (Finot, 2005).

The production of alcoholic beverages such as Tequila, Mezcal, whiskey, or beer includes the fermentation of a mash, or wort, containing Maillard reaction products. Excessive heating of the mash can lead to complications during the following fermentation step, so the impact of Maillard products on the metabolism of *Saccharomyces cerevisiae* was investigated by Tauer et al. (2004). They found that increasing amounts of Maillard products reduced the formation of ethanol by up to 80%. This effect was dependent on the pH value during the Maillard reaction, reaction time, as well as the carbohydrate and amino acid components used in the generation of Maillard reaction products. Another important factor is the pH value during fermentation: The inhibitory effect of Maillard products was not detectable at a pH of 4 and increased with higher pH values. Similarly, Coghe et al., (2005) found that the level of fermentable carbohydrates and amino acids in dark malts significantly decreased with increasing wort colour, probably as a result of the Maillard reaction during roasting of malt. Browning reactions might be so intensive that the resulting Maillard compounds are unavailable for enzymatic hydrolysis during mashing.

Texture

Although the effect on food texture has attracted less attention, increasing evidence suggests that within food systems the Maillard reaction can result in a crosslinking of proteins and that protein crosslinking has profound effects on food texture. Introduction of protein crosslinking into baked products has been shown to improve a number of properties that are valued by the consumers (Fayle and Gerrard, 2002; Miller and Gerrard, 2005).

Conclusions

The Maillard reaction is a cascade of consecutive and complex parallel reaction steps, and is exceptionally widespread. Ninety five years on, Maillard reaction has acquired an impressive number of disciplines, and there are few reactions that attract such attention from organic chemists, food scientists and medics. The reaction occurs in: humic substances in the soil and the sea, foodstuff particularly during processing at high temperatures and storage for prolonged periods, and medical implications, since it occurs in the body wherever there is contact between amino compounds and reducing sugars, particularly for longer periods

The ability to predict the properties of food products is of great importance to the food industry because it gives the manufacturer control over product quality. Therefore, study the effects of variation in the raw material and processing parameters for example, temperature and time heating, pH, moisture content, are required. Is difficult to generalize the conditions that enhance the Maillard reaction, because the variety of the raw material is huge, but increasing temperature, time of heating and pH, with an intermediate level of moisture in the food will improve the sugar/amino system reaction.

More appropriate extraction and analytical methods are needed to improve the knowledge and understanding of the chemistry taking place during the reaction. This represents a promising approach to help industry to maximise the acceptability and nutritional value of food and to minimise the levels of compounds with potentially toxic properties.

Exists a lot of studies about the different, pathways, intermediates compounds and products of the Maillard reaction, however, there are still many unanswered questions concerning the reaction. For example, more studies are needed to comprehend the structure of melanoidins.

Many of the properties that make food attractive are the products of the Maillard reaction, color, aroma and flavour, bioactivity, nutrition and texture. Color is often the first sensory quality by which foods are judged, and it may also provide an indication of the chemical changes suffered. In many instances, Maillard browning is highly desirable as part of food processing, and in other cases, Maillard browning is detrimental to product quality. More than 2500 different flavour compound are generated under the Maillard reaction, such us pyrroles, furans, furanones, pyrazines and pyradines in others. On modern flavour analysis, non-volatile sensory active compound should be taken into the research focus. Measuring beneficial effects of Maillard

products, such as antioxidants, is not a simple process, since some sort of bioassays is required before we can be confident that any predicted effect is real. The nutritional consequences of the reaction are the special interest in medical area, and a lot of efforts are made it to understand the beneficial and toxic compounds produced. More studies should be carrying on understanding the texture change in food under Maillard reaction.

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