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Maillard products” and “Maillard reactions” are much discussed in food science and technology, but do such products and reactions deserve their name?

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“Maillard products” and “Maillard reactions” are much discussed in food science and technology, but do such products and reactions deserve their name?

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Résumé

Alors que le nombre de publications scientifiques et technologiques consacrées aux « réactions de Maillard » augmente, en raison de l'importance des brunissements non enzymatiques lors de la production des aliments, les expressions « réaction de Maillard » ou « produits de Maillard » restent vagues. Une étude historique des travaux relatifs aux réactions des sucres et des composés contenant un groupe amine montrent que seules les condensations de sucres réducteurs, d'une part, et de peptides ou protéines, d'autre part, mériteraient de recevoir le nom de Maillard... si la dénomination internationalement reconnue n'était pas « réactions de glycation ». Surtout, afin de mieux comprendre le schéma réactionnel complexe qui fait suite à cette condensation, il y a lieu de quantifier les divers chemins réactionnels qui conduisent aux nombreux composés finalement engendrés par les diverses voies.

Abstract

Whereas the number of scientific and technical publications dealing with “Maillard reactions” is increasing in food science and technology, in particular due to the importance of non-enzymatic browning during food production, the expressions “Maillard reaction” and “Maillard products” remain vague, and different authors have different definitions. A historical study of the scientific researches on the reactions between sugars, on the one hand, and compounds containing an amino group on the other hand, shows that only the initial condensation of reducing sugars and peptides or proteins should be called Maillard reaction. The complex reaction pattern that follows this initial condensation should be explored more quantitatively, in order to assess the comparative kinetics of the various reaction pathways leading to the numerous compounds which are finally produced.

Mots-clés

Maillard reactions, saccharides, amino acids, peptides, proteins, history, glycation reactions

Keywords:

Maillard, réaction de Maillard, saccharides, acides aminés, peptides, protéines, histoire, réactions de glycation.

Introduction

“Maillard reactions” keep being more and more discussed in food science and medicine, mainly because such chemical processes are partly responsible for the flavour of cooked food, but they also generate compounds with possible toxicity and they are sometimes said to cause nutriment losses. What exactly are these processes? In many scientific and technical publications, there are references to “Maillard products”, but what are these “products”? It is frequently assumed that Maillard reactions are synonymous with non-enzymatic browning reactions, but we shall see that it is not that simple. And because the International Union of Pure and Applied Chemistry decided for “glycation reactions”, the expression “Maillard products” is faulty.

The chemist and physicist Louis Camille Maillard was born in Pont-à-Mousson, France, in 1878, and he died in Paris in 1936 (Rivail, 2005). He began his studies in Nancy, France before moving to Paris, then to Alger. Hagiographic documents indicate that the “Maillard reaction” (or “Maillard reactions”) was (respectively were) discovered by Maillard. They add that these reactions are characterized by a browning of food without any enzyme activity, and are also called “non-enzymatic browning” (Maillard, 2016). This sole characterization is ambiguous, because caramelizations in particular are also non-enzymatic browning reactions (Defaye and Fernandez, 1994), but they were not studied by Maillard and they do not involve amino groups. It is sometimes added, even on university websites and in scientific articles, that Maillard reactions

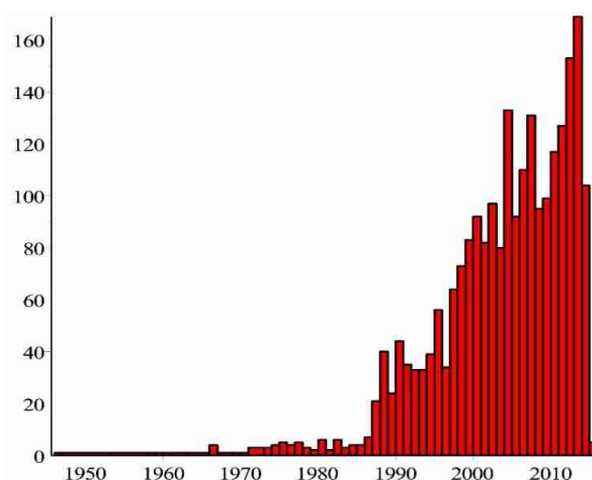


Figure 1. Number of citations of Maillard reactions through PubMed.

occur between “a carbonyl compound, such as a reducing sugar, react with an amine, such as an amino acid, a peptide or a protein” (This, 2002), but clearly the reactions are different when different moieties are involved. A distinction based on temperature can also be found between Maillard browning and caramelization, since the latter occurs between 120 °C and 150 °C whereas Maillard reactions can take place even at room temperature.

A superficial bibliographical research shows some France/UK opposition, with references to A.R. Ling (sometimes said “Ling and Malting”), who, in 1908, wrote that “during beer production, when the temperature is between 120 °C and 150 °C, odor and color are produced; amino acids probably react with sugars”. In some of these scientific publications, the fact that “Ling and Malting” are said to be the authors is particularly odd... since “malting” is only the name of the peculiar process that Ling was discussing, as appears in the right title of his publication: Ling, A.R. 1908. Malting, J. Inst. Brew., 14:494-521 (Ling, 1908).

Regardless, Maillard reactions are very popular today, an international society (IMARS) was formed by scientists and technologists interested in the glycation reactions (IMARS, 2015), and a growing number of scientific publications include

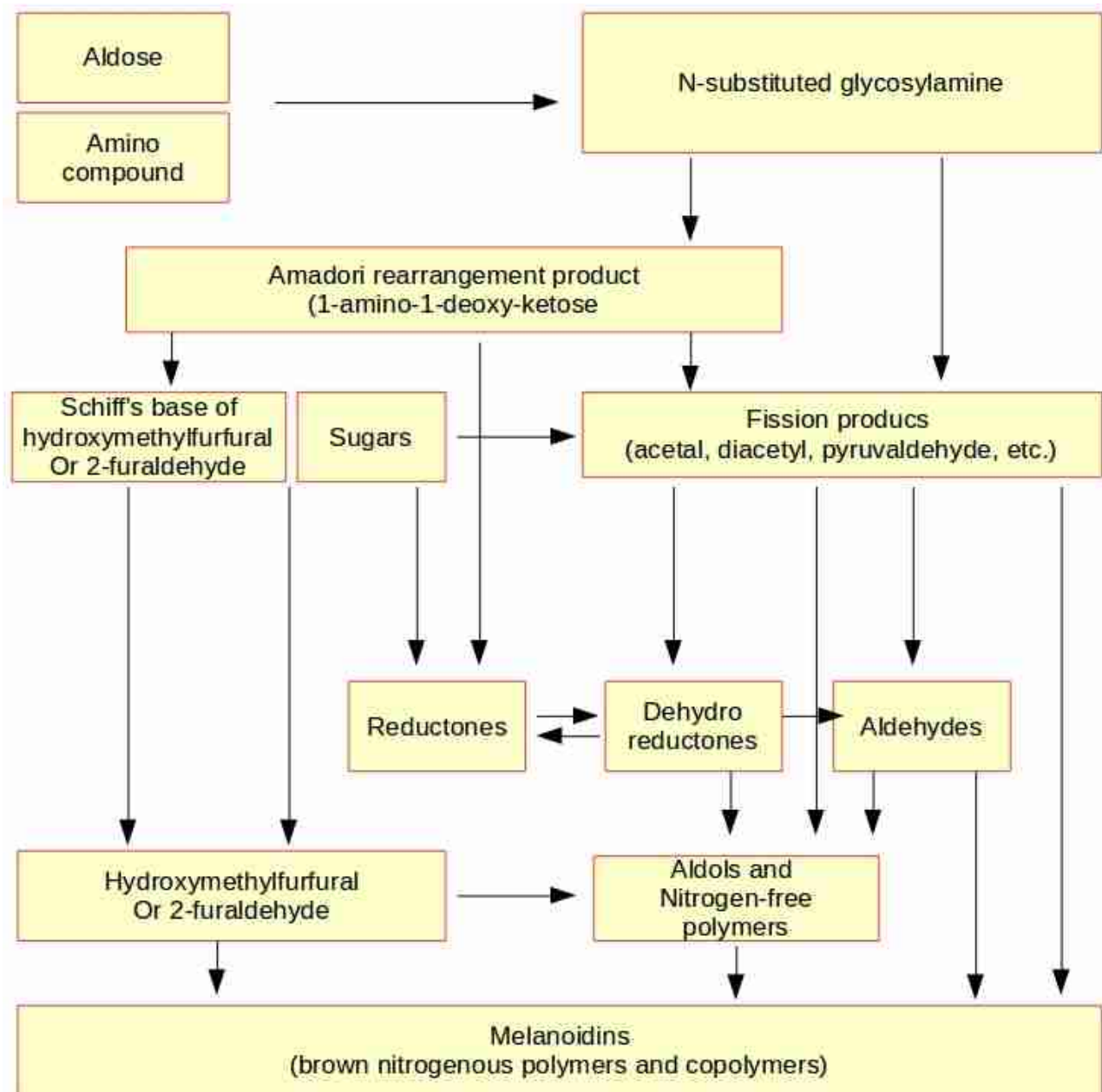


Figure 2. The general scheme of reactions occurring when reducing sugars react with amino compounds (adapted from Hodge, 1953).

“Maillard” in their title or keywords: in July 2015, the Google term search “Maillard reaction” yielded more than 45,000 answers, with more than 2,000 results in 2015 only (Google, 2015). PubMed also showed a large increase in quotations (PubMed, 2015) (Figure 1).

It is often explained that “Maillard reactions” are “a group of complex reactions”, which begins with the condensation of a reducing sugar and an amino acid, followed by a series of rearrangements, and ends with the formation of brown polymers called melanoidins. However,

examining the reaction patterns proposed as early as 1953 by Hodge (Hodge, 1953), Strecker reactions, decarboxylations, reductions, etc. are found along with an initial condensation (Figure 2). Hence the question: what exactly are Maillard reactions? And what are Maillard products? In order to clarify the situation, a serious historical research was made. It shows that our ideas on Maillard reactions and products should change.

Before Maillard

To our best knowledge, the history of Maillard reactions begins in 1866, when Hugo Schiff (1834-1915) published that aldehydes (including sugars) react with amines (including amino acids) to form dark compounds (Schiff, 1866; Qin, 2013). In his article, Schiff discussed D-glucose, aniline and p-toluidine (Figure 3). He proposed the formation of secondary imines (today called Schiff's bases) from aldehydes and aromatic amines. Then, in 1871, R. Sachsse studied the reaction of lactose with aniline (Sachsse, 1871), before Emil Fisher investigated the reactions of sugars and amino compounds in 1884 and 1886 (Fischer, 1884). Fischer focused on the reactions of D-glucose, D-fructose or sucrose with phenylhydrazine. He noted that D-glucose and D-fructose lead to the same compound, and he identified that the reaction of sucrose with phenylhydrazine was producing 1-amino-1-deoxyfructose.

In 1888, B. Sorokin studied the reaction of D-glucose and aniline (Sorokin, 1888), and in 1898 C. A. Lobry de Bruyn obtained D-glucosamine from D-fructose and ammonia; the reaction product was crystallized the same year by Breuer. From those discoveries, one can easily understand how Ling was able, in 1908, to propose a mechanism explaining the observed browning of malts: the reactions of sugars and amino acids were well-known at that time, *i.e.* about 30 years before Maillard published anything on the subject.

Maillard's results

In 1909, J. C. Irvine and R. Gilmour explored

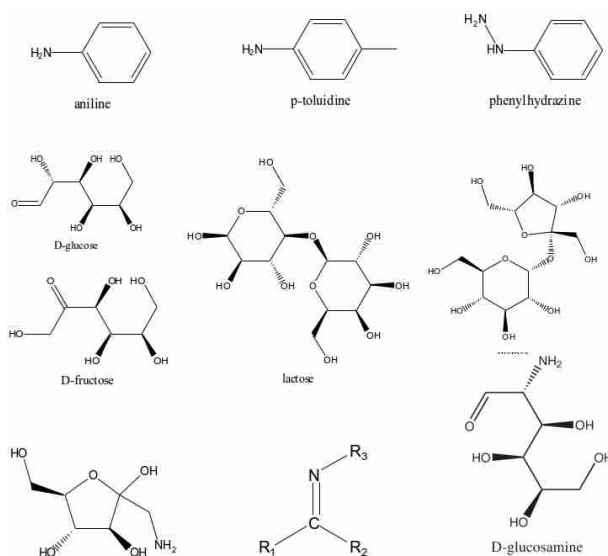


Figure 3. Some compounds which were considered in the history of the discovery of the reactions occurring after the condensation of a sugar and an amino compound.

the reaction of D-glucose and p-toluidine (as Schiff did before them), and Maillard began his studies. They are summarized in his 1913 academic report, under the title *Genèse des matières protéiques et des matières humiques* (Formation of proteic and humic matters) (Maillard, 1912).

In his articles or memoirs, Maillard recognized that his studies did not begin with him (in the following paragraph, a personal translation is given, keeping the old chemical names)¹: "The investigations that are shown in this document, *i.e.* my studies of the phenomena of condensation that α -amino acids undergo in presence of glycerine and sugars, are a small sample of the huge amount of work that has been

1 « Les recherches qui font l'objet du présent mémoire, et dans lesquelles j'ai étudié les phénomènes de condensation de divers ordres que subissent les acides α -aminés en présence de la glycérine et des sucres, ne sont qu'un très petit fragment de la somme énorme de travail qu'ont dû fournir les chimistes, depuis près d'un siècle bientôt, dans l'espoir d'élucider, par la double voie analytique et synthétique, le problème des albuminoïdes. »

performed by chemists, for about half a century, in the hope of solving, by the double way of analysis and synthesis, the issue of albuminoids" (by "albuminoids", Maillard meant proteins).

Indeed Maillard wanted to synthesize proteins²: "The study of the constitution of albuminoid matters, necessary for their synthetic reproduction, began quite early in the history of chemistry. In a few years, it will be one century since H. Braconnot, treating gelatine by concentrated sulfuric acid, could obtain a large quantity of the "sugar of gelatine", which was later recognized to be an amino acid (glycine, then named "*glycocolle*"), the first of a series. By attacking in the same way muscular tissues, Braconnot discovered leucine, less pure than the one we have today, but very important as well as the constitution of albuminoids is concerned. In 1820, H. Braconnot knew the first elements of a series which was later enlarged, both in numbers and function. During the XIXth century, much scientific effort showed finally that the decomposition of proteic matter was yielding more and more compounds that we now know to be amino acids."

However Maillard, who knew the important results from Fischer, wanted to synthesize proteins with

2 « L'étude constitutionnelle des matières albuminoïdes, préface obligatoire de leur reproduction synthétique, remonte assez haut dans l'histoire de la Chimie. Il y a aura dans quelques années un siècle déjà que notre compatriote H. Braconnot (1), en traitant la gélatine par l'acide sulfurique fort, sut obtenir en abondance le « sucre de gélatine », le glycocolle, qui devait être reconnu plus part comme un acide aminé, le type même de la série dont il est le premier terme. En attaquant de la même façon la matière musculaire, Braconnot découvrait la leucine, moins pure sans doute que celle dont nous pouvons disposer aujourd'hui, mais dont l'intérêt n'était pas moins considérable pour la constitution des albuminoïdes. H. Braconnot tenait en 1820 les premiers représentants de la série dont ses successeurs allaient accroître l'importance numérique et fonctionnelle. Au cours du XIX^e siècle se succèdent un grand nombre de recherches où toujours nous voyons la décomposition des matières protéiques fournir plusieurs de ces corps que nous rangeons aujourd'hui dans le groupe des acides aminés. »

softer conditions³: "It is considered nowadays that albuminoid matters are essentially composed, for a main part of their molecule at least, of a grouping of amino acids, linked by dehydration, between the -COOH carboxyl groups and the -NH₂ aminogen groups, *i.e.* by the -CO-NH- link, repeated a certain number of times inside the molecule. The repeated rupture of these -CO-NH-links by hydration leads to the progressive degradation of the albuminoid matter, under the action of reactants or ferments, through various stages of albumoses and peptones, until the last state of simple amino acids. The peptones themselves should be regarded as complex chains of amino acids, a conception that led E. Fischer to propose the name of peptides or polypeptides for the artificial chains of amino acids that Th. Curtius or E. Fischer himself succeeded to create by synthetic processes which will be discussed later."

In the paragraph VI entitled "Researches on the natural mechanisms of protein synthesis in living organisms", Maillard discusses his own results, based on the use of glycerol (called glycerin at that time)⁴: "The method from E. Fischer is both

3 « On considère aujourd'hui les matières albuminoïdes comme formées essentiellement, pour une partie prépondérante de leur molécule tout au moins, d'un semblable entassement d'acides aminés, enchaînés les uns aux autres par déshydratation, entre les groupes carboxyle -COOH et aminogène -NH₂, c'est-à-dire par le chaînon -CO-NH- répété un nombre plus ou moins grand de fois dans l'intérieur de la molécule. C'est la rupture successive de ces chaînons -CO-NH- par l'hydratation, qui produit la dégradation progressive de la matière albuminoïde sous l'action des réactifs ou des ferments, en l'amenant à travers des stades variés d'albumoses et de peptones, jusqu'à l'état définitif de simples aminoacides. Les peptones elles-mêmes doivent donc être considérées comme des chaînes complexes d'acides aminés, et c'est cette conception qui a suggéré à E. Fischer le nom de peptides ou polypeptides pour les chaînes artificielles d'aminoacides que Th. Curtius ou E. Fischer lui-même ont réussi à créer par des procédés synthétiques dont nous dirons plus loin quelques mots. »

4 « La méthode de E. Fischer a pour double

indirect and artificial. It is indirect, because it does not link together a series of preexisting amino acids; but in order to bind the n -th (terminal) amino acid at the end of a chain of rank $(n-1)$, it has to bring successively fragments which will make up the molecule only after many reactions. It is artificial, because it uses absolute ethanol, hydrochloric gas, bromine, thionyl chloride, concentrated ammonia, in short a whole set of violent reagents which are not present in the organism of human beings and of other living creatures. Nature makes it very differently. Our organism receives a set of separated amino acids, through digestion; and our intestinal mucosa has to re-associate such amino acids in order to constitute our own albuminoid materials. In order

caractère d'être indirecte et artificielle. Elle est indirecte, car elle ne soude pas ensemble une série d'acides aminés préexistants ; mais, pour construire le n -ième aminoacide à l'extrémité d'une chaîne de rang $(n-1)$, elle est obligée d'apporter successivement des fragments qui ne constitueront la molécule de l'aminoacide qu'après un ensemble de réactions. Elle est artificielle, car elle met en jeu l'alcool absolu, le gaz chlorhydrique, le brome, le chlorure de thionyle, l'ammoniaque concentré, bref tout un arsenal de réactifs violents dont ne disposent pas le corps humain ni les autres organismes vivants. La nature procède tout autrement. Notre organisme reçoit une série d'acides aminés séparés les uns des autres par le jeu de la digestion ; et ce sont ces aminoacides entiers que doit réassocier notre muqueuse intestinale pour en constituer nos propres matériaux albuminoïdes. Pour éclairer le mécanisme de ce processus direct et naturel, il est donc indispensable de trouver une nouvelle synthèse des polypeptides qui réponde aux deux conditions suivantes : 1° Partir, non point d'un chlorure d'acide halogéné ou de quelque autre fragment non biologique, mais bien des acides aminés eux-mêmes ; 2° Obtenir l'enchaînement peptidique de ces acides aminés sans recourir à l'intervention d'autres substances que celles dont dispose l'organisme. Tel est le problème auquel je me suis efforcé d'apporter une contribution. J'ai eu la bonne fortune de rencontrer dans une substance très répandue chez les êtres vivants, la glycérine, un agent de condensation qui m'a permis de réaliser déjà la synthèse d'un certain nombre de composés. »

to explain the mechanism of this direct, natural process, it is thus necessary to find a new synthesis of polypeptides with the two following conditions: 1° Start not from an halogenous acid chloride, or from some other non-biologic fragment, but from amino acids; 2° create peptide bonds between these amino acids without using any other substances than the ones the organism can provide. This is the problem that I tried to contribute to. I was lucky to find in a very common biological substance, glycerin, a condensing agent with which I could already synthesize a certain number of compounds."

It is in the fourth part of his report that Maillard discusses the action of sugars on α -amino acids⁵: "In spite of their alcoholic function, the sugars do not behave, toward α -amino acids, as peptidogenic condensing agents, such as glycerin. The presence of the aldehydic (or ketonic) function leads to phenomena of a very different kind. A mixture of *glycocolle* (glycine) and an apparent excess of glucose, in a concentrated aqueous solution heated at 100 ° C, gets brown in minutes, then foams because of an abundant gaseous production, leaving a dark brown amorphous residue, mainly insoluble in water". Maillard had few hints to interpret this phenomenon which, as we could see, was observed well before him. He only wrote: "The primitive reaction can be interpreted as a series of phenomena: a.- Condensation of the amino function of glycocoll with the aldehydic function of the sugar; b.- Rupture of the carboxyl; c.- Dehydration eliminating oxydrils and hydrogen atoms from glucose, making double bonds and probably molecular cycles".

5 « Malgré leurs fonctions alcooliques, les sucres ne se comportent pas, vis-à-vis des acides α -aminés, comme des agents de condensation peptidogènes, comparables à la glycérine. La présence de la fonction aldéhydrique (ou cétonique) donne lieu à des phénomènes d'un ordre tout différent. Un mélange de glycocolle avec un excès apparent de glucose, mis en solution aqueuse concentrée et porté à 100 °, brunit au bout de quelques minutes, puis mousse par suite d'un abondant dégagement gazeux, et laisse comme résidu une masse amorphe, d'un brun noir, en grande partie insoluble dans l'eau ».

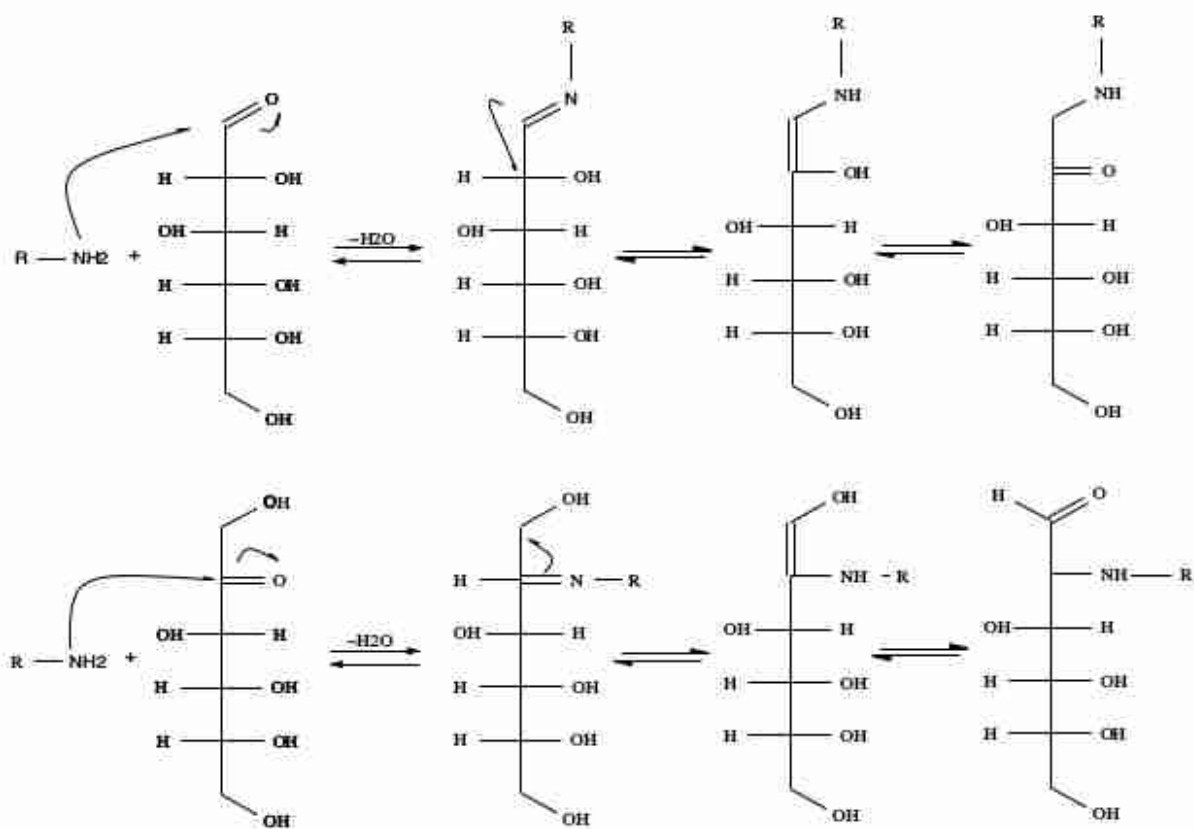


Figure 4. The initial condensation of a sugar and an amino compound.

After Maillard

After Maillard, Mario Amadori (1925-1941) identified the mechanism of the initial condensation of the α -hydroxyaldehydes with amines, leading to aminoketones, according to a mechanism different from the one that Maillard had conjectured (Wrodnigg and Eder, 2001). K. Heyns (whose name is sometimes wrongly written "Heyn") then also showed how hydroxyketones generate 2-amino-2-deoxyaldoses (Kawamura, 1983). The description of the first steps of the set of reactions beginning with the formation of a Schiff's base is now complete (Figure 4), and one can thus decide rationally of the names to be given to different reactions and compounds. Before coming back to the general description of reactions, it is interesting to look again at the general pattern given by Hodge, and to observe that some who say that Maillard reactions are "complex" tried to categorize the various reactions. In particular, it was proposed to group into "chemical pools" the compounds coming from specific precursors, i.e. sugars, amino acids or Amadori or Heyns products (Yaylayan, 1997). However we could see that this does not clarify

the whole pattern, because one must then name "Maillard reactions" the set of reactions, among which some are already known. For example, caramelization (Defaye, 1994), which would be included in the Maillard reactions, is not specific to sugars and amino acids, as well as pyrolysis, hydrolysis, intramolecular dehydration of hexoses, oxidations, Strecker degradation (Belitz and Grosch, 1999)... It was also proposed to distinguish initial, advanced and final Maillard reactions, but we could see that Maillard was unable to distinguish such steps (Eriksson, 1981). Finally some proposed to distinguish "fragmentation pools", but this is improper, as the various reactions following the initial condensation are not only fragmentations, but also condensations. Finally all this shows that we should restrict more than today's use what we call Maillard reactions or Maillard products, instead of enlarging them, and that we should keep in mind that the initial condensation of reducing sugars with amino acids was already known by Schiff, whose name is certainly appropriate for the secondary imines formed initially, whereas the names of Amadori and Heyns are right for the eponymous compounds, because they correctly analyzed them.

Maillard was apparently the first to observe that condensations such as the ones observed by Schiff and others could take place also with peptides, and this should lead us to give the name of Maillard only to such reactions (and also for the condensation of sugars and proteins). For the “Maillard products”, the issue is worse, as the products from the initial condensation already have names (after the chemists who discovered them), and the other products downward also have particular names, including the melanoidins, that Maillard called “brown matter soluble in water”, or “dark matter insoluble in water” (Maillard used the word “melanoidin, introduced by O. Schmieberg) (Schmieberg, 1897).

How much browning of the various kinds?

Having finally recognized that the name of Maillard should be given only to reactions between reducing sugars and peptides or polypeptides (not to reactions between sugars and amino acids because they were known before him), we can now focus on the scientific issue of understanding why food gets brown when heated. This question is complex when considered as a whole, but it could be made simpler if we consider precise elementary aspects, such as the appearance of the brown color, for example, or how much 5-hydroxymethylfurfural is formed from the initial Schiff's base, or how much melanoidins are formed, etc.

Indeed, concerning browning, many non-enzymatic reactions take place simultaneously, leading to identical or different compounds. It should be noted here that most organic compounds can turn brown through heating, and not only lemon juice from which “invisible ink” is frequently proposed to be made.

How could one control specific brownings, associated with specific compounds and specific food flavors? The question is of technological nature, but even without any application idea its science basis is important, as it covers the competition between different

mechanisms, sometimes from the same reagents (This, 2016 ; This, 2015 a ; This, 2015 b).

As regard to 5-HMF, the question presented above is only given as an example, and it also has some relationship with the browning question. 5-HMF can form through Schiff's condensation, through Maillard reactions, through dehydration from hexoses, from compounds such as cellulose, and more generally during many thermolyses and pyrolyses (Smets et al., 2014; Despax-Machefel, 2013; Lewkowski, 2001; Mednick, 1962). During food thermal processing (“cooking”), how much 5-HMF comes from each reaction?

When mankind is sending probes to Mars, it is strange that we cannot answer such questions. Obviously, more research is needed in order to assess more correctly what are Maillard reactions or products, and what they are not.

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