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## Why we should speak of glycation reactions, or of amino-carbonyl reactions, instead of Maillard reactions

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### **Abstract:**

As the number of scientific publications dealing with “Maillard reactions” is increasing, we have to admit that reactions between saccharides and amino compounds were discovered and investigated well before Louis-Camille Maillard, with scientists such as Lucien Dusart, Paul Thenard, Paul Schützenberger, Hugo Schiff, Emil Fischer or Oswald Schmiedeberg. After some limited work by Maillard, between 1911 and 1913, the mechanisms of the “amino-carbonyl reactions” were explored by Mario Amadori, John E. Hodge, and Kurt Heyns, among others.

### **Keywords:**

Maillard, glycation reactions, saccharides, amino acids, peptides, proteins, melanoidins, history, amino-carbonyl reactions.

Let's say it immediately: after having promoted for decades the idea that Louis Camille Maillard (1878-1936) deserved more recognition, I must confess that I was partly wrong: for sure, Maillard published some short articles and a long and thorough review of amino-carbonyl reactions, including a report of his own experiments (Maillard, 1911a; 1911b; 1912a; 1912b; 1912c; 1913a; 1913b), but a careful historical research shows that he was not the one who discovered these reactions, and he was not either the one who found their mechanisms. Accordingly, I propose that IMARS changes its

name for IACS, for example (which would also avoid the confusion with <https://imars-project.eu/>).

Most of what follows was published in two journals –first in the *Notes académiques de l'Académie d'agriculture de France* (This, 2016a) and also in a chapter of the *Handbook of Molecular Gastronomy* (This vo Kientza, 2021)–, but some historical mistakes remained in these two texts; here they are corrected after more historical information was found, based on articles which were often published in German.

### **Confusion in publications**

About reactions that sometimes get the name of Maillard in the scientific literature, commentaries have to be made, and in what follows, we give sentences from recent publications, without quoting the authors, in order to avoid hurting them.

Assuming temporarily that the amino-carbonyl reactions deserve the name of Maillard, for sure one could speak as well of “the Maillard reaction” or of “Maillard reactions”, because as for other classical reactions in organic chemistry (Würtz condensation, Grignard reaction, Diels-Alder reaction, etc.), a common result can be obtained from various reagents. However, sentences such as “The Maillard reaction is a very complex series of reactions” can be criticized from a grammar point of view, but also from a scientific point of view, as the adjective (complex) and the adverb (very) should be replaced by the answer to the question “how much?”.

A more serious inconsistency is the confusion between “Maillard reaction” (or “Maillard reactions”) and “non-enzymatic browning reactions” as a whole, because there are many non-enzymatic browning reactions that were not studied by Maillard (such as caramelization) and that do not begin with the condensation of an amino group (from an amino acid, or a peptide, or a protein) with a reducing saccharide (Defaye and Fernandez, 1994; Rao *et al.*, 2018). Also sentences such as “Naturally occurring reaction during food processing, glycation, also known as non-enzymatic browning or Maillard reaction, can improve food protein physicochemical properties and functionality” can be criticized in many regards. First food processing is not natural (according to dictionaries, something is “natural” when it is not the result of an action performed by human beings) (TLFi, 2023), and also reactions occurring during food processing are not “occurring naturally”, as they are triggered by the cook. Moreover,

such a sentence assimilates all non-enzymatic browning reactions to Maillard reactions, which is a wrong, and glycation is not the name of non-enzymatic browning, as we shall see later.

Sentences such as “Non-enzymatic Maillard reactions between reducing sugars and proteins, lipids, or nucleic acids” are also be disputable, because “Maillard reactions” are non-enzymatic: “non-enzymatic Maillard reactions” is pleonastic, and also because, in contrast, there are no “enzymatic Maillard reactions”. Less serious, but worth to be observed: “sugar” is a “loose term applied to monosaccharides and lower oligosaccharides” (IUPAC, 2019). Finally, a question is to know if Maillard ever considered reactions between reducing saccharides and lipids, or with nucleic acids: the answer is no, and the word “triglyceride”, for example, is only given in the conclusion of his 1913 memoir (p. 402) to designate compounds such as glycerol, and not triacylglycerols. In all this discussion, an important point is to know which kinds of reactions did Maillard really study: between reducing saccharides and peptides and proteins, or between reducing saccharides and amino acids? Here the history of chemistry has to be explored carefully, with only references to primary sources, as so many mistakes lie in later publications about this history (see below).

With sentences such as “The Maillard reaction is one of the most important chemical reactions occurring during heating or storage of food”, a question is to know how much “important” these so called Maillard reactions are: assuming that one can measure this “importance” (from a point of view that remains to be chosen: economy, frequency, effect on human health, etc.), this “importance” has to be compared quantitatively to other reactions such as lipid oxidation or protein hydrolysis, for example.

Sometimes it is added, even in university internet sites and in scientific articles, that “Maillard reactions are between a carbonyl compound, such a reducing sugar, and an amine, such an amino acid, a peptide or a protein” (This, 2002), but now the same question as before can be examined: which kind of amino compounds did Maillard study?

And finally it is sometimes written that “the difference between Maillard browning and caramelization is that the last one occurs at temperatures between 120 and 150 °C, whereas Maillard reactions take place even at room temperature”. However it is probably better to see the difference in the reagents: amines and reducing sugars

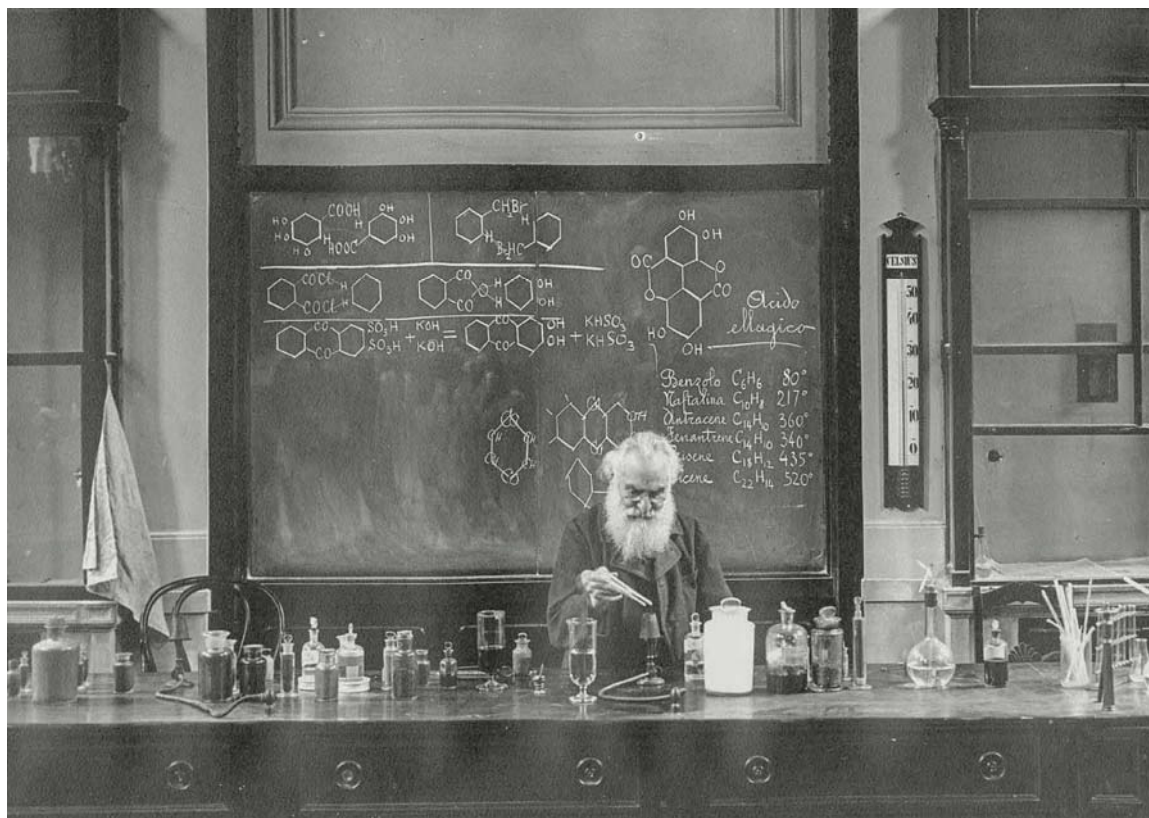
undergoing a condensation in the reactions studied by Maillard (he himself made the remark), whereas only saccharides undergo pyrolysis in caramelization.

### **The history of glycation**

In order to better understand what Maillard did and what he did not do, it seems appropriate to begin the history of amino-carbonyl reactions in 1856, *i.e.*, more than two decades before Maillard was born, when the pharmacist Lucien Dusart published in the *Archives de médecine* his results on the reaction of “glucose” and ammonia (Dusart, 1861) [personal translation]: “These comparisons get more interest when it is known that by heating glucose, or milk sugar or starch with liquid ammonia in a closed vessel at a temperature of about 150 °C, one get a nitrogenous material that can precipitate by the addition of alcohol, forming elastic filaments and making a non putrescible matter. In these experiments, which aimed at forming artificial albuminoid matters, I fixed up to 14 percent of nitrogen by heating in an oven for 12 to 15 days. I add that the product that formed was similar to gluten from a physical point of view, but did not react as natural albuminoids material do, except about the odor of burnt horn which is observed with other nitrogenous materials”. Here we can observe that the goal was the study of proteins, as for many chemists later.

Before moving to the next historical step, let us add that Lucien Pierre Joseph Dusart, born in Saint-Amand-les-eaux, in the north of France, the 28th July 1831, passed the competitive examination for hospitals internship in 1853, and was accepted as a pharmacist in 1859. He worked first in Paris, from 1868 to 1885, and one of his interns was Léon Midy (1847-1928), who created later the famous Midy Laboratories.

Four years after Dusart’s discovery, the French agronomist Paul Thenard (1819-1884) did independently the same kind of experiments while he explored “funic acid” (Thenard, 1861a; 1861b), writing [personal translation]: “When a flow of ammoniac is driven through glucose melted in its crystallization water at a temperature less than 100°, the gas is readily absorbed, and one gets only water as well as various brown substances are formed (with less than 10 % nitrogen)”. Thenard’s publication triggered fierce reclamations from Dusart (1861) and from Paul Schützenberger (1829-1897), who also studied the reaction of ammonia and “carbohydrates” (Schützenberger, 1861).

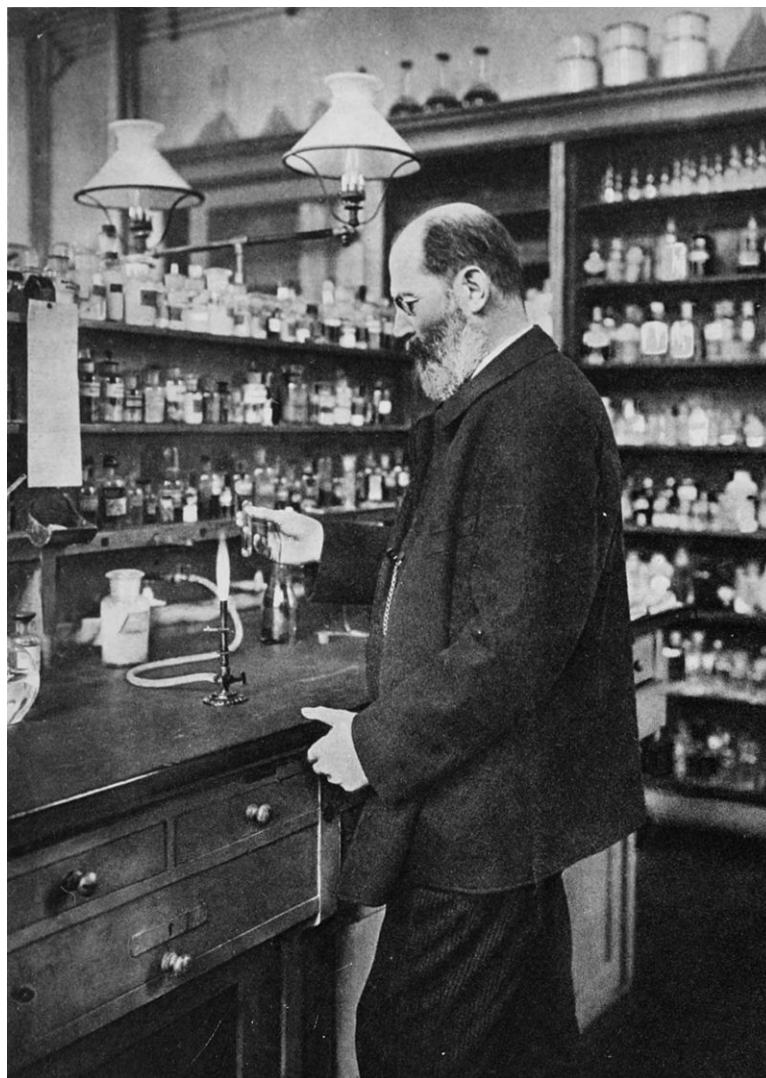


**Figure 1: Hugo Schiff (1834–1915) lecturing in Florence, the 26th of April 1915.**

Then, in 1866, the German born chemist, later Italian naturalized, Hugo Schiff (1834–1915, Figure 1) discovered that aldehydes (including monosaccharides) can react with amines - in particular with amino acids -, forming dark compounds (Schiff, 1866; Qin *et al.*, 2013). In his article, he discussed the reaction of D-glucose, aniline and *p*-toluidine, and he proposed the formation of imines (today called Schiff's bases) from aldehydes and amines.

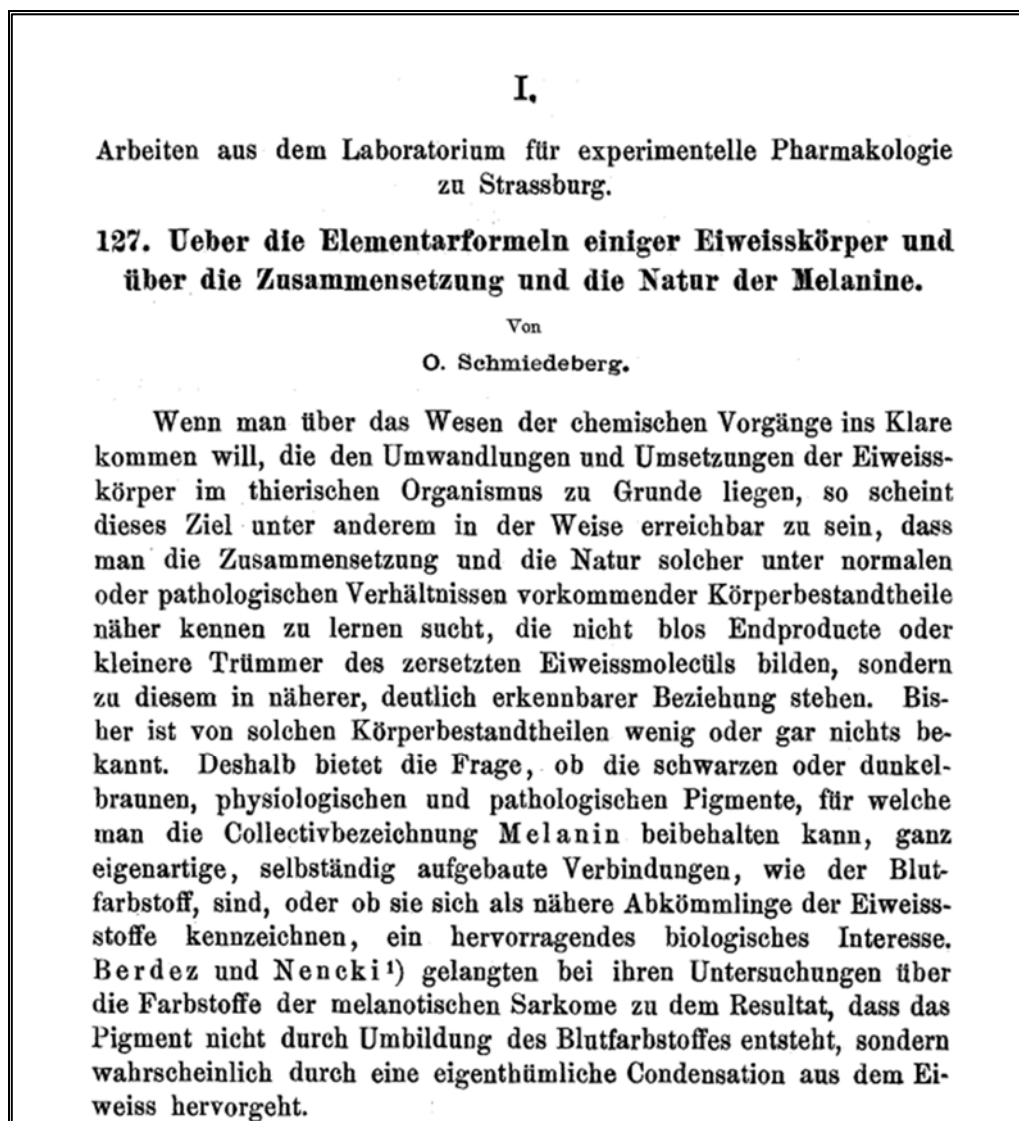
In 1871 (again, still before Maillard was born), Robert Sachsse based explicitly his works on Dusart, Thenard and Schützenberger, and reported the reaction between lactose and aniline (Sachsse, 1871). But much more work was performed in the 1880's by the German chemist Emil Fischer (1852-1919, Figure 2), who explored - among many other reactions - the reactions of saccharides and amino compounds, first D-glucose or D-fructose and phenylhydrazine, but also of sucrose and phenylhydrazine, obtaining 1-amino-1-deoxyfructose (Fischer, 1884). We shall see below the importance of Fischer in this history: it is much more than the short description given here (and for

reasons that we give below, it is proposed that all references about Fischer's work regarding amino-carbonyl reactions should be found in Maillard, 1913).



*Figure 2: Emil Fischer, in 1912.*

In 1888, B. Sorokin studied the reaction between D-glucose and aniline, and other researches took place, but an important step was made by Oswald Schmieberg (1838-1921) in 1897 (Schmieberg, 1897; Philippu and Seifert, 2022). In his article “Ueber die Elementarformeln einiger Eiweisskörper und über die Zusammensetzung und die Natur der Melanine” (On the elementary formulae of some bodies from egg white on the composition and nature of melanin, Figure 3), he introduced the word “melanoidin” [personal translation]: “If proteins are heated for a long time with concentrated mineral acids, they first dissolve in them, the liquid then turns violet or reddish, gradually taking



*Figure 3: The beginning of a publication where Schmiedeberg introduced "melanoidins".*

on a darker and darker brown colour, until finally black-brown flaky masses emerge, which in their properties correspond completely to the black animal pigments soluble in alkalis, but which seem to have originated from the egg white in a different way from the natural melanins and melanic acids and can therefore be called melanoidins and melanoidic acids”.

Also important because he was later quoted by Maillard (1913), Cornelis Adriaan Lobry van Troostenburg de Bruyn obtained in 1898 D-glucosamine from D-fructose and ammonia (crystallized by M. Breuer), a compound important in the reaction scheme later published by Hodge (1953).



Let us finish this brief history in 1908 with two results. First J. C. Irvine and R. Gilmour explored the reaction of D-glucose and *p*-toluidine (studied before by Schiff). Secondly the English beer technologist A. R. Ling observed 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” (Ling, 1908). This last assumption was easy to build on the well known work by Fischer and others. In passing, it is interesting to observe that some articles dealing with the history of amino-carbonyl reactions attribute this quotation to two authors “Ling and Malting”; but this is wrong, as “Malting” was not the co-author of Ling, but only the title of the article: “Ling AR. 1908. Malting, J. Inst. Brew., 14:494-521”.

### **The work done by Maillard**

Maillard published in French on amino-carbonyl reactions between 1911 and 1913, with the following articles:

Maillard LC. 1911. Condensation des acides aminés en présence de la glycérine : cycloglycylglycines et polypeptides [Condensation of amino acids in the presence of glycerine: cycloglycylglycines and polypeptides], Comptes rendus hebdomadaires de l'Académie des sciences, 1911, 153, 1078.

Maillard LC. 1911. Synthèse des peptides inférieurs par une méthode nouvelle et directe, voisine des réactions biologiques [Synthesis of small peptides by a new and direct method, similar to biological reactions], Comptes rendus de la Section de Biologie, 1911, 71, 546.

Maillard LC. 1912. Recherche du mécanisme naturel de formation des albuminoïdes [Research on the natural mechanism of the formation of albuminoids], Presse médicale, 1912, 20, 141.

Maillard LC. 1912. Action des acides aminés sur les sucres ; formation des mélanoidines par voie méthodique [Action of amino acids on sugars; methodological approach on the formation of melanoidins], Comptes rendus hebdomadaires de l'Académie des sciences, 1912, 154, 66.

Maillard LC. 1912. Réaction générale des acides aminés sur les sucres : ses conséquences biologiques [General reaction of amino acids on sugars: its biological consequences], Comptes rendus de la Section de Biologie, 1912, 72, 599.

Maillard LC. 1913. Formation de matières humiques par action de polypeptides sur les sucres [Formation of humic matter by action of polypeptides on sugars], Comptes rendus hebdomadaires de l'Académie des sciences, 1913, 156, 1159.

Maillard LC. 1913. Genèse des matières protéiques et des matières humiques (action de la glycérine et des sucres sur les acides  $\alpha$ -aminés) [Formation of proteinaceous and humic materials (action of glycerine and sugars on  $\alpha$ -amino acids)], Paris, Masson, 1913.

Maillard was a French physician, born in 1878 in Pont-à-Mousson, near Nancy, the city where the pharmacist Henry Braconnot (1780-1855) had worked (Rivail, 2005). He began his studies there, before moving to Paris, then to Alger (Algeria). In his large memoir published in 1913 (a mixture of a review and a report of personal experiments), Maillard recognized that his studies, focused on the synthesis of proteins, did not begin with him (personal translation): “The investigations that are shown in this document, *i.e.* my studies of the phenomena of condensation that  $\alpha$ -amino acids undergo in presence of glycerine and sugars, are a small sample of the huge amount of work that was performed by chemists, since about one half century, in the hope of solving, by the double way of analysis and synthesis, the issue of albuminoids” [by “albuminoid”, Maillard meant proteins]. [...] The study of the constitution of albuminoid matters, necessary for their synthetic reproduction, began quite early in the history of chemistry. In some years, it will make one century that H. Braconnot (1) [Braconnot, 1820], treating gelatine by concentrated sulfuric acid, could obtain a large quantity of the “sugar of gelatine”, glyocolle [glycine], which was later recognized to be an amino acid, 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, numerically and functionally. During the XIXth century, a large number of researches produced results showing that decomposition of protein matters was leading to more and more compounds that we know now to be amino acids.”

As said, many scientists, including Dusart, had studied the synthesis of proteins before him. In particular, Maillard, who knew the important results from Fischer, wanted to

synthesize proteins with softer conditions: "One considers today albuminoid matters as essentially formed, for a main part of their molecule at least, of a grouping of amino acids, linked by dehydration, between the carboxyl groups  $\text{-COOH}$  and the aminogen groups  $\text{-NH}_2$ , i.e. by the link  $\text{-CO-NH-}$ , repeated a certain number of times inside the molecule. The repeated rupture of these links  $\text{-CO-NH-}$  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 itself should be regarded as complex chains of amino acids, a conception that lead Fischer to propose the name of peptides or polypeptides for the artificial chains of amino acids that Curtius or Fischer himself succeeded to create by synthetic processes which will be discussed later."

Fischer is quoted 91 times in the 423 pages long document, with such footnotes as (personal translation): "I realized later that E. Fischer had already recommended, in a previous memoir (Ber. d.d.chem. Ges, 30, 1906, see p. 559) the replacement of hydrochloric acid by acetic acid or iodhydric acid during neutralization. My observations should then be considered only as simple confirmation of the data by E. Fischer itself".

What did Maillard by himself? At the end of his introduction, four parts are announced. The first deals with the reaction of glycerol with glycine, creating cyclo-glycyl-glycine and polycyclic compounds. The second deals with the reaction of glycerol with alpha amino acids. The third part discusses the reaction of glycerine with mixtures of amino acids. And the fourth part studies "the action of sugars on alpha amino acids; one observes the formation of very strange substances, identical to melanoidins or to nitrogenous humic matters, a general reaction whose discovery can explain the structure of these substances that remained mysterious in their origin as well as in their constitution".

Most of the memoir is based on using glycerol (called glycerine at that time) as did Theodor Curtius (1883) before him, and as did also extensively Fischer: "The method from E. Fischer is both 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 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 organisms. 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 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 a 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 one that 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, glycerine, a condensing agent with which I could already synthesize a certain number of compounds.”

It is in the fourth part of his memoir that Maillard discusses the action of sugars on  $\alpha$ -amino acids: "In spite of their alcoholic function, the sugars do not behave, towards  $\alpha$ -amino acids, as peptidogenic condensing agents, such as glycerine. The presence of the aldehydic (or cetonic) function leads to phenomena of a very different kind. A mixture of glycocoll [glycine] and an apparent excess of glucose, in a concentrated aqueous solution heated at 100 °C, gets brown in some minutes, then foams because of an abundant gaseous production, leaving a dark brown, amorphous residue, mainly insoluble in water”.

Maillard recognized that this was studied by Ling before him, but without focusing on CO<sub>2</sub> formation. He gave few hints in order 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 glycocolle with the aldehydic function of the sugar; b.- Rupture of the carboxyl; c.- Dehydration eliminating oxydriles and hydrogen atoms from glucose, making double bonds and probably molecular cycles”.

And finally let us observe that the memoir includes a chapter XX entitled: “Action of sugars on certain polypeptides”.

## After Maillard

Maillard did not explain the amino-carbonyl reactions, and indeed the mechanisms were only investigated later (between 1925 and 1931) by Mario Amadori (1886-1941), *i.e.*, reacting hydroxyaldehydes with amines to make  $\alpha$ -aminoketones according to a mechanism different from the one that Maillard had conjectured (Wrodnigg and Eder, 2001). He discovered that the condensation of D-glucose with an aromatic amine produces, depending on the particular experimental conditions, two “isomers”, one being more labile than the other (Amadori, 1929). In 1937, Richard Kuhn et Friedrich Weygand gave Amadori’s name to this rearrangement (Kuhn and Weygand, 1937).

Then in the 1950’s, Kurt Heyns – along with some colleagues, such as Hermann Breuer and Hans Paulsen – published extensively on these questions, exploring in particular  $\alpha$ -hydroxyketones to make 2-amino-2-deoxyaldoses (Heyns *et al.*, 1956; Heyns *et al.*, 1957; Heyns and Beilfuss, 1970; Kawamura, 1983), while John E. Hodge published his often quoted work (Hodge, 1953; 1979), using the right terminology “amino-carbonyl reactions”, which could be usefully used today.

Of course, more work remains to be done about these reactions, but we can now rationally try to decide which names are to be given to which mechanisms, reactions and compounds.

In order to take such important decisions, we have to recognize that Maillard was not the first to study non-enzymatic browning in food based on reactions between saccharides and amino compounds: Dusart seems to be the first one. Maillard was not either the first one to discover that amines (or amino acids, or peptides, or proteins) can react with saccharides. And he was not the one who explained correctly the mechanisms involved in these reactions. In the history of the study of amino-carbonyl reactions, the main names are probably Schiff, Fischer, Schmiedeberg, Amadori and Heyns.

Why was Maillard credited for something that he did not discover? For sure, he published a thorough and comprehensive work, but not more than Fischer, Curtius and others. In 1986, the term “glycation” was discussed by the International Union of Pure and Applied Chemistry (IUPAC) as a general term for saccharide–protein adducts, not to be confused with “glycosylation” being a subtype of glycation, *i.e.*, resulting from an enzyme-catalyzed reaction (Sharon, 1986). However, the term “glycation” applies to protein, as said, whereas one has often to describe all together the reactions between

saccharides, and proteins, peptides and amino acids. In this regard, the expression “amino-carbonyl reaction” that Hodge was using is probably the choice that we could make in the future.

To finish, one should remember that there was a strong opposition between France and Germany at the end the 19<sup>th</sup> century and at the beginning of the 20<sup>th</sup> century, with much nationalism, to the point that the Alsatian chemist Charles Adolphe Würtz declared in 1874 that “chemistry is a French science” (Würtz, 1874). Ironically this was published at a time when French chemistry was slowed down because of the reluctance of some influential French chemists (e.g., Marcelin Berthelot) to accept the modern idea of atoms and molecules (Jacques, 1987). It is now time to be more fair, and to better recognize the input of all who contributed to the understanding of the important field of “amino-carbonyl reactions” : personally, I shall now use this terminology, and distribute it widely.

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