IMARS
HIGHLIGHTS

Research Commentaries for the Members of The International Maillard Reaction Society

A Non-profit Research and Education Organization in Biomedicine and Food Science

Volume 17
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November 15, 2022

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IMARS
Established in 2005, the International Maillard Reaction Society gathers researchers and clinicians involved in the field of carbonyl reactions in foods, biology and medicine. It promotes research on Maillard Reaction and protein glycation and their numerous applications. It also organizes regular international congresses on the same theme, in addition to those that have been taken place since 1979.
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Editorial comments

For this November issue of IMARS Highlights, we would like to thank **Prof. Rodrigo Stephani** and **Dr. Mohsen Kerkeni** for their kind contributions.

In the first article, **Rodrigo Stephani**, researcher and coordinator of the Chemistry and Technology Laboratory (QUIMTEC) in the Chemistry Department at the Federal University of Juiz de Fora in Brasil, working together with **Prof. Ítalo Tuler Perrone** from the Pharmaceutical Department of the same University, present a food product special to South America that is quite prone to glycation: *Dulce de leche* is prepared by heating milk and sugar for a long time at high temperatures, but the chemical exploration of the product is beginning only now.

In the second article, **Dr. Mohsen Kerkeni** *(Laboratory of Research on Biologically Compatible Compounds, Faculty of Dental Medicine, University of Monastir, Tunisia)* covers the topic of non-invasive measurement of skin autofluorescence. As the results of this method are influenced by differences in skin color, novel data are still required for different populations worldwide. The authors show that a discrimination between metabolically healthy and non-healthy diabetic individuals is possible by applying skin autofluorescence measurements in a North African population.

We hope you enjoy this issue of IMARS Highlights. The IMARS Highlights editors always look forward to receiving your articles related to glycation research in food and medical sciences and any comment to the articles published in IMARS Highlights. Please feel free to contact us as per below:

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Outstanding aspects in the Maillard Reaction: glycation reactions in *dulce de leche* and relevance of this product in Latin America

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Introduction

The Maillard reaction was discovered in 1912 by Louis Camille Maillard while working on aminoacids and sugar systems, where he noticed upon heating, a brown product which he characterized as melanoidins and made it responsible for the dark end color (Maillard, 1912). Since then, this reaction has been the subject of investigations by many researchers. As it is shown in figure 1, the Maillard timeline, a lot of work and knowledge of chemistry has been necessary to better understand how this complex reaction happens.

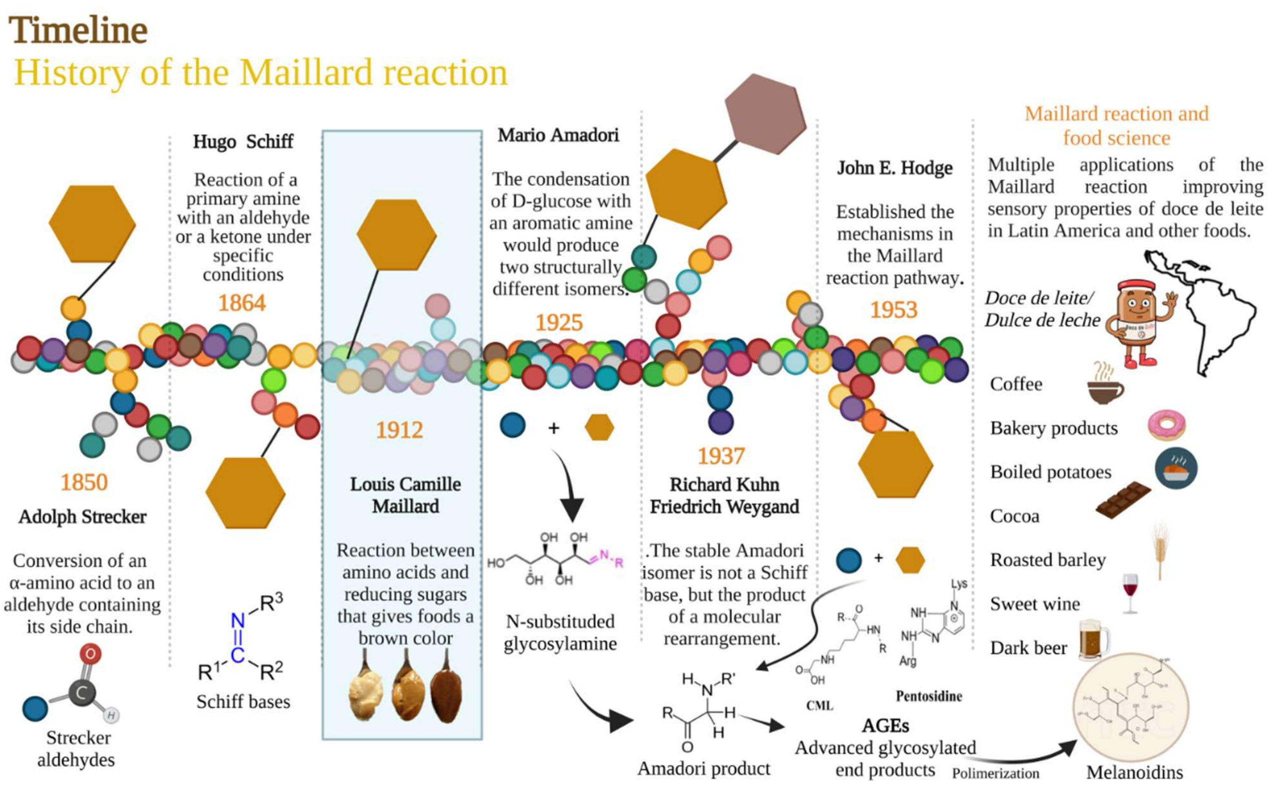

Figure 1. The history of the Maillard reaction in food Science: Understanding chemistry as a basis for applications in food technology.

The discovery of Schiff bases (Schiff, 1864) and Strecker aldehyde formation (Strecker, 1850), and many others, such as the products from Amadori rearrangement (Hodge, 1955), and advanced glycation end products formation (Hodge, 1953; Kuhn & Weygand, 1937), show that every little contribution was important to better understand the Maillard reaction (see figure 1) and how essential it is to continue studying it to extend and improve the applications that can be attributed to it. The Maillard reaction has gained a strong relevance not only in food science but also in medicine (Henle, 2008). Nowadays, for example, we know that glycation is the starting point for key reactions involved in the development of organoleptic properties of many common foods, like bread, cookies, meat, roasted coffee, and so on.

What is "*Dulce de Leche*"?

In Latin America a very important confectionery concentrated dairy product generally called *dulce de leche* is produced. According to the technical regulation, it is understood as the product, with or without the addition of other food substances, obtained by concentration and thermal treatment at normal or reduced pressure on milk or reconstituted milk; whereas the milk can be supplemented (or not) with solids of dairy origin and/or cream, but also with sucrose (partially substituted or not by monosaccharides and/or other disaccharides) (BRASIL, 1997).

In technological terms, we can define *dulce de leche* as a milk and sucrose mix where Maillard reactions occur during thermal and evaporation treatments. The Maillard reaction, therefore, defines *dulce de leche* main flavor, color, texture, and odor characteristics.

Depending on the Latin American country, the product can have different names: *Doce de Leite* (Brazil), *Dulce de Leche* (Argentina, Uruguay, Bolivia, Paraguay, Puerto Rico, Dominican Republic, Equator, and some regions in Colombia and Venezuela), *Cajeta* (Mexico), *Arequipe* (Venezuela, Guatemala and in some regions in Colombia), *Cajeta de Manjar de Leche* (Nicaragua), *Manjar* (Chile and Ecuador), *Manjar Blanco* (Bolivia, Peru, and Panama), and *Fanguito* (Cuba). It is also possible to find similar products in other countries around the globe, e.g., in France and Turkey, where it is called *Confiture de Lait* and *Sütreçeli*, respectively. In some English-speaking countries, it is called *Milk Jam*.

Dulce de leche is a sweet consequence of a glycation reaction; it will be found in many desserts and dishes in Latin America, such as cookies, ice cream, cakes, shakes, milkshakes, and much more. Brazil is almost the biggest world producer of *dulce de leche*, with over three hundred producers on its extension, including short, large, and even artisanal producers (Innovaleite, 2022). This reveals how the Maillard reaction can be useful and key for cultures and markets in Brazil and Latin America.

It is known that intrinsic color characteristics of *dulce de leche* are due to melanoidins (Mesías& Delgado-Andrade, 2017), a family of polymers produced at the later stages of the Maillard reaction. Darker or lighter products can be made depending on the progress of the reaction. Commonly at the beginning of *dulce de leche* production, an adjustment of the pH value to slightly alkaline conditions is done; this leads to a favored glycation reaction and as a consequence an increase in *dulce de leche* browning (Perrone et al., 2012). But not only the color of *dulce de leche* is affected by the reaction; some other sensorial properties can change, too: small volatile molecules such as acetic acid, furfurals, and diacetyl produced gives the product its characteristic aroma (Francisquini et al., 2016). In addition, milk protein modifications also give changes in texture development and rheological properties. All these properties depend on medium conditions during production, like pH, temperature, pressure, and of course, the raw material utilized in the formulation.

Research on *Dulce de Leche* – current status

Only a few research groups in Latina America (especially in Brazil) focus on studying *dulce de leche's* chemistry and technology, mainly in the industrial sector interface. Thus, there is a shortage of literature published in Portuguese, Spanish or English languages, albeit in the last years some research have been published in international journals. A general screening of the two main databases in scientific publications (Scopus and Web of Science) proved the small number of publications about the *dulce de leche* topic (Figure 2). The option made for using the *dulce de leche* or *doce de leite* filters (which are the most common terms used in international scientific documents because Spanish and Portuguese are the major languages spoken in Latin America) only led to 111 documents published in the SCOPUS system between 1984 and 2022 (38-year time-lapse): 102 articles, 5 book chapters and 4 different publications (notes, conferences, errata, and reviews). There were 150 documents identified between 1984 and 2021 in the Web of Science database. Data in Figure 2 depict that the number of publications increased over the years; 16 publications were found in 2021.

The research interest devoted to *dulce de leche* has not reached the level achieved for other concentrated and dehydrated products. However, the development of *dulce de leche* factories, production professionalization, the disregard of *dulce de leche* as an artisanal product, the new technologies allowing a decrease in its caloric value (which has been one of the main barriers for the consumer market), the demand for products with lower sugar content presenting remarkable milk features, besides products with higher added values (such as *dulce de leche* with different flavors - chocolate, strawberry, coffee, among others) and *dulce de leche* added with fruit jam (e.g., passion fruit, plum, strawberry, blackberry, peach), all these are factors making the action of expert professionals in the market even more significant.

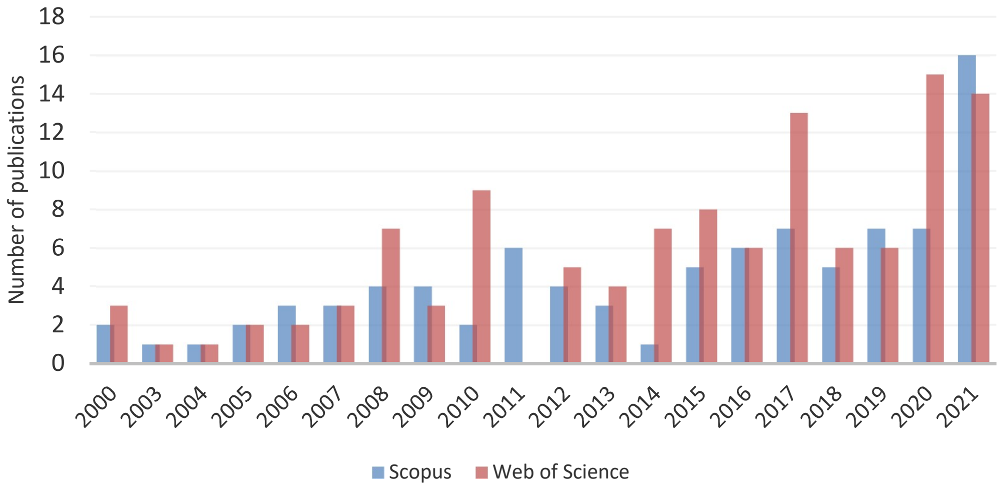

Figure 2. Number of publications (per year) in SCOPUS and Web of Science databases about dulce de leche or doce de leite between 1984 and 2021.

The developments achieved by dairies in other several production fields and especially those observed in *dulce de leche* manufacturing companies, seem to be a chasm to be overcome by a new generation of professionals. We also find a *dulce de leche* production sector in several well-developed dairy industries that have advanced technologies for cheese, fermented milk, and other derivatives, but that are obsolete to produce it. Their *dulce de leche* sectors are managed by candy makers who play an essential role in the production chain, but who are not supported by it or do not get technical support to have their problems or questioning answered.

Solid knowledge and deep understanding of the features of the milk to be used for *dulce de leche* production and knowing the desired product profile of the specific brand are the main skills one expects from individuals who will work in *dulce de leche* processing. On top, having at hand the necessary analytical chemical and engineering tools to control the manufacturing process are of utmost importance in this matter.

Glycation reactions: Future challenges in *dulce de leche* research

Studies about glycation and non-enzymatic cross-linking can lead to a better understanding of the role of the milk proteins and sugars, and consequently, products' structure and texture, in the case of *dulce de leche*. A recent study allowed observing that micellar casein or non-micellar sodium caseinate suspensions after heating, respectively, present formation similar to Amadori products with, or without, glucose for 0-4 hours at 100°C (conditions close to that of *dulce de leche* production). They were markers for the initial Maillard reaction stage and indicated that reactive amino acid side chains inside casein micelles are accessible for glucose (similarly to that of non-micellar casein). However, significant differences were observed in the formation of final advanced glycation products (AGEs), namely: N^{ε}-carboxymethyllysine (CML), pyrraline, pentosidine, and glyoxal-lysine dimer (GOLD). A larger number of CML could be observed in non-micellar casein, whereas pyrraline formation increased in micelles. Pentosidine and GOLD formation reached comparable amounts; moreover, the extension of protein cross-linking was significantly bigger in glycated casein micelles than in non-micellar casein samples (Möckel et al., 2016). Another study has shown that N^{ε}-carboxymethyllysine (CML) can face a second glycation event in its secondary amino group, a fact that leads to a new class of rearranged Amadori products (Hellwig et al., 2022).

Maillard's browning reactions are an integrating part of the manufacturing process applied to *dulce de leche*: milk heating with sucrose to produce browning products with pleasant flavor (O'Brien et al., 2009). These reactions are strongly influenced by several factors, with an emphasis on processing type, for which there are many. So far, no scientific literature focused on investigating the effect of different processes on e.g. the intensity and differences in Maillard stages.

Dulce de Leche features directly depend on the product/process interactions. It seems imperative to have more studies in *dulce de leche* dedicated to clarifying the processing effects, e.g. of the different production stages, on the physical-chemical changes occurring in the product as well as assessing the formation of routes, endogenous pool, etc., and the positive and/or negative influence of products already formed during the Maillard reactions.

From all the above, the need is clear for a research program and partnership with carbohydrate research experts, where specific expertise on Maillard reactions is available and well documented, and extend this knowledge to *dulce de leche*. Such an international project is already on its way to being opened and started, thanks to a partnership set with the research team of Prof. Dr. rer. nat. Dr. Ing. habil. Thomas Henle, from the *Chair of Food Chemistry* at the Faculty of Chemistry and Food Chemistry of *Technische Universität Dresden* (TUD) in Germany; the Brazilian counterpart is the team of Prof. Dr. Rodrigo Stephani, researcher and coordinator of the Chemistry and Technology Laboratory (QUIMTEC), of the Chemistry Department at the Federal University of Juiz de Fora, in Minas Gerais, Brazil. The group Inovaleite in Brazil, associated with UFV and UFJF, has proven competence in matters related to the processing and technology of *dulce de leche* and expects that eventually, this research will highlight the importance of the above international cooperation and the benefits of the scientific know-how on glycation reactions in *dulce de leche* production, a beloved product in many countries, with a significant contribution to the quality delivered to the consumer and the economies in Latin America.

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Skin autofluorescence of advanced glycation end products levels in Tunisian subjects with and without metabolic diseases

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The prevalence of cardiometabolic diseases, including diabetes mellitus, dyslipidemia and hypertension is currently increasing and represents a significant health problem in Tunisia [1,2]. It is well known that diabetes mellitus combined with the myriad of cardiovascular factors is responsible for the development of long-term microvascular and macrovascular complications, which contribute to the increased risk of morbidity and mortality [3]. Hyperglycemia induces glycation and oxidative pathways directly or indirectly on proteins, lipids, and nucleic acids [4, 5]. Measurement of serum glucose and glycated hemoglobin (HbA1c) concentrations are the standard biomarkers for detecting diabetes. Still, these parameters do not fully reflect the adverse effects in tissues for diabetic patients with or without vascular complications. Several studies have shown the role of accumulation of so-called advanced glycation end product (AGEs) in tissues as a biomarker for early detection, screening, and diagnosis of many diseases [6, 7]. The accumulation of AGEs contributes to tissue damage in several chronic and aging-related diseases, such as diabetes, atherosclerosis, chronic kidney disease, neurodegenerative disorders [8]. AGEs are a complex and heterogeneous group of non-fluorescent and fluorescent compounds that becomes irreversibly cross-linked adducts that bind to amino acids (lysine/arginine residues) of proteins and accumulate on proteins a slow turnover [9]. AGEs are formed by non-enzymatic glycation and oxidation during aging and are further increased in conditions of hyperglycemia. The degree of AGEs accumulation in the body is associated with increased production, decreased degradation and renal clearance [10].

There is no study about skin AGEs accumulation in Tunisian subjects with and without metabolic diseases. Previous studies have shown that non-invasive measurement of skin autofluorescence (SAF) with the AGE Reader can easily estimate AGEs accumulation and identify those at risk for developing diabetes and those at risk for diabetes-related complications [11]. In this cross-sectional study, we have measured SAF in healthy subjects and people with diabetes and/or hypertension and dyslipidemia. We have examined the relationship between SAF and various bio-clinical parameters. We have included 250 participants from the Monastir region in Tunisia for the current study. Participants were either healthy control subjects (n=88), individuals with type 2 diabetes (n=48), hypertension (n=62), or both (n=52), without a history of clinical cardiovascular or kidney disease. The medical ethical review committee of the hospital Fattouma Bourguiba at Monastir approved the study protocol, and all participants provided written informed consent.

This is the first study reporting skin autofluorescence measurements in a large cohort of individuals from an Arab population residing in Tunisia in northern Africa. We demonstrated increased SAF levels in individuals with diabetes and/or hypertension and dyslipidemia (2.42 ± 0.38) compared to healthy subjects (1.90 ± 0.29). The SAF *z*-score was increased in sujects with metabolic disease (0.65 ± 0.85) compared to healthy subjects (0.18 ± 0.55) as shown in Figure 1.

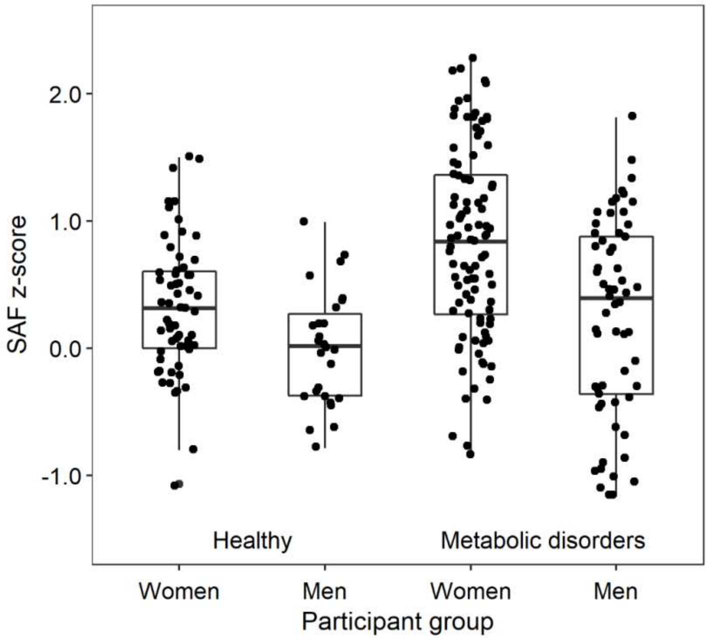

Figure 1: SAF z-score levels according the gender in healthy and subjects with metabolic disorders

Previously, it was shown that SAF values may vary in different ethnicities, as described by Mook-Kanamori et al [12]. People from Arab and Filipine descent had a significantly higher SAF than a South Asian population. Nevertheless, SAF levels were considerably higher in the Arab population with diabetes than healthy individuals [12]. Another study reported in more detail on the effects of seven different ethnicities: Arab, Central-East African, Eastern Mediterranean, European, North African, South Asian, and Southeast Asian on SAF. The highest SAF values were observed in the North African population, followed by East Mediterranean, Arab, South Asian, and European populations [13]. Two major reasons may explain the difference between the above Arab studies: the Tunisian population's lifestyle and habitual dietary adapted. For gender, our study showed no significant difference in healthy subjects. However, a significant difference was shown in subjects with metabolic disorders.Our results showed significantly increased SAF values in women compared to men.

In conclusion, this Tunisian population-based study shows an increased SAF level in subjects with diabetes and/or hypertension and dyslipidemia compared to healthy subjects. The AGE Reader device is a rapid and non-invasive tool in clinical practice to evaluate and screen metabolic disorders in people from Tunisia and more generally those with a North Africa phototype.

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Highlights of the glycation literature (Sep. 2022- Oct. 2022)

The publications were selected from **Web of Science** (Topic key word: glycation, Publication Date: September 2022~ October 2022, Document Type: Articles and Review Articles)

Papers of Editors' Choice

- Zhao, WB; Cai, PJ; Zhang, N; Wu, TT; Sun, AD; Jia, GL. **Inhibitory effects of polyphenols from black chokeberry on advanced glycation end-products (AGEs) formation.** *Food Chemistry*. 392. 133295 (2022)
- Wasim, R; Mahmood, T; Siddiqui, MH; Ahsan, F; Shamim, A; Singh, A; Shariq, M; Parveen, S. **Aftermath of AGE-RAGE Cascade in the pathophysiology of cardiovascular ailments**. *Life Sci*. 307. 120860 (2022)
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- Liu, HY; Wang, GQ; Wu, T; Hu, J; Mu, YM; Gu, WJ. **Association of skin autofluorescence with low bone density/osteoporosis and osteoporotic fractures in type 2 diabetes mellitus**. *J. Diabetes*. 14. 571-585 (2022)
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- Chen, Y; Yu, C; Jiang, S; Sun, L. **Japanese Flounder HMGB1: A DAMP Molecule That Promotes Antimicrobial Immunity by Interacting with Immune Cells and Bacterial Pathogen.** *Genes.* 14. 3598 (2022)
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- Liu, YY; Liu, XK; Zhou, W; Zhang, JY; Wu, JR; Guo, SY; Jia, SN; Wang, HJ; Li, JL; Tan, YY. **Integrated bioinformatics analysis reveals potential mechanisms associated with intestinal flora intervention in nonalcoholic fatty liver disease.** *Medicine.* 262. 110778 (2022)
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