



Non-enzymatic browning kinetics in sucrose-glycine aqueous and dehydrated model systems in presence of MgCl₂



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ABSTRACT

The development of non-enzymatic browning in the presence of MgCl₂ was evaluated in liquid and dehydrated sucrose-glycine model systems, in relation to interactions of the salt with water and/or with sucrose. In both systems, browning was accelerated by the presence of MgCl₂ because of the increased sugar hydrolysis (ten times faster) and the reduction of water mobility (¹H NMR T₂ relaxation times) caused by this salt (between 6 and 14% lower), counteracting the inhibitory effect of water on the Maillard reaction. MgCl₂ also provoked a 40% reduction on the fluorophores quantum yield, responsible also of the fluctuations observed in the fluorescence development as a function of time after 50 h at 70 °C. Molecular and supramolecular effects of the presence of MgCl₂ have been observed on the Maillard reaction kinetics. These results are of high technological interest when strategies to control the Maillard reaction rate are required for a particular application.

1. Introduction

Non-enzymatic browning (NEB) reactions are considered among the most important chemical reactions taking place during food processing or cooking and storage. They not only influence food quality attributes such as color and flavor, but also affects the nutritive value or toxicological implications of heat-induced food (Wang, Wang, Guo, Ma, & Yu, 2013). Thus, technological interest exists to find strategies to control Maillard reaction avoiding sulphites, because of their adverse clinical effects in sensitive individuals (asthmatic reactions, dermatitis, flushing, hypotension, among others) (Vally, Misso, & Madan, 2009). NEB reactions involve either caramelization, (the heat-induced decomposition reaction of sugars without amine participation) or the Maillard reaction in which reducing carbonyl groups sugars are condensed with the basic amino groups (Hodge & Osman, 1976). After several dehydration and rearrangement steps, NEB reactions lead to the formation of brown compounds (Nie et al., 2013; Tamanna & Mahmood, 2015). Several factors influence the NEB reactions rate, including the nature of the reactants, their molecular weight, number of carbonyl groups, pH, water activity, temperature and heating time (Mondaca-Navarro et al., 2017). In the case of sucrose, as a non-reducing sugar, its participation in NEB reaction can only occur after the molecule is hydrolyzed or cleaved to obtain reducing carbonyls.

Previous studies indicated that the presence of salts affect thermo-physical properties of concentrated aqueous sugar solutions and they provided evidence for the existence of sugar-metal complexes in solution (Miller, de Pablo, & Corti, 2000; Rongere, Morel-Desrosiers, & Morel, 1995). Salts affect also fluorescence and browning development from the Maillard reaction, kinetics of sugar crystallization and hydrolysis reaction (Matiacevich & Buera, 2006; Matiacevich, Santagapita, & Buera, 2010; Santagapita & Buera, 2006; Santagapita & Buera, 2008; Schebor, Burin, Buera, & Chirife, 1999). Sugar-cation (Morel-Desrosier, Lhermet, & Morel, 1991) and brown pigment-cation complexes can be formed in solution (O'Brien & Morrissey, 1997). Because of the inhibitory effect of water on the Maillard reaction, specifically when water activity is above 0.75 (Wong, Wijayanti, & Bhandari, 2015), modifications in water-solids interactions promoted by salts could be responsible for changes in the reaction rates. In this sense, Gökmen and Şenyuva (2007) reported that the addition of Ca²⁺ prevented the acrylamide formation in an asparagine-glucose system, but also increased hydroxymethylfurfural (HMF) and furfural formation, some intermediate compounds of the Maillard reaction.

The kinetics of fluorescent and browning development on glucose-lysine liquid model systems was delayed by MgCl₂. However, in liquid trehalose systems, the browning reaction was accelerated by the presence of MgCl₂ because of the increased sugar hydrolysis and the

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reduction of water mobility caused by this salt, counteracting the inhibitory effect of water on the Maillard reaction. On the other hand, in water restricted (freeze-dried) trehalose systems, MgCl_2 inhibited the Maillard reaction. In this case, the salt-sugar interactions, manifested by the delayed sugar crystallization, decreased the reaction rate by affecting the reactivity of reducing sugars (Matiacevich et al., 2010; Matiacevich & Buera, 2006). MgCl_2 dramatically accelerated sucrose degradation in sucrose concentrated solutions but to a lesser extent than did NaCl (Eggleston, Vercellotti, Edye, & Clarke, 1996). However, there is a lack of information on the effect of salts on sucrose hydrolysis in freeze dried systems and other possible effects on the kinetics of the above-mentioned interrelated phenomena.

The purpose of this work was to analyze the modification of non-enzymatic browning development in sucrose-glycine systems induced by the presence of MgCl_2 , in a wide range of water contents, evaluating solid-water interactions and water availability, which govern and modulate the kinetics of those reactions.

Non-enzymatic browning was followed by absorbance and fluorescence development. Complementary determinations of molecular mobility as T_2 values by proton nuclear magnetic resonance (^1H NMR) relaxometry, as well as fluorescence quantum yield were employed in order to accomplish a correct interpretation of the effects of environmental and compositional conditions on the reaction kinetics.

2. Materials and methods

2.1. Preparation of model systems

2.1.1. Liquid systems

Liquid systems were prepared from 5 to 70% w/v of sucrose (Merck, Darmstadt, Germany) and 0.5% w/v of L-glycine (Merck) with and without MgCl_2 (Mallinckrodt Chemical Works, St. Louis, USA), in a 5:1 sugar:salt molar ratio; controls without MgCl_2 and without glycine were also prepared in 0.1 M phosphate buffer at pH 5 (Merck). All reactants were analytical grade. 2.5 mL aliquots of each model were placed in 5 mL vials. Vials were hermetically sealed and stored at $70 \pm 1^\circ\text{C}$ in a forced air convection oven for different times between 0 and 212 h.

2.1.2. Solid systems

The solid systems were freeze-dried solutions obtained from solutions containing 20% w/v sucrose and 1% w/v L-glycine in 0.1 M phosphate buffer at pH 5. MgCl_2 was added in a 5:1 sugar:salt molar ratio. 2.5 mL aliquots of each model were placed in 5 mL vials, frozen at -26°C for 24 h and immersed in liquid nitrogen immediately before freeze-drying in order to freeze as much water as possible, close to complete freezing. Freeze-drying was performed in a Heto Holten A/S, cooling trap model CT 110 freeze-dryer (Heto Lab Equipment, Denmark) operating at a condenser plate temperature of -111°C and a chamber pressure of $4 \cdot 10^{-4}$ mbar. After freeze-drying, the systems were transferred for two weeks to vacuum desiccators and exposed to relative humidity conditions of 22 and 43% (Greenspan, 1977) at $25 \pm 1^\circ\text{C}$. Then, vials were hermetically sealed and stored at $70 \pm 1^\circ\text{C}$ in a forced air convection oven during different times between 0 and 212 h.

2.2. Determination of browning and fluorescence development

The progress of non-enzymatic browning (NEB) was followed by a browning index (B.I.), defined as absorbance units at 445 nm (model UV-Vis 1203, Shimadzu, Kyoto, Japan) multiplied by the dilution factor, and by fluorescence intensity with excitation at 340 nm/emission 492 nm (model USB 2000 spectrofluorometer, Ocean Optics Inc., FL, U.S.A). The samples employed for the determination of fluorescence were diluted to avoid inner filter effect, with absorbance values lower than 0.1 at the excitation wavelength (340 nm). Triplicate measurements for different times between 0 and 212 h were performed. The confidence intervals for browning and fluorescence values were 3% and

5% for a 95% certainty, respectively.

Browning development was analyzed as a function of time as follows:

$$\frac{db}{dt} = kb^n \quad (1)$$

where

b represents the absorbance units, n is the reaction order and k is the rate constant.

According to Petriella, Resnik, Lozano, and Chirife (1985), the reaction order n was obtained through the integration of Eq. (1) to give Eq. (2):

$$b = ((1 - n)kt)^{1/(1-n)} \quad (2)$$

and was close to 0.5, as obtained by non-linear analysis of data, employing Prism 6.01 Graph-Pad Software Inc. (San Diego, CA, USA).

2.3. Glucose release

The amount of glucose released during the incubation time was analyzed in triplicate by means of an enzymatic method based in the Trinder reaction (Wiener Lab. S.A.I.C., Rosario, Argentina) previously described by Bergmeyer & Grassi, 1983.

2.4. Fluorescence quantum yield

After storage, sugar-systems (70% w/v) were diluted to obtain absorbance values below 0.05 to avoid inner filter effects. Four dilutions were prepared for each time of incubation. Fluorescence quantum yields (Φ_F) was determined (in duplicate) following the same procedure described by Matiacevich et al., 2010.

2.5. Nuclear magnetic resonance (NMR) measurements

Transversal or spin-spin relaxation times (T_2) were measured in duplicate by low field-proton nuclear magnetic resonance (LF- ^1H NMR) in a Bruker Minispec mq20 (Bruker Biospin GmbH, Rheinstetten, Germany) with a magnetic field of 0.47 T operating at 20 MHz of frequency.

Liquid systems were analyzed using Carr-Purcell-Meiboom-Gill (CPMG) sequence with the following settings: $\tau = 0.5$, number of scans = 4, points = 256, dummy shots = 15, gain = 68 dB; phase cycling was used. Calibration was performed employing a 40% w/v sucrose system without thermal treatment.

A mono-exponential function was used to fit the experimental data adequately, from which transverse relaxation time constants (T_2) was obtained, as reported previously by Matiacevich et al., 2010.

All samples in test tubes were previously equilibrated at $25.00 \pm 0.01^\circ\text{C}$ in a thermal bath (Haake, model Phoenix II C35P, Thermo Electron Corporation GmbH, Karlsruhe, Germany).

2.6. Determination of water content

The total water content of the solid systems was determined gravimetrically by difference in weight before and after drying in a vacuum oven for 48 h at $62 \pm 2^\circ\text{C}$ (Mazzobre, Longinotti, Corti, & Buera, 2001; Schebor et al., 1999). Water content as an average of triplicate measurements was informed in dry basis.

2.7. Thermal transitions measurements

Melting events were determined by dynamic differential scanning calorimetry (DSC), in the temperature range from -120 to 160°C , by means of a Mettler Toledo model 822 equipment (Mettler Toledo AG, Greifensee, Switzerland) at a heating rate of $10^\circ\text{C}/\text{min}$. Melting temperatures values were taken as temperatures peaks. $40 \mu\text{L}$ inner volume

aluminum pans (Mettler Toledo AG) with 10–25 mg sample were hermetically sealed and measured in duplicate, using an empty pan as a reference, and average values are reported. Standard compounds (indium and zinc) of defined melting point and heat of melting (ΔH_m) were used for calibration.

2.8. Statistical analysis

Mean values of obtained data with their corresponding standard deviation were reported. Comparison of obtained mean values were analyzed using one-factor ANOVA with a 95% of confidence using Graph Pad Prism software (v 6.01).

3. Results and discussions

3.1. Maillard reaction and glucose release

No significant development of absorbance at 420 nm or fluorescence ($p < .05$) were detected in the model systems without aminoacid (data not shown). Thus, it could be concluded that there was no contribution to color or fluorescence due to caramelization in the experimental time frame of the experiments.

Browning and fluorescence development of the liquid sucrose systems increased as increasing storage time at 70 °C, for the whole composition range studied (5–70% w/v), as shown in Fig. 1(a–b) for 70% w/v sucrose systems (taken as an example). An accelerating effect of $MgCl_2$ was observed in sucrose liquid systems for both browning and fluorescence development (Fig. 1a and b, respectively). As expected, the corresponding visual color changes for all analyzed systems along time were: uncolored → yellow → golden → cinnamon → reddish brown. The compounds responsible for the browning development are melanoidins. They are formed by the condensation and polymerization of Maillard reaction intermediates (ketosamines, α -dicarbonyls, furans, furanones, and pyranones) (Hellwig & Henle, 2014; Hodge, 1953; Ledl & Schleicher, 1990) that proceed with loss of water, forming heterogeneous macromolecular structure. Even though, their structure is not unraveled in detail, substructures of lower molecular weight (Hofmann, 1998) or heterocycles polymerization products have been reported (Adams, Borrelli, Fogliano, & de Kimpe, 2005; Tressl, Wondrak, Garbe, Krüger, & Rewicki, 1998). Recently Mohsin et al. (2018) confirmed the general elemental composition suggested by Cämmerer, Jalyschko, and Kroh (2002), based in the combination of two 3-deoxyglucosone and one aminoacid, forming a stable carbohydrate backbone which is difficult to cleave.

Both sugar concentration and the presence of $MgCl_2$ were determinant for the rate of pigment and fluorescent compounds formation. Furthermore, glucose release from sucrose occurred during heat treatment at 70 °C, as confirmed by glucose analysis (Fig. 1c), was at least ten times faster in the systems containing $MgCl_2$, yielding > 80% of sucrose conversion after 212 h at 70 °C. It has to be also taken into account that the determined glucose concentration is the result from its release from sucrose and its consumption in the NEB reaction. Thus, sucrose cleavage could be underestimated by the direct measure of glucose concentration. However, this does not preclude to state that glucose release is accelerated in the presence of salt. This acceleration of sucrose cleavage can be considered the main responsible of the higher browning and fluorescence development observed in presence of $MgCl_2$. Richards (1988) attributed the increase of the degradation rate of sucrose in the presence of $MgCl_2$ to the enhanced withdrawal of electrons from the glycosidic oxygen of the sucrose molecule by protonation of the acetal oxygen with the hydrated magnesium ion. However, it has been demonstrated that in acidic methanol at 65 °C sucrose degrades into glucose and a very reactive fructofuranosyl cation, revealing that sucrose glycosidic bond may cleave without hydrolysis in food-related systems (Perez Locas & Yaylayan, 2008). In dry systems this cation can be effectively converted directly into HMF.

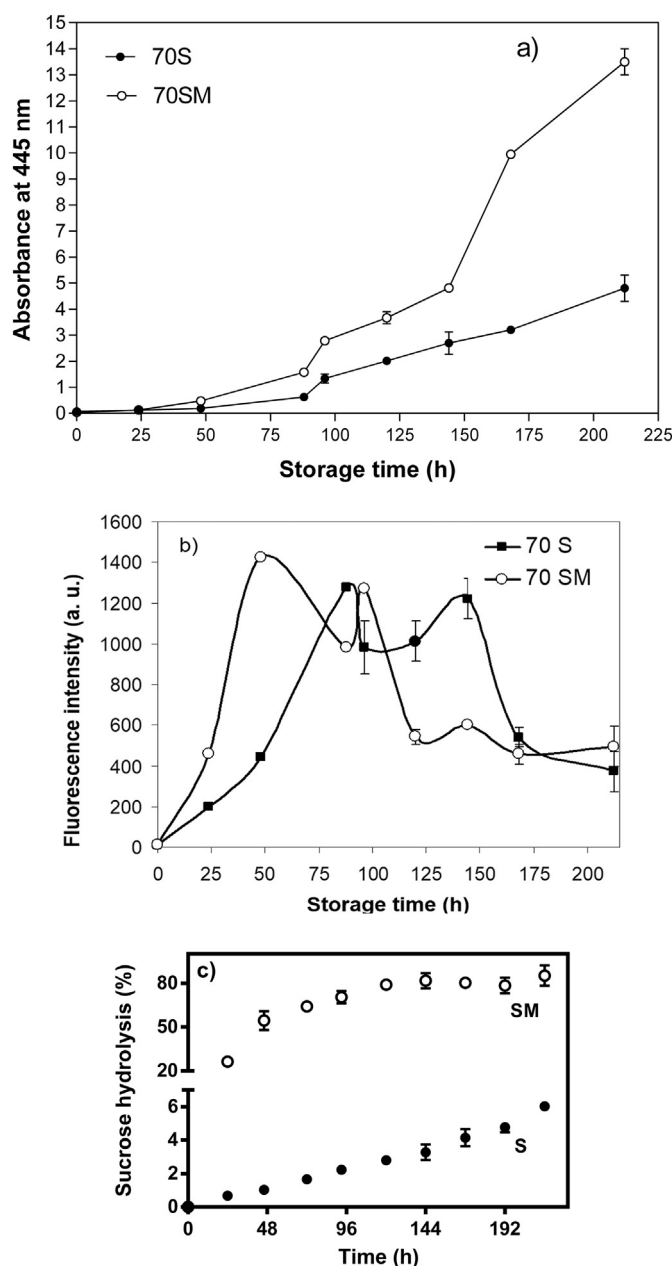


Fig. 1. a) Browning and b) fluorescence development for 70% (w/v) sucrose systems with $MgCl_2$ (SM) and without $MgCl_2$ (S) as a function of storage time at 70 °C. Excitation/emission at 340/450 nm. c) Hydrolysis development for 40% (w/v) sucrose systems with $MgCl_2$ (SM) and without $MgCl_2$ (S) as a function of storage time at 70 °C.

Then, the contribution from this path cannot be neglected.

In agreement with Karel and Labuza (1968), glucose release was detected even in the freeze-dried sucrose samples, humidified at relative humidities (RH) of 22 and 43%, in which the present water molecules interact strongly with solids and have very low mobility. Parallely, in these systems, fluorescence browning developed which were highly correlated (data not shown) and the rate of glucose release increased in the presence of salt, displaying a similar behavior that has been seen in the analyzed liquid systems (Fig. 1c).

Several works have analyzed the mechanisms behind sucrose pyrolysis. However, most of them are far from the conditions analyzed in present work: either they work at very high temperatures (Manley-Harris & Richards, 1993), or evaluate synthetic derivatives (Pelletier, Zwicker, Liana Allen, Schepartz, & Miller, 2017). It can be stated that

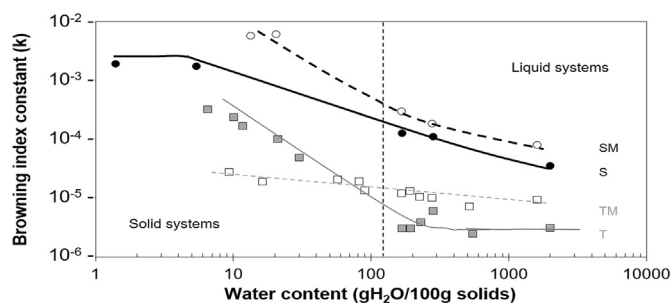


Fig. 2. Kinetic constants for the Maillard reaction (obtained by a quadratic model) as a function of water content for sucrose systems. Trehalose systems were included for comparison purposes, adapted from [Matiacevich et al., 2010](#). The trending lines were plotted, corresponding the solid lines to sugar systems without salt and the dashed lines to MgCl_2 -containing systems. S: sucrose; M: MgCl_2 ; T: trehalose.

sucrose, as a nonreducing sugar, does not undergo the sugar dehydration reaction until it is hydrolyzed to the reducing sugars ([Lee & Nagy, 1990](#); [Queneau, Jarosz, Lewandowski, & Fitremann, 2008](#)). Nevertheless, one of the most typical aspects of sucrose chemistry is the fragility of its glycosidic bond ([Queneau et al., 2008](#)). At conditions closed to those employed in the present work, acid-catalyzed hydrolysis of sucrose occurred even at water contents below 1% ([Karel & Labuza, 1968](#)), even though sucrose glycosidic bond can be cleaved without hydrolysis, as demonstrated [Perez Locas and Yaylayan \(2008\)](#).

In all cases, browning development was analyzed using Eqs (1) and (2), obtaining a reaction order of 0.5. It has to be noted that [Buera, Chirife, Resnik, and Lozano \(1987\)](#) and [Matiacevich and Buera \(2006\)](#) employed a two steps kinetic model based in contributions of 0 and 1 orders in liquid sugar or sugar-aminoacid systems. Thus, the obtained global fractional order in present work can be considered as the result of two independent contributions, one corresponding to a zero-order reaction and the other to a first-order one. It is also to be noted that the half order kinetics correspond to a quadratic dependence on time.

The obtained kinetic constants for NEB development in sucrose systems were plotted as a function of water content ([Fig. 2](#)). In both liquid and dehydrated systems, the presence of MgCl_2 increased the NEB reaction rate constant. In [Fig. 2](#) the kinetic constants for NEB development in trehalose systems obtained by [Matiacevich et al. \(2010\)](#) were included for comparative purposes. It is clearly shown that in trehalose systems MgCl_2 inhibited Maillard reaction at low water contents (solid systems), but, in liquid systems, the presence of this salt increased the reaction rate. Thus, while in sucrose systems, the MgCl_2 always accelerated NEB reaction, in trehalose samples the effect of MgCl_2 on browning development was dependent on physical state of the system.

3.2. Fluorescence characteristics and quantum yield study

In agreement with previous studies ([Matiacevich, Santagapita, & Buera, 2005](#), [Matiacevich et al., 2006](#), [Santagapita, Matiacevich, & Buera, 2008](#)), the maximum wavelength for fluorescence emission was 450 nm in all cases, and the fluorescence development at zero time of incubation was negligible. The spectral characteristics were independent of the studied system (data not shown), indicating that neither the sucrose concentration nor the presence of salt affected the type of fluorophore obtained in each system. Previous studies showed the same behavior in trehalose-glycine and glucose-glycine systems ([Matiacevich et al., 2010](#)).

The effect of MgCl_2 on fluorescent and pigment development was qualitatively similar in the analyzed sugar concentration range (5–70% w/v). It is also noticeable that in sucrose systems fluorescence intensity decreased at long incubation times, while browning continued to increase ([Fig. 1b](#)). The observed phenomena shown in [Fig. 1b](#), associated

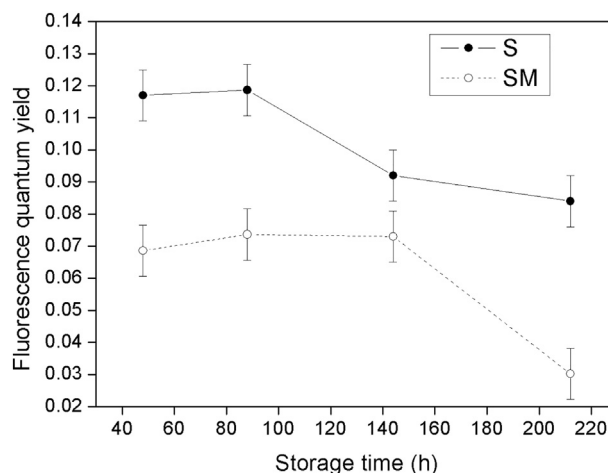


Fig. 3. Fluorescence quantum yield as a function of storage time for 70% (w/v) sucrose systems with MgCl_2 (SM) and without MgCl_2 (S). Excitation/emission at 340/450 nm.

to the effect of sugar type and their interactions with MgCl_2 on the Maillard reaction, were further analyzed by studying fluorescence characteristics in liquid systems.

Spectral characteristics of fluorescent products from simple systems (i.e. glucose-glycine) were observed to be similar to those of browned foods (bread, coffee, milk derivatives). Generally, the fluorophoric group is a Schiff-base in conjugation with an electron donating group. Even though some of the mechanisms involved in fluorescence development have been investigated, and some fluorescent compounds have been characterized, it is difficult to put fluorescence on a molar basis ([Bosch, Alegría, Farré, & Clemente, 2007](#); [Rizzi, 1997](#)). However, information on molecular structural properties of the fluorophores and their interactions could be obtained through the determination of the fluorescence quantum yield, which is defined as the ratio between the number of photons involved in the emission and the total number of excited photons. As shown in [Fig. 3](#), in sucrose systems fluorophores showed constant quantum yield until ~100–144 h and then it diminished as a function of time. A lower quantum yield was observed in the presence of MgCl_2 . This behavior was different to the one reported for trehalose and glucose systems, in which the presence of this salt did not affect the quantum yield ([Matiacevich et al., 2010](#)). Quantum yield also decreased significantly in sucrose systems as increasing reaction time, and was even lower in sucrose- MgCl_2 systems, probably by inner quenching mechanisms. According to the employed experimental procedure, the quenching mechanisms were not promoted by inner filter ([Lakowicz, 1999](#)) and could be due to interactions between Maillard reaction products from fructose and MgCl_2 , specifically with Heyns compounds, derived from the mechanisms involving ketoses as fructose ([Krause, Schlegel, Schwarzer, & Henle, 2008](#); [van Boekel, 2006](#); [Yuan, Sun, Chen, & Wang, 2017](#)), or interactions between reaction intermediates and free fructose ([Hemmler, Roullier-Gall, Marshall, Rychlik, & Taylor, 2017](#)) and MgCl_2 . It is to be noted that at 212 h of heat treatment a similar fluorescence value was observed in sucrose systems with and without MgCl_2 ([Fig. 1b](#)): since fluorophores have lower quantum yield in the MgCl_2 containing system than in the system without salt, this reveals a higher fluorophores concentration in the first. Thus, results confirm that the fluorescent compounds generated from fructose, after sucrose hydrolysis, are sensitive to the presence of MgCl_2 and this effect was not observed in glucose nor in trehalose systems ([Matiacevich et al., 2010](#)), evidencing the importance of the sugar type on the properties of their reaction products.

Table 1

^1H NMR spin-spin relaxation times (T_2) for S and SM as a function of sucrose concentration for untreated samples.

Sucrose (% w/v)	T_2 (ms)	
	S	SM
5	2273 \pm 6 ^a	2123 \pm 5 ^b
20	1652 \pm 8 ^c	1675 \pm 8 ^c
40	1141 \pm 9 ^d	1094 \pm 8 ^e
50	1012 \pm 8 ^f	996 \pm 7 ^f
60	823 \pm 6 ^g	807 \pm 6 ^g
70	687 \pm 5 ^h	627 \pm 5 ⁱ

*Lower-case superscript letters indicate significant difference with $p < .05$.

3.3. Proton mobility studies by NMR

In order to evaluate the possible influence of water-sugar/salt interactions in the results observed in Figs. 1 and 2, proton mobility in each system was studied by low field ^1H NMR, through T_2 values determination. In liquid systems, T_2 values were calculated after the CPMG sequence by a mono-exponential decay model and their values are shown in Table 1. With increasing sucrose concentration T_2 values diminished, as expected because of the relatively high efficiency of magnetic relaxing mechanisms in highly viscous or solid systems. T_2 values were between 6 and 14% lower in the presence of Mg^{2+} , reflecting that the ions imposed a certain local order to water molecules in sucrose systems, affecting water mobility. Since water, as a final product of the reaction, produces an inhibitory effect on Maillard reaction (Labuza, 1994), the acceleration of the browning reaction rate produced by MgCl_2 (Figs. 1 and 2) can be also associated to water-salt interactions, which reduce water availability in sucrose- MgCl_2 in comparison to sucrose systems.

Small or highly charged ions, such as Mg^{2+} , which attract water through strong ion-dipole forces act as nuclei for cluster formation by immobilizing the surrounding water molecules, and this attractive force extends beyond the nearest neighbors, resulting in high local water structure centered around the solute ion (Eggleston et al., 1996). According to Miller and de Pablo (2000), the local environment of ions contained in a sugar system has more water molecules respect to those corresponding to a uniform distribution of water, which can explain the lower T_2 values obtained in the disaccharide-salt containing systems. This effect was observed by You and Ludescher (2008), in amorphous sucrose films, in which MgCl_2 decreased the matrix molecular mobility, and promoted an extent of dynamic heterogeneity into the matrix. These phenomena are explained by the relatively strong Coulomb interactions among Mg^{2+} and water molecules, reducing the opportunity of the latter to construct cage-like clusters, leading to the hydrate formation near the ion-water interface (Yi, Liang, Zhou, Li, & Wang, 2014).

Fig. 4 shows the T_2 values as a function of thermal treatment time. In the liquid systems only slight changes of T_2 values were observed at the studied sucrose concentrations (5–70% w/v) for both S and SM systems. The overall effects which seems to predominate are a slight reduction of T_2 for the more diluted systems, and a virtual counteracting effect between hydrolysis (i.e. glucose and fructose presence, which reduces average molecular weight) and melanoidins (which increases average molecular weight) generation for the more concentrated ones.

3.4. Thermal analysis

MgCl_2 effects on the Maillard reaction kinetics were also studied by thermal transitions, which operate at supramolecular level. Fig. 5 shows the thermograms for 70% w/v S and SM systems before and after 212 h of thermal treatment at 70 °C. T_g values, ice melting and endothermic

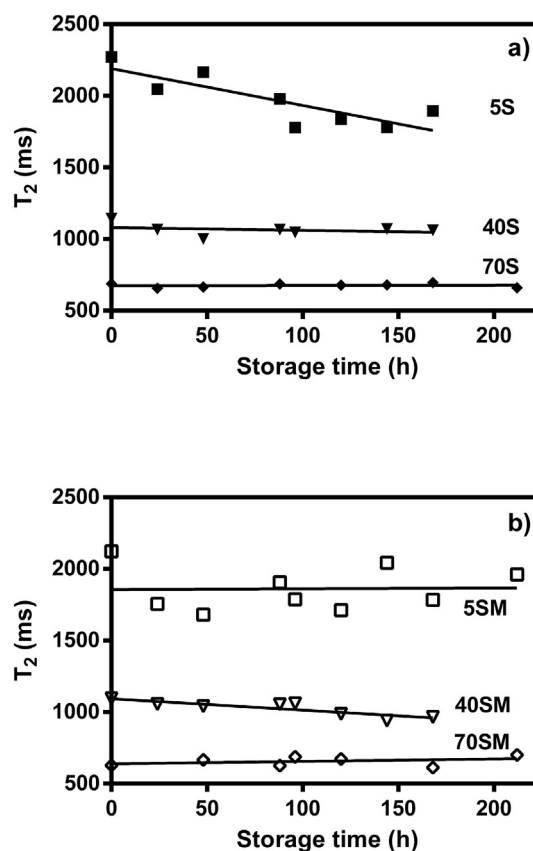


Fig. 4. ^1H NMR spin-spin relaxation times (T_2) for sucrose liquid systems with a) MgCl_2 (SM) and b) without MgCl_2 (S) at different sugar concentration as a function of storage time.

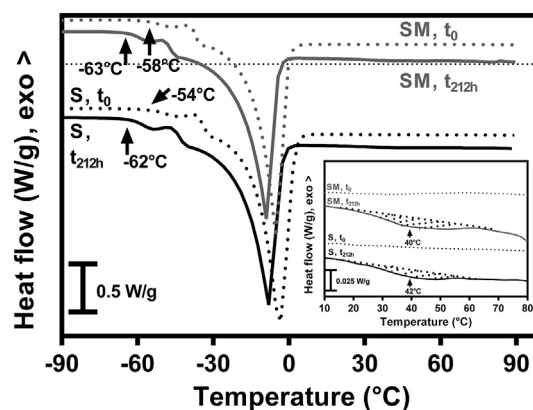


Fig. 5. Thermograms of 70% (w/v) sucrose systems with MgCl_2 (SM) and without MgCl_2 (S) at initial (solid lines) and after 212 h (dashed lines) of storage time at 70 °C. Glass transition temperature values (T_g) values are indicated. The inset highlights the small endothermic event (indicated by the dashed area) after ice fusion.

changes were observed. A reduction of T_g and T_m values occurred because of the MgCl_2 presence, as also reported for the disaccharide trehalose by Santagapita and Buera (2008). The change of T_m value is related to a cryoscopic effect and T_g decrease is related to the amount of non-frozen water retained in the matrix after freezing, connected with the magnitude of the sugar and salts influence on disrupting the tetrahedral hydrogen bond network of water and hence the reduction of freezable water. Ions that are large and have large polarizability such as Mg^{2+} impose more local ordering to the surrounding water molecules and strengthen the hydrogen bonds between them, enhancing the

tetrahedral coordinated hydrogen bond structure of water; these are known as structure makers or cosmotropic (Barreca et al., 2014).

A further reduced T_m value was observed in the thermally treated sucrose systems with or without $MgCl_2$, at 212 h, attributed to the presence of browning intermediates.

An endothermic event (peak around 40 °C) was observed in highly colored and concentrated samples after 212 h of thermal treatment at 70 °C. This endothermic event could be attributed to the disruption of the polymeric network of the melanoidins of high molecular weight generated in the Maillard reaction. This event was not observed at lower thermal treatment times, and in more diluted systems. It should be noticed that a deeper analysis of this transition may help to understand how the real conformation of the melanoidins is and how it changes with temperature. The reduction of T_g values after 212 h of thermal treatment was attributed to the hydrolysis of sucrose to glucose and fructose, both of lower T_g ' values (Saavedra-Leos et al., 2012) and to the water released during the Maillard reaction.

4. Conclusion

The addition of $MgCl_2$ provokes an increase of browning development in both liquid and dehydrated sucrose-glycine systems. This behavior was principally promoted by the increased sucrose hydrolysis due to the presence of $MgCl_2$, and to the higher reactivity of fructose in comparison to glucose.

$MgCl_2$ also provoked the change on fluorophores quantum yield, responsible of the fluctuations observed in the fluorescence development as a function of time. It is important to note that the presence of this salt did not affect the quantum yield in glucose and trehalose-glycine liquid systems, revealing that the composition of the systems is critical, since affects the reactivity of the involved sugar.

Besides, $MgCl_2$ affected the protons mobility in the media, which is reflected by the lower proton magnetic transversal relaxation times (T_2), which decreased between 6 and 14%, in the presence of $MgCl_2$. This indicates that the ions imposed a certain local order to water molecules in sucrose systems. Since water, as a final product, promotes an inhibitory effect on Maillard reaction, the acceleration of the browning reaction rate produced by $MgCl_2$ can be also associated to water-salt interactions, which reduce water availability in sucrose- $MgCl_2$ in comparison to sucrose systems.

Supramolecular effects of $MgCl_2$ were also observed, since T_g and T_m values decreased in the presence of this salt. The change of T_m value is related to a cryoscopic effect and T_g decrease is related to the amount of non-frozen water retained in the matrix after freezing, connected with the magnitude of the sugar and salts influence on disrupting the tetrahedral hydrogen bond network of water and hence the reduction of freezable water.

Therefore, molecular and supramolecular effects of $MgCl_2$ have been observed that change Maillard reaction kinetics. These results are of high technological interest to find strategies to inhibit or enhance the Maillard reaction rate for a particular application.

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Declarations of interest

None.

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