



A kinetic study on the effect of hyperbaric storage on the development of Maillard reaction in glucose-glycine model systems

Federico Basso^a, Lara Manzocco^{a,*}, Jorge A. Saraiva^b, Maria Cristina Nicoli^a

^a Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via Sondrio 2/A, 33100 Udine, Italy

^b LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

The effect of pressure (0.1, 15, 50 and 100 MPa) and temperature (43, 53, 63 °C) on the formation rate (k) of Maillard α -dicarbonyls (absorbance at 294) and melanoidins (absorbance at 420 nm), was studied during hyperbaric storage (HS) of glucose-glycine model solutions (pH 6).

While increasing storage temperature increased k values according to the Arrhenius equation ($E_a \sim 85$ kJ mol⁻¹), increasing pressure decreased reaction rates as predicted by the Eyring model ($V_a \sim 11$ mL mol⁻¹). Pressure did not affect reaction temperature sensitivity, indicating no significant mechanism changes under hyperbaric conditions. A combined model predicting the effect of concomitant changes of temperature and pressure on Maillard reaction rate was implemented and validated within and outside (20–25 °C, 20–200 MPa) its building range.

Results indicate HS to limit Maillard browning in food, with possible practical applications, and the potential to develop predictive models based on temperature-accelerated HS trials.

Industrial relevance: The capability of hyperbaric storage to impair Maillard reaction rate extends the scope of this multi-tasking technology to the prevention of color alterations due to non-enzymatic browning. The latter is expected to be of industrial relevance in the case of perishable foods affected by this phenomenon, such as thermally-treated milk. In these matrices, the technology could concomitantly guarantee microbiological safety, protein functionalization, and Maillard browning impairment.

1. Introduction

Hyperbaric storage (HS) is a novel, non-thermal food technology based on the application of hydrostatic pressure (Santos et al., 2021). From a technical perspective, HS is very similar to high hydrostatic pressure processing (HHP), as the steps involved in the application of both technologies are essentially the same (i.e., foods are packaged inside plastic pouches, inserted into pressure-holding steel vessels, and pressurized by pumping a liquid inside the latter) (Basso, Manzocco, & Nicoli, 2022). However, the technologies substantially differ in their scope. In particular, HHP is mainly applied as a non-thermal pasteurization technology, whereas HS is primarily intended as a sustainable alternative to refrigerated storage (Santos, Fidalgo, Pinto, et al., 2021). For this reason, while HHP relies on brief (5–30 min), intense pressurizations (400–800 MPa), HS involves the application of moderate pressure (up to 250 MPa) for times ranging between days to several months

(Basso, Manzocco, & Nicoli, 2022).

Over the last decade, HS has been increasingly investigated due to its low energetic cost when applied at room temperature (Bermejo-Prada, Colmant, Otero, & Guignon, 2017). Several studies have proven HS capable to: i) almost indefinitely guarantee the microbiological safety of perishable foods (e.g., meat, milk, egg white, fruit juices), ii) achieve pathogens inactivation levels on par with those of thermal pasteurization (i.e., 5–6 log units), iii) enhance the techno-functionality of food by protein structural modification, and iv) inhibit the catalytic activity of enzymes associated with food quality decay (Basso, Maifreni, Innocente, Manzocco, & Nicoli, 2022; Basso, Manzocco, Maifreni, & Nicoli, 2021; Bermejo-Prada, Segovia-Bravo, Guignon, & Otero, 2015; Duarte, Pinto, Gomes, Delgadillo, & Saraiva, 2022; Lemos, Ribeiro, Delgadillo, & Saraiva, 2020; Manzocco, Basso, & Nicoli, 2023; Pinto et al., 2017; Santos et al., 2021; Santos, Castro, Delgadillo, & Saraiva, 2020; Santos, Fidalgo, Pinto, et al., 2021). Although the body of evidence available

* Corresponding author.

E-mail addresses: federico.basso@uniud.it (F. Basso), lara.manzocco@uniud.it (L. Manzocco), jorgesaraiva@ua.pt (J.A. Saraiva), mariacristina.nicoli@uniud.it (M.C. Nicoli).

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about HS has been primarily focused on these aspects, a limited number of studies have also reported the effects of the technology on chemical phenomena occurring in food. In particular, according to the Le Chatelier principle and to the Transition State Theory (Evans & Polanyi, 1935; Eyring, 1935), HS (25–200 MPa, 18–25 °C, up to 25 days) was shown to boost the development of volume-reducing reactions, like lipid peroxidation in raw meat and fish, and oxidative color fading in watermelon and strawberry juice (Bermejo-Prada & Otero, 2016; Fidalgo, Lemos, Delgado, & Saraiva, 2018; Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014; Pinto et al., 2017; Santos, Matos, Casal, Delgado, & Saraiva, 2021). On the other hand, the production of volatile compounds, which is knowingly associated with a volume expansion, was found to be suppressed by HS (50–200 MPa, 18–25 °C, up to 60 days) in meat, strawberry juice and milk (Bermejo-Prada, Vega, Pérez-Mateos, & Otero, 2015; Duarte et al., 2022; Martínez-Monteagudo & Saldaña, 2014; Santos, Matos, Casal, et al., 2021). Besides this limited evidence, the comprehension of the effect of HS on the kinetics of chemical reactions is still scarcely understood. In particular, no information is available regarding the effect of this technology on the development of the Maillard reaction, despite its relevance in food storage contexts (van Boekel, 2001; van Boekel, 2008). To the best of our knowledge, the phenomenon was mentioned in the literature only to tentatively explain color changes occurring in raw skim milk and egg white during HS at room temperature (150–200 MPa, 6–28 days) (Basso et al., 2021; Basso, Maifreni, et al., 2022).

Maillard browning is a term designating a complex scheme of interdependent chemical reactions originating from the condensation of an amino compound (e.g., amino acids, proteins) with a molecule containing a carbonylic moiety (e.g., reducing sugars) (Martins, Jongen, & van Boekel, 2000). After several steps, the stable products of the Maillard reaction are comprised of organic acids, volatile compounds, α -dicarbonyls, and brown polymers (Martins & van Boekel, 2005a; van Boekel, 2001). Due to its complexity, the effect of compositional and environmental variables on Maillard browning has been primarily studied in model solutions containing amino acids and sugars (Guan, Wang, Yu, Yu, & Zhao, 2012; Hill, Ledward, & Ames, 1996; Martins & van Boekel, 2005a, 2005b; Shen, Tseng, & Wu, 2007; Yoshimura, Iijima, Watanabe, & Nakazawa, 1997). In this framework, the application of HHP (400–600 MPa) has been found to hamper the development of non-enzymatic browning due to the positive volume change associated with the phenomenon (Hill et al., 1996; Hill, Isaacs, Ledward, & Ames, 1999; Isaacs & Coulson, 1996; Martínez-Monteagudo & Saldaña, 2014). Based on its kinetic effect, the application of hydrostatic pressure was also shown to limit the formation of harmful Maillard-generated compounds such as acrylamide, furan and its derivatives, and 5-hydroxymethylfurfural in several matrices subjected to high-pressure/high-temperature thermal treatments (De Vleeschouwer, Van der Plancken, Van Loey, & Hendrickx, 2010; Palmers, Grauwet, Kebede, Hendrickx, & Van Loey, 2014; Sevenich, Rauh, Belkova, & Hajslova, 2020; Vervoort et al., 2012). Despite this evidence, it must be highlighted that the kinetics of the Maillard reaction under pressure could be influenced by many other factors, with particular reference to temperature (Lund & Ray, 2017). It is in fact well known that the rate of Maillard browning is exponentially dependent on temperature according to the Arrhenius equation (Arrhenius, 1901; Martins & van Boekel, 2005b). Nevertheless, to our knowledge, the combined effect of temperature and pressure on the kinetics of the Maillard reaction has never been investigated. In the HS context, this knowledge would be of utmost importance, since, due to the remarkably prolonged pressurization times applied for HS processes (e.g., weeks-months), even small changes in temperature and pressure could result in dramatical changes in Maillard browning.

To date, kinetic studies about the combined effect of temperature and pressure on the rate of chemical reactions are only available with reference to brief pressurizations (up to 900 MPa, 20–130 °C) and phenomena other than non-enzymatic browning (e.g., starch swelling and oxidative reactions) (Ahromrit, Ledward, & Niranjana, 2007; Huang,

Zhang, & Xiong, 2019; Verbeyst, Oey, van der Plancken, Hendrickx, & van Loey, 2010; Verbeyst, van Crombruggen, van der Plancken, Hendrickx, & van Loey, 2011). Since hydrostatic pressure and temperature can be regarded as independent variables (i.e., negligibly affecting each other), the prediction of their combined effect on the rates of these reactions was performed adopting a simple regression approach based on the combination of Arrhenius and Eyring equations. This modelling procedure was also shown to provide information regarding the combined effects of the variables at a mechanistic level, by estimating the influence of pressure and temperature on activation energy and volume, respectively (Ahromrit et al., 2007; Huang et al., 2019; Verbeyst et al., 2010, 2011). Although such an approach has never been applied to predict the rate of Maillard reaction, nor that of any other chemical or physical event occurring during HS, its potential feasibility for such scope is evident.

The aim of the present work was to study the combined effect of pressure and temperature on the kinetics of Maillard reaction in sugar-amino acid model solutions during hyperbaric storage. To this aim, glucose-glycine model systems with pH 6 were selected as representative of foods capable to support microbial growth (i.e., perishable), which are the most suitable ones for HS applications (Santos, Fidalgo, Pinto, et al., 2021). An experimental design comprising two phases was implemented. The first phase had the goal of studying the individual and combined effects of HS pressure and temperature on the kinetics of Maillard reaction. For this reason, samples were subjected to hyperbaric storage (0.1, 15, 50 and 100 MPa) for up to 9 days at 43, 53 and 63 °C. The choice of an experimental plan based on 4 pressures and 3 temperatures was selected in agreement with the literature (Verbeyst et al., 2010). At increasing time during HS, absorbances at 294 and 420 nm were measured as indicators of the formation of Maillard α -dicarbonyls and melanoidins, respectively. Although resulting from the superposition of several compounds in solution, absorbance at these wavelengths is generally recognized as the fastest and most appropriate indicator of the development of Maillard intermediates and late brown compounds (Benjakul, Lertittikul, & Bauer, 2005; Lertittikul, Benjakul, & Tanaka, 2007). The dependence of first-order reaction rates on temperature and pressure was described using the Arrhenius and the Eyring equations, respectively. Then, a combined model based on these equations was fitted to the data to describe the effect of concomitant changes in temperature and pressure.

The second phase of the experiment had two goals: validating the kinetic information obtained by fitting data obtained at 43, 53 and 63 °C, and demonstrating the capability of the combined Arrhenius-Eyring model in predicting Maillard browning rate during HS. To this aim, the accuracy of the model was tested against experimental data obtained at pressure and temperature conditions external to the range used for its development, and that are typically employed for food HS (20–200 MPa, 20–25 °C, up to 68 days).

2. Materials and methods

2.1. Samples preparation

Glucose-glycine model solutions were obtained by dissolving D-glucose monohydrate (1.71 M, Labchem, Lisbon, Portugal) and glycine (2.05 M, Biochem Chemopharma, Cosne-Cours-sur-Loire, France) in MilliQ water. Reactants concentration was selected based on those reported by Nicoli, Elizande, Pitotti, and Lerici (1991). Complete solubilization was ensured by vigorous stirring at room temperature for up to 1 h. Solutions were divided into two aliquots whose pH was adjusted at 6 by addition of 1 M NaOH or HCl. Aliquots of 1 mL of each solution were then introduced in 5 × 3 cm polyamide/polyethylene plastic pouches and heat-sealed with minimum headspace using a SK heat-sealing bar (Albipack, Águeda, Portugal).

2.2. Hyperbaric storage

Depending on the temperature applied during hyperbaric storage, different working units were used, as described below. In all cases, a 60:40 (v:v) mixture of propylene glycol (96% propylene glycol and 4% of water containing mold inhibitors, Dowcal N fluid, Dow Chemical) and tap water was used as pressure-mediating fluid in the hyperbaric working unit.

2.2.1. Hyperbaric storage for model building

A U33 high-pressure system (Unipress Equipment Division, Institute of High Pressure Physics, Warsaw, Poland) equipped with a 0.1 L jacket-thermostated pressure vessel was used to store samples for times comprised between 30 and 216 h (1.25 and 9 days, respectively) at 0.1, 15, 50 and 100 MPa, at 43, 53 and 63 °C. In this case, the 60:40 (v:v) propylene glycol:water mixture employed as pressurizing fluid was also used to control the temperature in the vessel thermostating jacket. To this aim, the fluid was heated and circulated in the vessel jacket by a FA 90 thermostatic bath (FALC Instruments, Treviglio, Italy).

2.2.2. Hyperbaric storage for model validation

A pilot-scale hyperbaric storage unit (Comer S.r.l., Bologna, Italy) was used to store samples at 200 MPa for up to 1632 h (68 days). The hyperbaric unit consisted of a 2 L steel vessel (Hystat, Slaithwaite, Huddersfield, UK) pressurized by a pneumatic multiplier (Haskel International, Burbank, CA, USA). The pressurizing fluid temperature was maintained at 20 ± 1 °C by placing the working unit in a thermostated room.

For samples storage at 20, 30 and 40 MPa for up to 456 h (19 days) at 25 ± 1 °C, an SFP FPG13900 hyperbaric storage experimental equipment (Stansted Fluid Power, Stansted, UK) with three independent 0.4 L steel vessels was used. Pressure was generated inside the vessels using the integrated pneumatic pressure multiplier of the equipment coupled with a DPC10QTC (capacity = 9.4 L, maximum pressure = 12 bar, engine power = 1.5 cv) air compressor (DeWalt, Baltimore (MD), U.S.A.). Storage temperature was maintained constant by placing the equipment in a thermostated room.

2.3. Sample absorbance

At increasing time during hyperbaric storage, the working units were depressurized and samples were collected from the pressure vessels. Samples stored at 43, 53 and 63 °C were quickly cooled with fresh running tap water and kept at 4 °C, in order to avoid the reaction to proceed under uncontrolled conditions until absorbances were read. Samples absorbance at 294 and 420 nm was measured at 25 °C in UV-grade microplates using a Multiskan Go microplate spectrophotometer (Thermo Scientific, Waltham, EUA). Samples showing absorbance values higher than 0.7 were appropriately diluted in MilliQ water to reach absorbance values in scale.

2.4. Kinetic modelling

Kinetic modelling was performed by adapting the approach previously described by Verbeyst et al. (2010). The increase in sample absorbance at 294 and 420 nm as a function of storage time (t , h) at increasing pressure (0.1–200 MPa) and temperatures (20–63 °C) was fitted using a first-order model (Eq. (1)).

$$\ln \frac{A}{A_0} = k^* t \quad (1)$$

where k is the kinetic rate constant (h^{-1}).

The dependence of k on storage temperature (T , K) at isobaric conditions was modelled in the 43–63 °C temperature range using the reparametrized Arrhenius equation (Eq. (2)).

$$\ln k = -\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) + \ln k_{T_{ref}} \quad (2)$$

where E_a is the activation energy (kJ mol^{-1}), R is the universal gas constant ($8.314 \cdot 10^{-3} \text{ kJ K}^{-1} \text{ mol}^{-1}$), T_{ref} is the reference temperature, which was selected as the midpoint of the temperature experimental range (53 °C, i.e. 326 K), and $k_{T_{ref}}$ is the frequency factor (i.e., kinetic rate at T_{ref}).

The dependence of k on storage pressure (P , MPa) at isothermal conditions was modelled in the 0.1–100 MPa pressure range using the reparametrized Eyring equation (Eq. (3)).

$$\ln k = -\frac{V_a}{R^* T} (P - P_{ref}) + \ln k_{P_{ref}} \quad (3)$$

where V_a is the activation volume (mL mol^{-1}) at the temperature T , P_{ref} is the reference pressure, which was selected as the midpoint of the pressure experimental range (50 MPa), and $k_{P_{ref}}$ is the kinetic rate at P_{ref} .

The combined temperature–pressure dependence of k was further investigated using a combined Arrhenius-Eyring model (Eq. (4)), adapted from Verbeyst et al. (2010).

$$\ln k = \left[\frac{-E_a^*}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] + \left[\frac{-V_a^*}{R^* T} (P - P_{ref}) \right] + \ln k_{ref}^* \quad (4)$$

where E_a^* , V_a^* , and k_{ref}^* represent the activation energy, activation volume and frequency factor of the reaction, respectively, estimated using Eq. (4).

2.5. Model validation

The capability of Eq. (4) to predict the development of the absorbance at 294 and 420 nm of glucose-glycine solutions subjected to hyperbaric storage was validated on external data. The latter were obtained by storing analogous samples at 20, 30 and 40 MPa at 25 °C (i.e., outside the experimental temperature range but within the experimental pressure range), and at 200 MPa at 20 °C (i.e., completely outside the experimental range). These conditions were selected among those typically applied during food hyperbaric storage. Model accuracy was evaluated by linear correlation between the predicted absorbance values and the ones that were experimentally obtained under the storage conditions used for validation.

2.6. Statistical analysis

Absorbance data were obtained by at least three determinations per sample. Measurements accuracy was evaluated based on the variation coefficient (CV). Data with $CV > 5\%$ were discarded from the dataset. Kinetic modelling based on the first-order model, on the reparametrized Arrhenius and Eyring equations, and on the combined Arrhenius-Eyring model were performed using Origin Pro 2021 (OriginLab, Northampton, MA, USA). Calculated parameters were expressed as estimate \pm standard error and goodness of fit was assessed based on the determination coefficient (R^2) and root-mean square error (RMSE). Spearman correlation test for model validation was performed using R (v. 4.2.2) for Windows (The R foundation for statistical computing). Goodness of correlation was evaluated based on the coefficient of correlation (r) and the p -value.

3. Results and discussion

3.1. Maillard reaction during hyperbaric storage

Hyperbaric storage in a wide range of pressure (0.1, 15, 50 and 100

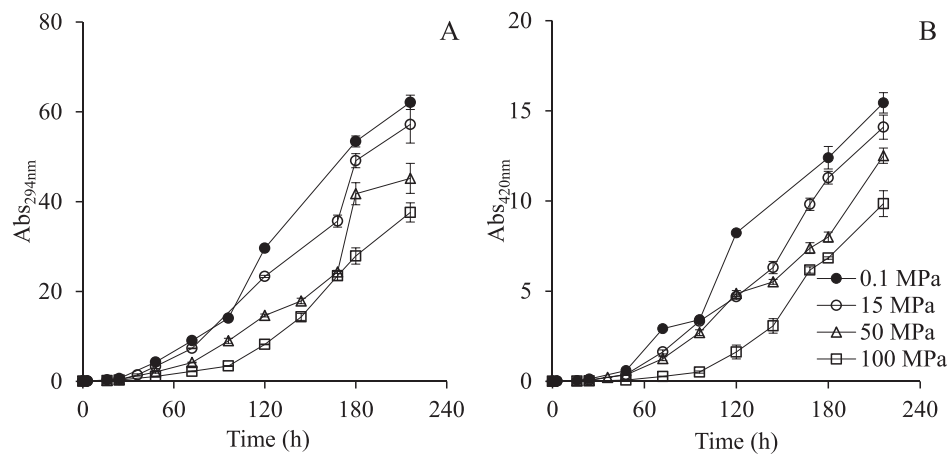


Fig. 1. Absorbance at 294 (A) and 420 (B) nm of glucose-glycine solution at pH 6 stored at 43 °C at increasing pressure.

MPa) and temperature (43, 53 and 63 °C) conditions was applied to the glucose-glycine solution. At increasing time during storage, sample absorbance at 294 and 420 nm was registered to monitor the development of Maillard α -dicarbonyls and melanoidins, respectively (Nicoli et al., 1991). As an example, Fig. 1 shows the increase in samples absorbance at 294 (A) and 420 (B) nm during storage at 43 °C. Data relevant to samples stored at 53 and 63 °C are shown in Fig. S1.

As clearly visible from Fig. 1 and S1, the absorbance at 294 and 420 nm of all samples readily increased according to a biphasic trend resembling a sigmoidal curve, which is frequently observed for complex autocatalytic reactions like Maillard browning (Quintas, Brandão, & Silva, 2007). When storage pressure was increased, a reduction of the absorbance values at 294 and 420 nm was observed at all storage times, indicating that HS hampered Maillard browning. These results are in agreement with abundant literature reporting the capability of HHP (400–600 MPa) to impair color development deriving from non-enzymatic browning phenomena (Martinez-Monteaugudo & Saldana, 2014).

3.2. Kinetic modelling of Maillard reaction during hyperbaric storage

3.2.1. Kinetic rates

Based on the hampering effect of pressure on the Maillard reaction in the glucose-glycine solution, data were modelled to estimate the rate of the increase in absorbance at 294 and 420 nm in the tested experimental conditions. The first-order kinetic equation (Eq. (1)) was selected for this scope based on its reported efficacy in fitting data relevant to non-

enzymatic browning reactions (Ajandouz & Puigserver, 1999; Baisier & Labuza, 1992; Tamaoka, Itoh, & Hayashi, 1991). This equation also provided the further advantage of estimating Maillard reaction rate in the exponential phase of the phenomenon, during which α -dicarbonyls and melanoidins formation is fastest (Quintas et al., 2007). To this aim, samples absorbance curves were considered up to the storage times that allowed to optimally fit (*i.e.*, highest R^2 and lowest RMSE) data with Eq. (1). Results are shown in Fig. 2.

It can be noted that, in agreement with the literature, the first-order model adequately fitted experimental data (Fig. 2) (van Boekel, 2001). This equation was thus used to estimate the reaction rate (k , $R^2 > 0.96$; RMSE < 0.34) in samples stored at increasing temperature (43–63 °C) and pressure (0.1–100 MPa) conditions (Table 1).

In accordance with the literature, the increase in storage temperature caused an increase in reaction rate, whereas an opposite effect was obtained by increasing pressure at constant temperature (Table 1) (Martinez-Monteaugudo & Saldana, 2014; van Boekel, 2001). The boosting effect of temperature on the kinetics of Maillard browning is known to be well described by the Arrhenius equation (Martins & van Boekel, 2005b). On the other hand, hyperbaric conditions are known for hampering non-enzymatic browning phenomena based on two independent mechanisms. The first and most relevant one is related to the compression of the reaction environment, which impairs any chemical event associated with a volumetric expansion (Bristow & Isaacs, 1999; Isaacs & Coulson, 1996; Tamaoka et al., 1991). To this regard, evidence relevant to glucose-tryptophan and glucose-lysine model systems suggests that the formation and the decomposition of Amadori compounds

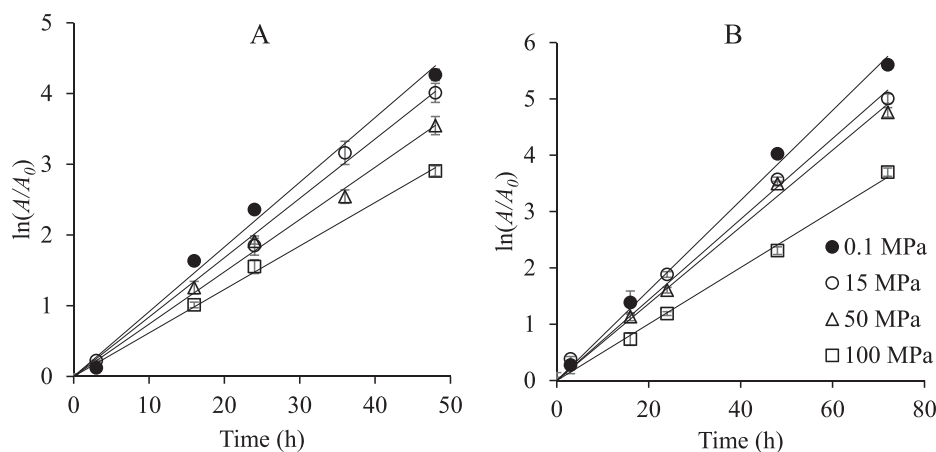


Fig. 2. Absorbance at 294 (A) and 420 (B) nm, expressed as normalized logarithmic ratios, of glucose-glycine solution at pH 6 stored at 43 °C at increasing pressure. Regression lines (—) obtained according to first-order kinetic model are also shown.

Table 1

First-order kinetic rates (k) of the formation of Maillard α -dicarbonyls (294 nm) and melanoidins (420 nm) in glucose-glycine solutions stored at increasing temperature and pressure.

Reaction step	Temperature (°C)	Pressure (MPa)	$k \pm SE$ (h ⁻¹)	
Formation of Maillard α -dicarbonyls (294 nm)	43	0.1	0.092 \pm 0.001	
		15	0.084 \pm 0.001	
		50	0.074 \pm 0.001	
		100	0.061 \pm 0.001	
		53	0.1	0.261 \pm 0.003
	53	15	0.229 \pm 0.004	
		50	0.191 \pm 0.001	
		100	0.177 \pm 0.002	
		63	0.1	0.741 \pm 0.011
		15	0.632 \pm 0.013	
	Formation of Maillard melanoidins (420 nm)	43	50	0.580 \pm 0.014
			100	0.458 \pm 0.008
			0.1	0.080 \pm 0.001
			15	0.071 \pm 0.001
			50	0.068 \pm 0.001
53		100	0.051 \pm 0.001	
		0.1	0.217 \pm 0.003	
		15	0.194 \pm 0.002	
		50	0.168 \pm 0.003	
		100	0.151 \pm 0.003	
63	0.1	0.606 \pm 0.012		
	15	0.546 \pm 0.016		
	50	0.431 \pm 0.008		
	100	0.369 \pm 0.008		
	0.1	0.008		

would be the reaction steps mostly affected by pressurization (80–400 MPa, up to 5 h) (Isaacs & Coulson, 1996; Moreno, Molina, Olano, & López-Fandiño, 2003). Secondly, pressure-promoted dissociation of weak acid moieties (e.g., amino acids carboxylic group) has been suggested to cause a slight local pH decrease (Neuman, Kauzmann, & Zipp, 1973). As a consequence, early-stage sugar isomerization would become slower due to the decreased reactivity of the protonated amino acids, ultimately affecting the rate of all the subsequent Maillard reaction steps (Hill et al., 1996).

3.2.2. Dependence of kinetic rates on temperature and pressure

The dependence of Maillard reaction rate on storage temperature and pressure was modelled according to Arrhenius (Eq. (2)) and Eyring (Eq. (3)) equations, respectively. The Arrhenius model well-fitted experimental data ($R^2 > 0.96$, RMSE < 0.09), allowing to accurately estimate E_a and $k_{T_{ref}}$ at each storage pressure (Table 2 A). Arrhenius and Eyring plots are shown in Fig. S2.

Activation energy values were coherent with those reported by

Table 2

Activation energy (E_a) and Arrhenius frequency factor ($k_{T_{ref}}$) at isobaric conditions (A), and activation volume (V_a) and Eyring frequency factor ($k_{P_{ref}}$) at isothermal conditions (B), relevant to kinetic rates of the increase in absorbance at 294 and 420 nm in glucose-glycine solutions stored at increasing temperature (43–53 °C) and pressure (0.1–100 MPa).

A				
Wavelength (nm)	Pressure (MPa)	$E_a \pm SE$ (kJ mol ⁻¹)	$k_{T_{ref}} \pm SE$ (h ⁻¹)	
294	0.1	92.30 \pm 1.60	0.266 \pm 0.004	
	15	89.32 \pm 1.73	0.234 \pm 0.004	
	50	90.85 \pm 5.68	0.206 \pm 0.011	
	100	88.89 \pm 1.20	0.174 \pm 0.002	
	420	0.1	89.59 \pm 2.18	0.223 \pm 0.004
420	15	89.99 \pm 2.48	0.200 \pm 0.005	
	50	81.44 \pm 2.38	0.174 \pm 0.004	
	100	87.94 \pm 3.51	0.144 \pm 0.005	
	B			
	Wavelength (nm)	Temperature (°C)	$V_a \pm SE$ (mL mol ⁻¹)	$k_{P_{ref}} \pm SE$ (h ⁻¹)
294	43	10.30 \pm 0.58	0.074 \pm 0.001	
	53	10.17 \pm 2.45	0.205 \pm 0.007	
	63	12.40 \pm 1.75	0.571 \pm 0.014	
420	43	11.20 \pm 1.94	0.064 \pm 0.002	
	53	9.46 \pm 1.62	0.175 \pm 0.004	
	63	13.89 \pm 1.93	0.459 \pm 0.013	

several authors for the development of Maillard compounds in near-neutral, glucose-glycine model systems at 20–60 °C (i.e., 80–96 kJ mol⁻¹) (van Boekel, 2001). The temperature sensitivity of both Maillard reaction indicators (absorbance at 294 and 420 nm) was only slightly affected by pressure, as shown by very similar E_a values (Table 2 A). These results are in agreement with circumstantial evidence reporting high pressure (600 MPa) not to affect the activation energy of Maillard browning in glucose-lysine solutions maintained at 40–60 °C for up to 22 h after pressure processing (Hill et al., 1996). Differently from E_a , the application of hyperbaric conditions caused a significant decrease in the Arrhenius frequency factor ($k_{T_{ref}}$). From a kinetic point of view, this result suggests that the application of hyperbaric conditions decreased the overall amount of compounds formed during the development of the reaction, without substantially modifying its pathway (Hill et al., 1996; Piskulich, Mesele, & Thompson, 2019). The dependence of k on pressure was thus modelled using the Eyring equation. Similar to the Arrhenius model, good fitting accuracy ($R^2 > 0.89$, RMSE < 0.07) was obtained, allowing a precise estimation of V_a and $k_{P_{ref}}$ at each storage temperature (Table 2B). It is interesting to note how a clear parallelism can be found in the physical meaning of Arrhenius and Eyring parameters. In particular, while the Arrhenius E_a quantifies the sensitivity of a phenomenon kinetics on temperature at a given pressure, the activation volume (V_a , mL mol⁻¹) of the Eyring equation defines the dependence of a phenomenon on pressure at a given temperature (Zamora & Leyva, 2021). To this regard, V_a can be intended as a mathematical representation of the volume change associated with the phenomenon (Evans & Polanyi, 1935). Also, the Arrhenius parameter $k_{T_{ref}}$ has a physical meaning analogous to the one of the Eyring parameter $k_{P_{ref}}$. In particular, the former identifies the number of effective collisions among reactants at the selected reference temperature T_{ref} (326.15 K, i.e. 53 °C), whereas $k_{P_{ref}}$ represents the frequency of collisions between reactant molecules at the reference pressure (50 MPa) (Piskulich et al., 2019). In accordance with the literature about the Maillard reaction in sugar-amino acid model systems under pressure, the V_a of both α -dicarbonyls and melanoidins formation was 11 \pm 2 mL mol⁻¹ (Table 2B) (Hill et al., 1996; Hill et al., 1999; Isaacs & Coulson, 1996; Moreno et al., 2003). Such order of

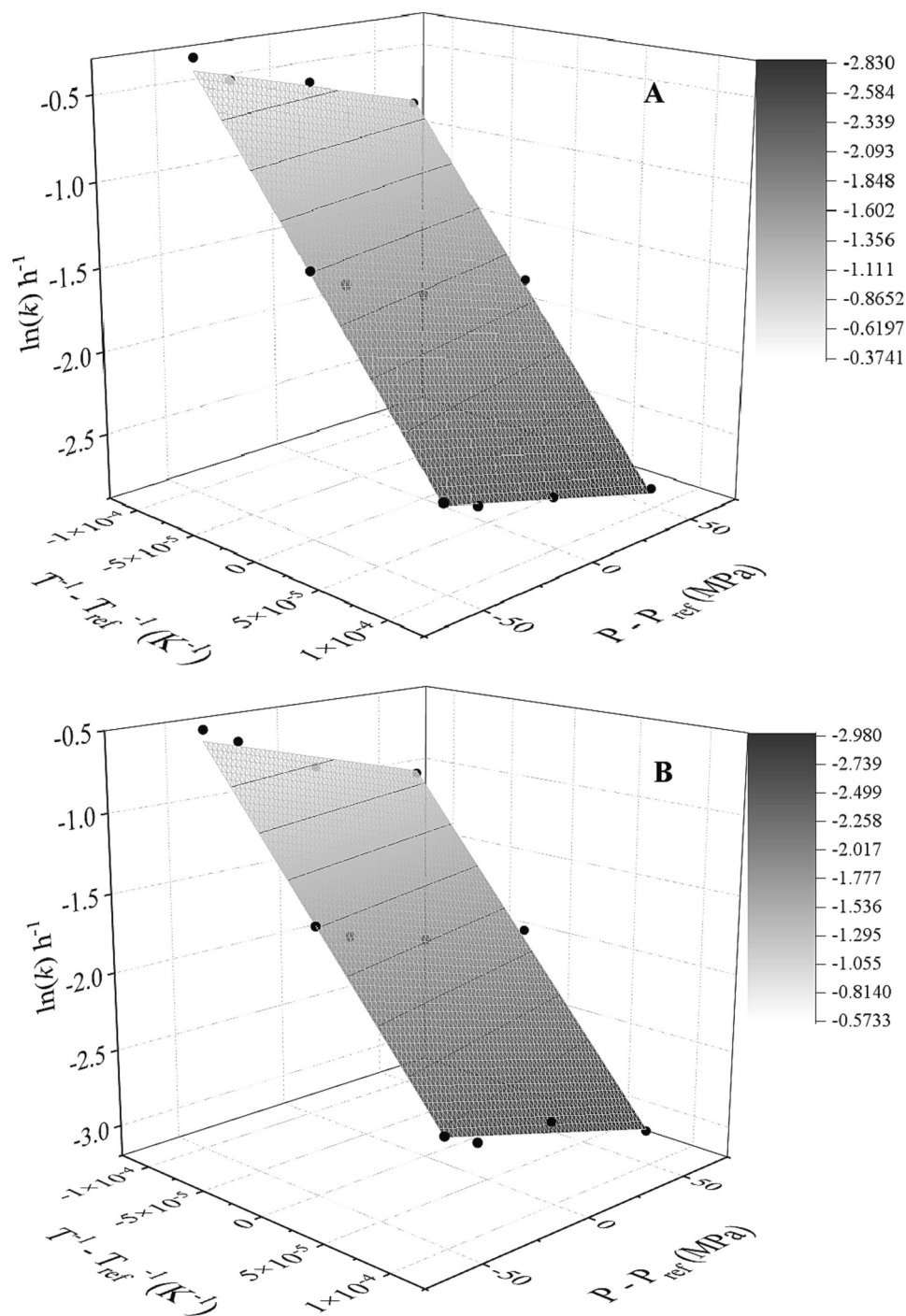


Fig. 3. Reaction rates of the increase in absorbance at 294 (A) and 420 (B) nm in glucose-glycine solutions as a function of storage temperature (43–53 °C) and pressure (0.1–100 MPa). Fitting surfaces relevant to the combined Arrhenius-Eyring model are also shown.

magnitude is typical of chemical reactions that are moderately hampered by pressure (Martinez-Monteagudo & Saldaña, 2014). As indicated by data reported in Table 2B, the V_a of the increase in absorbance at both 294 and 420 nm was only slightly affected by temperature. This indicates that the influence of temperature on the pressure sensitivity of the reaction would be negligible in the studied conditions range (43–63 °C). In other words, the efficacy of HS in limiting the development of Maillard browning would be scarcely affected by temperature changes. On the other hand, and in agreement with the Arrhenius dependence of Maillard k values (Table 2 A), $k_{p,ref}$ significantly increased with increasing storage temperature (Table 2B).

It is also worth highlighting that the V_a of the formation of α -dicarbonyls was very similar to that of melanoidins (Table 2B). From a mechanistic point of view, this could be due to the hampering effect of pressure being exerted on very early steps of the Maillard reaction (e.g., Amadori rearrangement) (Moreno et al., 2003). In other words, under pressure, these early events would become limiting reactions in the Maillard pathway, consequently hampering the development of all the subsequent reaction steps to a similar extent.

Based on the accurate fitting provided by the Arrhenius equation at isobaric conditions and by the Eyring equation at isothermal conditions (Table 2, Fig. S2), it is likely that the effect of concomitant changes in

Table 3

E_a^* , V_a^* and k_{ref}^* estimated using the combined Arrhenius-Eyring model relevant to the increase in absorbance at 294 and 420 nm in glucose-glycine solutions stored at increasing temperature (43–53 °C) and pressure (0.1–100 MPa).

Wavelength (nm)	$E_a^* \pm SE$ (kJ mol ⁻¹)	$V_a^* \pm SE$ (mL mol ⁻¹)	$k_{ref}^* \pm SE$ (h ⁻¹)
294	90.44 ± 1.52	10.91 ± 0.99	0.201 ± 0.003
420	87.34 ± 1.64	11.46 ± 1.07	0.176 ± 0.003

storage temperature and pressure on the reaction rate could be predicted using a single equation. To test this hypothesis, a combined model based on the Arrhenius and Eyring equation (Eq. (4)) was fitted to the data. Results relevant to this elaboration are shown in Fig. 3.

The combined model fitted experimental data with very high accuracy ($R^2 > 0.99$; RMSE < 0.05). Furthermore, as visible from Table 3, E_a^* and V_a^* values estimated using the combined Arrhenius-Eyring model were very similar to E_a and V_a obtained by separately fitting Arrhenius and Eyring equations at isobaric and isothermal conditions data (Table 2).

3.3. Model validation and prediction of reaction rate

The reliability of the combined Arrhenius-Eyring model in predicting the formation rate of Maillard dicarbonyls and melanoidins under conditions typical of hyperbaric storage was finally assessed. External validation was performed using data obtained from samples stored at temperature and pressure conditions other than those used for model development. In particular, samples were stored for up to 19 days at 25 °C, at 20, 30 and 40 MPa, and for up to 68 days at 20 °C, at 200 MPa. Absorbance values predicted by using Eq. (4) were thus compared to the experimental data by linear correlation (Fig. 4). Despite the compact experimental design, comprising only 3 temperatures and 4 pressures, the combined Arrhenius-Eyring model predicted the formation of both Maillard intermediates and melanoidins in glucose-glycine systems in all the conditions considered for validation. In fact, as visible from Fig. 4, very good correlations were found for data relevant to the formation of both Maillard products ($r > 0.94$; $p < 0.005$). It is not excluded that model accuracy could be improved by expanding the experimental plan for model building, through the addition of further temperature and pressure conditions.

The applicability of the classical models in describing the kinetic effect of HS in food has been already demonstrated for enzymatic food-spoiling events. In particular, Manzocco et al. (2023) and Bernejo-Prada, Segovia-Bravo, et al. (2015) have demonstrated that the effect of HS pressure (0.1–200 MPa) at 20 °C on polyphenoloxidase inactivation

and pectin-methylesterase activation can be accurately described using the Bigelow and the Eyring models, respectively.

4. Conclusions

This work studied for the first time the development of Maillard intermediates (α -dicarbonyls) and melanoidins during hyperbaric storage of a glucose-glycine model solution. The rate of the reaction increased with storage temperature and decreased with storage pressure. The obtained data were then used to develop a model based on the Arrhenius and Eyring equations, which was able to predict the rate of Maillard reaction at temperature and pressure conditions outside its building range. The model also provided information about the influence of temperature and pressure on temperature and pressure sensitivity, as well as on frequency factors of the reaction under HS. Based on these results, this model could likely be applied to predict the development of Maillard reaction in food submitted to hyperbaric storage.

The obtained results suggest that the applicability of the employed modelling approach could extend even further. In particular, it could represent the foundation for the development of experimental protocols involving the use of high temperature to perform accelerated HS trials in real foods. This would actually allow to make kinetic assays under HS significantly less time-consuming, and could represent a very useful tool for future investigations about this technology. However, it must be noted that the structural and compositional complexity of real food matrices will probably be a critical source of variability, and could be potentially capable to induce deviations from both the Arrhenius and Eyring equations predicted data. To tackle this issue, the accurate selection of an adequate experimental domain for model building will be crucial. In particular, a number of temperature and pressure conditions higher than that used in the present work on simple solutions should be included in the experimental design.

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CRediT authorship contribution statement

Federico Basso: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lara Manzocco:** Writing – review & editing, Validation, Resources, Methodology, Data curation, Conceptualization. **Jorge A.**

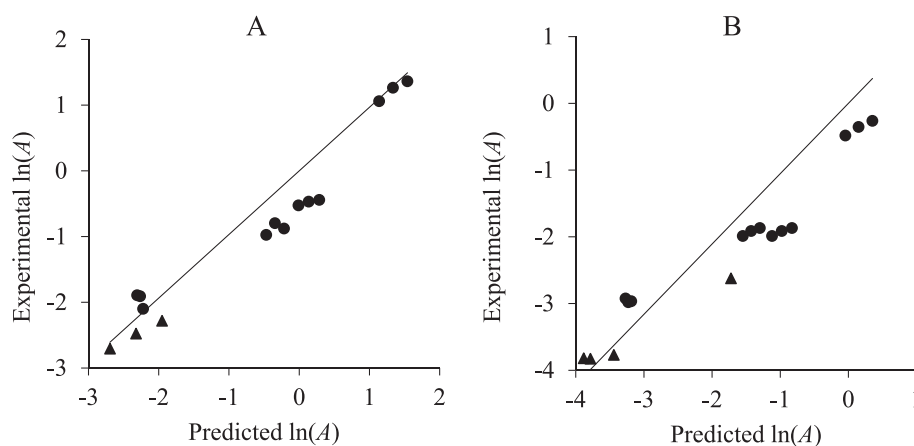


Fig. 4. Experimental and predicted (Eq. (4)) logarithmic absorbance values at 294 (A) and 420 (B) nm of glucose-glycine solutions with pH 6 stored for up to 68 days at 200 MPa and 20 °C (▲), and at 20, 30 and 40 MPa at 25 °C (●).

Saraiva: Conceptualization, Validation, Resources, Writing – review & editing. **Maria Cristina Nicoli:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ifset.2024.103603>.

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