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PII: S0308-8146(97)00176-3

Food Chemistry, Vol. 62, No. 3, pp. 309–313, 1998

Printed in Great Britain
0308-8146/98 $19.00+0.00

Reducing sugars effect on available lysine loss of casein by moderate heat treatment

Gabriela B. Naranjo,* Laura S. Malec & María S. Vigo

Departamento de Química Orgánica, Area Bromatología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428-Buenos Aires, Argentina

(Received 3 February 1997; revised version received and accepted 2 July 1997)

In order to investigate the effect of various reducing sugars on the available lysine loss by Maillard reaction, four model systems were prepared by mixing casein with glucose, fructose, lactose or maltose, followed by storage at 37°C, 50°C and 60°C. The available lysine contents were monitored periodically. Highest and lowest reaction rates were observed in the model systems containing glucose and fructose, respectively, at the three temperatures. The two disaccharides behaved very similarly, with reaction rates between those of the monosaccharides studied. The activation energies of glucose, lactose and maltose systems were similar (116–132 kJ mol^{-1}), while that of fructose was somewhat higher (166 kJ mol^{-1}). This difference was supposed to be in part due to the different mechanism of tautomerization of fructose, highly dependent on temperature. Therefore, though the potential nutritional damage at moderate temperatures is lower when fructose is used instead of other reducing sugars, its higher activation energy can reverse the effect at higher temperatures. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The most important nutritional effect of the Maillard reaction during processing and storage of foods is the reduction of protein quality. This reaction between free amino groups and reducing sugars leads to the biological unavailability of amino acids by modification of their side chains or destruction of their carbon skeleton, with an overall reduction in protein digestibility (Hurrell, 1990; Mauron, 1990).

When heating conditions are not extremely severe, the Maillard reaction stops at early stages. This may not cause browning but can seriously reduce the nutritional value, lysine being the most affected amino acid because of the blockage of its free-amino group (Erbersdobler, 1989; Hurrell, 1990).

Milk proteins are an excellent source of lysine and other essential amino acids, with casein comprising 82% of them. However, milk products are prone to lysine damage, particularly through its reaction with lactose. Other sugars can react with milk proteins as well (e.g. in infant formulas, lactose-hydrolyzed milk, regional products such as 'dulce de leche', mixtures with cereals or formulated foods).

Browning rate is significantly influenced by the type of reducing sugar involved in the reaction. Some investigators reported the following order of reactivity: aldopentoses > aldohexoses > ketohexoses > disaccharides (Spark, 1969; Mauron, 1981). Nevertheless, results are often difficult to compare because of the great diversity of conditions of treatment and indicators of the degree of reaction used.

For a better understanding of the differences in reactivity of sugars it is necessary to take note that, in most chemical and biochemical reducing sugar reactions, the major pathway involves only one of its tautomeric forms, usually the acyclic form (free aldehyde or ketone). This is considered the central intermediate in the interconversion of the cyclic anomers of a sugar and is generally present in a very low proportion at equilibrium (less than 1%) (Pigman and Isbell, 1968; Cockman *et al.*, 1987; Yaylayan *et al.*, 1993). If the reactive form reacts faster than it is generated from the other forms, the rate of tautomeric interconversion dominates the reaction rate (Angyal, 1984). Thus, in the Maillard reaction, the concentration of open chain form might be a crucial factor in determining the rate of glycation if the interconversion rate is slower than the reaction rate (Yaylayan *et al.*, 1993).

Some authors tried to find a simple association between both rates to predict the behaviour of different

*To whom correspondence should be addressed.

sugars in the initial steps of the Maillard reaction. In some studies a log/log correlation was found between the amino-carbonyl condensation rate and the extent to which the sugars exist in the acyclic or open form, considering aldoses and ketoses separately (Bunn and Higgins, 1981; Labuza and Baisier, 1992).

Wolf *et al.* (1982) examined the influence of the mutarotation constants of some sugars and reported a linear relationship between this parameter and the lysine loss rate coefficients of xylose, glucose, lactose and maltose.

Most of the results previously reported on available lysine loss in casein-reducing sugar systems were obtained using glucose as the only reacting carbohydrate (Lea and Hannan, 1949; Warmbier *et al.*, 1976; Warren and Labuza, 1977; Smith and Friedman, 1984) and only few using other sugars such as lactose (Keyes and Hegarty, 1979; Morales *et al.*, 1995). Lewis and Lea (1950) reported the reaction rates of several sugars and sugar derivatives with casein at 25°C. However, only in a few studies were the nutritional consequences of the Maillard reaction of different sugars with proteins considered.

This paper compares the effect of several sugars commonly used in the food industry on the kinetics of available lysine loss of casein under moderate heat treatment.

MATERIALS AND METHODS

Samples preparation

Four model systems were prepared. They were composed of purified casein and one of the following sugars: fructose, glucose, lactose and maltose. The initial sugar/available lysine molar ratio was 3:1. Potassium sorbate was added as an antimicrobial agent.

The dry components were blended and then slurried with pH 6.5 phosphate buffer solution. The slurried mixtures were quick-frozen and freeze-dried. The samples were equilibrated for five days at 4°C in evacuated desiccators over a saturated $Mg(NO_3)_2$ solution ($a_w = 0.52$). Each system was sealed in glass flasks and stored at 37°C, 50°C and 60°C. A pair of duplicate flasks was periodically removed and held at −18°C until analysis.

Analytical methods

Total nitrogen was determined in duplicate by the Kjeldahl method using a Büchi assembly of a digestor mod.430 and a nitrogen distillation unit mod.320.

Available lysine was measured by the spectrophotometric o-phthaldialdehyde (OPA) method modified by Vigo *et al.* (1992) to be used in milk products and related systems. Samples were dissolved in 2% sodium dodecylsulfate solution and the absorbance was measured at 340 nm with a Hewlett Packard spectrophotometer mod.HP 8451. Six replicate measurements of each sample were done. The available lysine content was obtained from a standard curve prepared with purified casein dissolved in pH 9.0 sodium tetraborate buffer solution in the range 1.0–10.0 mg ml^{-1}.

The possible interference of the free amino groups of amino acids, small peptides and amines was checked in the supernatant of samples dissolved in pH 9.0 sodium tetraborate buffer solution after treatment with 10% trichloroacetic acid solution (Goodno *et al.*, 1981), and was always negligible.

The water activity was checked with an electric hygrometer Vaisala Humicap with sensor modified by Driesen and Kern.

Statistical analysis

The standard curve data were analyzed by linear regression according to the method of least squares ($r^2 = 0.9995$). The analytical values were submitted to analysis of variance (ANOVA). Zero and first order kinetic rate constants were calculated from the average value of replicate measurements of each data point. Activation energies were estimated by the point by point analysis suggested by Labuza (1984).

RESULTS AND DISCUSSION

Available lysine losses of casein in the four model systems at 37°C, 50°C and 60°C are shown in Figs 1–3, respectively. In all cases the decrease in available lysine correlated well with a first order kinetics. So the regression curves (least squares criteria) were plotted on a semi-log scale. This reaction order agrees with that indicated in a great number of studies performed at high (Wolf *et al.*, 1977, 1978) and moderate temperatures (Warmbier *et al.*, 1976; Warren and Labuza, 1977; Labuza and Saltmarch, 1981; Labuza and Massaro, 1990; Baisier and Labuza, 1992).

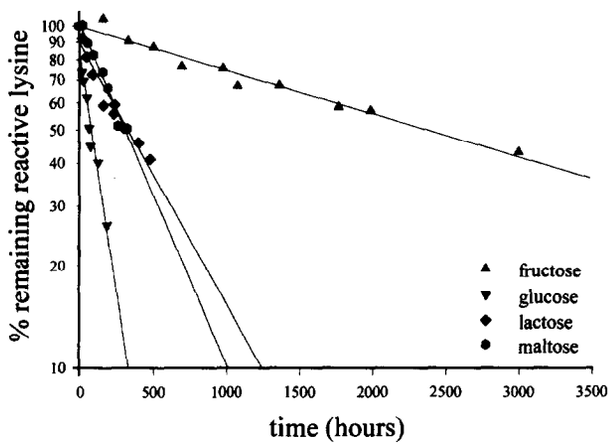

Fig. 1. Available lysine losses in model systems of casein with glucose, fructose, lactose and maltose during heating at 37°C.

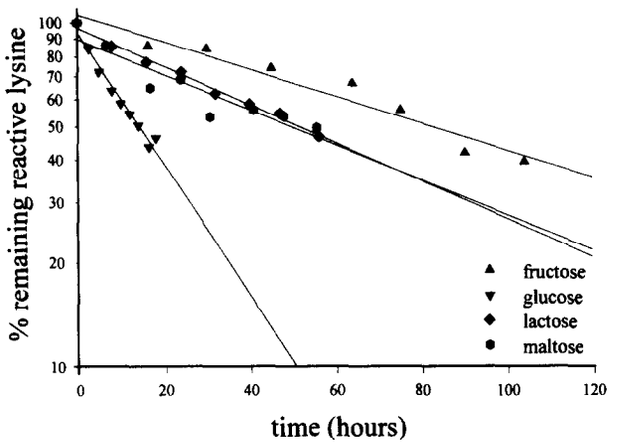

Fig. 2. Available lysine losses in model systems of casein with glucose, fructose, lactose and maltose during heating at 50°C.

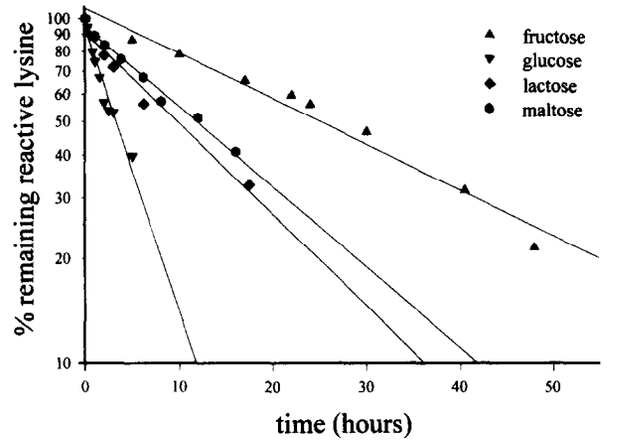

Fig. 3. Available lysine losses in model systems of casein with glucose, fructose, lactose and maltose during heating at 60°C.

The 'no loss' period reported by various researchers (Wolf *et al.*, 1977; Warren and Labuza, 1977; Labuza and Massaro, 1990; Baisier and Labuza, 1992) was not observed in this study, even though the available lysine losses always reached at least 50%, attaining 80% in some cases. Nevertheless, it can be inferred from the studies of Thompson and Wolf (1979) and Wolf *et al.* (1982) that the deviation from the first order depends on the composition of the model system or food used. In particular, no reports were found about this deviation in systems with casein.

The reaction rate constants (k_1) of the model systems at the three temperatures and their respective correlation factors (r^2) are listed in Table 1.

As expected, glucose (an aldohexose) was the most reactive. The reactivities of the reducing disaccharides, maltose and lactose, were similar at the three temperatures, in agreement with the reports of Lewis and Lea (1950) and Kato *et al.* (1990). These reactivities were found to be between those of glucose and fructose. Fructose (a ketohexose) had the lowest reaction rate constant, in opposition to some reports reviewed by

Table 1. First order rate constants and activation energies for available lysine loss

	$k_1 \times 10^3$ (h^{-1})[a]			E_a (kJ/mole)
	37°C	50°C	60°C	
Fructose	− 0.29 (0.9695)	− 9.1 (0.9503)	− 30.3 (0.9742)	166 ± 4.6
Maltose	− 2.31 (0.9755)	− 11.8 (0.8671)	− 53.4 (0.9847)	132 ± 4.2
Lactose	− 1.76 (0.9402)	− 12.8 (0.9900)	− 60.7 (0.9679)	125 ± 4.2
Glucose	− 6.52 (0.9393)	− 44.2 (0.9714)	− 190 (0.9464)	116 ± 2.5

[a] r^2 in brackets.

Dills (1993), but in accordance with the observations of Lewis and Lea (1950), Spark (1969) and Sakai *et al.* (1990). Baxter (1995) achieved similar results with glucose and fructose in mixtures of amino acids and sugars, though the reactivities of both disaccharides were slightly lower than that of fructose.

The reaction rate of each sugar depends on the percentage of the acyclic form (see Introduction), but also on the electrophilicity of the carbonyl group, as stated by Bunn and Higgins (1981). Among the four sugars studied here, fructose has the highest proportion of open chain form, but aldoses would react faster than ketoses because they are more electrophilic.

The differences between the reactivities of fructose and aldoses observed among several studies may be due to the diversity of the composition of the systems and of the conditions of the reaction, since parameters such as pH, solvent, inorganic salts and temperature affect not only the Maillard reaction rate, but also the tautomerization rate of sugars (Avigad *et al.*, 1970; Angyal, 1984; Goux, 1985).

The activation energy values for each system are shown in Table 1. They were within the wide range found in other studies for available lysine loss in food and model food systems (50–238 kJ mol^{-1}) (Lea and Hannan, 1949; Labuza and Saltmarch, 1981; Wolf *et al.*, 1982; Kessler and Fink, 1986; Morales *et al.*, 1995).

It is remarkable that, though glucose, maltose and lactose had similar activation energy values, that of fructose was about 30% higher. In fact, the mechanism of tautomerization of fructose is different from that of most aldoses. Glucose, lactose and maltose show mainly interconversions between pyranose anomers, a mechanism of first order known as simple mutarotation. However, fructose also exhibits interconversions with furanose anomers, a tautomerization that is not of first order, but a complex mechanism (Pigman and Isbell, 1968; Angyal, 1984). In contrast to simple mutarotation, complex tautomerization is highly dependent on temperature (Wertz *et al.*, 1981; Cockman *et al.*, 1987). This could be one of the reasons why the influence of temperature on lysine loss rate in the system with fructose was greater than in those with aldoses.

On account of the higher activation energy of the fructose system, the difference between the rate constants of available lysine loss by this sugar and the others decreases with rise of temperature. Consequently, at higher temperatures, the reaction rate of fructose with free amino groups would exceed those of other sugars more reactive at moderate temperatures, if there were no mechanistic changes.

It can be estimated that the reaction rate constant for available lysine loss in the fructose system would equal those of lactose and maltose at about 80°C and that of glucose at about 100°C.

It can be concluded, that from the nutritional standpoint, the damage to lysine is least with fructose under the conditions of this study. So it should be preferred to the other sugars in the processing or formulation of food products. However, it must be noted that variations in the composition or the conditions of the system may drastically modify the effect, in particular if the process involves high temperatures.

ACKNOWLEDGEMENT

This work was financial supported by grant UBACYT No. EX178 from Secretaría de Ciencia y Técnica de la Universidad de Buenos Aires.

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