ELSEVIER

PII: S0308-8146(96)00257-9

Food Chemistry, Vol. 59, No. 1, pp. 143–147, 1997

0308-8146/97 $17.00+0.00

Maillard reaction as influenced by buffer type and concentration

Leonard N. Bell*

Nutrition and Food Science, 328 Spidle Hall, Auburn University, Auburn, AL 36849, USA

(Received 7 April 1996; accepted 30 June 1996)

Buffering agents are added to many diverse foods to control the pH of the system. Limited data exist on the effects these buffers have on the Maillard reaction. The objective of this study was to investigate the effects of buffer type and concentration on the Maillard reaction. The loss of glycine and the formation of brown pigment were evaluated in phosphate and citrate buffer solutions of various concentrations at pH 7 and 25°C. No glycine loss nor brown pigment formation was observed in the citrate buffer solutions. However, citrate did not appear to be functioning as an inhibitor of the reaction. The rates of glycine loss and browning increased with increasing phosphate buffer concentration. The bifunctional catalytic ability of the phosphate anion was proposed as an explanation for the differing effects of the buffers. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The reaction between carbonyls and amines, known as the Maillard reaction, plays a major role in food stability, flavor development, nutrition, and health (Waller & Feather, 1983; Fujimaki *et al.*, 1986; Finot *et al.*, 1990; Labuza *et al.*, 1994). Many aspects of the Maillard reaction have been investigated. The type of amine and carbonyl influence the rate of reaction as well as the products formed, which ultimately are brown melanoidin pigments (Willits *et al.*, 1958; Pomeranz *et al.*, 1962; Wolfrom *et al.*, 1974; Bunn & Higgins, 1981; Ashoor & Zent, 1984; Feather & Nelson, 1984; Labuza & Massaro, 1990). The concentration and ratio of reactants also have a significant impact on the reaction (Wolfrom *et al.*, 1974; Warmbier *et al.*, 1976*b*; Baisier & Labuza, 1992). The pH of the system significantly influences both the reaction rate and the type of products formed; generally the reaction rate increases as pH increases (Wolfrom *et al.*, 1953; Willits *et al.*, 1958; Underwood *et al.*, 1959; Lento *et al.*, 1960; Lee *et al.*, 1984; Ashoor & Zent, 1984; Apriyantono & Ames, 1993). Numerous studies have demonstrated a browning rate maxima between water activities 0.5 and 0.8 in solids (Eichner & Karel, 1972; Warmbier *et al.*, 1976*a*; Karmas & Karel, 1994). However, in solid systems, differing extents of mobility associated with the plasticization effect of water (i.e. lowering the glass transition temperature) appear to have a greater effect on the rate of browning than the water activity of the system (Karmas *et al.*, 1992; Karmas & Karel, 1994; Buera & Karel, 1995; Bell, 1995).

Of the 12 previously mentioned solution studies, seven used phosphate buffer to control the pH, while four used no buffer. Citrate buffer seldom was employed to control the pH during the Maillard reaction. A survey of the literature finds only three studies have attempted to evaluate the effect of buffer on the Maillard reaction. Burton and McWeeny (1963) noted that color development was higher in the presence of phosphates (which may come from buffer salts) compared to solutions not containing phosphates; the phosphates were suggested to lower the stability of the aldose-type sugar. A comparison to other buffer types was not mentioned. Saunders and Jervis (1966) evaluated browning at 121°C in citrate and phosphate buffer and noted higher absorbance readings in citrate compared to phosphate at pH 3. At pH 8 under refluxing conditions, browning in the presence of phosphate buffer was faster than in a similar unbuffered solution. At the elevated temperatures employed by Saunders and Jervis (1966), the exact pH values and how they differed between buffer types were unknown, because temperature has a dramatic effect on the pK of buffers and thus buffer pH (Barnes *et al.*, 1966; Clever, 1968). In addition, the high temperatures utilized by Saunders and Jervis (1966) limit the application of the data to food processing rather than to typical storage conditions of products. Potman and van Wijk (1989) showed that, at pH 5.6 and 100°C in the presence of glycine, the rate of glucose loss increased

*To whom correspondence should be addressed.

with increasing phosphate concentration; the potential effect of other buffer types was not investigated. Again, the high temperature used makes the reported pH value questionable. A more complete study of the Maillard reaction as influenced by buffer is therefore warranted, especially at less extreme temperatures.

Thus, the objectives of the current project were to investigate the effects of buffer type and concentration on two aspects of the Maillard reaction: the initial degradation of amino acid and the ultimate formation of brown pigmentation. The study was performed at a temperature typical of long-term product storage (25°C).

MATERIALS AND METHODS

Samples

Glucose, glycine, citrate buffer salts, and phosphate buffer salts were obtained from Fisher (Fair Lawn, NJ, USA). The reagents were classified as certified or certified ACS. Solutions were prepared containing 0.1 M glucose and 0.1 M glycine in citrate and phosphate buffers initially at pH 7.02 ± 0.02. The buffer concentrations were 0.02, 0.05, 0.2, and 0.5 M. To help prevent microbial contamination, 0.2% sodium azide was added, and the solutions were filtered through a 0.2 μm syringe filter into multiple vials. The vials were stored at 25°C and removed for analysis approximately every 10–15 days for 160 days. A single sample of each solution type was analyzed at each time point with a minimum of nine samples analyzed over the experimental time period.

Glycine analysis

The experimental systems were diluted 1/9 (v/v) with deionized water. A 200 μl aliquot of the diluted sample was added to 2 ml of 0.05 M phosphate or citrate buffer at pH 7. To this solution, 5 ml of 3.5% (w/w) fluorodinitrobenzene (Sigma, St, Louis, MO, USA) in methanol was added. The sample was vortexed 30 s and stored in darkness at room temperature overnight. The derivatized glycine (dinitrophenyl-glycine) was analyzed using isocratic reverse phase high performance liquid chromatography. The column used was a 150×4.6 C-18 NovaPak column (Waters, Milford, MA, USA). The mobile phase consisted of 28/72 (v/v) acetonitrile/water acidified to pH 3 with phosphoric acid. Absorbance was read at 436 nm. Glycine in standard solutions were derivatized as described for the experimental solutions; these derivatized standard solutions were prepared fresh for the analysis at each time interval. A recovery study of a known glycine solution resulted in a 98% average recovery and a coefficient of variation of 1.1%, indicating that the method of analysis was acceptable.

Browning

The remaining portion of the experimental solution was filtered through a 0.2 μm syringe filter. The absorbance was read at 420 nm using a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, NY, USA). The pH of this solution also was measured.

Data analysis

While the loss of glycine is a second-order reaction, the initial period of loss (i.e. < 15% loss) can be modeled as a pseudo-zero order reaction (Labuza, 1984). Brown pigment formation also has been modeled using pseudo-zero order kinetics (Warmbier *et al.*, 1976*b*; Karmas *et al.*, 1992; Baisier & Labuza, 1992). Thus, for the initial period of glycine loss and brown pigment formation, pseudo-zero order rate constants with 95% confidence limits were calculated using computerized least-squares analysis as discussed by Labuza and Kamman (1983).

Additional experiment

To understand better the role of citrate with respect to the Maillard reaction, an additional experiment was performed. Solutions were prepared containing 0.25 M phosphate buffer, 0.2 M each of glucose and glycine, and 0.2% sodium azide; one solution also contained 0.2 M citrate buffer while the other did not. Both solutions were initially at pH 7.00 and were stored at 37°C. Loss of glycine and formation of brown pigment were determined as discussed above.

RESULTS AND DISCUSSION

Table 1 lists the rates of glycine loss and brown pigment formation. As shown in this table, the rates of glycine loss and brown pigment formation in citrate buffer were not significantly different from zero ($P > 0.05$) while in phosphate buffer both rates increased significantly ($P < 0.05$) with increasing buffer concentration. A plot of glycine concentration as a function of time (Fig. 1)

Table 1. Rates of glycine loss and brown pigment formation with 95% confidence limits in 0.1 M glucose/glycine solutions at pH 7 and 25°C containing different buffer types and concentrations

Buffer	Rate of glycine loss (M/month)	Rate of browning (OD/month)
0.02 M Citrate	0[a]	0
0.05 M Citrate	0	0
0.2 M Citrate	0	0
0.5 M Citrate	0	0
0.02 M Phosphate	0.00055 ± 0.00050	0
0.05 M Phosphate	0.00082 ± 0.00039	0
0.2 M Phosphate	0.0019 ± 0.0003	0.017 ± 0.007
0.5 M Phosphate	0.0033 ± 0.0009	0.076 ± 0.012

[a]Rates are not significantly different from zero ($P > 0.05$).

demonstrates the faster glycine loss at higher phosphate buffer concentrations. Similarly, the faster brown pigment formation at higher phosphate buffer concentrations is shown in Fig. 2. Reactants in citrate buffer, regardless of the buffer concentration, displayed neither glycine loss nor the development of brown pigmentation.

The results of this study support the Burton and McWeeny (1963) conclusion that phosphates enhance the Maillard reaction. The mechanism by which phosphates do so may not only be associated with sugar stability. Potman and van Wijk (1989) suggested that phosphate did not form a reactive intermediate with glucose, but that phosphate was a base catalyst for the Amadori rearrangement. However, the Amadori rearrangement may not be the only step of the reaction that phosphate is catalyzing. Potman and van Wijk (1989) demonstrated that glucose was consumed more rapidly as the phosphate concentration increased, but did not provide a clear reason for the phenomena.

Both aspartame degradation at pH 7 and the Maillard reaction involve an unprotonated amine nucleophilically attacking a carbonyl group. Bell & Wetzel (1995) showed that the catalytic effect of phosphate on the degradation of aspartame compared to four other buffers was not due to differences in the pK of the buffers, but that the phosphate anion showed unique behavior. Bell and Wetzel (1995) suggested that, in contrast to citrate buffer, the increased degradation of aspartame in phosphate buffer was due to the ability of the small phosphate anion to simultaneously donate and accept the proton necessary for the ultimate conversion into diketopiperazine. The same type of mechanism is possible for the initial steps of the Maillard reaction as shown in Fig. 3 and may explain the enhanced glycine loss in phosphate buffer as compared to citrate buffer. After the initial nucleophilic attack of the amine on to the aldehyde, a charged intermediate is formed. The small phosphate anion, which has both hydrogen-accepting and -donating groups in close proximity, simultaneously accepts and donates the protons necessary for the conversion of this intermediate into the glycosylated amine (Fig. 3). The glycosylated amine then proceeds through the complex series of reactions, some of which also require either the addition or removal of protons (Hodge, 1953), to ultimately yield the melanoidin pigments. The faster formation of the glycosylated amine and the continued catalytic potential of the phosphate anion during the subsequent reactions, as suggested by Potman and van Wijk (1989), would enable brown pigment to develop faster than in the citrate buffer, as was observed in this study.

Saunders and Jervis (1966) noted that browning at pH 8 under reflux conditions was faster in the presence

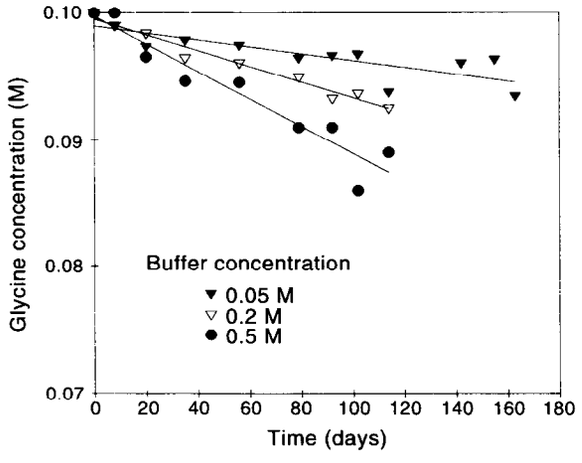

Fig. 1. Effect of phosphate buffer concentration on the loss of glycine in 0.1 M glucose/glycine solutions at pH 7 and 25°C.

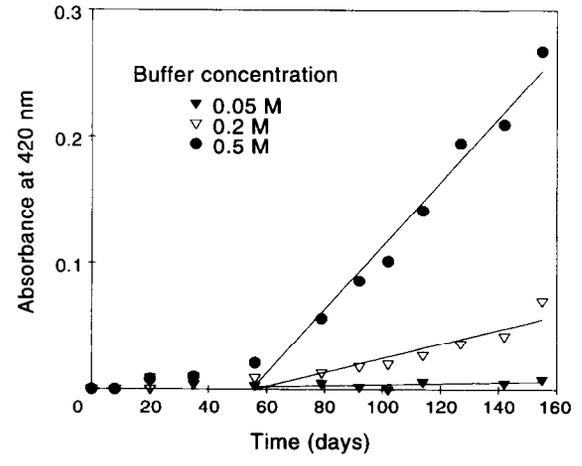

Fig. 2. Effect of phosphate buffer concentration on brown pigment formation in 0.1 M glucose/glycine solutions at pH 7 and 25°C.

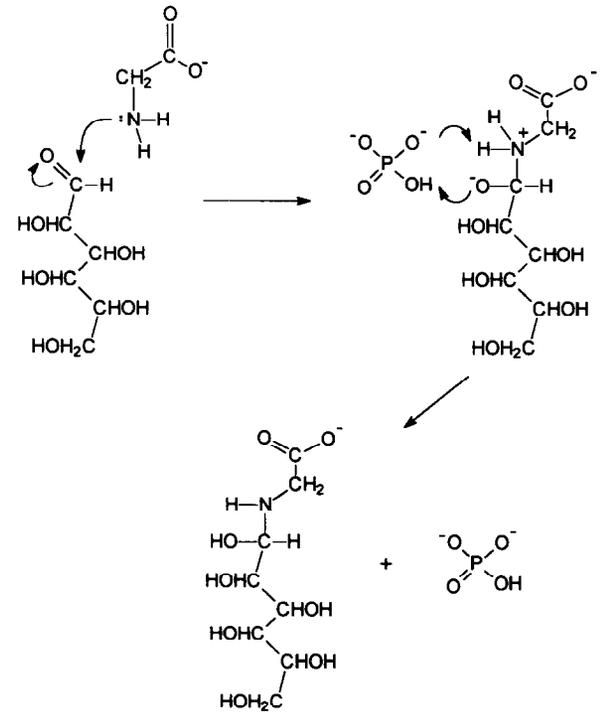

Fig. 3. Proposed effect of the phosphate anion on the reaction of glycine with glucose.

of phosphate buffer as compared to an unbuffered solution. The findings in the current study where reaction rates increased with increasing phosphate buffer concentration were similar to that previously mentioned. However, Saunders and Jervis (1966) also found that browning at pH 3 and 121°C in citrate buffer was faster than in phosphate buffer; at pH 7 and 25°C, the current study found no loss of glycine nor browning in citrate buffer. The elevated temperature and low pH in the Saunders and Jervis (1966) study probably changed the mechanism of the reaction as compared to the current study which may explain the differences in results.

During the Maillard reaction, pH frequently decreases (Lee *et al.*, 1984; Apriyantono & Ames, 1993). This decrease in pH can lead to a slowing of the reaction (Wolfrom *et al.*, 1953; Willits *et al.*, 1958; Ashoor & Zent, 1984). To better understand the reaction rate differences as influenced by buffer type and concentration, the pH also needed to be monitored. Table 2 lists the measured pH for each solution during the coarse of the experiment. As shown, the pH change was limited to ±0.2 pH units. Interestingly, the pH of the solutions containing citrate actually increased, which would be expected to have an accelerating effect on the reaction while the pH of the phosphate solutions decreased, which would be expected to slow the reaction. However, neither pH change appeared to affect the rates in the current study.

Table 2. Change in pH during the Maillard reaction

	Citrate buffer (M)				Phosphate buffer (M)			
Time (d)	0.02	0.05	0.2	0.5	0.02	0.05	0.2	0.5
0	7.03	7.03	7.02	7.01	7.01	7.02	7.03	7.00
35	7.09	7.08	7.08	7.08	7.04	7.02	7.03	7.02
79	7.13	n.d.[a]	n.d.	7.08	7.02	n.d.	n.d.	7.01
102	7.19	7.19	7.05	7.04	7.04	7.01	6.93	7.05
114	7.15	7.14	7.02	6.98	7.01	6.99	6.92	6.87
142	7.20	7.18	7.13	7.14	7.01	6.99	6.91	6.86

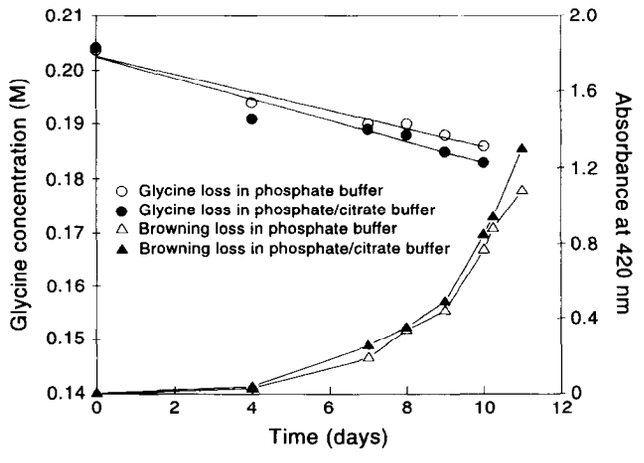

Fig. 4. Effect of added citrate on glycine loss and brown pigment formation in a solution containing 0.2 M glucose, 0.2 M glycine, and 0.25 M phosphate buffer at pH 7 and 37°C.

The lack of the browning reaction in citrate buffer at pH 7 has not been observed previously. Additional experiments were performed to determine whether citrate was an inhibitor of the reaction or simply having a non-observable effect on the reaction. Figure 4 shows that when 0.2 M citrate was added to a 0.25 M phosphate buffer solution containing 0.2 M glucose and glycine at pH 7 and 37°C, the rate of glycine loss and the extent of browning was slightly higher than in the solution without citrate. These results indicate that citrate is not acting as an inhibitor, but appears to have only minimal effects on the Maillard reaction.

CONCLUSION

The catalytic effect of phosphate on the Maillard reaction found in the current study is in agreement with previous studies. Phosphate buffer enhanced the rates of amino acid loss and brown pigment formation in solutions at pH 7 and 25°C while citrate buffer showed no effect during the course of the experiment. In addition to considering the most frequently discussed variables (i.e. amino acid and sugar type, amino acid and sugar concentration, amine–sugar ratio, pH, temperature), the buffer type and concentration also have a significant impact on the Maillard reaction and should not be ignored. From a practical perspective, products susceptible to the Maillard reaction may be more likely to lose nutrients, suffer from discoloration, and produce off-flavors if formulated with phosphate buffer compared to citrate buffer.

ACKNOWLEDGEMENTS

This study was supported by project ALA-13-005 of the Alabama Agricultural Experiment Station and is AAES journal # 10-965196. The author also is appreciative for the laboratory assistance provided by Clinton Wetzel and Alexandra Grand.

REFERENCES

Apriyantono, A. & Ames, J. M. (1993). Xylose–lysine model systems: the effect of pH on the volatile reaction products. *J. Sci. Food Agric.*, **61**, 477–484.

Ashoor, S. H. & Zent, J. B. (1984). Maillard browning of common amino acids and sugars. *J. Food Sci.*, **49**, 1206–1207.

Baisier, W. M. & Labuza, T. P. (1992). Maillard browning kinetics in a liquid model system. *J. Agric. Food Chem.*, **40**, 707–713.

Barnes, H. L., Helgeson, H. C. & Ellis, A. J. (1966). Ionization constants in aqueous solutions. In *Handbook of Physical Constants*, ed. S. P. Clark, Jr. The Geological Society of America, Inc., New York, pp. 401–413.

Bell, L. N. (1995). Kinetics of nonenzymatic browning in amorphous solid systems: distinguishing the effects of water activity and the glass transition. *Food Res. Intl*, **28**, 591–597.

Bell, L. N. & Wetzel, C. R. (1995). Aspartame degradation in solution as impacted by buffer type and concentration. *J. Agric. Food Chem.*, **43**, 2608–2612.

Buera, M. P. & Karel, M. (1995). Effect of physical changes on the rates of nonenzymatic browning and related reactions. *Food Chem.*, **28**, 359–365.

Bunn, H. F. & Higgins, P. J. (1981). Reaction of monosaccharides with proteins: possible evolutionary significance. *Science*, **213**, 222–224.

Burton, H. S. & McWeeny, D. J. (1963). Non-enzymatic browning reactions: consideration of sugar stability. *Nature*, **197**, 266–268.

Clever, H. L. (1968). The ion product constant of water. *J. Chem. Educ.*, **45**, 231–235.

Eichner, K. & Karel, M. (1972). The influence of water content and water activity on the sugar–amino browning in model systems under various conditions. *J. Agric. Food Chem.*, **20**, 218–223.

Feather, M. S. & Nelson, D. (1984). Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde, and glycine and methionine. *J. Agric. Food Chem.*, **32**, 1428–1432.

Finot, P. A., Aeschbacher, H. U., Hurrell, R. F. & Liardon, R. (1990). *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*. Birkhäuser Verlag, Basel, Switzerland.

Fujimaki, M., Namiki, M. & Kato, H. (1986). *Amino-Carbonyl Reactions in Food and Biological Systems*. Elsevier, Amsterdam.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *J. Agric. Food Chem.*, **1**, 928–943.

Karmas, R., Buera, M. P. & Karel, M. (1992). Effect of glass transition on rates of nonenzymatic browning in food systems. *J. Agric. Food Chem.*, **40**, 873–879.

Karmas, R. & Karel, M. (1994). The effect of glass transition on Maillard browning in food models. In *Maillard Reactions in Chemistry, Food and Health*, ed. T. P. Labuza, G. A. Reineccius, V. M. Monnier, J. O'Brien & J. W. Baynes. The Royal Society of Chemistry, Cambridge, England, pp. 182–187.

Labuza, T. P. (1984). Application of chemical kinetics to deterioration of foods. *J. Chem. Educ.*, **61**, 348–358.

Labuza, T. P. & Kamman, J. F. (1983). Reaction kinetics and accelerated tests simulation as a function of temperature. In *Computer-Aided Techniques in Food Technology*, ed. I. Saguy. Marcel Dekker, Inc., New York, pp. 71–115.

Labuza, T. P. & Massaro, S. (1990). Browning and amino acid loss in model total parenteral nutrition solutions. *J. Food Sci.*, **55**, 821–826.

Labuza, T. P., Reineccius, G. A., Monnier, V. M., O'Brien, J. & Baynes, J. W. (1994) *Maillard Reactions in Chemistry, Food and Health*. The Royal Society of Chemistry, Cambridge, England.

Lee, C. M., Sherr, B. & Koh, Y. (1984). Evaluation of kinetic parameters for a glucose–lysine Maillard reaction. *J. Agric. Food Chem.*, **32**, 379–382.

Lento, H. G., Underwood, J. C. & Willits, C. O. (1960). Browning of sugar solutions. IV. The effect of pH on the volatile products of reducing sugars. *Food Res.*, **25**, 750–756.

Pomeranz, Y., Johnson, J. A. & Shellenberger, J. A. (1962). Effect of various sugars on browning. *J. Food Sci.*, **27**, 350–354.

Potman, R. P. & van Wijk, T. A. (1989). Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. In *Thermal Generation of Aromas*, ed. T. H. Parliament, R. J. McGorrin, & C.-T. Ho. American Chemical Society, Washington, D. C., pp. 182–195.

Saunders, J. & Jervis, F. (1966). The role of buffer salts in non-enzymatic browning. *J. Sci. Food Agric.*, **17**, 245–249.

Underwood, J. C., Lento, H. G. Jr & Willits, C. O. (1959). Browning of sugar solutions. 3. Effect of pH on the color produced in dilute glucose solutions containing amino acids with the amino groups in different positions in the molecule. *Food Res.*, **24**, 181–184.

Waller, G. R. & Feather, M. S. (1983). *The Maillard Reaction in Foods and Nutrition*. American Chemical Society, Washington, D.C.

Warmbier, H. C., Schnickles, R. A. & Labuza, T. P. (1976). Effect of glycerol on nonenzymatic browning in a solid intermediate moisture model food system. *J. Food Sci.*, **41**, 528–531.

Warmbier, H. C., Schnickles, R. A. & Labuza, T. P. (1976). Nonenzymatic browning kinetics in an intermediate moisture model system: effect of glucose to lysine ratio. *J. Food Sci.*, **41**, 981–983.

Willits, C. O., Underwood, J. C., Lento, H. G. Jr & Ricciuti, C. (1958). Browning of sugar solutions. I. Effect of pH and type of amino acid in dilute sugar solutions. *Food Res.*, **23**, 61–67.

Wolfrom, M. L., Kashimura, N. & Horton, D. (1974). Factors affecting the Maillard browning reaction between sugars and amino acids — studies on the nonenzymatic browning of dehydrated orange juice. *J. Agric. Food Chem.*, **22**, 796–800.

Wolfrom, M. L., Kolb, D. K. & Langer, A. W. Jr (1953). Chemical interactions of amino compounds with sugars VII. pH dependency. *J. Am. Chem. Soc.*, **75**, 3471–3473.