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Effect of pH on non-enzymatic browning reaction during γ-irradiation processing using sugar and sugar–glycine solutions

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Abstract

Effects of pH on the non-enzymatic browning reaction caused by γ-irradiation processing were investigated. The brown colour development of irradiated sugar–glycine solutions was greater in a buffer system than in deionized distilled water (DDW) with higher pH. Although browning of irradiated sugar solution without glycine was highly increased was in alkaline buffer, no browning was developed in DDW. The maximum browning of the solutions of sugar and sugar–glycine were observed at pH 10 and pH 8, respectively. The browning intensity was in the following order: sucrose ⩾ fructose > glucose. Non-constant pH was observed in DDW (dropped pH between 1.58 and 2.03 units); however, the use of buffers was partially effective in keeping pH constant (pH dropped between 0.03 and 0.56 units). When the irradiated solutions of sugar with and without glycine were analyzed using reverse phase high performance liquid chromatography with diode-array detection (HPLC-DAD), three peaks were separated at 3.37 ± 0.04, 4.60 ± 0.02, and 2.53 ± 0.26 min, and the λ_{max} values of these peaks were the range 259–288 nm. The sum of the areas of these peaks at 260 nm increased with increasing pH. The results of this study indicated that conditions of the system, such as pH and media, can influence the non-enzymatic browning reaction during γ-irradiation.

Keywords: γ-Irradiation; Non-enzymatic browning; pH; Phosphate buffer; HPLC-DAD

1. Introduction

The browning of food during processing is a reaction of importance with regard to the quality of foods, as it affects colour, flavour, taste and nutritional quality (van Boekel, 2001). Non-enzymatic browning, during heat treatment of foods, has been widely studied, but very little research is available on browning developed during γ-irradiation. Irradiation processing of food is now recognized as another method of preserving food and ensuring its wholesomeness by sterilization or cold pasteurization, and it has diverse application worldwide (Olson, 1995).

Non-enzymatic browning is greatly influenced by temperature, pH, water activity and the concentrations of components (Ajandouz & Puigserver, 1999; Petriella, Resnik, Lozano, & Chirife, 1985; Renn & Sathe, 1997). The pH of the system significantly influences both the reaction rate and the type of products formed. Nursten (1986) reported that furfural (from pentoses) or 5-hydroxymethylfurfural (from hexoses) was formed in Maillard reactions at low pH, while higher pH values

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favour the formation of furanones. Generally, at higher pH, more colour was produced by both Maillard (carbonyl-amino reaction) and caramelization (non-amino carbonyl reaction) reactions (Andrews, Godshall, & Moore, 2002; van Boekel, 2001). Ashoor and Zent (1984) reported that no browning was observed in any amino acid–glucose mixtures below pH 6.0 and the maximum browning occurred at pH 10.0. The pH affects the activation energy (E_a) of the browning reaction; the lower the pH, the higher is the E_a for the formation of brown polymers (Lee, Sherr, & Koh, 1984). We studied the effect of pH on the non-enzymatic browning reaction that occurred by γ-irradiation processing using the aqueous model solutions. For this purpose, a solution of sugar alone or in the presence of amino acid was γ-irradiated at 30 kGy at pH values ranging from 4.0 to 10.0. The sugars chosen were glucose, fructose, and sucrose, and the amino acid used was the neutral amino acid, glycine.

2. Materials and methods

2.1. Materials

D (+)-Glucose (>99.5%), D (−)-fructose (>99.5%), α-D-sucrose (>99.5%) and L-glycine (>98%) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Sodium acetate, disodium hydrogenphosphate, sodium dihydrogenphosphate, sodium carbonate, sodium bicarbonate and acetic acid were purchased from the Junsei Chemical Co., Ltd. (Tokyo, Japan). Acetonitrile (99.8%, HPLC grade) was purchased from the Tedia Co., Inc.(Symmes Road, Fairfield, USA).

2.2. Preparation of aqueous solutions

The sugar solutions were prepared as follows: each sugar (glucose, fructose, or sucrose) was dissolved in deionized distilled water (DDW) or buffer to final concentrations of 0.1 M. The sugar–glycine solutions were prepared as equimolar (0.1 M) mixtures. The following buffers were used: 0.05 M sodium acetate adjusted to pH 4.0 with 1 M acetic acid, 0.05 M sodium phosphate adjusted to pH 6.0, 7.0, and 8.0, using either monobasic or dibasic sodium phosphate, 0.05 M sodium carbonate bicarbonate adjusted to pH 10.0, using either sodium carbonate or sodium bicarbonate. Ten millilitres of each sample were placed in 15 ml glass tubes. The glass tube was sealed with a Teflon-lined septum and screw cap, and stored at 5 °C overnight before irradiation.

2.3. γ-Irradiation of prepared solutions

The samples were irradiated in a cobalt-60 irradiator (IR-7P, MDS Nordion Intl., Ottawa, Ontario, Canada). The source strength was about 100 kCi with a dose rate of 10 kGy/h at 15 ± 0.5 °C. Dosimetry was performed using 5 mm dia alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The absorbed doses in the present study were 30 kGy and the actual doses were within 2% of the target doses.

2.4. Browning and pH measurements

The browning intensity of the irradiated samples was measured at 420 nm in a spectrophotometer (UV-1601 PC, Shimadzu Co., Tokyo, Japan) using the same untreated solution as a reference; pH measurement was carried out with a pH meter (MP 220, Mettler-Toledo International Inc., Greifensee, Zurich, Switzerland).

2.5. Analysis using reverse-phase HPLC-DAD

The HPLC systems used were the following: separation modules (Waters 2690, Milford, MA, USA), a millennium 32 chromatography manager (System Software, Workstation version 3.0, Waters Co.), a photodiode array detector (PDA, Waters 996, Waters Co.) and C_{18} 5 μm column (4.6 mm × 250 mm i.d., Shiseido Co., Ltd., Tokyo, Japan). A linear acetonitrile/water gradient of 0–49% acetonitrile was applied upto 30 min. At the end of the gradient, the column was reequilibrated to initial conditions for 5 min with a final composition of 100% DDW. The flow rate employed was 1 ml/min, and samples (20 μl) were applied to the column with no pre-treatment. Chromatograms were monitored at 260 and 420 nm with raw spectral data collection between 210 and 450 nm.

2.6. Statistical analysis

Pearson's correlation coefficients, and significance defined at $P < 0.05$ and $P < 0.01$ levels, were analyzed by Windows SPSS 10.0 (SPSS, 1999).

3. Results and discussion

3.1. Effect of initial pH on brown colour development of γ-irradiated solutions

Fig. 1 shows the brown colour development, measured at 420 nm, of the sugar solutions (glucose, fructose or sucrose), with and without glycine, in a buffer system at initial pH values ranging from 4.0 to 10.0 after irradiation at 30 kGy. The browning of irradiated sugar solutions in buffer was slightly increased below initial pH 8.0 and rapidly increased at the initial pH of 10.0. In the

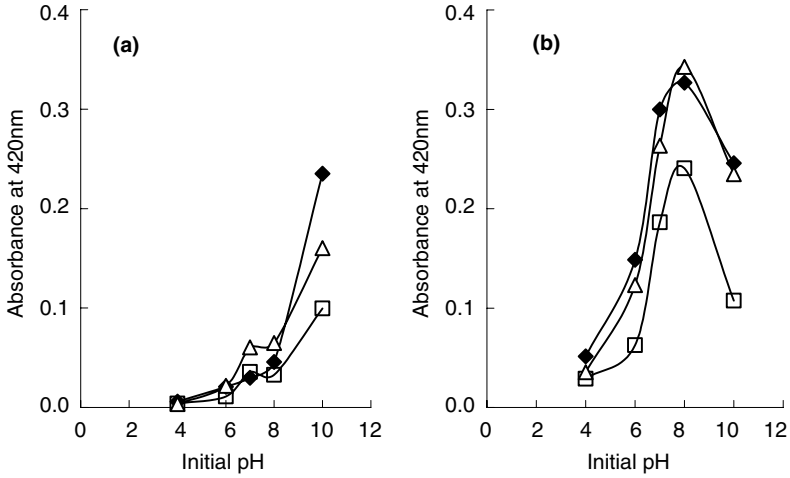

Fig. 1. Brown colour development of the sugar solutions in buffer, with and without glycine, after irradiation at 30 kGy. A, sugar solution without glycine; B, sugar solution with glycine. Glucose (□), fructose (△), and sucrose (◆).

case of irradiated sugar–glycine solutions in buffer, the brown colour increased initially at pH 8.0, while it decreased initially at pH 10.0. The brown colour development of irradiated sugar solutions with and without glycine, increased more in the buffer system, especially at alkaline pH. This is a similar result to the study previously reported for non-enzymatic browning during heat treatment (Andrews et al., 2002; van Boekel,

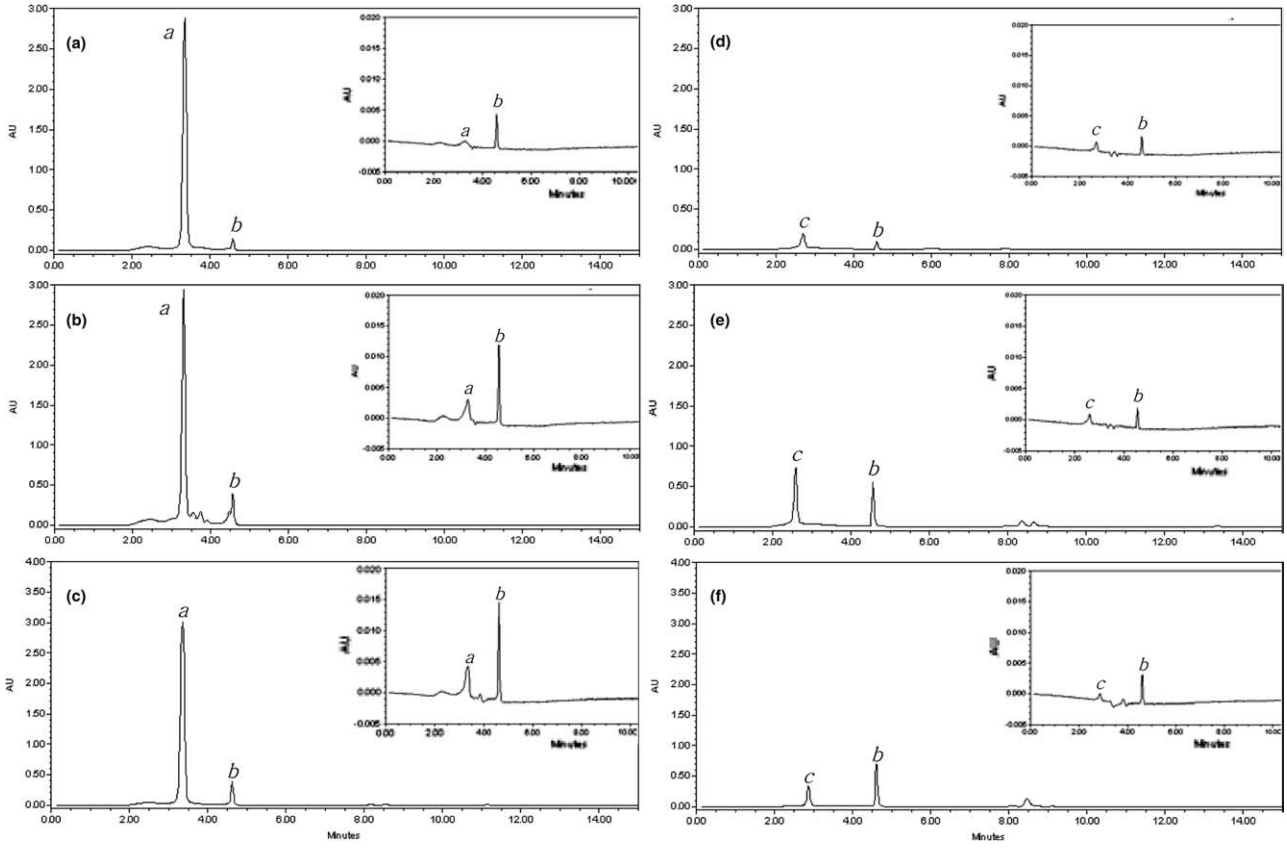

Fig. 2. Chromatograms of irradiated sugar solutions in buffered (pH 10) and DDW monitored at 260 and 420 nm. A, glucose solution at pH 10; B, fructose solution at pH 10; C, sucrose solution at pH 10; D, glucose solution in DDW; E, fructose solution in DDW; F, sucrose solution in DDW. Inset: chromatogram monitored at 420 nm.

2001; Lee et al., 1984; Martins, Jongen, & van Boekel, 2001). However, there should be further clarification of the non-enzymatic browning reaction caused by γ-irradiation, i.e., whether the brown colour formation is a result of Maillard reaction or caramealization or other reaction. The browning of irradiated sugar solution, with or without glycine, had the following order of intensity: sucrose ⩾ fructose > glucose. When γ-irradiation passes through matter, such as a solution or food, energy is absorbed, and it leads to the ionization or excitation of the atoms and molecules of the matter which results in chemical changes (Stewart, 2001). The pH of an aqueous system can affect the end result of irradiation. An acid medium (excess H^+) favours the disappearance of aqueous electrons (e^-_{aq}), while an alkaline medium favours their formation (Brewer, 2004). The primary radiolytic species of water, such as aqueous electrons (e^-_{aq}), hydroxy radicals ($^{\bullet}OH$), hydrogen atoms ($^{\bullet}H$) and superoxide anion radical ($^{\bullet}O_2^-$) can lead to radiolysis and chemical change of the matter (Stewart, 2001).

As mentioned previously, the pH significantly influences the non-enzymatic reaction as well as irradiation processing. Andrews et al. (2002) reported that as pH is increased, greater amount of colour is developed in sugar solution during processing at 85 °C. They explained that, under alkaline conditions, sugars undergo condensation polymerization reactions with ion intermediates (e.g., 3-carbon), then polymerize to high-molecular weight colorants. In the Maillard reaction, the reactivity of the sugar and the amino group is highly influenced by pH. The open chain form of the sugar and unprotonated form of the amino group, considered to be the reactive forms, are favoured at higher pH (Martins et al., 2001).

3.2. Analysis of total reaction products of irradiated solutions using HPLC-DAD

In order to observe the formation of coloured and colourless non-enzymatic browning reaction products, the irradiated sugar solutions, with and without glycine, were analyzed using reverse phase HPLC-DAD. As shown Figs. 2 and 3, three major peaks (Peak *a*, *b*, and *c*) were separated, which had mean retention times of 3.37 ± 0.04, 4.60 ± 0.02, and 2.53 ± 0.26 min, respectively, and their λ_{max} values were in the range 259–288 nm (data not shown). These peaks may be mainly composed of the intermediate reactants, having

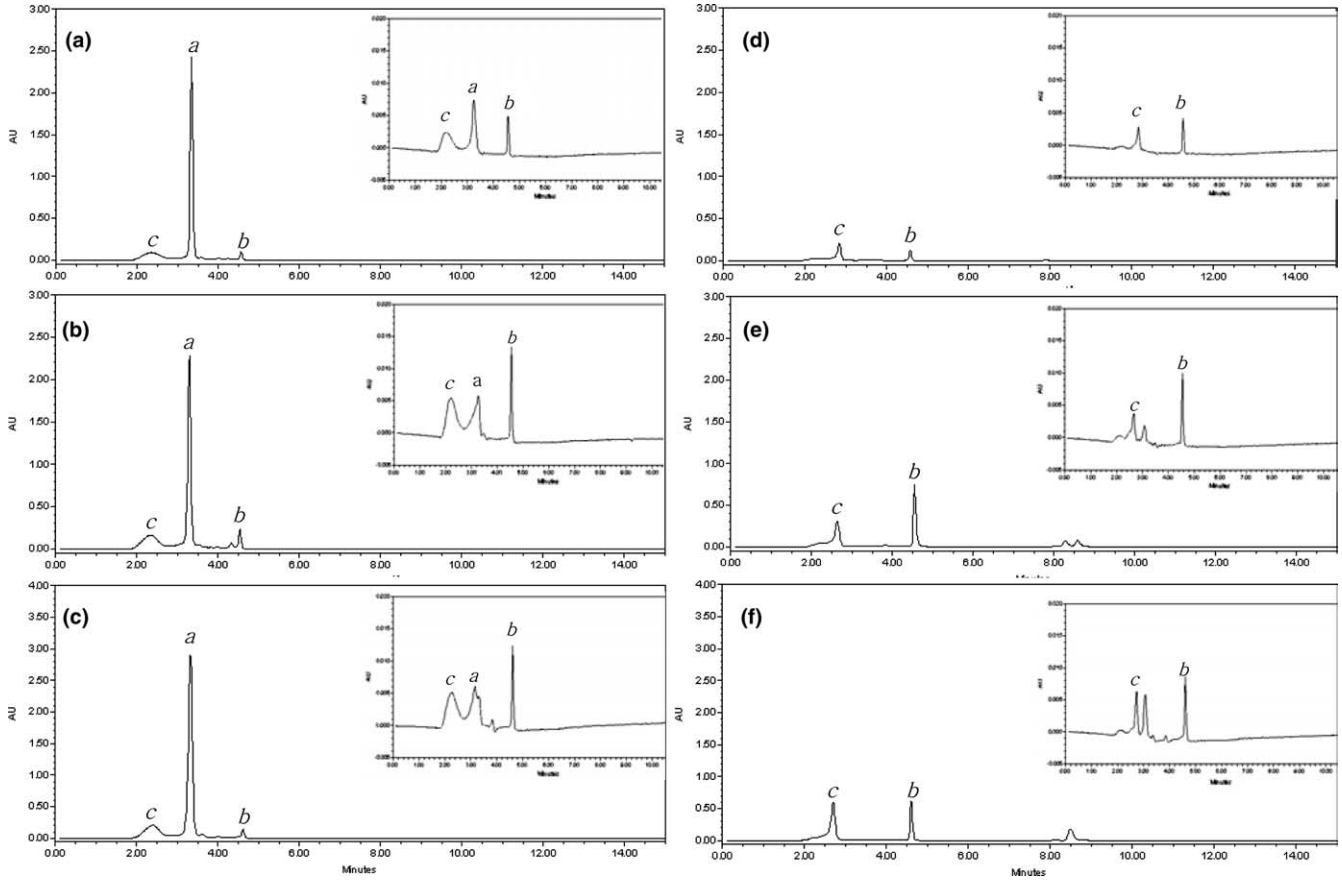

Fig. 3. Chromatograms of irradiated sugar–glycine solutions in buffered (pH 10) and DDW, monitored at 260 and 420 nm. A, Glucose–glycine solution at pH 10; B, fructose–glycine solution at pH 10; C, sucrose–glycine solution at pH 10; D, glucose–glycine solution in DW; E, fructose–glycine solution in DW; F, sucrose–glycine solution in DW. Inset: chromatogram monitored at 420 nm.

Table 1
Areas of the main resolved peaks of the chromatogram monitored at 260 and 420 nm from irradiated sugar solutions without glycine at various pH values (unit: AU s)

Sugar type	Initial pH	260 nm				420 nm			
		Peak *a*	Peak *b*	Peak *c*	Sum[a]	Peak *a*	Peak *b*	Peak *c*	Sum
Glucose	4[c]	0.729	0.914	–[b]	1.643	0.008	0.019	–	0.027
	6	5.156	0.046	–	5.202	0.033	0.030	–	0.063
	7	9.880	1.417	–	11.297	0.057	0.045	–	0.102
	8	11.320	1.144	–	12.464	0.047	0.031	–	0.078
	10	22.205	0.030	–	22.235	0.039	0.028	–	0.067
	DDW[d]	–	0.047	2.611	2.658	–	0.028	0.029	0.057
Fructose	4	0.863	3.099	–	3.962	–	0.019	–	0.019
	6	5.613	4.672	–	10.285	0.066	0.064	–	0.130
	7	11.970	3.432	–	15.402	0.111	0.076	–	0.187
	8	13.208	2.705	–	15.913	0.086	0.065	–	0.151
	10	24.548	2.995	–	27.543	0.076	0.084	–	0.160
	DDW	–	2.585	7.390	9.975	–	0.029	0.034	0.063
Sucrose	4	2.220	4.689	–	6.909	0.015	0.034	–	0.049
	6	11.022	5.143	–	16.165	0.028	0.055	–	0.083
	7	14.714	3.307	–	18.021	0.046	0.053	–	0.099
	8	16.946	3.060	–	20.006	0.055	0.044	–	0.099
	10	25.379	2.053	–	27.432	0.104	0.081	–	0.185
	DDW	–	3.256	3.023	6.279	–	0.043	0.023	0.066

[a] Sum: the sum of three peak areas.
[b] Below limit of detection.
[c] The sugar solutions in buffer of pH values ranging from 4.0 to 10.0.
[d] The sugar solutions in deionized distilled water.

strong absorption in the near-ultraviolet that formed during the non-enzymatic browning reaction, e.g., by sugar dehydration, sugar fragmentation, or amino acid degradation (Hodge, 1953). Tables 1 and 2 show peak areas of peak *a*, *b*, and *c* in the chromatogram obtained from irradiated solutions of sugar, with and without glycine, solutions. Peak *a*, separated from irradiated sugar solution with and without glycine in buffered system, was higher with increasing initial pH. Peak *b* was separated from all the irradiated sugar and sugar–glycine solutions, and peak *c* was separated from the irradiated sugar solution, with and without glycine in DDW, and irradiated sugar–glycine solution in the buffered system at initial pH 10. The sum of these peaks areas obtained from chromatograph monitored at 260 nm, increased with increasing pH. This observation may suggest that the non-enzymatic browning reaction was enhanced in alkaline conditions during γ-irradiation. These peaks were also monitored at 420 nm, as an index of the brown polymers formed in the more advanced stages of non-enzymatic browning reaction (Hodge, 1953). This indicated that the peaks might include coloured reactants. However, no direct correlation between these peak areas, detected at 420 nm, and browning of each irradiated solution was observed (Table 3). In the present study, although no unresolved broad band was present, reaction mixtures were not well separated, especially for coloured reactants. In the course of the Maillard reaction, not only the complexity but also the diversity of the reaction mixture is increased (Davidek, Clety, Devaud, Robert, & Blank, 2003). HPLC-DAD is most commonly used; however, it may offer neither sufficient resolution nor satisfactory sensitivity for simultaneous analysis of reaction products, because the complex mixtures formed of coloured and colourless react products possess a wide range of polarities and molecular weights (Ames, Arnoldi, Bates, & Negroni, 1997; Royle, Bailey, & Ames, 1998), and parent sugars and Amadori compounds can be analyzed in the same run (Davidek et al., 2003). In this study, these separated peaks (peaks *a*, *b*, and *c*) seem to be composed of non-enzymatic browning reactant mixture, such as Amadori compounds and melanoidins. Therefore more research is needed that includes other analytical techniques in order to separate and detect the non-enzymatic browning reaction products during γ-irradiation.

3.3. Effect of media on the non-enzymatic browning reaction during γ-irradiation

Table 4 shows the brown colour development, measured at 420 nm, of the sugar solutions (glucose, fructose, or sucrose) with or without glycine in DDW after

Table 2
Areas of the main resolved peaks of the chromatogram monitored at 260 and 420 nm from irradiated sugar solutions with glycine at various pH values (unit: AU s)

Sugar type	Initial pH	260 nm				420 nm			
		Peak *a*	Peak *b*	Peak *c*	Sum[a]	Peak *a*	Peak *b*	Peak *c*	Sum
Glu–Gly	4[c]	0.993	0.933	–[b]	1.926	0.009	0.024	–	0.034
	6	4.995	0.826	–	5.821	0.149	0.009	–	0.158
	7	9.375	0.957	–	10.332	0.347	0.065	–	0.412
	8	11.802	1.315	–	13.117	0.377	0.089	–	0.466
	10	12.883	0.707	2.835	16.425	0.106	0.043	0.096	0.245
	DDW[d]	–	0.615	1.919	2.534	–	0.038	0.057	0.095
Fru–Gly	4	2.190	3.027	–	5.217	0.011	0.0328	–	0.044
	6	9.654	4.082	–	13.736	0.292	0.137	–	0.429
	7	13.696	3.455	–	17.151	0.579	0.203	–	0.782
	8	17.407	3.010	–	20.417	0.644	0.183	–	0.827
	10	15.290	1.149	4.620	21.059	0.153	0.078	0.180	0.411
	DDW	–	3.737	4.233	7.970	–	0.063	0.064	0.127
Suc–Gly	4	3.616	4.512	–	8.128	0.007	0.045	–	0.052
	6	12.738	3.489	–	16.227	0.267	0.138	–	0.405
	7	17.925	2.907	–	20.832	0.501	0.168	–	0.669
	8	18.821	2.471	–	21.292	0.535	0.081	–	0.616
	10	24.173	0.977	5.791	30.941	0.189	0.063	0.177	0.429
	DDW	–	2.877	6.806	9.683	–	–	0.069	0.069

[a] Sum: sum of three peaks area.
[b] Below limit of detection.
[c] The sugar–glycine solutions in buffer of pH values ranging from 4.0 to 10.0.
[d] The sugar–glycine solutions in deionized distilled water.

Table 3
Pearson's correlation coefficients between the peak areas of chromatogram monitored at 420 and the brown colour development, measured at 420 nm, of irradiated solutions

	Sugar solution			Sugar–glycine solution		
	Glucose	Fructose	Sucrose	Glu–Gly	Fru–Gly	Suc–Gly
Peak *a*	0.458[a]	−0.044	0.948*	0.949*	0.835	0.896*
Peak *b*	0.221	0.763	0.904*	0.892*	0.789	0.751
Peak *c*	–	–	–	1.000**	1.000**	1.000**
Sum[b]	0.407	0.622	0.961**	0.986**	0.907*	0.971**

[a] Values with significant correlation with $*P < 0.05$ and $**P < 0.01$.
[b] Sum: Pearson's correlation coefficients between sum of three peaks area and brown colour development.

Table 4
The brown colour development, measured at 420 nm, of the sugar solutions irradiated at 30 kGy in deionized distilled water, with and without glycine

	Sugar type		
	Glucose	Fructose	Sucrose
Without glycine	0.002	0.005	0.010
With glycine	0.027	0.035	0.046

irradiation at 30 kGy. In contrast to the irradiated sugar solutions in buffer (Fig. 1), no browning was observed with irradiated sugar solution without glycine in DDW. With these contradictory results between alkaline buffer and DDW, there should be further clarification of the non-enzymatic browning reaction of the irradiated solution of sugar alone (without other ion or compound). In present study, the brown colour of irradiated sugar solutions, with and without glycine, was more developed in buffer than in DDW. This may be due to the effect of phosphate ions present in the buffer. The acceleration effects of phosphate on Maillard browning reaction have been reported after first being studied in detail by Reynolds (1958), and this effect has been explained by a catalytic role (Bell, 1997; Rizzi, 2004). Davidek, Clety, Auin, and Blank (2002) reported that phosphate ions, which act as a base, abstracting a proton during the Amadori rearrangement, were found

Table 5
The pH changes of sugar solutions, with and without glycine, after γ-irradiation at 30 kGy

Initial PH	Sugar solution			Sugar–glycine solurion		
	Glucose	Fructose	Sucrose	Glu–Gly	Fru–Gly	Suc–Gly
4[a]	−0.04	−0.03	−0.04	−0.15	−0.15	−0.14
6	−0.45	−0.29	−0.25	−0.23	−0.40	−0.31
7	−0.13	−0.09	−0.08	−0.17	−0.22	−0.18
8	−0.49	−0.43	−0.37	−0.56	−0.51	−0.50
10	−0.44	−0.34	−0.28	−0.11	−0.18	−0.11
DDW[b]	−2.03	−1.94	−2.04	−1.58	−1.69	−1.45

[a] The sugar solutions in buffer of pH values ranging from 4.0 to 10.0.
[b] The sugar solutions in deionized distilled water.

to be the principal catalytic species leading to the enhanced degradation of Amadori compounds. They also enhance the browning and fragmentation of sugar during processing (Rizzi, 2004).

The constant pH of the system is important during reaction because the non-enzymatic browning reaction is dependent on pH (Monti, Bailey, & Ames, 1998). However, as shown in Table 5, changes of pH were observed in the solutions of sugar, with and without glycine, during irradiation. When sugar solution was irradiated, the pH value dropped due to the production of sugar acids, such as gluconic acid, glyceric acid, deoxyketohexonic acid, and 2-deoxygluconic acid (Diehl, Adam, Delincée, & Jakubick, 1978; Stewart, 2001). Irradiation-induced pH decrease was also reported in real food system, for instance in apple juice (Baraldi, 1973) and in cooked rice (Lee et al., 2004). During the Maillard reaction, sugar isomers and organic acids, namely formic and acetic acid, were produced and they also cause reduction in pH (Martins et al., 2001). These decreased pH values (non-constant pH) during an experiment consequently cause a slowing down of the non-enzymatic browning reaction (Martins et al., 2001). In the present study, the use of buffers was partially effective for maintaining constant pH (pH units dropped from 0.03 to 0.56), compared with DDW (pH units decreased from 1.58 to 2.03). It should be considered, however, that buffers also have an influence on the non-enzymatic browning reaction (Bell, 1997; Davidek et al., 2002; Rizzi, 2004).

4. Conclusion

Non-enzymatic browning, reaction during γ-irradiation processing, was greatly influenced by pH and reaction medium. The brown colour development of irradiated sugar solutions, with and without glycine, is more increased in a buffer system, especially at alkaline pH, than DDW. These results indicated that when food is irradiated, an off-colour such as browning, can be produced, due to the non-enzymatic browning reaction and it is influenced by other ions and/or pH of the system. This suggests that the browning of irradiated food might be retarded by lowering the pH of the system. However, more research is needed to provide adequate information for various complex food systems.

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