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The effect of γ-irradiation on the non-enzymatic browning reaction in the aqueous model solutions

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Abstract

Aqueous solutions of sugars (xylose, arabinose, fructose, glucose or sucrose), alone or in the presence of amino acid (lysine), were γ-irradiated at 0, 5, 10, 20 and 30 kGy at room temperature. Also evaluated were differences between irradiation and heat treatment. Absorbances at 420 nm, of the irradiated sugar–amino acid solutions, were increased although no browning was observed in the irradiated sugar or amino acid alone. The degree of browning of the irradiated sugar–amino acid solution increased with increasing irradiation dose and was dependent on the type of sugar. The non-reducing sugar, sucrose, did not react with lysine by heating for 4 h at 80 °C; however, the irradiated sucrose–lysine solution showed the non-enzymatic browning reaction and it had a higher reactivity than other sugars. For the sugar–lysine solution irradiated at 30 kGy, browning was in the following order of intensity: sucrose > fructose > arabinose > xylose > glucose. Furfural compounds (5-hydroxymethylfurfural and 2-furaldehyde) were not detected in any irradiated samples.

Keywords: Irradiation; Non-enzymatic browning; Furfural compound; Non-reducing sugar

1. Introduction

Irradiation processing has been demonstrated to be a safe and effective means for reducing or eliminating biological hazards in foods (WHO, 1999). This process has been shown, in the pasteurization or sterilization of foods, to effectively control the many biological hazards associated with foods without compromising the nutritional properties (Skala, McGown, & Waring, 1987; Thayer, Lachica, Huhtanene, & Wierbicki, 1986). However, high-doses of irradiation can lead to a change of the sensory characteristics, such as flavour (Ahn, Jo, & Olson, 2000; Zhu, Mendoca, & Ahn, 2003), texture (Prakash, Manley, De Costa, Caporaso, & Foley, 2002; Yoon, 2003), and colour (Fan & Thayer, 2002; Kim et al., 2004; Roushdi, Harras, El-Meligi, & Bassim, 1981). Some researchers have observed that colour intensity of irradiated foods, increases as the irradiation dose is raised (Kim et al., 2004; Lee et al., 2003; Roushdi et al., 1981). They hypothesized that these observations were due to the forming of coloured compounds by the Maillard reaction. Nicoli, Casadei, Guerzoni, and Lerici (1994) suggested that irradiation leads to non-enzymatic browning reactions similar to those induced in heat-treatment food. However, there are few scientific reports of the mechanism of the change of the colour in irradiated foods.

When food is irradiated, chemical changes may arise as a result of direct action of the food components or by indirect action through reactive intermediates formed by

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radiolysis of water (Hayes, Murano, Murano, Olson, & Sapp, 1995). Irradiation of low molecular weight sugars and their derivatives, in the solid or aqueous state, causes changes in their physical and chemical properties. When sugars are irradiated in their solid states, melting points decrease, optical rotation is reduced, and browning can be observed (Stewart, 2001).

We studied the non-enzymatic browning reaction which is caused by irradiation using aqueous model solutions. For this purpose, a solution of sugar alone, or in the presence of essential amino acid at an equimolar concentration, was irradiated from 5 to 30 kGy. The sugars chosen were pentoses (D-xylose and D-arabinose) the ketohexose, D-fructose, the aldohexose, D-glucose and disaccharide, sucrose. Lysine was used as it has a high reactivity for browning (Ajandouz & Puigserver, 1999). It is also one of the most important nutritional factors in food because it is an essential amino acid and is usually used as an indicator of the potential biological value of food protein (Naranjo, Malec, & Vigo, 1998).

2. Materials and methods

2.1. Materials

Sucrose (>99.5%), D (+)-glucose (>99.5%), D (−)-fructose (>99.5%), D (+)-xylose (>99%), D (−)-arabinose (>99%), L-lysine (>98%), 5-(hydroxymethyl)furfural (HMF, >99%) and 2-furaldehyde (F, >99%) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). 3,5-Dinitrosalicylic acid, sodium hydroxide and potassium sodium tartarate (Rochelle salt) were purchased from the Junsei Chemical Co., Ltd. (Japan). Acetonitrile (99.8%), HPLC grade, was purchased from the Tedia Co., Inc. (Symmes Road, Fairfield, USA).

2.2. Sample preparation

2.2.1. Sugar solutions and amino acid solution

Each sugar (sucrose, glucose, fructose, xylose or arabinose) and L-lysine were dissolved in deionized distilled water to final concentrations of 1.0 M. Ten millilitres of each sample was placed in 15-ml glass tubes. The glass tube was sealed with Teflon-lined septa and screw caps and stored at 5 °C overnight before irradiation.

2.2.2. Sugar–amino acid solutions

A molar sugar–lysine ratio of 1:1 was used in the experiments. Each sugar (sucrose, glucose, fructose, xylose or arabinose) and lysine was dissolved at a final concentration of 0.1 M in deionized distilled water, and then five millilitres of the sugar solution and amino acid solution were mixed. Ten millilitres of the sugar–amino acid solution containing 0.05 M sugar and 0.05 M lysine was placed in 15-ml glass tubes. The glass tubes were sealed with Teflon-lined septa and screw-caps and stored at 5 °C overnight before irradiation or heating.

2.3. γ-irradiation or heat treatment

The samples were irradiated in a cobalt-60 irradiator (IR-7P, MDS Nordion Intl., Ottawa, Ont., Canada). The source strength was about 100 kCi with a dose rate of 10 kGy/h at 15 ± 0.5 degrC. Dosimetry was performed using 5-mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The absorbed doses in this study were 0, 5, 10, 20 and 30 kGy and the actual doses were typically within 2% of the target doses.

The samples were heated at 80 degrC in a water bath for 4 h and then rapidly cooled in ice water.

2.4. Absorbance measurements

Absorbance of the irradiated and/or heated solutions was measured at 284 and 420 nm in a spectrophotometer (UV-1601 PC, Shimadzu Co., Tokyo, Japan) using the same untreated solution as a reference. Absorbance was measured at 284 nm as an indication of the formation of the intermediate products (by sugar dehydration, sugar fragmentation, or amino acid degradation) of the non-enzymatic browning, and at 420 nm, as index of the brown polymers formed in the more advanced stages (Hodge, 1953). When necessary, appropriate dilutions were made in order to have an optical density of less than 1.5.

2.5. Determination of the reducing power of the sugar

Reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). One millilitre of the sample was transferred into 15-ml glass tubes and 2 ml of the modified DNSA regent (0.5 g dinitrosalicylic acid, 8 g sodium hydroxide and 150 g Rochelle salt in distilled water up to 500 ml) was added. The solution was mixed with a vortex mixer for 5 s and was boiled for 10 min, and then cooled in ice. The reducing power of the sample was analyzed by spectrophotometer in reference to the glucose standard.

2.6. Determination of the sugars using HPLC

The sugar contents of the treated solutions were analyzed by high performance liquid chromatography (HPLC). The HPLC systems used were the following: separation modules (Waters 2690, Waters Co., Milford, MA, USA), a millennium 32 chromatography manager (System Software, Workstation version 3.0, Waters Co.), a refractive index detector (RI detector, Waters

2410, Waters Co.), and a high performance carbohydrate column (4 μm, 4.6 mm × 250 mm, Waters Co.). Separations were carried out isocratically at room temperature, using a mixture of acetonitrile–water (75:25, v/v) at a flow-rate of 1.4 ml/min for the mobile phase. The injection volume was 15 μl.

A standard stock solution containing 2% sugars was used to prepare the working standard solutions (the range of 0.2–1.0%). Each sugar (xylose, arabinose, fructose, glucose and sucrose) was completely separated out in 14, 15, 16, 17 and 20 min, respectively, and the run time was 25 min.

2.7. Determination of the furfural compounds (HMF and F)

Furfural compounds were measured by HPLC with UV detection at 284 nm, according to the method proposed by Ferrer, Algría, Farré, Abellán, and Romero (2002). The treated solutions were directly injected into the liquid chromatograph. The HPLC systems were composed of: separation modules (Waters 2690, Waters Co., 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.) at 284 nm, and C_{18} 5 μm column (4.6 mm × 250 mm, Shiseido Co., Ltd., Tokyo, Japan). Separations were carried out isocratically at room temperature, using a mixture of acetonitrile–water (5:95, v/v) at a flow-rate of 1 ml/min for the mobile phase. The injection volume was 20 μl.

The 5-(hydroxymethyl)furfural (HMF) and 2-furaldehyde (F) concentrations were obtained from the calibration curve in the range of 0.01–2 μg/ml in the assay. The HMF and F were completely separated out in 8 and 10 min, respectively, and the run time was 15 min.

2.8. Statistical analysis

For the statistical analysis of samples we used the Windows SPSS 10.0 (SPSS, 1999). The general linear model procedure was processed and the Student–Newman–Keul's multiple range test was used to compare the differences in the mean values. Mean values and pooled standard errors of the mean (SEM) were reported, and significance was defined at $P < 0.05$.

3. Results and discussion

3.1. Irradiation effects on the browning, reducing power and furfural compounds of the sugar solutions

Table 1 shows the absorbances at 284 and 420 nm of 1.0 M each sugar solutions (sucrose, glucose, fructose, xylose, arabinose) after γ irradiation. Browning of the irradiated sugar solution, measured at 420 nm, was not increased when compared with the untreated solution. Absorbance at 284 nm, however, was increased with increasing irradiation dose. This increased absorbance in the ultraviolet may be due to the decomposition of the sugar substrates by dehydration and sugar fragmentation (Hodge, 1953) during irradiation. The colour did not change when the sugar solutions (Table 1) or amino acid solution (data are not shown) alone were irradiated. However, Liggett, Feazel, and Ellengerg (1959) reported that the irradiated 50% aqueous solutions of the sugars (glucose, fructose, sucrose) were colourless immediately after an irradiation dose of 5.0 Mrad (50 kGy), but they usually developed a brown colour upon standing for several hours at room temperature. In this study, we used the 14–34% (1.0 M) sugar or amino acid solution.

Table 1
Absorbance at 284 and 420 nm of the sugar solutions (1.0 M) after γ-irradiation

	Irradiation dose (kGy)				SEM[A]
	0	10	20	30	
A_{284} (Dil 1/10)					
Sucrose	0.000d[B]	0.202c	0.292b	0.313a	0.0061
Glucose	0.000d	0.490c	0.852b	1.038a	0.0292
Fructose	0.000d	0.192c	0.308b	0.376a	0.0150
Xylose	0.000d	0.100c	0.128b	0.164a	0.0032
Arabinose	0.000d	0.005c	0.009b	0.122a	0.0081
A_{420}					
Sucrose	0.000b	0.002ab	0.002ab	0.003a	0.0007
Glucose	0.000	0.002	0.002	0.002	0.0058
Fructose	0.000	0.004	0.017	0.005	0.0058
Xylose	0.000b	0.001b	0.002ab	0.001a	0.0005
Arabinose	0.000b	0.003a	0.002a	0.004a	0.0008

[A] SEM: pooled standard errors of the mean ($n = 12$).
[B] Different letters (a–d) within a row differ significantly ($P < 0.05$).

Table 2
Changes of the reducing level (%) in the sugar solutions (1.0 M) after γ-irradiation [A] or heat treatment [B]

	Irradiation does (kGy)				SEM [C]
	0	10	20	30	
Sucrose	0.00c[D]	1.06b	1.61ab	2.06a	0.236
Glucose	18.4	18.4	18.3	18.2	0.271
Fructose	18.0a	17.6a	17.2ab	16.6b	0.312

[A] Irradiation: the sugar solutions were irradiated at 0, 10, 20 or 30 kGy at 15 °C.
[B] Heat treatment: the sugar solutions were incubated for 4 h at 80 °C.
[C] SEM: pooled standard errors of the mean ($n = 12$).
[D] Different letters (a–c) within a row differ significantly ($P < 0.05$).

Table 2 shows the changes of the reducing power (%) of the sugar solutions after irradiation. In this study, we determined the reducing level of the sugar samples by the DNSA method, but this method cannot determine the reducing level of xylose and arabinose. Sucrose, a non-reducing sugar, showed an increase in reducing power as the irradiation dose level was raised. Several investigators (Lee et al., 2003; Liggett et al., 1959; Roushdi et al., 1981; Sokhey & Hanna, 1993) have reported the irradiation induced reducing power in non-reducing sugar such as sucrose (disaccharide) and starch (polysaccharide). Irradiation-induced scission of the glycosidic linkage leads to the generation of a free radical at the C1 position on the glucose molecule in starch or disaccharide in the presence of water and, consequently, these radiolytic end-products had a reducing power (Stewart, 2001; Raffi et al., 1985). Other irradiated reducing sugar solutions have a decreased reducing level, which may be due to decomposition of the sugar by irradiation.

Furfural derivatives, such as 5-(hydroxymethyl)furfural (5-HMF) and 2-furaldehyde (2-F), are intermediate products of the Maillard reaction (Ferrer et al., 2002) and are also formed by acidic degradation of the sugars at a high temperature (Feather & Harris, 1973). No furfural compound was found in the irradiated sugar solutions (data are not shown).

3.2. Irradiation effects on the browning, sugar loss and furfural compounds of the sugar–amino acid solutions

Figs. 1 and 2 show the development of the absorbance at 284 and 420 nm of the irradiated sugar–amino acid solutions. Hodge (1953) suggested that the sugar–amine browning reaction (Maillard reaction) may be classified into three stages of development: initial stage, intermediate stage and the final stage. The intermediate stage of browning reaction has a strong absorption near the ultraviolet by sugar or amino acid dehydration, sugar fragmentation, or dehydration of the (recombinded) fragments, even though it appears colourless. In the final stage of the browning reaction, the intermediates polymerize and coloured polymers are formed. Absorbance at 420 nm is frequently used to determine browning (Fernández-Artigas, Guerra-Hernández, & García-Villanova, 1999).

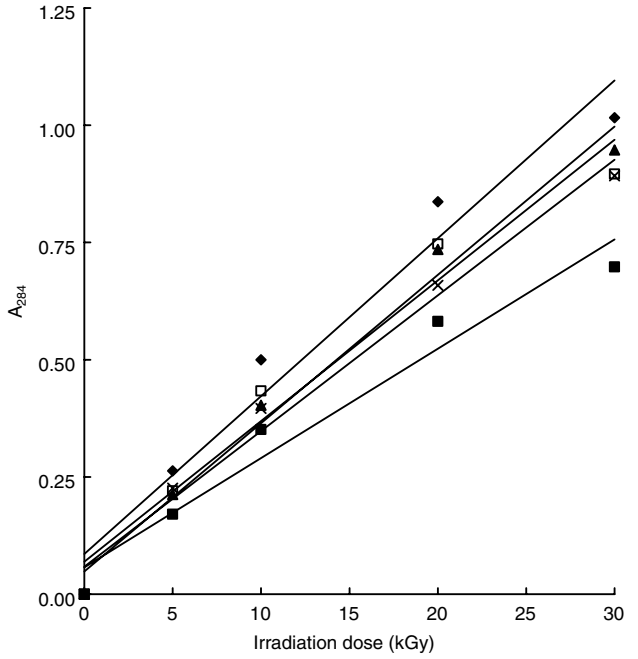

Fig. 1. Absorbance at 284 nm (dilution 1/10) of the sugar–amino acid (sugar–amino acid solution: the samples contained sugar (sucrose, glucose, fructose, xylose or arabinose) and lysine at molar ratio of 1:1, at a final concentration of 0.05 M in deionized distilled water. Sucrose (◆), glucose (■), fructose (□), xylose (▲) and arabinose (×).) solutions after γ irradiation.

As shown in Figs. 1 and 2, at the absorbance of 284 and 420 nm, the irradiated sugar–amino acid solutions have an increased optical density with increasing irradiation. Although the irradiated sugar or amino acid solutions alone were colourless, the irradiated sugar–amino acid solutions formed coloured products. This suggests that irradiation may lead to non-enzymatic browning reactions, similar to those induced by heating (Nicoli et al., 1994). This reaction may influence the colour of the irradiated foods. The browning rates of the irradiated sugar–amino acid solutions are listed in Table 3. The rates of the browning reaction decreased in the following order of reactivity: sucrose > xylose > fructose > arabinose > glucose.

Table 4 shows the remaining sugar and sugar loss of the sugar–amino acid solutions after irradiation. The sugar loss increased, depending on the irradiation dose. When the sugar–amino acid solutions were irradiated at 30 kGy, the losses of the sugars, glucose, fructose, sucrose, xylose and arabinose, were 16.8, 11.3, 10.4, 9.5 and 6.7%, respectively. These sugar losses could be attributable to the degradation by the irradiation or participation in the sugar–amine reaction (Cerrutti, Resnik, Seleds, & Ferrofontan, 1985). No furfural com-

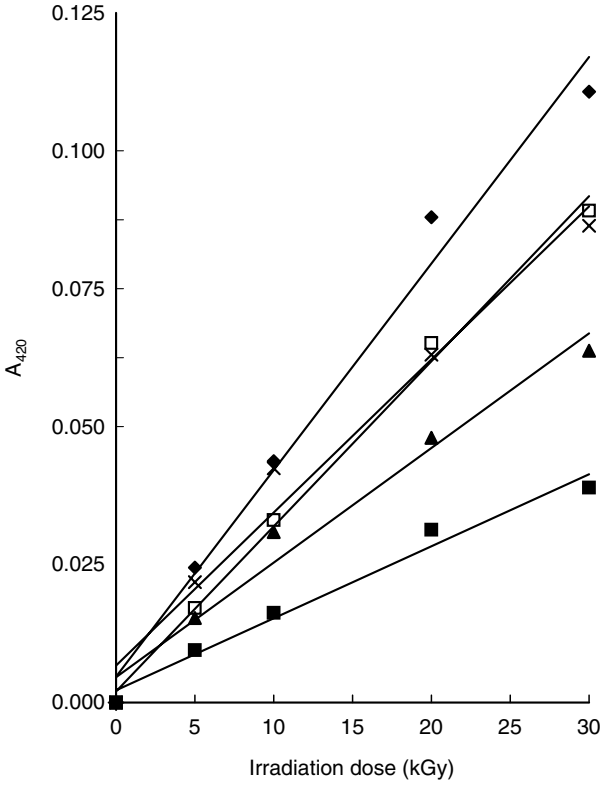

Fig. 2. Brown colour development in the sugar–amino acid solutions (sugar–amino acid solution: the samples contained sugar (sucrose, glucose, fructose, xylose or arabinose) and lysine at molar ratio of 1:1, at a final concentration of 0.05 M in deionized distilled water. Sucrose (◆), glucose (■), fructose (□), xylose (▲) and arabinose (×.)) after γ irradiation measured spectrophotometrically at 420 nm.

pounds (5-HMF or 2-F) were found in the irradiated sugar–amino acid solutions, whereas, in heated glucose-, fructose- and xylose–amino acid solutions, the compounds were detected in the concentration range 0.12–0.29 mg/l (data are not shown). This result is supported by previous reports (Fernández-Artigas et al., 1999), though the data obtained form the experiments are slightly different.

Table 3
Intermediate reaction rate and browning rate of the sugar–amino acid solutions[A] after irradiation

	Intermediate reaction rate (A_{284}/kGy, r^2)	Browning rate (A_{420}/kGy, r^2)
Sucrose	0.3366 (0.96)[B]	0.0337 (0.96)
Glucose	0.2331 (0.96)	0.0233 (0.96)
Fructose	0.3000 (0.96)	0.0300 (0.96)
Xylose	0.3162 (0.98)	0.0316 (0.98)
Arabinose	0.2893 (0.98)	0.0289 (0.98)

[A] Sugar–amino acid solution: the samples contained sugar (sucrose, glucose, fructose, xylose or arabinose) and lysine at molar ratio of 1:1, at a final concentration of 0.05 M in deionized distilled water.
[B] r^2, regression coefficient.

3.3. Comparison between irradiation and heat treatment

Table 5 shows the properties, such as absorbance, sugar loss, and the reducing power of the sugar, in the irradiated and heated solutions. As shown in the Table, the browning rate was significantly influenced by the type of the sugar involved in both the irradiated and heated reactions. In the heated solution, no browning was observed with the non-reducing disaccharide, sucrose, while the formation of coloured products was observed in the irradiated sucrose–lysine solution. This may be explained on the basis that irradiation causes a release of energy, which promotes the breakdown of the glycosidic linkages of the disaccharide and the interaction between the liberated carbonyl and amino compounds, forming coloured compounds (Lee et al., 2003; Roushdi et al., 1981). It was demonstrated that the irradiated sucrose solutions had reducing power, while the heated one showed none.

The browning of the irradiated sugar–lysine solutions was in the following order of intensity: sucrose > fructose, arabinose, xylose > glucose, and in the heated ones, xylose > fructose, arabinose, glucose > sucrose. Some investigators have reported the following order of browning reactivity by heat treatment: aldopentoses > aldohexoses > ketohexoses > disaccharides (Mauron, 1981; Spark, 1969; Pomeranz, Johnson, & Shellenberger,

Table 4
Remaining sugar (mg/ml) and sugar loss (%) of the sugar–amino acid solutions [A] after irradiation

	Irradiation dose (kGy)					SEM [B]
	0	5	10	20	30	
Sucrose	17.0a[C]	16.3b (4.4)[D]	16.1b (5.5)	15.5c (8.8)	15.2c(10.4)	0.239
Glucose	8.95a	8.33b (6.9)	8.0bc(11.2)	7.66bc(14.4)	7.45c (16.8)	0.265
Fructose	8.93a	8.52ab (4.6)	8.40ab (5.9)	8.18c (8.4)	7.92c (11.3)	0.238
Xylose	7.40a	7.13ab (3.7)	7.13ab (3.7)	6.88ab (7.0)	6.70b (9.5)	0.189
Arabinose+	7.46a	7.34ab (1.6)	7.29b (2.3)	7.11c (4.7)	6.96d (6.7)	0.067

[A] Sugar–amino acid solution: the samples contained sugar (sucrose, glucose, fructose, xylose or arabinose) and lysine at molar ratio of 1:1, at a final concentration of 0.05 M in deionized distilled water.
[B] SEM: pooled standard error of the mean ($n = 15$).
[C] Different letters (a–d) within a row differ significantly ($P < 0.05$).
[D] Sugar loss of the samples.

Table 5
A comparison of the absorbance at 284 and 420 nm, sugar losses, and reducing powers of the sugars between the irradiated and heated solutions [A]

	Sugars					
	Sucrose	Glucose	Fructose	Xylose	Arabinose	SEM[B]
A_{284}(Dil 1/10)						
Irradiation[C]	1.02a[E]	0.698b	0.896ab	0.947ab	0.892ab	0.0847
Heat[D]	0.007c	1.05a	0.387bc	0.318bc	0.621b	0.160
A_{420}						
Irradiation	0.111a	0.0390b	0.089ab	0.064ab	0.086ab	0.0172
Heat	0.002b	1.89ab	2.43ab	4.25a	2.50ab	1.34
Sugar loss (%)						
Irradiation	10.8ab	16.6a	11.2ab	9.30ab	6.69b	2.36
Heat	3.92b	44.0a	51.2a	50.1a	24.3ab	11.8
Reducing power (%)						
Irradiation	2.06c	18.2a	16.6b			0.302
Heat	0.004c	18.1a	17.4b			0.161

[A] The values used are the absorbances and sugar losses of the irradiated or heated sugar–lysine solutions and the reducing power of the sugar of the irradiated or heated sugar solutions.
[B] SEM pooled standard errors of the mean ($n = 15$).
[C] Irradiation: the solutions were irradiated at 30 kGy at room temperature (15 °C).
[D] Heat: the solutions were incubated for 4 h at 80 °C.
[E] Different letters (a–c) within a row differ significantly ($P < 0.05$).

1962). Nevertheless, the results are often difficult to compare because of the great diversity of the conditions of the treatment and the indicators of the degree of the reaction used (Hodge, 1953; Naranjo et al., 1998). Naranjo et al. (1998) reported that, at higher temperatures, the reaction rate of fructose with the free amino groups would exceed those of the other sugars which are more reactive at moderate temperatures. In this study, the browning reactivity of disaccharide, ketohexose and pentose with the amino group were higher than aldohexose or glucose by irradiation. Furthermore, research is needed on the browning reaction by γ irradiation involving various conditions.

4. Conclusion

γ irradiation leads to a non-enzymatic browning reaction (carbonyl–amine reaction) in an aqueous system similar to those induced by heating. This reaction may influence the changes of the colour in irradiated foods. The intensity of the reaction was dependent on the type of the sugar, whether by irradiation or by heating. There was a difference in the browning reaction between irradiation and heating. Although no browning was observed in the heated solution of the non-reducing sugar, formation of coloured products was observed in the irradiated sucrose–lysine solution. This could be explained on the basis that irradiation promotes the breakdown of the glycosidic linkages of the disaccharide, sucrose and increases reducing power. More research is needed on the non-enzymatic browning reaction by γ-irradiation, concentrating on various factors, such as the concentration and/or molar ratios of the sugar/amino acid, pH, and water activity.

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