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Food Chemistry 82 (2003) 465–468

Food Chemistry

www.elsevier.com/locate/foodchem

Radiolytic characteristics of nitrite by gamma irradiation

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Received 14 July 2002; received in revised form 6 December 2002; accepted 6 December 2002

Abstract

The characteristics of nitrite radiolysis by gamma rays were investigated. Sodium nitrite in deionized distilled water was irradiated at 0, 5, 10, 15, 20, 25, 30 and 40 kGy, and the degradation rate constant was calculated. The sodium nitrite was significantly reduced by irradiation in a dose-dependent manner ($P < 0.05$). The degradation rate of sodium nitrite fitted a first-order model; a high linear correlation ($r^2 > 0.9$) was observed and the degradation rate constant was 0.009 min^{-1}. When nitrite was nitrosated in different pH ranges after irradiation, the irradiated nitrite could not form the carcinogenic N-nitrosodimethylamine. Results indicated that the gamma irradiation may be effective for breakdown of nitrite, and the radiolysis products did not act a precursor for N-nitrosodimethylamine formation.

Keywords: Gamma irradiation; Nitrite; Radiolysis; Kinetics; Nitrosation

1. Introduction

Gamma irradiation is used in many countries for inhibition of sprouting, disinfestation of insects and parasites, delay of physiological ripening, extension of shelf-life or improvement of the technological properties of food (Loaharanu, 1989; Thayer, 1994). Recently, irradiation technology has been applied to new fields, besides the sanitation of foods, such as destruction or reduction of pesticides or anti-nutrients in foods (Lepine, 1991; Villavicencio, Mancini-Filho, Delincee, & Greiner, 2000), production of oligosaccharides for the preparation of conjugate vaccines (Pawlowski & Svenson, 1999), chlorophyll breakdown in oil (Byun, Jo, Lee, & Kim, 2002) and the breakdown of carcinogenic N-nitrosamines (Ahn, Yook, Rhee, Lee, Cho, & Byun, 2002).

Sodium nitrite plays important roles in meat products for the development of desirable colour, flavour and texture and for protection against oxidative rancidity and pathogenic microorganisms, especially, *Clostridium botulinum* (Cassens, 1995). Besides the practice of adding nitrite for cured meat, however, scientific research has cast nitrite and its reaction products both in a negative light because of epidemiological suggestions that hot dog consumption is linked to childhood cancer, a risk of leukaemia and a positive light because of synthesis of nitric oxide for physiological functions (Culotta & Koshland, 1992; Maugh, 1994). Another problem for the use of nitrite in cured meat was the formation of carcinogenic N-nitrosamines. Generally, the chemistry of nitrosation of amines in foodstuffs is a complex process in which oxides of nitrogen, formed through nitrite activation during processing, preservation and preparation, can react with amino compounds to produce carcinogenic and volatile N-nitrosamines (Francis, 2000). Therefore, it might be important to reduce nitrite residues to the minimum needed after processing. Wierbicki and Brynjolfsson (1979) reported, previously, that irradiation sterilization with ^{60}Co and ^{137}Cs reduced nitrite and preformed volatile nitrosamine levels in cured meat products. Afterwards, Jo, Ahn, Son, Lee, and Byun (2003) reported that gamma irradiation was effective in reducing nitrite level in emulsion-type cooked pork sausage during storage. The authors suggested that vacuum- or CO_2-packaging were more effective for level reductions than aerobic

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doi:10.1016/S0308-8146(02)00591-5

packaging. These results implied possibilities for reducing nitrite in a wide range of food systems.

This study was designed to investigate the effect of irradiation in the radiolysis of nitrite in an aqueous medium, and to illuminate radiolytic characteristics. Accordingly, the purpose of the present study is (1) to investigate the degradation rate of standard sodium nitrite in an aqueous model system by gamma rays and (2) to evaluate the ability of irradiated nitrite to act as a precursor of N-nitrosamine formation.

2. Materials and methods

2.1. Sample preparation

Sodium nitrite was purchased from Sigma Co. (St. Louis, MO, USA). The sodium nitrite was dissolved in deionized distilled water for a final concentration of 100 mg kg^{-1} of standard solution.

The sample was irradiated in a cobalt-60 irradiator (Nordion International, Canada). The source strength was ca. 100 kCi with a dose rate of 5 kGy h^{-1} at 10 ± 0.5 °C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Brüker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Brüker EMS 104 EPR Analyser. The irradiation doses in this study were 0, 5.0, 10, 15, 20, 25, 30 and 40 kGy, and the actual doses were within ±2% of the target dose.

2.2. Nitrite determination and degradation rate constant

The concentration of sodium nitrite was determined according to AOAC methods no. 973.31 (AOAC, 1995). Then, the degradation of sodium nitrite by irradiation was kinetically studied (Chiu & Chen, 2000). The absorbent dose at a unit time was converted to irradiation time for calculating the degradation rate constant. The correlation coefficient (r^2) was calculated from the plot of the logarithm of the concentration of sodium nitrite standard versus irradiation time. The degradation rate constant (k, min^{-1}) was obtained from the following equation: $k = -\ln (CA/CA_0)/t$, where CA = the concentration of sodium nitrite standard after irradiation, CA_0 = the initial concentration of sodium nitrite standard, t = irradiation time (83.3 Gy min^{-1}).

2.3. Nitrosation reaction

The sodium nitrite irradiated at 0 and 40 kGy was nitrosated in vitro as described by Mirvish et al. (1972) with modification. The irradiated or non-irradiated sample (50 mM sodium nitrite, 1 ml) and 50 mM dimethylamine (1 ml) were adjusted up to 10 ml with 50 mM sodium citrate and the reaction mixture was maintained at 37 °C. The nitrosation was started by adding 60% perchloric acid to the mixture to make the pH value 2, 3, 4 or 6. The pH value of the reaction mixture was carefully monitored and, when necessary, it was adjusted by the addition of either perchloric acid or sodium hydroxide. After an hour, the pH of the mixture was adjusted to pH 13 with 5 N sodium hydroxide to stop the reaction. Then, the mixture was extracted with dichloromethane, using a separatory funnel, after addition of 1 g sodium chloride and N-nitrosodipropylamine (NDPA, Sigma Co., St. Louis, MO, USA). NDPA (1 mg kg^{-1}) was used as an internal standard for extraction efficiency. The mean recovery values for the internal standards were $94.6\pm3.29\%$. The concentration of dichloromethane solution was extracted up to 10 ml using an evaporator and the dichloromethane solution was dried with sodium sulphate. The sodium sulphate residue was removed by filtration.

2.4. N-Nitrosodimethylamine determination

After the nitrosation reaction, the concentration of N-nitrosodimethylamine (NDMA) was determined quantitatively by gas chromatography (GC, model 5890II, Hewlett-Packard Co., Wilmington, DE, USA) coupled to a thermal energy analyser (TEA, Thermo Electron model 502B, Waltham, MA, USA). Analyses were carried out with a non-polar SPB-5 fused silica capillary column (0.53 mm i.d.×30 m, Supelco Co., Bellefonte, PA, USA), which was introduced into the ceramic pyrolysis tube at the end of TEA. Helium was used as the carrier gas at a flow rate of 3.5 ml/min. The injection port was set at 220 °C and the temperature of the column port was ramped: 50 °C for 5 min, increased to 150 °C at 5 °C/min. The injection volume was 2 μl. The NDMA, which was identified by pure standards, was detected at levels of 0.1 μg kg^{-1}.

2.5. Statistical analysis

The experiment was replicated twice and data was analysed by SAS software (SAS, 1985). The ANOVA was processed, and Student–Newman–Keuls test was used to compare mean values. Significance was defined at $P<0.05$.

3. Results and discussion

Changes of sodium nitrite concentrations by irradiation are shown in Table 1. A significant difference was observed in nitrite reduction by irradiation dose ($P<0.05$). The sodium nitrite was reduced about 50% by 10 kGy irradiation, and the complete degradation was shown over 40 kGy. The correlation coefficient was 0.9995; therefore, the degradation occurred in a dose

Table 1
Degradation of sodium nitrite in deionized distilled water by gamma irradiation (unit: mg kg^{-1})

	Irradiation dose (kGy)								SEM[a]	r^2[b]	F-value	Pr > F
	0	5	10	15	20	25	30	40				
Sample[c]	100a[d]	63.6b	50.8c	35.9d	20.7e	12.5f	4.57g	0.28h	0.684	1.00	2481	0.0001

[a] Standard error of the means ($n = 24$).

[b] Correlation coefficient.

[c] The concentration of sodium nitrite was 100 mg kg^{-1} in deionized distilled water.

[d] Different letters (a–h) within a same row differ significantly ($P < 0.05$).

dependent manner. Ahn, Kim, Jo, Lee, and Byun (2002) reported that residual nitrite in pork sausage was reduced approximately 30% by 30 kGy irradiation, and accordingly there is somewhat of a difference between the real foods and model system. Water plays an important role in radiolytic degradation, because water is required for hydrophotolysis of nitrite by UV irradiation (Reszka, Bilski, & Chignell, 1996). Chemically, NO_2 produces NO radical by UV irradiation; however, the NO radical is difficult to detect. It might be that the amounts and life of the NO radical vary according to method of irradiation, including UV, α-, β-, λ- or X-rays, due to the differences of their energies and wavelengths. Reduction of nitrite to NO can occur under acidic, highly reduced conditions, or energy input. NO is a free radical, formed endogenously in many biological roles, such as neurotransmission, blood clotting, blood pressure control and in the immune system's ability to kill tumor cells as a biological messenger (Cassens, 1995), while a neurotoxic effect, as a precursor of peroxynitrite anion formation, was also reported (Tabrizi-Fard, Lee, & Fung, 1999).

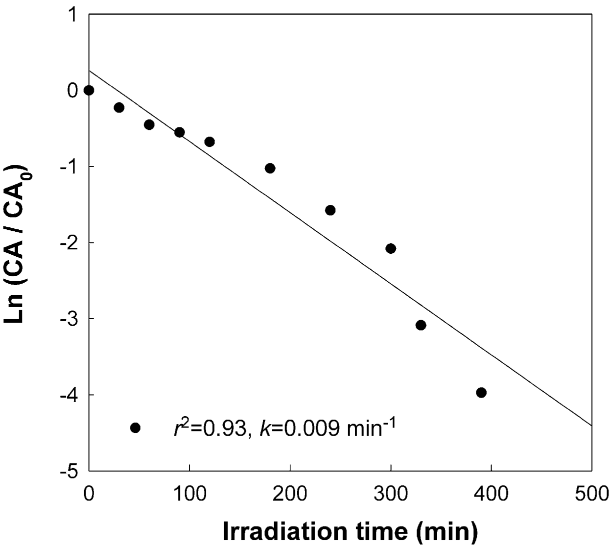

Fig. 1. First-order plot for degradation of sodium nitrite in deionized distilled water by gamma irradiation at 0–40 kGy (r^2; correlation coefficient, k; degradation rate constant, CA; the concentration of sodium nitrite after irradiation, CA_0; the initial concentration of nitrite).

The degradation rate constant (k), calculated from the plot of the logarithm of the concentration of sodium nitrite, is shown in Fig. 1. The degradation rate of sodium nitrite fit a first-order model with a high linear correlation ($r^2 > 0.9$) between irradiation time and ln (CA/CA_0). These results indicated that a high dose of irradiation was used for breakdown of sodium nitrite in the model system.

NDMA formation by nitrite irradiated at 0 or 40 kGy was investigated at pH 2, 3, 4, or 6 (Table 2). Immediately after irradiation, residual nitrite levels were determined, and about 50% reduction was observed in 50 mM nitrite solution. This result was different from Table 1 due to the dilution effect.

Non-irradiated nitrite formed 166 and 157 μg kg^{-1} NDMA at pH 2 and 3, respectively, whereas half of the NDMA formation occurred at pH 4. Generally, the nitrosation reaction is predominant in acidic conditions (pH 2–4) by activated nitrite (Francis, 2000). Otherwise, 40 kGy-irradiated samples formed low concentrations of NDMA, and about 49, 44, 44, or 39% of NDMA formed at pH 2, 3, 4 and 6, respectively, compared with the non-irradiated control. Only the residual nitrite after irradiation was nitrosated to NDMA. This indicated that irradiated nitrite cannot form the carcinogenic NDMA in the model system because the NO radical might rapidly disappear in the aqueous state. Fiddler, Gates, Pensabene, Phillips, and Wierbicki (1981) sug-

Table 2
N-Nitrosodimethylamine formation of nitrite irradiated at 0 and 40 kGy at different pH (unit: μg kg^{-1})

Treatment[a]	pH				
	2	3	4	6	SEM[b]
0 kGy	166a[c]x[d]	157ax	75.3b	22.9c	7.66
40 kGy	81.5ay	69.6ay	33.7b	9.26c	5.89
SEM[e]	7.419	2.78	10.39	4.255	

[a] Sodium nitrite in deionized distilled water was irradiated at 0 and 40 kGy.

[b] Standard error of the means ($n = 8$).

[c] Different letters (a–c) within a same row differ significantly ($P < 0.05$).

[d] Different letters (x,y) within a same column differ significantly ($P < 0.05$).

[e] Standard error of the means ($n = 4$).

gested that irradiation sterilization at 30 kGy reduces residual nitrite in bacon prior to frying, thereby reducing volatile nitrosamines after frying, and destroying preformed volatile nitrosamines, if present, in the bacon prior to irradiation. Actually, gamma irradiation is effective for reducing residual nitrite level in meat products, and the low concentration of N-nitrosamine by irradiation is maintained after storage (Ahn et al., 2002).

In conclusion, gamma irradiation can be used for reducing nitrite and furthermore, radiolytically destroyed nitrite could not form carcinogenic N-nitrosamine, even in a model human stomach condition. However, further study will be needed to elucidate the fate of NO radical induced by gamma irradiation.

Acknowledgements

This research was supported under the Nuclear R&D Program by the Ministry of Science and Technology in Korea.

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