Reduction of 4-methylimidazole in caramel coloring by γ-irradiation

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▸ ***Short report***

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ABSTRACT

4-Methylimidazole (4-MEI) is a simple nitrogen-containing heterocyclic compound, and recently classified as group 2B by International Agency for Research on Cancer (IARC). Caramel coloring is widely applied food coloring in many food products including coffee, carbonated beverages, beer and wine. In present study, γ-irradiation was applied to Caramel Color III to reduce the level of 4-MEI without color changes. Caramel coloring III was γ-irradiated with 1, 3, 5, 10, 30, 50 and 100 kGy. Non-irradiated caramel coloring was served as a control. The level of 4-MEI and color were analyzed on all caramel coloring. The concentration of 4-MEI in the non-irradiated caramel coloring was 714.9 mg/kg. The level of 4-MEI in caramel coloring was significantly reduced from 3 kGy γ-irradiated caramel coloring ($p<0.05$). After 100 kGy irradiation, the amount of 4-MEI in caramel color was at 658 mg/kg, which equates 7.9% reduction from non-irradiated sample. No color change was observed on irradiated and non-irradiated caramel coloring ($p>0.05$). Therefore, γ-irradiation could be used as an alternative processing method to improve the quality of caramel coloring by reducing 4-MEI content without its color change.

Keywords: *4-methylimidazole, γ-irradiation, Caramel coloring.*

INTRODUCTION

4-Methylimidazole (4-MEI) is a simple nitrogen-containing heterocyclic compound, and is widely used in pharmaceuticals, photographic chemicals, food colorings such as dyes and pigments [(1)]. In food application, caramel coloring is well-known coloring ingredient that high amount of 4-MEI was detected. Caramel coloring is widely consumed food coloring agent worldwide by consumption weight. Typical manufacturing process of caramel coloring involves browning reaction between carbohydrates and ammonium compounds with and/or without sulfite [(2)]. Therefore, 4-MEI can be generated intentionally and unintentionaly during food processing including home-cooking. Most common food commodity, known to contain caramel coloring included carbonated beverages, beer and wine, soy sauce, molassess, and cracker [(3)].

The toxicity of 4-MEI has been reported in many International agencies including the National Toxicology Program [(4)], The Office of Environmental Health Hazard Assessment [(1)], and International Agency for Research on Cancer [(5)]. In 2007, NTP reported "clear evidence of carcinogenic activity" of 4-MEI in animal studies, based on increased incidences of alveolar/bronchiolar neoplasms [(4)]. In 2011, OEHHA added 4-MEI as a carcinogen and "No Significant Risk Level (NSRL)" for 4-MEI was calculated as 29 μg/day (Health and Safety Code, OEHHA, 2011). Recently, IARC classified 4-MEI as a group

2B compound, possibly carcinogenic to humans [5]. Considering the fact that a high level of 4-MEI is found in the caramel coloring, a reduction of 4 -MEI in food commodities including carbonated beverages, beer and wine, by reducing the use of caramel coloring has been a recent focus in food industry.

γ-irradiation has recently become an alternative processing technology to preserve food with minimum interruption of its nutritional and sensory properties [6]. Besides the traditional purpose of using γ-irradiation, significant reduction of pathogenic bacteria for sanitary purposes, new perspectives of γ-irradiation have been reported, that is, reduction of chemical toxicants such as N-nitrosamines in sausage [7-8], biogenic amines in fermented soybean paste [9], allergenicity of foods [10-12]. To this date, no studies were conducted previously to apply γ-irradiation on reduction of carcinogenic 4-MEI in food application. Therefore, the objective of this study was to evaluate the effects of γ-irradiation on reduction of 4-MEI in caramel coloring III.

MATERIALS AND METHODS

Preparation of caramel coloring

Caramel coloring III was created in the lab according to the method described in the previous study [3, 13]. Briefly, a 30-ml of aqueous solution containing 0.1M D-glucose (purity >99.5%, Sigma-Aldrich Chemical Co. St. Louis, MO) and ammonium hydroxide (purity >29%, Sigma-Aldrich Chemical Co. St. Louis, MO) were prepared in a swingtop bottle and heated at 100 °C for 2 hr in the oven. After that, the samples were cooled to room temperature and reacted samples were diluted with deionized water at 10% solution. Samples were stored at 4°C til the irradiation treatment. One-ml of caramel coloring in tightly capped ependorf tube were irradiated at 0, 1, 3, 5, 10, 30, 50, and 100 kGy in a cobalt-60 irradiator (point source AECL, IR-221, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) with 11.1 peta-becquerel (PBq) source strength and operated at a dose rate of 10 kGy/h. The dosimeter was calibrated using an International Atomic Energy Agency (Vienna, Austria) standard.

Analysis of 4-methylimidazole (4-MEI)

Quantitative analysis of 4-MEI in caramel coloring III was conducted using a Hewlett Packard 1100 liquid chromatograph interfaced to an Applied Biosystems API 2000 MS/MS via an electrospray ionization (ESI) source operating in the positive ion mode at 400°C with nitrogen gas. Chromatographic separation was accomplished with a 100 × 4.6 mm Varian Polaris RP column (Varian, Walnut Creek, CA, USA) with a 3 μm particle size. The mobile phase was water (15 mmol ammonium hydroxide, solvent A) and acetonitrile (15 mmol ammonium hydroxide, solvent B). A linear gradient that follows at 0 – 3 min, A/B= 98/2; at 10 – 13 min, 60/40; and at 15 – 25 min, 98/2 was used with flow rate at 0.4 mL/min. Under these conditions, 4-MI was eluted at 9.1 min.

The mass spectrometeric data were acquired in multiple reactions monitoring (MRM) mode. The 4-MEI peak area from the transition of m/z 83 to m/z 56 was used for the conformation and quantification. A standard calibration curve was prepared in 0.25, 0.5, 1.0, 5.0, and 10.0 μg/mL concentrations by diluting the standard solution (10 mg/L) with HPLC grade water and each solution (20 μL) was injected to LC-MS. Recovery efficiency of 4-MEI was examined using 10 μg/mL aqueous solutions.

Color measurement of caramel coloring

Caramel coloring dissolved in distilled water (10 mg/mL) were transferred into a glass cell (CM A-98, 10 mm in width) and measured with a Color Difference Meter (Spectrophotometer CM-3500d, Minolta Co., Ltd. Osaka, Japan). The instrument was calibrated to standard black and white tiles before an analysis. A large size aperture was used and the measurements were triplicated.

Statistical Validation

Means and standard deviations for results were calculated using the SPSS software version 10.0 (2000). Results are expressed as the mean ±

standard deviation of triplicate analyses of data. Analysis of Variance (ANOVA) followed by duncan's multiple range test was conducted for means separation at α=0.05.

RESULTS AND DISCUSSION

The concentration of 4-MEI in caramel coloring treated with different level of γ-irradiation

A significant change was observed on the amount of 4-MEI in caramel coloring III according to different dosage levels of γ-irradiation ($p<0.05$; figure 1). A control caramel coloring that did not receive irradiation treatment, 714.92 mg/kg of 4-MEI was detected. No significant difference of 4-MEI level was seen on caramel coloring received 1 kGy and non-treated sample ($p>0.05$). From the caramel colorings treated with 3 kGy irradiation, significant reduction of 4-MEI was observed in comparison to non-treated caramel colorings ($p<0.05$), and further increasing irradiation dose also significantly decreased the amount of 4-MEI in caramel coloring. The maximum irradiation dose (100 kGy) in this study decreased 4-MEI concentration down to 658.34 mg/kg, which equates 7.91% reduction, compare to the non-irradiated sample.

Caramel coloring III is manufactured by the controlled heat treatment of rich carbohydrate sources with ammonium compounds and this caramelization process lead to formation of a wide range of by-product including 2- and 4-methylimidazole. The analytical evidence suggests the presence of 4-methylimidazole in the range of 50-700 mg/kg in caramel colors depending upon the process of manufacture [13]. Due to the toxicity of 4-MEI, The European Commission [14] and JECFA [15] have established the maximum level of 4-MEI in class III caramel coloring as lower than 250 mg/kg [4]. In current study, the caramel coloring was created by simulating the condition for Mailard reaction, therefore the high level of 4-MEI in the caramel coloring is evident. In current caramel coloring model system, current result indicated that γ-irradiation reduced 4-MEI. Therefore, γ-irradiation can be utilized as an alternative method to improve the usage of caramel coloring.

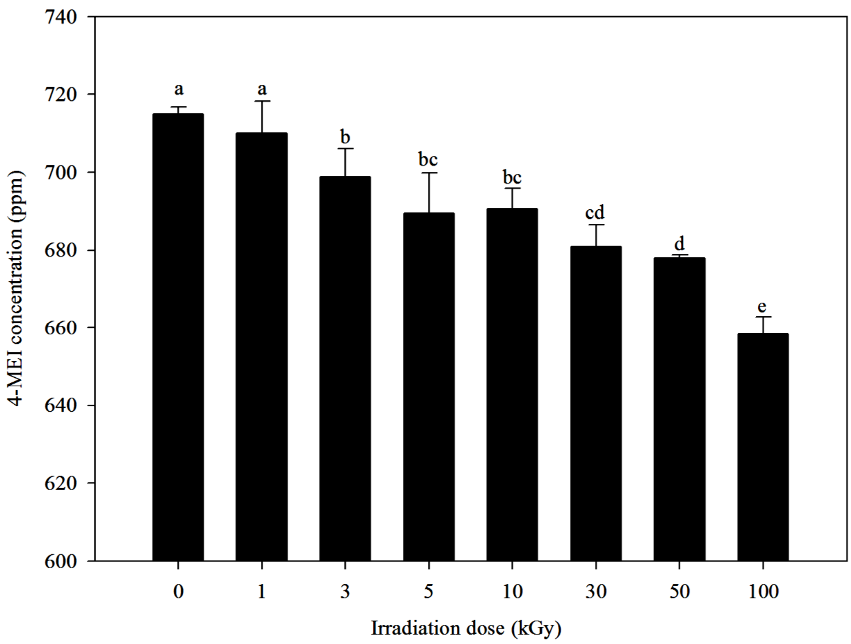

Figure 1. The concentration of 4-Methylimidazole in caramel coloring according to the irradiation dosage (kGy) Alphabetical letter above the bar that does not share the same letter represent a significant difference at $p<0.05$.

Color measurement result of caramel coloring treated with different level of γ-irradiation

Current work monitored the color changes of irradiated caramel coloring, in order to meet the purpose of adding caramel coloring in food and beverage products. Color measurement result of caramel coloring according to different irradiation dosage can be found in table 1. No significant changes were observed in L* (brightness), a* (redness) and b* (yellowness) values of caramel colorings treated with γ-irradiation, regardless of its dosage level, when comparing the non-irradiated caramel coloring ($p>0.05$). Overall color difference (DE) also did not show significant changes until the maximum γ-radiation dose (100 kGy). These results indicated ɣ-irradiation did not induce the color changes of caramel coloring III after γ-irradiation.

A major chemical reaction involved in manufacturing caramel coloring III is maillard reaction, non-enzymatic browning reaction between carbohydrate and amine compound during heating process [3]. Previous work suggested that γ-irradiation could lead to non-enzymatic browning reactions similar to those induced by heat-treated food, by observance of increasing color intensity of irradiated foods compared to non-irradiated foods [16]. Others also reported that the non-enzymatic browning reaction by γ-irradiation was influenced by the conditions of a system such as the reactant type, pH, medium, or temperature in a γ-irradiated aqueous model solution [17-19]. In this study, γ-irradiation applied to final product of caramel coloring, in which Maillard reaction was already taken place during manufacturing process prior to irradiation treatment. Therefore, caramel coloring from current study may not experience the further Maillard reaction during γ-irradiation treatment; hence no color change by γ-irradiation was observed, while significant 4-MEI reduction was taken place in caramel coloring.

Table 1. Color measurement result of caramel coloring according to different irradiation dosage.

Irradiation dose (kGy)	Hunter color values[a]			ΔE[b]
	L*-value	a*-value	b*-value	
0	0.44 ± 60.39	0.13 ± 1.32	0.41 ± 13.56-	
1	0.30 ± 60.70	0.08 ± 1.26	0.23 ± 13.82-	0.39 ± 0.80
3	0.48 ± 60.50	0.08 ± 1.52	0.46 ± 13.40-	0.37 ± 0.69
5	0.54 ± 60.75	0.54 ± 1.48	0.04 ± 13.47-	0.55 ± 0.83
10	0.65 ± 60.33	0.36 ± 1.67	0.17 ± 13.10-	0.07 ± 0.69
30	0.20 ± 60.36	0.20 ± 1.29	0.57 ± 13.28-	0.16 ± 0.45
50	0.58 ± 60.87	0.39 ± 1.51	0.23 ± 13.02-	0.19 ± 0.90
100	0.07 ± 60.71	0.07 ± 1.56	0.29 ± 13.25-	0.37 ± 0.76

[a] Numbers in the table represent Mean ± standard deviation of triplicate analyses of color measurement
[b] Overall color difference $((DL^*)^2+(Da^*)^2+(Db^*)^2)^{1/2}$
No significant differences in a column was observed among caramel colorings treated with different level of gamma irradiation ($p>0.05$).

CONCLUSION

Thereby, γ-irradiation could be used as an alternative processing method to improve the quality of caramel coloring by reducing 4-MEI content without its color change. However, further studies are necessary to verify the exact reducing mechanism of 4-MEI by γ-irradiation and/or application to food model system studies for industrial application.

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Conflict of interest: *Declared none.*

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