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Irradiation-induced Maillard reaction with glucosamine: an advance method to modify chitosan

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Abstract: In this study, Maillard reaction with glucosamine (GA) induced by gamma irradiation was applied for improving the stability and antibacterial activity under alkaline condition of chitosan (CTS) and oligochitosan (OC). The mixture solutions of CT and/or OC with GA were irradiated at 25 kGy to form the Maillard reaction products (MRPs), respectively. The formations of MRPs were determined by UV-vis spectrophotometric analyses at the wavelength of 284 nm and 420 nm. The stability improvement of the MRP solutions in alkaline condition was evaluated via the increase of pH value at the precipitation point. The antibacterial activity of the solutions against *Escherichia coli* was also investigated. The results showed that the CTS-GA MRPs and OC-GA MRPs solutions could remain their stability at pH 7, and the pH values at the precipitation point were found about 7.4 and 11.5, respectively. Moreover, at pH 7, the MRPs solutions exhibited high antibacterial activity with the reduction of ~ 4 log CFU/ml over control sample. Furthermore, 5-hydroxymethylfurfural (5-HMF), a known cytotoxic product formed by heat-induced Maillard reaction was not detected in both irradiated CTS-GA and OC-GA solution. These results proved that the irradiation-induced Maillard reaction is an effective strategy to modify chitosan, and the MPRs of CT/OS with GA had a great potential to use as a natural preservative for food applications.

Keywords: *Maillard reaction, gamma irradiation, chitosan, solution stability, antibacterial activity*

I. INTRODUCTION

Chitin, a linear polymer of β-(1-4)-N-acetyl-D-glucosamine, is the second-most abundant and distributed polysaccharide in nature after cellulose. It is a major component of fungal cell wall and exoskeleton of insects, cephalopods and crustaceans [1]. In practice, the extraction of chitin is mainly exploited from marine processing waste, an abundant and renewable source [2]. Chitosan is a polycationic biopolymer derived by deacetylation of chitin under strong alkaline condition. It is biodegradable, nontoxic and non-allergenic. Besides, chitosan also exhibits several interesting biological activities such as antioxidative, antimicrobial, and anticancer activity as well as technical properties such as fat uptake, gelation and emulsification [1]. Therefore, chitosan and it derivatives have gained great interest as a potentially renewable resource, and have been widely applied in agriculture, food industry, pharmacy, medicine and environment field [3]. In terms of safety,

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shrimp-derived chitosan has been approved by US-FDA, EU and K-FDA for dietary use and granted GRAS (Generally Recognized as Safe) status which removes some of the earlier regulatory restriction on its use in foods [4-6]. However, the application of chitosan in many fields is still restricted because of its insolubility as well as its decrease in biological activities at neutral or basic pH [7]. Therefore, there are many efforts to improve the solubility and biological activities of chitosan based on chemical or enzymatic modifications, in which chemical modifications are generally not preferred in food application [8].

The Maillard reaction is a non-enzymatic reaction, in which the carbonyl group of reducing ends in carbohydrates links with the amino groups of amino acid, proteins or any nitrogenous compounds. This reaction also lead to the formation of a myriad products termed Maillard reaction products (MRPs) including antioxidant and antibacterial compounds, which make it an idea strategy to improve chitosan's properties. Favorably, the reaction can take place more rapidly by irradiation method at room temperature without forming any toxic by-products, such as 5-hydroxymethylfurfural [9]. There have been several studies focusing on synthesis of chitosan-sugar MRPs by different methods and their biological activities [1]. However, up to now, there have been few reports on the application of gamma irradiation to induce the Maillard reaction between chitosan and glucosamine for the improvement of its solubility and antibacterial activity. In this study, the MRPs of CTS or OC with GA were prepared by gamma irradiation method, and their solution stability and antibacterial activity against *Escheriachia coli* at alkaline pH were investigated.

II. CONTENT

A. Material and methods

Materials: Chitosan from shrimp shell with the average molecular weight (Mw) of ~120 kDa and the degree of deacetylation of ~ 90 was supplied by Sun Eco Green Import Export Company Limited, Vietnam. Glucosamine was purchased from Merk (Germany). The *E. coli* ATCC 51813 was provided by Metabolic Biology Laboratory, University of Science, Ho Chi Minh City. The Luria- Bertani medium and agar plates used for bacteria incubation were purchased from Himedia, India. 5-hydroxymethylfurfural was purchased from Sigma-Aldrich, USA. Other chemicals such as lactic acid, NaOH… were used in analytical grade. Distilled water was used for all experiments.

Preparation of CT-GA and OC-GA MRPs

The preparation of CTS-GA and OC-GA MRPs solutions was carried out according to the method of Rao et al. (2011) with some modifications [9]. A solution of 4% OC (w/v) was obtained by gamma irradiation degradation method of chitosan solution containing 0.5% (v/v) H_2O_2 and lactic acid (2%) at the dose of 21 kGy and then diluted in water for the final OC 2% solution. Similarly, a solution of 2% CTS in lactic acid (1%) was prepared. In addition, a solution of 1% glucosamine in distilled water was also prepared. The CTS or OC solutions were mixed with the GA solution in the ration 1:1 (v/v) separately in order to obtain two mixture solutions, namely A solution, containing of CTS 1% and GA 0.5%, and B solution, containing 1% OC and 0.5% GA. All solutions were exposed to γ-irradiation with the dose of 25 kGy by a Gamma-cell 5000 (BRIT, Mumbai, India) with a dose rate of 1.3 kGy/h. A and B solutions after irradiation were named as A25 and B25, respectively.

Spectrophotometric analyses

The irradiated solutions were characterized by spectrophotometric analyses as described by Chawla et al. (2007) [10]. The as-prepared solutions were appropriately diluted and the absorbance was measured at 284 nm (early Maillard reaction products) and 420 nm (late Maillard reaction products) for determining UV absorbance and browning intensity, respectively by a UV–vis spectrophotometer, Jasco-V630, Japan.

pH stability test

pH stability represents the pH range for stable solubility of the CTS/OC solutions. The stability of as-prepared solutions in different pH ranges was investigated by the method of Nguyen et al. (2017) with some modifications [11]. Briefly, the pH of four solutions: A, B, A25 and B25 were adjusted to the precipitate point by adding 0.1 N NaOH solution drop-wise until absorbance of the solutions at 600 nm was higher than 0.1 and at this point the solubility was deemed unstable. The absorbance of the solution was monitored by a UV-vis spectrophotometer, Jasco-V630, Japan. The pH value of solution at the precipitate point was measured by pH Meter, Mettler Toledo SevenExcellence S400, USA.

Detection of 5-hydroxylmethylfurfural

5-hydroxylmethylfurfural was detected by using reverse phase high performance liquid chromatography (RP-HPLC) using UV detector at 284 nm, according to the method described by Theobal et al. (1998) with some modifications [12]. Chromatography analyses were carried out with an Agilent 1260 Infinity HPLC system, which comprises a degasser (ElmaSonic Untrasound Vessel), UV-vis detector and autosampler. Separation was performed on a Restek Ultra Aqueous C18 Column (250 mm × 4.6 mm), with a 5 μm particle size. The samples were filtered through a 0.45 μm membrane and then injected directly to the chromatograph. The separations were carried out isocratically at room temperature, using a mixture of methanol-water (15:85, v/v) at a flow-rate of 1 ml/min for the mobile phase. The injection volume was 10 μl and the run time was 20 min. Monitoring of the analytes was carried out by a UV detector at 284 nm wavelength. A 5-HMF solution (20 μg/ml) was used as standard and heat-induced CT-GA MRPs solution was used as positive control.

Antibacterial activity

Escherichia coli ATCC 51813 was used to evaluate the antibacterial activity of as-prepared MRPs solutions formed by 25 kGy in both qualitative and quantitative tests. Un-irradiated solutions, the A and B solution, were used as positive controls.

In qualitative test, the agar well diffusion method was used as described by Balouiri et al. (2016) [13]. The LB agar plates, after being spread by *E. coli* (~ 10^4 CFU/ml) were punched aseptically with a sterile tip to form wells with a diameter of ~5 mm. 100 μl of A25 and B25 solutions were introduced to wells separately. Sterile distilled water, GA solution and un-irradiated solutions were also added to other four wells as the controls. Then the plates were incubated overnight at 37°C and monitored colony formation.

In quantitative test, the antibacterial activity against *E. coli* was investigated at pH 7 to prove the advantage of MRPs in alkaline condition over native CTS and/or OC solution. Briefly, 1 ml of A, B, A25 and B25 solution was added separately into 19 ml *E. coli* suspensions (10^7 CFU/ml), in which the pH was already adjusted to 7 by NaOH 0.1N solution. Then the mixtures were shaken at 150 rpm for 4 hours and subsequently

determined the survival cell density by spread plate technique. The control sample only containing bacteria suspension and water was carried out in parallel. The antimicrobial activity of the solutions was expressed by the reduction of bacteria density (log CFU/ml) of the testing mixture in comparison with that of the control sample.

B. Results and discussion

Preparation of the MRPs and Spectrophotometric analyses

In Fig.1 (i) the visual colors of the solutions were changed and become browner after irradiation treatment. This result was also confirmed by UV-vis spectrophotometric analyses, where there were the increase of absorbance intensity at 284 and 420 nm (Fig. 1 (ii)). The same results were also recorded in other studies where the protein/sugar solutions were treated by heating [14] or irradiating [9, 15]. In Maillard reaction, the intermediate stage products can be detected by UV-absorbance at 284 nm while absorbance at 420 nm prefers to the detection of the final stage products [16]. Therefore, the obtained results confirmed that MRPs were formed effectively by 25kGy irradiation treatment.

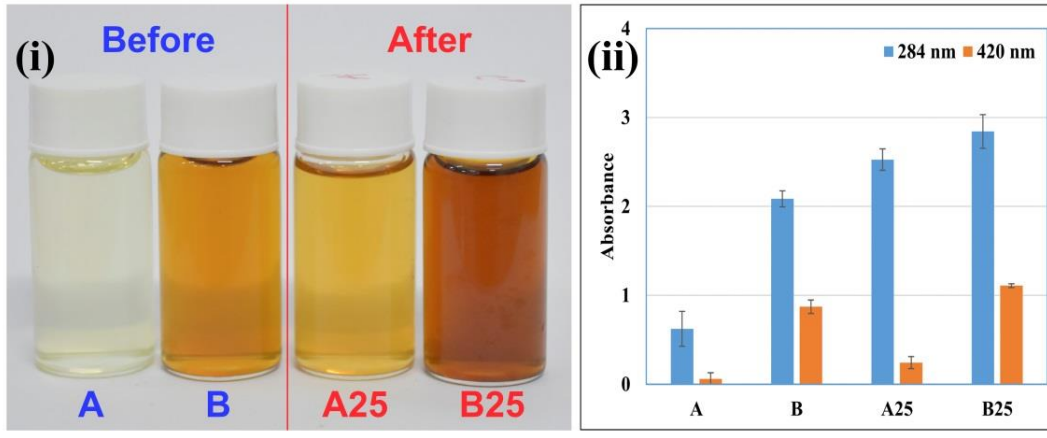

Fig. 1. The visual color (i) and absorbance intensity at 284 and 420 nm (ii) of the solutions: A, B, A25 and B25

pH stability test

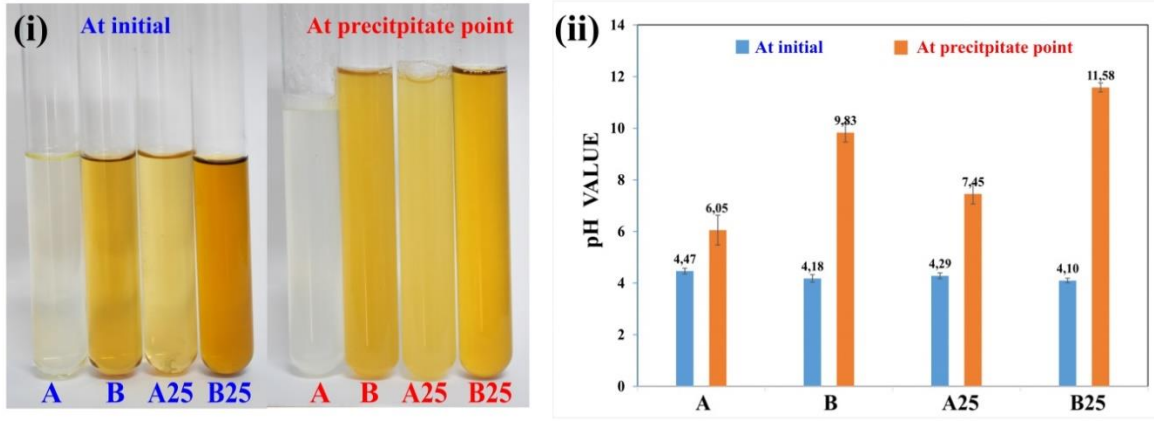

Fig. 2. The visual color (i) and pH values (ii) of the solutions at initial and at precipitate point

The Fig. 2 presented the visual color and pH values of the solutions at initial and at precipitate point. At the precipitate point, the coagulation was formed in the solutions (Fig. 2 (i)) with an increase of their absorbance density at 600 nm. The results in Fig. 2 (ii) revealed that irradiation treatment could increase the pH values at precipitate point of the solutions. Particularly, after irradiation pH value at precipitate point of the CTS-GA solution was increased from 6.05 to 7.45 while that of the OC-GA solution was increase from 9.83 to 11.58. These results indicated that the Maillard reaction could improve the solubility of CT/OC solution effectively. The same results were also reported in the study of Chung et al. (2005), in which, heat-induced MRP solution of chitosan-glucose could maintain the solubility up to pH 10 [17].

Detection of 5-hydroxylmethylfurfural

5-Hydroxylmethylfurfural is one of intermediate products of the Maillard reaction [18]. 5-HMF is cytotoxic at high concentration, causing irritation to eyes, upper respiratory tract, skin and mucous membranes; its reported oral LD50 value in rats was 3.1 g/kg body weight [19]. After the RP-HPLC separation, the characteristic chromatographic peak of 5-HMF in the standard solution appeared at the retention time of ~7.0 min (data not shown). The HPLC chromatograms of MRPs solutions prepared by heating and irradiating method are shown in Fig. 3. A peak corresponding to 5-HMF was recorded at the retention time of ~7.0 min in case of heat-induced CTS-GA MRPs solution, whereas there was no 5-HMF peak in chromatograms of both A25 and B25 solution. Oh et al. (2006) reported that no furfurals were detected in irradiated sugar-amino acid solution, whereas these compounds were found in heated sugar-amino acid solutions [15]. The results in this study again confirmed that irradiation does not cause 5-HMF in sugar-amino acid solution.

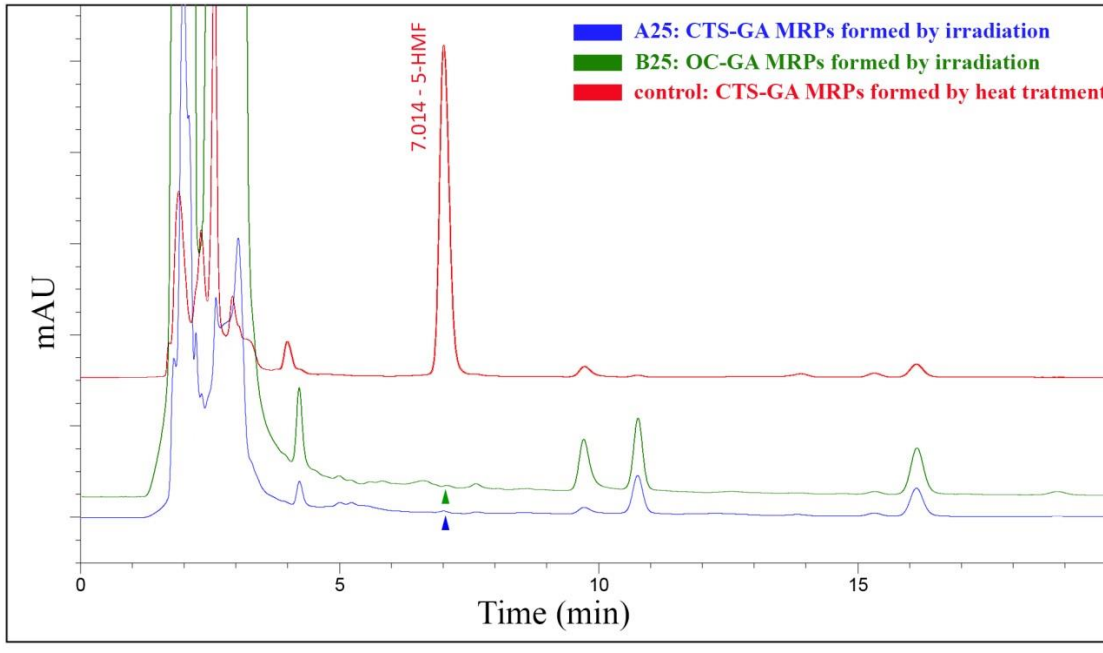

Fig. 3. HPLC chromatograms of MRP solutions formed by heat treatment (control) and by irradiation (A25 and B25)

Antibacterial activity

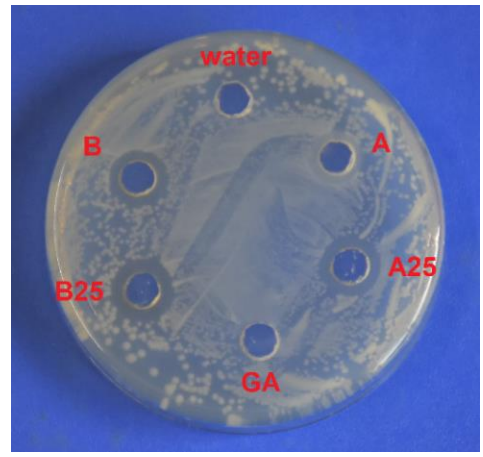

Fig. 4. The results of agar well diffusion test (water and GA solution) and CTS/OC-contained solutions (A, B, A25 and B25) and the control (GA solution and water)

In well diffusion test, the A, B, A25 and B25 solutions were able to form inhibition growth zone against *E. coli* while water and the GA solution were not (Fig. 3). This result meant that water and GA solution did not exhibit antibacterial activity. Therefore, the antibacterial abilities of the CTS-GA and OC-GA solution were come from the role of CTS and OS, which had been reported in previous studies [20-22]. The antibacterial ability of solutions could be predictable through the diameters of their growth inhibition zones formed on the plate, briefly the larger the diameters represent the higher antibacterial activity [13]. Hence, B25 could be the solution with the strongest antibacterial activity on the plate. In addition, the growth inhibition zone diameters of irradiated solutions were obviously larger than that of un-irradiated ones. This result proved that the modifications formed upon irradiation-induced Maillard reaction have significantly antibacterial potential.

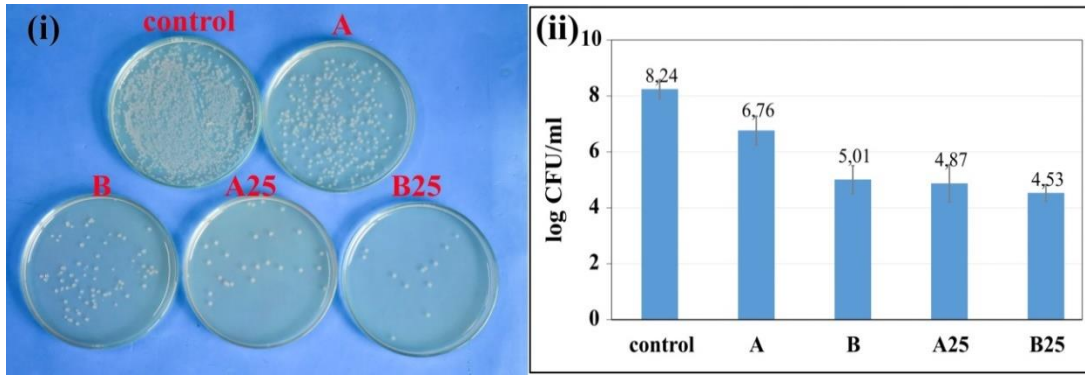

Fig. 5. The viable colonies on plates (i) and the viable bacteria density of the mixtures after the exposure in pH 7 (ii)

In Fig. 4, there was a decrease in bacterial density of tested mixtures in comparison with the control. Because the lower the viable bacteria density represents the higher antibacterial ability, the result indicated that at pH 7, B25 solution expressed the highest antibacterial activity, followed by A25, B and A solutions. Moreover, the irradiated solutions

exhibited higher antibacterial activity than un-irradiated ones. These results are consistent with the prediction result from qualitative test. As discussion above, the antibacterial ability of the A and B solution was mainly due to CTS and OS, however, at pH 7, CTS solution was precipitated while OS solution was not, hence the antibacterial activity of the B solution was higher than that of the A one. In the study of Rao et al. (2011), chitosan-glucose MPRs solution prepared by irradiation method also showed the higher antibacterial activity against *E. coli* at pH 7.2 than that of native chitosan one [9]. Thus, these results demonstrated that irradiation-induced Maillard reaction has effectively improved the antibacterial activity of chitosan/oligochitosan as well as maintained this effect even in alkaline pH.

III. CONCLUSIONS

Gamma irradiation from Co-60 source was effectively applied to induce Maillard reaction in CTS/OC-GA solutions. The obtained MRPs solutions possessed higher alkaline pH tolerance ability than that of the native CTS/OC ones. Moreover, the MRPs solutions without containing cytotoxic 5-hydroxymethylfurfural exhibited the significantly antibacterial activity against *E. coli* at pH 7. This study has also demonstrated the effectiveness and safety of irradiation-induced Maillard reaction as a novel method to enhance the solubility and antibacterial activity of CTS. Further studies are needed to explore potential application of such MRPs solution in food products.

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