STUDY ON pH TOLERANCE ABILITY AND ANTIBACTERIAL ACTIVITY OF MAILLARD REACTION PRODUCTS OF CHITOSAN AND OLIGOCHITOSAN WITH GLUCOSAMINE PREAPARED BY GAMMA IRRADIATION METHOD

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Abstract: The Maillard reaction products (MRPs) were synthesized during the gamma irradiation of mixtures of chitosan (CT) and/or oligochitosan (OC) with glucosamine (GA) at the dose of 25 kGy and investigated their some characteristics. The formations of MRPs were determined by UV-vis spectromectric analyses at the wavelength of 284 nm and 420 nm. The pH tolerance abilities of these solutions were determined through pH values at precipitate point. The antibacterial activity of the solutions against Escherichia coli was also investigated. The results showed that the CT-GA MRPs and OC-GA MRPs could be remain soluble at pH 7, and the pH values at precipitate-point were 7.4 and 11.5 respectively. Moreover, at pH 7, the MRPs solutions exhibited high antibacterial activity with Log 4 CFU/mL reduction in compared with the control. These results prove that the gamma-induce Mallard reaction is an effective strategy to modify chitosan, and the MPRs of CT/OC with GA had a great potential to replace synthesis additives as a natural preservative for food applications.

Keywords: Antibacterial activity, Chitosan, Maillard reaction, Oligochitosan, pH tolerance

1. INTRODUCTION

Today food safety is a global concern. The principles of food safety aim to prevent food from becoming contaminated and causing food poisoning. Many kinds of food are perishable by natural causes and need to be protected from spoilage during their preparation, storage and distribution. There are many preservation techniques to prevent the spoilage and pathogen in foods, such as: heat treatment, salting, acidification, drying or simply adding food preservatives. These are substances that can slow or prevent food spoilage caused by microorganisms or oxidation. The most common food preservative are synthetic chemicals, such as sorbates, benzoates, nitrates and nitrites [7]. However, nowadays there are more and more consumer awareness and concern regarding consuming these synthetic chemical additives even below the recommended limits as defined by regulatory agencies, such as the FDA. Therefore, there are numerous efforts to find alternative food preservatives and natural additives are supposed the most potential candidate because of their safety. The natural sources of these compounds are very diverse, including plant-extracted essential oil, animal enzyme, microbial-source bacteriocins and natural polymers [11]. Among these compounds, chitosan, a deacetylated derivative of chitin, has received tremendous interest due to its excellent properties such as non-toxicity, biocompatibility and biodegradability [10], especially its unique biological activities, including anti-flammatory, antimicrobial and antioxidative activity [9]. Therefore, there have been many studies to apply chitosan in food preservation, such as in fruit and vegetable [7], seafood [12],

meat and meat product [5]. However, the applications of chitossan are limited because of its poor solubility at neutral or basis pH [6]. For this reason, a numerous researches have been carried out to improve the solubility and/or increase the biological activities of chitosan upon chemical or enzymatic modifications.

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. Therefore, the Maillard reaction has been considered a possible strategy to improve chitosan's properties. More interesting, 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 bioactivities. However, up to now, there has been barely report on preparation of chitosan-glucosamine MRPs by irradiation method for food applications. In this study, the MRPs of chitosan (CT) or oligochitosan (OC) with glucosamine (GA) were firstly prepared by gamma irradiation method and then investigated their antibacterial activity against *Escheriachia coli*.

2. MATERIALS AND METHODS

Materials: Chitosan from shrimp shell with the average molecular weight (Mw) of 123.5 kDa and the degree of deacetylation of 93.3 % was supplied by a factory in Vung Tau province, Vietnam. Glucosamine was purchased from Merk (Germany). The *E. coli* ATCC 6538 was provided by Metabolic Biology Laboratory, University of Science, Ho Chi Minh City and preserved at Research and Development Department, VINAGAMMA, Ho Chi Minh City. The Luria- Bertani medium and agar plates used for bacteria incubation were purchased from Himedia, India. Other chemicals such as: lactic acid, NaOH, etc. are used in analytical grade. Distilled water is used for all experiments.

Preparation of CT-GA and OC-GA MRPs

The preparation of CT-GA and OC-GA MRPs solutions was carried out according to the method of Rao et al. (2011) with some modification [9]. A OC 4% (w/v) solution was obtained by gamma Co-60 ray irradiation degradation method of chitosan solution containing 0.5 % (w/v) H_2O_2 at the dose of 21 kGy and then diluted by water for the final OC 2 % solution. A 2% solution of chitosan in acetic acid (1%) was prepared. Similarly, 1 % (w/v) solutions of glucosamine in distilled water were prepared. The CT or OC solutions were mixed to the GA solutions with the ration 1:1 (v/v) separately in order to obtain two mixture solutions, namely A solution: CT 1% - GA 0.5%; B solution: OC 1% - GA 0.5 %. All solutions were exposed to γ-irradiation with the dose rate of 2.2 kGy/h for the dose of 25 kGy by a Gamma-cell 5000 (BRIT, Mumbai, India). A and B solution after irradiated were named as A25 and B25, respectively.

Spectrophotometric analyses

The irradiated solutions were characterized by spectrophotometric analyses described by Chawla et al. 2009 [2]. The 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

The solubility of as-prepare solutions in different pH levels was investigated by the method of Nguyen et al. 2017 with some modifications [8]. Briefly, the pH levels of four solutions: A, B, A25 and B25 were respectively adjusted by 0.1 N NaOH solution to the precipitate point, which was determine by by the change of solution transmittance at 600 nm on a UV-vis spectrophotometer, Jasco-V630, Japan. The pH values of these solutions before and after adjustment were measured by pH Meter, Mettler Toledo SevenExcellence S400, USA.

Antibacterial activity

Escherichia coli ATCC 6538 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, A and C solution, were used as positive controls.

In qualitative test, the agar well diffusion method was used [1]. The LB agar plates, after being spread by *E. coli* (~ 10^4 CFU/ml) on the surface, were punched aseptically with a sterile tip to form wells with a diameter of ~6 mm. 100 µl of A25 and B25 solutions were respectively introduced to separate wells. Water, glucosamine 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 the 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 chitosan/oligochitosan solutions. Briefly, 1 ml of A, B, A25 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 simultaneously. The antimicrobial activity of the solutions were expressed by the reduction of bacteria density (log CFU/ml) in the testing mixture in comparison with the control sample.

3. Results and discussion

Preparation of the MRPs and Spectrophotometric analyses

The A and B solutions were irradiated at 25kGy to form A25 and B25. Colors of the solutions were changed and become browner after irradiation treatment (Fig. 1 (i)). This result was also confirmed by spectrophotometric analysis, namely there was the increase of absorbance intensity at 284 and 420 nm (Fig. 1 (ii)). The same results were recorded in other studies where the protein/sugar solutions were treated by heating [2] or irradiating [11]. In Maillard reaction, the intermediate stage products can be detected by UV-absorbance at 284 nm while absorbance at 284 nm prefer to the detection of the final stage products [8]. Therefore, the obtained results confirm that MRPs were formed effectively by 25 kGy irradiation treatment.

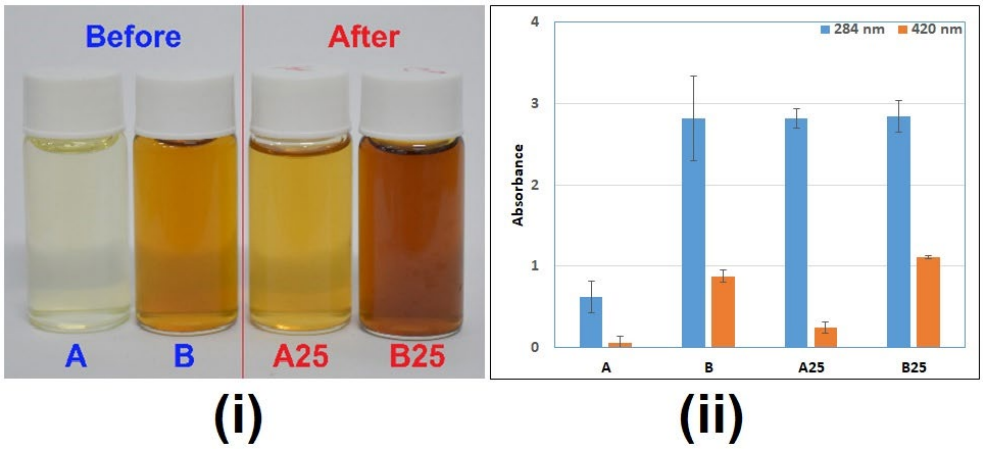

Fig. 1. The visual color (i) and Absorbance intensity at 284 and 420 nm (ii) of the solutions the solutions before and after irradiated (A: CT 1%- GA 0.5 %, B: OC 1% - GA 0.5 %, A25 and B25: A and B solution after irradiated at the dose of 25kGy respectively)

pH stability test

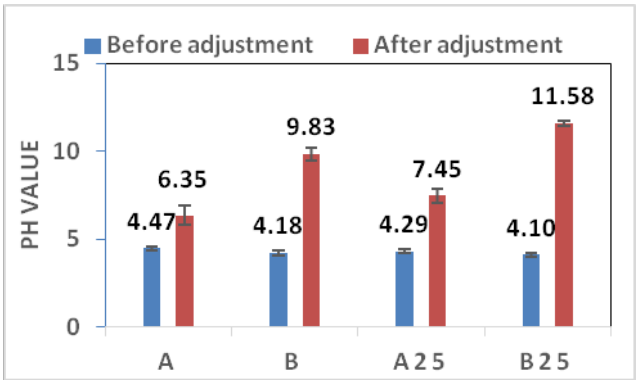

Fig. 2. pH values of the solutions before and after adjustment

(A: CT 1%- GA 0.5 %, B: OC 1% - GA 0.5 %, A25 and B25: A and B solution after irradiated at the dose of 25kGy respectively)

The Fig. 2. presented the pH values of the solutions at initial and at precipitate point. The results revealed that irradiation treatment could increase the pH values at precipitate point of the solutions, namely pH values at precipitate point of the CT-GA solution were increased from 6.35 to 7.45 while the increase from 9.83 to 11.58 were belong to OC-GA solution. These results indicate that the Maillard reaction could improve the solubility of CT/OC solution in alkaline pH condition effectively. The same results were also reported in the study of Chung et al., in which, chitosan-glucose MRPs prepared by heating treatment could be remain soluble at pH 10 [4].

Antibacterial activity

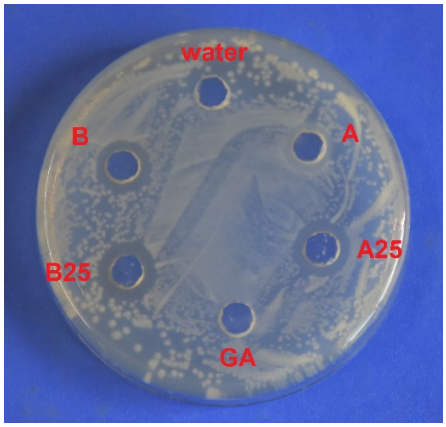

Fig. 3. The result of agar well diffusion test

(A: CT 1%- GA 0.5 %, B: OC 1% - GA 0.5 %, A25 and B25: A and B solution after irradiated at the dose of 25kGy respectively)

In well diffusion test, A, B, A25 and B25 were able to form inhibition zone against *E. coli* while water and the GA sample were not (Fig. 3). This meant that water and glucosamine did not exhibit the antibacterial activity in contrast to other samples. Therefore, the antibacterial abilities of the A and B sample were due to the role of CT and OC respectively. Moreover, the inhibition zone diameter of B25 sample was largest while the diameter of A sample was smallest. The antibacterial ability of samples could be primarily compared through the diameters of their inhibition zones formed on the plate [1], therefore the result indicated that the antibacterial activity of B25 sample is highest and A sample is lowest.

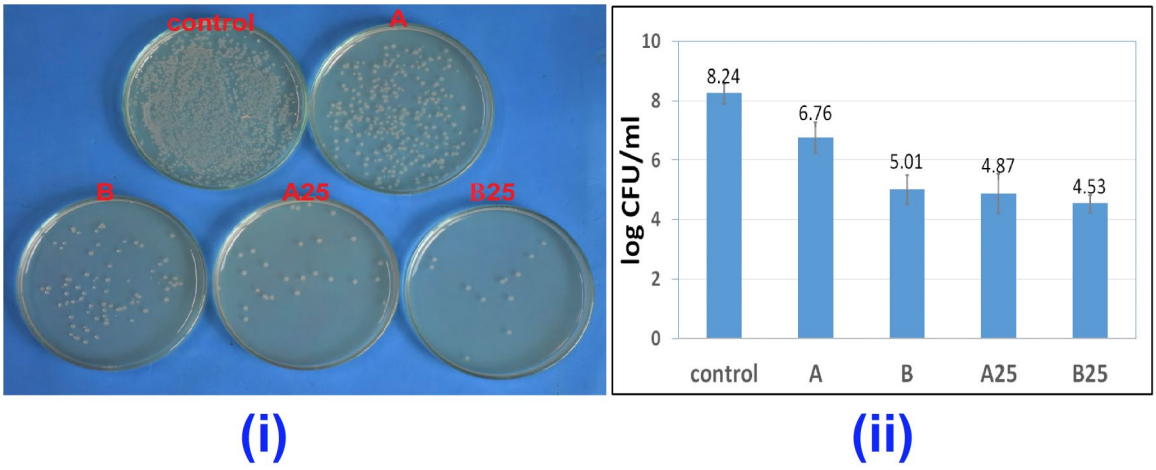

Fig. 4. The viable colonies on plate (i) and the viable bacteria density of the mixture after exposing time of 4 hours at pH 7 (ii) (A: CT 1%- GA 0.5 %, B: OC 1% - GA 0.5 %, A25 and B25: A and B solution after irradiated at the dose of 25kGy respectively)

In Fig. 4, there was an increase in bacterial density of tested samples over control. The lower the viable bacteria density is, the higher antibacterial ability of sample is. Therefore, the results revealed that at pH 7, B25 sample expressed the highest antibacterial activity, followed by A25, B and A sample. This result is consistent with the prediction from qualitative test. Moreover, as discussion above, the A and B sample exhibited their antibacterial ability due to the role of CT and OC, and in pH

7, CT was precipitated while OC was not so the antibacterial activity of B sample was high than A sample. More interesting, the irradiated solutions (A25 and B25) presented higher antibacterial activity than un-irradiated solutions. This result matches with the report of Rao et al., in which chitosan-glucose MPRs prepared by irradiation-induced Maillard reaction showed the higher antibacterial activity against *E. coli* at pH 7 after shaking 24 hours [9]. Hence, this result could demonstrate that Maillard reaction is able to improve effectively the antibacterial activity of chitosan and help it maintain the effect even in alkaline pH.

4. Conclusion

CT-GA solution and OC-GA solution were positively modified by gamma irradiation-induce Maillard reaction at 25 kGy successfully. By this method, the pH tolerance and antibacterial activity of irradiated solutions were improve significantly in comparison to the native one. Moreover, the MPRs solutions exhibited the high antibacterial activity against *E. coli* at pH 7. These findings indicate the the gamma-induce Mallard reaction is an effective strategy to extend the applications of chitosan as well as its derivatives, and furthermore the MPRs of CT/OC with GA have a great potential to replace synthesis additives as a natural preservative for food applications.

5. References

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NGHIÊN CỨU KHẢ NĂNG THÍCH ỨNG pH VÀ HOẠT TÍNH KHÁNG KHUẨN CỦA CÁC SẢN PHẨM PHẢN ỨNG MAILLARD GIỮA CHITOSAN VÀ OLIGOCHITOSAN VỚI GLUCOSAMINE TẠO ĐƯỢC BẰNG PHƯƠNG PHÁP CHIẾU XẠ GAMMA

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Tóm tắt: Các sản phẩm phản ứng Maillard (SPM) được chế tạo bằng cách chiếu xạ gamma các hỗn hợp dung dịch của chitosan (CT) và/hoặc oligochitosan (OC) với glucosamin (GA) tại liều xạ 25 kGy và đánh giá một số đặc tính của chúng. Sự hình thành của SPM được xác nhận bằng phân tích quang phổ tử ngoại tại bước sóng 284 nm và 420 nm. Khả năng thích ứng pH của các dung dịch này được xác định thông qua giá trị pH tại điểm bắt đầu kết tủa. Hoạt tính kháng khuẩn của các dung dịch chitosan đối với *Escherichia coli* cũng được khảo sát. Kết quả cho thấy CT-GA SPM và OC-GA SPM vẫn có thể tan trong nước tại pH 7, và giá trị pH kết tủa của các dung dịch này lần lượt là 7,4 và 11,5. Ngoài ra, tại pH 7, các dung dịch SPM còn thể hiện hoạt tính kháng khuẩn mạnh với khả năng làm giảm mật độ tế bào 4 bậc lũy thừa (Log CFU/ml) so với dung dịch đối chứng. Các kết quả này chứng minh rằng phản ứng Maillard gây bởi chiếu xạ là một cách thức hữu hiệu để nâng cao hoạt tính sinh học của chitosan, đồng thời các SPM của CT/OC với GA cũng cho thấy tiềm năng ứng dụng rất lớn trong việc thay thế các chất bảo quản hóa học trong thực phẩm.

Từ khóa: Chitosan, thích ứng pH, hoạt tính kháng khuẩn, Oligochitosan, phản ứng Maillard.