Antibacterial activity and preservative effect for pork tenderloin of chitosan - glucosamine Maillard reaction products prepared by gamma irradiation

Anh Quoc Le[1, 2, 3*], Van Phu Dang[3], Ngoc Duy Nguyen[3], Chi Thuan Nguyen[3], Quoc Hien Nguyen[4]

[1]Faculty of Biology - Biotechnology, University of Science, Vietnam National University - Ho Chi Minh City, 227 Nguyen Van Cu Street, District 5, Ho Chi Minh City, Vietnam

[2]Vietnam National University - Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

[3]Research and Development Center for Radiation Technology, Vietnam Atomic Energy Institute, 202A Street 11, Linh Xuan Ward, Thu Duc City, Ho Chi Minh City, Vietnam

[4]Vietnam Atomic Energy Institute, 59 Ly Thuong Kiet Street, Tran Hung Dao Ward, Hoan Kiem District, Hanoi, Vietnam

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Abstract:

A mixed solution of chitosan (CTS) (1.6%) and glucosamine (GA) (0.4%) was irradiated by gamma rays from a Co-60 source to form Maillard reaction products (MRPs). Upon gamma-ray irradiation, the colour of the mixture solution became browner. The resultant MRP solution was characterised by spectrophotometric analyses at the wavelengths of 284 nm and 420 nm. The antibacterial activity of the CTS-GA MRP solution was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, and the minimum inhibitory concentration (MIC) value against these bacteria was also determined. The results showed that the CTS-GA MRP solution exhibited high antibacterial activity against both bacteria. Furthermore, pork tenderloin soaked in CTS-GA MRP solution for 10 min could extend the storage time by 8 days at 5°C, with higher protein content and lower total aerobic microorganism count than the control. Therefore, this CTS-GA MRP solution has the potential to be applied as a natural preservative agent for meat and meat products.

Keywords: **antibacterial effect, chitosan, glucosamine, Maillard reaction, pork tenderloin.**

Classification numbers: **3.4, 3.5, 3.6**

1. Introduction

Currently, foodborne illnesses caused by pathogens represent a global challenge with significant implications for human health. According to the World Health Organisation (WHO), foodborne illnesses are responsible for 420,000 deaths worldwide annually from 2007 to 2015 [1]. Bacteria, especially *Salmonella typhi*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, are the causative agents for two-thirds of human foodborne illnesses. Additionally, meat, dairy products, and eggs are the main foods that expose people to these zoonotic bacteria [2]. On the other hand, food waste and food loss, estimated at 1.3 million tonnes annually, pose challenges to food security [3]. Hence, more effort is needed to develop new preservation methods, prevent foodborne illnesses, and reduce food waste. Due to the widespread use of artificial preservatives and consumers' growing concerns about health issues, natural antibacterial agents have been explored as a potential solution for food preservation.

Chitosan, a cationic polysaccharide commercially prepared by alkaline deacetylation of chitin, has been receiving significant attention due to its excellent biological properties [4, 5]. Besides being biodegradable, biocompatible, and non-toxic, this polysaccharide also exhibits antibacterial, antioxidant [6, 7], fat uptake [8], emulsification [9], and edible film-forming properties [10]. Furthermore, CTS derived from shrimp has been recognised as Generally Recognized as Safe (GRAS) in the USA since 2001, thus broadening its applications in

*Corresponding author: Email: anhquoc1704@gmail.com

many fields, including food applications [7]. In fact, in recent decades, the applications of CTS as a preservative for many types of food have been widely reported in many studies, such as for fruits and vegetables [11, 12], seafood [13], and meat and meat products [14, 15]. Nevertheless, applications of CTS in many fields are still restricted due to its insolubility in water and its reduced biological activities at neutral or basic pH [4]. Therefore, many efforts have been made to improve the solubility and biological activities of CTS through chemical or enzymatic modifications, with chemical modifications generally being less preferred in food applications [5].

The Maillard reaction (MR), a non-enzymatic browning reaction, is a condensation reaction between the carbonyl groups of reducing sugars and the amino groups of amino acids, proteins, or any nitrogenous compounds through heating or irradiation [16]. This complex reaction occurs spontaneously during thermal food processing and forms a myriad of MRPs, which contribute to flavour formation and generate antibacterial and antioxidant compounds for food [17]. Additionally, it is generally agreed that a substantial amount of MRPs is taken up in the human daily diet [18]. Hence, MR is considered a desirable green method to improve the properties of CTS for food applications. Among the MRPs of CTS with various reducing polysaccharides, CTS-GA MRPs prepared by heat-induced MR were reported to exhibit impressive solubility and antibacterial activity in comparison with other MRPs and the native CTS [4]. More interestingly, it was also found that besides the heat-induced method, MR could take place rapidly by gamma-ray irradiation at room temperature without forming undesirable toxic by-products, such as 5-hydroxymethylfurfural [19-21]. In this study, the CTS-GA MRPs were prepared by the gamma Co-60 ray irradiation method, and the *in vitro* antibacterial activity and the preservative effect on fresh pork tenderloin during refrigerated storage were investigated.

2. Materials and methods

2.1. Materials

Shrimp-shell CTS with an average molecular weight of ~97 kDa and a degree of deacetylation of ~90% was supplied by Sun Eco Green Import Export Company Limited, Vietnam. GA was purchased from Merck, Germany. *Escherichia coli* ATCC 51813 and *Staphylococcus aureus* ATCC 25923 were provided by the Metabolic Biology Laboratory, University of Science, Vietnam National University - Ho Chi Minh City. The Mueller Hinton (MH) medium and agar were purchased from Himedia Laboratories Ltd, India. 5-Hydroxymethylfurfural was supplied by Sigma-Aldrich, USA. Other chemicals such as lactic acid, NaOH in analytical grade, and distilled water were used for all experiments.

2.2. Preparation of chitosan-glucosamine maillard reaction product solution

The CTS-GA MRP solution was prepared according to our previous study [22]. Briefly, CTS was dissolved in a 1% lactic acid solution to obtain a 2% CTS solution. A 2% GA solution in distilled water was also prepared. Four volumes of 2% CTS solution were mixed with one volume of 2% GA solution to obtain a mixture of CTS (1.6%) and GA (0.4%). This mixture was then exposed to γ-rays from a Co-60 source of a Gamma-cell 5000 (BRIT, India) with a dose of 25 kGy at a dose rate of 1.3 kGy/h to perform the MR. The irradiated solution was characterised by spectrophotometric analyses, with the absorbance of the solution after appropriate dilution measured respectively at 284 nm (intermediate MRPs) and 420 nm (late MRPs) using a UV-Vis spectrophotometer (Jasco-V630, Japan) [19, 20].

2.3. Evaluation of antibacterial activity

E. coli ATCC 51813 and *S. aureus* ATCC 25923 were used to evaluate the antibacterial activity of the CTS-GA MRP solution in both qualitative and quantitative tests. In the qualitative test, the antibacterial activity was investigated by the agar well diffusion method [23]. Briefly, MH agar plates, after being spread with *E. coli* or *S. aureus* (~10^4 CFU/ml), were punched aseptically to form wells with a diameter of ~5 mm. CTS-GA MRP solution (100 µl) was introduced into a well. GA solution and CTS-GA mixture solution were also added to other wells. The plates were then incubated overnight at 37°C and monitored for inhibition-zone formation. In the quantitative test, the antibacterial activity was evaluated by the MIC determination method at an alkaline pH condition (pH 7) using the broth dilution technique [24]. MIC is defined as the lowest concentration of antimicrobial agent required to prevent the growth of microorganisms after subculture on antibiotic-free media [25]. The CTS-GA MRP solution was dispensed into sterile MH broth by a two-fold serial dilution technique to obtain broths with dilutions from 2^{-1} to 2^{-8}. A broth containing only the nutrient broth without the MPRs served as the control. The 0.1 ml bacterial suspension containing about 10^8 CFU/ml was aseptically inoculated

into 10 ml of dilution broths separately. The broths were then incubated at 37°C for 24 h and observed for turbidity development by unaided eyes or by measuring the optical density at a wavelength of 600 nm (OD_{600}). The MIC was determined as the lowest CTS-GA MRP concentration in the tube that prevents visible growth [24].

2.4. Preservative effect of chitosan-glucosamine Maillard reaction product solution on pork tenderloin at cold storage

The pork tenderloin was sliced with an average weight of 25±5 g. The CTS-GA MRP solution was diluted in water to final concentrations of 500 and 1000 mg/l, respectively. Pork slices were soaked in the diluted solutions for 15 minutes before being packed in plastic containers. Water-soaked pork slices were performed in parallel to serve as the control. The pork slices were then stored in a refrigerator at 5±1°C. After 0, 7, and 14 days, the pork slices were tested for total crude protein and total plate count (TPC) using the Kjeldahl method [26] and the pour plate technique [27], respectively. The shelf-life or storage time of meat and meat products is the duration during which they retain their qualitative characteristics until the arrival of spoilage phenomena, including off-flavours, discolouration, gas production, and slime formation [28].

3. Results and discussion

3.1. Preparation of the chitosan-glucosamine Maillard reaction product solution and UV-Vis spectrophotometric analyses

The changes in colour and UV-Vis absorbance of the CTS-GA mixture solution after irradiation are presented in Fig. 1. Particularly, upon gamma-ray irradiation, the colour of the mixture solution became browner (Fig. 1A). This result was also confirmed by UV-Vis spectrophotometric analyses shown in Fig. 1B, where the absorbance intensity at 284 nm and 420 nm of the irradiated CTS-GA mixture solution increased in comparison to the non-irradiated one. Similar results have been obtained in other studies where protein-sugar mixture solutions were treated by heat [5, 7] or gamma-ray irradiation [19-21]. In the MR, intermediate-stage products can be detected by UV absorbance at 284 nm, while absorbance at 420 nm is preferred for detecting final-stage products [20]. Therefore, the increase in absorbance at 284 and 420 nm indicates that MRP was effectively formed by irradiation treatment at 25 kGy.

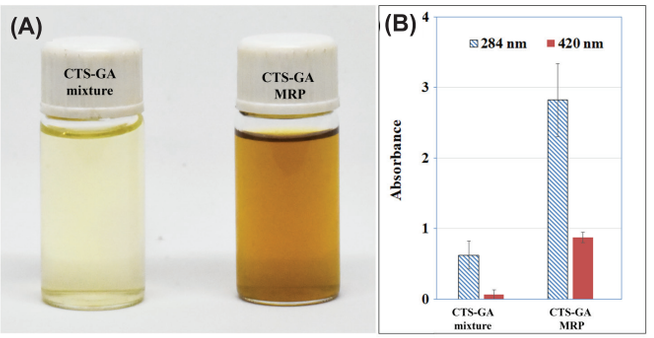

Fig. 1. Visual colour (A) and absorbance intensity (B) of the chitosan-glucosamine mixture and chitosan-glucosamine Maillard reaction product solution.

3.2. Antibacterial activity

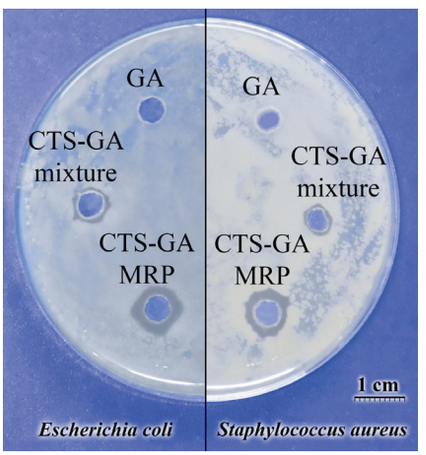

Fig. 2. Photograph of inhibition zone formation on *E. coli* and *S. aureus* plates by agar well diffusion test.

The antibacterial activity of the CTS-GA mixture and CTS-GA MRP solution against *E. coli* and *S. aureus* is displayed in Fig. 2. On both plates of *E. coli* and *S. aureus*, the CTS-GA mixture or CTS-GA MRP solution were able to form growth inhibition zones, while the control (the GA solution) did not. Interestingly, although the GA solution did not show antibacterial activity, the CTS-GA mixture exhibited this activity against both tested bacteria. This result proves that the antibacterial ability of the CTS-GA mixture is due to the role of CTS, as reported in previous studies [6, 29, 30]. Moreover, the antibacterial ability of different antibiotics can be relatively evaluated by the diameter of their growth inhibition zones formed on a bacterial plate, with larger diameters representing higher antibacterial activity [23]. Therefore, results in Fig. 2 indicate that the antibacterial activity of CTS was enhanced by the irradiation-induced MR.

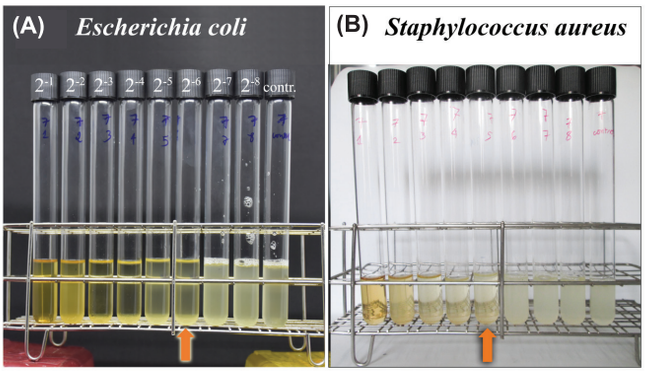

Fig. 3. Serial dilution tubes (A) and minimum inhibitory concentration values (B) of chitosan-glucosamine Maillard reaction product solution against *E. coli* and *S. aureus*.

Table 1. Minimum inhibitory concentration values of chitosan-glucosamine Maillard reaction product solution against *E. coli* and *S. aureus*.

	E. coli	*S. aureus*
Dilution ratio for Minimum inhibitory concentration	2^{-6}	2^{-5}
Minimum inhibitory concentration (mg/l)	312.5	625

The antibacterial activity of CTS and its derivatives has been widely studied. Generally, there are many factors affecting their antibacterial activity, including the type of microorganism [30]. This study investigated the antibacterial activity of the CTS-GA MRP solution on both *E. coli* and *S. aureus*. The MIC values of the CTS-GA MRP solution against these bacteria at pH 7 are shown in Fig. 3 and Table 1. After incubation, *E. coli* did not cause turbidity from the dilution $\geq 2^{-6}$, while that of *S. aureus* was observed from dilution $\geq 2^{-5}$ (Fig. 3). Hence, the MIC values of the CTS-GA MRP solution against *E. coli* and *S. aureus* were evaluated as 312.5 and 625 mg/l, respectively (Table 1). The higher MIC value represents the lower antibacterial activity. Thus, the results in Table 1 reveal that the CTS-GA MRP solution exhibited higher antibacterial activity against *E. coli* than against *S. aureus*. A similar phenomenon has also been reported in other studies [4, 19], where *E. coli* was found to be more sensitive to MRP of CTS and glucose/GA than *S. aureus*.

3.3. Preservative effect of chitosan-glucosamine Maillard reaction product solution on pork tenderloin at cold storage

During storage at 5°C, the pork tenderloin slices treated by the diluted CTS-GA MRP solutions at 500 and 1000 mg/l became discoloured and formed slimy liquid as well as foul odour on the 12th and 14th day, respectively, while the control showed spoilage on the 6th day (Fig. 4). This result indicated that the diluted

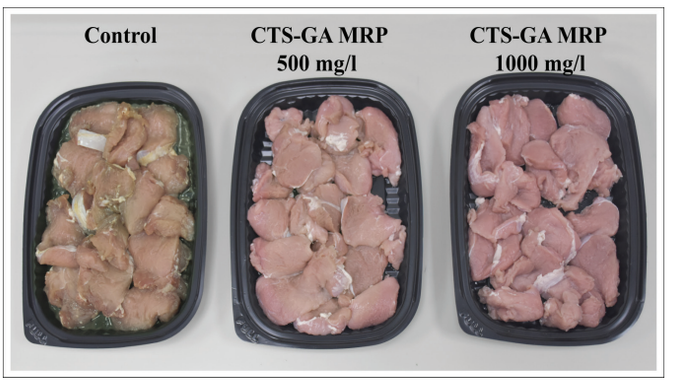

Fig. 4. Pork tenderloin slices treated by the diluted chitosan-glucosamine Maillard reaction product solutions after 6 days of storage at 5°C.

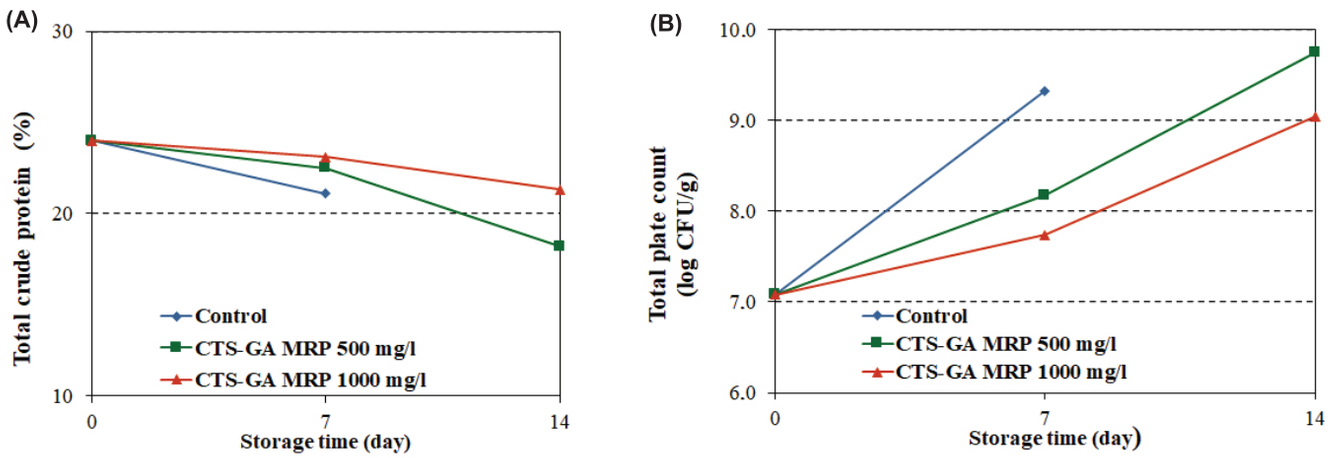

Fig. 5. Total crude protein (A) and total plate count of treated and untreated pork (B) tenderloin as a function of storage time at 5°C.

CTS-GA MRP solutions at 500 and 1000 mg/l could extend the shelf-life of the pork tenderloin at 5°C by 6 and 8 days, respectively, compared to the control. Furthermore, the total crude protein and TPC of the tenderloin, as presented in Fig. 5, also revealed that the diluted CTS-GA MRP solutions reduced protein loss and effectively inhibited microbial growth. These findings are consistent with the results of other studies [5, 31]. S.R. Kanatt, et al. (2008) [5] reported that adding a heat-induced CTS-glucose complex increased the shelf-life of lamb meat by more than two weeks during chilled storage and extended the shelf-life of pork cocktail salami to 28 days.

4. Conclusions

MR is considered a green strategy to improve the biological activities of CTS. In this study, the CTS-GA MRP solution prepared by the gamma Co-60 ray irradiation exhibited high antibacterial activity against *E. coli* and *S. aureus* at pH 7. Furthermore, the diluted CTS-GA MRP solutions at 500 and 1000 mg/l effectively extended the shelf-life of pork tenderloin for 6 and 8 days at cold storage, respectively. Therefore, the CTS-GA MRP solution could be used as a novel natural preservative for the food industry, particularly for meat and meat products. In addition, the gamma-ray irradiation method is considered a green method, which is favourable for large-scale production, including the production of MRPs.

CRediT author statement

Anh Quoc Le: Conceptualisation, Performed the experiment, Writing - Original draft preparation; Van Phu Dang: Performed the experiment, Original draft preparation; Ngoc Duy Nguyen: Performed the experiment; Chi Thuan Nguyen: Formal analysis, Writing, Reviewing and Editing; Quoc Hien Nguyen: Conceptualisation, Formal analysis, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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