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Synthesis of the Bioactive Conjugates of Nisin-Xanthan through the Maillard Reaction at Moderate Temperature

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ABSTRACT

Nisin is a natural heat resistance preserver with a wide range of applications in food industries. The main drawback of nisin is its weak activity against the most of Gram-negative bacteria. In this study, the antibacterial activities of nisin against Salmonella typhimurium, Klebsiella pneumoniae, Citrobacter freundii, and Escherichia coli improved via the Maillard reaction with xanthan. The nisin-xanthan conjugates analyzed by the ultraviolet, fluorescence, and Fourier transform infrared spectroscopies. The results showed temperature, the reaction duration, and the nisin-to-xanthan ratio affected the quality of the obtained conjugates. The antibacterial activity of 100 ppm of the conjugates was increased and reached 88.8, 98.7, and 97.7% respectively against S. aureus, S. typhimurium, and E. coli, when the nisin to xanthan ratio was increased from 1:1 to 4:1. The increase in temperature from 90 °C to 110 °C enhanced the antibacterial effects against all test bacteria, especially for persistent Gram-negative cells, namely C. freundii and K. pneumoniae. The longer Maillard reaction after 110 min at 110 °C did not improve the antibacterial activity of the conjugates against all test bacteria. The best antibacterial activity was observed at the temperature of 110 °C for 110 min for the nisin-to-xanthan ratio of 4:1.

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1. Introduction

Nisin is the most commercial lantibiotic bacteriocin that is produced mainly by strains of *Lactococcus lactis* [1]. Nisin has high antibacterial activity, especially for Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* and as well as the spores of *Bacillus* and *Clostridium* [2]. Nisin inhibits the biosynthesis of the cell wall via binding to the cell wall precursor lipid

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II [3, 4]. Nisin is heat resistant, active at a broader pH range, and is digestible by the proteases of the human gut. It has been widely used as a natural preservative in dairy, meat, and fish products, liquid eggs, bakery, and vegetables [5, 6]. The World Health Organization (WHO) in 1969 and US Food and Drug Administration (US-FDA) in 1980 approved nisin as a safe bacteriostat at a maximum level of 250 ppm in food industries [7, 8].

Although nisin has a high bactericidal activity, there are still some drawbacks to its weak activity against the most of Gram-negative bacteria [8, 9]. Previous studies showed that the modifications with protein engineering may harm the expression during the fermentation and also purification of nisin from cultivation media [10]. However in the post-production modification, the change of nisin can be made after the expression by various natural and synthetic materials. The studies to improve the protein functionality have commonly involved chemical modifications, including esterification, alkylation, deamidation, amidation, covalent attachment to carbohydrates and fatty acids, thiol-disulfide exchange, and enzymatic modification [11, 12]. The covalent binding of carbohydrates through the Maillard reaction is among modifications applied to food proteins to improve their antimicrobial activities [9, 13]. The Maillard reaction involves the covalent attachment of the carbonyl groups of sugars to the free amino acid groups in protein [14]. This reaction is ordinarily used at the high temperature ranging 140-180 ^{o}C [15]. Previously, the Maillard glycation of lysozyme with dextran showed modified antimicrobial activities against Gram-negative bacteria [16, 17]. However, the opposite results have been reported for the glycated nisin. Abdullah et al. [18] reported that the glycation of nisin with glucose adversely affected the antibacterial activity of nisin. Other researchers also made the same observation by he glycation of nisin with lactose, dextran, and maltodextrin [19]. Muppalla et al. [9] observed that radiation-induced nisin-carbohydrate (glucose or dextran) conjugates had higher activities against Gram-positive and Gram-negative bacteria.

The movement of nisin against Gram-positive bacteria comes from the positive charges of lysine residues [20, 21]. The lysine is among primary sites of the Maillard reaction, and thus it is possible that the decrease of the positive charges after glycation resulted in reducing the antimicrobial activity of nisin towards Gram-positive bacteria. Also, previous findings showed the conjugation of proteins to polysaccharides led to a more significant enhancement in their specific physicochemical and functional properties in comparison with mono- and disaccharides [22]. The whey protein isolate properly conjugated to gum acacia via the aided ultrasound Maillard reaction at 50-70 ^{o}C [13]. Also, the conjugation of lysozyme to xanthan showed a high antibacterial activity against Gram-positive and Gram-negative bacteria when prepared at the pH of 8.5 and 60 ^{o}C [23].

This study aimed to covalently bind nisin to xanthan, a water-soluble polysaccharide, via the Maillard reaction at moderate temperature. It is expected that the glycation of nisin with xanthan, due to the presence of low reducing ends in the polysaccharide molecule, to result in reducing the positive charge less (saving the antibacterial activity against Gram-positive bacteria) and also enhance its surface characteristics to interact with Gram-negative bacteria [13, 19]. Because, nisin due to the amphiphilic nature can bind with lipids, proteins, and other components in food matrices, which could cause a loss of its activity [24]. To the best of our knowledge no report is available for the glycation of nisin

with xanthan in literatures. The effect of the parameters, such as the temperature, reaction time, and nisin to xanthan ratio, was examined. The antibacterial activity of the nisin-xanthan conjugates was investigated against a collection of Gram-positive and Gram-negative bacteria.

2. Materials and Methods

2.1 Chemicals

Nisin, xanthan, Mueller Hinton Broth, Muller-Hinton Agar, methylene blue, safranin, barium chloride, and sulfuric acid were purchased at analytical grade from local suppliers.

2.2. Microorganisms

Bacterial strains of *Staphylococcus aureus*, *Bacillus coagulans*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii* were obtained from the microbial collection of Kermanshah University of Medical Sciences. The bacterial strains were cultivated on Muller Hinton agar (MHA) and Muller-Hinton Broth (MHB) at 37 ^{o}C for 24 h in an incubator. Previously, these media were sterilized by an autoclave with a temperature of 121 ^{o}C for 20 min. The McFarland method was used to standardize the inoculum concentration in the microbial susceptibility test. For this purpose, 0.5 mL of barium chloride ($BaCl_2$) with a concentration of 0.048 mol L^{-1} was added to 99.5 mL of the sulfuric acid solution (1 vv^{-1}%), and a suspension was obtained. The standard optical density of the resulting solution was determined spectrophotometrically at 625 nm.

2.3. Preparation of nisin-xanthan conjugates by the Maillard reaction

The preparation of nisin-xanthan conjugates was performed via the Maillard reaction between nisin and xanthan according to the previously described method [23] with slight modifications. Initially, nisin and xanthan powders were mixed at different nisin-to-xanthan ratios (1:1, 2:1, 4:1) and then added to the phosphate buffer solution 0.1 M at pH 8.5. The Maillard reaction is strongly influenced by pH and increases by increasing the pH. At low pHs, the amino group was protonated, so only a few amino groups were available for the Maillard reaction [25]. On the other hand, the solubility of nisin at alkaline solutions is limited, so the nisin at the concentration ranging 1-4 g L^{-1} was used. The sample solutions were agitated on a magnetic stirrer at room temperature for 1 h to completely dissolve the mixture. The obtained solution was poured in a 1 mL micro-vial and then transferred to a micro-reactor with a temperature accuracy of 0.1 ^{o}C. According to literatures, the best bioactivity of nisin-glucose conjugates was obtained at 90 ^{o}C after 120 min [18]. Therefore, to evaluate the effect of the nisin-to-xanthan ratio (1:1, 2:1, 4:1), the temperature of the micro-reactor was set at 90 ^{o}C for 110 min. The further increase of temperature at a long time negatively affects the preparation of nisin-xanthan conjugation mainly due to the denaturation process before the Maillard reaction especially at high nisin-to-xanthan ratios [18]. The Maillard reaction at the temperatures of 60, 75, 90, and 110 ^{o}C was examined in this study where the nisin-to-xanthan ratio and reaction time were 1:1 and 110 min respectively. The temperature range was selected based on the previous work reported for the glycation of lysozyme [23]. Also, the effect of the reaction time at 10, 30, 60, 90, 110, 180, 240, and 300 min was investigated at 90 ^{o}C and a nisin-to-xanthan ratio of 4:1. The nisin-to-xanthan ratio of 4:1 was chosen based on the results of the previous stage in this study. A control sample, in which nisin and xanthan were not treated by heating, was also prepared. In each case, upon completing the reaction, the solution was

cooled and then lyophilized. The lyophilized powder was used for the antibacterial tests.

2.4. Analysis methods

2.4.1. Characterization of nisin-xanthan conjugates

The chemical bond formed among nisin-xanthan conjugates was analyzed by ultraviolet (UV) (200-400 nm) [9], fluorescence [13], and Fourier transform infrared (FT-IR, 400-4000 cm^{-1}) spectroscopies [23].

2.4.2. Antibacterial activity evolutions

ELISA plate manufactured by Zhejiang Sofa Life Science Research Company was used to measure the population of bacterial . Gram-positive (namely: *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus coagulans*) or Gram-negative (namely: *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Escherichia coli*) species were used to investigate the effect of the presence of nisin, xanthan, and nisin-xanthan conjugates (produced under different conditions) on the bacterial growth. A 100 µL sterile Mueller-Hinton broth and 30 µL of each solution of nisin, xanthan or nisin-xanthan conjugates were added to each well. Then each well was inoculated with 30 µL of the 24-h grown culture of each strain. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (the MTT dye from Sigma Aldridge Co.) was added to the wells to study the growth of microorganisms [26]. The change from the initial yellow to dark blue colour indicated bacterial growth in the well. The absorbance was measured at 630 nm [27].

3. Results and discussion

3.1. Effect of operational parameters on the formation of nisin-xanthan conjugates by the Maillard reaction

The present study was performed to evaluate the impact of the Maillard reaction on improving the antimicrobial properties of nisin against Gram-positive and Gram-negative bacteria by the thermal treatment of nisin with xanthan. The temperature, reaction duration, and nisin-to-xanthan ratio showed significant effects on the nisin-xanthan conjugates. To investigate the effect of temperature, heating at 60, 75, 90, and 110 °C was applied for 110 min at the nisin-to-xanthan ratio of 1:1. The results of the fluorescence spectroscopy analysis are given at Fig. 1.

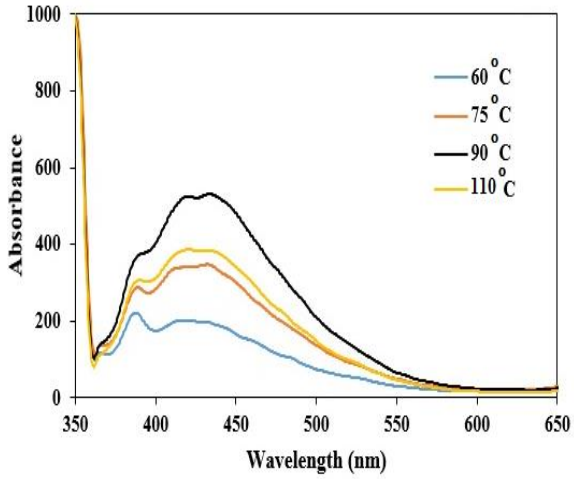

Figure 1. The effect of temperature on the results of the fluorescence spectroscopy analysis of the produced conjugates at the nisin-to-xanthan ratio of 1:1 after 110 min.

Accordingly, the fluorescence spectrum in the 400-440 nm region shows two peaks, which indicate the formation of a bond between nisin peptide and xanthan carbohydrate during the Maillard reaction [18, 23]. In fact, the progress of the Maillard reaction results in generating brown pigments. The increase in absorbance at 420 nm of Fig. 1 indicated the formation of Amadori linkage [9]. By increasing temperature from 60 °C to 90 °C, the intensity of these peaks was improved. However, with further increase in temperature to above 90 °C, a decline in the intensification of these peaks

can be seen. Similar trends are observed in Fig. 2 and Fig. 3 for other nisin-to-xanthan ratios. Temperatures above 90 °C are likely to cause the thermal degradation of the raw materials [28], thus reducing the possibility of the bond formation. Nisin consists of three molecules of lysine at positions 12, 22, and 34. These positions easily participated in glycation with sugars. Increasing temperature to above a certain level caused the denaturation of these active parts in nisin. Generally, the Maillard reaction is involved in four sequential steps [18]: 1) the formation of N-glucosamine from sugar-amino bonding, 2) the rearrangement of glucosamine via Amadori mechanism, 3) the degradation and fragmentation of the Amadori product and amino acid degradation, and 4) the condensation and polymerization of aldehyde and amino compounds forming melanoildins. It was expected that the first stage of the Maillard reaction was taken place at the temperature of about 90 °C. The range of intermediate compounds and brown pigments have developed at this stage. However, the content of these substances has decreased at higher temperatures. It is possible that the Maillard reaction has entered the next stages. Since a clear peak is still visible at 110 °C, it shows that the Maillard reaction has not stopped at 110 °C and follows to the next stages. So, the heating at 90 °C is chosen for further studies.

Another noteworthy point observed from Fig. 2 is the significant increase in the peak intensity of the Maillard reaction products by increasing the ratio of nisin to xanthan. This result shows that by increasing nisin, the number of amino acid groups participating in the Maillard reaction increased. In other words, the concentration of amino acid as a reaction limiter compared with the carbonyl groups in xanthan polysaccharide effectively controls the Maillard reaction [23]. For this reason, the nisin to xanthan ratio equal to 4:1 was used for continuing this study.

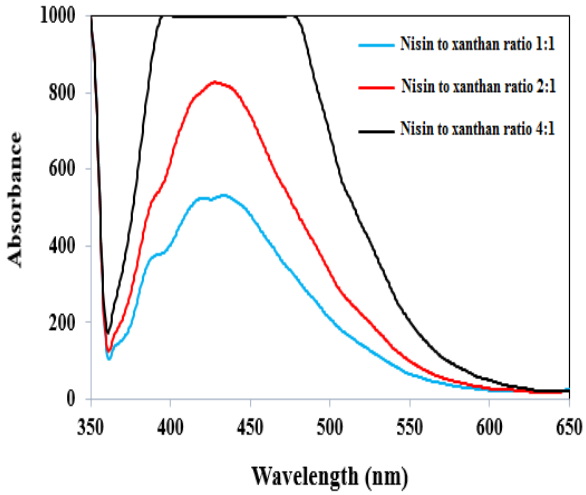

Figure 2. The effect of the nisin to xanthan ratio on the results of the fluorescence spectroscopy analysis of the produced conjugates at 90 °C for 110 min.

The result of the Maillard reaction duration at 30, 60, 90, 110, 180, 240, and 300 min was investigated by applying heating at 90 ℃ in the nisin to xanthan ratio of 4:1. The obtained results are shown in Fig. 3. As it can be seen, the highest peak of the product is obtained after 110 min. In other words, increasing the reaction time to 110 min has developed the Maillard reaction, but further increase led to a reduction in the formation of the Maillard reaction products between nisin and xanthan. It is to be noted that without applying heat (control experiment), the product peak at 440 nm is not visible and indicates that applying heat is essential to perform the reaction. Based on the data, the reaction time of 110 min was selected for further studies. The results of the ultraviolet spectroscopy confirmed the results of the fluorescence spectroscopy. The resulting peaks at about 280-290 nm indicate the formation of a bond between the amino acids of nisin and the carbonyl groups in xanthan. As it can be seen, increasing time to up to 240 min shows a constant behaviour of

the system. Still, by increasing the reaction time to 300 min, the UV spectrum shows a noticeable difference, which indicates the formation of new products.

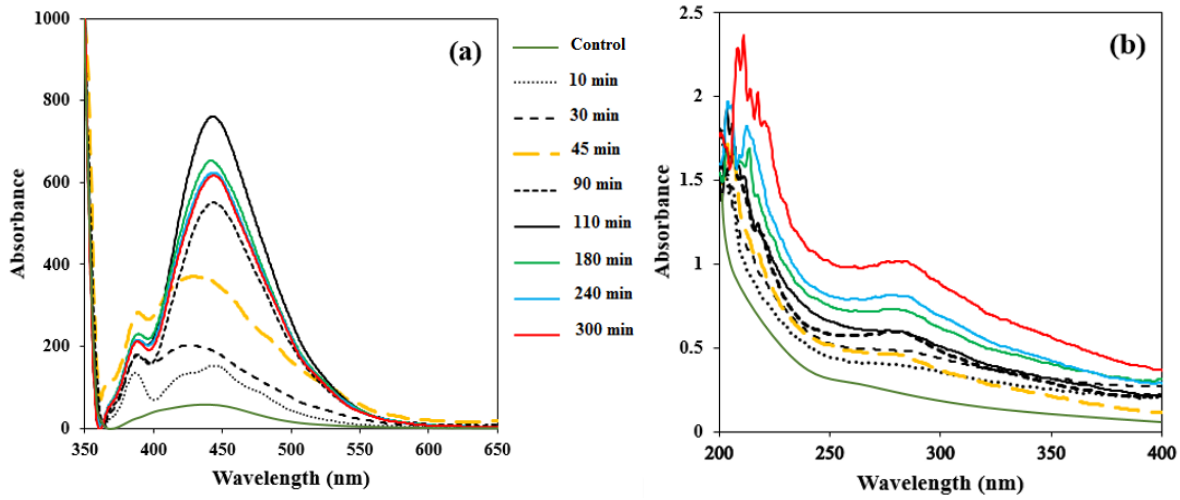

Figure 3. The effect of the reaction time on the results of (a) the fluorescence spectroscopy and (b) the ultraviolet spectroscopy analysis of the produced conjugates at 90 °C and the nisin-to-xanthan ratio of 4:1

The results of the FT-IR analysis on nisin, xanthan, and the produced conjugate (the temperature = 90 °C, nisin to xanthan ratio = 4:1, and reaction time =110 min) are presented in Fig. 4. Several peaks are easily identified at these FT-IR spectrums at the region of 800-1800 cm^{-1}. The bonds correspond to the formation of amide groups (CO, CN, and NH), which confirmed the presence of the Maillard reaction products such as amorphous compounds, shift compounds, and pyrazines. The most distinctive spectral feature of proteins is the presence of solid amide bands 1 and 2, approximately between 1530 cm^{-1} and 1650 cm^{-1} respectively [29 , 30]. These prominent peaks obtained at 1528 cm^{-1} and 1643-1670 cm^{-1} in this study. The initial amines of the two extremes are poorly adsorbed at 3540 cm^{-1} and 3450 cm^{-1}, indicating asymmetric and symmetric N-H tensions respectively. Thus, a broad band at 3300 cm^{-1} appeared for nisin due to the stresses of the free amino group. A set of overlapping peaks is located at carbohydrate spectrums in 953-1180 cm^{-1} [31]. In this study, the xanthan spectrum shows a rise at 1028 cm^{-1}, which can be attributed to the C-C and C-O tension and C-H curvature in this polysaccharide. Also, the specific rise at 3400 cm^{-1} can be attributed to the stress under the influence of the intermolecular and extra-molecular forces of hydrogen bonds in xanthan [32]. The peak at about 2927 cm^{-1} is associated with the C-H tension. The adsorption at 1411-1666 cm^{-1} related to the symmetric and asymmetric tensile vibrations of the carboxylate anion. The reaction between xanthan and nisin is expected to result in the loss of functional groups, including NH_2, especially lysine in the area of 1531 cm^{-1}. In addition, the amount of Maillard reaction products such as amorphous compounds (C=O) and shift base (C=N) enhanced. These chemical changes lead to several changes in the mid-range of the FT-IR spectrum. For instance, the disappearance of bonds in 1027 cm^{-1} and the appearance of a new peak at 1251 cm^{-1} may be due to the formation of a shift base between the reductive

end of xanthan and the amino group of nisin. In addition, vibrational tensile frequencies for C-N at the bottom of the shift, depending on the ligand, were observed at 645-1610 cm^{-1}. Since the frequencies overlap those of the peptide bonds, their formation as a shift base can be shown in Fig. 8. However, the disappearance of the 3400-3500 cm^{-1} bands in the conjugate spectrum indicates the involvement of the amide groups of nisin in the Maillard reaction. The nisin-xanthan conjugate in the range of 1643 cm^{-1} and 1531 cm^{-1}, referred to the tensions of C-N, C-O in amide I and amide II, having changed in the Maillard reaction. Amide III is known to be a very complex compound that is mainly due to the C-N tension and N-H deformation. The relevant bonds in nisin were found at 1460-1280 cm^{-1}. The decrease in the amide III spectrum in 1410 cm^{-1} showed that this change was due to the tension in the bands, including the C-N curvature having been observed in the structure of conjugates.

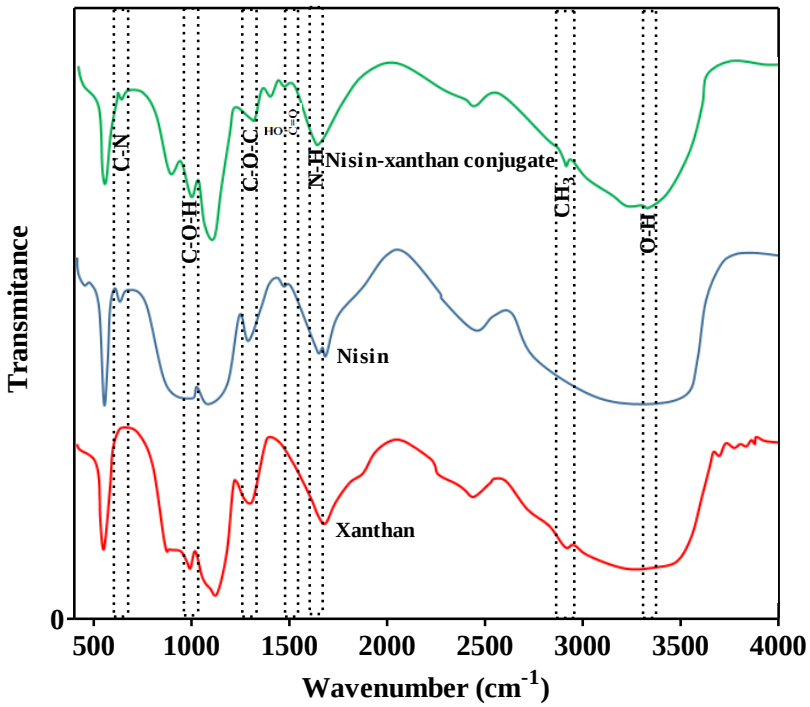

Figure 4. The results of the FT-IR spectrums from nisin, xanthan and the nisin-xanthan conjugate produced at temperature 90 °C for 110 min and nisin-to-xanthan ratio of 4:1.

3.2. Antimicrobial activities of the nisin-xanthan conjugates

The inhibitory activities of nisin and xanthan gum on the growth of test Gram-positive and Gram-negative bacteria are presented in Fig. 5. It can be seen that nisin had a proper antibacterial effect on all test Gram-positive bacteria. For instance, 100 ppm of nisin inhibited the growth of *S. aureus*, *B. subtilis*, and *B. caogulance* by 75.6, 98.6, and 97.8 % respectively. While only the growth of *E. coli* as a Gram-negative bacterium was inhibited by 22.8% in the presence of 100 ppm of nisin. It did not affect other test Gram-negative bacteria at this concentration. Since the cell envelope of Gram-negative bacteria contains a significant amount of hydrophobic substances such as lipopolysaccharide, nisin cannot destroy them when it is added to the cell suspension [16]. The results of Fig. 5 show that the antibacterial activities of nisin were enhanced by increasing its concentration, where the growth of Gram-

negative bacteria such as *E. coli*, *S. typhimurium*, and *C. freundii* was respectively affected by 94.4, 58.8, and 24.7% at the nisin concentration of 400 ppm. Notice that the WHO and US-FDA approved nisin as a safe bacteriostatic substance at a maximum level of 250 ppm to food industries, and thus the 400 ppm was not applicable in foods. Nisin had no significant inhibitory role on the growth of *K. pneumoniae* at all test concentrations. Likewise, the antibacterial activity was not observed in any of test bacterial strains by raw xanthan. In further experiments, the simultaneous addition of nisin (100, 200, and 400 ppm) and xanthan (100 ppm) did not change the antibacterial activities of the substances (data not presented here).

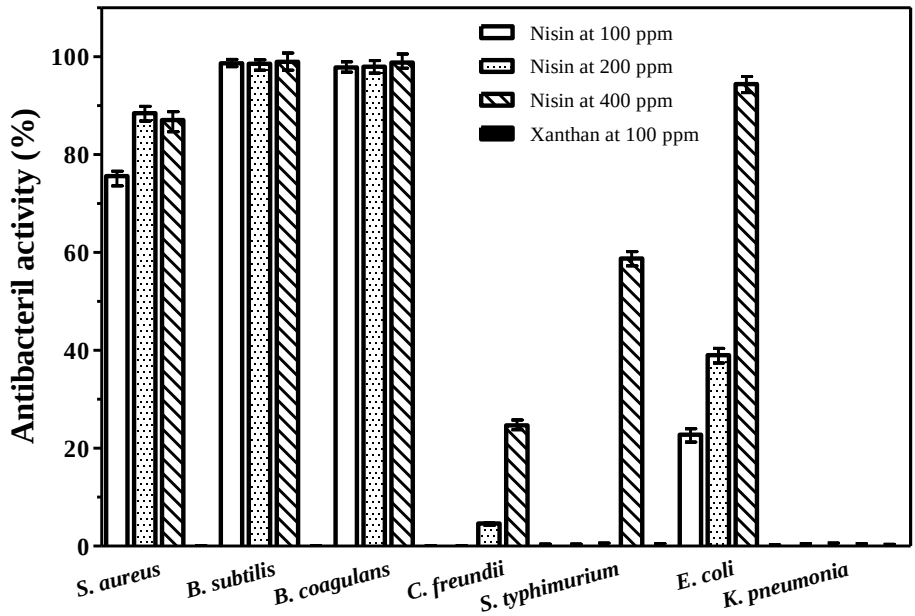

Figure 5. The antibacterial activities of raw nisin and xanthan on test Gram-positive and Gram-negative bacteria.

The results of Fig. 6 depict that the antibacterial activities of nisin on the test Gram-positive bacteria endured after thermal heating at 121 and 180 ^{o}C. It indicates the thermal resistance of nisin as a polypeptide molecule preserver, which could be successfully utilized in heat-sterilized food industries. The antibacterial activities of nisin on some Gram-negative cells varied after heating. For instance, the antibacterial activity towards *S. typhimurium* and *E. coli* was sharply increased after preheating nisin at 121 ^{o}C for 90 min. Also, nisin showed antibacterial activity of about 4% on the growth of *K. pneumoniae* cells after preheating at 121 and 180 ^{o}C. The comparison of antibacterial actions against *S. typhimurium* and *E. coli* indicated that the activities were decreased when the preheating was carried out at 180 ^{o}C. It is mainly due to the degradation of the active part of the polypeptide chain in the nisin molecule at high temperatures [2].

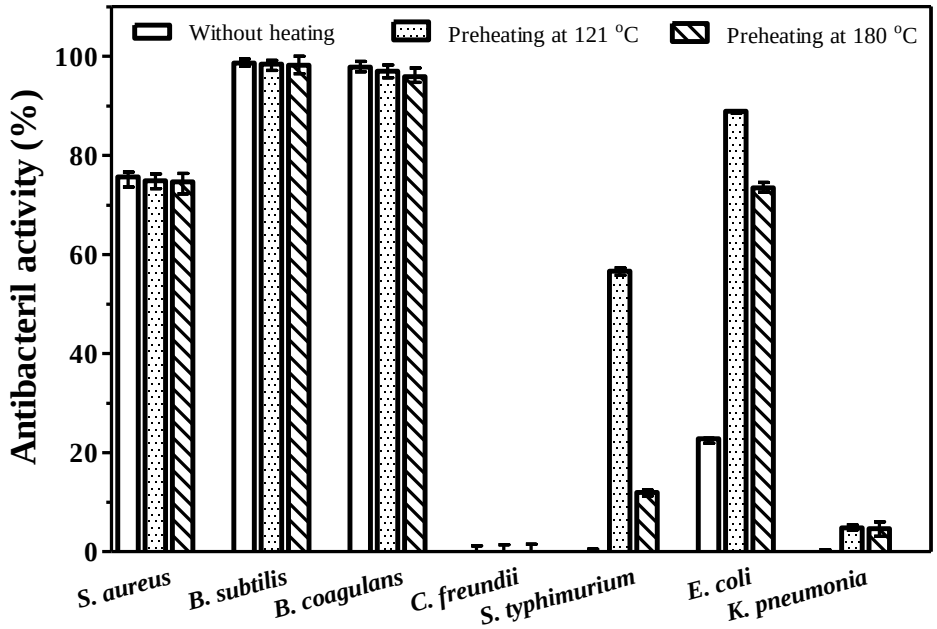

Figure 6. The effect of thermal preheating on the antibacterial activities of nisin (the concentration of the test nisin was 100 ppm and the preheating treatment was performed for 90 min).

The Maillard reaction at different nisin to xanthan ratios of 1:1, 2:1, and 4:1 were also carried out. The antibacterial activities of the obtained conjugates are examined, and the relevant results are presented in Fig. 7. The results showed the antibacterial activities of the nisin-xanthan conjugates are higher than those of the raw nisin, especially against the Gram-negative cells. It is noticeable about the performance of the conjugates, the enhancement of the antibacterial activity by increasing nisin to xanthan ratio in the preparation of conjugates as postulated before. By growing nisin, the number of amino acid groups that can participate in the Maillard reaction increases. So, the number of influential groups interacting with bacterial cell walls increases [33]. The modification of nisin during the Maillard reaction led to improving its antibacterial activities against both Gram-positive and Gram-negative bacteria. The growth of *B. subtilis* and *B. coagulans* was inhibited by 98% at the presence of the nisin-xanthan 1:1 conjugate with a concentration of 100 ppm. The antibacterial activities of the nisin-xanthan conjugate obtained at a ratio of 2:1 were 82.7, 97.4, and 94.2% against *S. aureus*, *S. typhimurium,* and *E. coli* respectively. These activities were increased to 88.8, 98.7, and 97.7% when the nisin-xanthan conjugate obtained at the ratio of 4:1. Some Gram-negative cells, namely *C. freundii* and *K. pneumoniae* were persistent against the nisin-xanthan conjugates and only 43.3 and 31.2% were inhibited in the presence of the nisin-xanthan conjugate obtained at the ratio of 4:1.

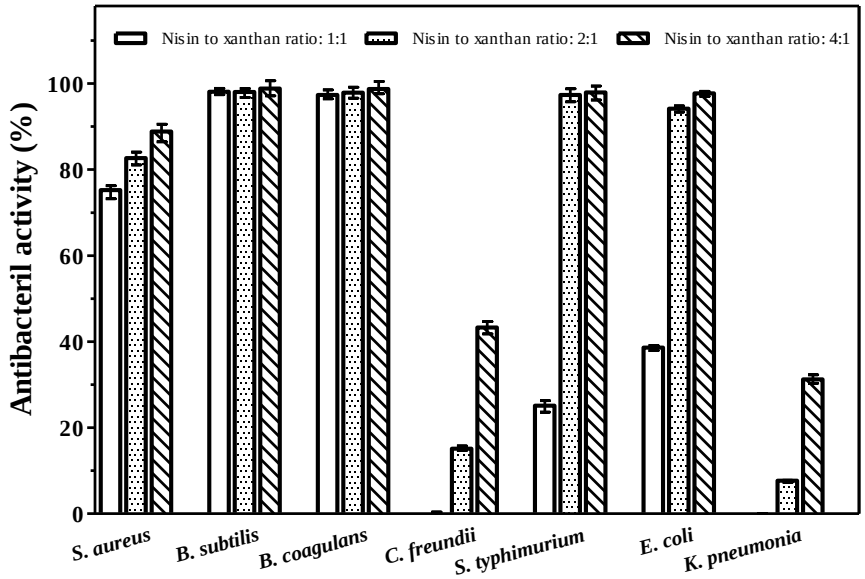

Figure 7. The antibacterial activities of the nisin-xanthan conjugates (at the concentration of 100 ppm) obtained at different nisin to xanthan ratios (Maillard reaction carried out at 110 ° C for 90 min).

The results of the ultraviolet and fluorescence spectrophotometry showed that temperature played a significant role in the development of the Maillard reaction. Thus, the antibacterial activities of the nisin-xanthan conjugates produced at different temperatures of 90, 110, and 180 °C were examined. The results of Fig. 8 indicate that the increase in temperature from 90 °C to 110 °C increased the antibacterial effects against all test bacteria, especially for Gram-negative cells, namely *C. freundii* and *K. pneumoniae*. The development of chemical bonds between nisin and xanthan by the Maillard reaction progressed by increasing temperature from 90 °C to 110 °C. Therefore, the antibacterial activity of the conjugates improved toward a broader range of microbial cells. However, a further increase in temperature for the Maillard reaction to 180 °C had a reverse effect on the antibacterial properties of the conjugates. The growth inhibition obtained by the conjugates was vigorously decreased from 79% to 58.7% against *C. freundii* cells when the Maillard reaction was accomplished at 180 °C. the denaturation of the sensitive parts of the nisin peptide, such as tryptophan amino acid, reduced the potential of the Maillard reaction occurrence at 180 °C in comparison with the same at 110 °C.

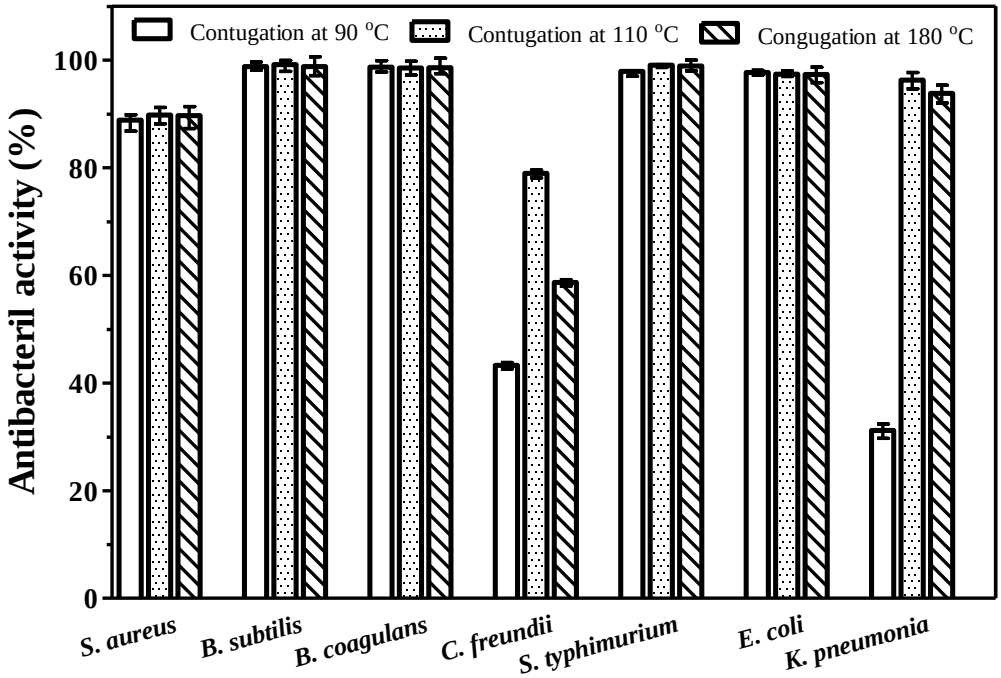

Figure 8. The antibacterial activities of the nisin-xanthan conjugates (at the concentration of 100 ppm) obtained at different Maillard reaction temperatures (Maillard reaction was carried out at the nisin to xanthan ratio of 4:1 and the reaction time of 90 min).

Finally, the effect of the duration of the Millard reaction on the antibacterial activities of the conjugates was investigated at 110, 240, and 300 min at a temperature of 110 °C. The results of the experiments are presented in Fig. 9. It can be seen that the more extended Maillard reaction did not improve the antibacterial activity of the conjugates against all test bacteria. The continuation of the Maillard reaction after 110 min decreased the antibacterial activity of the nisin-xanthan conjugates against persistent Gram-negative bacteria cells, i.e. *C. freundii* and *K. pneumoniae.*

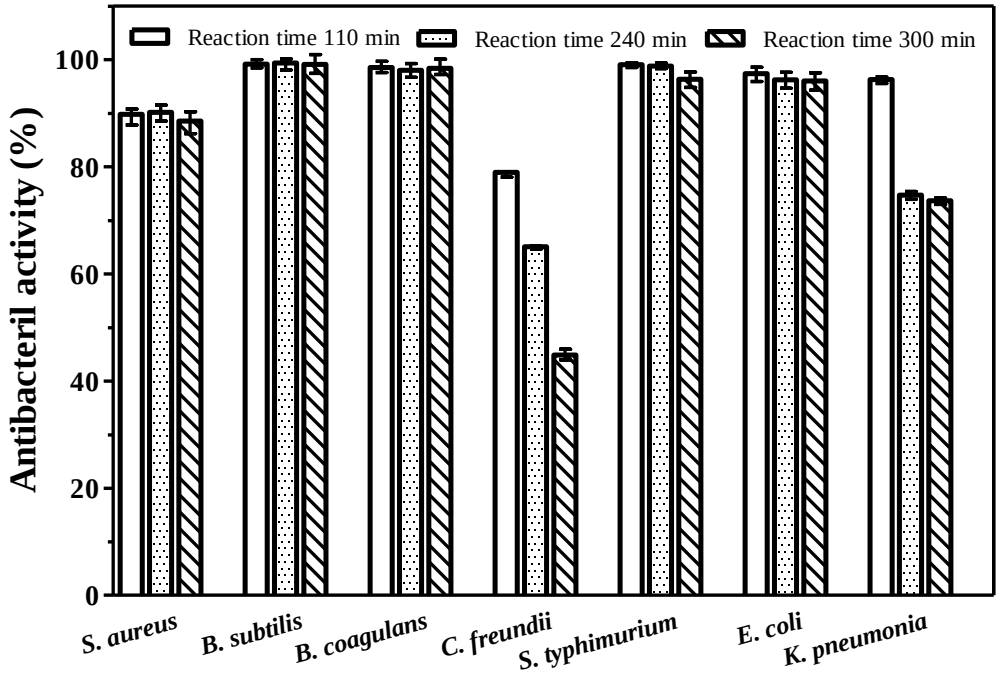

Figure 9. The antibacterial activities of the nisin-xanthan conjugates (at the concentration of 100 ppm) obtained at different Maillard reaction times (Maillard reaction was carried out at the nisin to xanthan ratio of 4:1 and the temperature of 110^{o} C).

4. Conclusion

The results of this work showed that the conjugation of nisin with xanthan via the Maillard reaction at a moderate temperature (110 ^{o}C) could improve the antibacterial activities against pathogenic Gram-negative bacterium such as *S. typhimurium, K. pneumoniae*, *C. freundii*, and *E. coli* at a concentration of 100 ppm. This concentration is lower than the level (250 ppm) approved by the FDA and WHO in the food and cosmetic industries. So, it indicates the potential application of the nisin-xanthan conjugate for some food manufacturers, including fruit jams, prepared soup, and catchup.

References

[1] Slootweg, J. C., van der Wal, S,. van Ufford, H. Q., Breukink, E., Liskamp, R. M. and Rijkers, D. T., "Synthesis, antimicrobial activity, and membrane permeabilizing properties of C-terminally modified nisin conjugates accessed by CuAAC", *Bioconjugate Chem.*, **24** (12), 2058 (2013)

[2] Gharsallaoui, A., Oulahal, N., Joly, C. and Degraeve, P., "Nisin as a food preservative: Part 1: Physicochemical properties, antimicrobial activity, and main uses", *Crit. Rev. Food Sci. Nutr.*, **56** (8), 1262 (2016)

[3] Cui, H. Y., Wu, J., Li, C. Z. and Lin, L., "Anti-listeria effects of chitosan-coated nisin-silica liposome on Cheddar cheese", *J. Dairy Sci.*, **99** (11), 8598 (2016)

[4] Ahmad, V., Khan, M. S., Jamal, Q. M. S., Alzohairy, M. A., Al Karaawi, M. A. and Siddiqui, M. U., "Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation", *Int. J. Antimicrob. Agents*, **49** (1), 1 (2017)

[5] Vadyvaloo, V., Hastings, J. W., van der Merwe, M. J. and Rautenbach, M., "Membranes of class IIa bacteriocin-

resistant *Listeria monocytogenes* cells contain increased levels of desaturated and short-acyl-chain phosphatidylglycerols", *Appl. Environ. Microb.*, **68** (11), 5223 (2002)

[6] Bahrami, A., Delshadi, R., Jafari, S. M. and Williams, L., "Nanoencapsulated nisin: an engineered natural antimicrobial system for the food industry", *Trends Food Sci. Technol.*, **94**, 20 (2019)

[7] Zhao, X., Zhen, Z., Wang, X. and Guo, N., "Synergy of a combination of nisin and citric acid against *Staphylococcus aureus* and *Listeria monocytogenes*, *Food Addit. Contam. A*, **34** (12), 2058 (2017)

[8] Qian, J., Chen, Y., Wang, Q., Zhao, X., Yang, H., Gong, F. and Guo, H., "Preparation and antimicrobial activity of pectin-chitosan embedding nisin microcapsules. *Eur. Polymer J.*, **157**, 1106 (2021)

[9] Muppalla, S. R., Sonavale, R., Chawla, S. P. and Sharma, A., "Functional properties of nisin–carbohydrate conjugates formed by radiation induced Maillard reaction", *Radiat Phys. Chem.*, **81** (12), 1917 (2012)

[10] Minten, I. J. and Abello, N., Schooneveld-Bergmans, M. E., van den Berg, M. A., "Post-production modification of industrial enzymes", *Appl. Microb. Biotechnol.*, 98 (14), 6215 (2014).

[11] Kato, A., Murata, K. and Kobayashi, K., "Preparation and characterization of ovalbumin-dextran conjugate having excellent emulsifying properties", *J. Agric. Food Chem.* **36** (3), 421 (1988)

[12] Scaman, C., Nakai, S. and Aminlari, M., "Effect of pH, temperature and sodium bisulfite or cysteine on the level of Maillard-based conjugation of lysozyme with dextran, galactomannan and mannan", *Food Chem.* **99** (2), 368 (2006)

[13] Chen, W., Ma, X., Wang, W., Lv, R., Guo, M., Ding, T., Ye, X., Miao, S. and Liu, D., "Preparation of modified whey protein isolate with gum acacia by ultrasound maillard reaction", *Food Hydrocolloids*, **95**, 298 (2019)

[14] Bi, B., Yang, H., Fang, Y., Nishinari, K. and Phillips, G. O., "Characterization and emulsifying properties of β-lactoglobulin-gum Acacia Seyal conjugates prepared via the Maillard reaction", *Food Chem.*, **214**, 614 (2017)

[15] Zheng, X., He, Y., Zhou, H. and Xiong, C., "Effects of chitosan oligosaccharide–nisin conjugates formed by Maillard reaction on the preservation of *Collichthys niveatus", J. Food Process Preserv.* **43** (10), e14116 (2019)

[16] Nakamura, S., Kato, A. and Kobayashi, K., "New antimicrobial characteristics of lysozyme-dextran conjugate", *J. Agric. Food Chem.*, **39** (4), 647 (1991).

[17] Amiri, S., Ramezani, R. and Aminlari, M., "Antibacterial activity of dextran-conjugated lysozyme against *Escherichia coli* and *Staphylococcus aureus* in cheese curd", *J. Food Prot.*, **71** (2), 411 (2008)

[18] Abdullah, S. U., Badaruddin, M., Ali, R. and Riaz, M. N., "Effect of elementary and advanced glycation products of nisin on its preservative efficacy and digestibility", *Food Chem.* **122** (4), 1043 (2010)

[19] Chen, H., Davidson, P. M. and Zhong, Q., "Antimicrobial properties of nisin after glycation with lactose, maltodextrin and dextran and the thyme oil emulsions prepared thereof", *Int. J. Food Microb.*, **191**, 75 (2014)

[20] Shu, Y. W., Sahara, S., Nakamura, S. and Kato, A., "Effects of the length of polysaccharide chains on the functional properties of the Maillard-type lysozyme−polysaccharide conjugate", *J. Agric. Food Chem.* **44** (9), 2544 (1996)

[21] Thorpe, S. and Baynes, J., "Maillard reaction products in tissue proteins: new products and new perspectives", *Amino Acids*, **25** (3), 275 (2003)

[22] Niu, L. Y., Jiang, S. T., Pan, L. J., and Zhai, Y. S., "Characteristics and functional properties of wheat germ protein glycated with saccharides through Maillard reaction. *Int. J. Food Sci. Technol*., **46** (10), 2197 (2011)

[23] Hashemi, M. M., Aminlari, M., and Moosavinasab, M., "Preparation of and studies on the functional properties and bactericidal activity of the lysozyme–xanthan gum conjugate", *LWT-Food Sci. Technol.*, **57** (2), 594 (2014)

[24] Solomakos, N., Govaris, A., Koidis, P., and Botsoglou, N., "The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157: H7 in minced beef during refrigerated storage", *Meat Sci.* **80** (2), 159 (2008)

[25] El-Ghorab, A.H., Ashraf, I.F., Mohamed, A.F., Shaaban, H.A., El-massry, K.F., and Farouk, A.., "The effect of pH on flavor formation and antioxidant activity of amino acid and sugars interaction products", *JASMR*, **5**(2), 131 (2010)

[26] Sylvester, P.W., "Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability, *Methods Mol Biol*. **716**, 157 (2011)

[27] Ghasemi, M., Turnbull, T., Sebastian, S., and Kempson, I., "The MTT Assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis., *Int J Mol Sci.* **22**(23) 12827 (2021)

[28] Badr, S., Karem, H.A., Husseini, H., and D. El-Hadedy, D., "Characterization of nisin produced by *Lactococcus lactis*, *Int. J Agric. Biol.* **7**(3), 499 (2005)

[29] Gu, F. L., Kim, J. M., Abbas, S., Zhang, X. M., Xia, S. Q., and Chen, Z. X., "Structure and antioxidant activity of high molecular weight Maillard reaction products from casein–glucose", *Food Chem*., **120** (2), 505 (2010)

[30] Yu, X., Lu, N., Wang, J., Chen, Z., Chen, C., Regenstein, J. M., and Zhou, P., "Effect of N-terminal modification on the antimicrobial activity of nisin", *Food Control*., **114**, 107227 (2020)

[31] Liu, P., Li, Y., Gao, L., Zhou, X., Ma, P., and Wang, Q., "Effect of different carbohydrates on the functional properties of black rice glutelin (BRG) modified by the maillard reaction", *J. Cereal Sci.* **93**, 102979 (2020)

[32] Meng, Z., Qi, K., Guo, Y., Wang, Y.,and Liu, Y., "Physical properties, microstructure, intermolecular forces, and oxidation stability of soybean oil oleogels structured by different cellulose ethers", *Eur. J. Lipid Sci. Technol.*,**120** (6),1700287 (2018)

[33] Friedman, M., "Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins", *J. Agric. Food Chem.* **47** (4), 1295 (1999)