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Antimicrobial properties of nisin after glycation with lactose, maltodextrin and dextran and the thyme oil emulsions prepared thereof

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ABSTRACT

To clarify the reported conflicting antimicrobial activities of nisin after glycation, nisin was glycated with lactose, maltodextrin, and dextran at 70 °C and 50% relative humidity for 1–24 h. Nisin before and after glycation was studied for the first time to prepare thyme oil emulsions. The activity of glycated nisin and the thyme oil emulsions was tested in both tryptic soy broth (TSB) and 2% reduced fat milk. Results showed that nisin glycated with a smaller saccharide for a longer duration had a higher degree of glycation and the reduced number of positive charges lowered its antibacterial activity. The emulsified thyme oil had an additive effect with either glycated or native nisin against *Listeria monocytogenes* Scott A and *Bacillus subtilis* in TSB and 2% reduced fat milk. However, emulsions were less effective against *L. monocytogenes* Scott A in milk than same units of native nisin and same concentration of free thyme oil, likely due to the reduced availability of thymol and carvacrol, the main components of thyme oil. These results showed that glycation of nisin cannot broaden its antimicrobial activity and nisin is not a good compound to prepare emulsions of essential oils.

1. Introduction

Nisin is a cationic peptide produced by certain strains of *Lactococcus lactis* ssp. *lactis* (Bonev et al., 2000; Jozala et al., 2009) and is a well-known bacteriocin. It has 34 amino acids and a molecular weight of around 3.5 kDa (Abdullah et al., 2010). The three lysine residues contribute to its positive charges that make nisin active against gram-positive bacteria such as *Listeria* and *Staphylococcus* as well as the outgrowth of spores of *Bacillus* and *Clostridium* (Jozala et al., 2009). Currently, nisin is the only bacteriocin that is recognized as safe for food applications in over 50 countries (Thomas and Delves-Broughton, 2010). In the United States, nisin has been listed as a generally recognized-as-safe (GRAS) food preservative by the Food and Drug Administration since 1988 and has been widely used in cheeses, sausages, and ready-to-eat meat products (Jozala et al., 2009).

Some problems with nisin are its weak activity against gram-negative bacteria and variable antimicrobial activity in foods. Several studies have described attempts to improve the activity of antimicrobial peptides and proteins in food matrices. Glycation of proteins with reducing saccharides by the Maillard reaction has been used to improve physical properties of proteins, such as solubility and emulsification ability, as well as the activity of antimicrobial proteins and peptides. For example, lysozyme glycated with dextran improved its antimicrobial activity against gram-negative bacteria (Amiri et al., 2008; Nakamura et al., 1991). Additionally, there have been a few studies on the activity of glycated nisin that reported opposite results. Abdullah et al. (2010) reported that glycation of nisin with glucose actually reduced its antimicrobial activity against several bacteria. Conversely, nisin glycated with glucose or dextran in aqueous solutions by a gamma irradiation-induced Maillard reaction showed improved activity against both gram-positive and gram-negative bacteria (Muppalla et al., 2012).

Because the activity of nisin against gram-positive bacteria comes from positive charges provided by lysine residues that are also the major sites for the Maillard reaction (Shu et al., 1996; Thorpe and Baynes, 2003), we could hypothesize that the reduction of positive charges after glycation will reduce the antimicrobial activity of nisin. Furthermore, the amphiphilic nature of nisin can result in binding with lipids, proteins and other components in food matrices, which could cause loss of activity (Solomakos et al., 2008b). Because glycation reduces the positive charge and the hydrophobicity of nisin, glycated nisin may bind with food components to a lesser extent which, in turn, may improve the antimicrobial activity in food matrices (Shu et al., 1996; Thorpe and Baynes, 2003).

Combination of nisin with essential oils or essential oil components has been shown to cause synergistic improvement of antimicrobial activity against foodborne pathogens (Ettayebi et al., 2000; Olasupo et al., 2004). Since essential oils are lipophilic, preparing them as emulsions is needed for dispersion in aqueous foods. Although many reports have demonstrated the improved emulsifying property of proteins after glycation (Ho et al., 2000; Li et al., 2013; Shah et al., 2012b), the

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properties of glycated nisin emulsifying essential oils and the impacts on antibacterial activity have not been studied.

The first objective of this work was to study the physicochemical properties of nisin after glycation with a disaccharide (lactose), oligosaccharide (maltodextrin), or polysaccharide (dextran). The second objective was to characterize the antibacterial activity of the glycated nisin compounds alone and thyme oil emulsified by the glycated nisin compounds in microbiological culture medium and 2% reduced fat milk against several common gram-negative and gram-positive bacterial strains.

2. Materials and methods

2.1. Materials

Commercial nisin (1000 IU/mg solids) was purchased from MP Biomedicals, LLC (Solon, OH). The powdered product contained 2.5% nisin, NaCl, and denatured milk solids. Nisin solution was prepared by dissolving the powder in 0.02 M HCl. Lactose was purchased from Fisher Scientific (Pittsburgh, PA), maltodextrin (dextrose equivalent of 18) was from Grain Processing Corp. (Muscatine, IA) and dextran (from *Leuconostoc* spp.; MW ca. 40,000 Da) and thyme oil were from Sigma-Aldrich Corp. (St. Louis, MO). Tryptic soy broth (TSB, Remel®™, Fisher Scientific) medium was prepared according to manufacturer instructions by dissolving 30 g of powder in 1000 mL water. Tryptic soy agar (TSA) was prepared by adding 12 g of agar (Fisher Scientific) into the TSB medium. Ultra-high-temperature (UHT) processed 2% reduced fat milk (Simple Truth Organic™, San Diego, CA) was purchased from a local grocery store.

2.2. Glycation of nisin with lactose, maltodextrin, and dextran

Nisin power was dissolved at 72 mg/mL in 50 mL of 20 mM HCl and then diluted to 6 mg/mL with 550 mL deionized water. Lactose, maltodextrin, or dextran was dissolved at 6 mg/mL in the nisin solution at room temperature (~21 °C), followed by adjusting pH to 7.0 before freeze drying. The freeze-dried powder was collected and stored at −20 °C before use.

To prepare glycated nisin, the method in our earlier study was modified (Wang and Zhong, 2014). The freeze-dried powder was spread as a thin layer on a plastic tray and incubated for 1, 3, 6, and 24 h in a forced-air environmental chamber (model IG420U environmental chamber, Yamato Scientific America Inc., Santa Clara, CA) which was pre-equilibrated at 70 °C and 50% relative humidity. The glycated powder was ground by a mortar and a pestle and then collected and stored at −20 °C before use.

2.3. Determination of the degree of glycation

The degree of glycation was measured using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) method (Tainturier et al., 1992) with some modification. Briefly, glycated nisin (0.1% w/v) was mixed with 0.1 M sodium bicarbonate (pH 8.5) using an end-to-end shaker overnight at room temperature (21 °C). Immediately prior to assays, a TNBS working solution was prepared by diluting the aqueous 5% TNBS solution (Sigma-Aldrich) to 0.01% w/v TNBS in 0.1 M sodium bicarbonate (pH 8.5). The 250 μL working solution of TNBS was mixed well with 500 μL of a nisin sample. After incubation at 37 °C for 2 h, the reaction was stopped by adding 250 μL of 10% (w/w) sodium dodecyl sulfate (SDS) solution and 125 μL 1 N HCl to the mixture, followed by measuring the absorbance at 335 nm using a UV–Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA). Native nisin was used as a control to determine the glycation degree using the following equation.

$$\text{Degree of glycation } (\%) = (A_c - A_a)/A_c \times 100\% \quad (1)$$

where A_c and A_a are the respective absorbance values when native nisin (control) and glycated nisin solutions were used in reaction.

2.4. Estimation of nisin activity

Nisin activity was determined using a standard agar diffusion assay described in our earlier work (Xiao et al., 2011) with some modification. *Micrococcus luteus* ATCC 10240 was used as an indicator microorganism. An agar solution was prepared by mixing 980 mL deionized water, 1% peptone, 0.2% sodium chloride, 0.15% yeast extract, 0.1% D-glucose, 1% Tween® 20 and 0.75% agar and heating at 100 °C. The cooled agar solution (30 mL) containing 6 log CFU/mL *M. luteus* was added to a sterile Petri dish and allowed to solidify at −4 °C for 2 h before making wells for nisin solutions. A standard curve was established using nisin standard solutions in agar wells at 50, 100, 250, 500, 800 and 1000 IU/mL with sterile water. Inhibition zone diameters after incubation at 32 °C for 24 h were measured and correlated to nisin concentration as in Eq. (2).

$$D = a \log[\text{nisin}] + b \quad (2)$$

where D is the diameter (cm) of the inhibition zone after subtracting the well diameter (0.7 cm); [nisin] is the concentration of nisin (IU/mL); a and b are the slope and intercept from the linear regression of data from nisin standard solutions, respectively.

2.5. Preparation of thyme oil emulsions for antimicrobial tests

To prepare emulsions for microbial tests, the native or glycated nisin was dissolved in 10 mM PBS (pH 7.0) at a nisin activity of 5000 IU/mL. Thyme oil was emulsified at 0.2–1% v/v into the nisin solution using a homogenizer (model Cyclone I.Q.$_2$, The VirTis Company, Inc., Gardiner, NY) at 12,000 rpm for 2 min.

2.6. Bacterial culture preparation

Four gram-negative *Escherichia coli* O157:H7 strains, including ATCC 43895, 43889, 43894 and K3995, and two gram-positive strains, *Listeria monocytogenes* Scott A and *Bacillus subtilis*, were tested. Nisin is known to be effective in inhibiting the growth of *L. monocytogenes* and *B. subtilis* but ineffective against *E. coli* (Jozala et al., 2009). Four *E. coli* O157:H7 strains were selected in order to understand the antibacterial activity of glycated nisin against gram-negative bacteria. All bacteria were stock cultures from the Department of Food Science and Technology at the University of Tennessee (Knoxville, TN). Cultures were grown in TSB and stored at −20 °C in 40% glycerol as stocks. The cultures were activated by inoculating the stock culture in TSB and transferring for two consecutive days at 37 °C for *E. coli* O157:H7 or 32 °C for *L. monocytogenes* Scott A and *B. subtilis*. The working culture was obtained by diluting the activated stock culture and growing for another 18 h to ca. 5 log CFU/mL for *E. coli* O157:H7 and 6 log CFU/mL for *L. monocytogenes* and *B. subtilis*.

2.7. Antimicrobial activity of glycated nisin and thyme oil emulsions

2.7.1. Determination of minimum inhibitory (MIC) and bactericidal concentrations (MBC)

A two-fold serial broth dilution method was used to determine the MIC/MBC against the above six bacterial strains (Ma et al., 2013). Nisin before and after glycation was prepared at 5000 IU/mL and then serially diluted to 2500–78 IU/mL. For emulsion treatments, 2 mg/mL thyme oil was homogenized in the 5000 IU/mL nisin solution, followed by the same dilution scheme as above. Similar to an early study (Chen et al., 2014), free thyme oil was dissolved in 100% ethanol at 100 mg/mL and diluted to 4 mg/mL in TSB, which was used for further dilution in TSB to determine MIC and MBC.