



Assessment of the inhibitory effects of sodium nitrite, nisin, potassium sorbate, and sodium lactate on *Staphylococcus aureus* growth and staphylococcal enterotoxin A production in cooked pork sausage using a predictive growth model

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Abstract

This study was conducted to analyze the effects of sodium nitrite, nisin, potassium sorbate, and sodium lactate against *Staphylococcus aureus* (*S. aureus*) growth and staphylococcal enterotoxins (SEs) production in cooked pork sausage by inoculating sausage samples containing preservative with an *S. aureus* strain producing staphylococcal enterotoxin A (SEA) and then storing them at 37 °C for 36 h. Samples were analyzed every 3 h to count the *S. aureus* colonies and to detect SEA. The modified Gompertz model was used to describe *S. aureus* growth in the samples under various conditions, and the preservatives with a significant antimicrobial effect were selected. In addition, the antimicrobial effects of the selected preservatives under various concentrations were tested. Results showed that sodium nitrite, nisin, and potassium sorbate had a weak effect against *S. aureus* growth and had no effect against SEA production, whereas sodium lactate could significantly inhibit *S. aureus* growth and SEA production. Moreover, the antimicrobial effect of sodium lactate was concentration-dependent, wherein sodium lactate concentration <12 g/kg showed no inhibitory effect, but when the concentration was increased to 24 g/kg, sodium lactate could effectively inhibit *S. aureus* growth and SEA production, and at 48 g/kg, sodium lactate had a significant inhibitory effect.

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Keywords: *Staphylococcus aureus*; Staphylococcal enterotoxin A; Cooked pork sausage; Preservative; Sodium lactate

1. Introduction

Staphylococcal food poisoning (SFP) is caused by staphylococcal enterotoxins (SEs) produced during massive growth of

Staphylococcus aureus in food [1–3]. SFP is one of the most common foodborne illnesses in the United States [4], and it is also very common in China [5–7]. *S. aureus* has the ability to grow and produce SEs over an extensive range of pH, water activity (A_w), sodium chloride concentration, and temperature, leading to intoxication from an equally extensive range of foods [1]. SFP is often associated with protein-rich food, such as meat and dairy products, which may be subject to extensive manual handling, inadequate heating, or inappropriate storage [8].

Being a type of popular meat product, cooked pork sausage has shown a fast growing market in China [9]. It is produced by blending minced raw pork meat with other ingredients, mix-

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ing and cutting, filling into a casing, and then cooking using water tank. Like most of the cooked meat products sold at delis in China, cooked pork sausage was found to be stored at delis without packaging, thereby making it susceptible to contamination by microorganisms, including pathogens such as *S. aureus*, from the environment during storage and distribution; moreover, its high water activity and rich nutrition encourage *S. aureus* to grow and produce SEs, and even to cause SFP [10,11]. Several studies on food safety monitoring conducted in China [12–14] have indicated that *S. aureus* was one of the most common foodborne pathogens associated with cooked meat products. Therefore, controlling the growth of *S. aureus* is an important issue for ensuring the safety of cooked meat products. Indeed, inhibiting *S. aureus* growth and SEs production in cooked meat sausage will be beneficial, and one possible solution is to identify effective preservatives that can be added into the product, so that whenever the product is contaminated with *S. aureus*, the growth could be effectively inhibited or at least the production of SEs could be delayed. Thus, the safety of the cooked pork sausage could be significantly improved.

According to the Chinese National Food Safety Standard for Uses of Food Additives (GB2760-2014), sodium nitrite, nisin, potassium sorbate, and sodium lactate are legally approved to be added into cooked meat products as preservatives. The maximum levels of sodium nitrite, nisin, and potassium sorbate allowed to be added into meat products are 0.15, 0.5, and 0.15 g/kg, respectively, while there is no use limitation on the level of sodium lactate. The United States Department of Agriculture and Food Safety and Inspection Service allows the use of sodium lactate as a preservative in processed meat products, and the maximum level is 48 g/kg [15]. Several studies have reported about the effects of preservatives on the growth of *S. aureus* [16–20]. However, the results of these studies were sometimes inconsistent, as the antimicrobial activity is affected by a number of environmental factors, including pH, inoculum size, and interaction with food components. Moreover, as SEs are considered as the causative agents of SFP, the detection of SEs is essential for assessing the risk of SFP; however, till date, only a few studies have reported about the applicability of SEs detection for assessing the effect of preservatives against *S. aureus* growth.

The growth of microorganisms is known to be greatly influenced by environmental factors. Food preservatives could be considered as a type of environmental factor that affects the growth of microorganisms [21]. Since the microbial predictive model can be used to assess the impact of food handling and storage conditions on the risk of foodborne pathogens in food and public health [22], we assumed that it could also be used to quantify the effects of preservative factors on the growth of *S. aureus*. The objective of this study was to assess the inhibitory effects of four preservatives on the growth of *S. aureus* and SEs production in cooked pork sausage; an *S. aureus* strain producing staphylococcal enterotoxin A (SEA), the most common type of SEs found in food products [3,23], was chosen to be inoculated into the prepared cooked pork sausage containing one of the preservatives. The growth predictive model of *S. aureus* in cooked pork sausage coupled with the detection of SEA was

used to estimate the inhibitory effects of the four preservatives on the growth of *S. aureus* and SEA production. The results from this study may be beneficial for manufacturers to formulate cooked pork sausages that limit the ability of *S. aureus* to produce SEs, thereby reducing the risk of SFP.

2. Materials and methods

2.1. *S. aureus* strain and preparation of inoculum

S. aureus strain (SA14966) was preserved in Shanghai Food Research Institute; this strain is known to produce SEA. To prepare the inoculum, 0.1 mL of thawed bacteria was transferred into 50 mL of Luria–Bertani (LB) medium and were grown to the desired cell density of 8.0–9.0 log CFU/mL in a thermostatic oscillation incubator at 100 rpm at 37 °C for 18–20 h. Single colonies of *S. aureus* were obtained from plate streaking, which were incubated at 37 °C for 24 h. Following the method of Peter and Robert [18], a loopful of bacteria was picked from a single colony, transferred into 50 mL of LB medium, and incubated in a thermostatic oscillation incubator at 100 rpm at 37 °C for 18–20 h. Then, the bacteria were centrifuged at 10,000 rpm at 4 °C for 20 min and washed with sterile 0.1 mol/L potassium phosphate buffer (pH 7) solution two times and diluted to the desired density of 2.0–3.0 log CFU/mL. Two times' washing guarantees that the resulting solution contains only *S. aureus* cells, without carrying SEA.

2.2. Preparation of samples with different preservatives and the experimental design

The cooked pork sausage samples were prepared based on regular process and formulation; the ingredients of the samples consist of lean and fat pork meat, soy protein, starch, and sodium tripolyphosphate.

In this study, two steps were designed to estimate the effects of the four preservatives against *S. aureus* growth and SEA production. First, five cooked pork sausage samples were prepared according to the mentioned process and formulation, of which four samples contained maximum levels of 0.15 g/kg sodium nitrite, 0.5 g/kg nisin, 0.15 g/kg potassium sorbate, and 48 g/kg sodium lactate, respectively, and one sample contained no preservative (blank). Second, to better understand the inhibitory effects of the preservatives under various concentrations, additional cooked pork sausage samples containing the preservative that showed a significant inhibitory effect in the first step were prepared, some of them containing full, half, quarter, and eighth concentration of the maximum level of the selected preservative, and one sample containing no preservative (blank).

After cooking and cooling, the pH and A_w values of the samples were tested using a pH meter (Delta 320, Mettler Toledo, China) and an A_w meter (HygroLab2, Rotronic, Switzerland), respectively.

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