



## Effects of vacuum tumbling with chitosan nanoparticles on the quality characteristics of cryogenically frozen shrimp



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### ABSTRACT

A chitosan-sodium tripolyphosphate (CH-TPP) nanoparticle solution was developed and applied to shrimp through vacuum tumbling, and the quality characteristics during frozen storage were evaluated. Four solutions were prepared: (1) a 1% acetic acid (AA) solution, (2) a 0.5% chitosan (CH) solution in the 1% AA solution, (3) a 0.167% sodium tripolyphosphate (TPP) solution in the 1% AA solution, and (4) a CH-TPP solution, prepared by adding 0.167% TPP to the CH solution. Shrimp meat was separately vacuum tumbled with the solutions, cryogenically frozen, and evaluated for quality characteristics during 120 days of frozen storage ( $-20\text{ }^{\circ}\text{C}$ ). Shrimp meat vacuum tumbled with distilled water and shrimp meat without vacuum tumbling were used as controls. CH and CH-TPP treated shrimp had lower aerobic plate counts compared to other treatments during the entire storage time. CH and CH-TPP treated shrimp retained their color, texture, and moisture contents. CH and CH-TPP treatments generated the highest reduction in lipid oxidation compared to other treatments at 120 days of storage at  $-20\text{ }^{\circ}\text{C}$ . This study showed that a CH or CH-TPP solution combined with vacuum tumbling was effective at reducing aerobic plate counts and lipid oxidation in shrimp during frozen storage while maintaining desired physico-chemical properties.

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### 1. Introduction

Chitosan is a widely used polysaccharide in edible films and coatings due to its non-toxicity, antimicrobial activity, and antioxidant properties (Elsabee & Abdou, 2013; Jeon, Kamil, & Shahidi, 2002; Kucukgulmez, Kadak, & Gokcin, 2013; No, Meyers, Prinyawiwatkul, & Xu, 2007). Chitosan is derived from chitin, a natural biopolymer found in the shells of crustaceans and cell walls of fungi. Chitin is the second most available biopolymer on earth (Shahidi, Arachchi, & Jeon, 1999). While chitin is mainly composed of poly  $\beta$ -(1-4)-2-acetamido-D-glucose, chitosan is a copolymer that contains  $\beta$ -(1-4)-2-acetamido-D-glucose and  $\beta$ -(1-4)-2-amino-D-glucose units (Elsabee & Abdou, 2013). Chitosan is typically produced from the partial deacetylation of chitin with sodium

hydroxide. At a degree of deacetylation of over 50%, chitin becomes soluble in acidic solutions and can then be classified as chitosan. The degree of deacetylation of chitosan, in addition to molecular weight, strongly influences its antimicrobial activity (Tsai, Su, Chen, & Pan, 2002). Some inherent characteristics of chitosan, such as its large particle size (Qi, Xu, Jiang, Hu, & Zou, 2004) and high viscosity in solution (Jo, Lee, Lee, & Byun, 2001), may limit its penetration into shrimp muscle tissues.

Reduction of particle size to sub-micron levels can allow for deep penetration into tissues through fine capillaries and fenestration in the epithelial lining. Chitosan nanoparticles are formed by instant ionic gelation in which positively charged chitosan is combined with an anion, such as sodium tripolyphosphate (Gan, Wang, Cochrane, & McCarron, 2005). Chitosan nanoparticles have been used in drug delivery systems due to their mucoadhesivity and ability to augment large molecule penetration across mucosal surfaces (Xu & Du, 2003). It has been reported that chitosan nanoparticles may exhibit greater antimicrobial activity than chitosan particles of larger sizes as a result of their small size and the

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quantum size effect (Qi et al., 2004). Many studies have been conducted using chitosan as a coating for seafood preservation (Fan et al., 2009; Sathivel, Liu, Huang, & Prinyawiwatkul, 2007; Soares, Oliveira, & Vicente, 2015; Solval, Espinoza Rodezno, Moncada, Bankston, & Sathivel, 2014), although limited research has been done on the efficacy of vacuum tumbling seafood with chitosan. Chitosan penetration into shrimp may be facilitated by vacuum tumbling with a low viscosity chitosan nanoparticle solution.

Tumbling is used to increase brine uptake and protein extraction in meat through the transfer of kinetic energy with a rotating drum or paddles. (Lin, Mittal, & Barbut, 1990; Price & Schweigert, 1987). This process has been shown to enhance tenderness, ensure juiciness, effectively promote cohesion of meat pieces during cooking, and develop a uniform product with increased yield and desirable slicing characteristics (Cassidy et al., 1978; Chow, Ockerman, Cahill, & Parrett, 1986; Gillett, Cassidy, & Simon, 1981; Krause, Ockerman, Krol, Moerman, & Plimpton, 1978). Vacuum is an important component of the vacuum tumbling process and its function is to prevent air from diffusing into the protein gel structure, ensuring that a tacky exudate is present on the surface of meat proteins after tumbling, rather than frothy foam (Price & Schweigert, 1987). In addition to improving tenderness and decreasing cooking losses, vacuum tumbling can enhance the water holding capacity of meat (Rejt, Kubicka, & Pisula, 1978). Also, pulsed vacuum brining has been reported to aid mass transfer in meat by increasing solution uptake through the pores (Deumier, Trystram, Collignan, Guedider, & Bohuon, 2003). The objective of this study was to evaluate the effects of vacuum tumbling with a chitosan nanoparticle solution on the quality characteristics of cryogenically frozen shrimp.

## 2. Materials and methods

### 2.1. Proximate composition of fresh white shrimp

Fresh, medium size (36/40) head-on white shrimp (*Litopenaeus setiferus*) were obtained from a local seafood store in Baton Rouge, LA. The shrimp were placed on ice and transported to the Food Processing Pilot Plant, Louisiana State University Agricultural Center. They were stored at 4 °C in the cold processing room before they were manually de-headed and de-shelled. Fresh white shrimp meat was analyzed for moisture, lipid, protein, and ash content. The moisture content was determined according to AOAC (1995). Lipid content was analyzed according to the Bligh and Dyer (1959) method. Protein content was determined using the Dumas combustion method with a Leco TruSpec nitrogen analyzer (Leco Corporation, St. Joseph, MI) at the Soil Testing and Plant Analysis Laboratory, Louisiana State University Agricultural Center. A protein to nitrogen conversion factor of 6.25 was applied. Ash content was identified with a Thermolyne Type 6000 muffle furnace (Thermo Scientific, Lawrence, KS) at 549 °C (AOAC, 1999).

### 2.2. Preparation of treatment solutions

Medium molecular weight chitosan (CH) and technical grade sodium tripolyphosphate (TPP) was purchased from Sigma Aldrich (St. Louis, MO). Reagent-grade acetic acid (AA) was obtained from EMD Chemicals Inc. (Gibbstown, NJ). Solutions used for vacuum tumbling with shrimp were formulated according to the process described by Solval et al. (2014). As shown in Fig. 1, four solutions were prepared for this study: (1) a 1% AA solution, (2) a 0.5% CH solution in the 1% AA solution, (3) a 0.167% TPP solution in the 1% AA solution, and (4) a chitosan-sodium tripolyphosphate (CH-TPP) solution, prepared by adding 0.167% TPP to the CH solution. With the addition of TPP to the CH solution, CH-TPP nanoparticles were instantaneously formed by ionotropic gelation. This occurs due to

an interaction between TPP anions and the positively charged amino groups of CH (Fernandez-Urrusuno, Calvo, Remunan-Lopez, Vila-Jato, & Alonso, 1999). The solutions were stirred for 1 h at room temperature. The mixtures were then sonicated for 30 min in an ice bath at 4 °C with amplitude of 80% and pulser set to 2 s using an ultrasonic processor (Model WU-04711-70, Cole-Parmer Inc., Vernon, IL) fitted with a 22 mm tip diameter. To further reduce particle size, the solutions were processed using an ultra-homogenizer (Omni, Ultra shear M, Omni International, Kennesaw, GA) at 25000 rpm for 30 min.

### 2.3. Vacuum tumbling with treatment solutions and freezing of shrimp

The shrimp processing and storage methods are shown in Fig. 2. Fresh white shrimp meat was separately vacuum tumbled with treatment solutions (AA, CH, TPP, or CH-TPP) at a 1:1 ratio by weight in a 2270 g capacity Reveo MariVac vacuum tumbler (Eastman Outdoors, Flushing, MI) in a cold processing room (4 °C) for 10 min. Shrimp meat vacuum tumbled with distilled water and untreated shrimp meat were used as controls. After vacuum tumbling, excess solution was allowed to drain for 30 s. The shrimp were then frozen with liquid nitrogen using a cabinet type cryogenic freezer (Air Liquide, Houston, TX). During cryogenic freezing, thermocouples attached to a data logger (Comark, Comark Ltd. Stevenage, Herts, UK) were embedded into the middle of the second abdominal segment of the shrimp to record temperature changes until a core temperature of 20 °C was reached. Frozen shrimp samples were packed into one quart Ziploc freezer bags (SC Johnson, Racine, WI) and stored at -20 °C for 120 days, undergoing physicochemical analysis every 30 days and microbiological analysis in 60 day intervals. Frozen shrimp were thawed at 4 °C for 22 h prior to analysis.

### 2.4. Microbial counts of thawed shrimp

Aerobic plate counts (APC), yeast and mold counts (YMC), and total coliform counts (TCC) were quantified using 3M Petrifilms (3M Microbiology, St. Paul, MN) according to Solval et al. (2014) with some modifications. Whole shrimp samples were placed in 18 oz Whirl-Pak bags, from Weber Scientific (Nasco, Fort Atkinson, WI), and diluted to 10<sup>-1</sup> concentration with 0.85% NaCl solution. The shrimp samples in the Whirl-Pak bags were homogenized using a Stomacher lab-blender type 400 (Tekman Co., Cincinnati, OH) for 2 min. Duplicated serial dilutions of shrimp samples in 0.85% NaCl solution were produced and separately plated on Petrifilms for APC, YMC, and TCC. Using a flat surface, 1 mL of shrimp dilution was placed on the bottom film and the inoculums were covered with the top film and spread with 3M plastic spreaders. APC Petrifilms were incubated for 48 h at 35 °C and the red colonies were counted. For YMC Petrifilms, the blue-green colonies were counted after incubation for 72 h at 22 °C, while TCC Petrifilms were incubated for 24 h at 35 °C and counted for red gas forming colonies.

### 2.5. pH and moisture content of thawed shrimp

The pH of shrimp was analyzed according to the method described by Sundararajan et al. (2011) with some modifications. Ten grams of shrimp was homogenized in a Waring 51BL32 commercial blender (Torrington, CT) with 40 mL distilled water for 15 s. The mixture was then poured into a 100 mL beaker and sonicated for 1 min at 4 °C with amplitude of 82% and pulser set to 2 s. The pH of the homogenized sample was measured with a VWR Symphony SB70P pH meter (VWR Scientific, Singapore). Moisture content of

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