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ScienceDirect
LWT 40 (2007) 1108–1111
LWT
www.elsevier.com/locate/lwt

Research Note

Decimal reduction times of *Salmonella* Typhimurium in *guinataang kuhol*: An indigenous Filipino dish

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Received 24 January 2006; accepted 9 June 2006

Abstract

This study established the thermal inactivation of *Salmonella* Typhimurium (E1366) in a mixture of golden apple snail (*Pomacea conaliculata* Linn.) meat and coconut cream medium—a dish native to the Philippines locally known as *guinataang kuhol.* Artificially inoculated *S.* Typhimurium were heated to 60, 75 and 90 °C for 0–1.5 min. Survivor cells were enumerated using a selective and a nonselective enumerating medium, bismuth sulphite agar (BSA) and nutrient agar (NA), respectively. The thermal inactivation rates of *Salmonella* Typhimurium were expressed in terms of decimal reduction times (*D*-values). Results showed that in both enumerating media and in all heating temperatures, the number of surviving cells significantly ($P<0.05$) decreased with increasing treatment times. Survivor cell populations enumerated on NA were always significantly greater than those enumerated on BSA. The *D*-values calculated from the survivor counts on both media were however not significantly different. The calculated *D*-values ranged from 0.22 to 0.48 min.

Keywords: Coconut cream; *D*-value; Golden apple snail; Indigenous foods; Thermal inactivation

1. Introduction

Regarded as culinary specialties, snails are favoured by an increasing number of consumers (Schubring & Meyer, 2002). Presently, the main consumers of snails and its products are the populations of West Africa and West Europe (Adeyeye & Afolabi, 2004). In the Philippines, snails are consumed mostly in rural areas where the mollusks are harvested from their natural habitats and prepared as ingredient in many indigenous dishes. The consumption of golden apple snail or *kuhol* as fish and meat substitute has become popular in the provinces where there are vast rice fields (Fernandez & Alegre, 1988). A number of restaurants in the metropolitan have also started serving snails as part of their regular menus. Among the popular dishes include *adobong kuhol,* cooked with soy sauce, vinegar, garlic and peppercorn; and *chicharong kuhol,* prepared by marinating the snail flesh with soy sauce, vinegar and spices before sun drying and deep frying (Management Options for the Golden Apple Snail (MOGAS), 2003). The more popularly known snail dish, *guinataang kuhol*, is prepared by boiling the golden apple snails in coconut cream and spices (General, 1994). The nutritional composition of snail meats sufficiently meets required levels for a healthy and well-balanced diet (Milinsk, das Gracas Padre, Hayashi, de Souza, & Matsushita, 2003).

Snail consumption on the other hand, has been recently associated with outbreaks of foodborne illnesses (Serrano, Medina, Jurado, & Jodral, 2004). Snails are substrate feeders that inhabit a wide range of ecosystems including swamps, ditches and ponds where they accumulate a broad array of microorganisms, including pathogens autochthonous to these habitats. Serrano et al. (2004) reported the presence of mesophilic aerobic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus* and coliforms among ready-to-eat snails. It was also found out that snails can serve as an intermediate host of the liver fluke *Fasciola* spp. (Legaspi & Jovellanos, 1990). Andrews, Wilson, Romero and Poelma (1975) and Serrano et al. (2004) have also reported that

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0023-6438/$30.00
doi:10.1016/j.lwt.2006.06.003

Salmonella is a frequent contaminant among uncooked snails of the species *Achatina achatina* in Nigeria and in *Helix aspersa* in Morocco. Foodborne illnesses due to consumption of snails may occur when the mollusks that contain pathogenic microorganisms are consumed raw or improperly cooked. Therefore, establishment of suitable thermal process schedules for food products that will ensure complete inactivation of pathogens that may be present should be done. This study was conducted to determine the decimal reduction times (*D*-values) of *Salmonella* Typhimurium in golden apple snail and coconut cream mixture. The *D*-value of any biological entity in a food system is equivalent to the number of minutes required to reduce the initial population of the organisms by 1 $\log_{10}$ unit (Jay, 2000). The thermal resistance characteristics of microorganisms are used in establishing proper thermal process designs and operations (Murphy, Marks, Johnson, & Johnson, 2000). The results obtained in the study may be used in establishing appropriate thermal process schedules for golden apple snails based on *Salmonella* Typhimurium inactivation.

2. Materials and methods

2.1. Microbial culture and inoculum preparation

Salmonella Typhimurium E1366 in nutrient agar (NA) slant was obtained from the culture collection division of the Natural Sciences Research Institute (NSRI), University of the Philippines, Diliman, Quezon City, Philippines. The culture was enriched by making two successive loop transfers into sterile 100 ml Nutrient Broth (NB) (Hi-Media, Mumbai, India) and incubating at 37 °C for 24 h. The *Salmonella*-NB suspension was aseptically spun in a Laboratory Centrifuge (R4C, REMI, India) at 1400 rpm for 20 min. The supernatant liquid was decanted and the pelleted cells were resuspended in 100 ml sterile phosphate buffer.

2.2. Suspending medium preparation and Salmonella inoculation

Live golden apple snails were purchased from a wet market in Cubao, Quezon City, Philippines. The snails were transported to the laboratory where the mollusks were individually cleaned and depurated for 24 h in a sanitized plastic container filled with tap water. The depurated snails were washed in running water and drip dried for 10 min. The snail meats were removed from the shells using sterile laboratory forceps and mixed with coconut cream, coconut milk, onion, garlic and ginger at 20:3:8:1:1:1 weight ratios (General, 1994). The mixture was homogenized in a blender for 2 min at medium speed. *Salmonella* Typhimurium inoculation was done by mixing the homogenized snail meat mixture with the *Salmonella*-buffer suspension at 10:1 vol/vol ratio. The mixture was gently stirred with a sterile glass rod for 2 min. Five grams aliquots of the resulting inoculated snail meat medium with approximately 9.00 $\log_{10}$ CFU g^{-1} *Salmonella* were transferred into duplicate sterile screw-capped tubes prior to the thermal inactivation studies. The tubes containing the inoculated medium were set aside for not more than 60 min prior to the thermal inactivation studies.

2.3. Thermal inactivation studies and D-value calculations

Duplicate tubes containing the inoculated snail meat medium were prepared and exposed to a specific thermal treatment. A tube containing uninoculated medium was inserted with a probe-type digital thermometer to monitor temperature. The tubes were immersed in a water bath (Buchi, Germany) and heated to 60, 75 and 90 °C for 0, 0.5, 1.0 and 1.5 min. The heat-treated tubes were immediately immersed into an ice bath after the thermal exposure and were kept cool until withdrawn for survivor cell enumeration. The heated snail meat media were serially diluted with sterile 0.1% peptone water before surface plating onto bismuth sulphite agar plates (BSA, Hi-Media, Mumbai, India) for selective enumeration and NA for the non-selective enumeration. The plates were incubated at 37 °C for 24–48 h prior to colony counting.

Salmonella Typhimurium *D*-values per exposure temperature were calculated from the survivor curves. The survival curve was elucidated by plotting $\log_{10}$ of the surviving population against exposure time. The equation of the best-fitted line was obtained through linear regression. The *D*-values were determined as the number of unit time required for the survivor curve to traverse 1 $\log_{10}$ cycle (Mossel, Corry, Struijk, & Baird, 1995; Jay, 2000) and graphically equal to the negative inverse of the slope of the regressed straight line. Survivor counts and *D*-values were subjected to single-factor analyses of variance (ANOVA) using the General Linear Model Procedure (PROC GLM) of the SAS statistical software version 8.0 (SAS Institute, Cary, NC). Duncan multiple range test was used as post hoc determination of significant differences when $P<0.05$.

3. Results and discussion

Surviving *Salmonella* Typhimurium counts on NA have been observed to be significantly greater ($P<0.05$) than the populations counted on BSA in all heating temperatures (Table 1). The differences between the enumerated survivor populations may be attributed to the sublethally injured cells on NA (Jay, 2000). Despite the significant differences between the enumerated populations, the *D*-values calculated from the selective and nonselective media were not significantly ($P>0.05$) different in all heating temperatures. The calculated *D*-values from the survivor counts on BSA were 0.48, 0.28 and 0.22 min for 60, 70 and 90 °C, respectively. The *D*-values determined from populations on NA were 0.42, 0.39 and 0.22 min for 60, 70 and 90 °C, respectively.