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Synergistic effect of nisin and garlic shoot juice against *Listeria monocytogenes* in milk

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Abstract

The aim of this research was to determine the synergistic effect of nisin and garlic shoot juice (GSJ) against *Listeria monocytogenes* ATCC 19118 found in whole (3.5%), low (1%) and skim (no fat content) milk. Garlic shoot juice (GSJ) at concentrations of 2.5%, 5% and 10% revealed strong and similar patterns of antilisterial effect against *L. monocytogenes* ATCC 19118 in all categories of milk. Nisin only at concentrations of 62.5, 125, 250 and 500 IU/ml displayed a strong antilisterial effect as compared to the control group. Also, the synergistic combinations of GSJ (2.5%, 5%) and nisin (62.5, 125, 250 and 500 IU/ml) had a remarkable antilisterial activity in all categories of whole, low and skim milk after 14 days. Results of this study indicated the synergistic effect of GSJ and nisin as a potential antilisterial agent for the food industry.

Keywords: Synergistic effect; Antilisterial activity; Garlic shoot juice (GSJ); Nisin; *Listeria monocytogenes* ATCC 19118

1. Introduction

Listeria monocytogenes has been implicated in several food-borne outbreaks associated with the consumption of pasteurized milk (Fleming et al., 1985). The pathogen can cause bovine mastitis and is occasionally found in raw milk (Liewen & Plautz, 1988). The Food and Drug Administration (FDA) reported that 5% of raw milk samples tested was found positive for *Listeria*, and 18% of the milk from lactating Holstein cows contained *L. monocytogenes* serotype 4b. Raw milk was the source of the first case of food-borne listeriosis reported in 1953 in Germany, and the first listeriosis outbreak associated with milk in the United States occurred in 1979 (Ryser, 1999). Although *L. monocytogenes* is destroyed by pasteurization, several studies have reported its heat resistance and its ability to survive pasteurization due, in part, to the protective nature of the leukocyte in which the pathogen may be present (Doyle et al., 1987).

L. monocytogenes has the ability to grow at temperatures ranging from 1 to 45 °C (George, Lund, & Brocklehurst, 1988). It also grows at higher rates due to temperature abuses which may be encountered during warehouse storage, transportation, retail display, consumer transportation and consumer storage at home. A variety of different chemical and synthetic compounds have been used as antimicrobials to inhibit *L. monocytogenes* in food systems. It is very important to inhibit the growth of such microorganisms that cause decay and transmutation in stored food. Currently there are conflicting opinions concerning the possible heat resistance of *L. monocytogenes*. Some have reported that *L. monocytogenes* is more thermotolerant than most nonspore-forming bacterial pathogens and may in some instances survive at minimum pasteurization treatment, as supported by the findings of others (Garayzabal, Rodriguez, Boland, Cancelo, & Fernandez, 1986). Recently, consumers are concerned about the safety of foods containing preservatives. Therefore, antimicrobial substances from

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natural sources like plants have been investigated to achieve higher levels of food safety standards (Hao, Brackett, & Doyle, 1998).

Garlic is a widely distributed plant used in all parts of the world not only as a spice but also as a popular remedy for several diseases. A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations (Cavallito, Bailey, & Suter, 1944; Delaha & Garagusi, 1985; Ghannoum, 1988). Moreover, garlic has been reported to reduce blood lipid and cholesterol levels (Gebhardt & Beck, 1996), possess anticancer effects and prevent aging (Hong, Ham, Choi, & Kim, 2000; Sheen, Li, Sheu, Meng, & Tsai, 1996). The analysis of nutritional and volatile flavor compounds of garlic shoots has already been reported (Kim & Chung, 1997), but there are few reports that have been published so far on the biological effect of garlic shoots. Indeed, it is necessary to study the potential applications of garlic shoot juice, a by-product of garlic.

Nisin is a well-known broad spectrum bacteriocin active against Gram-positive pathogens associated with foods. Its use as a food biopreservative is limited by the lack of effect against Gram-negative bacteria; moreover, the development of nisin resistance has been reported in sensitive Gram-positive pathogens (Ming & Daeschel, 1993). The combination of bacteriocins with other preservation mechanisms has been reported to reduce the selection for resistance to bacteriocins in target strains or to extend its inhibitory activity to Gram-negative species (Stevens, Sheldon, Klapes, & Klaenhammer, 1991). Recent studies have shown that the spectrum of activity of nisin may also be extended to Gram-negative bacteria by using it in combination with other agents (Cutter & Siragusa, 1995). Many reports have been published on the synergistic antimicrobial effects of nisin with sucrose fatty acid esters (Thomas, Davies, Delves-Broughton, & Wimpenny, 1998), the lactoperoxidase system (Boussouel et al., 1999), thymol (Ettayebi, Yamani, & Rossi-Hassani, 2000) and carbon dioxide (Nilsson et al., 2000). Therefore, this study was performed to determine the synergistic effect of nisin and garlic shoot juice against *L. monocytogenes* present in the varied categories of whole, low and skim milk, as a potential preservative for use in the food industry.

2. Materials and methods

2.1. Preparation of garlic shoot juice (GSJ)

Garlic shoots were purchased from a local market in Gyungsan City, Korea. The samples were prepared by our method (Kim, Choi, & Kang, 2007). The collected samples of garlic shoot were washed in running water followed by rinsing in distilled water. After the removal of water content from garlic shoot samples by using sterilized gauge, the garlic shoots were finally dried at room temperature for 2–3 h. For sample preparation, the garlic shoot samples were mixed with distilled water at the ratio of 1:1 (w/v), then ground by using a mixer (ARTLON, Model DA282-2, Korea); 50% (w/v) juice of garlic shoot (GSJ) was obtained. The product was centrifuged at 18,000 rpm for 30 min at 4 °C. The supernatant of garlic shoot juice was collected and passed through a 0.45 μm filter (Millipore Co., USA), and stored at 4 °C until further analysis.

2.2. Chemicals

Nisin was purchased from Sigma Chemical Co. (N5764; Sigma, St. Louis, Missouri, USA). Nisin stock solution was prepared with 0.02 N HCl (6.25×10^4 IU/ml) and was sterilized by autoclaving at 121 °C for 15 min, and kept in a refrigerator at 4 °C until used.

2.3. Preparation of milk samples

Pasteurized and homogenized grade A milk samples consisting of whole (3.5% fat), low (1% fat) and skim milk (no fat) were obtained from a local market.

2.4. Preparation and maintenance of L. monocytogenes

The strain *L. monocytogenes* ATCC 19118 used in this study was collected from the Korean Agricultural Culture Collection, Suwon, Republic of Korea. The strain was maintained on BHI agar (brain heart infusion, Difco) at 4 °C and was grown in BHI broth at 37 °C for 24 h.

2.5. Inhibitory effect of garlic shoot juice (GSJ)

The varied concentrations of garlic shoot juice (GSJ) (2.5%, 5% and 10%) were added to whole, low and skim milk and inoculated with initial population of approximately 2×10^4 cfu/ml of *Listeria* strain ATCC 19118, respectively. Controls were inoculated with *Listeria* strain without GSJ, and stored at 0, 2, 4, 6, 8, 10, 12 and 14 days at 4 °C. The colonies were counted in NA (nutrient agar, Difco) at 37 °C after 24 h of incubation.

2.6. Inhibitory effect of nisin

To determine the inhibitory effect of nisin, varied concentrations of nisin (62.5, 125, 250 and 500 IU/ml) were added to whole, low and skim milk samples and were inoculated with an initial population of approximately 2×10^4 cfu/ml of *Listeria* strain ATCC 19118, respectively. Controls were inoculated with *Listeria* strain without nisin, and stored at 0, 2, 4, 6, 8, 10, 12 and 14 days at 4 °C. The colonies were counted in NA (nutrient agar, Difco) at 37 °C after 24 h of incubation.

2.7. Synergistic effect by GSJ and nisin

To determine the synergistic effect of garlic shoot juice (GSJ) and nisin, two concentrations of GSJ (2.5% and 5%) in combination with 62.5, 125, 250 and 500 IU/ml of