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Research Note

Microbial Growth and the Effects of Mild Acidification and Preservatives in Refrigerated Sweet Potato Puree†,‡

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ABSTRACT

Refrigerated sweet potato puree is a convenient form of sweet potato that can be used as an ingredient in formulated foods. The microbiology of refrigerated sweet potato puree during storage for up to 5 weeks was evaluated. Because the puree was made by comminuting steam-cooked sweet potatoes before refrigeration, no naturally occurring vegetative bacterial cells were detected during a 4-week period of refrigerated storage at 4°C. However, if postprocessing microbial contamination of the puree were to occur, contaminating microorganisms such as *Listeria monocytogenes* could grow during refrigerated storage. The effects of acidification or the addition of potassium sorbate and sodium benzoate on a population of *L. monocytogenes* inoculated into refrigerated (4°C) sweet potato puree were determined. Inoculation of the refrigerated puree with *L. monocytogenes* at 10^6 CFU/ml resulted in a 3-log increase after 3 weeks storage of nonsupplemented puree. Supplementation of the sweet potato puree with 0.06% (wt/vol) sorbic acid or benzoic acid plus mild acidification of the sweet potato puree with citric acid to pH 4.2 prevented growth of *L. monocytogenes* during storage at 4°C.

Sweet potato is a nutritious vegetable with high levels of dietary fiber, vitamins, minerals, and bioactive phytochemicals such as beta-carotene and polyphenols *(14, 18).* It is a healthy food choice for consumers and can serve as a functional ingredient in formulated foods and nutraceuticals. Sweet potato roots often are processed into purees that can be subsequently incorporated in various products such as baby food, casseroles, puddings, pies, cakes, bread, restructured fries, patties, soups, and beverages *(15–18).*

Canning and freezing are well-established methods for the processing and distribution of sweet potato purees. Canned purees typically receive excessive thermal treatment during retorting *(10),* which results in high nutrient losses and poor-quality products. Freezing preservation can provide better nutrient retention and high-quality products. However, the frozen purees require considerable investment in frozen distribution and storage and must be thawed before use. Because of these constraints on frozen purees, production of refrigerated sweet potato purees for limited distribution to institutional and retail clients would be desirable. Refrigerated puree is made by peeling, slicing, and steam blanching sweet potato roots. After blanching, the roots are ground into puree, which is then pumped through a series of chillers to quickly cool the product to 4°C. Once the puree is cool, it is placed in plastic containers, which are sealed and stored at 4°C for distribution under refrigeration. The product remains refrigerated until it is used, usually within 2 to 3 weeks. Unlike the frozen sweet potato puree, the refrigerated puree is convenient for incorporation into processed products because there is no need for a lengthy defrosting process.

Although vegetative microorganisms present on the raw product will be killed during steam blanching, there is a potential for postheating contamination by both nonpathogenic and pathogenic bacteria, such as *Listeria monocytogenes.* Postheating contamination and temperature abuse can pose a food safety risk when refrigerated products are not properly stored and handled *(2, 12).* Treatments based on refrigeration combined with sanitizers and preservatives have been studied as microbial safety measures for fresh-cut fruits and vegetables *(1),* asparagus puree *(12),* and green beans *(9).* The objectives of this research were to investigate microbial growth in sweet potato purees during refrigeration and to evaluate the effectiveness of preservatives or mild acidification for preventing growth of the psychrotrophic pathogen *L. monocytogenes* in the refrigerated puree. The preservatives tested were potassium sorbate and sodium benzoate. Both are unsaturated monocarboxylic salts, which are expected to have optimum antimicrobial activity when their components are in the protonated (undissociated) forms *(5, 7).* Although both preservatives are used as antifungal agents, sorbic acid is also effective against certain bacteria, excluding lactic acid bacteria *(5).* Acidification was achieved by the addition of either hydrochloric acid or citric acid, both of which have been used commercially for acidification of a variety of foods.

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MATERIALS AND METHODS

Preparation of sweet potato puree. Two lots of fresh sweet potatoes (Beauregard cultivar) were obtained 3 weeks apart from a sweet potato packing company (Wayne E. Bailey Produce Co., Chadbourn, N.C.). The harvested roots were cured, as commonly practiced, at 30°C and 85 to 90% relative humidity for 7 days and stored at 13 to 16°C at 80 to 90% relative humidity. The roots were stored in the company's facilities for 22 to 24 weeks before processing. Sweet potato puree was prepared in the Fruit and Vegetable Pilot Plant at the Department of Food Science (North Carolina State University) following the method described by Coronel et al. *(4).* Roots were washed, hand-peeled, trimmed, and cut into 0.95-cm-thick slices with a mechanical slicer (Louis Allis Co., Milwaukee, Wis.), and the slices were steamed for 20 min in a thermoscrew cooker (Rietz Manufacturing Co., Santa Rosa, Calif.). The temperature of the cooked sweet potato pieces was 100°C. The cooked pieces were rapidly transferred to a hammer mill (model D, Fitzpatrick Co., Chicago, Ill.) fitted with a 0.15-cm-mesh screen and comminuted to a puree. The temperature of the puree coming out of the hammer mill was 70 to 75°C. The puree was then pumped through a series of chillers to quickly cool to 4°C. The puree was placed in plastic pails, which were sealed and stored at 4°C. The same procedure was followed for both lots of sweet potatoes.

Microbiology of sweet potato puree during storage. Microbial counts of the sweet potato puree were determined immediately after preparation and then every 7 days for 1 month. Triplicate 100-g samples were taken aseptically from the refrigerated pails of each of the two lots of sweet potato puree and placed in sterile filtered stomacher bags (Spiral Biotech, Norwood, Mass.) with 100 ml of saline solution. Samples were homogenized for 1 min at normal speed in a laboratory blender stomacher (Tekmar, Cincinnati, Ohio), serially diluted with saline solution, and plated on plate count agar (PCA), yeast malt extract agar supplemented with 0.21 mM chlortetracycline and 0.31 mM chloramphenicol (YMA), violet red bile agar with 1% glucose (VRBG), and lactobacilli deMan Rogosa Sharpe agar (MRS) (all from Becton Dickinson, Sparks, Md.). YMA, PCA, and VRBG plates were incubated at 25, 30, and 37°C, respectively, under aerobic conditions for 1 week. MRS plates were incubated at 30°C under anaerobic conditions for 3 days. Counts for thermophilic microorganisms were obtained from PCA plates incubated under aerobic and anaerobic conditions at 45°C for 1 week.

Sweet potato puree as a substrate for growth of *L. monocytogenes*. The ability of *L. monocytogenes* to survive and grow in the refrigerated sweet potato purees under anaerobic conditions was evaluated by inoculating the puree with a five-strain *L. monocytogenes* cocktail (Silliker Research Center Collection, Chicago, Ill.). All isolates were obtained from human outbreaks caused by this bacterium via pepperoni, yogurt, ice cream, cabbage, and diced coleslaw, respectively. The strains were activated in tryptic soy agar (Becton Dickinson) containing 1% glucose (TSG agar) at 30°C under anaerobic conditions. Mid-log cells from cultures transferred twice were harvested (10 ml) and washed with 3 ml of saline solution. Cocktails of *L. monocytogenes* strains were prepared by mixing the washed cell suspensions in sterile 50-ml conical centrifuge tubes. Cells were harvested, washed, and resuspended in 10 ml of oxygen-free saline solution in an anaerobic chamber (Coy Laboratory Products, Grass Lake, Mich.). Samples (5 g) of sweet potato puree were preincubated in an anaerobic jar for 48 h before inoculation to remove oxygen. Triplicate 5-g samples from each of the two of sweet potato purees were inoculated with 500 μl of a 100× dilution of the cocktail resuspension (10^6 CFU/g) in an anaerobic hood. The inoculated samples were incubated at 4°C in an anaerobic jar for 36 days. Every 7 days, the anaerobic jars were placed inside the anaerobic chamber to process samples for the determination of counts of viable *L. monocytogenes*. Samples (100 μl) were collected with a 1-ml pipette from each tube, diluted in saline solution, and plated on TSG agar with a Spiral Plater (Spiral Biotech). Plates were incubated at 30°C under anaerobic conditions, and after 72 h of incubation colonies were counted with an automated colony counting system (Q-count, Spiral Biotech).

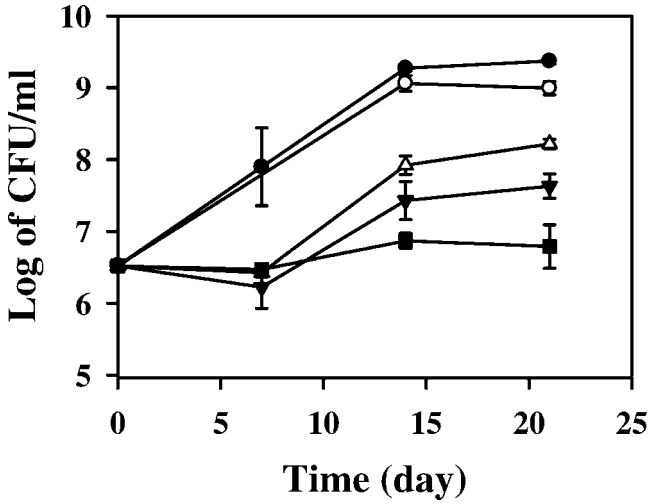

FIGURE 1. *Growth of* Listeria monocytogenes *in mildly acidified sweet potato puree at 4°C: without pH adjustment (●); pH adjusted to 5.0 with HCl (○); pH adjusted to 4.2 with HCl (▼); pH adjusted to 5.0 with citric acid (△); and pH adjusted to 4.2 with citric acid (■).*

Growth of *L. monocytogenes* in mildly acidified sweet potato puree. The amounts of HCl and citric acid required to adjust the pH of triplicate 5-g samples of the two sweet potato purees to 5.0 and 4.2 were determined by titration with 1 M solutions (Fisher Scientific, Pittsburgh, Pa.). Acidified samples were prepared, inoculated, incubated, and analyzed as described.

Growth of *L. monocytogenes* in sweet potato puree containing preservatives. Triplicate 5-g samples of sweet potato puree from each of the two lots were supplemented with 12.0, 5.0, and 8.2 or 8.9 mM potassium sorbate or sodium benzoate, respectively. All samples were subsequently prepared, inoculated, stored, and analyzed as described.

RESULTS AND DISCUSSION

Sweet potato puree was prepared from steam cooked sweet potato slices. As a result, there was no evidence of microbial growth in noninoculated samples from two separately prepared lots of sweet potato puree during storage for 4 weeks at 4°C. The observation of mold growth on the surface of puree that was exposed to air during refrigerated storage in one of the pails indicates that packaging that prevents the entry of oxygen into the head space of the package is necessary to prevent fungal growth.

If postprocessing contamination of sweet potato puree by *L. monocytogenes* were to occur, the data presented here indicate that this microorganism could grow extensively under anaerobic conditions, assuming a 4-week product shelf

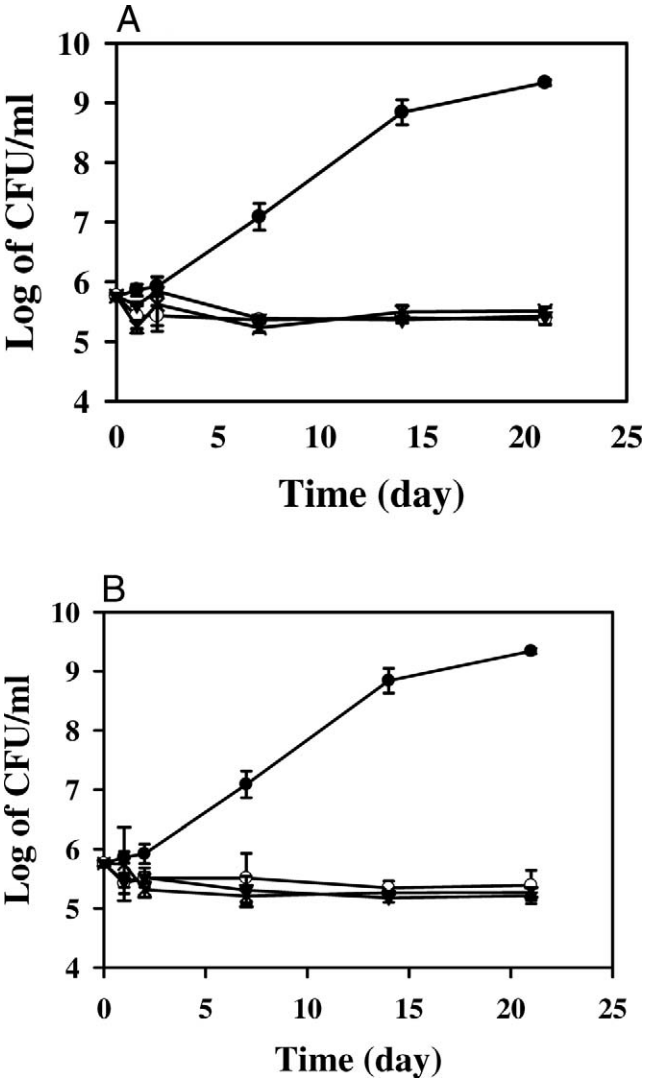

FIGURE 2. *Growth of* Listeria monocytogenes *in sweet potato purees containing potassium sorbate and sodium benzoate.* L. monocytogenes *grew in sweet potato puree (●) at 4°C under anaerobic conditions, but growth differed in sweet potato purees supplemented with 12.0 mM (○), 8.9 or 8.2 mM (×), or 5.0 mM (▼) potassium sorbate (A) or sodium benzoate (B).*

life (Figs. 1 and 2). However, acidification of the vegetable puree to pH 4.2 with citric acid effectively prevented growth of *L. monocytogenes* under refrigerated conditions (4°C). Acidification with HCl to pH 4.2 and with citric acid to pH 5.0 reduced but did not eliminate growth of *L. monocytogenes.* The addition of citric acid was expected to result in development of some sour taste in the puree *(11)* because more than 99% of the citric acid molecules would have at least one protonated carboxyl group at 4°C and pH 4.2 *(6).*

A minimum concentration of 0.06% (5 mM) potassium sorbate or sodium benzoate prevented growth of *L. monocytogenes* in sweet potato puree (pH 6.5 ± 0.2) stored at 4°C (Fig. 2). However, at 17°C even the highest concentration of the preservatives (12 mM) did not prevent growth of *L. monocytogenes.* At this higher temperature, the *L. monocytogenes* population increased from 10^6 to 10^7 CFU/g during the first 10 days of incubation. Therefore, proper temperature control is critical even with the addition of these preservatives. The ability of these preservatives to inhibit growth of *L. monocytogenes* in refrigerated sweet potato puree was somewhat unexpected because at the pH and temperature tested only 1.5% of the sorbate molecules and 0.5% of the benzoate molecules would be protonated *(6).* However, Eklund *(7, 8)* suggested that the dissociated molecules in preservatives make some contribution to the ability of these compounds to inhibit bacteria. Stratford and Anslow *(13)* provided some evidence to suggest that in yeast sorbic acid may act as a membrane-active substance rather than as a weak-acid preservative. Bracey et al. *(3)* also presented evidence suggesting that sorbic acid does not act as the classic weak-acid inhibitor. Their studies suggest that the inhibitory action of sorbic acid is due to the induction of an energetically expensive protective mechanism that compensates for any disruption of internal pH, resulting in a reduced amount of energy available for growth.

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