Effect of High Hydrostatic Pressure on *Salmonella* Inoculated into Creamy Peanut Butter with Modified Composition

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MS 14-062: Received 3 February 2014/Accepted 11 April 2014

ABSTRACT

Peanut butter has been associated with several large foodborne salmonellosis outbreaks. This research investigates the potential of high hydrostatic pressure processing (HPP) for inactivation of *Salmonella* in peanut butter of modified composition, both by modifying its water activity as well by the addition of various amounts of nisin. A cocktail of six *Salmonella* strains associated with peanut butter and nut-related outbreaks was used for all experiments. Different volumes of sterile distilled water were added to peanut butter to increase water activity, and different volumes of peanut oil were added to decrease water activity. Inactivation in 12% fat, light roast, partially defatted peanut flour, and peanut oil was also quantified. Nisaplin was incorporated into lactis strains (11). Mbandaka linked to contaminated *Salmonella* that is highly effective against gram-positive *Lactococcus lactis* Bredeney in 2012, from consump-

Peanut butter has been associated with several large foodborne salmonellosis outbreaks. The first recorded outbreak of salmonellosis resulting from the consumption of peanut butter occurred in 1996 when 15 persons were infected with *Salmonella* Mbandaka linked to contaminated roasted peanuts that were processed into peanut butter and sold in South Australia (11). An outbreak of *Salmonella* Agona infection in Israel, England, Wales, and the United States in 1994 through 1995 infected 2,200 people and was associated with a peanut butter–coated snack produced in Israel (9). More than 600 persons infected with *Salmonella* Tennessee were reported in 47 states in 2006 and 2007 due to consuming contaminated peanut butter processed in a single facility in Georgia (2). Another large multistate outbreak of *Salmonella* infection associated with peanut butter and peanut butter products occurred in 2008 and 2009, infecting 529 persons from 43 states, with eight confirmed deaths (3). *Salmonella* Typhimurium–contami-

history. Most recently, 42 persons from 20 states were infected with *Salmonella* Bredeney in 2012, from consumption of peanut butter manufactured by Sunland, Inc. This recall, expanded to include almond butter and both in-shell and shelled, raw and roasted peanuts, eventually led to the U.S. Food and Drug Administration’s (FDA’s) suspension of the food facility registration of Sunland Inc. (1).

The ability of *Salmonella* to survive in low water activity foods, such as peanut butter, and its increased thermal resistance at temperatures even as high as 90°C (12) have led to the exploration of technologies other than thermal pasteurization. One such nonthermal technology is high hydrostatic pressure processing (HPP). However, HPP seems to have limited effectiveness against *Salmonella* under low–water activity conditions (5, 7). Irrespective of the pressure time conditions of HPP used, as well as pressure cycling conditions used, the achieved log reduction was less than 2 at a mean initial concentration of 6.5 log CFU/g (5).

Other possible technologies suitable for use in combination with HPP to inactivate *Salmonella* in peanut butter include the application of antimicrobial peptides such as nisin. Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* that is highly effective against gram-positive bacteria and spores (4). It is a natural, toxicologically safe
food preservative. Nisin was approved by the FDA in 2001, with generally recognized as safe status for usage as a preservative at levels ranging from ~1 to 25 ppm in dairy products, meat products, and canned foods (4). Although nisin is not commonly known to be effective against gram-negative bacteria, such as Salmonella, recent studies have shown that nisin, in combination with HPP, may inactivate Salmonella (10) and Escherichia coli (6), perhaps by causing alterations in the outer cytoplasmic membrane of gram-negative bacteria, facilitating penetration of nisin into the cell.

This research investigates the potential of HPP for inactivation of Salmonella in peanut butter of modified composition, both by modifying its water activity with the addition of distilled water and 100% peanut oil in different proportions, as well by the addition of various amounts of nisin.

MATERIALS AND METHODS

Bacterial cultures, inoculum preparation, and HPP. Cultures were obtained and prepared, as described elsewhere (5). Briefly, six Salmonella strains associated with peanut butter and nut-related outbreaks (Salmonella Enteritidis PT30, also known as LJI 608 or ATCC BAA-1045; Salmonella Tennessee, isolated from a patient from an outbreak associated with peanut butter; Salmonella Oranienburg, isolated from pecans; Salmonella Anatum, isolated from an almond survey; Salmonella Enteritidis PT 9c, a clinical isolate from an outbreak associated with raw almonds; and Salmonella Montevideo, isolated from an almond survey) were maintained in glycerol stocks and stored at −85°C. Cultures were activated by inoculation into tryptic soy broth and incubated at 37°C for 18 to 24 h. Aliquots of overnight culture were combined in equal amounts to produce the desired cocktail at ~10⁹ CFU/ml. Samples for each experimental condition outlined below were high pressure processed in a 10-l HPP vessel (Elmhurst Research, Inc., Albany, NY), as described elsewhere (5). Experiments were replicated twice, and repeated samples were plated in duplicate.

Peanut butter with higher water activities. Different volumes of sterile distilled water were added and blended into peanut butter to increase its water activity. Peanut butter water activity (a_w) was measured using a digital a_w meter (Rotronic Instrument Corporation, Hauppauge, NY) calibrated as per manufacturer’s directions using saturated salt solutions. Experiments were carried out with 10, 15, 25, 35, 50, 75, and 90% (wt/wt) of peanut butter replaced with added water. For example, a jar of peanut butter (16.3 oz; 462 g) was emptied into the blender jar, and 10% of the weight of peanut butter was replaced with sterile distilled water (46.2 ml) and blended, until no phase separation was observed, to produce the 10% moisture product. Modified peanut butters for the other added moisture content experiments were produced in a similar manner. For each added moisture content sample, the water activity was measured in duplicate using the digital a_w meter. The overnight culture of the Salmonella cocktail was inoculated into the modified peanut butter (1% by weight of the modified peanut butter) and blended to achieve homogeneity. The inoculated peanut butter was distributed into pouches, and the pouches were vacuum packaged using the FoodSaver Vacuum Sealer (Sunbeam Products, Inc., Boca Raton, FL) and high pressure processed at room temperature at 600 MPa for 18 min. For comparison with our earlier work (5) and as an upper practical limit for commercialization, we selected 18 min. The average initial process temperature was 26 ± 1°C, the average maximum process temperature was 43 ± 1°C, and the average final temperature after depressurization was 24 ± 1°C. Control and high pressure–processed samples were diluted and plated on xylose lysine Tergitol 4 agar. Plates were incubated at 37 ± 1°C for 24 h, and putative Salmonella colonies enumerated.

Peanut butter with decreased water activities. A bottle of Planters 100% Peanut Oil (24 fluid oz [710 ml]; New Century, KS) was purchased from a local supermarket. The bottle of peanut oil was stored at room temperature before and after opening. Measured volumes of peanut oil were added and blended into peanut butter to decrease its water activity. Resulting water activities were measured using the digital a_w meter. Experiments were carried out with 50 and 75% of the peanut butter replaced by peanut oil (wt/wt), using similar procedures described previously for the increased water activity experiments, e.g., for the 50% added peanut oil experiment, 50% of the weight of peanut butter (231 ml of peanut oil) was added and blended until no phase separation is observed. Inoculation, high pressure processing, plating, and enumeration occurred as previously detailed.

Peanut butter components. A 1-kg sample of 12% fat, light roast, partially defatted peanut flour was obtained from Golden Peanut Company (Alpharetta, GA). Peanut flour (500 g) was weighed into a 2,000-ml blender jar. A cocktail of Salmonella strains was prepared as described in the section “Bacterial cultures, inoculum preparation, and HPP,” and 5 ml of the cocktail inoculated into the peanut flour (1% by weight of peanut flour) and blended. The inoculated peanut flour was distributed into four pouches (Fisher Scientific, Pittsburgh, PA) and high pressure processed at 600 MPa for 18 min at room temperature. Control (unprocessed inoculated peanut flour), and high pressure–processed samples were serially diluted and plated as previously described.

One-tenth milliliter of the Salmonella cocktail was inoculated into 10 ml of peanut oil in plastic vials (Fisher Scientific). Control vials of Salmonella-inoculated peanut oil were stored at room temperature for 2 h (equivalent to the time to prepare samples and process them under high pressure), and test vials of Salmonella-inoculated peanut oil were vacuum packed using a FoodSaver Vacuum Sealer prior to high pressure processing. Vials were high pressure processed at 600 MPa for 18 min at room temperature. The ambient temperature control vials and high pressure–processed vials were vortexed, diluted, and plated as described previously.

Nisin incorporation into peanut butter. Nisin was incorporated into peanut butter in its food-grade formulation Nisaplin (Danisco, Waukesha, WI) at four concentrations: 100, 200, 500, and 1,000 ppm. These levels of Nisaplin correspond to 2.5, 5.0, 12.5, and 25.0 ppm of pure nisin, which is within the range of recommended dosage levels for food applications (4). Nisin has increased solubility in an acidic environment and optimal stability in the pH range of 3.0 to 3.5 (4); thus, was dissolved in 0.02 N HCl prior to incorporation into the peanut butter. For example, to achieve 100 ppm of Nisaplin (2.5 ppm of nisin), 100 mg of Nisaplin/1,000 g of peanut butter is needed. Thus, for 462 g of peanut butter, 0.046 g of Nisaplin was dissolved in 3 ml of 0.02 N HCl. The resulting solution was vortexed and filtered to produce a nonparticulate solution. This Nisaplin solution was added to peanut butter and blended until thoroughly mixed (~2 min). The overnight culture of cocktail was then added to the peanut butter (1% by weight of peanut butter) and blended. The inoculated nisin-containing peanut butter was then distributed into multiple pouches and high pressure processed as detailed previously. Control
Salmonella samples (25 g of inoculated unprocessed peanut butter with nisin) and two pressure-treated pouches were weighed out in filter bags 1 h after high pressure processing. The 1-h delay was to allow time for nisin to permeate through the membranes of the Salmonella cells after high pressure processing. Peptone water (25 ml) was added to each 25-g sample of peanut butter and stomached for 2 min, followed by dilution and plating on xylose lysine Tergitol 4 agar plates. Plates were incubated at 37°C for 24 h, and colonies enumerated. Other pressure-treated pouches were stored at room temperature to sample over a longer time. High pressure–processed pouches containing 2.5 and 5.0 ppm of nisin were sampled 1 day after high pressure processing, samples containing 12.5 ppm of nisin were sampled after 1 and 3 days, and samples containing 25.0 ppm of nisin were sampled 1, 3, 5, and 7 days.

To determine if Nisaplin alone played any role on the inactivation of Salmonella in peanut butter over time without high pressure processing, an experiment was carried out using 12.5 ppm of nisin (500 ppm of Nisaplin). All experimental details were as described previously. Inoculated nisin-containing peanut butter was sampled immediately after inoculation and after 1 h, 1 day, 3 days, 5 days, and 7 days.

Statistical analysis of data. We used single-factor analysis of variance tests and t tests in Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA) to compare the Salmonella counts at varying compositions of peanut butter after HPP at a level of significance of 95% (P < 0.05).

RESULTS AND DISCUSSION

Effect of HPP on Salmonella in increased water activity formulations of peanut butter. Figure 1 shows the effect of HPP on Salmonella in formulations of peanut butter with differing added moisture. The initial level of Salmonella recovered in each different moisture content peanut butter is represented by the black bars, while the grey bars represent the level of Salmonella in the water activity–modified samples of peanut butter after HPP at 600 MPa for 18 min. Bars with the same letter above are not statistically significantly different (P ≤ 0.05). Figure 1 shows that there is no difference for 10, 15, or 25% added moisture (corresponding to water activities of 0.67, 0.79, and 0.87, respectively). The initial Salmonella level is constant ~6.7 log CFU/g, and the effect of HPP was the same, producing about a 0.8-log CFU/g reduction, resulting in a final concentration of 5.9 log CFU/g. Although the log reduction is of little practical significance, the difference is statistically significant. When added moisture is 35%, the starting concentration does not change significantly, but log reduction does increase significantly to 1.2 log CFU/g. When the added moisture is raised to 50, 75, or 90% (corresponding to water activities of 0.93, 0.94, and 0.96, respectively), the initial concentration of the Salmonella increases slightly but is statistically significantly to ~7.3 log CFU/g. This may be due to slight variations in inoculum preparation or to improved recovery efficiency from the more liquid food matrices present in the higher-moisture formulations. These higher concentrations are unlikely to be due to Salmonella growth as the time from inoculation to sample recovery is quite short (<2 h). Of more practical importance is the statistically significant increases in log reduction seen as added moisture increases from 50 to 75 to 90%. After high pressure processing, the color of all peanut butter samples containing added moisture contents darkened, with the degree of darkening correlated to the amount of added moisture (data not shown). It should also be noted that the texture of peanut butter changed significantly due to the excess moisture present. Although increasing the moisture content of peanut butter increased the log reductions of Salmonella from HPP, the texture and color of the resulting product make this technique impractical, to say the least.

Effect of HPP on Salmonella in decreased water activity formulations of peanut butter. Figure 2 shows the effect of HPP on Salmonella in formulations of peanut butter modified to have lower water activities by the
addition of peanut oil. The black bars represent concentrations of *Salmonella* in unprocessed samples, and the grey bars represent samples after high pressure processing at 600 MPa for 18 min. At 50% added peanut oil (a~w~ 0.16), a statistically (but not practically) significant, 0.8-log reduction was achieved. At 75% added peanut oil (a~w~ 0.13), no significant log reduction was observed. These experiments show that the addition of peanut oil to lower the water activity of peanut butter further reduces the effectiveness of HPP.

**Effect of HPP on *Salmonella* in peanut butter components.** Figure 3 shows the effect of HPP on *Salmonella* in 12% fat, light roast, partially defatted peanut flour and 100% peanut oil. Interestingly, just over a 1-log reduction was obtained in peanut flour, while inactivation to below detection limits (2 log CFU/g) was observed in peanut oil. These results are consistent with the observations of Grasso et al (8), where a single strain (*Salmonella Typhimurium* ATCC 53647) in 100% peanut oil was inactivated to below detection limits after high pressure processing at 600 MPa for 5 min at 45°C. The mechanism for such an effect is as yet undiscovered but may relate to the nature of the bacterial membrane when it is in a pure oil rather than a protein-oil or protein-oil-water matrix.

**Effect of nisin and HPP on *Salmonella* in peanut butter.** Figure 4 shows the effect of nisin incorporation at the two lowest levels studied (2.5 and 5.0 ppm), before processing (black bars), immediately after processing (grey bars), as well as 1 day after processing (white bars). In both levels, a clear trend is seen in which processing produces an immediate ~0.8-log reduction and a ~1.1-log reduction after 1 day when 2.5 ppm of nisin is used and a similar immediate log reduction and slightly greater (~1.4) log reduction after 1 day when 5.0 ppm is used. As the letters indicate in Figure 4, the effect of processing is statistically significant at both concentrations, and the additional decline after 1 day is significant at 5.0 ppm.

Figure 5 shows the effect of nisin at the two higher levels of incorporation (12.5 and 25.0 ppm) over a longer time (7 days), as well as the effect of nisin at 12.5 ppm without high pressure processing. Black bars represent unprocessed peanut butter, with progressively lightened bars representing 1 h, 1 day, 3 days, 5 days, and 7 days after processing. The first set of bars shows that nisin at 12.5 ppm without HPP results in no significant change to the concentration over 1 week of storage. When nisin is incorporated at 12.5 or 25.0 ppm and subject to HPP
(600 MPa for 18 min), the *Salmonella* concentration is reduced by a statistically significant amount such that the concentration 1 h and 1 day after processing is different from the unprocessed sample. The *Salmonella* concentration declines further such that at 12.5 ppm, the concentrations at 3 and 5 days are significantly lower. The same trend is seen at 25.0 ppm but at 5 and 7 days after processing.

In summary, plating after nisin and HPP treatment at varying times did show some effect on increase in the log reduction of *Salmonella* with time. The maximum log reduction of *Salmonella* achieved was 1.7 log CFU/g, which was comparable to that achieved by noncycling pressure treatment alone (5), which used a time interval from treatment to measurement of >1 day. In any event, it can be concluded that the application of nisin in combination with HPP does not enhance the inactivation of *Salmonella* in peanut butter that it would likely be worth the effort or the cost, despite recent studies showing that nisin, in combination with HPP, may inactivate *Salmonella* (10) and *E. coli* (6) in high-water activity environments.

Nisin is a water-soluble molecule but also is able to bind to cell membranes. It has been shown in micellar systems that the didehydroalanine and leucine of ring A (residues 5 and 6) will insert themselves to the lipid phase (13). The results from our experiments with nisin and HPP on *Salmonella*-inoculated peanut butter may indicate that most of the nisin added to the peanut butter inserts itself into the lipid phase of peanut butter. This may have a negligible effect on inactivation of *Salmonella* cells that likely reside in the small water phase pockets of the peanut butter matrix.

Our research has shown that in peanut butter modified to water activities of 0.96 (or in 100% peanut oil matrices), *Salmonella* can be inactivated to below detection limit. In the case of the former condition, such a modified product is significantly different from peanut butter and darkens considerably after HPP. Nisin, in combination with HPP, is not effective in enhancing inactivation of *Salmonella* in peanut butter. The maximum log reduction after HPP at 600 MPa and 18 min was 1.7 log CFU/g, which is closely comparable to the result obtained with HPP alone. The peanut butter matrix supports the survival of *Salmonella* in peanut butter during and after HPP. High pressure processing alone or with added nisin is not a suitable technology to manage the microbiological safety of *Salmonella*-contaminated peanut butter. HPP can be explored in combination with other technologies for achieving greater inactivation of *Salmonella* in peanut butter and/or be the final step of a multiple-hurdle approach to ensure the microbiological safety of peanut butter.

**ACKNOWLEDGMENTS**

This research was supported by the Center for Advanced Food Technology, Rutgers—The State University of New Jersey. We thank Danisco for providing Nisaplin and Dr. Linda J. Harris, University of California, Davis, for providing us with the pathogenic *Salmonella* strains.

**REFERENCES**


