



The Potential Benefit of Yellow Mustard to Control *Salmonella* spp. in a Pork Fermented Sausage

ABSTRACT

It has been shown that deodorized yellow mustard powder has the potential to inhibit the growth of common foodborne pathogens such as *Escherichia coli* O157:H7 and *Listeria monocytogenes* in dry fermented sausages. However, the time needed to achieve a significant reduction could be longer than what a commercial process requires. In the present study, the effect of yellow mustard on a *Salmonella* cocktail in a pork sausage was determined to explore the potential use of this antimicrobial in pork sausage production. For this, yellow mustard powder was added to the pork sausage batter during manufacturing, and the sausage samples were analyzed for any effects on *Salmonella*, starter cultures, and physico-chemical characteristics. The results showed that the yellow mustard powder in the dry fermented sausage tested was able to achieve a 5-log reduction in numbers of *Salmonella* within 14 days of drying. The yellow mustard powder had no effect on the starter cultures and did not affect the

water activity or pH of the dry fermented sausages, compared to the sausages without yellow mustard.

INTRODUCTION

During food production, food products could be contaminated with *Salmonella* (21). In 2016, the Centers for Disease Control and Prevention (CDC) reported 7 recalls because of contamination with *Salmonella* in food (13), and the Canadian Food Inspection Agency (CFIA) handled 18 food recalls due to *Salmonella* (8). These recalls show that it is a challenge for the food industry to reduce the risk of contamination with *Salmonella*. In addition, since *Salmonella* can survive at relatively low pH and water activity (a_w) conditions, it can cause foodborne illness associated with the consumption of non-heat-treated food products such as dry-fermented foods. In fact, several salmonellosis outbreaks have been due to the consumption of fermented sausages. For example, there was an outbreak of *S. Typhimurium* associated with Lebanon bologna-semi-dry fermented sausage in the U.S. in 1995 (48), an outbreak of *S. Goldcoast* linked to fermented sausage in Germany in

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2001 (5) and a food recall of dry-fermented sausages due to possible *Salmonella* contamination in Canada in 2014 (6). As a result, the CFIA made it mandatory in 2017 for producers to achieve a 5-log reduction of *Salmonella* in processed meat products (except for poultry) that do not receive a heat treatment (10). In addition, current requirements in the U.S. mandate achieving at least a 5-log reduction of *Salmonella* in these products (50). Several studies have shown that foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. can contaminate dry fermented sausages and raw ingredients and may survive the processing of dry fermented sausages (15).

Salmonella spp. are commonly found in animals such as poultry and swine (21, 43). *Salmonella* can grow at temperatures ranging from 5 to 47°C, at pH values from 4.0 to 9.0, and down to a water activity of 0.94 (4). *Salmonella* is one of most common causes of foodborne illness in the world. Approximately 1.3 billion cases and 3 million deaths are due to non-typhoid salmonellosis per year (44). In the U.S., such infections cause an estimated 1.2 million cases of illness and 450 deaths annually (12, 43). In Canada, the Public Health Agency of Canada estimates that 87,500 cases of illness, 925 hospitalizations, and 17 deaths yearly are caused by non-typhoidal *Salmonella* (23). Since hosts of *Salmonella* can be animals and humans, the organism has been found in humans, poultry, cattle, swine, soil, and water and even on surfaces of factory equipment (21).

Strategies to enhance food safety while inhibiting pathogenic and spoilage microorganisms in dry fermented sausages include the use of modified-atmosphere packaging or active packaging, as well as new preservation technologies such as high hydrostatic pressure (29). In addition, natural antimicrobial compounds such as bacteriocins and plant-based derivatives (such as essential oils) can be used to reduce and/or inactivate pathogens and spoilage bacteria (22, 29). Recent studies have focused on natural antimicrobials, as food producers become more interested in using these natural compounds as the result of increasing concern about the quality and safety of food as well as increasing consumer demand for “natural,” “functional,” “healthier” food (24, 39). One such natural compound is found in mustard. Mustard has therefore been proposed as an alternative to natural inhibitors for the control of foodborne pathogens because of its chemical composition (25). Mustard is a significant source of the compound glucosinolate, which contributes to its hot flavor (11, 20) and which is the stable precursor of the isothiocyanates, known for their antimicrobial activity (4, 19). Yellow mustard has a mild flavor after hydrolysis of sinalbin (a glucosinolate found in yellow mustard) by endogenous myrosinase, with production of isothiocyanate (14, 20, 42, 50). Isothiocyanate in mustard was found to have strong antimicrobial activity against foodborne pathogens such as *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. (40, 45). The application of yellow mustard as a food ingredient

is limited, however, because of its pungent characteristics (17, 31, 38). Nevertheless, thermally treated or deodorized (i.e., with inactivated myrosinase activity) mustard powder can be used as a high-protein ingredient with flavorless characteristics after denaturing the enzyme myrosinase (32, 38). Deodorized mustard in the form of an oriental mustard extract has been found to decrease the numbers of *Salmonella* on chicken breast by 2.3 log CFU/g after 21 days at 4°C (40, 41). However, the production process for some fermented sausages could be less than 21 days. The current study was carried out to determine the conditions necessary for yellow mustard to control *Salmonella* spp. in a pork fermented sausage.

MATERIALS AND METHODS

Bacterial strains

The batter for the dry fermented sausages was inoculated with a cocktail of five strains of *Salmonella* spp. (Enteritidis, Typhimurium, Agona, Heidelberg, Hadar), which were a kind donation of Dr. Sandeep Tamber, Bureau of Microbial Hazards, Health Canada (Table 1). All strains were stored in tryptic soy broth (TSB) (Difco, Sparks, MD, USA) containing 20% glycerol at -80°C. The starter culture used for fermentation was a commercial mix containing *Lactobacillus sakei*, *Pediococcus pentosaceus* and *Staphylococcus carnosus*.

Inoculum preparation

The procedures for the inoculation of the *Salmonella* spp. cocktail were similar to the one described by Mataragas et al. (35), with some modifications. In brief, the *Salmonella* serotypes were streaked onto XLD selective medium (Difco, Sparks, MD, USA), and single colonies were transferred into 240 ml of TSB, which was incubated for 18–24 h at 37°C and 200 rpm to obtain stationary phase cells. The OD₆₀₀ of each strain was measured in a spectrophotometer (Beckman Coulter, Inc., Fullerton, California) until it reached a value of 0.9 (late log phase). The cultures were then centrifuged (7800 × g at 4°C for 10 min) to collect the cell pellets. The supernatants were removed, and pellets were washed with 0.1% (wt/vol) peptone water (Oxoid, Hampshire, England) and centrifuged again. After centrifugation, the cultures were suspended in 40 ml 0.1% (wt/vol) peptone water. All five *Salmonella* strains were combined for the inoculation cocktail, using the same volume of each strain. *Salmonella* levels in the cocktail were enumerated by surface plating on Brilliant Green Sulfa Agar (BGA) (Difco, Sparks, MD, USA). The final concentration of *Salmonella* was 2×10^7 CFU/g in the meat batter.

Fermented sausage manufacture

The procedures for dry fermented sausage manufacture were similar to those of Coroller et al. (18). Briefly, for each batch, there were 2 treatments for dry

TABLE 1. *Salmonella* strains used in a bacterial cocktail to inoculate the pork batter

Strain Name	Source of Isolation
<i>Salmonella</i> Enteritidis	pork
<i>Salmonella</i> Typhimurium	pork
<i>Salmonella</i> Agona	pork
<i>Salmonella</i> Heidelberg	pork muscle
<i>Salmonella</i> Hadar	pork

TABLE 2. Composition of the sausages prepared for this study

Pork Sausage	3 kg Pork Meat
Starter culture	0.45 g
Dextrose	0.45 g
Spices	118.81 g
Water	9 ml
Deodorized mustard	84 g
Mustard (hot)	8.4 g
	Total 3,221.21 g of batter

fermented sausage samples, one with yellow mustard (kindly donated by Montana Specialty Mills, Great Falls, MT, USA) and one without yellow mustard. The mustard was comparable to the deodorized mustard used by Cordeiro et al. (17) in terms of its sinalbin concentration. For each treatment, the mixture included meat, curing agents, starter cultures, yellow mustard and hot mustard powder, as well as the 5-strain cocktail of *Salmonella* (Table 2). After thorough mixing, the batter was stuffed into a natural casing (approximately 35 mm in diameter; Marini Foods). The length of each sausage was approximately 15 cm and the weight of each sausage was 200 g. After the sausages were stuffed, they were hung on aluminum sticks and put into a fermentation chamber for approximately 23 h until a pH of ≤ 5.3 was reached at a constant temperature of 34°C. After fermentation, the sausages were moved to a chamber at 25°C with a fan blowing on the products for about 15 h. Subsequently, the sausages were transferred to an environmental chamber at 80% relative humidity at a constant temperature of $15 \pm 2^\circ\text{C}$ and dried for 21 days. The a_w of the final product was less than 0.90, while the final pH was less than 5.3 (7).

Microbial analyses

For the Aerobic Plate Count (APC), 25 g duplicate samples of raw meat was taken from multiple locations from each batch before the sausages were manufactured. The 25 g meat sample was placed into a Stomacher™ bag containing 225 ml of 0.1% sterile peptone water and then stomached for 2 min at 200 rpm. Serial dilutions in 0.1% peptone water were then surface plated onto plate count agar (Difco, Sparks, MD, USA) and incubated at $35 \pm 1^\circ\text{C}$ for 24 h. To enumerate *Salmonella*, samples were plated onto BGA. Two sausage sticks were collected from each treatment at the determined sampling time. Similar to the process used for raw meat, random samples from each dried sausage stick (25 g total) were placed into a Stomacher bag with peptone water. Samples were stomached and plated on agar. All plates were incubated at $35 \pm 1^\circ\text{C}$ for 18–24 h. When the levels of *Salmonella* were below the detection limit ($0.8 \log_{10}$ CFU/g), 25 g samples were enriched in 225 mL TSB, which was then incubated at $35 \pm 1^\circ\text{C}$ for 18–24 h. After enrichment, samples were plated onto BGA to detect viable cells. For the enumeration of the starter cultures, meat samples were collected from multiple locations of sausages at the end of the fermentation. Samples were surface-plated onto De Man-

Rogosa-Sharpe (MRS) (Sigma Aldrich, St. Louis, MO, USA) and Yeast Peptone Dextrose (YPD) agar (Fisher Scientific Co., Fair Lawn, NJ, USA), and then incubated at 35°C for 18 h. The viable population of *Salmonella* and starter cultures in the sausages were expressed as mean \log_{10} values.

Physicochemical analyses

The pH of each sample (stuffed and dried sausages) was measured using a calibrated Denver Instrument model AP5 pH meter (Fisher Scientific Co., Fair Lawn, NJ, USA) according to the manufacturer's instructions. Data were collected from different positions of the sausage in duplicate by insertion of the electrode directly into the meat. The a_w of the sausages was measured using a calibrated Aqualab model CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA, USA), following manufacturer's instructions. For this, random samples were taken from different parts of the sausage (stuffed and dried sausages) to cover the bottom of the a_w sample cups. Sample cups were then placed into the equipment chamber for measurement.

Statistical analysis

All values presented are averages from 3 separate trials, using duplicate samples ($n = 6$). Statistical differences among treatments were compared using Student's *t*-test (Excel for Mac 2011, version 14.4.2, Microsoft Corporation, Redmond, WA, USA). Differences between treatments were considered significant when the *P* value was < 0.05 .

RESULTS

Antimicrobial activity of yellow mustard

At the end of the fermentation, the average population of the starter cultures in samples without mustard was $9.11 \pm 0.05 \log_{10}$ CFU/g on MRS and $8.86 \pm 0.14 \log_{10}$ CFU/g on YPD. For samples with mustard, the population of the starter cultures was $8.88 \pm 0.21 \log_{10}$ CFU/g on MRS and $9.14 \pm 0.08 \log_{10}$ CFU/g on YPD. In terms of the populations of *Salmonella*, the sausages without mustard powder showed a $3.3 \log_{10}$ CFU/g reduction of *Salmonella* after 21 days of drying. The samples with yellow mustard demonstrated a $3.3 \log_{10}$ CFU/g reduction of *Salmonella* at the end of 7 days, and by day 14, *Salmonella* populations had further decreased, to below the detection limit ($< 0.8 \log_{10}$ CFU/g) (Fig. 1), showing a significant difference ($P < 0.05$) between the sausage with mustard powder and the sausage without mustard.

Changes in pH and a_w during sausage drying

Initially, the average pH of sausages with no added mustard was 5.88 ± 0.04 before fermentation. For samples with mustard, the average pH was 5.76 ± 0.04 . However, the pH values of these sausages decreased rapidly to less than 5.0 by the end of fermentation. After 21 days, the final pH values of the dry fermented sausages ranged from 4.90 to 4.66 with mustard and from 5.03 to 4.87 without mustard (Fig. 2A). The pH was not significantly different ($P > 0.05$) for the two treatments from day 0 to day 21. The initial a_w of the meat batter was 0.9678 in sausages without mustard and

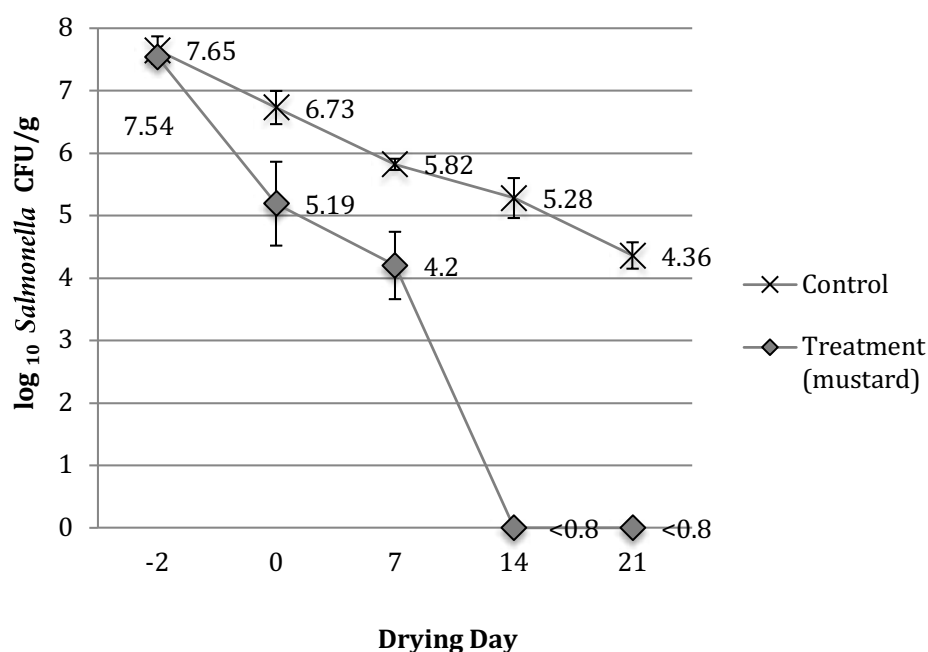


Figure 1. *Salmonella* levels in the pork fermented sausages with (treatment) and without mustard (control).

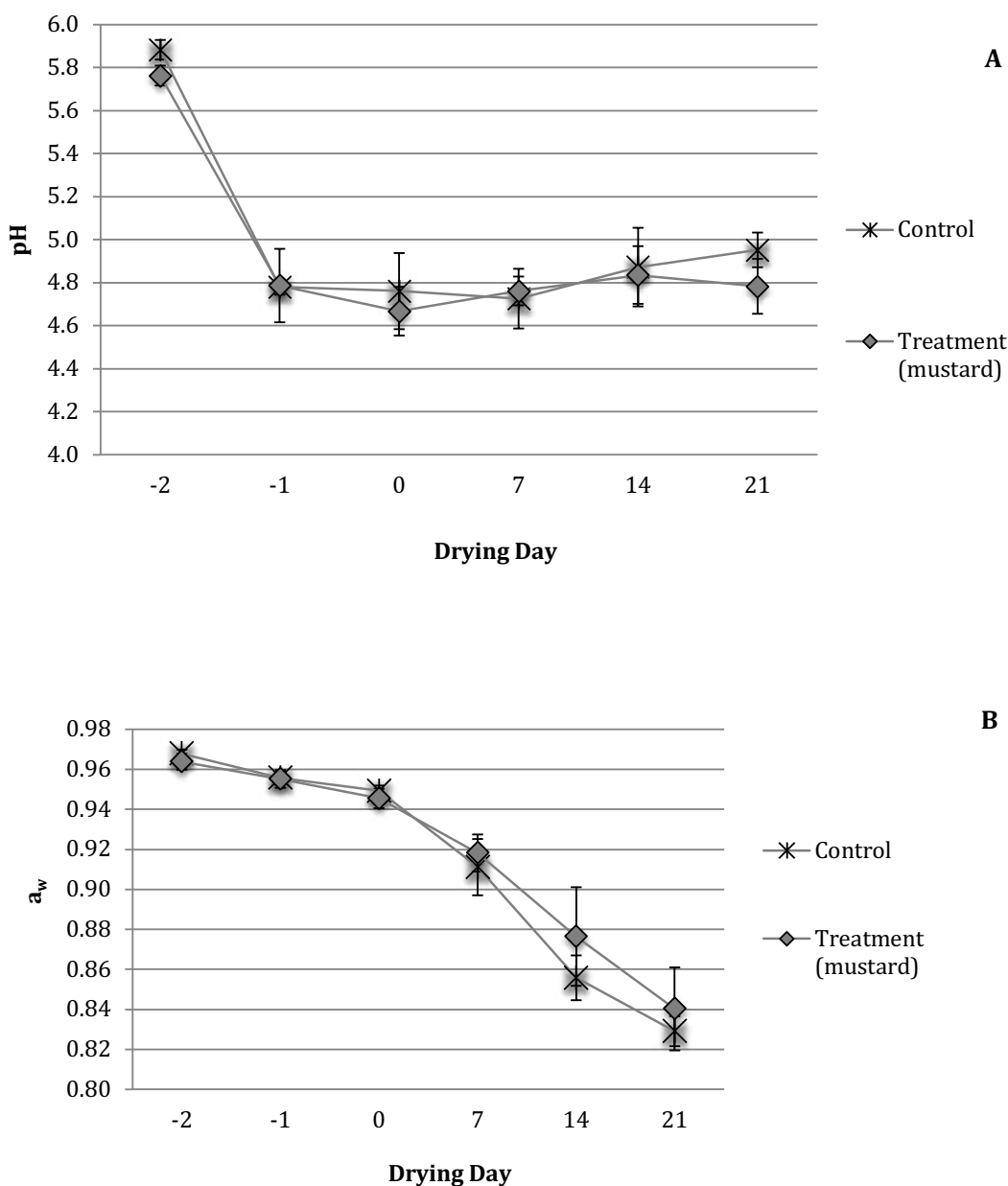


Figure 2. pH (A) and water activity (B) values in pork fermented sausages with (treatment) and without mustard (control).

0.9638 in sausages with mustard; the a_w then decreased to less than 0.95 at day 0 in all batches (Fig. 2B). At 21 days, the a_w of the final products ranged from 0.8215 to 0.8365 in sausages without mustard and from 0.8195 to 0.8609 in sausages with mustard.

DISCUSSION

Survival of *Salmonella* during drying in the presence of yellow mustard powder

Our results showed that sausages made with yellow mustard achieved a 5-log reduction of *Salmonella* in less than 21

days of drying. It was previously found that *Salmonella* is capable of metabolizing glucosinolate to isothiocyanates because the cells have myrosinase-like activity, which could potentiate the antimicrobial effects of mustard (26). In addition, Luciano et al. (32) stated that some starter cultures used during sausage production (lactic acid bacteria and *S. carnosus*), also have the ability to convert glucosinolate to isothiocyanates during drying (32). The viability of starter cultures during drying and ripening is essential for production, organoleptic, sensory, and microbial properties of the sausage. In similar sausage studies in which *E. coli* cocktails were used instead of *Salmonella*, the

authors reported that the populations of starter cultures were slightly reduced by the addition of mustard, but not significantly (16, 25, 32, 33). In the present study, the overall populations of the starter cultures were not decreased by the presence of the yellow mustard. Since the specific populations of each starter culture was not measured, it will be important to determine whether the organoleptic properties of the sausage were affected. The starter culture is a commercial mix that is used for the commercial production of sausages; therefore, the specific population of each starter microorganism might be difficult to obtain. The 5-log reduction of *Salmonella* observed at the end of the sausage drying was likely due to a combination of the starter cultures and the ability of *Salmonella* to degrade glucosinolate and was similar to findings of a previous study in which oriental mustard extract with EDTA was observed to have antimicrobial activity against *Salmonella* on chicken breast (41). In addition, Hwang et al. (28) showed that the populations of *Salmonella* could be reduced by 3 log₁₀ CFU/g after fermentation and drying in soudjouk-style fermented sausages (28). The reduction in populations of *Salmonella* in our experiments could also be attributed to the combined effects of curing agents such as salt, spices, nitrate and nitrite as well as the rapid decreases in pH and a_w during the fermentation (25). It is noteworthy that the current fermentation and drying processes without yellow mustard for the type of sausage described in this study achieved only a 3.3 log₁₀ CFU/g reduction of *Salmonella*, while we observed a greater than 7-log reduction in populations of *Salmonella* in sausages with added mustard.

Impact of physicochemical properties on *Salmonella* survival

After 24 h of fermentation, the pH of the sausages with and without mustard powder both were below 5.3 at 34°C, which is the critical pH and temperature needed to stop the growth and toxin production of *Staphylococcus aureus* (8). The pH of sausages with mustard powder treatment was slightly, but not significantly, lower than the pH of sausages without mustard powder. A similar antimicrobial effect was observed when deodorized mustard powder was used in dry fermented sausage to control *E. coli* O157:H7 (16). The low pH value of the sausages with mustard might be due to the growth of the lactic acid bacteria in the starter cultures which are using mustard as an additional source of fermentable sugar and thus producing more acid (51). There were no differences in the water activity between product with and without mustard. Because these fermented sausages are “shelf stable” and do not require storage at refrigeration temperatures, they need to meet the minimum concentrations of curing agents such as salt and nitrite/nitrate, the degree hours requirements and specific requirements for pH and a_w (7, 46). Since our results showed a greater than 5-log reduction of *Salmonella* spp. in sausages containing yellow mustard, but no significant differences in pH and a_w values in comparison

with the sausages that did not have mustard, this suggests that the antimicrobial effect of the mustard contributed to the additional inactivation of *Salmonella*, beyond the pH and a_w effects. The size and type of sausage could also affect the physicochemical and microbiological characteristics of the final products (49). According to Tabanelli et al. (49), the diameter of the products used in the present study belong to a smaller type and size of dry fermented sausage. Because of the kinetics of water loss, the a_w of the small sausages is lower than the a_w of the medium and large size sausages (49). This size difference might have caused the lower a_w of sausages observed in our studies, compared with results of previous studies that used bigger sausages such as salami (25). In addition, the flavor of the sausages can be affected by product size and starter culture mix as the result of acidification, oxygen availability and kinetics of water loss (34, 37). Therefore, sensory studies with this specific type of sausage should be done to investigate what concentration of yellow mustard would be suitable for small size sausages so as to be considered acceptable by consumers (31).

The maximum reduction of *Salmonella* in the sausages without mustard was < 3.3 log₁₀ CFU/g in the final product. This shows that there were some combined hurdle-type effects, e.g., starter culture competition, low pH (fermentation), and low a_w (drying) that helped to play a role in reducing the numbers of *Salmonella*. However, the addition of mustard to the sausage batter appeared to enhance the synergistic interactions, e.g., showing a > 5 log reduction in levels of *Salmonella* by day 14 of drying, as compared to a 1.45 log reduction for sausage without mustard. Compared with other antimicrobial compounds such as plant essential oils or spice extracts such as garlic powder in chicken sausages (3, 47), mustard had a much greater inhibitory effect on *Salmonella*. Essential oils from spices and herbs were able to achieve a > 2-log reduction in levels of *Salmonella* on fermented sausages (27). However, because of costs, strong aroma, flavor and potential toxicity, the application of essential oils in the food industry is restricted (1, 2). With regard to mustard powder, a major disadvantage of its use in foods is that it is a potential allergen (9). More specifically, proteins of the 2S albumin class, which are heat-stable and are not degraded by myrosinase, are major sources of food allergens in mustard seeds (30, 36). This limits the use of yellow mustard in the production of fermented sausages, although it could be used in processed meat products for its high protein content and binding capability. The availability of other natural compounds with antimicrobial activity which have no effect on the organoleptic characteristics of the product will help improve the safety of non-heat-treated products. Other considerations such as potential allergens and commercial availability should be taken into consideration when exploring the use of novel natural antimicrobials.

CONCLUSIONS

Yellow mustard, when added to the pork sausages, was able to control *Salmonella* in fermented sausages. A process using a fermentation at 34°C followed by two stages of drying was able to achieve a greater than 5-log reduction of *Salmonella* spp. after 14 days of drying, while reductions of only 2.4 and 3.3 log units were achieved after 14 and 21 days of drying, respectively, when mustard was not added to the fermented product. The yellow mustard used on the fermented pork sausages is a common, stable, easy-to-use and natural antimicrobial agent that can be used in the meat industry. Therefore, the application of yellow mustard powder to control *Salmonella* could be a good alternative

method for dry fermented sausage manufacturers to use in their operations to achieve a minimum of a 5-log reduction of *Salmonella*.

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