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Research Paper

Effect of cranberry pomace on the physicochemical properties and inactivation of *Salmonella* during the manufacture of dry fermented sausages

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ABSTRACT

The effect of cranberry pomace (CP) incorporation on *S. enterica* serovars inactivation, starter culture population, and physicochemical properties of sausages during the manufacture of dry fermented sausages (DFS) was studied. Sausages containing a five-strain cocktail of *S. enterica* serovars at 7-log CFU/g, with different levels of CP (control, 0%; low, 0.55%; medium, 1.70%; high, 2.25% wt/wt), or liquid lactic acid (0.33% vol/wt, LA) were subjected to typical fermentation and drying conditions. A significant ($P < 0.05$) reduction in initial pH was observed in all CP treatments on day 0 as a result of CP native acidity. All treatments except low CP showed a significantly lower pH than the control throughout the study. Water activity (a_w) was not significantly affected by CP level during fermentation. However, sausages containing medium and high CP levels showed a significantly lower final product a_w than the control. DFS with CP exhibited a significantly ($P < 0.05$) faster and greater *Salmonella* inactivation during the first 5 days; reduction rate and level directly correlated to CP level. In the presence of medium and high levels of CP, *Staphylococcus* spp. growth was suppressed, while *Lactobacillus* spp. and *Pediococcus* spp. exhibited a stimulatory response. All treatments except low CP had no significant effect on product chemical composition, and Moisture Protein ratio (MPr). Low CP level yielded DFS with a slightly higher ($P < 0.05$) moisture content and MPr. Medium and high CP levels resulted in darker, duller and redder DFS with a softer texture. Findings suggest that low CP levels can be utilized by DFS manufacturers as a natural functional ingredient to further minimize the risk associated with *Salmonella* during DFS production without altering final product characteristics.

1. Introduction

Cranberry has emerged as one of the super food due to its broad nutrient content and high levels of bioactive phenolic compounds. Specifically, these constituent bioactive phenolics, such as anthocyanins, flavonols, and proanthocyanidins (PACs), have gained considerable interest among the food and pharmaceutical industry as they have been shown to exhibit a wide range of potential biological health benefits including antioxidation, antimicrobial, antiviral, anticancer, and anti-inflammatory implications (Caillet et al., 2012; Côté et al., 2011; Howell et al., 2005; Manach et al., 2004; McKay and Blumberg, 2007; Nile and Park, 2014; Puupponen-Pimia et al., 2001). The antimicrobial activity of phenolic compounds of cranberry origin have been widely studied, as there is growing consumer demand for healthier, natural, and minimally-processed food products free from chemical preservatives. These phenolic constituents have the potential of being utilized as a natural food preservative as they were found to hold excellent antimicrobial activity against several foodborne pathogens *in vitro*, without significantly affecting or in some cases even enhanced the growth of beneficial microorganisms such as lactic acid bacteria (Caillet et al., 2012; Côté et al., 2011; Lacombe et al., 2013; Nohynek et al., 2006; Puupponen-Pimiä et al., 2005; Puupponen-Pimia et al., 2001; Wu, Qiu, Bushway and Harper, 2008; Wu, Qiu, de los Reyes, Lin and Pan, 2009; Yin Lau, Barbut, Ross, Diarra and Balamurugan, 2019).

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Cranberry pomace (CP) is the main by-product of cranberry processing and consists of the pulps, seeds, peels and other fruit structures of cranberry. As the polyphenol-rich fractions are not lost and a large amount of moisture and soluble solids are removed during fresh cranberry pressing, CP and its subsequent extracts show high levels of vitamins, dietary fibers, and phenolic compounds (Harrison et al., 2013; Ross et al., 2017; White et al., 2010). Nevertheless, due to its acidic nature, the potential use of CP as a value-added resource was not fully explored and hence is often treated as solid fruit waste (Vattem and Shetty, 2002). Being a rich source of bioactive phenolics with antimicrobial activity, researching the potential use of CP as a functional ingredient in food systems would not only fulfill consumers' expectation for food products with natural ingredients but would also allow economical utilization of valuable solid fruit waste. The use of cranberry press cake and cranberry juice powder as antioxidants in meat and poultry products has been explored by several researchers (Larrain et al., 2008; Raghavan & Richards, 2006, 2007). Chloroform extract of cranberry juice powder showed significant inhibition of lipid oxidation and lower rancidity scores compared to ethyl acetate or ethanol extracts of cranberry cake press in vacuum-packed mechanically separated turkey and cooked ground pork products. Similarly, inclusion of water soluble extracts of cranberry concentrates and/or ethanol extracts of cranberry pomace in ground beef or pork hamburger and in cooked ham resulted in significant reductions in several foodborne pathogens without affecting quality (Stobnicka and Gniewosz, 2018; Tamkutė et al., 2019; Wu et al., 2009). However, to the best of our knowledge, there are no studies to date examining the potential application of whole CP as a functional ingredient in meat systems.

Dry fermented sausages (DFS) are a category of dry meat products manufactured from a mixture of ground meat, animal fat, water, salt, curing salt, spices, and starter culture containing lactic acid-producing bacteria. The sausage batter is then stuffed into casings and subjected to fermentation followed by drying under controlled relative humidity and temperature. Dry fermented sausages represent one of the oldest forms of meat preservation and are traditionally manufactured without the use of thermal treatments, thus their preservation mainly relies on a combination of acidification, controlled drying (low a_w), addition of chemical preservatives and curing agents such as salt and nitrite. Regulations stipulate that DFS processors that do not use heat as a lethality step during their manufacture, utilize fermentation and dry curing conditions that have been scientifically validated to result in a 5-log reduction in *E. coli* O157:H7 and/or *Salmonella* spp. to produce a shelf-stable product (Canadian Food Inspection Agency, 2016; Health Canada, 2000; USDA-FSIS, 2005). Thus CP being a rich source of bioactive polyphenols that show preferential antimicrobial activity against several pathogens while enhancing growth of LAB could be used as a functional ingredient in DFS to enhance pathogen inactivation and safety. This is specifically the objective of the present study, would CP incorporation at varying levels enhance/hasten the inactivation of *S. enterica* serovars during the manufacture of DFS? The effect of CP incorporation at varying levels on *S. enterica* serovars inactivation, meat starter culture growth, and physicochemical properties of DFS were examined. The findings of the study should provide useful knowledge regarding the use of CP as a functional ingredient for DFS, specifically as an antimicrobial agent.

2. Materials and methods

2.1. Cranberry pomace powder

Physical properties and chemical composition of the CP used in the present study was previously determined and reported by Ross et al. (2017). The CP produced and used in the characterization study by Ross et al. (2017) was kindly provided by these authors for use in the present study. Briefly, the CP used in the study has a total phenolic content, pH, and sugar content of 24.87 ± 0.66 mg gallic acid eq./g, 2.74 ± 0.02, and 22.76 ± 0.49%, respectively.

2.2. Bacteria and growth condition

A five-strain cocktail of *S. enterica* serovars was used to inoculate the raw meat batter in the study. Strain name, serogroup, and source of each isolate are listed in Table 1. Strains were stored frozen at −80 °C individually in Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) containing glycerol in a 1:1 vol ratio. Prior to experiment use, each strain was streaked on Tryptic Soy Agar (TSA; Becton, Dickinson and Company) and incubated aerobically for 24 h at 37 °C. A single colony was used to inoculate TSB and incubated at 37 °C and 120 rpm for 24 h. An aliquot was transferred to TSB +1.0% glucose (Sigma Chemical Co., St. Louis, MO, USA) resulting in a final 100-fold dilution and incubated at 37 °C and 120 rpm for 24 h to acid adapt the cultures and ensure high cell growth and density prior to inoculation. Ahead of inoculation, individual strains of the *S. enterica* serovars cocktail were harvested by centrifugation at 10,000 rpm and 4 °C for 10 min, and the resulting pellet was washed twice, and re-suspended in sterile distilled water. Each strain was then combined and thoroughly mixed to make a five-strain cocktail containing equal concentrations of each strain, prior to meat batter inoculation at a level of approximately 7-log CFU/g in each treatment. All inocula were stored on ice prior to inoculation.

2.3. Dry fermented sausage production

Pork fat, lean pork trims, and lean beef trims were obtained from University of Guelph Meat Abattoir. The trims were ground through a 3.175 mm (1/8 inch) plate. The ground trims were then thoroughly mixed to form a homogenous mass, portioned into individual 7.5 kg vacuum packed packages, and stored at −20 °C. Meat was then defrosted at 4 °C as needed, prior to the experimental trials.

All DFS were manufactured in a dedicated biosafety level 2 containment food processing pilot plant at the Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada. All equipment were chilled at 4 °C overnight to prevent fat smearing during production. Pre-weighted meat mix for each treatment were loaded to a stand food mixer (KitchenAid®, Mississauga, ON, Canada) and mixed for 30 s to break down large meat clumps, before adding *S. enterica* serovars inoculum cocktail resulting in an initial inoculation level of approximately 6.5-log CFU/g. Curing salt (HeLa Spice Canada Inc., Uxbridge, ON, Canada), salami seasoning (HeLa Spice Canada Inc.), starter culture (B-LC-007 SafePro®, Chr Hansen, Denmark), and/or CP powder (GRDC, Guelph, ON, Canada) at selected levels or liquid lactic acid (LA; ≥ 90%; Sigma Chemical Co.) were added to the meat batter following the addition of *S. enterica* serovars inoculum. The four CP levels (control: 0.00% wt/wt; low: 0.55% wt/wt; medium: 1.7% wt/wt; high: 2.25% wt/wt) used in the study were based on the results from our preliminary trials and formulation for each

Table 1
Serovars and origin of the five strain *Salmonella enterica* strains used in the study.

Pathogen	Source and Application
Salmonella enterica subsp. *enterica* serovar Heidelberg ATCC 8326	American Type Culture Collection; Enteric disease research, Infectious disease research
Salmonella enterica subsp. *enterica* serovar Enteritidis ATCC 13076	American Type Culture Collection; Enteric disease research, Infectious disease research
Salmonella enterica subsp. *enterica* serovar Berta ATCC 8392	American Type Culture Collection; Enteric disease research, Infectious disease research
Salmonella enterica subsp. *enterica* serovar Typhimurium ATCC 14028	American Type Culture Collection; Tissues from pools of heart and liver from 4-wk-old chicken; Food testing research, Enteric disease research, Infectious disease research
Salmonella enterica subsp. *enterica* serovar Newport ATCC 6962	American Type Culture Collection; Food poisoning fatality; Enteric disease research, Infectious disease research

treatment are provide in Table S1. Mixing apparatuses (e.g. bowls, paddles) were changed between batches to prevent cross-contamination. The meat batter was mixed for an extra 5 min before being stuffed into moistened pre-cut 33 mm caliber size fibrous cellulose casings (Nalo Fibrous, Kalle Gmbh, Wiesbaden, Germany) at a length of at least 100 mm (i.e., at least twice the size of casing diameter as required by Canadian Food Inspection Agency (CFIA) "Meat Hygiene Manual of Procedures" (Canadian Food Inspection Agency, 2016) using a pre-chilled stuffer (LEM dual gear stuffer, West Chester, OH, USA). Each sausage was clipped shut using a pneumatic clipper (Poly-Clip®, Koch equipment, Kansas City, MO, USA), hung on horizontal aluminum rods, and spaced evenly to ensure uniform airflow throughout fermentation and maturation.

Sausages were transferred to a programmed fermentation cabinet (Stagionello STG100 MTO, Crotone, Italy) conditioned at 25 °C and at a relative humidity (RH) of 88%. Temperature was decreased stepwise at 2 °C every 24 h until it reached 20 °C, while RH was reduced to 80% after 24 h and subsequently 2% drop every 24 h. After the fermentation period, the temperature was reduced by 2 °C every 12 h until a final temperature of 14 °C and RH of 75% was reached. Fermented sausages were then transferred to a drying cabinet fitted with a heatless dryer (Caron Environmental Chamber 6020, Caron Products and Services Inc., Marietta, OH, USA) and dried at 14 °C and 75% RH for the next 28 days of the study.

Two batches of sausages for each treatment, each with approximately 15 kg of salami meat batter, were manufactured one batch each on two separate days, resulting in a total of 10 batches. Two sausages per batch were randomly selected on day 0, 1, 2, 5, 12, 19, 26 and 33 of the process for different analyses.

2.4. Microbiological analysis

The sampling of DFS for microbial analysis was carried out for all treatments. Briefly, 225 ml of 0.1% sterile peptone water (Difco Peptone Water, Becton, Dickinson and Company) was added to 25 g of aseptic composite sausage (De Souza, Ahmed, Strange, Barbut and Balamurugan, 2018) in a sterile stomacher bag (Filtra-bag, VWR Canada, AB, Canada) and homogenized using a stomacher (Stomacher 400 circulator, Seward Laboratory Systems Inc., FL, USA) for 2 min at 230 rpm. The stomached sample was then serially diluted with 0.1% sterile peptone water, and 100 μl of appropriate dilution were surface plated onto selective agar plates including De Man, Rogosa and Sharpe Agar (MRSA; Becton, Dickinson and Company), Mannitol Salt Agar (MSA; Becton, Dickinson and Company), and Xylose-Lysine-Tergitol 4 (XLT-4; Becton, Dickinson and Company), and incubated aerobically at 37 °C for 24–72 h for enumeration of presumptive *Pediococcus* spp. (small pinpoint cream colonies after 48 h), *Staphylococcus* spp. (pink/red colonies by 24 h) and *S. enterica* serovars (black colonies by 24 h), respectively. Another set of MRSA plates were incubated anaerobically at 30 °C for 72 h for enumeration of presumptive *Lactobacillus* spp. which form pale straw colonies after 48 h. Colony forming units (CFU) were enumerated with a detection limit of 25–250 CFU/g. When plates had fewer than 25 colonies the actual plate counts were used for calculation of inactivation and denoted with an "*". The reduction in *S. enterica* serovars numbers was represented as log reduction, and was calculated using the formula below:

$$\log \text{ reduction} = \log \frac{N_t}{N_0}$$

where N_t is the average CFU/g at time t and N_0 is the average CFU/g at time zero. Two random sausages per batch were analyzed at each sampling day and final bacterial count were presented as mean log CFU/g ± standard deviation obtained from duplicate plated samples from each sausages.

In order to account for injured *S. enterica* serovars cells, 10 g of composite sample were enriched in Selenite Cystine Broth (SCB; Selenite Cystine Broth, Becton, Dickinson and Company) and incubated for 24 h at 37 °C when no colony growth was noted throughout the 33 days sampling plan. The enriched samples were then spread-plated in duplicate onto XLT-4 agar plates for *S. enterica* serovars detection.

2.5. Physicochemical analysis

Approximately 15 g of composite sausage sample was ground into small uniform particles using a food processor (Blixer 2, Robot Coupe U. S.A., Jackson, MS, USA) and analyzed for water activity (a_w), pH, protein, moisture, and fat content. Water activity was analyzed using a Dew point water activity meter 4 TE (Aqua Lab, Pullman, WA, USA) calibrated at 25 °C, while pH was assessed using a bench-top flat surface pH probe (B10P Benchtop Meter, VWR, Radnor, PA, USA) after a 2-point calibration with buffers at different pH (pH 7.01 and 4.01). The protein content was determined by using a CEM Sprint Rapid protein analyzer (AOAC Method 967.12, 930.33, and 930.29. CEM Corporation, Matthews, NC, USA). The moisture and fat level were analyzed using a Meat Trac Fat and Moisture Analyzer, microwave moisture analyzer and LF-NMR, respectively (AOAC Method, 2008.06, CEM Corporation). Two sausages per batch were analyzed at each sampling point and physicochemical results were presented as mean ± standard deviation of the two batches.

2.6. Color measurement

Color analysis was conducted on all treatments, except the LA treatment. The color of the DFS samples was evaluated with a Nix™ Pro Color Sensor (Nix™ Pro Color Sensor, Nix sensor Ltd., Hamilton, ON, Canada) based on the CIE L*a*b* color space with a D-65 illuminant source setting, and 2° standard observer angle. The device was controlled wirelessly using an iOS device through Bluetooth and has its own light-emitting diode (LED) light source located within the concave base of the sensor about 1 cm above the field of view. Both the exterior and interior color of DFS were evaluated. The exterior color of DFS was measured immediately after removing the sausage casing, whereas the interior color was measured after slicing the sausages into slices of 1 cm thick. Since color redness is highly influenced by color yellowness, the L*a*b* color space was transformed and reported as L*, C*, and h^o value, which represents color lightness, chroma, and hue angle of the CIE Lab color space, respectively, by the following equations:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$h^o = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

All exterior and interior color measurements were performed four times for each sausage at room temperature and two sausages were analyzed for each batch.

2.7. Textural analysis

Textural Profile Analysis (TPA) was carried out on all treatments, except the LA treatment, on days 1, 2, 5, 12, 19, 26 and 33 of the process. The TPA was performed to evaluate the effect of CP on product textural parameters using a textural analyzer equipped with a 30 kg load cell (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). Three cylindrical samples (1.0 cm tall with a diameter of 1.5 cm) from the core of each sausage were compressed twice to 50% of their original height with a cross-head speed of 1.5 mm/s. All textural parameters except cohesiveness and springiness were modified by dividing the sample cross-section area (cm^2), and presented as the following: Hardness (N/cm^2) = the maximum force required to compress the sample; Springiness (cm) = the vertical distance the sample recovered after the deformation force is removed; Cohesiveness = ratio of the area of the second force-

displacement curve to the area of the first curve; Gumminess (N/cm^2) = force to disintegrate a semi-solid meat sample for swallowing (Hardness x Cohesiveness); Chewiness (N/cm) = work to masticate the sample for swallowing (Springiness x Gumminess). Two sausages were analyzed for each treatment on each sampling day at room temperature.

2.8. Statistical analysis

Microbial counts were transformed to log CFU/g. The value 1 was added before logarithm transfer in order to accommodate the value of zero CFU/g. Analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test was carried out to evaluate the difference between the treatments at each sampling point using R 3.2.3 (R foundation for Statistical Computing, Vienna, Austria) at a confidence level of 95% ($P < 0.05$). All data were reported as mean ± standard deviation.

3. Result and discussion

3.1. Effect of CP on changes in pH, a_w and Moisture Protein ratio

Changes in pH, a_w, and MPr of DFS of varying treatments during sausage fermentation and drying are presented in Fig. 1. Initial pH of sausages was significantly affected by CP incorporation and was found to be CP level-dependent ($P < 0.05$; Fig. 1A). Sausages with high CP levels showed the lowest initial pH (5.04 ± 0.01) compared to control sausages which showed the highest initial pH (5.65 ± 0.05). This fast reduction of pH can cause some textural challenges in meat products which will be discussed in section "effect of CP on DFS textural parameters". It is interesting to point out that in the present study, the initial pH of sausages with high CP level was similar to the final pH of conventional DFS products with a similar manufacturing process (Balamurugan et al., 2017; De Souza et al., 2018). All DFS exhibited a gradual drop in pH during the fermentation process and can be related to the starter culture activity, which metabolized the sugars, dextrose present in the meat mix into lactic acid. All CP-added sausages showed a markedly lower ($P < 0.05$) pH than the control by the end of the 5-day fermentation process and throughout the drying process. It is important to point out that in the present study, all sausages containing CP except low CP sausages reached a pH of <5.2 on Day 0 (Fig. 1A), before fermentation even started, while low CP sausages had a pH of 5.37 (Fig. 1A). Such dramatic change in pH profile appears to be related to the acidic nature of CP (pH 2.74 ± 0.02). This could explain the relatively higher *Salmonella* inactivation rate (i.e., the main objective of trying CP in this kind of uncooked dry sausage), lower meat starter culture population, and the soft texture observed, which will be addressed in the later sections. All DFS reached a pH of <4.9 at the end of the DFS production process. The liquid lactic acid (LA) treatment resulted in a pH profile that was not statistically different ($P > 0.05$) from the medium CP level sausages throughout the DFS production stages. A slight elevation in pH towards the end of the drying stage was observed in all treatments, which is characteristic of DFS and could be attributed to the generation of ammonia and amide compounds as a result of proteolytic activities (Balamurugan et al., 2017; De Souza et al., 2018; Pérez-Alvarez et al., 1999).

The change in a_w was not significantly different ($P > 0.05$) between treatments during fermentation and all treatments achieved a a_w of ≤0.9 by the end of the drying process (Fig. 1B). The a_w level of the treatments ranged from approximately 0.96 on day 0–0.71–0.75 at the end of the drying period. The final product a_w was not significantly different ($P > 0.05$) between treatments except the medium and higher CP level sausages, which showed a considerably lower ($P < 0.05$) final a_w. The lower a_w observed could perhaps be related to the additional amount of sugar and dietary fiber provided by the CP. Similar a_w reduction in DFS enriched with fiber has also been reported by others (Eim et al., 2008; Fernández-López et al., 2008; Garcia et al., 2002).

All DFS reached a shelf-stable MPr of <1.9 by day 10 of the DFS production process and continued to steadily drop to a final MPr of 1.10 to 0.97 after the additional 4-weeks drying (Fig. 1C). There was no significant difference ($P > 0.05$) seen among the treatments except the low CP treatment, which showed a final MPr approximately 0.15 higher ($P < 0.05$) than that of the control. The outcome for low CP addition was possibly due to the enhanced moisture retention ability in the presence of dietary fiber-rich CP (Garcia et al., 2002).

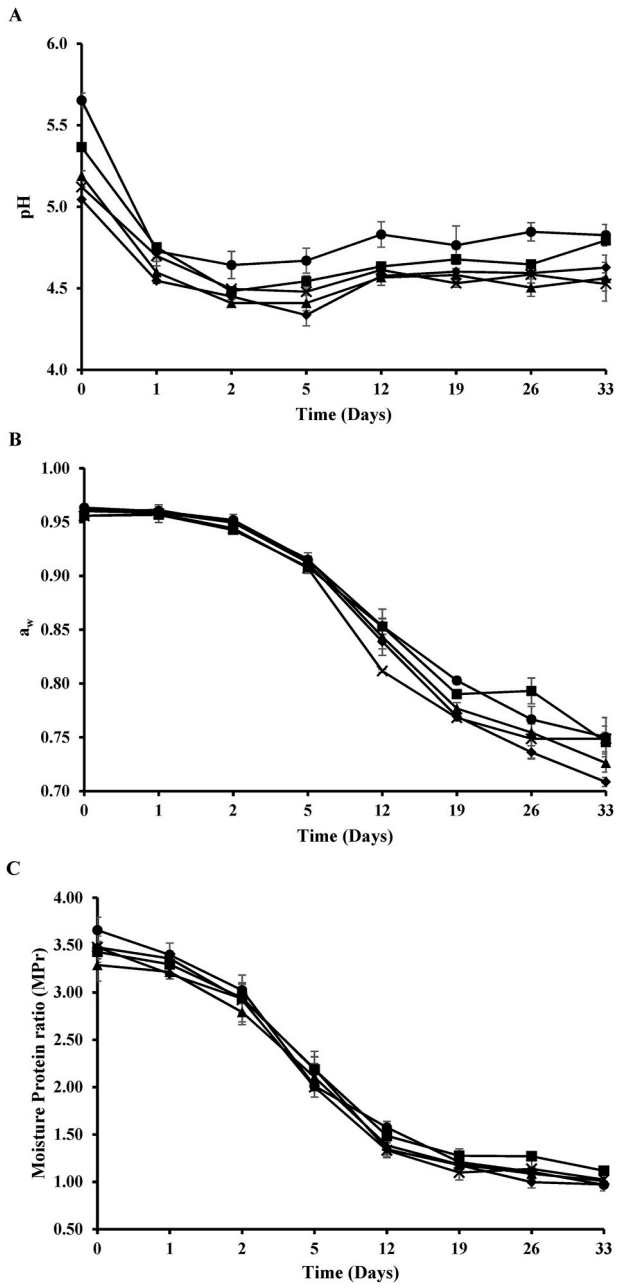

Fig. 1. Changes in pH, a_w and Moisture Protein ratio (MPr) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; × : 0.33% liquid lactic acid). Each data point is a mean of four observations. Error bars denote ± one standard deviation.

3.2. Effect of CP on DFS moisture, protein, and fat levels

Overall, all treatments showed a stepwise reduction in moisture content, and increase in protein and fat content throughout the study as

a result of drying. The relative percentage of moisture, protein, and fat changed from approximately 54-57% to 19–23%, 16–18% to 26–29%, and 21–23% to 38–41%, respectively (moisture, protein, and fat content data not presented; $P > 0.05$). Such typical changes in DFS chemical compositions during manufacturing are consistent with earlier studies with similar manufacturing process (Balamurugan et al., 2017; Chacon et al., 2006; Graumann and Holley, 2008; Holck et al., 2011). All treatments, except the low CP level, showed no significant ($P > 0.05$) effect on product chemical composition when compared to the control. Low CP level yielded DFS with a moisture content approximately 2.04% higher than the control and is in agreement with Garcia et al. (2002) and Sánchez-zapata et al. (2013), who also reported a similar 2–4% higher final water content in DFS with fruit fiber, and tiger nut fiber, respectively. On the other hand, Eim et al. (2008) found that increased level of carrot dietary fiber yielded DFS with a lower moisture and fat content. In fact, it is believed that such changes in moisture content are linked to the type of fiber studied and in any case have minimal impact on DFS organoleptic properties.

3.3. Effect of CP on S. enterica serovars inactivation

To the best of our knowledge, there are limited studies to date examining the practicality of cranberry processing by-products as functional antimicrobial agents in a processed meat matrix, especially models that involve other biological factors (e.g. meat starter culture bacteria) that are crucial to the development of end-product structure, organoleptic characteristics, and microbiologically safety. In the present work, DFS added with CP resulted in a significantly ($P < 0.05$) greater *S. enterica* serovar reduction than the control (Table 2). After the 5-day fermentation stage a mean reduction of 3.57 ± 0.10, 4.78 ± 0.20, 5.55 ± 0.28, >6.59 ± 0.10, and 4.80 ± 0.30 log of *S. enterica* serovar was observed in the control, low, medium, high CP, and LA treated DFS, respectively. This suggests that both inactivation level and rate are positively related to CP incorporation level as well as sausage acidity. Our findings are in agreement with Gniewosz and Stobnicka (2018), who reported that the application of 2.5% water-soluble CP extract to minced pork effectively reduced the number of *S. enterica* by 4-log CFU/g after 6 days. Nonetheless, a non-fermented meat system was employed in their case. Moreover, in our experiment, CP shortened the duration required to achieve a 5-log reduction of *S. enterica* serovar required for validation studies for non-thermally processed meat products (Canadian Food Inspection Agency, 2016). For instance, the regulated inactivation level was achieved by around day 12, 5, 5, and 2 of the manufacturing process for the control, low, medium, and high CP DFS, respectively (Table 2). The progressively greater ($P < 0.05$) reduction observed, in DFS with increasing levels of CP, is likely to be related to the pH-decreasing characteristic of CP, as it has long been hypothesized that the low pH of cranberry plays an important role in foodborne pathogen inhibition via creating an osmotic stress that causes sublethal injury to bacterial membranes (Lacombe et al., 2013; Lin et al., 2004; Wen et al., 2003; Wu et al., 2008). In fact, LA treatment in our work also strengthened the significance of CP-induced acidification on *Salmonella* inactivation when compared to the control. Furthermore, the smaller log reduction value in LA treatment ($P < 0.05$; 4.80 ± 0.30-log CFU/g) compared to medium (5.55 ± 0.28-log CFU/g) and high (>6.59 ± 0.13-log CFU/g) CP treatments observed at the end of the five day fermentation process suggests that key ingredients in CP which include bioactive compounds such as anthocyanins, flavonols, PACs, and phenolic acids works in synergy with the acidification of sausages by the natural organic acids (i.e. citric acid, quinic acid, malic acid, etc.) in CP to produce the observed enhanced *Salmonella* inactivation. Various researchers pointed out that the antimicrobial action of cranberry, against foodborne pathogens, not only comes from its acidity but can also be attributed to certain cell structure destabilization and downregulation actions manifested by bioactive phenolics as well as synergistic effects among the several components (Caillet et al., 2012; Côté et al., 2011; Lacombe et al., 2013; Lacombe et al., 2010; Puupponen-Pimia et al., 2001; Wu et al., 2008; Wu et al., 2009). For instance, Wu et al. (2008) reported that cranberry concentrates hold greater antibacterial activity than acidic solutions, and their transmission electron microscope analysis revealed that pH standardized cranberry phenolics were able to disintegrate bacterial cell wall, cell membrane and cause subsequent cell death. Lacombe et al. (2010), (2013) also noticed a similar outer membrane weakening effect against *E. coli* O157:H7 by pH neutral cranberry phenolic and anthocyanin components. Although the antimicrobial activity of CP greatly depends on the cranberry variety, preparation method, and chemical composition, our work demonstrated a pronounced concentration-dependent effect of CP on *S. enterica* serovar inactivation in an acidified meat matrix and further supports the idea of the relationship between cranberry natural acidity, phenolic compounds and foodborne pathogen inhibition. It is important to note that the reported days to reach the required 5-log reduction are only a guideline duration as it varies widely among DFS recipes, manufacturing conditions, and CP preparation procedures. Results from the enrichment samples showed that all DFS remained positive for *S. enterica* serovar at the end of the study. In fact, similar observations were reported in other challenge studies involving *E. coli* O157:H7 (Balamurugan et al., 2017; De Souza et al., 2018).

Table 2
Log reduction number of *S. enterica* serovars in dry fermented sausages (DFS) added with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and drying.

Time (days)	Log (N_t/N_0) ± SD				LA
	Control	Low	Medium	High	
Fermentation					
1	2.06 ± 0.14[a]	2.23 ± 0.14[a]	2.20 ± 0.10[a]	2.70 ± 0.26[b]	2.12 ± 0.16[a]
2	2.78 ± 0.27[a]	3.41 ± 0.02[b]	3.63 ± 0.20[b]	4.68 ± 0.42[c]	3.17 ± 0.13[ab]
5	3.57 ± 0.10[a]	4.78 ± 0.20[b]	5.55 ± 0.28[c][a]	ND/+[d]	4.80 ± 0.30[b]
Drying					
12	4.63 ± 0.20[a]	ND/+[b]	ND/+[b]	ND/+[b]	ND/+[b]
19	ND/+	ND/+	ND/+	ND/+	ND/+
26	ND/+	ND/+	ND/+	ND/+	ND/+
33	ND/+	ND/+	ND/+	ND/+	ND/+

Means ± standard deviation in the same row followed by different letters are significantly different ($P < 0.05$).
Control: 0% CP; Low CP: 0.55%; Medium CP: 1.70%; High CP: 2.25%; LA: 0.33% liquid lactic acid.
ND indicates no colonies detected with a reduction level greater than the detection limit.
+ indicates positive results for Selenite Cystine Broth Enrichment.
Mean initial inoculation level in control, low, medium, high, and LA treatment was 6.62 ± 0.06, 6.60 ± 0.08, 6.60 ± 0.04, 6.59 ± 0.13, and 6.58 ± 0.14, respectively.
[a] *S.enterica* serovars less than 25 CFU per plate.

3.4. Effect of CP on meat starter culture

3.4.1. Lactobacillus spp. and Pediococcus spp.

The growth and viability of lactic acid-producing bacteria is essential for DFS as they are responsible for the formation of organic acids, mainly lactic acid, which contributes to the development of product flavor, color, aroma, textural characteristics, as well as microbiological stability. In the present study, the control treatment demonstrated the highest *Lactobacillus* spp. and *Pediococcus* spp. initial population size, whereas the medium and high CP treatments showed a progressive reduction in initial population size and time to reach maximum population, especially as the CP level increased (Fig. 2A and B). Overall, the *Lactobacillus* spp. and *Pediococcus* spp. population of the low CP treatment and the control were not statistically different throughout the study ($P > 0.05$).

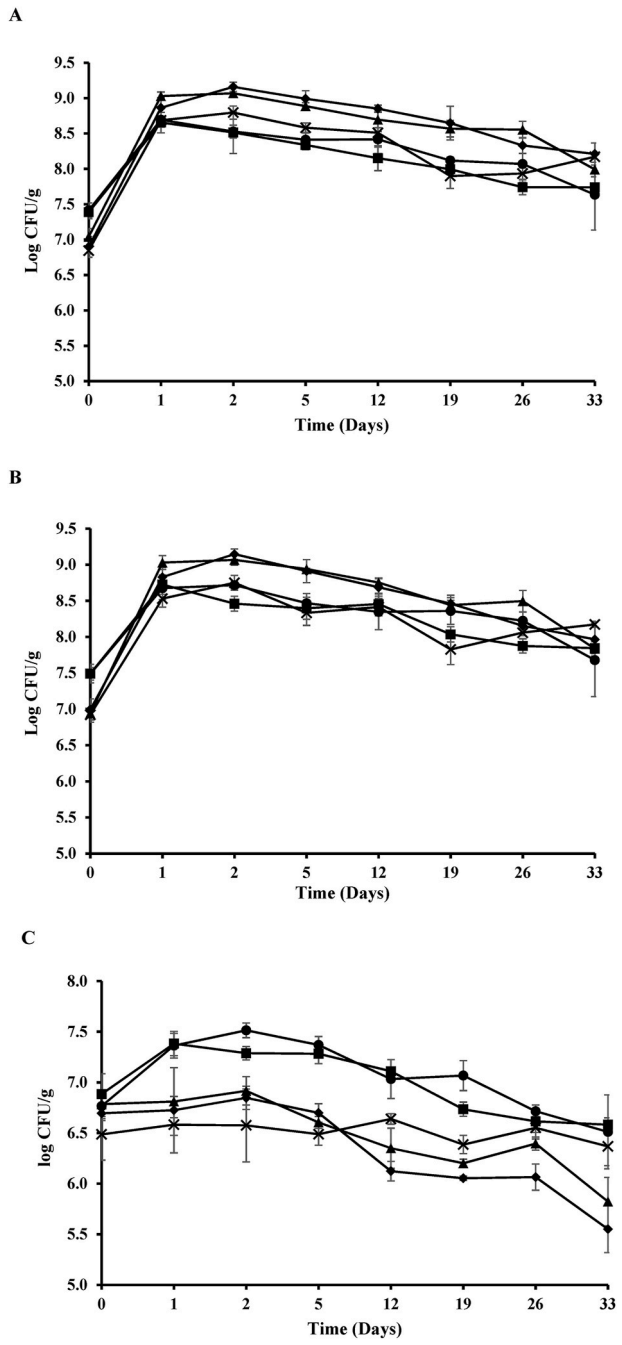

Fig. 2. Changes in *Lactobacillus* spp. (A), *Pediococcus* spp. (B), and *Staphylococcus* spp. (C) cell count (Log CFU/g) in dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; × : 0.33% liquid lactic acid). Each data point is a mean of four observations. Error bars denote ± one standard deviation.

The lower initial population size observed for higher CP level treatment could perhaps be related to the relatively lower meat batter pH mentioned previously, as LA treatment also showed a similar trend. Nevertheless, both *Lactobacillus* spp. and *Pediococcus* spp. population in the medium and high CP treatments were able to rebound, and attained a significantly ($P < 0.05$) higher number than the control after 2 days, and remained higher ($P < 0.05$) than control throughout the reminder of the DFS production process (Fig. 2A and B). Yalınkılıç et al. (2012) also reported a higher lactic acid bacteria count in Turkish-type DFS (Sucuk) produced with 4% orange fiber. In fact, the observed higher population size, after 2-days and onwards, could be attributed to the presence of growth stimulators and/or additional growth substrates (e.g. sugars) from CP. Our results suggest a stimulatory response of CP specifically on *Lactobacillus* spp. and *Pediococcus* spp. similar to that observed by Yin Lau et al. (2019) with cranberry extract (CE) where an adaptation period to CE and its natural acidity was required prior to the observed enhanced growth of these bacteria.

3.4.2. *Staphylococcus spp.*

Changes in the *Staphylococcus* spp. population during DFS manufacturing are presented in Fig. 2C. All treatments demonstrated a similar initial population on production day ($P > 0.05$). Among all treatments, the control showed the fastest increase in cell number and reached the greatest ($P < 0.05$) population size at around 7.52 ± 0.07-log CFU/g after 2 days of fermentation, and then remained relatively stable until the end of the study. Similarly, low CP level seemed not to have any impact on *Staphylococcus* spp. growth ($P > 0.05$) throughout the entire study. A significant ($P < 0.05$) decrease in population was observed in DFS added with medium and high levels of CP after 24 h of fermentation; the number remained significantly ($P < 0.05$) lower than that of the control until the end of the process. For instance, cell count for the medium and high CP treatment on day 33 was approximately 0.68- and 0.95-log CFU/g lower than that of the control, respectively. Our results are in agreement with Yalınkılıç et al. (2012), who similarly reported a reduction in *Staphylococcus* spp. population in Sucuk added with increasing levels (2–4%) of orange fiber. Such phenomenon could perhaps be explained by the pH-decreasing effect of CP. It has been previously reported that rapid acidification in DFS could reduce the viability of certain acid-sensitive functional microorganisms such as *Micrococcus* spp. and *Staphylococcus* spp. (Fernández-López et al., 2006; Lizaso et al., 1999; Rantsiou and Cocolin, 2006). *Staphylococcus* spp. growth profile in sausages acidified using LA were similar ($P > 0.05$) to that in sausages containing medium CP level during the fermentation process, but increased and remained at a considerably higher ($P < 0.05$) level during the 4-week drying stage. This suggests that the reduction in *Staphylococcus* spp. population might not solely be caused by the CP acidity, but also a range of phenolic compounds present in CP. Such finding is in line with another work by the authors, where increasing levels of cranberry bioactive phenolics and acidity were found to be the growth-limiting factors for *S. carnosus and S. xylosus* (Yin Lau et al., 2019). The major functions of these microorganisms consist of limiting lipid oxidation, color formation and stabilization as well as aromatic profile development by means of their nitrate reductase, proteolytic and lipolytic activities (Hammes and Hertel, 1998; Simonova et al., 2006; Talon et al., 1999). For this reason, the impact on DFS sensorial attributes due to reduced *Staphylococcus* spp. population should be further researched.

3.5. *Effect of CP on DFS color parameters*

Color of DFS is a very important characteristic that influences consumer acceptability and, eventually, associated with products' sensorial quality when purchased. It is important to keep in mind that when attempting to incorporate a new ingredient into a traditional product, consumers might reject the product if it does not retain its original characteristics. The results for both exterior and interior color measurements are shown in Figs. 3 and 4, respectively. A significant ($P < 0.05$) reduction in L*, C*, and h^o value for both exterior and interior color was observed for all CP treatments on day 0; and could mainly be attributed to the presence of purple-red cranberry pomace pigment compounds such as anthocyanins and proanthocyanidins. Despite the bright red hue of CP alone, the darker purplish-red color observed was probably due to the partial color shift of anthocyanin ions from red to blue under increased pH when added to the meat batter (von Elbe and Swartz, 1996), yielding a dark and purplish mixture of the CP and raw meat. The observed spike in exterior C* value (14.94 on day 0–19.68 on

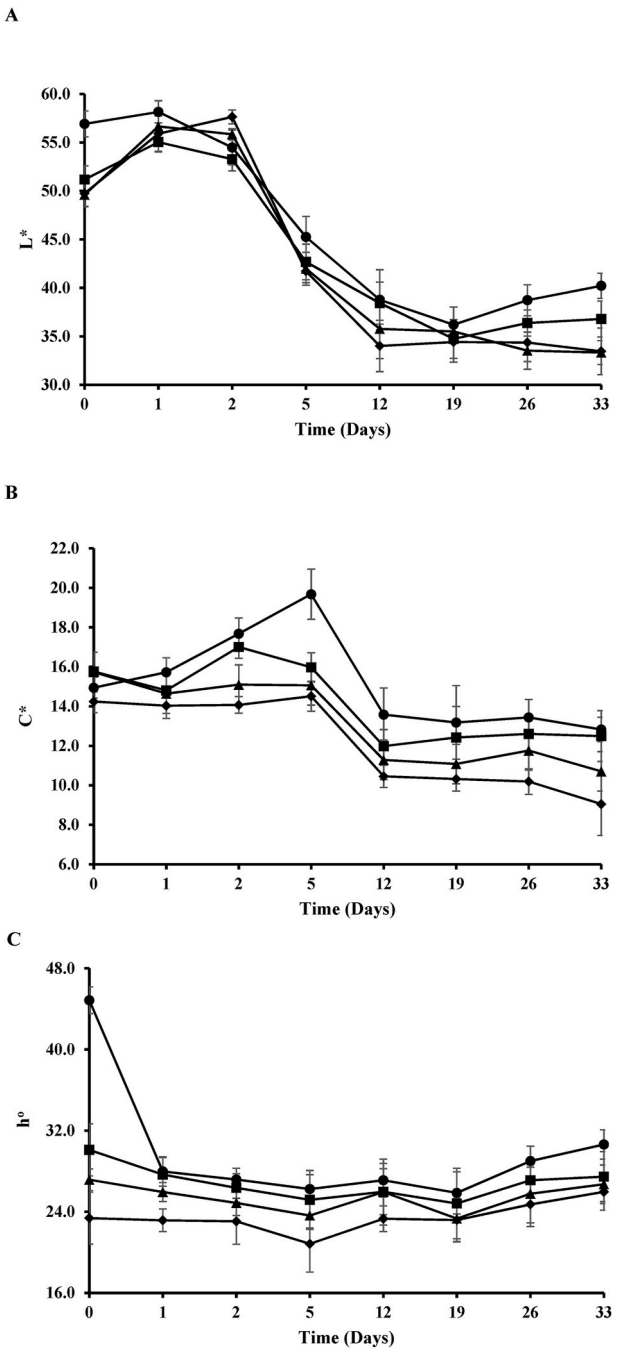

Fig. 3. Changes in exterior lightness L*(A), chroma C* (B), and hue angle h^o value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%). Each data point is a mean of four observations. Error bars denote ± one standard deviation.

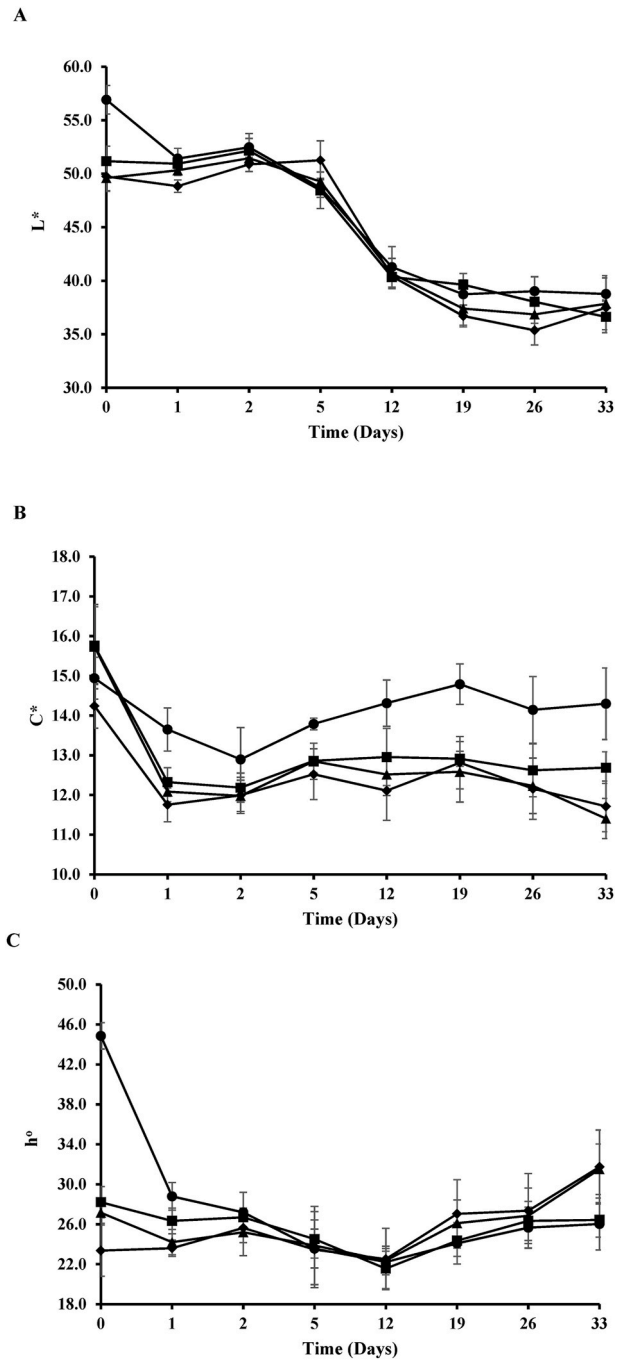

Fig. 4. Changes in interior lightness L*(A), chroma C* (B), and hue angle h^o value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%). Each data point is a mean of four observation. Error bars denote ± one standard deviation.

day5), and reduction in h^o value (44.85 on day 0–26.24 on day5) in the control after the 5-days fermentation was due to the formation of bright red nitrosylmyoglobin pigment; i.e. between myoglobin and nitric oxide, which essentially turned the DFS from pale yellow to bright red ($P < 0.05$; Fig. 3B and C). In fact, such drastic change in color parameters was not observed in CP-added DFS as their darker hue might have had masked the brightness and intensity of the newly formed meat color pigments. By the end of the process, all treatments showed a lower L* value than day 0 as a result of stepwise moisture loss. Both medium and high CP treatments showed a progressively ($P < 0.05$) lower exterior L*, C* and h^o value than the control during the 4-week drying stage (Fig. 3A, B, 3C). Medium and high CP sausages after 4-week drying (end-product) had a significantly ($P < 0.05$) lower exterior L* (Medium: 33.33; High: 33.45), C* (Medium: 10.71; High: 8.88), and h^o (Medium: 26.71; High: 26.60) value than the control (L*: 40.21; C*: 12.68; h°: 29.52), suggesting a darker, duller, as well as redder end-product color. Low CP level yielded a final exterior color slightly darker (lower L* value; $P < 0.05$) than the control while color dullness and redness was not impacted ($P > 0.05$). End-product interior C* values were also significantly reduced ($P < 0.05$; Fig. 4A and B), while a greater h^o value ($P < 0.05$; Fig. 4C) was observed for the medium and high CP treatments;

indicating a more yellow final interior. In contrast, low CP level only reduced interior color saturation (lower C* value; $P < 0.05$) but not lightness and redness. Our findings suggest that the influence of CP on DFS color characteristics is related to the incorporation level; with low CP treatment showing the most similar color to the control. In any case, such differences in end-product color require future research to validate consumer acceptability.

3.6. Effect of CP on DFS textural parameters

Texture of DFS is considered an important attribute of consumer acceptance and is often employed as a parameter to determine the quality of the finished product (Herrero et al., 2007). Low CP level showed no significant effect on the textural characteristics of DFS throughout the entire manufacturing process ($P > 0.05$; Table 3) compared to control. Contrarily, a significantly lower hardness, springiness, cohesiveness, gumminess, and chewiness ($P < 0.05$) were observed for the medium and high CP DFS after 24 h into the fermentation stage, and values remained lower ($P < 0.05$) in quite a few of the dates along the process compared to the control; indicating that the addition of higher levels of CP can impart a softer texture due to initial fast pH reduction (pH < 5.2) and protein denaturation. However, this can be resolved by utilization of encapsulated CP, as previously demonstrated by Barbut (2005) with encapsulated LA and Chacon et al. (2006) with allyl isothiocyanate, which will result in slow release of CP and acidification after a few hours/days (after stuffing and initial lactic acid bacteria fermentation). However, our results show conflicting trends compared to earlier reports examining the effect of fiber or pomace addition to DFS. Calvo et al. (2008), Eim et al. (2008), and Sánchez-zapata et al. (2013) reported an increase in hardness of DFS enriched with tomato peel, dried carrot pomace, and Tiger nut fiber, respectively. The addition of 1.5–3.0% peach, orange or apple fiber was also found to decrease DFS firmness only, however not springiness, cohesiveness, gumminess, and chewiness (Garcia et al., 2002). Backers and Noli (1997) suggested that incorporation of dietary fiber can enhance the integrity of meat products by forming a more ordered three-dimensional network, or by reinforcing the existing meat protein network which can subsequently alter the rheological properties as well as the water-holding capacity of meat emulsions. However, the variation in our case could be due to the difference in type and/or chemical composition of the pomace used. In the present study at which a significant pH-decreasing phenomenon was observed, the softer texture could be attributed to the significant acidification effect seen in the medium and high CP treatments. When a meat batter is subjected to instant and drastic pH reduction, rapid protein denaturation and dis-ordered aggregation occurs, causing clumping of ground meat particles, loss of liquid, and irreversible reduction in product integrity (Barbut, 2005). Similarly, Fernández-López et al. (2008) reported a reduction in textural acceptability in DFS supplemented with 2% orange fiber (pH 3.28 ± 0.02) due to excessive low product pH. The observed phenomenon in our work could also be the result of meat protein cross-linking disruption and subsequent network weakening in the presence of high levels of dietary fiber from CP (Ktari et al., 2014; Wang et al., 2017).

4. Conclusion

The present study is the first comprehensive research examining the antimicrobial effect of CP in DFS as well as its impact on product quality attributes. The results show that CP has a significant effect on *Salmonella* inactivation in a fermented meat matrix, and the level and inactivation rate are concentration-dependent. Overall the growth of lactic acid bacteria was stimulated in the presence of medium to high levels of CP while *Staphylococcus* spp. growth was inversely linked to the CP level. Addition of low levels of CP not only resulted in enhanced *Salmonella* inactivation, but also yielded DFS with physicochemical, color, and

Table 3
Effect of cranberry pomace (CP) at varying levels on the textural properties of dry fermented sausages (DFS) during fermentation and drying.

Time (days)	Hardness (N/cm^2)				Cohesiveness (ratio)				Springiness (mm)				Gumminess (N/cm^2)				Chewiness (N/cm)			
	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
Fermentation																				
1	8.77 ±1.5^{a}	9.15 ±0.8^{a}	5.48 ±0.9^{b}	4.94 ±0.7^{b}	0.79 ±0.03^{a}	0.78 ±0.01^{a}	0.75 ±0.02^{b}	0.72 ±0.02^{b}	8.39 ±0.5^{ab}	8.24 ±0.4^{b}	7.60 ±0.3^{a}	7.71 ±0.5^{a}	6.88 ±1.1^{a}	7.14 ±0.7^{a}	4.09 ±0.7^{b}	3.56 ±0.5^{b}	5.80 ±1.1^{a}	5.89 ±0.7^{a}	3.11 ±0.5^{b}	2.75 ±0.5^{b}
2	10.01 ±1.2^{a}	8.77 ±1.6^{ab}	7.85 ±0.6^{b}	7.21 ±1.4^{b}	0.78 ±0.01^{a}	0.78 ±0.01^{a}	0.73 ±0.01^{b}	0.70 ±0.03^{c}	8.40 ±0.4^{a}	8.11 ±0.4^{a}	7.49 ±0.6^{b}	6.81 ±0.4^{c}	7.90 ±0.9^{a}	6.80 ±1.2^{a}	5.74 ±0.4^{b}	5.05 ±0.8^{b}	6.54 ±0.9^{a}	5.52 ±1.0^{a}	4.29 ±0.3^{b}	3.46 ±0.7^{b}
5	19.91 ±1.3^{a}	21.12 ±1.9^{a}	18.66 ±1.4^{a}	17.66 ±1.6^{a}	0.70 ±0.01^{a}	0.69 ±0.01^{a}	0.65 ±0.02^{b}	0.63 ±0.03^{b}	7.39 ±0.4^{a}	7.17 ±0.2^{b}	6.46 ±0.3^{c}	6.23 ±0.2^{c}	13.19 ±0.8^{a}	14.54 ±1.4^{a}	13.20 ±1.4^{ab}	11.66 ±0.9^{b}	9.76 ±0.9^{a}	10.42 ±1.1^{a}	8.55 ±1.2^{b}	7.27 ±0.6^{b}
Drying																				
12	49.48 ±10.4^{a}	57.21 ±10.9^{a}	58.35 ±11.7^{a}	49.40 ±8.1^{a}	0.57 ±0.01^{a}	0.59 ±0.03^{a}	0.57 ±0.02^{a}	0.57 ±0.02^{a}	6.54 ±0.1^{a}	6.39 ±0.4^{ab}	6.26 ±0.2^{ab}	5.98 ±0.5^{b}	28.79 ±5.6^{a}	33.74 ±6.5^{a}	33.22 ±6.1^{a}	26.92 ±5.1^{a}	18.75 ±3.8^{a}	21.53 ±4.5^{a}	20.72 ±3.4^{b}	16.18 ±3.8^{b}
19	78.67 ±6.7^{ab}	85.33 ±5.2^{a}	70.30 ±9.5^{b}	69.44 ±5.3^{b}	0.54 ±0.01^{a}	0.53 ±0.02^{a}	0.47 ±0.02^{b}	0.46 ±0.03^{b}	5.39 ±0.2^{a}	5.33 ±0.2^{a}	4.87 ±0.1^{a}	5.08 ±0.7^{a}	42.38 ±3.6^{a}	46.15 ±4.5^{a}	32.66 ±3.9^{b}	32.57 ±1.9^{b}	22.85 ±2.1^{a}	24.57 ±2.4^{a}	15.92 ±2.1^{b}	16.47 ±1.8^{b}
26	119.76 ±7.5^{a}	121.71 ±8.0^{a}	97.97 ±8.6^{b}	99.48 ±8.1^{b}	0.54 ±0.01^{a}	0.54 ±0.02^{a}	0.48 ±0.03^{b}	0.47 ±0.02^{b}	5.49 ±0.2^{a}	5.52 ±0.2^{a}	5.17 ±0.1^{b}	5.12 ±0.2^{b}	64.76 ±4.5^{a}	65.62 ±3.6^{a}	47.28 ±4.9^{b}	46.32 ±4.0^{b}	35.64 ±3.3^{a}	36.21 ±2.5^{a}	24.43 ±2.7^{b}	23.68 ±1.9^{b}
33	134.43 ±7.0^{a}	142.15 ±5.8^{a}	113.67 ±7.6^{b}	106.39 ±8.2^{b}	0.55 ±0.02^{a}	0.55 ±0.01^{a}	0.45 ±0.01^{b}	0.46 ±0.03^{b}	5.55 ±0.2^{a}	5.66 ±0.1^{a}	5.34 ±0.2^{b}	5.24 ±0.2^{b}	73.91 ±4.1^{a}	78.41 ±4.0^{a}	51.30 ±3.4^{b}	48.38 ±2.9^{b}	41.59 ±3.6^{a}	44.36 ±2.3^{a}	27.40 ±2.3^{b}	25.3 ±1.8^{b}

Means ± standard errors in the same row followed by different letters are significantly different ($P < 0.05$).
C: control, 0% CP; L: low, 0.55% CP; M: medium, 1.70% CP; H: high, 2.25% CP.

textural characteristics similar to conventional DFS. DFS incorporated with medium and high CP levels resulted in sausages with a softer texture and a darker, duller, yet redder color. Increase in CP levels causes an immediate (i.e., prior to fermentation) acidification of the raw meat batter to a pH of 5.2 and negatively affects the texture of the products which would make it unacceptable for consumers. Therefore, any processor considering the inclusion of a polyphenolic antioxidant compound with preferential antimicrobial activity in a product such as DFS, would first have to determine an appropriate concentration of the compound to use that would not negatively affect the product while still eliciting the antimicrobial activity. Additionally, application of micro-encapsulation technologies to enable slow release of CP in to the meat matrix could assist with enabling slow acidification and along with the antimicrobial and antioxidant contributions of CP could promote the utilization of this by-product stream by the meat industry. Future studies on product flavor characteristics and consumer acceptability are recommended.

CRediT authorship contribution statement

Alex Tsun Yin Lau: Data curation, Formal analysis, designed all experiments and carried out all studies including data collection, analysis, interpretation and preparation of manuscript with guidance from SBarbut and SBalamurugan. **Laura Arvaj:** Data curation, provided significant technical support during inoculation, DFS manufacture, sampling and data collection. **Philip Strange:** Data curation, provided significant technical support during inoculation, DFS manufacture, sampling and data collection, technical support during inoculation, DFS manufacture, sampling and data collection. **Madison Goodwin:** Data curation. **Shai Barbut:** Data curation, Formal analysis, designed all experiments and carried out all studies including data collection, analysis, interpretation and preparation of manuscript with guidance from SBarbut and SBalamurugan. **S. Balamurugan:** Data curation, Formal analysis, Investigation, Funding acquisition, Supervision, designed all experiments and carried out all studies including data collection, analysis, interpretation and preparation of manuscript with guidance from SBarbut and SBalamurugan. the principal investigator of the project and was responsible for preparing the research proposal, acquire funds, assemble and supervise the team, provide overall guidance and mentorship throughout the scope of the project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2021.09.001.

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