Effects of salt purity on lipid oxidation, sensory characteristics, and textural properties of fresh, ground pork patties

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ABSTRACT

The objective was to evaluate salts of varying purity levles on lipid oxidation, texture, and sensory properties of fresh ground pork patties. Approximately 160 kg of fresh boneless pork trimmings was used to test a salt typical to industry (treatment A), 3 specialty salts (B, C, and D), and a control (no added salt). Salts were analyzed for Na, Cl, Fe, Cu, Mg, Ca, and Mn content. Experimental treatments were replicated 6 times, for a total of 30 independent batches. Analysis was conducted using the MIXED procedure of SAS as a repeated measure in a complete randomized design. After 11 days of refrigerated storage, there were no differences in lipid oxidation among salts A, C, or D ($P \geq 0.15$), but salt B had less ($P \leq 0.04$) lipid oxidation than salts A, C, and D. However, no differences in oxidized flavor or odor ($P \geq 0.95$) were detected. Overall, salts of varying impurities differed in lipid oxidation but sensory panelists were not able to detect differences in oxidized odors or flavors.

Keywords: Lipid oxidation, Pork, Salt, Sensory, Sodium Chloride

1. Introduction

Salt can act as a preservative by inhibiting microbial growth in food products. It can also accelerate oxidation of lipids (Romans et al., 2001; Ruiz, 2007) and fresh meat pigment (Devatkal and Naveena, 2010). This is particularly true in fresh meat products where the primary role of salt in the formula is not as a preservative, but as a flavoring or an aid in protein extraction. In such scenarios salt is rarely included in concentrations greater than 3.0% of the final product. Salt at concentrations below 3.0% increases the activity of lipoxygenase in fresh pork, contributing to the development of rancidity (Jin et al., 2011). Thus, when including salt in product formulations, it is important to consider the positive and potential negative effects that it may have on shelf-life. The term "salt" is typically associated with sodium chloride; however some commercially available salts contain metallic components such as iron, copper, and magnesium, and other transition metals. Sodium chloride alone can act as a prooxidant in meat systems (Kanner et al., 1991), but the other metallic components associated with salt also contribute to oxidation. Unrefined salts often contain a greater concentration of mineral impurities than more refined salts (Kaufman, 1960; Bess et al., 2013). Even so, the market for naturally harvested, unrefined salts is projected to grow by 6.3% to over $1.34 billion in revenue by 2019 (Markets and Markets, 2014).

Bess et al. (2013) evaluted the rate of lipid oxidation and sensory characteristics of fresh and frozen pork patties manufactured using commercial salts but, reported no difference in lipid oxidation or oxidized flavor attributes between the salts. However, the salts investigated by Bess et al. (2013) did not represent the full spectrum of impurity level found in unrefined salts that have, traditionally, not been used in food processing. The unrefined salt varieties used in the present experiment represent a greater concentration of impurities known to influence the rate of

lipid oxidation than what have been used in previous experiments, yet are representative of salt varieties available in the market. Therefore, the objective of this experiment was to evaluate the effects of unrefined salt varieties containing greater porportions of impurities than would be found in salts typically used in commercial food processing on textural properties, lipid oxidation, and sensory characteristics of fresh ground pork patties.

2. Materials and methods

2.1 Raw materials

The experiment closely followed the experimental design described by Bess et al. (2013). Approximately 160 kg of fresh boneless pork trimmings were obtained from pigs slaughtered at the University of Illinois Meat Science Laboratory. Carcasses were fabricated 24 hours after slaughter and the generated trimmings were stored at 4°C overnight and thus used in formulation at 2 d postmortem. A single-sourced master meat block was used to control the variation of response variables due to variation of the meat block. It was then divided into independent batches, prior to salt inclusion. This approach has been used previously as a means to control variation due to raw materials (Hęś et al., 2012; Bess et al., 2013; Comi et al., 2015).

The salt treatment groups included a salt typical to the meat industry, 3 varieties of unrefined salt, and a control group (no added salt; Table 1). A salt representative of what would typically be used in food manufacturing was purchased from a commercial food processing ingredient supplier (Salt A). The 3 unrefined salts, 2 rock salts (B and D) and a sea salt (C) were purchased from grocery stores in Champaign, IL. Salts were selected in order to represent varying concentrations of proxidant metals such as; copper, iron, manganese, calcium, and magnesium, and were based on previous analyses of similar products. Concentrations of known

112 metal prooxidants, as well as Na^+ and Cl^- ions were later quantified (Table 1) using inductively
113 coupled plasma-optical emission spectroscopy (ICP-OES) (method 985.01; AOAC Int., 2007).

114 *2.2 Formulation, packaging, and storage*

115 Five treatment groups (control, Salt A, B, C, and D) were replicated 6 times for a total of
116 30 independent experimental units in the same manner described by Bess et al. (2013). A master
117 meat block was used to control variation due to raw materials and then divided into 30 separate
118 batches (Hęś et al., 2012; Bess et al., 2013; Comi et al., 2015). Initially, the entire meat block
119 was ground in an industrial meat grinder (model 7552 H12, Biro MFG. CO, Marblehead, OH,
120 U.S.A) through a 1.32 cm plate, thoroughly mixed, and then ground through a 0.32 cm plate
121 using an industrial mixer (model 900E Mixer-Grinder, Hollymatic Corporation, Countryside, IL,
122 U.S.A). After grinding, the master meat block was separated into 30 independent, 5 kg batches
123 prior to salt inclusion. Batch served as the experimental unit because salt treatment was applied
124 independently to each experimental unit (batch) prior to salt inclusion (Hęś et al., 2012; Bess et
125 al., 2013; Comi et al., 2015).

126 A 227 g sample from each batch was collected before the addition of salt for determination of
127 lipid, moisture, and salt-soluble protein analysis of each and for each salt treatment. Each batch
128 was standardized to 4.325 kg and placed in a bowl chopper (TALSA, model C40P, Xirivella,
129 Valencia, Spain). Salt inclusions were added independently to each experimental unit (batch) at a
130 rate of 1.5 g/100 g of meat by mixing with 5 revolutions of the bowl chopper. The control group
131 was also placed in a bowl chopper for 5 revolutions, but no salt was added. No other ingredients
132 were included in the formulation in order to prevent confounding effects of salt driven oxidation
133 with oxidation or anti-oxidation effects from additional ingredients. This resulted in 30

independently formulated batches that represented 6 replications (n = 6) per salt treatment. Sixty patties per treatment-storage time combination, each weighing approximately 110 g were made using a manual patty press (patty Moulding Machine, MH-120, Manica USA, St. Louis, MO, U.S.A). Two patties were placed side by side on polystyrene trays (Bush Brothers Inc., Champaign, IL) identified and placed in a freezer (-40°C). After 1 hour in the freezer, the trays were over-wrapped with polyvinylchloride (PVC) film (oxygen transmission rate = 1,627.9 cc/m^2/day; moisture vapor transmission rate = 170.5g/m^2/day). Patties were subsequently stored at 4°C with the entire surface of the packaged patties exposed to full light (1075 lx) for 1, 6, or 11 days.

Extractible lipid and moisture percentage of each experimental unit (batch), prior to salt inclusion, was determined using the methods described by Novakofskiet al. (1989).

2.3 Salt-soluble protein extractibility

Salt-soluble protein extractibility of each salt and the control was evaluated using the procedure described by Boler et al. (2011). Extraction buffer was prepared by mixing 0.01M 2-[N-Morpholino]ethanesulfonic acid (MES) in distilled (nanopure) water. This extraction master mix was used to make each extraction buffer, which contained increasing salt concentrations of 0.09 mol/L, 0.26 mol/L, 0.43 mol/L or 0.60 mol/L. Samples were quantified with a BCA Protein Assay Kit (Pierce Protein Research Products, Rockford, IL) and absorbance values were measured at 562 nm using a Synergy HT Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT.). Amount of soluble proteins were calculated using a second order polynomial quadratic equation and were expressed as a percentage of tissue.

2.4 Break strength

Two fresh patties from each batch for each storage period were evaluated (n=6) using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) to determine the amount of force required to break the patty in half. Patties were cooked at 191°C for 14 minutes in an oven (South Bend Convection Oven, Model V-15, South Bend, IN, U.S.A.). Patties were allowed to cool to approximately 22 °C for 1 hour. Break strength was evaluated using a protocol described by Souza et al. (2011). Continous force was applied directly to the midline of each patty at a rate of 3.33 mm/s with a crossbar (10 mm diameter), at platform gap of 3.2 cm, and a travel distance of 70mm. Break strength values were expressed as Newtons of force averaged between the two patties.

2.5 Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid (TBA) values correlate with sensory evaluations of oxidized flavor and odor in meat products (Fernández et al., 1997), thus the TBARS assay was conducted in order to complement sensory evaluation of oxidized flavor and odor. Two patties representing each batch and storage time combination were evaluated (n=6). After each storage time (1, 6, or 11 days), two patties representing each batch were removed from fresh storage, placed in vacuum packaged bags and frozen at -40°C for two days prior to TBARS assessment.

Thiobarbituric acid reactive substances were evaluated using the procedure described by Leick et al. (2010). Samples were analyzed for malanaldedyde (MDA) content using a 96-well plate in a Synergy HT Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT). A standard concentration curve was plotted with TEP (1,1,3,3-tetraethoxypropane; 0-7.5 µM) to obtain the MDA concentration. Results were expressed as mg MDA/g extractable lipid in order to account for any differences in extracable lipid of batches.

178 *2.6 Color evaluation*

179 Objective CIE *L** (lightness), *a** (redness), and *b** (yellowness; Commission
180 Internationale de l'Eclairage (CIE), 1978) scores were collected with a Minolta CR-400 Chroma
181 meter (Minolta Camera Co., Ltd., Osaka, Japan) utilizing a D_{65} light source and a 0° observer
182 with an aperture size of 8 mm. Measurements were collected at four locations from one patty
183 from each batch at each storage period (n=6), with the aperture placed directly on the patty's
184 surface, and the mean of the four measurements were recorded as the objective color score for
185 *L**, *a**, and *b**. Hue angle, a measurement related to the state of pigments (Ripoll et al., 2011),
186 was calculated using the following equation and reported in degrees: hue angle = $\tan^{-1}(b^* / a^*) \times 57.296$. Chroma was calculated using the following equation: chroma = $\sqrt{a^{*2} \times b^{*2}}$.

188 Brown discoloration was measured by a visual evaluation by three trained panelists on
189 the same patty as objective color evaluation for each storage period. Discoloration was evaluated
190 immediately before objective color measurements were recorded, with the overwrap still intact in
191 order to best represent what a consumer would encounter in a store. Discoloration was evaluated
192 on a 10 point scale with a score of zero representing 0% brown discoloration and 10 representing
193 100% brown discoloration. Simulated retail storage was terminated when the mean discoloration
194 of the patties exceeded a score of 5 (50% discolored).

195 *2.7 Sensory evaluation*

196 Two patties representing each batch and storage time combination (n = 180) were used
197 for sensory evaluation by a trained sensory panel. After each storage time (1, 6, or 11 days), two
198 patties representing each batch were removed from fresh storage and placed in vacuum packaged
199 bags and frozen at - 40°C until sensory evaluation. Panelists were selected from departmental

students and staff and trained according to American Meat Science Association Guidelines (AMSA, 1995). Sensory evaluations were conducted in individual booths under ambient conditions of temperature and humidity and under red light. Before evaluation, panelists participated in a training session to orient them toward scale attributes and anchors. Panelists were presented with salt solutions containing 0-4g/100g of salt for saltiness training. Potato puffs cooked in oxidized oil were used for oxidized odor and flavor training. Panelists rated attributes on a 15 cm line scale with anchors at 0, 7.5, and 15 cm with 0 cm representing no oxidized flavor, odor, or salty taste. A score of 15 cm indicated that the sample was extremely intense for each of the characteristics.

A total of 15 sensory evaluation sessions were conducted over the course of 10 days with each session having 6 samples evaluated by 6 trained panelists. No more than two sessions occurred per day and concurrent sessions were held at least 1 hour apart. Samples were allocated to sessions such that all three storage time points for a specific batch were represented in each session, but each salt treatment was not necessarily represented. Sessions were organized such that each salt treatment group was directly compared with each of the other salt varieties during at least one session. This allowed for the control of variation in sensory parameters due to a random session effect.

Sensory patties were thawed 12-16 hours at 4° C prior to evaluation. Two patties, representing each experimental unit and time point, were wrapped in aluminum foil and cooked at 191°C for 14 minutes in a convection oven (South Bend Convection Oven, Model V-15, South Bend, IN, USA). Immediately after cooking, patties were cut into 2.54 cm by 2.54 cm portions and placed in small plastic cups with lids, identified with randomized single digit codes, and presented to panelists in numerical order.

2.8 Statistical analyses

Because salt treatments were applied independently to each batch, batch (n = 6) served as the experimental unit for all statistical analyses (Hęś et al., 2012; Bess et al., 2013; Comi et al., 2015). Least square means were calculated for moisture and extractible lipid percentage using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Salt treatment was the fixed effect and means were separated using the PDIFF option. Statistical analyses for salt-soluble protein extractability, objective and subjective color evaluation, break strength, and TBARS, were conducted using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as repeated measures. Fixed effects were salt treatment, storage time, and the interaction of salt treatment and storage time. Storage time was included in the repeated statement. An autoregressive covariance matrix was selected for each dependent variable based on Akaike's information criteria to minimize variance. Single degree of freedom contrast statements were used to determine differences between pooled salt treatments and the control for each storage time. Least square means for main effects of salt treatment and storage time were separated using PDIFF option of the MIXED procedure of SAS. Replication was included in the models as a random variable for all analyses and panel were used as random variables for analysis of sensory data to account for variation in panelists between sessions.

3. Results

3.1 Analysis of Salts

Salt A had the lowest concentration of measured impurities followed by Salt C (Table 1). This was expected as Salt A was a salt typical for use in food manufacturing. Salts B and D had the greatest amount of total impurities due in large part to the greater concentrations of iron (43.9

and 129.4 mg/kg, respectively) compared with Salts A and C (Table 1). Overall, Salt D contained the most impurities as it had the greatest concentration of iron and manganese and proportions of copper, magnesium, and calcium similar to the other 3 salt treatments. Salt A also had the greatest proportion of pooled Na^+ and Cl^- ions (99.41%) followed by Salt B (99.06%), Salt D (97.14%) and Salt C (95.32%).

3.2 Break strength, & Salt-soluble proteins

Patties that did not contain salt (control) had lesser break strength values than all salt treatments ($P < 0.0001$). There was no interaction of treatment and storage time for break strength ($P = 0.66$) and there were no differences among the salt treatments ($P = 0.53$; Fig 1). Break strength increased the longer the patties were stored ($P < 0.0001$) with break strength, on average, increasing ($P < 0.0001$) 7.05 N from d 1 to d 11 of storage.

All salt treatments had greater amounts of extracted salt-soluble proteins than the no-salt buffer control ($P < 0.001$; Fig 2). Among salt treatments, there was no interaction of concentration and salt treatment ($P = 0.77$). Salt A and salt B extracted a greater amount of salt-soluble proteins than either salts C or D ($P \leq 0.03$) overall but, were not different from one another ($P = 0.14$). Extracted salt-soluble protein from salts C and D were not different ($P = 0.90$). When salt concentration was increased from 0.09 mol/L to 0.26 mol/L extracted salt-soluble protein increased by 2.42 g/100 g ($P < 0.01$) regardless of salt treatment. However, as salt concentration was increased to greater than 0.26 mol/L, extracted salt-soluble proteins did not increase ($P \geq 0.19$).

3.3 TBARS

All salt treatments had greater concentrations of TBARS than the control at each storage time ($P < 0.0001$). Among salt treatments there was an interaction of salt treatment and storage time ($P < 0.01$) for TBARS as values of Salt B did not increase at the same rate as the other 3 treatments. Patties treated with Salt B, at each storage time, had less ($P < 0.01$) TBARS than patties treated with Salt C or D. TBARS were lesser in Salt B compared with C and D after d 1 ($P < 0.01$) and d 11 of fresh storage ($P < 0.01$). At d 1 and 6 of storage, TBARS of Salts A and B were similar ($P \geq 0.07$) though at d 11. Salt B had less TBARS than salt A ($P = 0.04$). At 1 and 11 d of storage, Salts C or D did not differ from Salt A ($P \geq 0.14$), while at d 6, Salt A TBARS were lesser ($P \leq 0.0001$) compared with Salt C and D.

3.4 Color evaluation

Salt inclusion decreased L^* (lightness), a^* (reddness), b^* (yellowness), and hue angle values ($P \leq 0.04$) compared with the control (Table 2). There were no signifcant differences in L^*, a^*, or b^* among salt treatments ($P > 0.12$). There was an effect of storage time on L^*, a^*, and b^* values among salt treatments ($P < 0.02$). L^* values were unchanged from days 1 to 6 ($P = 0.49$) but increased from days 6 to 11 for all salt-included treatments ($P < 0.0001$). Among salt treatments, patties decreased in redness from day 1 to 6 and from day 6 to 11 by 1.45 and 4.47 units, respectively ($P < 0.0001$). There was no difference in yellowness of salt treatments between 1 and 6 days of storage ($P = 0.37$) however, day 11 samples were more yellow than samples stored for 6 days ($P < 0.01$), but there was no difference ($P = 0.29$) between patties stored 1 or 11 days. Among salt treatments, there was no significant interaction between salt treatment and duration of storage for L^*, b^*, or hue angle values ($P \geq 0.08$) however there was an interaction of treatment and storage time on redness ($P = 0.02$) This was attributed to the fact

288 that the a^* values for the salt B treatments after 1 and 6 days were not significantly different (P
289 = 0.40) but all other treatments were less red at day 6 than at day 1 ($P \leq 0.01$).

290 Browning discoloration was used as a metric for evaluating the development of
291 metymyoglobin on the surface of the patties. Color is the primary metric by which consumers
292 decide the quality of meat products (Tikk et al., 2008). The simulated retail storage of the patties
293 in the present study was terminated when the mean discoloration exceeded a score 5 (50%
294 discoloration). As expected, discoloration increased over time as all treatments were more
295 discolored with each successive duration of storage time ($P < 0.0001$). Overall, the inclusion of
296 salt increased the development of discoloration ($P < 0.0001$). Among salt treatments, there was
297 an interaction ($P < 0.0001$) of treatment and storage time as there were no differences after 1 or 6
298 days of storage ($P \geq 0.40$), but at d 11 Salt A was more discolored ($P = 0.01$) than Salt C, Salt C
299 was more discolored than Salt D ($P < 0.01$), and Salt D was more discolored than Salt B ($P =$
300 0.02, Fig. 5).

301 *3.5 Sensory evaluation*

302 At d 1 oxidized odor and flavor evaluations were low (Oxidized odor < 4.1; Oxidized
303 flavor < 4.5) and would not be considered oxidized by the calibration used for this panel. There
304 were no differences between the control and salt treatments after 1 or 6 days of storage ($P \geq$
305 0.34) for oxidized flavor, however control patties had less oxidized flavor after 11 days of
306 storage ($P < 0.0001$) compared with the pooled salt treatments. There was no interaction of of
307 storage time and salt treatment ($P = 0.86$) and there were no differences in oxidized flavor
308 among salt treatments ($P = 0.54$) of storage (Fig 6a). All treatments increased in oxidized flavor

309 over time ($P < 0.0001$). Oxidized flavor did not increase ($P = 0.41$) between d 1 and d 6 but did
310 increase from d 6 to d 11 ($P < 0.0001$).

311 Panelists were unable to detect any differences in oxidized odor between the control and
312 the salt treatments ($P \geq 0.29$) after 1 or 6 d of storage (Fig 6b). After 11 days of fresh storage
313 control patties had a less oxidized odor than salt treatments ($P < 0.0001$). There was no
314 interaction of salt treatment and storage time ($P = 0.98$) among salt treatments. Furthermore,
315 there were no differences in oxidized odor among the salt treatments ($P = 0.94$) after 1, 6, or 11 d
316 of storage. There was no difference in oxidized odor between days 1 and 6 of storage among salt
317 treatments ($P = 0.36$) but, oxidized odor increased between day 6 and 11 ($P < 0.0001$).

318 Perceived saltiness was increased by the inclusion of salt in the ground pork patties ($P <$
319 0.0001) compared with the control regardless of storage day (Fig 6c). There was no interaction
320 of salt treatment and storage time ($P = 0.89$). There were no differences in the panelists
321 evaluations for saltiness among salt treatments ($P = 0.47$). Among salt treatments, panelists
322 found no difference in saltiness between patties stored 1 day or 6 days ($P = 0.14$) or between day
323 6 and 11 ($P = 0.11$), and found that patties stored for 11 days were saltier than those stored for 1
324 day ($P < 0.01$).

325 4. **Discussion**

326 The role of salt as a proxidant was confirmed in the present experiment, in agreement
327 with previous reports (Kanner et al., 1991; Devatkal and Naveena, 2010; Bess et al., 2013). The
328 role of transition metals such as iron and copper as prooxidants has also been documented
329 (Ladikos & Lougovois, 1990; St. Angelo et al., 1996). Bess et al. (2013) investigated the
330 characteristics of a variety of commercial salts of varying purity, but were unable to detect

differences in lipid oxidation despite the differences in concentrations of known prooxidants. The objective of the present experiment was to investigate the effects of salts that contained impurity levels beyond the concentrations used in previous studies, but would still be representative of unrefined salts on the market.

Salts A and B were able to extract more salt soluble proteins than either Salt C or D, but the greater extraction of myofibrillar proteins did not result in differences in break strength which indicates that regardless of salt purity, there was sufficient concentrations of chloride ions to aid extraction and binding of myofibrillar protein. As patties were stored longer, break strength increased, similar to Hand et al. (1992), that reported the cohesiveness, a measure of binding between meat particles, of coarse-ground sausage patties increased as preblended batters were held for longer periods of time.

Salih et al. (1989) reported there was no difference in lipid oxidation between pure salt and rock salt in ground turkey breast, despite the fact that the rock salt contained 37 mg/kg more iron than the pure salt. The salts used in this study represented a wider spectrum of iron than used in previous work, with Salt B having 43.9 mg/kg and Salt D having 129.4 mg/kg of iron, compared to with < 0.1 mg/kg in either Salt A or Salt C. With such a wide spectrum of impurity levels, particularly in repsect to iron, it was expected that Salts A and C would be least susceptible to lipid oxidation and Salts B and D, which contained the greatest levels of iron, would have had the most lipid oxidation products. However, Salts A, C, and D did not differ in TBARS after d 1 or d 11 of fresh storage and most surprisingly, Salt B had consistently lesser TBARS than Salt C throughout the duration of the study. The differences in TBARS may be the result of prooxidants that were not quantified in the salts. Despite the differences in lipid oxidation, panelists were unable to detect differences in oxidized flavor or odor among the salt

354 treatments, similar to the report of Bess et al. (2013). The lack of difference in salty flavor was
355 expected as previous experiments had reported similar results in comparing sensory attributes of
356 unrefined salts used in marinated chicken breast (Broadway et al., 2011).

357 During fresh storage the patties treated with salt increased in lightness and browning
358 discoloration while decreasing in redness, in agreement with previous studies (Devatkal and
359 Naveena, 2010) but did not differ in hue angle. There was no effect of salt treatment on *L**, *a**,
360 or ***b**,*** however there was an interaction of salt treatment and storage time for *a** values, with Salt
361 B being more red than Salts C and D after 11 days of storage while having lesser TBARS than
362 Salts C or D. Similarly, Salt A had less TBARS at 11 days than Salts C and D. Browning
363 discoloration also followed a pattern similar to the results for TBARS at d 11 with Salt B being
364 the least discolored of the treatments.

365 The majority of salts evaluated in this study would not be used in the commercial meat
366 processing industry as they would be considered novel or gourmet in nature, and likely cost
367 prohibitive. Even so, the results of this study show that although there were differences among
368 the salt treatments in terms of lipid oxidation and color, those differences did not result in
369 differnces in sensory characteristics. Previous studies have shown that elevated levels of
370 impurities beyond what would be found in most commercial salts increase lipid oxidation rates.
371 The levels of prooxidant impurities in unrefined salts used in this experiment did not differ
372 enough to result in differences in lipid oxidation that would be detectable to the consumer and
373 would likely be even less importance in formulations including antioxidants. In conclusion, the
374 impurity levels in salts used in meat products should be of minimal concern to processors when
375 formulating products.

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References

AMSA. (1995). Research Guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Chicago, IL, USA.

AOAC. 2007. Official methods of analysis. 18th ed. Rev.2. Hortwitz, W. and Latimer Jr., G.W. editors. *Association of Official Analytical Chemists*. Gaithersburg, MD.

Bess, K., Boler, D. D., Tavarez, M. A., Johnson, H. K., McKeith, F. K., Killefer, J., and Dilger, A. C. (2013). Texture, lipid oxidation and sensory characteristics of ground pork patties prepared with commercially available salts. *LWT - Food Science and Technology*, 50, 408-413.

Boler, D. D., Holmer, S. F., Duncan, D. A., Carr, S. N., Ritter, M. J., Stites, C. R., Petry, D. B., Hinson, R. B., Allee, G. L., McKeith, F. K., and Killefer, J. (2011). Fresh meat and further processing characteristics of ham muscles from finishing pigs fed Ractopamine hydrochloride (Paylean®). *Journal of Animal Science*, 89, 210-220.

Broadway, P.R., Behrends, J.M., and Schilling, M.W. (2011). Effect of alternative salt us on broiler breast meat yields, tenderness, flavor, and sodium concentration. *Poultry Science*, 90, 12, 2869 – 2873.

Comi, G., Tirloni, E., Andyanto, D., Manzano, M., and Iacumin, L. 2015. Use of bio-protective cultures to improve the shelf-life and the sensorial characteristics of commercial hamburgers. *LWT-Food Science and Technology*, 62, 1198 – 1202.

Commission internationale de l'eclairage (CIE). (1978). Recommendations on uniform color spaces-color equations, psychometric color terms. Supplementary No.2 to CIE Publication No. 15 (E-1.3.L), 1971 (9TC-1-3). Paris, France.

Devatkal, S.K. and Naveena, B.M. (2010). Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. *Meat Science*, 85, 306 – 311.

Fernández, J., Pérez-Álvarez, J.A., and Fernández-López, J.A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry*, 59, 3, 345-353.

406 Hand, L.W., Mandigo, R.W., and Calkins, C.R. (1992). The effects of preblending time on
407 physical and textural properties of coarse ground sausages. *Meat Science*, 31, 13-24.

408 Hęś, M., Waszkowiak, K., and Szymandera-Buszka, K. 2012. The effect of iodine salts
409 on lipid oxidation and changes in nutritive value of protein in stored processed meats.
410 *Meat Science*, 92, 139 – 143.

411 Jin, G., Zhang, J., Yu, X., Lei, Y., and Wang, J. (2011). Crude lipoxygenase from pig muscle:
412 Partial characterization and interactions of temperature, NaCl and pH on its activity. *Meat*
413 *Science*, 87, 257 – 263.

414 Kanner, J., Harel, S., and Jaffe, R. (1991). Lipid Peroxidation of muscle food as affected by
415 NaCl. *Journal of Agricultural and Food Chemistry*, 39, 1017-1021.

416 Kaufman, D. (1960). Sodium Chloride: The Production and Properties of Salt and Brine. New
417 York, NY: Reinhard Publishing Corporation.

418 Ladikos, D., and Lougovois, V. (1990). Lipid oxidation of muscle foods: a review. *Food*
419 *Chemistry*, 35, 295-314.

420 Leick, C. M., Puls, C. L., Ellis, M., Killefer, J., Carr, T. R., Scramlin, S. M., England, M. B.,
421 Gaines, A. M., Wolter, B. F., Carr, S. N., and McKeith, F. K. (2010). Effect of distillers
422 dried grains with solubles and ractopamine (Paylean) on quality and shelf-life of fresh
423 pork and bacon. *Journal of Animal Science*, 88, 2751-2766.

424 Marketsandmarkets.com. (2014). Gourmet Salts Market by Type (Fleur de Sel, Sel Gris,
425 Himalayan Salt, Flake Salt, Specialty Salt), Application (Bakery & Confectionary, Meat
426 & Poultry Products, Seafood Products, Sauces & Savory) & Geography - Global Trend &
427 Forecast to 2019. marketsandmarkets.com.
428 http://www.marketsandmarkets.com/PressReleases/gourmet-salts.asp. Accessed 2
429 February, 2015.

430 Novakofski, J., Park, S., Bechtel, P. J., and McKeith, F. K. (1989). Composition of cooked pork
431 chops - effect of removing subcutaneous fat before cooking. *Journal of Food Science*, 54,
432 15-17.

433 Ripoll, G., Joy, M., and Muñoz, F. (2011). Use of dietary vitamin E and selenium (Se) to
434 increase the shelf life of modified atmosphere packaged light lamb meat. *Meat Science*,
435 87, 88-93.

436 Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., and Jones, K.W. (2001). *The Meat*
437 *We Eat*. (14th ed.) Interstate Publishers. Danville, IL.

438 Ruiz, J. (2007). Ingredients. In F. Toldrá, editor, *Handbook of fermented meat and poultry.*
439 Blackwell Publishing. Ames, IA.

Salih, A. M., Price, J. F., Smith, D. M., and Dawson, L. E. (1989) Lipid oxidation in turkey meat as influenced by salt, metal cations, and antioxidants. *Journal of Food Quality*, 12, 71-83.

Souza, C. M., Boler, D. D., Clark, D. L., Kutzler, L. W., Holmer, S. F., Summerfield, J. W., Cannon, J. E., Smit, N. R., McKeith, F. K., Killefer, J. (2011) The effects of high pressure processing on pork quality, palatability, and further processed products. *Meat Science*, 87, 419-427.

St. Angelo, A. J., Vercellotti, J., Jacks, T., and Legendre, M. (1996). Lipid oxidation in foods. *Critical Reviews in Food Science and Nutrition*, 36, 175-224.

Tikk, K., Lindahl, G., Karlsson, A.H., and Andersen, H.J. (2008). The significance of diet, slaughter weight and aging time on pork colour and colour stability. *Meat Science*, 79, 806 – 816.

Tables

Table 1. Characteristics of each salt variety and proximate composition of batches prior to salt inclusion

	Control	Salt A	Salt B	Salt C	Salt D	SEM[1]	P-value
Composition of Salts							
Sodium, %	-	41.61	40.46	40.12	40.04	-	-
Chlorine, %	-	57.80	58.60	55.20	57.10	-	-
Iron, ppm	-	< 0.1	43.9	< 0.1	129.4	-	-
Copper, ppm	-	0.8	< 0.3	< 0.3	0.4	-	-
Magnesium, ppm	-	< 0.01	1000.00	500.00	200.00	-	-
Calcium, ppm	-	100.00	1600.00	200.00	1600.00	-	-
Manganese, ppm	-	< 0.01	< 0.01	< 0.01	8.79	-	-
Proximate composition[2]							
Moisture, g/100 g	64.64[b]	63.02[a]	62.82[a]	62.88[a]	63.84[ab]	0.40	0.02
Lipid, g/100 g	18.22	19.21	19.53	18.95	18.68	0.35	0.11

[ab]Means within a row lacking a common superscript differ (P < 0.05)

[1]Data are LSmeans and reported SEM is the maximum SEM among treatments

[2]Proximate composition of ground pork batches

Table 2. Main effects of salt and storage time on texture, objective color, and sensory characteristics of ground pork patties

	Salt						Storage time				*P*-values			
Item,	No Salt	A	B	C	D	SEM[1]	1	6	11	SEM[1]	Control vs Salt[2]	Salt	Storage time	Salt x storage time
n	18	18	18	18	18		30	30	30					
Texture														
Break strength, N	1.41	2.45	2.30	2.31	2.32	0.08	1.94^{c}	2.38^{b}	2.71^{a}	0.07	< 0.0001	0.53	< 0.0001	0.66
Objective Color[3]														
*L**	56.94	54.87	24.34	54.63	54.50	0.39	54.12^{b}	53.92^{b}	55.71^{a}	0.26	< 0.0001	0.80	< 0.0001	0.15
*a**	12.80	11.31	12.09	11.97	11.85	0.24	14.26^{a}	12.81^{b}	8.35^{c}	0.24	< 0.01	0.13	< 0.001	0.02
*b**	9.67	8.74	8.95	9.13	8.96	0.16	8.96^{ab}	9.14^{a}	8.74^{b}	0.20	< 0.01	0.34	0.01	0.48
Hue Angle, °	37.22	38.70	36.99	38.39	37.91	0.45	32.00^{c}	35.53^{b}	46.38^{a}	0.52	0.11	0.21	< 0.0001	0.08
Chroma	16.06	14.40	15.10	15.15	14.95	0.26	16.85^{a}	15.75^{b}	12.11^{c}	0.30	< 0.01	0.18	< 0.0001	0.09
Sensory														
Oxidized Flavor	3.47	5.24	4.62	5.46	5.01	0.42	4.09^{b}	4.33^{b}	6.83^{a}	0.33	< 0.01	0.54	< 0.001	0.86
Oxidized Odor	4.07	5.19	4.95	5.10	5.25	0.41	4.42^{b}	4.71^{b}	6.25^{a}	0.32	0.01	0.95	< 0.0001	0.98
Saltiness	1.42	5.14	5.05	5.50	4.99	0.36	4.81^{b}	5.17^{ab}	5.54^{a}	0.38	< 0.0001	0.56	0.02	0.91

^{abc}LS means within row under main effects lacking a common superscript are different ($P < 0.05$).

[1]Data are presented as least square means and reported SEM is the maxium SEM among treatments.

[2]*P*-value of single degree of freedom contrast comparing LS mean of No Salt (control) with LS mean of pooled salt treatments.

[3]*L** = Lightness, *a** = redness, *b** = yellowness, hue angle = $\tan^{-1}(b^*/a^*) \times 57.296$, chroma = $\sqrt{(a^{*2} \times b^{*2})}$

[4]Units were assigned by trained panelists using a 15 cm anchored, unstructured line scale where 0 = no oxidized flavor, oxidized odor, or salt flavor and 15 = extreme oxidized flavor, oxidized odor, or saltiness

Figures

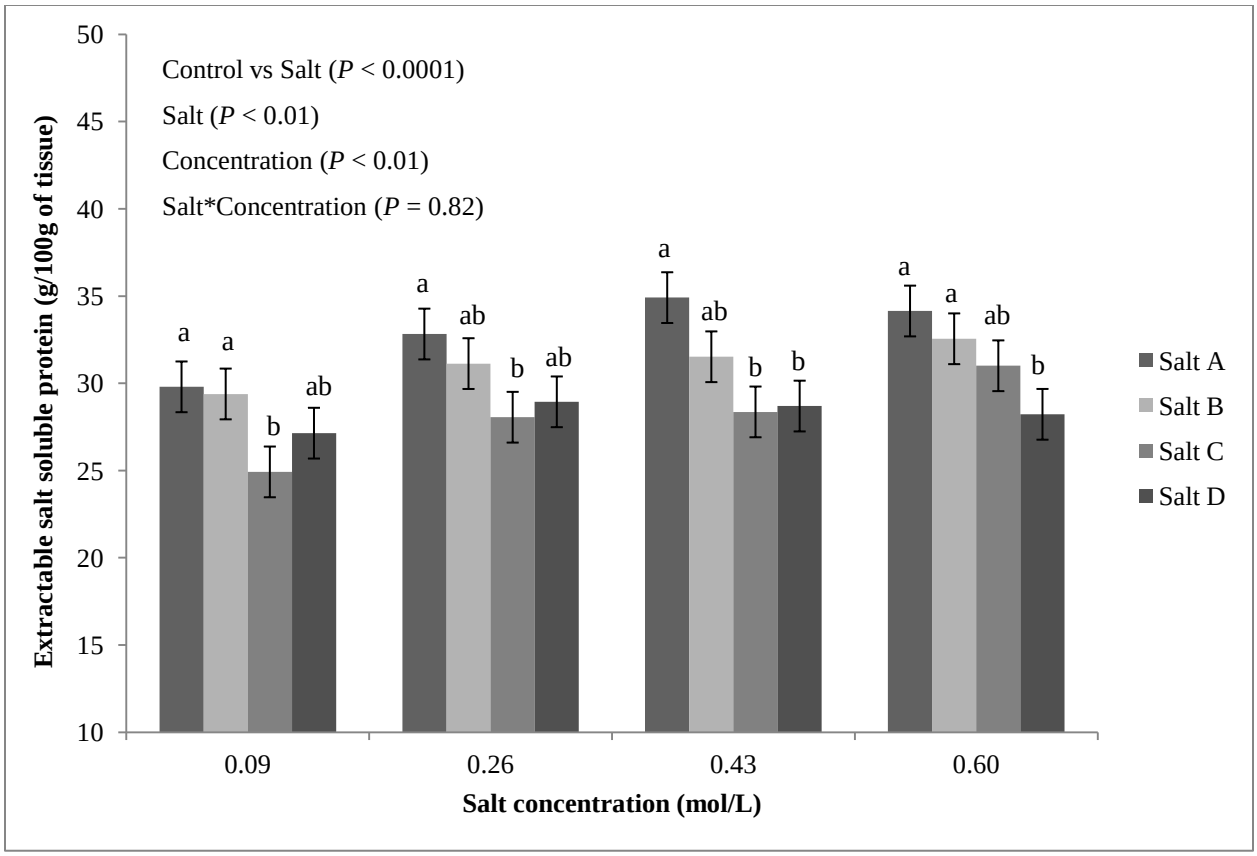

Figure 1. Effects of salt treatment and salt concentration on salt soluble protein extraction of fresh ground pork. Salt treatments within concentration not sharing a common superscript differ ($P < 0.05$). Inset displays *P*-values of single degree of freedom contrast comparing no salt with pooled salt treatments, fixed effects of salt treatment, salt concentration, and salt*concentration interaction.

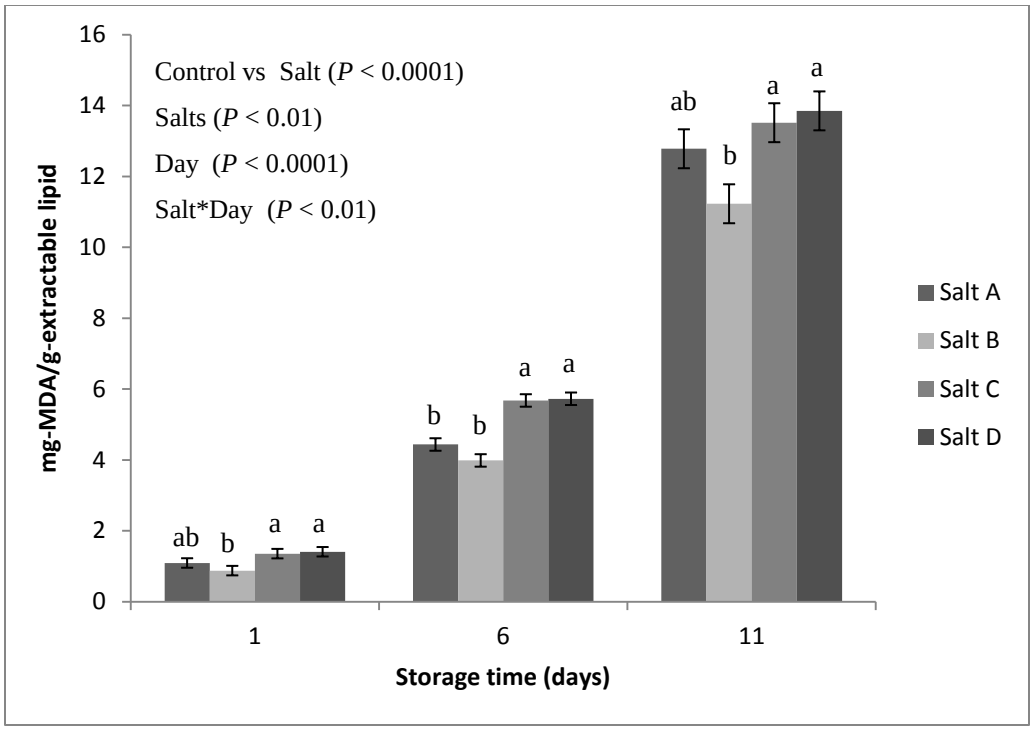

Figure 2. Effects of salt variety and storage time on lipid oxidation (TBARS) of fresh, ground pork patties stored for 1, 6, or 11 days. Salt treatments within storage time not sharing a common superscript differ ($P < 0.05$). Inset displays *P*-value of single degree of freedom contrast comparing no salt with the pooled salt treatments, fixed effects of salt treatment, storage time, and salt*storage time interaction.

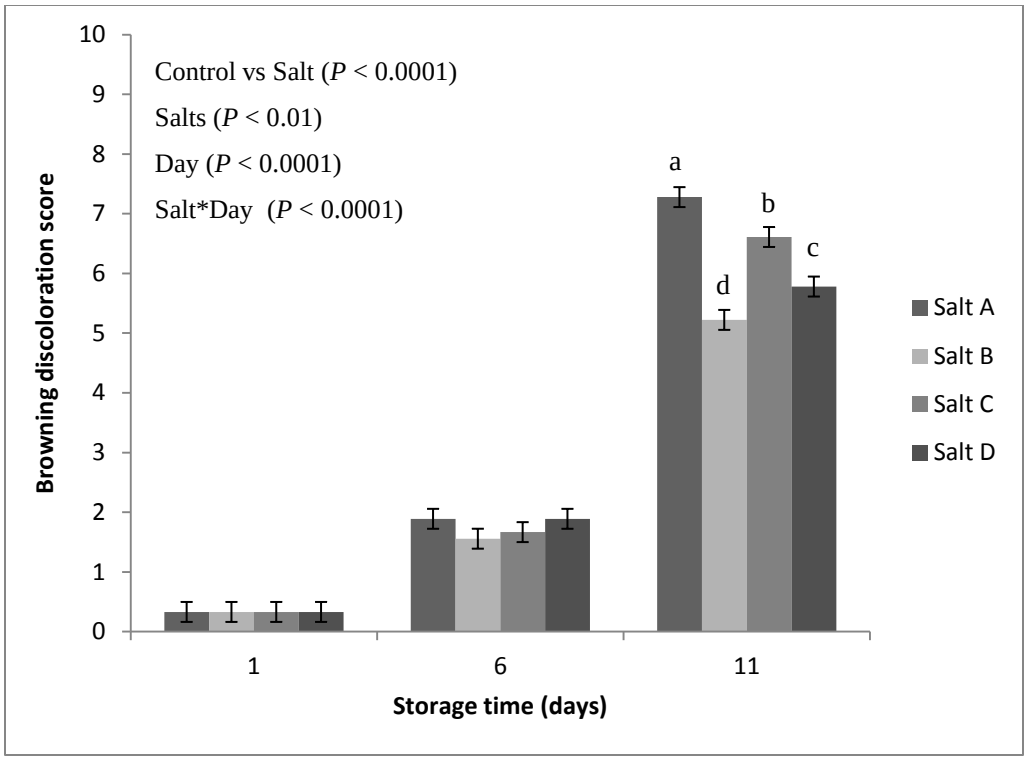

Figure 3. Effects of salt variety and storage time on brown discoloration of fresh, ground pork patties stored for 1, 6, or 11 days. Salt treatments within storage time not sharing a common superscript differ ($P < 0.05$). Inset displays *P*-value of single degree of freedom contrast comparing no salt with pooled salt treatments, fixed effects of salt treatment, storage time, and salt*storage time interaction.