INFLUENCE OF SALT CONCENTRATION AND SALT IONS ON NON-HEME IRON CONCENTRATION AND LIPID OXIDATION IN GROUND PORK

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BACKGROUND: Sodium chloride has been established as a prooxidant in meat (Kanner et al., 1991). However, its exact mechanism as a prooxidant is unclear. Kanner et al. (1991) proposed that NaCl enhances the activity of iron ions in lipid oxidation. They postulated that when free iron ions were added to ground turkey, a large portion of the added iron interacted with protein macromolecules. Sodium chloride interrupts the interaction between iron ions and protein macromolecules. Therefore, more free iron is available to interact with the lipid fraction and catalyze lipid oxidation. Alkali and alkali-earth halides salts (LiCl, NaCl, NaF, KCl, NaBr and NaI) have been examined for different catalytic activity in lipid oxidation in meat. The consensus in the literature regarding their relative catalytic activity in lipid oxidation indicates that extensive differences exist. It is not understood why these differences occur as the prooxidant effect of various salts is studied. It is also not clear whether these salts share the same prooxidant mechanism as NaCl, and if their catalytic effects on lipid oxidation are due to their varying ability to release iron ions.

OBJECTIVES: The first objective of this study was to examine the effect of NaCl concentration on lipid oxidation and non-heme iron release in ground pork. The second objective of this research was to study the effect of NaCl, KCl, NaBr and KBr on non-heme iron concentration and lipid oxidation.

EXPERIMENTAL METHODS: Pork (legs) from market hogs were obtained from a local meat company within 48 hr of slaughter. They were boned and skinned. All visible fat was removed and the pork was ground twice through a 9 mm and a 3 mm plate, respectively. For the NaCl concentration study, 150g ground pork and 15 ml of NaCl solutions of varying concentrations (1.65 M, 3.27 M or 4.91 M) were hand mixed using a spoon in a 250 ml beaker for 1.5 min at 4 °C to give target NaCl concentrations of 0.15 M, 0.30 M and 0.45 M NaCl in meat. For the control, 15 ml of distilled water were added to the pork and mixed in a similar manner. For the second study, 150 g ground pork were mixed with 15 ml of 1.65 M salt solutions (NaCl, KCl, NaBr and KBr) to reach a target of 0.15 M salt concentration in the meat. The control was handled as described above. In the cooked studies, 165 g samples were left in the 250 ml beaker after salt incorporation, covered with aluminum foil and a thermometer was inserted to the center of the meat to monitor the internal temperature during cooking. The samples were immersed in a 83 ~ 2 °C water bath and cooked to an internal temperature of 70 °C. Samples were subsequently stored in a refrigerator (4 °C). Lipid oxidation was monitored using both thiobarbituric acid-reactive substances (TBARS) and peroxide values methods. The TBARS measurement was based on the method of Tarladgis et al. (1960) as modified by Crackel et al. (1988), and TBARS were expressed as mg malonaldehyde/Kg sample. Peroxide values were measured according to Shantha and Decker (1994). Total iron fractions were prepared as described by Igene et al. (1979). Non-heme iron was determined according to the modified Schricker method (Rhee and Ziprin, 1987) and quantitated using atomic absorption spectroscopy. Lipid oxidation and non-heme iron were monitored on days 0, 3 and 6 for raw samples and days 0, 1 and 2 for cooked samples. Storage time designated as day 0 represents analyses immediately after the salt or distilled water was mixed with the meat.
The experiment utilized a split-plot design with repeated measurements (Gill, 1978). Means, standard errors, sum of squares, mean square errors and the least significant difference (LSD) test were calculated using the MSTAT-C microcomputer statistical program. Three replicates were conducted in each study.

RESULTS AND DISCUSSION: Effect of NaCl concentrations on TBARS and peroxide values. For the raw study, the addition of NaCl significantly ($p<0.05$) increased TBARS and peroxide values during the 6 day refrigerated storage (4 °C) period. The treatment without NaCl (control) produced almost constant values during the storage period. At day 0 there were no significant differences ($p<0.05$) in the extent of lipid oxidation between pork sample treatments monitored by both TBARS and peroxide values. After 3 days of refrigerated storage, increasing NaCl concentrations produced greater ($p<0.05$) TBARS values (0.45 M NaCl > 0.30 M > 0.15 M > control). These data are consistent with those reported by other researchers indicating that NaCl is a prooxidant. Trends established by peroxide value measurements were similar to those obtained by measuring TBARS. However, peroxide values are not as sensitive a measure of lipid oxidation as the TBARS in this study.

For cooked samples, increasing NaCl concentrations significantly ($p<0.05$) increased lipid oxidation as measured by both TBARS and peroxide values during two days of refrigerated storage. However, the most significant differences in the lipid oxidation between treatments occurred at the early stage of storage (day 0) rather than at the late stage of storage as in the raw studies (day 6). The NaCl concentration effect in TBARS and peroxide values are either less apparent or not significant between cooked treatments when compared to the raw treatments. This phenomenon is consistent with reports by other researchers who indicate that NaCl or other salts promote lipid oxidation in raw samples but may or may not in cooked samples. Several explanations for differences in data obtained by investigators regarding the effect of NaCl on lipid oxidation in cooked meat are possible. First, lipid oxidation is a free radical reaction and the initiation reaction is the rate-determining step. Once the reaction is initiated, the importance of the presence of the prooxidant may be reduced. Cooking can provide the energy to initiate lipid oxidation in meat. Although NaCl has a prooxidant effect by itself, the NaCl effect was confounded with cooking effects and the NaCl effect on lipid oxidation is less dramatic for cooked samples. Second, it is possible that heating and NaCl may have similar mechanisms for promoting lipid oxidation. For example, both NaCl and heating break down the meat microstructure and release iron ions. Therefore, the prooxidant effect of NaCl will be less significant after heating because the microstructure has already been broken down with or without NaCl addition. The exception may be when a high concentration of NaCl is used. A third possibility is attributed to the experimental error due to differences in the meat systems or species examined, complexity of the model system, and concentrations of salt used

(Srinivasan and Xiong, 1996).
Effect of NaCl on non-heme iron concentration. There were no differences in total iron concentrations within raw or cooked samples. This indicates that salt or other materials used in this study do not contribute to the total iron concentrations in the meat systems. In raw control samples (no salt), the trends of non-heme iron concentration increases were not significant during 6 days of refrigerated storage. In raw

samples containing salt, non-heme iron concentrations significantly increased during the refrigerated storage period after 6 days. There were no non-heme iron concentrations that were significantly ($p<0.05$) greater than the control after 3 days. In the cooked samples, NaCl increased non-heme iron concentrations in ground pork at day 0. However, only treatments with 0.30 M and 0.45 M NaCl were significantly ($p < 0.05$) greater than the control.

The relationship between lipid oxidation non-heme iron concentration and NaCl concentration. As NaCl concentrations increased in the cooked samples at day 0, the nonheme iron concentration increased and also measures of lipid oxidation increased proportionately. Greater NaCl concentrations resulted in greater lipid oxidation and nonheme iron concentrations. The cooked study data support the hypothesis that the prooxidant mechanism of NaCl is to increase the availability of iron to catalyze lipid oxidation in meat. In the raw study, increasing NaCl concentrations produced significantly higher TBARS and peroxide values than the control after 3 days of refrigerated storage. However, NaCl appeared to only affect non-heme iron concentrations in samples stored for 6 days at 4 °C. Based on our hypothesis, it was expected that there would be differences in the non-heme iron concentrations between treatments before or at the same time a significant difference in lipid oxidation was detected.

Although TBARS and peroxide values were significantly different between raw treatments before significant differences in non-heme iron concentrations were detected, the hypothesis may be valid because it is unknown how large a difference in the non-heme iron concentration is needed to observe significant changes in lipid oxidation. Treatments with higher NaCl concentrations generally had greater non-heme concentrations compared to the control (although the difference was not significant). Differences of less than 1 ppm iron were not detected due to the level of sensitivity of the method.

The effect of various salts on lipid oxidation and non-heme iron concentration in pork. In the raw study, there were no differences in lipid oxidation monitored by TBARS and peroxide values as well as non-heme iron concentration between various salt treatments at day 0. After day 3, lipid oxidation in treatments with the various salts (NaCl, KC1, NaBr and KBr) was significantly ($p<0.05$) higher than the control when monitored by TBARS. However, only NaC1 and NaBr treatments were significantly ($p<0.05$) higher than the control when monitored by the peroxide values. For the corresponding non-heme iron concentrations at day 3, only the 0.15 M NaC1 treatment was significantly different from the control. The other three salt treatments had higher, but not significant, non-heme iron concentrations than the control. There were no significant differences in TBARS and nonheme iron concentrations between various salt treatments. In general, these data demonstrated that salts (KC1, NaBr and KBr) other than NaC1 can also promote lipid oxidation and increase the non-heme iron concentrations in meat systems. However, the differences in prooxidant activity and non-heme iron concentration were not significant between these various salts. For cooked samples, various salt treatments had higher TBARS and peroxide values than the control at day 0. After 1 day of refrigerated storage, the differences in lipid oxidation between control and various salt treatments were not significant. However, all TBARS values were above 3.5 and all peroxide values were above 8.0. The salt effect was less significant in the cooked samples compared to the raw samples as was discussed previously. The corresponding non-heme iron data at day 0, treatments with various salts had a trend of higher non-heme iron concentrations than the control. However, only the KBr treatment was significant ($p<0.05$). After 2 days of refrigerated storage, NaCl, KCl and KBr treatments had significantly higher non-heme iron concentrations than control.

SUMMARY AND CONCLUSIONS: The addition of NaCl increased lipid oxidation in both raw and cooked samples as well as non-heme iron concentrations. The corresponding non-heme iron analyses indicated that non-heme iron concentration increased significantly immediately for cooked samples and after 6 days of storage for raw samples. Salts (KCl, NaBr and KBr), other than NaCl, also had similar effects as NaCl with respect to lipid oxidation and non-heme iron concentration. Based upon data presented, it is possible that the prooxidant effect of salt is to make more iron available to catalyze lipid oxidation. The results also suggest that more than one prooxidant mechanism is responsible for lipid oxidation in raw pork.

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