

RESEARCH ARTICLE

How does reducing sodium impact the proteolysis and texture in salted meat along 180 days of shelf life?

Vitor Andre Silva Vidal^{1*}, Fabiane Manke Nachtigall², Andressa Baseggio¹,
Mario Roberto Marostica Junior¹, Leonardo Silva Santos³, Marise Aparecida Rodrigues Pollonio¹

¹University of Campinas, Faculty of Food Engineering, 13083-862, Campinas, São Paulo, Brazil, ²Instituto de Ciências Químicas Aplicadas, Universidad Autónoma de Chile, 3467987, Talca, Chile, ³Instituto de Química de Recursos Naturales, Universidad de Talca, 3462227, Talca, Chile

ABSTRACT

Sodium reduction in salted meat is a major technological challenge due to the technological functions of NaCl, responsible for the reduction of water activity and consequent microbiological safety, stability during shelf life, sensory characteristics, proteolysis and lipolysis. The objective of this research is to evaluate the effects of partial replacement of NaCl by KCl and CaCl₂ in the texture properties during 180 days of storage in salted meat. During 180 days of storage, a significant decrease ($P < 0.05$) of shear force values have been observed in all treatments. The electrophoretic images suggest that CaCl₂ can promote denaturation of high molecular weight proteins resulting in lower molecular weight proteins. The results showed the intense effects of CaCl₂ on texture and proteolysis reactions and the blend NaCl + KCl produced a similar impact to salted meat product compared to control treatment (100% NaCl).

Keywords: Calcium chloride; Potassium chloride; Proteolysis; Salted meat; Sodium reduction

INTRODUCTION

The salted meat is widely consumed worldwide due to its unique sensorial characteristics besides its relevance in regions where the cold chain is not fully established (Ishihara and Madruga, 2013). The elaboration of these products is based on hurdle technology, using mainly dehydration by salt and vacuum packaging (Leistner, 1987; Vidal et al., 2019a). Thereby, the growth of microorganisms is inhibited and the product is stable for up to 180 days at room temperature.

The excessive intake of sodium is associated with several health problems such as increasing the of risk developing hypertension and cardiovascular diseases besides certain types of cancer (Pires et al., 2017). Although the salted meat has the technological benefits already mentioned, the salting used to elaborate the product provides a high sodium content, needing a desalting process for consumption. As this procedure is not always standardized by consumer due to domestic procedures, the partial

substitution of NaCl as the main component of the salting steps by other chloride salts is an excellent strategy to effectively guarantee sodium reduction in this category of products (Vidal et al., 2020a).

Proteolysis is a result of the action of microbial and tissues enzymes, which is affected by water activity, processing conditions, and mainly salt levels. Salt regulates the activity of proteolytic enzymes, and the reduction of NaCl can activate muscular proteases, being able to increase the proteolytic activity and consequent release of free amino acids (Toldrá et al., 1992). Thus, to understand the effect of NaCl reduction and/or substitution by other salts on the proteolysis is one of the main problems to be approached and explained.

In this context, the objective of this study was evaluated the effect of NaCl replacement by KCl and CaCl₂ blends on texture properties by comparing electrophoretic profile and shear force analysis of salted meat, aiming to understand the effect of different salts.

*Corresponding author:

Vitor Andre Silva Vidal, University of Campinas, Faculty of Food Engineering, 13083-862, Campinas, São Paulo, Brazil. Tel: (+55) 19 98435-8853. E-mail: vitorandrevidal@gmail.com

Received: 21 June 2020; Accepted: 29 August 2020

MATERIALS AND METHODS

Treatments, material and additives

The additives sodium nitrite and sodium erythorbate were donated by the company Kerry of Brazil. The NaCl, KCl and CaCl₂ salts added in the treatments are food grade (Anidrol, Brazil). The selected meat cut (bovine raw meat) was the *biceps femoris* obtained from slaughterhouses with assured sanitary quality at Campinas, São Paulo, Brazil.

Salted meat processing

Four treatments of salted meat were performed. The NaCl replacement by KCl and CaCl₂ was based on ionic strength, obtaining the same ionic strength in all treatments. The additive sodium nitrite and sodium erythorbate was added at fixed content in the wet step. The variable was the salt used during the wet and dry salting steps (as described at Table 1).

The salted meat processing was performed according to Vidal et al. (2019b). In wet salting step, the meat pieces of each treatment were submerged in saturated solution with respective salts and 0.015% of sodium nitrite and 0.05% of sodium erythorbate for 1 hour at 13 °C.

For dry salting step, a chamber at 13 °C was used for a period of 144 hours (6 days), putting the treatments in contact with respective salts. The ripening step was carried out in a controlled climatic chamber (Instala Frio, Paraná, Brazil) with 55% humidity, 25 °C and 0.5 m/s forced ventilation for 24 hours. After the process, the final products were vacuum packed in polyethylene bags (Spel, São Paulo, Brazil) and stored at room temperature. (25 °C).

Desalting, cooking and shear force

The desalting and cooking procedures were conducted according to Vidal et al. (2020b). Cooking was done in a water bath (RSA-1708, RSA, Campinas, Brazil) at 80 °C.

The desalted and cooked samples were stored under refrigeration for 24 hours prior to the analysis of Warner-Bratzler shear force (Stock and Board, 1995). The shear force was measured in a TA-XT 2i texture analyzer (Texture Technologies Corporation / Stable Micro Systems, Hamilton, UK). For Warner-Bratzler shear force analysis,

the distance to the platform was 25 mm and the ascent/descent speed was 200 mm/min. The analysis of Warner-Bratzler shear force was performed in six replicates for each replicate process. The shear force was measured at 0, 45, 90, 135 and 180 days of storage. The analysis was performed in six replicates in each process replicate.

Myofibrillar and sarcoplasmic proteins extraction

The sarcoplasmic and myofibrillar proteins extraction were performed according to the methodology described by Hughes et al. (2002), with some modifications.. Accordingly, 5 g of sample were homogenized with 35 mL (phosphate buffer 0.03 mol/L, pH 7.4) for 2 min at 13500 rpm at 4 °C using an Ultra-Turrax T25 (IKA, Leicestershire, UK). Then, the sample was centrifuged at 10000 g for 20 minutes at 4 °C to extract the sarcoplasmic protein. The process described above was repeated twice to remove any remaining sarcoplasmic proteins. After the washes, the resulting pellet was homogenized with a 25 mL solution of 8 mol/L urea and 1% β-mercaptoethanol for 2 min at 13500 rpm at 4 °C using an Ultra-Turrax T25 (IKA, Leicestershire, UK), followed by centrifugation at 10000 g for 20 minutes at 4 °C. The concentration of myofibrillar and sarcoplasmic proteins were determined by the method of Bradford (1976). Samples at 0, 45, 90, 135 and 180 days of storage were evaluated.

SDS-PAGE electrophoresis

To evaluate the proteolysis, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli (1970) using a Mini Protean II apparatus (Bio Rad, California, USA) and 8% and 17.5% polyacrylamide gradient gels. The extracted sarcoplasmic and myofibrillar proteins with 4 mg protein/mL were dissolved in a sample buffer (2% SDS and 5% β-mercaptoethanol) and heated at 96 °C for 10 min. An aliquot of 10 μL was loaded onto the gels and the electrophoresis conditions were 10 mA/gel and 250 V. The gels were stained using 0.1% Coomassie Blue, and destained in a solution of acetic acid: methanol: distilled water (1:4:5). A 10,000 to 250,000 relative molecular masses (Dalton) marker kit (Precision Plus Protein Kaleidoscope Standards, Biorad, California, USA) was used as the standard. The molecular weights of the products of proteolysis were estimated based on the relative mobility of the protein standards.

Statistical analysis

The process was performed in three replicates on different days using the same formulation, methodology and technology. For each process, at least three samples were taken for each analysis. The general linear model (GLM) considering the treatments as a fixed effect and the replicates as a random effect using 5% of significance

Table 1: Salts used to performed the salted meat treatments

| Treatments | NaCl (%) | NaCl (mg)* | KCl (%) | KCl (mg)* | CaCl ₂ (%) | CaCl ₂ (mg)* |
|------------|----------|------------|---------|-----------|-----------------------|-------------------------|
| FC1 | 100 | 1000 | - | - | - | - |
| F1 | 50 | 441 | 50 | 560 | - | - |
| F2 | 50 | 614 | - | - | 50 | 387 |
| F3 | 50 | 513 | 25 | 326 | 25 | 162 |

The amount of salt added was based on the ionic strength, all treatments obtained the same ionic strength. * Salt proportion added according to ionic strength, for each 1000mg of bovine raw meat was utilized 2000mg of salt

was used. The Tukey's test at the 5% level of significance was utilized to analyze significant differences. The GLM and Tukey's test were performed using commercial software Statistica v. 8 (Statsoft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Shear force determination

The high content of salts applied in the salting steps directly change texture characteristics due to modification of the solubility of myofibrillar proteins and swelling of muscle fibers (Offer et al., 1989), being this change related to effects on water retention and binding capacity by different concentration of salt and pH (Offer and Trinick, 1983; Oliveira et al., 2012).

In this study, the impact of using different salts on texture can be analyzed by the values of shear force (N) presented in Table 2. At day 0, the treatments containing NaCl (FC1: 100% NaCl) and NaCl + KCl (F1: 50% NaCl + 50% KCl) differed ($P < 0.05$) from the treatments containing CaCl_2 (F2: 50% NaCl + CaCl_2 and F3: 50% NaCl + 25% KCl + 25% CaCl_2) for this texture parameter. During

Table 2: Shear force (N) values in salted meat treatments during storage

| Treatments | 0 day | 45 days | 90 days | 135 days | 180 days |
|----------------|--------------------|---------------------|----------------------|----------------------|--------------------|
| FC1 | 39.6 ^{bA} | 38.7 ^{bAB} | 37.9 ^{bAB} | 31.3 ^{bcBC} | 27.5 ^{bC} |
| F1 | 41.9 ^{bA} | 37.5 ^{bAB} | 35.6 ^{bABC} | 30.9 ^{bcBC} | 28.2 ^{bC} |
| F2 | 54.2 ^{aA} | 52.9 ^{aA} | 51.6 ^{aA} | 39.9 ^{abB} | 40.5 ^{aB} |
| F3 | 58.8 ^{aA} | 48.7 ^{aBC} | 50.8 ^{aAB} | 42.0 ^{aBC} | 38.5 ^{aC} |
| Standard error | 1.6 | 1.1 | 1.4 | 1.9 | 2.2 |

Values are means. ^{a,b,c,d} Values in the same column with the same lowercase letters do not differ significantly ($P < 0.05$) according to Tukey's test. ^{A,B,C,D} Values in the same line with the same capital letters do not differ significantly ($P < 0.05$) according to Tukey's test. FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% CaCl_2 ; F3: 50% NaCl + 25% KCl + 25% CaCl_2

180 days of storage, a significant decrease ($P < 0.05$) of shear force values have been observed in all treatments. It can be explained, possibly due to the intense proteolytic reactions promoted by the different salts. On the other hand, treatments containing CaCl_2 (F2: 50% NaCl + 50% CaCl_2 and F3: 50% NaCl + 25% KCl + 25% CaCl_2) had the highest shear force values during 180 days of storage in relation to treatments containing NaCl (FC1: 100% NaCl) or NaCl + KCl (F1: 50% NaCl + 50% KCl). These results may have obtained due to the higher dehydrating effect of CaCl_2 in relation to NaCl and KCl. Many authors have described an increase in the rate of dehydration using calcium salts in various foods (Lewicki et al., 2002; Mastrantonio et al., 2005; Pereira et al., 2007) which directly affect the texture characteristics (Aliño et al., 2010; Offer et al., 1989; Offer and Trinick, 1983; Oliveira et al., 2012). Along the storage, the treatments with CaCl_2 in blend salt composition, shown a hard surface layer resulted by fast and higher rate of water released.

SDS-PAGE electrophoresis

Electrophoresis gels containing the profile of myofibrillar proteins can be seen in (Fig. 1) and sarcoplasmic proteins in (Fig. 2) The average intensities of the main bands in (Fig. 1) corresponding to myosin heavy chain (~205 kDa), C-protein (~137 kDa), actin (~43 kDa), tropomyosin (~36 kDa) and myosin light chain (~24 kDa) (dos Santos et al., 2015). Analyzing the electrophoretic profile of myofibrillar proteins of salted meat treatments with different salts, is possible to verify the disappearance of some bands of high molecular weight proteins during the storage simultaneously in all treatments. However, after 135 days, treatments containing CaCl_2 (F2: 50% NaCl + 50% CaCl_2 e F3: 50% NaCl + 25% KCl + 25% CaCl_2) decreased the intensity of bands with molecular weights

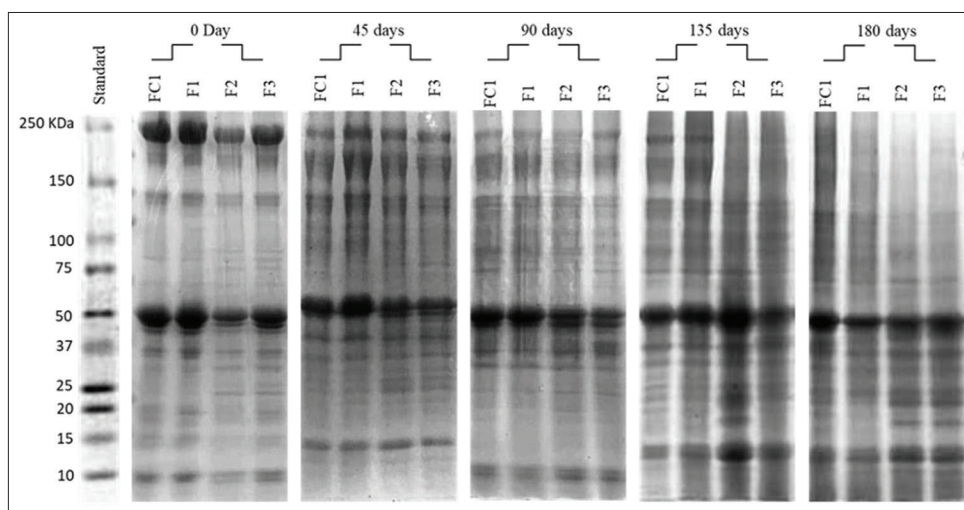


Fig 1. 8 and 17.5% SDS-PAGE gels of myofibrillar proteins in the salted meat treatments. Standards: BioRad molecular weight standards (Da: 10000 to 250000). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% CaCl_2 ; F3: 50% NaCl + 25% KCl + 25% CaCl_2

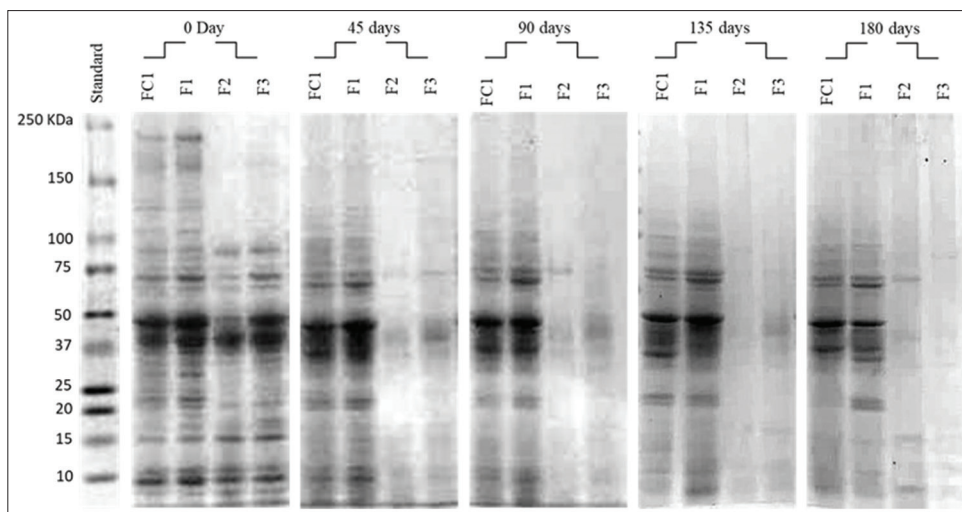


Fig 2. 8 and 17.5% SDS-PAGE gels of sarcoplasmic proteins in the salted meat treatments. Standards: BioRad molecular weight standards (Da:10000 to 250000). FC1: 100% NaCl; F1: 50% NaCl + 50% KCl; F2: 50% NaCl + 50% CaCl₂; F3: 50% NaCl + 25% KCl + 25% CaCl₂

greater than 100 kDa as myosin heavy chain and C-protein and increased the intensity of bands with lower molecular weights such as actin, tropomyosin and myosin light chain. This suggests that CaCl₂ can promote denaturation of high molecular weight proteins resulting in lower molecular weight proteins.

Observing the (Fig. 2) there was a significant change in the electrophoretic profile of sarcoplasmic proteins during storage of salted meat treatments with the addition of CaCl₂ (F2: 50% NaCl + 50% CaCl₂ and F3: 50% NaCl + 25% KCl + 25% CaCl₂), promoting the disappearance of several bands compared to treatments FC1 (100% NaCl) and F1 (50% NaCl + 50% KCl). It can be noted that after 45 days of storage, CaCl₂ caused an intense proteolysis of sarcoplasmic proteins of all weights. According to Lorenzo et al. (2015), salt type influences chemical and biochemical reactions such as proteolysis.

In addition, the electrophoretic profile both myofibrillar and sarcoplasmic proteins of treatment containing 100% NaCl (FC1) were similar to the treatment containing partial replacement of NaCl by KCl (F1: 50% NaCl + 50% KCl).

CONCLUSION

The use of CaCl₂ to elaborated salt blends aiming to reduce sodium in salted meat intensely affected texture and proteolysis reactions during 180 days of storage. In contrast, the blend containing NaCl + KCl had a similar impact to salted meat samples compared to NaCl control treatment, being a promising alternative for reducing or replacing NaCl in salted meat products without impact in texture properties.

ACKNOWLEDGMENT

The authors would like to thank National Council for Scientific and Technological Development (CNPq, Brazil) (process numbers: 140533/2015-0 and 406671/2016-8) for the financial support.

Authors' contributions

Conceptualization and funding acquisition, M.A.R.P.; investigation, V.A.S.V.; methodology, V.A.S.V., F.M.N., A.B.; resources, M.R.M.J, L.S.S.; writing-review and editing, V.A.S.V., M.A.R.P.

REFERENCES

- Aliño, M., R. Grau, A. Fuentes and J. M. Barat. 2010. Influence of low-sodium mixtures of salts on the post-salting stage of dry-cured ham process. *J. Food Eng.* 99: 198-205.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- dos Santos, B. A., P. C. B. Campagnol, R. N. Cavalcanti, M. T. B. Pacheco, F. M. Netto, E. M. P. Motta, R. M. S. Celeguini, R. Wagner and M. A. R. Pollonio. 2015. Impact of sodium chloride replacement by salt substitutes on the proteolysis and rheological properties of dry fermented sausages. *J. Food Eng.* 151: 16-24.
- Hughes, M. C., J. P. Kerry, E. K. Arendt, P. M. Kenneally, P. L. H. McSweeney and E. E. O'Neill. 2002. Characterization of proteolysis during the ripening of semi-dry fermented sausages. *Meat Sci.* 62: 205-216.
- Ishihara, Y. M. and M. S. Madruga. 2013. Indicadores de maciez em carnes salgadas e dessecadas: Uma revisão. *Ciênc. Agrár.* 34: 3721-3738.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227: 680.
- Leistner, L. 1987. Shelf stable product and intermediate moisture foods based on meat. In: L. Rockland and L. B. Beauchat (Eds.),

- Water Activity Theory and Application to Food. Marcel Dekker Inc., New York. p295-328.
- Lewicki, P. P., H. Vu Le and W. Pomarańska-Lazuka. 2002. Effect of pre-treatment on convective drying of tomatoes. *J. Food Eng.* 54: 141-146.
- Lorenzo, J. M., A. Cittadini, R. Bermúdez, P. E. Munekata and R. Domínguez. 2015. Influence of partial replacement of NaCl with KCl, CaCl₂ and MgCl₂ on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. *Food Control.* 55: 90-96.
- Mastrantonio, S. D. S., L. M. Pereira and M. D. Hubinger. 2005. Osmotic dehydration kinetics of guavas in maltose solutions with calcium salt. *Alim. Nutr.* 16: 309-314.
- Offer, G. and J. Trinick. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Sci.* 8: 245-281.
- Offer, G., P. Knight, R. Jeacocke, R. Almond, T. Cousins, J. Elsey, N. Parsons, A. Sharp, R. Starr and P. Purslow. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Struct.* 8: 17.
- Oliveira, H, S. Pedro, M. L. Nunes, R. Costa and P. Vaz-Pires. 2012. Processing of salted cod (*Gadus* spp.): A review. *Compr. Rev. Food Sci. Food Saf.* 11: 546-564.
- Pereira, L. M., S. M. Carmello-Guerreiro, H. M. Bolini, R. L. Cunha and M. D. Hubinger. 2007. Effect of calcium salts on the texture, structure and sensory acceptance of osmotically dehydrated guavas. *J. Sci. Food Agric.* 87: 1149-1156.
- Pires, M.A., P. E. S. Munekata, J. C. Baldin, Y. J. P. Rocha, T. Carvalho, I. R. dos Santos, J. C. Barros and M. A. Trindade. 2017. The effect of sodium reduction on the microstructure, texture and sensory acceptance of Bologna sausage. *Food Struct.* 14: 1-7.
- Stock, N. L. and M. Board. 1995. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat. American Meat Science Association, Savoy, Illinois, United States.
- Toldrá, F., E. Rico and J. Flores. 1992. Activities of pork muscle proteases in model cured meat systems. *Biochimie.* 74: 291-296.
- Vidal, V. A. S., O. D. Bernardinelli, C. S. Paglarini, E. Sabadini and M. A. R. Pollonio. 2019a. Understanding the effect of different chloride salts on the water behavior in the salted meat matrix along 180 days of shelf life. *Food Res. Int.* 125: 108634.
- Vidal, V. A. S., J. M. M. Lorenzo, P. E. S. Munekata and M. A. R. Pollonio. 2020a. Challenges to reduce or replace NaCl by chloride salts in meat products made from whole pieces a review. *Crit. Rev. Food Sci. Nutr.* 4: 1-13.
- Vidal, V. A. S., J. P. Biachi, C. S. Paglarini, M. B. Pinton, P. C. B. Campagnol, E. A. Esmerino, A. G. Cruz, M. A. Morgano and M. A. R. Pollonio. 2019b. Reducing 50% sodium chloride in healthier jerked beef: An efficient design to ensure suitable stability, technological and sensory properties. *Meat Sci.* 152: 49-57.
- Vidal, V. A. S., J. B. Santana, C. S. Paglarini, M. A. A. da Silva, M. Freitas, E. A. Esmerino, A. G. Cruz and M. A. R. Pollonio. 2020b. Adding lysine and yeast extract improves sensory properties of low sodium salted meat. *Meat Sci.* 159: 107911.