Effect of sodium, potassium and calcium chloride on proteolysis in dry fermented sausages

Dos Santos, B. A.[1], Campagnol, P. C. B.[2], Wagner R.[3] and Pollonio M.A.R.[1]

[1] Department of Food Technology, Faculty of Food Engineering, University of Campinas, Brazil

[2] Federal Institute of Triângulo Mineiro, Brazil

[3] Department of Food Science and Technology, Federal University of Santa Maria, Brazil

The reduction of added NaCl in fermented meat products may cause excessive proteolysis. The NaCl contents in dry fermented sausages were reduced by 50% or were substituted by KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1). The proteolysis was evaluated during the ripening period (0, 7 and 19 days), using SDS-PAGE gradient gels (8 to 17.5%). The results showed that the reduction or replacement of NaCl by other chloride salts did not affect the proteolytic reactions of myofibrillar and sarcoplasmic proteins in the product. Thus, with respect to the proteolysis, the study showed that the reduction or replacement of NaCl by KCl and / or $CaCl_2$ did not alter the protein degradation during the ripening period of dry fermented sausages.

I. INTRODUCTION

Sodium chloride (NaCl) is the main source of sodium in dry fermented sausages, and therefore, in order to obtain healthier products, this ingredient must be eliminated or reduced. However, tackling this is a huge challenge, since in addition to being a low cost ingredient, NaCl significantly affects technological quality.

Proteolysis reactions constitute an important biochemical mechanism that occurs during the fermentation and maturation of fermented meat products, which significantly influences the final quality of the product. The main products of proteolysis are small peptides and free amino acids that can contribute to the flavor and texture, as well as products derived from free amino acids resulting from enzymatic and non-enzymatic reactions [1,2]. Among the factors that influence the proteolysis are the characteristics of the raw material, age of animals, product formulation, including salt content and type of starter culture [1,2], processing technology, temperature and ripening period. Considering the current need to reduce sodium levels in fermented meat products, the purpose of this study was to evaluate the effect of salt substitutes (KCl and $CaCl_2$) on the proteolysis of low-sodium dry fermented sausages.

II. MATERIALS AND METHODS

Treatments

Dry fermented sausages with 50% of their NaCl content reduced or substituted by KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1) were produced according to Table 1.

Table 1. Levels of sodium chloride, potassium chloride, and calcium chloride used in dry fermented sausage formulations.

	Treatments (%)				
	Control	F1	F2	F3	F4
NaCl	2.5	1.25	1.25	1.25	1.25
KCl	-	-	1.25	-	0.625
$CaCl_2$	-	-	-	1.25	0.625

* Control- 100% NaCl, F1- 50% NaCl, F2- 50% NaCl and 50% KCl, F3- 50% NaCl and 50% $CaCl_2$, F4- 50% NaCl, 25% KCl and 25% $CaCl_2$.

Dry fermented sausages processing

The dry fermented sausages were produced using the following main ingredients: pork meat beef and pork back fat. The raw material was ground with a disk (8 mm) and mixed with the correct amount of NaCl and other ingredients for each treatment described in table 1. The following ingredients were added to the meat mixture in each treatment: glucose (5 g/kg), sucrose (5 g/kg), sodium nitrate (0.15 g/kg), sodium nitrite (0.15 g/kg), sodium ascorbate (0.25 g/kg), white pepper (2 g/kg), garlic (3 g/kg), nutmeg (0.02 g/kg) and starter culture (0.25 g/kg; SPX Floracarn, Chr Hansen). Artificial collagen casings, 60 mm in diameter, were stuffed with the dry fermented sausage meat and cut into pieces of approximately 25 cm in length, with a total of approximately 60 pieces of 300 g per treatment. After the sausages were formed, each one was immersed in a 20%

potassium sorbate solution and placed in a climatized chamber, at a controlled temperature and relative humidity, where they remained for 19 days, during which period, the water activity of the control remained below 0.900. The temperature and relative humidity (Tº/UR%) were set as follows: first day, temperature 25°C/95%; second day, 24°C/93%, third day, 23°C/90%, fourth day, 22°C/85%, fifth day, 21°C/80%, sixth day, 20°C/75%, and from the seventh day though the nineteenth day, 18°C/75%. The air speed remained at 5 m/s throughout the processing.

Determination of proteolysis:

The proteolysis in dry fermented sausages was determined throughout the ripening period (0, 7 and 19 days), in triplicate. Myofibrillar and sarcoplasmic proteins were extracted as reported by Diaz [3] and quantified according to Bradford [4], and sodium dodecyl sulphate polyacrylamine gel electrophoresis (SDS-PAGE) was performed under reducing conditions [5] using a Mini Protean II apparatus (Bio Rad, CA, USA) and 8 and 17.5% polyacrylamide gradient gels. The samples (4 mg/mL) were prepared in a buffer (2% SDS and 5% b-mercaptoethanol) and subsequently boiled for 10 min at 96 °C. An aliquot of 10 µL (4 mg of protein/mL) was applied to each well. The gels were stained with 0.1% Coomassie Blue. Destaining was performed in acetic acid: methanol: distilled water (1:4:5). A 10 a 250 kDa molecular mass (MM) marker kit (Precision Plus Protein Kaleidoscope Standards, Biorad, USA) was used as standard.

Data analysis

Three independent manufacturing processes were Carried out with the same formulation and technology. In each manufacture, three sample units (dry fermented sausage) were taken per sampling day (n = 9). The analyzes of SDS-PAGE gels were performed by triplicate. The choice of SDS-PAGE gels for the preparation of the figures presented were chosen based on the best resolution of bands of myofibrillar and sarcoplasmic proteins. The molecular weights of the products of proteolysis were estimated by reference to the relative mobilities of standard proteins [6].

III. RESULTS AND DISCUSSION

Myofibrillar and sarcoplasmic proteins were extracted from dry fermented sausages with 50% reduced salt and / or replaced by KCl and / or $CaCl_2$. Figures 1 and 2 show that in general there was no difference in the behavior of myofibrillar and sarcoplasmic proteins throughout the ripening period evidencing that the reduction of NaCl and the addition of KCl and $CaCl_2$ did not affect the electrophoretic profile of the dry fermented sausages. Figure 1 shows a severe degradation of the myofibrillar protein myosin (200KDa) and actin (45kDa) during the ripening period, for all treatments, evidencing a typical proteolytic activity of fermented meat products resulting from the release of polypeptides and free amino acids [7]. During the process, low molecular weight proteins were observed in all treatments. Sun et al [7] have reported that the degradation of high molecular weight proteins may be due to either the proteolysis or ripening period, which causes a reduction in pH and salt concentration, and salami dehydration. The bands between 15-100 kDa indicate the presence of the sarcoplasmic proteins in dry fermented sausages over the ripening period (Fig. 2). As occurred with the myofibrillar proteins, with a decrease in pH, polypeptides of molecular weight between 37-70kDa and 10-30kDa were observed. This behavior remained until the end of processing (19 days).

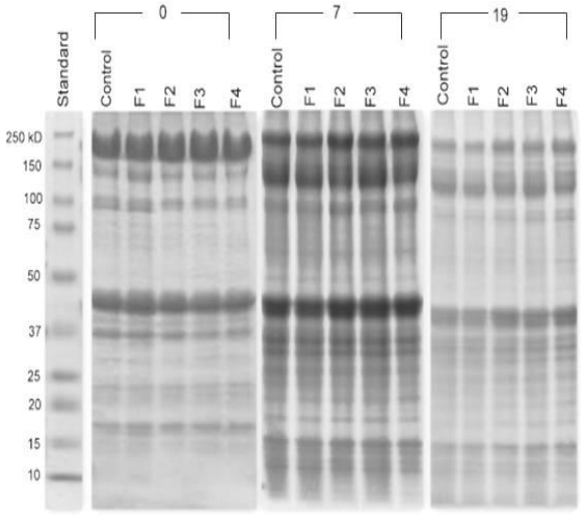

Figure 1: 8 and 17.5% SDS-PAGE gels of myofibrillar proteins in the dry fermented sausage submitted to five types of salt treatments. Standards: BioRad molecular weight standards. Treatments as described in Table1.

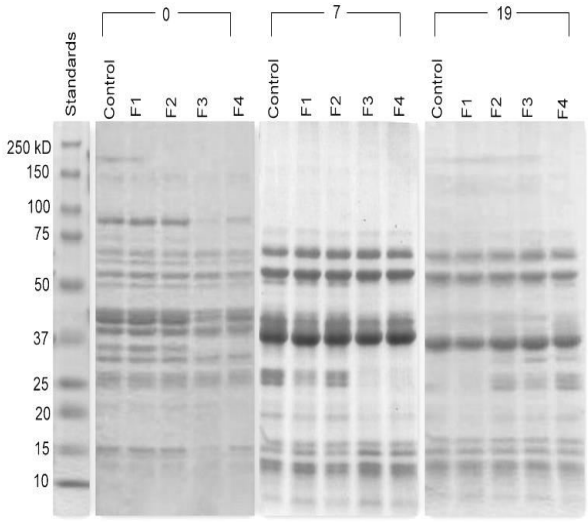

Figure 2: 8 and 17.5% SDS-PAGE gels of sarcoplasmic proteins in the dry fermented sausage submitted to five types of salt treatments. Standards: BioRad molecular weight standards. Treatments as described inTable1.

IV. CONCLUSION

This study indicates that the reduction of NaCl and the addition of chloride salts such as $CaCl_2$ and KCl did not affect the proteolysis of dry fermented sausages during the ripening period.

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