Journal of Food Engineering 151 (2015) 16–24

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Impact of sodium chloride replacement by salt substitutes on the proteolysis and rheological properties of dry fermented sausages

Bibiana A. dos Santos [a,*], Paulo C.B. Campagnol [b], Rodrigo N. Cavalcanti [a], Maria T.B. Pacheco [c], Flávia M. Netto [a], Eliana M.P. Motta [a], Renata M.S. Celeguini [a], Roger Wagner [d], Marise A.R. Pollonio [a]

[a] Universidade Estadual de Campinas, CEP 13083-862, Campinas, São Paulo, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, CEP 38064-300, Uberaba, Minas Gerais, Brazil
[c] Instituto de Tecnolgia de Alimentos, CEP CEP 13070-178, Campinas, São Paulo, Brazil
[d] Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil

ARTICLE INFO

Article history:
Received 2 August 2014
Received in revised form 27 October 2014
Accepted 2 November 2014
Available online 3 December 2014

Keywords:
Proteolysis
Free amino acids
Rheology
Sodium chloride
Potassium chloride
Calcium chloride

ABSTRACT

The effect of a 50% reduction of NaCl and its replacement by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ (1:1) on the proteolysis and rheological properties of dry fermented sausages was investigated. The reduction or replacement of NaCl by KCl did not cause changes in the electrophoretic profile and the addition of $CaCl_2$ decreased the degradation of sarcoplasmic proteins during the manufacturing process. Samples with a 50% reduction of NaCl showed a higher content of the amino acids Arg, Glu, His, Val, Cys, Lys, and Trp, whereas the samples containing $CaCl_2$ had a higher content of the amino acids Asp, Thr, Ala, Met, Leu, Ile, and Phe. The reduction or replacement of NaCl by KCl decreased sample firmness, whereas the addition of $CaCl_2$ increased the hardness of the samples.

1. Introduction

A diet high in sodium increases the risk of hypertension and the incidence of cardiovascular disease (Desmond, 2006) and is considered a risk factor for other diseases, such as obesity (He and MacGregor, 2010), kidney stones, osteoporosis, and certain cancers (Cappuccio et al., 2000). According to the World Health Organization (WHO, 2010), meat products contribute approximately 20–30% of the sodium in a person's diet. Thus, reducing sodium in these products may be useful to help people reduce their sodium intake by up to 2 g, as recommended by governmental authorities (WHO, 2010).

Dry fermented sausages are among the meat products that have the highest NaCl content, which can reach as high as 6% (Campagnol et al., 2011). Because these products are consumed worldwide, several studies have evaluated the effect of sodium reduction on their physical chemical and biochemical (Gelabert et al., 2003; Flores et al., 2006; Campagnol et al., 2011; Dos Santos et al., 2014; Aaslyng et al., 2014).

Despite sodium reduction being widely covered in the scientific literature, the effects of the reduction or replacement of NaCl by other chloride salts on the proteolysis reactions that occur during the manufacturing process of dry fermented sausages remain minimally studied. These reactions are very important to the quality of the final product, as low-molecular-weight compounds are formed during proteolysis, including peptides and free amino acids, which have a great impact in flavor and texture development (Toldrá, 2008), in addition to being important precursors to the formation of aroma compounds (Toldrá, 2006a). Proteolysis is a result of the action of tissue and microbial enzymes, whose activity is altered by processing conditions, low water activity, and especially by sodium chloride, because this ingredient regulates the activity of proteolytic enzymes, inhibiting the enzyme activity when its concentration increases during the drying stage (Toldrá, 2002). Thus, the reduction of NaCl in a formulation may increase the activity of proteolytic enzymes, resulting in a more soft and brittle texture due to the higher degradation of myofibrillar proteins (Toldrá, 2006b).

Another important issue that has received little attention thus far is the effect of the reduction or replacement of NaCl by other chloride salts on the rheological properties of dry fermented sausages. Although some authors have reported that salt reduction may interfere with the texture (Gimeno et al., 2001; Dos Santos

* Corresponding author. Tel.: +55 19 3521 4016; fax: +55 19 3289 3617.
E-mail address: bialvesantos@gmail.com (B.A. dos Santos).

http://dx.doi.org/10.1016/j.jfoodeng.2014.11.015
0260-8774/

et al., 2014), rheological properties in uniaxial compression and creep tests during the storage of dry fermented sausages have not been explored. The uniaxial compression and creep tests are fundamental tests to evaluate viscoelastic properties, and have been widely used in the rheological studies of food products. They permit a rapid characterization of the material behavior (Gunasekaran and Ak, 2002) and provide information on the permanent cross-linking of the protein network structure (Fox et al., 2000; Andrés et al., 2008). Thus, these tests can help to elucidate the texture changes in low-sodium dry fermented sausages. This study aimed to investigate the effect of a 50% reduction of NaCl, or a 50% replacement of NaCl by KCl, $CaCl_2$, and a blend of KCl and $CaCl_2$ (1:1) on proteolysis and rheological properties of dry fermented sausages. The myofibrillar and sarcoplasmic proteins and free amino acids were determined along the processing and the rheological properties (texture profile, uniaxial compression and creep compliance tests) were determined during the storage of dry-fermented sausages.

2. Materials and methods

2.1. Dry fermented sausage processing

The formulations were prepared with a 50% reduction of NaCl or a 50% replacement by KCl, $CaCl_2$, or a blend of KCl and $CaCl_2$ (1:1), as shown in Table 1. The dry fermented sausages were produced using the following ingredients: pork (650 g/kg; 76.08 ± 0.26% moisture; 18.00 ± 0.82% protein; 4.43 ± 0.10% lipids; 1.07 ± 0.61% ash), beef (200 g/kg; 77.12 ± 0.22% moisture; 18.57 ± 0.12% protein; 2.20 ± 0.02% lipids; 1.01 ± 0.61% ash), and pork back fat (150 g/kg; 17.28 ± 1.24% moisture; 6.54 ± 0.94% protein; 75.39 ± 0.51% lipids; 0.36 ± 0.55% ash). The raw material was ground with a disk (8 mm) and mixed with the respective amount of NaCl and other ingredients for each treatment as 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). After complete homogenization, the treatments were stuffed in collagen casings (diameter of 60 mm), and they were cut into slices of approximately 15 cm in length. In total, 60 pieces (approximately 300 g each) were prepared for each treatment. After being stuffed, the samples were subjected to a bath containing a 20% solution of potassium sorbate, and the samples were then ripened in a laboratory ripening cabinet (Menoncin, Erechim, Brazil). The temperature and relative humidity (T °C/RH%) were set as follows: first day, 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 to the nineteenth day, 18 °C/75%. The air speed remained constant at 5 m/s throughout the processing.

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

	Treatments (%)				
	Control*	F1	F2	F3	F4
Sodium chloride (NaCl)	2.5	1.25	1.25	1.25	1.25
Potassium chloride (KCl)	–	–	1.25	–	0.625
Calcium chloride ($CaCl_2$)	–	–	–	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% $CaCl_2$.

2.2. Proteolysis analysis

The proteolysis in the dry fermented sausages was determined in triplicate throughout the manufacturing process (0, 7, and 19 days). The extraction of myofibrillar and sarcoplasmic proteins was performed according to the methodology described by Díaz et al. (1997), with some modifications. Accordingly, 5 g samples were homogenized with a 35 mL phosphate buffer (0.03 mol/L, pH 7.4) for 2 min at 4 °C using an Ultra Turrax (13,500 rpm). Then, the sample was centrifuged at 10,000g for 20 min at 4 °C. The supernatant containing the sarcoplasmic proteins was filtered through glass wool and kept in refrigeration (4 °C ± 1 °C). The pellet was washed twice under the conditions described above to remove any remaining sarcoplasmic proteins. The resulting pellet was homogenized with a 25 mL solution of 8 mol/L urea and 1% β-mercaptoethanol for 2 min at 4 °C using an Ultra Turrax (13,500 rpm), followed by centrifugation at 10,000g for 20 min at 4 °C. The supernatant containing the myofibrillar proteins was filtered through glass wool, and kept in refrigeration (4 °C ± 1 °C) until analysis.

The concentration of myofibrillar and sarcoplasmic proteins was determined by the method of Bradford (1976) using bovine serum albumin (Sigma–Aldrich, USA) as a protein standard.

The proteolysis was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions (Laemmli, 1970) using a Mini Protean II apparatus (Bio-Rad, CA, USA) and 8% and 17.5% polyacrylamide gradient gels. The samples (4 mg/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 (4 mg of protein/mL) was loaded onto the gels. 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 (M_r) marker kit (Precision Plus Protein Kaleidoscope Standards, Bio-Rad, USA) was used for the standard. The molecular weights of the products of proteolysis were estimated based on the relative mobility of the protein standards.

2.3. Determination of free amino acids

The free amino acids in the dry fermented sausages were determined at 0, 7, and 19 days after the manufacturing process. Samples were triturated (Retsch, USA), and 5 g were homogenized with 0.1 mol/L HCl 1:5 (w/v) for 30 min using an orbital shaker. The supernatant was filtered using Whatman filter paper No. 1 (1001125), and a 5 mL filtrate was passed through a filter membrane with 0.45 μm pores (HPLC grade). Then, a 1 mL aliquot was transferred to a glass tube (6 mm × 50 mm) for derivatization using a phenyl isothiocyanate (PITC) reagent according to the method described by Hagen et al. (1989). Amino acids were determined by RP-HPLC using a Shimadzu HPLC system (Shimadzu Corporation, Tokyo, Japan) equipped with a UV detector (254 nm) and a C18 column Luna-Phenomenex (250 mm × 4.6 mm, 5 μm) (Phenomenex Inc., Torrence, CA, USA). The identification of amino acids was performed by the external standard method (Pierce/PN 20088), and quantification was performed as described by White et al. (1986) and Hagen et al. (1989).

2.4. Texture measurements

Texture profile analysis (TPA) was performed at the end of the manufacturing process and every 30 days during refrigerated storage (4 °C ± 1 °C) for 90 days using the TA-XT21 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) with a load cell of 25 kg. Each sample was cut into 3 cm cylinders, and stretched axially into two consecutive circles compressed at 30% with a probe of 30 mm

in diameter, moving at a constant test velocity of 1 mm/s. The data were collected, and the texture profile curves were drawn using the program Texture Expert, version 1.11 (Stable Micro Systems Ltd.). The following texture parameters were calculated: hardness, springiness, cohesiveness, resilience and chewiness. For each treatment, 5 pieces of dry fermented sausages were used in the instrumental texture analysis.

2.5. Rheological measurements

The viscoelastic behavior of the dry fermented sausage formulations was evaluated after the manufacturing process (day 0), and at 30, 60 and 90 days of refrigerated storage (4 °C ± 1 °C). The formulations were intended to reduce and/or partially replace the amount of sodium in dry fermented sausages. The rheological analyses were performed using a TA-XT21 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), fitted with a 25 kg load cell. The texture analyzer was equipped with a fixed platform and an aluminum plate 35 mm in diameter. No lubrication of the plate or platform was required because the exudate formed during the test was sufficient to reduce the friction between the sample surface and the plate/platform to insignificant levels. The samples were cut into cylinders (25 mm in diameter, 20 mm in height), wrapped individually in PVC film and placed in waterproof plastic bags, which were then maintained in a refrigerated bath at 10 °C for at least 15 min before testing. All rheological analyses were performed at least five times.

2.5.1. Uniaxial compression test

The uniaxial compression was performed according to the Karlsson et al. (2007) methodology. The breakdown pattern of the dry fermented sausage samples was observed by compressing the samples to 20% of their initial sample height at a cross-head speed of 1 mm s^{-1}. Force–displacement data were converted to true stress (σ_t) (Eq. (1)) and true strain (ε_t) (Eq. (2)) as described by Wium and Qvist (1997).

$$\sigma_t = \frac{F(t)}{A(t)} = \frac{F(t)H(t)}{A_0 H_0} \tag{1}$$

$$\varepsilon_t = \left|\ln\left(\frac{H(t)}{H_0}\right)\right| = \left|\ln\left(\frac{H_0 - \Delta H}{H_0}\right)\right| \tag{2}$$

where $F(t)$, $A(t)$ and $H(t)$ are the applied force, cross-sectional area and displacement at any time, respectively; A_0 and H_0 are the initial sample cross-sectional area and initial sample height, respectively; and ΔH is the absolute deformation.

The following parameters were calculated from the true stress–true strain curve (σ_t–ε_t): Young's modulus (E); fracture stress (σ_f); fracture strain (ε_f); fracture work (W_f); and maximum stress (σ_{max}) (Wium and Qvist, 1997). The fracture point corresponds to the first local maximum point ($d\sigma/dt = 0$) of the data, where the σ_f and ε_f values are obtained. Young's modulus was calculated as the angular coefficient from the linear regression of the σ_t–ε_t data until the fracture point. The fracture work corresponds to the total energy of the fracture represented by the area behind the curve until the fracture point. The maximum stress was determined as the true stress at maximum compression (80%) (Fox et al., 2000).

2.5.2. Creep compliance test

The creep test was performed according to Chattong et al. (2007) by measuring the deformation from applying a constant force of 0.89 N for 180 s. Then, the force was removed and the sample recovery was measured for an additional 180 s. The force–displacement data were converted to true stress (σ_t) (Eq. (1)) and strain (ε) (Eq. (3)).

$$\varepsilon = \frac{H(t)}{H_0} \tag{3}$$

where $H(t)$ is the height at any time and H_0 is the initial height of the samples.

The results were expressed as the ratio between the strain measured and the initial stress applied, referred to as compliance ($J(t) = \gamma(t)/\sigma_0$). The experimental compliance data during the creep phase was fitted by the four-component Burgers model, which is expressed in Eq. (4) (Burgers, 1935).

$$J(t) = J_0 + J_1\left(1 - e^{-t/\tau}\right) + \frac{t}{\eta_N} \tag{4}$$

where $J(t)$ is the creep-recovery compliance as a function of time; J_0 is the instantaneous compliance of the Maxwell spring; J_1 is the viscoelastic compliance that represents the retarded compliance related to the Kelvin–Voigt element; τ is the mean retardation time associated to the Kelvin–Voigt element; and η_N is the Newtonian viscosity associated to the Maxwell dashpot.

2.6. Statistical Analysis

Three independent manufacturing processes were performed using the same formulation and technology. For each manufacturer, three sample units (dry fermented sausages) were taken per sampling day ($n = 9$). All analyses were performed in triplicate. The results reported in this study are the mean obtained from all data recorded for each parameter.

An analysis of variance (one-way ANOVA) and Tukey's test at $P < 0.05$ were used to verify the statistical significance of the results using the statistical software XLSTAT v.5.02 (Addinsoft, Paris, France). The commercial software Statistica v.8 (Statsoft, Inc., Tulsa, OK, USA) was used for a linear and non-linear regression analysis to estimate the model parameters of the uniaxial compression and creep compliance tests, respectively.

3. Results and discussion

3.1. Effect of reduction or replacement of NaCl on proteolysis reactions

The myofibrillar and sarcoplasmic proteins were extracted from the dry fermented sausages with NaCl reduced by 50% and/or replaced by KCl and/or $CaCl_2$. There were no differences in the behavior of the myofibrillar proteins during the manufacturing process (Fig. 1). The proteins myosin (200,000 M_r) and actin (45,000 M_r) showed a severe degradation during the manufacturing process for all treatments. This behavior is normal and expected (García de Fernando and Fox, 1991), and the evidenced proteolytic activity is characteristic of fermented meat products, which involve the release of free amino acids and polypeptides (Sun et al., 2009). After seven days of manufacture, an increase in the number of bands with a molecular weight between 50,000 and 75,000 M_r and 10,000 and 37,000 M_r was observed, which remained until the end of the manufacturing process (19 days). According to Toldrá (1998), the fermentation process of dry fermented sausages provides an increase in the degradation of myofibrillar proteins due to pH values being lower than 5.00. This is the case of myosin and actin proteins, which are degraded into fragments of 135,000 and 38,000 M_r, respectively. Other protein fragments are also formed, with molecular weights of 29,000 and 13,000 M_r. This fact has been reported in other studies on the proteolysis of dry fermented sausages (Aro et al., 2010; Ikonić et al., 2013) and ham (Flores et al., 2006). Other authors have found bands with a molecular weight less than 150,000 M_r during the manufacturing process of dry fermented sausages (Hughes et al., 2002).

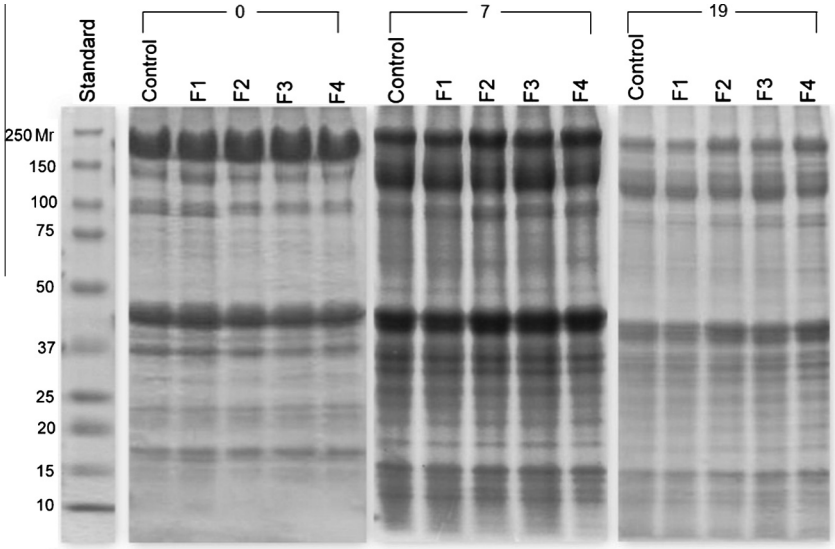

Fig. 1. 8% and 17.5% SDS–PAGE gels of myofibrillar proteins in the dry fermented sausages submitted to five types of salt treatments. Standards: Bio-Rad molecular weight standards (M_r = 250,000 a 10,000). Treatments as described in Table 1.

The bands with a molecular weight between 15,000 and 100,000 M_r indicated the presence of sarcoplasmic proteins in dry fermented sausages throughout the manufacturing period (Fig. 2). Polypeptides with a molecular weight between 37,000 and 70,000 M_r and 10,000 and 30,000 M_r were formed from the seventh day of manufacture. Sun et al. (2011) reported that the degradation of high-molecular weight proteins might be due to proteolysis or the manufacturing process leading to pH reduction, increased salt concentration, and the dehydration of dry fermented sausages. This behavior continued until the end of the manufacturing process and was similar to that found by other authors on the proteolysis in dry fermented sausages (García de Fernando and Fox, 1991; Hughes et al., 2002; Sun et al., 2011).

The reduction of NaCl and the addition of $CaCl_2$ altered the electrophoretic profile of the sarcoplasmic proteins during the manufacturing process. On day 0, the bands near Mr 90,000, 45,000, 35,000 and 15,000 appeared with low intensities in the treatments containing $CaCl_2$ (F3 and F4). On day 7, the treatments F3 and F4 also presented lower bands with molecular weight near Mr 25,000. At the end of the process (19 days), bands near Mr 25,000 were observed with low intensities in the control sample, and in the treatments F1 (50% NaCl) and F2 (50% NaCl and 50% KCl), and reappeared in the treatments F3 (50% NaCl and 50% $CaCl_2$) and F4 (50% NaCl, and 25% KCl and $CaCl_2$ 25%). The lower degradation of sarcoplasmic proteins observed in the samples containing $CaCl_2$ may be due to the slower growth of lactic acid bacteria in these treatments (data not shown), since these microorganisms have shown good ability for protein degradation (Fadda et al., 1999).

The total free amino acids released during the manufacturing of fermented meat products, including dry fermented sausages, depends on the decrease in pH, the salt concentration, the addition of a starter culture and the processing conditions (time, temperature and lower water activity), as these parameters mainly affect

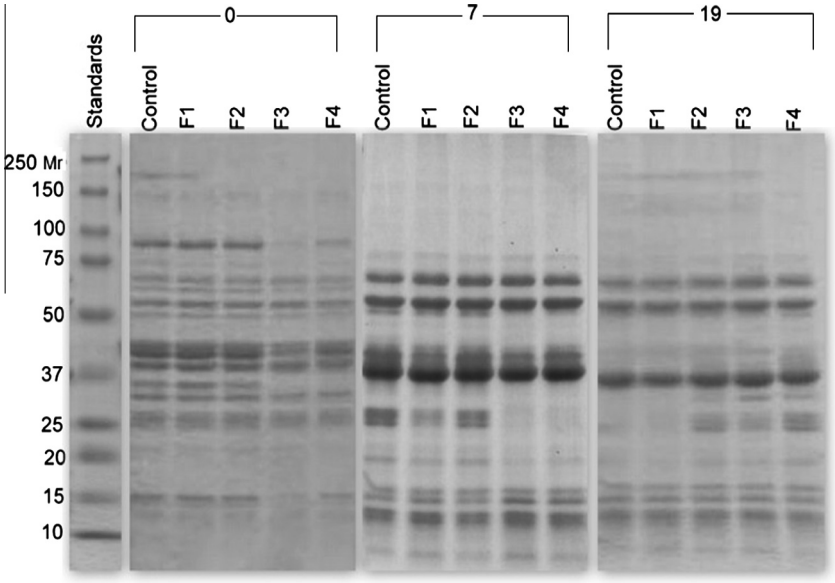

Fig. 2. 8% and 17.5% SDS–PAGE gels of sarcoplasmic proteins in the dry fermented sausages submitted to five types of salt treatments. Standards: Bio-Rad molecular weight standards (M_r = 250,000 a 10,000). Treatments as described in Table 1.

the activity of aminopeptidase enzymes (Sanz and Toldrá, 2002). In our study, an increase in the total free amino acids was observed during the manufacturing process (Table 2) for all treatments, and a 50% replacement of NaCl with KCl and/or $CaCl_2$ did not hinder the release of free amino acids. The change occurred during fermentation and ripening process indicating that the highest enzymatic activity took place during these stages (Verplaetse et al., 1989). Several authors have reported a major release of free amino acids at the beginning of the process in coincidence with the fermentation stage (Díaz et al., 1997; Aro et al., 2010).

In this study, dry fermented sausages produced with a 50% NaCl reduction (F1), containing 1.25% NaCl, had the highest amount of total free amino acids (2230.7 mg/100 g), which may be due to the reduced salt content. Within the literature there are reports that reduced sodium chloride levels (<2%) may activate most of the muscle proteases, which would increase the proteolytic activity and, therefore, the release of free amino acids (Toldrá, 1992). The treatments with KCl and/or $CaCl_2$ had a similar amount of total free amino acids from the control. Similarly, Armenteros et al. (2012) partially replaced NaCl with a blend of chloride salts (KCl, $CaCl_2$, and $MgCl_2$) in ham and found no difference in the total free amino acids. Also, no difference was observed in the total free amino acids in cured loins prepared with a partial replacement of NaCl by KCl (Armenteros et al., 2009).

The concentration of all free amino acids increased over the 19 days of manufacture, except glycine (Gly) and tyrosine (Tyr), which decreased on the seventh day and increased at the end of the manufacturing process. The decrease in the concentrations of amino acids may indicate that their metabolism by bacteria was more intense than their production during the latter stages of fermentation (Ordóñez et al., 1999; Hughes et al., 2002). Of the 18 free amino acids, a higher arginine concentration was observed in all treatments, and the samples prepared with 50% NaCl (F1) differed significantly from the control. According to Toldrá (2002), aminopeptidases are activated in the presence of salt, and may contribute to the generation of free amino acids in the ripening processes, including arginine. It is worth mentioning that this amino acid is responsible for the flavor development that is characteristic of dry-cured meat products. No significant difference was observed between the treatments and control for the concentration of the free amino acids serine (Ser), glycine (Gly), and tyrosine (Tyr). In contrast, aspartic acid (Asp), threonine (Thr), alanine (Ala), methionine (Met), leucine (Leu), isoleucine (Ile), and phenylalanine (Phe) concentrations were higher than the control ($P \leqslant 0.05$) in the treatments with 50% NaCl (F1), 50% NaCl and 50% $CaCl_2$ (F3), and 50% NaCl, 25% KCl, and 25% $CaCl_2$ (F4). This result can have a negative impact on the sensory quality, as high concentrations of Met, Leu and Ile may increase the bitter taste of dry fermented sausages (Toldrá, 2006a). In contrast, higher concentrations of the free amino acids glutamic acid (Glu), histidine (His), valine (Val), cysteine (Cys), lysine (Lys) and tryptophan (Trp) were observed in the treatment with a 50% reduction of NaCl (F1), which differed significantly from the control.

3.2. Effect of reduction/replacement of NaCl on rheological properties

3.2.1. Texture profile

The effect of sodium reduction or replacement on the textural properties, such as hardness, springiness, cohesiveness, resilience, and chewiness, of dry fermented sausage samples is shown in Table 3. The reduction and replacement of NaCl did not significantly ($P < 0.05$) affect the texture parameters, except for hardness values, which decreased in the F1 (50% NaCl) formulation. Similar behavior was observed during the shelf life of dry fermented sausage samples for all formulations.

A comparison of the TPA and rheological results of this study with those in the literature was not possible because no study has investigated the effect of the composition of dry fermented sausages on its texture profile and/or viscoelastic parameters. Therefore, these results were compared with those obtained for sausages (Houben and van 't Hooft, 2005; Andrés et al., 2008) and model system meat emulsions (Yilmaz et al., 2012). Springiness denotes how well a product physically springs back after it has been deformed during the first compression (Bourne, 1978). Springiness values were related to the elastic properties of the samples; thus, an increase in the springiness value indicates that elasticity of the sample is enhanced.

In this study, the dry fermented sausages tended to increase their resilience values for all formulations during 30 days of storage with no further significant ($P > 0.05$) modification after that storage time. Cohesiveness, how good the sample retains its structure after compression (Bourne, 1978), tended to increase with time, which is probably due to the effect of fat on cohesiveness. A similar trend was also found in the literature (Papadina and Bloukas, 1999; Saricoban et al., 2009).

3.2.2. Uniaxial compression test

The values for Young's modulus, fracture stress, fracture strain, work for fracture, and maximum stress calculated for the low-sodium dry fermented sausages during the 90 days of refrigerated storage are shown in Table 4. Young's modulus (E) provides a useful indication of how easily a sample can be contracted and stretched, sometimes referred to as the stiffness of the material, and corresponds to the slope of the linear portion of the σ_t–ε_t curves, where only Hooke's law is valid (Gunasekaran and Ak, 2002; Fox et al., 2000). The fracture stress (σ_f) is related to sample toughness, whereas the maximum stress (σ_{max}) and the work for fracture (W_f) are directly related to sample firmness and hardness (Fox et al., 2000). The fracture strain (ε_f) denotes the strain needed to cause a rupture point in the sample and may be associated with the crumbliness of the samples (Cunha et al., 2006).

The values for Young's modulus, fracture stress, fracture strain, maximum stress and work for fracture did not decrease significantly ($P > 0.05$) with storage time, demonstrating the preservation of the rigidity of the samples and, consequently, a well-structured peptide matrix during shelf life. Neither sample F3 (50% NaCl and 50% $CaCl_2$) nor F4 (50% NaCl, 25% KCl and 25% $CaCl_2$) presented enough resistance to cause a fracture point after 90 days of storage.

Regarding the formulations of salts, the 50% reduction and/or replacement of the sodium content significantly affected all viscoelastic parameters, except fracture strain, which indicates that there was no difference between the dry fermented sausage formulations with respect to crumbliness. The 50% reduction of NaCl in F1 caused a decrease in the parameters. This behavior indicates a loss of rigidity due to the weaker intermolecular interaction formed in the protein network, which is probably caused by an ion charge reduction. However, the incorporation of potassium in F2 (50% NaCl and 50% KCl) and F4 (50% NaCl, 25% KCl and 25% $CaCl_2$) increased the viscoelastic parameters, demonstrating a symbiotic effect between sodium and potassium in the formulations of the dry fermented sausage, thus resulting in tougher samples. All dry fermented sausage samples exhibited a higher rigidity when compared to low-fat chicken sausage (Andrés et al., 2008), semi-dry fermented sausage (Houben and van 't Hooft, 2005) and meat emulsions (Yilmaz et al., 2012). This characteristic could be associated with a well-structured protein network resulting in a more solid-like and less viscoelastic behavior.

3.2.3. Creep compliance test

The main characteristic of viscoelastic materials is their continuous deformation when they are exposed to constant tension. The

Table 2
Free amino acids content during the manufacturing process of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and/or $CaCl_2$.

Free amino acids (FAA) (mg/100 g)	Days	Treatments					
		Control	F1	F2	F3	F4	SEM*
Asp	0	4.0^{aC}	4.0^{aC}	3.0^{bC}	2.0^{cC}	4.7^{aC}	0.3
	7	18.0^{bB}	13.0^{cB}	15.4^{cB}	22.0^{aB}	35.0^{aB}	0.9
	19	35.0^{cA}	45.3^{bA}	35.0^{cA}	46.0^{bA}	58.0^{aA}	2.3
Glu	0	16.0^{cC}	19.7^{aC}	13.0^{eC}	14.3^{dC}	17.7^{bC}	0.7
	7	86.0^{cB}	99.0^{aB}	92.0^{bB}	89.7^{bB}	98.0^{aB}	1.4
	19	171.0^{eA}	199.3^{aA}	178.0^{dA}	185.3^{cA}	192.3^{bA}	2.7
Ser	0	8.7^{bC}	10.7^{aC}	8.3^{bC}	8.0^{bC}	9.0^{bC}	0.3
	7	21.3^{bB}	21.7^{bB}	21.3^{bB}	20.7^{bB}	23.4^{aB}	0.2
	19	51.0^{aA}	53.3^{aA}	44.0^{aA}	32.0^{aA}	50.0^{aA}	3.4
Gly	0	17.7^{aA}	17.7^{aB}	15.0^{bB}	17.7^{aB}	18.3^{aA}	0.3
	7	11.0^{abB}	10.0^{bC}	11.3^{aC}	9.7^{cC}	11.7^{aB}	0.2
	19	17.3^{aA}	20.0^{aA}	16.0^{aA}	23.3^{aA}	20.0^{aA}	1.2
His	0	31.0^{abC}	36.3^{aC}	29.0^{bC}	30.7^{abC}	31.0^{abC}	0.7
	7	65.3^{eB}	81.7^{aB}	71.3^{cB}	67.7^{dB}	74.3^{bB}	1.5
	19	135.7^{cA}	179.3^{aA}	135.7^{cA}	135.7^{cA}	148.0^{bA}	4.6
Arg	0	246.8^{abC}	231.7^{cC}	207.7^{dC}	244.7^{bC}	249.7^{aC}	4.1
	7	338.0^{dB}	353.0^{bB}	362.0^{aB}	337.7^{dB}	349.7^{cB}	2.5
	19	605.7^{bA}	632.0^{aA}	565.3^{cA}	603.3^{bA}	605.0^{bA}	5.7
Thr	0	8.3^{aC}	8.7^{aC}	7.3^{aC}	7.7^{aC}	8.3^{aC}	0.2
	7	22.3^{cB}	25.7^{bB}	24.3^{bB}	28.3^{aB}	29.3^{aB}	0.7
	19	45.0^{cA}	56.0^{aA}	43.7^{cA}	52.0^{bA}	52.0^{bA}	1.3
Ala	0	34.8^{cC}	38.7^{aC}	32.3^{cC}	33.7^{cdC}	36.3^{bC}	0.6
	7	77.3^{eB}	91.3^{aB}	83.4^{cB}	81.3^{dB}	87.4^{bB}	1.3
	19	151.7^{dA}	184.3^{aA}	150.3^{dA}	158.7^{cA}	165.7^{bA}	3.3
Pro	0	7.7^{abC}	9.0^{aC}	6.7^{bC}	7.3^{bC}	7.7^{abC}	0.2
	7	21.3^{aB}	20.7^{aB}	20.7^{aB}	16.7^{bB}	19.7^{aB}	0.4
	19	38.0^{aA}	37.0^{aA}	35.7^{abA}	28.3^{cA}	33.0^{bA}	1.0
Tyr	0	2.0^{aA}	1.0^{aB}	1.3^{aB}	2.0^{aB}	1.4^{aB}	0.1
	7	0.3^{aB}	0.3^{aB}	0.7^{aB}	1.3^{aB}	1.3^{aB}	0.2
	19	3.0^{aA}	5.0^{aA}	2.0^{bA}	5.0^{aA}	5.0^{aA}	0.4
Val	0	6.7^{cC}	10.0^{aC}	6.7^{cC}	7.3^{bcC}	8.7^{abC}	0.3
	7	27.3^{dB}	34.3^{aB}	30.3^{cB}	31.7^{bcB}	32.7^{abB}	0.6
	19	52.0^{cA}	67.7^{aA}	56.0^{bA}	56.7^{bA}	58.3^{bA}	1.4
Met	0	5.3^{aC}	5.3^{aC}	4.3^{aC}	4.3^{aC}	4.7^{aC}	0.1
	7	11.7^{bB}	15.7^{aB}	13.3^{bB}	15.3^{aB}	15.3^{aB}	0.3
	19	24.3^{cA}	32.0^{aA}	25.0^{cA}	28.7^{bA}	28.7^{bA}	0.8
Cys	0	10.3^{abC}	11.7^{aC}	7.3^{cC}	9.4^{bC}	9.7^{bC}	0.4
	7	48.3^{bB}	60.3^{aB}	30.0^{cB}	32.7^{cB}	34.3^{cB}	3.1
	19	126.0^{cA}	155.3^{aA}	107.7^{dA}	124.3^{cA}	135.3^{bA}	4.2
Ile	0	6.3^{bC}	8.3^{aC}	6.3^{bC}	6.4^{bC}	8.3^{aC}	0.3
	7	24.3^{bB}	29.7^{aB}	26.7^{bB}	29.7^{aB}	29.7^{aB}	0.6
	19	46.3^{eA}	62.0^{aA}	50.7^{dA}	59.0^{abA}	57.7^{cA}	1.6
Leu	0	12.3^{bC}	15.7^{aC}	11.3^{bC}	12.3^{bC}	14.3^{aC}	0.5
	7	42.7^{bB}	68.7^{aB}	55.7^{abB}	68.3^{aB}	67.7^{aB}	1.8
	19	106.7^{eA}	144.7^{aA}	110.7^{dA}	136.7^{bA}	133.0^{cA}	4.0
Phe	0	7.3^{bcC}	9.7^{aC}	6.7^{cC}	7.3^{bcC}	8.3^{abC}	0.3
	7	30.3^{cB}	39.7^{aB}	33.3^{bB}	39.3^{aB}	38.7^{aB}	1.0
	19	58.3^{cA}	78.7^{aA}	61.3^{cA}	77.0^{aA}	72.0^{bA}	2.3
Lys	0	13.3^{aC}	14.3^{aC}	9.7^{bcC}	8.7^{cC}	11.4^{bC}	0.6
	7	39.3^{cB}	44.7^{aB}	42.7^{bB}	39.3^{cB}	40.7^{cB}	0.6
	19	87.3^{cA}	111.0^{aA}	92.3^{cA}	92.0^{cA}	99.0^{bA}	2.2
Trp	0	ND**	6.3^{aC}	ND**	ND**	3.3^{bC}	0.7
	7	70.8^{cB}	77.9^{aB}	77.4^{aB}	67.3^{dB}	74.3^{bB}	1.0
	19	127.0^{dA}	167.8^{aA}	137.3^{cA}	136.0^{cA}	149.0^{bA}	3.7
Total FAA	0	438.5	458.8	375.9	423.8	452.8	
Total FAA	7	955.5	1087.4	1011.8	998.7	1063.2	
Total FAA	19	1881.3	2230.7	1846.7	1980.0	2062.0	

Averages in the same row followed by the same letter are not significantly different by Tukey's test ($P \geqslant 0.05$). Averages in the same column followed by the same uppercase letter are not significantly different by Tukey's test ($P \geqslant 0.05$). 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% $CaCl_2$.

* SEM – Standard error of the mean.

** ND – Not detected.

Table 3
Texture profile analysis during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and/or $CaCl_2$.

Texture parameter	Storage time (days)	Control	F1	F2	F3	F4	SEM*
Hardness (*N*)	0	47.35^{abc}	37.11^{c}	58.16^{a}	42.84^{c}	51.38^{ab}	1.90
	30	43.30^{ab}	36.37^{b}	52.46^{a}	37.73^{b}	52.12^{a}	1.65
	60	39.95^{a}	23.52^{b}	39.16^{a}	27.23^{b}	42.17^{a}	1.76
	90	45.74^{a}	27.52^{b}	50.08^{a}	33.71^{b}	48.52^{a}	1.85
Springiness	0	0.64^{ab}	0.66^{a}	0.62^{b}	0.65^{ab}	0.63^{ab}	0.01
	30	0.68^{a}	0.70^{a}	0.64^{a}	0.69^{a}	0.69^{a}	0.02
	60	0.71^{a}	0.65^{a}	0.68^{a}	0.74^{a}	0.70^{a}	0.02
	90	0.70^{a}	0.68^{a}	0.65^{a}	0.66^{a}	0.71^{a}	0.01
Cohesiveness	0	0.61^{a}	0.61^{a}	0.59^{a}	0.60^{a}	0.59^{a}	0.02
	30	0.67^{a}	0.65^{a}	0.67^{a}	0.64^{a}	0.65^{a}	0.02
	60	0.64^{a}	0.59^{a}	0.62^{a}	0.71^{a}	0.64^{a}	0.03
	90	0.65^{a}	0.65^{a}	0.65^{a}	0.64^{a}	0.64^{a}	0.01
Resilience	0	0.175^{a}	0.182^{ab}	0.177^{a}	0.182^{ab}	0.174^{a}	0.20
	30	0.212^{a}	0.211^{a}	0.212^{a}	0.217^{a}	0.214^{a}	0.58
	60	0.206^{ab}	0.216^{a}	0.213^{a}	0.211^{a}	0.210^{a}	0.75
	90	0.211^{ab}	0.185^{a}	0.200^{a}	0.193^{a}	0.210^{ab}	0.35
Chewiness (*N*)	0	18.28^{ab}	14.96^{b}	21.29^{a}	16.60^{ab}	19.04^{ab}	0.65
	30	19.51^{abc}	16.41^{c}	22.37^{ab}	16.62^{bc}	23.26^{a}	0.75
	60	18.21^{a}	9.08^{a}	17.07^{a}	16.86^{a}	18.97^{a}	1.43
	90	20.71^{a}	12.10^{a}	21.53^{a}	14.35^{a}	22.06^{a}	0.91

Averages in the same row followed by the same letter in lowercase are not significantly different by Tukey test ($P > 0.05$). 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% $CaCl_2$.
* SEM – Standard error of the mean.

Table 4
Uniaxial compression parameters content during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and/or $CaCl_2$.

Uniaxial compression parameter	Storage time (days)	Control	F1	F2	F3	F4
E (kPa)	0	245.3^{cA}	185.0^{dA}	271.5^{aA}	214.3^{bA}	290.1^{aA}
	30	251.1^{aA}	160.0^{cB}	232.1^{bC}	174.7^{cB}	252.7^{aB}
	60	221.4^{bB}	167.0^{cdAB}	290.6^{aAB}	166.8^{cdB}	175.4^{cC}
	90	212.9^{bB}	167.0^{cAB}	246.9^{aBC}	-	-
σ_f (kPa)	0	319.7^{cA}	154.9^{dA}	430.1^{aA}	228.5^{eA}	347.7^{bA}
	30	297.3^{bB}	115.6^{eB}	345.6^{aB}	168.7^{dB}	227.1^{cB}
	60	237.6^{bC}	155.0^{cdA}	288.0^{aC}	128.0^{dC}	120.9^{deC}
	90	296.3^{aB}	117.7^{cB}	211.6^{bD}	-	-
ε_f (–)	0	1.283^{abA}	1.077^{bA}	1.168^{abAB}	1.073^{bA}	1.073^{bA}
	30	1.287^{abA}	0.778^{cB}	1.644^{abA}	1.053^{bA}	1.074^{bA}
	60	1.158^{abA}	0.977^{abAB}	1.059^{abB}	0.867^{bAB}	0.757^{bAB}
	90	1.358^{abA}	0.977^{bAB}	1.079^{abAB}	-	-
W_f (kJ/m^3)	0	212.6^{bcA}	84.3^{dA}	252.0^{abB}	121.2^{cA}	192.2^{bA}
	30	195.4^{bB}	45.7^{eD}	316.0^{aA}	89.8^{dB}	121.4^{cB}
	60	142.6^{bC}	76.0^{cB}	153.8^{aC}	55.5^{cdC}	46.7^{cdC}
	90	220.8^{aA}	58.0^{cC}	117.8^{bD}	-	-
σ_{max} (kPa)	0	384.8^{cdB}	314.8^{deA}	572.5^{aA}	368.0^{cdB}	469.0^{bA}
	30	410.7^{bA}	268.3^{cB}	438.0^{aB}	289.8^{cC}	438.8^{aA}
	60	326.3^{bC}	324.4^{bA}	326.3^{bC}	404.3^{aA}	257.6^{cC}
	90	404.0^{aA}	271.2^{cB}	334.2^{bC}	334.3^{bB}	375.9^{abB}

$^{a-e}$ Different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied dry fermented sausages formulations for the same period of storage. $^{A-D}$ Different superscript uppercase letters denote significant differences ($P < 0.05$) among different periods of storage for the same studied dry fermented sausages formulation. 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% $CaCl_2$.

experimental creep compliance data of the dry fermented sausage samples were fitted to a four-component Burgers model ($0.9641 < R^2 < 0.9985$) by non-linear regression. The parameters adjusted are comprised by the instantaneous elastic compliance (J_0), the retarded compliance (J_1), retarded time (τ) and Newtonian viscosity (η_N).

The Levenberg–Marquardt estimation method was used with 5000 interactions and 10^{-6} least squares as the convergence criterion for the loss function. The adjusted model parameters are shown in Table 5. These parameters indicate some rheological properties, in which the main factor of influence is probably the composition of the dry fermented sausage and processing characteristics. Furthermore, these parameters are good indications not only of the possible texture properties but also of the behavior of the dry fermented sausage throughout the shelf life as they may suffer deformation and changes in composition during storage time.

The formulations significantly influenced ($P < 0.05$) all creep parameters, with the exception of the retardation time (for which $P > 0.05$). The refrigerated storage also significantly ($P < 0.05$) affected these parameters. According to Olivares et al. (2009), the instantaneous elastic compliance (J_0) represents the value of instantaneous shear creep compliance at an initial time, and it may be related to the undisturbed protein network structure (Ma et al., 1997). A higher value of J_0 reflects a higher degree of non-retarded Hookean-type (elastic) deformation, indicating that the

Table 5
Creep viscoelastic parameters of Burger's model during refrigerated storage of dry fermented sausages with 50% reduction of NaCl, and replacement with KCl and / or $CaCl_2$.

Creep viscoelastic parameters	Storage time (days)	Control	F1	F2	F3	F4
J_0 (10^{-22} Pa^{-1})	0	3.47^{bB}	3.36^{bA}	6.33^{aA}	0.57^{cBC}	0.01^{dA}
	30	5.59^{aA}	3.51^{bA}	2.22^{cD}	2.07^{cA}	0.13^{dA}
	60	0.16^{bC}	0.13^{bC}	3.42^{aC}	0.13^{bC}	0.11^{bA}
	90	3.39^{bB}	2.44^{cB}	4.07^{aB}	1.97^{dB}	0.14^{eA}
J_1 (10^{-5} Pa^{-1})	0	2.75^{bC}	3.36^{aD}	2.66^{bB}	3.58^{aC}	3.13^{abB}
	30	3.52^{bB}	4.77^{aC}	2.12^{cC}	4.72^{aB}	2.07^{cC}
	60	2.24^{dC}	5.61^{aB}	2.83^{dB}	4.26^{bB}	3.49^{cB}
	90	5.51^{bA}	6.91^{aA}	3.53^{dA}	5.88^{bA}	4.42^{cA}
τ (s)	0	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	30	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	60	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
	90	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}	0.10^{aA}
η_N (10^7 Pa s)	0	2.94^{aB}	1.63^{bA}	2.61^{aB}	1.86^{bA}	2.90^{aA}
	30	2.53^{bC}	1.66^{cA}	3.57^{aA}	1.82^{cA}	2.77^{bA}
	60	3.70^{aA}	1.62^{cA}	3.19^{abA}	1.84^{cA}	2.80^{bA}
	90	2.12^{aC}	1.18^{cA}	1.70^{bC}	1.34^{cA}	2.24^{aA}

$^{a-e}$Different superscript lowercase letters denote significant differences ($P < 0.05$) among the different studied dry fermented sausages formulations for the same period of storage. $^{A-D}$Different superscript uppercase letters denote significant differences ($P < 0.05$) among different periods of storage for the same studied dry fermented sausages formulation. 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% $CaCl_2$.

polypeptide strands in the network are relatively free to rearrange between cross-links (Ma et al., 1997). In other words, the instantaneous compliance (J_0) is inversely related to the rigidity of dry fermented sausages (Olivares et al., 2009).

Among the dry fermented sausages studied, the formulation F4 exhibited the lowest J_0 value, followed by F3, F1, the control and F2. This behavior indicates that the $CaCl_2$ addition in F4 and F3 enhanced the dry fermented sausages' rigidity, resulting in more solid-like samples (Lobato-Calleros et al., 2000). By contrast, the use of KCl as a 50% substitute of NaCl in F2 produced softer and less firm samples. These results are probably due to a higher ionic charge of $CaCl_2$, which caused stronger interactions among salt, protein and water molecules fortifying the polypeptide network. An increasing J_0 with storage time indicates that the dry fermented sausages become less rigid. It is worth mentioning that J_0 showed significant differences ($P < 0.05$) with storage time only for the formulations F3 and F4. All dry fermented sausage samples showed very low values of J_0, exhibiting relatively higher rigidity when compared to Thai sausages (Chattong et al., 2007). According to Olivares et al. (2009), the retarded compliance (J_1) represents the principal component of the viscoelastic behavior of dry fermented sausage. It was observed that J_1 significantly ($P < 0.05$) increased with storage time for all formulations. The increase of this parameter is associated with a less solid and more viscoelastic behavior, which confirms the results observed for J_0. In addition, Newtonian viscosity (η_N), which is derived from the slope of the curve at large values of time, may be attributed to the breakdown of protein network structures (Messens et al., 2000). A decrease in η_N was observed with a decrease in sodium concentration for F1 (50% NaCl) and F3 (50% NaCl and 50% $CaCl_2$), indicating a lower resistance to flow. Thus, both formulations show a less solid-like behavior in dry fermented sausage samples.

3.2.4. A comparison of texture and rheological measurements

At first sight, TPA results may appear inconsistent with creep and uniaxial compression tests. However, resilience, a measurement of how the sample recovers from deformation, is the parameter that is able to provide a real indication of the elasticity of a product. Indeed, the best correlation among texture and rheological measurements can be observed between resilience and J_1 values and between resilience and E values, specially with respect to formulations ranging from 0 to 60 days. Resilience is how well a product resists regaining its original shape (Bourne, 1978). Considering the results obtained by the creep test, this phenomenon may be indicative of the loss of rigidity that unfortunately could not be measured. As mentioned in the creep tests, this likely loss of elasticity may be associated to proteolysis during storage. The reduction and/or replacement of sodium caused significant changes in the firmness, elasticity and texture of the samples compared to the control. The reduction of NaCl in F1 had a relative decreasing effect on the firmness of the dry fermented sausages when compared to the control, whereas the addition of $CaCl_2$ in formulations F3 and F4 caused an increase in the hardness of the dry fermented sausages. In addition, those samples showed pronounced decay in their firmness over storage time.

4. Conclusion

The reduction of 50% NaCl increased the total free amino acids released during the manufacturing and had a relative decreasing effect on the firmness of the dry fermented sausages. The addition of $CaCl_2$ modified the total free amino acids released and decreased the degradation of sarcoplasmic proteins during the manufacturing process. In addition, $CaCl_2$ increased the hardness of the dry fermented sausages and those samples showed pronounced decay in their firmness over storage time. Such changes are undesirable, as they can interfere with the sensory consumer's acceptability and/or preference.

The 50% replacement of NaCl by KCl did not influence the proteolysis and exhibited the most similar viscoelastic properties to the control and a greater stability during storage, demonstrating a potential application of this formulation in the marketplace. However, sensory analysis should be performed to evaluate consumer acceptability.

Acknowledgments

The present study was carried out with the support of the National Council for Scientific and Technological Development (CNPq) – Brazil and funding from the Foundation for Research Support of the State of São Paulo (FAPESP) – Brazil.

References

Aaslyng, M.D., Vestergaard, C., Koch, A.G., 2014. The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. Meat Sci. 96, 47–55.
Andrés, S.C., Zaritzky, N.E., Califano, A.N., 2008. Stress relaxation characteristics of low-fat chicken sausages made in Argentina. Meat Sci. 79, 589–594.

Armenteros, M., Aristoy, M.C., Barat, J.M., Toldrá, F., 2009. Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. Food Chem. 117, 627–633.
Armenteros, M., Aristoy, M.C., Barat, J.M., Toldrá, F., 2012. Biochemical and sensory changes in dry-cured ham salted with partial replacements of NaCl by other chloride salts. Meat Sci. 90, 361–367.
Aro, J.M.A., Nyam-Osor, P., Tsuji, K., Shimada, K.-I., Fukushimac, M., Sekikawa, M., 2010. The effect of starter cultures on proteolytic changes and amino acid content in fermented sausages. Food Chem. 119, 279–285.
Bourne, M.C., 1978. Texture profile analysis. Food Technol. 32, 62–66.
Bradford, M.M., 1976. A rapide and sensitive method for quantification of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72, 248–251.
Burgers, J.M., 1935. Mechanical considerations-model systems – phenomenological theories of relaxation and viscosity. In: First report on viscosity and plasticity. Nordemann, New York.
Campagnol, P.C.B., Santos, B.A., Wagner, R., Terra, N.N., Pollonio, M.A.R., 2011. The effect of yeast extract addition on quality of fermented sausages at low NaCl content. Meat Sci. 87 (3), 290–298.
Cappuccio, F.P., Kalaitzidis, R., Duneclift, A., Eastwood, J.B., 2000. Unravelling the links between calcium excretion, salt intake, hypertension, kidney stones and bone metabolism. Journal of Nephrology 13 (3), 169–177.
Chattong, U., Apichartsrangkoon, A., Bell, A.E., 2007. Effects of hydrocolloid addition and high pressure processing on the rheological properties and microstructure of a commercial ostrich meat product "Yor" (Thai sausage). Meat Sci. 76, 548–554.
Cunha, C.R., Viotto, W.H., Viotto, L.A., 2006. Use of low concentration factor ultrafiltration retentates in reduced fat "Minas Frescal" cheese manufacture: effect on composition, proteolysis, viscoelastic properties and sensory acceptance. Int. Dairy J. 16, 215–224.
Desmond, E., 2006. Reducing salt: a challenge for the meat industry. Meat Sci. 74, 188–196.
Díaz, O., Fernández, M., Garcia de Fernando, G.D., De La Hoz, L., Ordóñez, J.A., 1997. Proteolysis in dry fermented sausages; the effect of selected exogenous proteases. Meat Sci. 46, 115–128.
Dos Santos, B.A., Campagnol, P.C.B., Morgano, M.A., Pollonio, M.A.R., 2014. Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. Meat Sci. 96, 509–513.
Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M.C., Oliver, G., Toldrá, F., 1999. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. Appl. Environ. Microbiol. 65, 3540–3546.
Flores, M., Barat, J.M., Aristoy, M.-C., Peris, M.M., Grau, R., Toldrá, F., 2006. Accelerated processing of dry-cured ham. Part 2. Influence of brine thawing/salting operation on proteolysis and sensory acceptability. Meat Sci. 72, 766–772.
Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., 2000. Cheese rheology and texture. In: Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. (Eds.), Fundamentals of Cheese Science. Gaithersburg, Aspen, pp. 305–340.
García de Fernando, D.G., Fox, P.F., 1991. Study of proteolysis during the ripening of a dry fermented pork sausage. Meat Sci. 30, 367–383.
Gelabert, J., Gou, P., Guerrero, L., Arnau, J., 2003. Effect of sodium chloride replacement on some characteristics of fermented sausages. Meat Sci. 65, 833–839.
Gimeno, O., Astiasarán, I., Bello, J., 2001. Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausages: effect on colour, texture and hygienic quality at different concentrations. Meat Sci. 57, 23–29.
Gunasekaran, S., Ak, M.M., 2002. Cheese Rheology and Texture. CRC Press, Boca Raton, FL.
Hagen, S.R., Frost, B., Augustin, J., 1989. Pre-column phenylisothiocyanate derivatization and liquid chromatography of amino acids in food. J. Assoc. Official Anal. Chemists 72 (6), 912–916.
He, F.J., Macgregor, G.A., 2010. Reducing population salt intake worldwide: from evidence to implementation. Prog. Cardiovasc. Dis. 52, 363–382.
Houben, J.H., van 't Hooft, B.J., 2005. Variations in product-related parameters during the standardised manufacture of a semi-dry fermented sausage. Meat Sci. 69, 283–287.
Hughes, M.C., Kerry, J.P., Arendt, E.K., Kenneally, P.M., McSweeney, P.L.H., O'Neill, E.E., 2002. Characterization of proteolysis during the ripening of semidry fermented sausages. Meat Sci. 62, 205–216.
Ikonić, P., Tasi, T., Petrović, L., Skaljac, S., Jokanović, M., Mandić, A., Ikonić, B., 2013. Proteolysis and biogenic amines formation during the ripening of Petrovská Klobása, traditional dry-fermented sausage from Northern Serbia. Food Control 30, 69–75.
Karlsson, A.O., Ipsen, R., Ardo, Y., 2007. Influence of pH and NaCl on rheological properties of rennet-induced casein gels made from UF concentrated skim milk. Int. Dairy J. 17, 1053–1062.
Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (15), 685–689.
Lobato-Calleros, C., Aguirre-Mandujano, E., Vernon-Carter, E.J., Sanchez-Garcia, J., 2000. Viscoelastic properties of white fresh cheese filled with sodium caseinate. J. Texture Stud. 31 (4), 379–390.
Ma, L., Drake, M.A., Barbosa-Canovas, G.V., Swanson, B.G., 1997. Rheology of full-fat and low-fat Cheddar Cheeses as related to type of Fat Mimetic. J. Food Sci. 62 (4), 748–752.
Messens, W., Van de Walle, D., Arevalo, J., Dewettinck, K., Huyghebaert, A., 2000. Rheological properties of high-pressuretreated Gouda cheese. Int. Dairy J. 10 (5/6), 359–367.
Olivares, M.L., Zorrilla, S.E., Rubiolo, A.C., 2009. Rheological properties of mozzarella cheese determined by creep/recovery tests: effect of sampling direction, test temperature and ripening time. J. Texture Stud. 40, 300–318.
Ordóñez, J.A., Hierro, E.M., Bruna, J.M., de la Hoz, L., 1999. Changes in the components of dry-fermented sausages during ripening. Crit. Rev. Food Sci. Nutr. 39, 329–367.
Papadina, S.N., Bloukas, J.G., 1999. Effect of fat level and storage conditions on quality characteristics of traditional Greek sausages. Meat Sci. 51, 103–113.
Sanz, Y., Toldrá, F., 2002. Purification and characterization of an arginine aminopeptidase from *Lactobacillus sakei*. Appl. Environ. Microbiol. 68 (4), 1980–1987.
Saricoban, C., Yilmaz, M.T., Karakaya, M., 2009. Response surface methodology study on the optimisation of effects of fat, wheat bran and salt on chemical, textural and sensory properties of patties. Meat Sci. 83, 610–619.
Sun, W., Zhao, H., Zhao, Q., Zhao, M., Yang, B., Wu, N., et al., 2009. Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. Innovat. Food Sci. Emerg. Technol. 10, 558–563.
Sun, W., Cui, C., Zhao, M., Zhao, Q., Yang, B., 2011. Effects of composition and oxidation of proteins on their solubility, aggregation and proteolytic susceptibility during processing of Cantonese sausage. Food Chem. 124, 336–341.
Toldrá, F., 1992. The enzymology of dry-curing of meat products. In: Smulders, J.M., Toldrá, F., Flores, J., Prieto, M. (Eds.), New technologies for meat and meat products. The Netherlands, Audet, Nijmegen, pp. 209–231.
Toldrá, F., 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Sci. 49 (1), S101–S110.
Toldrá, F., 2002. Manufacturing of dry-cured ham. In: Toldrá, F. (Ed.), Dry-Cured Meat Products. Trumbull, pp. 27–62.
Toldrá, F., 2006a. Biochemical proteolysis basis for improved processing of dry-cured meats. In: Nollet, L.M.L., Toldrá, F. (Eds.), Advanced Technologies for Meat Processing. CRC Press, Boca Raton, FL, pp. 329–351.
Toldrá, F., 2006b. Dry-cured ham. In: Hui, Y.H. (Ed.), Handbook of Food Science Technology and Engineering. CRC Press, Boca Raton, FL, pp. 164–1 – 164–11.
Toldrá, F., 2008. Meat biotechnology. In: Toldrá, F. (Ed.), Biotechnology of Flavor Generation in Fermented Meats. Springer, New York, pp. 199–215.
Verplaetse, A., De Bosschere, M., Demeyer, D., 1989. Proteolysis during dry sausage ripening. In: Proceedings of the 35th International Congress on Meat Science and Technology, Copenhagen, Denmark.
White, J.A., Hart, R.J., Fry, J.C., 1986. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J. Automat. Chem. 8 (4), 170–177.
World Health Organization (WHO), 2010. Strategies to Monitor and Evaluate Population Sodium Consumption and Sources of Sodium in the Diet. Report of a Joint Technical Meeting Convened by WHO and the Government of Canada. <http://whqlibdoc.who.int/publications/2011/9789241501699_eng.pdf>.
Wium, H., Qvist, K.B., 1997. Rheological properties of UF-Feta cheese determined by uniaxial compression and dynamic testing. J. Texture Stud. 28, 435–454.
Yilmaz, M.T., Karaman, S., Dogan, M., Yetim, H., Kayacier, A., 2012. Characterization of O/W model system meat emulsions using shear creep and creep recovery tests based on mechanical simulation models and their correlation with texture profile analysis (TPA) parameters. J. Food Eng. 108 (2), 327–336.