

The Influence of Marination with Different Salt Concentrations on the Tenderness, Water Holding Capacity and Bound Water Content of Beef

Nesimi AKTAŞ, Muhammet İrfan AKSU, Mükerrerem KAYA
Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum - TURKEY

Received: 19.08.2002

Abstract: *Longissimus dorsi* muscle obtained from beef carcasses was used in this study. Meat cores 2.54 cm in diameter were marinated in 0.34, 0.68 and 1.02 M sodium chloride (NaCl) and 0.05, 0.1 and 0.15 M calcium chloride (CaCl₂) solutions at 5 °C for 72 h. Warner Bratzler Shear (WBS) values were significantly different between samples. The samples marinated with CaCl₂ held less water than those marinated in NaCl. Cooking losses were lower in the control samples than in the marinated samples. Differential scanning calorimetry was employed to determine the amount of bound water in the meat samples. The latent heat of melting (ΔH_m) and bound water were found to be a function of moisture content.

Key Words: Beef, marination, tenderness, bound water, differential scanning calorimetry.

Sığır Etinin Gevrekliği, Su Tutma Kapasitesi ve Bağlı Su İçeriğine Farklı Tuz Konsantrasyonlarının Etkisi

Özet: Araştırmada 4 yaşındaki sığır karkaslarından elde edilen *Longissimus dorsi* kasları kullanılmıştır. Etler 2,54 cm çapa sahip olacak şekilde doğranmış ve 0,34, 0,68 ve 1,02 M sodyum klorür (NaCl), 0,05, 0,1 ve 0,15 M kalsiyum klorür (CaCl₂) çözeltileri ile 1:4 (et: marinasyon çözeltisi) oranında karıştırılarak 5 °C'de 72 saat süreyle marinasyon işlemine tabi tutulmuşlardır. Warner Bratzler Shear (WBS) ile yapılan gevreklik ölçümlerinde örnekler arasındaki WBS değerlerinin önemli ölçüde farklı olduğu tespit edilmiştir. NaCl ile karşılaştırıldığında CaCl₂ ile marinasyona tabi tutulmuş örneklerin daha az su tuttukları belirlenmiştir. Kontrol örneklerinde pişirme kayıplarının tuzlarla marinasyona tabi tutulmuş etlerden daha az olduğu tespit edilmiştir. Differential Scanning Calorimetry etlerde bağlı su içeriğini belirlemede kullanılmış ve bağlı su içeriği ile erime gizli ısısının (ΔH_m) etlerdeki nem içeriğinin bir fonksiyonu olduğu belirlenmiştir.

Anahtar Sözcükler: Sığır eti, marinasyon, gevreklik, bağlı su, differential scanning calorimetry.

Introduction

Many of the technological and sensory properties of meat and meat products depend on the capacity of muscle tissue to bind and hold water. All these properties are associated with changes that take place in meat after slaughter and the application of substances added to meat in the course of technological processes (1). NaCl and CaCl₂ are substances commonly applied in meat technology and meat marination, although CaCl₂ is more frequently used in meat marination. The increase in water holding capacity caused by the application of these salts is attributed to the rise in the solubility of meat proteins as well as to the increase in ionic strength (2,3).

Meat tenderness is one of the main attributes of meat quality. Currently, marination is widely used by consumers to improve meat tenderness and flavour (4). As a mechanical property, it is related to final pH, post-mortem temperature, sarcomere length and enzymatic proteolysis of myofibrillar proteins, particularly troponin and desmin (5,6). Many studies have been undertaken with the aim of reducing the ageing time of meat and the variability in meat tenderness between animals. In particular, salt solutions used in the infusion, injection or marination of meat have a positive effect on tenderness (7-11).

Although a great amount of research has dealt with the effects of NaCl and CaCl₂ on tenderness, no study has analysed their effects on bound water. The present study was carried out to determine the influence of different solutions of NaCl and CaCl₂ on the weight gain, cooking loss, Warner Bratzler Shear (WBS) and bound water content of meat.

Materials and Methods

Materials

The meat samples were obtained from the *Longissimus dorsi* muscle of 4-year-old beef carcasses obtained from a major slaughterhouse in Erzurum, Turkey. The carcasses were chilled for 24 h in a cooling room (5 ± 1 °C). Following the chilling, the *Longissimus dorsi* muscles were removed from the carcasses, and all trimmable fat and connective tissue (epimysium) removed.

Marination of meats with NaCl and CaCl₂ solutions

Since the NaCl concentration in various meat products ranges between 2-6% (0.34, 0.68 and 1.02 M) and the CaCl₂ concentration in meat marination ranges between 0.05, 0.1 and 0.15 M, the same limits were maintained in this study.

1. NaCl: 0.34, 0.68 and 1.02 M solutions.
2. CaCl₂: 0.05, 0.1 and 0.15 M solutions.

Cores 2.54 cm in diameter were removed parallel to the muscle fibre. Each core was weighed and placed in a polyethylene plastic bag. Each core was marinated with NaCl solutions of 0.34, 0.68 and 1.02 M and CaCl₂ solutions of 0.05, 0.1 and 0.15 M for 72 h at 5 °C. The ratio of sample to marinating solutions was 1:4 (meat: marinating solutions).

pH and Moisture Analysis

Moisture percentages were determined from minced meat based on the methods outlined by Gökalp et al. (12) from the cooked and uncooked cores from each treatment. All core pH values were evaluated on, before and after marinating and cooking.

Weight gain and cooking loss

The meat was removed from the marinade, blotted with paper towels to remove excess surface moisture,

and weighed. The blotted sample weight was subtracted from the initial sample weight to obtain the weight gain value. The percentage of weight gain was determined by dividing the weight gain by the initial sample weight. Then, the blotted samples were cooked in cooking bags in an oven for 30 min at 200 °C. The cooked cores were cooled to room temperature, blotted dry, and weighed in order to calculate the cooking loss. The percentage of cooking loss was determined by dividing the difference between the blotted uncooked and cooked weights by the weight of the blotted sample (uncooked).

Warner-Bratzler Shear

After being cooked and weighed, three cores 2.54 cm in diameter were sheared with a WBS device (Model 5KH29GK58, Manhattan, Kansas).

Bound water by differential scanning calorimetry

The bound water contents of the meat samples were determined, using a DSC-50 (Shimadzu Corporation, Kyoto, Japan), as the amount of unfrozen water within a sample after being cooled to -80 °C with liquid nitrogen. Meat samples (10 mg) were weighed into aluminium hermetic cells (Shimadzu 201-53090) and sealed with a crimper. An empty sealed cell was used as a reference. A nitrogen gas flow of 30 ml/min (99.9% N₂) was used to avoid water condensing in the measuring cell. Each sample was cooled to -80 °C and then heated at 5 °C/min to 40 °C. Temperature calibration was carried out with Indium (Mettler standard, mp: 156.6 °C) and water (mp: 0 °C deionised, distilled) using a heating rate of 5 °C/min, as in the measurement. The heat flow was calibrated by using the heat of fusion of Indium (28.45 J/g). The most common method for the determination of the unfrozen water comes from the latent heat of melting (ΔH_m). The melting curve was integrated to determine the ΔH_m . The ΔH_m was divided by the value of water (333 J/g). The unfrozen water content is the difference between the moisture content and the water content obtained from the latent heat of melting (13,14).

Statistical analysis

This experiment was conducted according to a completely randomised block design with three replicates. Analysis of variance of all data was conducted using the general linear models (GLM) procedure (15).

Results

pH and moisture

The proximate compositions of the raw materials used in the study were as follows: moisture, 76.59%; protein, 21.26%; fat, 1.03% and ash 1.12%. All the values in the raw materials were close to the values for fresh meat.

The pH and total moisture content of the meats are shown Table 1. The average pH values of meat marinated with NaCl are higher than those obtained from the control group and other samples marinated with CaCl₂. Similar results were obtained from marinated/cooked samples. The pH values of the samples marinated with NaCl were significantly ($P < 0.05$) higher than in the control samples (Table 1). However, the pH values of the samples marinated with CaCl₂ were significantly ($P < 0.05$) lower than in the control samples (Table 1).

Weight gain and cooking loss

Weight gain values following marinating and cooking loss following marinating/cooking with salts are shown in Table 2. Marinated meats with different concentrations of NaCl and CaCl₂ created a statistically significant change in weight gain ($P < 0.05$, Table 2). The samples marinated with CaCl₂ held less water than the NaCl samples (Table 2). Salt solutions led to statistically significant changes in cooking losses, at the level of $P < 0.05$ (Table 2). The controls exhibited less cooking loss than the samples marinated with NaCl and CaCl₂ solutions (Table 2).

Warner-Bratzler Shear

The WBS values of cooked meats are shown in Table 2. The results of WBS measurements of meat marinated with NaCl and CaCl₂ showed lower WBS values than the

Table 1. Results of Duncan's multiple comparisons test of pH, moisture content, enthalpy and bound water.

Concentration (M)	NaCl									
	After marination					After marinating/cooking				
	pH	Total moisture (%)	Bound water (%)	Latent heat (J/g)	Concentration (M)	pH	Total moisture (%)	Bound water (%)	Latent heat (J/g)	
0	5.46 ^a ± 0.05	76.52 ^a ± 0.33	30.76 ^a ± 1.40	152.37 ^a ± 4.66	0	5.75 ^a ± 0.06	59.68 ^a ± 0.28	39.80 ^b ± 4.06	66.20 ^a ± 3.54	
0.34	5.50 ^b ± 0.07	81.16 ^d ± 0.09	27.30 ^a ± 0.30	179.35 ^a ± 0.99	0.34	5.85 ^c ± 0.09	63.13 ^b ± 0.29	31.35 ^a ± 4.18	105.81 ^b ± 3.92	
0.68	5.58 ^c ± 0.05	79.52 ^b ± 0.06	29.89 ^a ± 1.12	165.27 ^a ± 5.02	0.68	5.86 ^d ± 0.07	63.42 ^{bc} ± 0.05	31.42 ^a ± 3.06	106.54 ^b ± 2.21	
1.02	5.47 ^a ± 0.04	80.21 ^c ± 0.06	31.10 ^a ± 3.34	163.55 ^a ± 1.12	1.02	5.80 ^b ± 0.05	64.36 ^c ± 0.63	32.69 ^a ± 2.08	105.46 ^b ± 2.95	
Concentration (M)	CaCl ₂									
	After marination					After marinating/cooking				
	pH	Total moisture (%)	Bound water (%)	Latent heat (J/g)	Concentration (M)	pH	Total moisture (%)	Bound water (%)	Latent heat (J/g)	
0	5.46 ^d ± 0.05	76.52 ^a ± 0.33	30.76 ^a ± 1.40	152.37 ^a ± 4.66	0	5.75 ^d ± 0.06	59.68 ^a ± 0.28	39.80 ^b ± 4.06	66.20 ^a ± 3.54	
0.05	5.40 ^c ± 0.08	82.40 ^d ± 0.04	25.77 ^a ± 2.79	188.59 ^b ± 5.31	0.05	5.55 ^b ± 0.08	60.98 ^b ± 0.07	24.58 ^a ± 1.98	121.23 ^c ± 3.62	
0.10	5.36 ^b ± 0.07	81.61 ^c ± 0.19	29.24 ^a ± 1.24	174.38 ^{ab} ± 4.13	0.10	5.60 ^c ± 0.06	60.86 ^b ± 0.16	36.50 ^b ± 2.15	81.13 ^b ± 3.17	
0.15	5.29 ^a ± 0.05	80.41 ^b ± 0.07	25.87 ^a ± 3.86	181.64 ^{ab} ± 6.21	0.15	5.41 ^a ± 0.07	64.72 ^c ± 0.07	22.85 ^a ± 2.67	139.48 ^d ± 3.83	

^{a-d} values in a column with the same superscript are not significantly different by Duncan's multiple range test ($p < 0.05$)
±: Standard deviation for three replicates

Table 2. Results of Duncan's multiple comparisons test of WBS, weight gain, and cooking loss.

Concentration (M)	NaCl			CaCl ₂			
	WBS (kg)	Weight gain (%)	Cooking loss (%)	Concentration (M)	WBS (kg)	Weight gain (%)	Cooking loss (%)
0	16.26 ^c ± 0.09	2.14 ^a ± 0.27	46.19 ^a ± 2.34	0	16.26 ^c ± 0.09	2.14 ^a ± 0.27	46.19 ^a ± 2.34
0.34	11.23 ^b ± 0.48	11.77 ^b ± 1.27	52.07 ^c ± 1.12	0.05	12.81 ^b ± 0.86	8.05 ^b ± 1.35	52.68 ^c ± 0.61
0.68	7.68 ^a ± 0.41	14.01 ^c ± 1.60	49.55 ^b ± 1.45	0.10	11.77 ^b ± 0.22	7.86 ^b ± 1.89	53.56 ^c ± 0.65
1.02	9.97 ^{ab} ± 1.60	19.31 ^d ± 2.59	50.32 ^b ± 2.26	0.15	10.16 ^a ± 0.38	10.73 ^c ± 1.42	47.98 ^b ± 0.62

^{a-d} values in a column with the same superscript are not significantly different by Duncan's multiple range test ($p < 0.05$)
±: Standard deviation for three replicates

controls. Samples marinated with NaCl exhibited lower WBS values than CaCl₂-treated samples. The lowest WBS value for marinated samples was obtained at 0.68 M NaCl, and the highest WBS value for marinated samples was obtained at 0.05 M CaCl₂. The results obtained indicated that when the addition of salt to beef ranged from 0.05 to 1.02 M there were significant differences in the WBS values (Table 2).

Bound water by differential scanning calorimetry

The bound water contents and ΔH_m of the meats are given Table 1, which also shows the results of a comparison of the means. The moisture content of the meats was 59.68%-82.40% (Table 1). The ΔH_m determined from the peak area under the curves ranged from 66.20 to 188.59 J/g. The marination of meats with salts had a significant effect on the bound water and ΔH_m values after marinating/cooking ($P < 0.05$, Table 1). This means that the bound water of meat is influenced by the type of additives used. The research showed that an increase in NaCl concentration caused an increase in the amount of bound water (Table 1). The samples marinated with CaCl₂ had less bound water content than the NaCl samples.

Discussion

pH is an important criterion for changes brought about by marination in salts. NaCl and CaCl₂ caused different responses in the pH values in this study. Offer and Knight (3) stress that NaCl depolymerises the thick filament shafts by weakening the interactions between the tails, presumably by favouring the exposure of previously buried charged and/or hydrophilic groups. According to Puolanne et al. (18), this changes the amount and nature of the titratable group accessible to the solvent, probably revealing new groups with pKa values. CaCl₂, either infused into the carcass or injected directly into the muscle following exsanguination, is known to activate the calpain system and enhance tenderisation (7,8,19). The elevation of pH may play a role in the increased activation of calpains and subsequent improvement in meat tenderness.

As a rule increased the pH value of cooked meat was higher than the value of the samples that were not subjected to cooking. The increase of pH values in cooked meats is probably caused by the reduced amount of the available carboxylic group of proteins, and also by the

liberation of calcium and magnesium ion proteins, as proposed by Medynski et al. (1).

Water holding capacity is an important attribute of meat. There was a decrease in water holding due to marination with CaCl₂. This is probably due to proteolysis of the myofibrillar proteins and the decrease in pH shifting towards the isoelectrical point of the myofibrillar proteins (approximately 5.2). These results are parallel to those of Perez et al. (6), Wheeler et al. (9) and Aktaş and Kaya (20). Wheeler et al. (9) indicated that the activities of calpains increased with the addition of exogenous calcium to post-rigor meat, and that proteolysis of myofibrillar proteins had occurred.

NaCl has been shown to increase the water holding capacity of meat, which results in lower cooking losses. In addition, meat pH treated with CaCl₂ before cooking is nearer the isoelectric point of myofibrillar proteins. This explains why there are more cooking losses in meat marinated with CaCl₂.

NaCl may improve tenderness in different ways, such as the solubilisation of proteins from myofilaments. Wu and Smith (21) presented evidence that an increase in ionic strength was effective in solubilising many proteins in myofilaments. Accordingly, NaCl might increase the ionic strength of myofibrils so that tenderisation occurs through the solubilisation of muscle protein. Once the necessary calculations had been made, the WBS values showed a 21%, 27% and 37% improvement over the control with 0.05 M, 0.1 M and 0.15 M CaCl₂ treatments, respectively. Improvements in WBS with CaCl₂ injection treatments in control beefsteaks have been extensively reported by Koohmaraie et al. (7), Morgan et al. (8) and Eilers et al. (22). The hypothesis for this effect has been constructed as CaCl₂ injections sufficiently increasing intracellular calcium concentration to activate the calcium-dependent calpain system and subsequently increasing muscle fibre fragmentation.

It is accepted that high-quality meat should contain a high percentage of bound water. Increasing NaCl concentrations caused an increase in the amount of bound water. This may be explained on the basis of hydrated-ion binding strength, as discussed by Medynski et al. (1). Terrell (23) reported that the addition of both NaCl and CaCl₂ decreases expressible moisture loss (a measurement of free water) significantly, although the decrease in NaCl-added concentrations is much higher than in CaCl₂.

This is parallel to the relationship between the increase in NaCl concentration and bound water. Increasing the salt concentration up to 0.6 M NaCl augments the electrostatic repulsion to such an extent that the myofibrillar structure disintegrates. This effect is utilised in meat curing and emulsion preparation in sausage manufacture, as the salt allows more water to be bound and liberates the myofibrillar proteins so they can act as emulsifiers for fat particles.

There was clearly a decrease in the bound water content of meats treated with CaCl₂ compared with the control samples. The reason for this lies in CaCl₂-induced destabilisation of proteins. Ca⁺² is particularly effective in decreasing the stability of the native conformation of fibrillar proteins in water (promoting unfolding) and is

also an effective destabiliser of the native conformation of globular proteins. Koohmaraie et al. (7) reported that CaCl₂-induced destabilisation of proteins mainly occurs due to the effect of Ca⁺² ions.

Meats marinated in NaCl and CaCl₂ solutions had lower WBS values and were much tender than control samples. We conclude that marination is beneficial in improving the tenderness of meat. Unlike with NaCl, increasing concentrations of CaCl₂ after marination caused a decrease in the percentage of bound water. Additionally, another contribution to the beef industry is that the incorporation of calcium into the meat will also provide an additional source of this mineral without introducing undesirable flavours or any chemical additives that might be harmful to human health.

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