Myofibrillar protein gel properties are influenced by oxygen concentration in modified atmosphere packaged minced beef

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Abstract

Minced beef was stored for 8 days and myofibrillar protein (MP) was extracted to investigate the effect of oxygen concentration (0, 20, 40, 60, and 80%) in modified atmosphere packaging (MAP) on heat-induced gel properties. Compression force of gels was lower when prepared from beef packaged in 0% oxygen, intermediate in 20 to 60% oxygen and greater in 80% oxygen. Total water loss of gels prepared from beef packaged with oxygen (20–80%) was higher and rheology measurements presented higher G' and G'' values. Additionally, gels from beef packaged without oxygen exhibited higher $J(t)$ values during creep and recovery tests, demonstrating that oxygen exposure of meat during storage in MAP affect MP in such a way that heat-induced protein gels alter their characteristics. Generally, storage with oxygen in MAP resulted in stronger and more elastic MP gels, which was observed already at a relative low

oxygen concentration of 20%.

Key words:

Protein oxidation, Water-holding capacity, Gel strength, Rheology, Viscoelasticity

1. Introduction

Modified atmosphere packaging (MAP) is used widely to improve the color and prolong the shelf life of fresh meat. The atmosphere in the packages usually contains mixtures of two or three gases: O_2, CO_2 and N_2. Commercially, high oxygen (70% to 80%) is used as an effective method to maintain the desirable red color of the fresh meat (McMillin, 2008). However, it also promotes oxidation of lipids and proteins, which subsequently decreases the eating quality for instance by promoting the development of a rancid off-flavor, and by reducing tenderness and juiciness (Clausen, Jakobsen, Ertbjerg, & Madsen, 2009; Lagerstedt, Lundstrom, & Lindahl, 2011; Zakrys-Waliwander, O'Sullivan, Allen, O'Neill, & Kerry, 2010; Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012). Therefore, an increased focus is emerging on the effect of the oxygen concentration and on the balance between increased color stability and the negative consequences of lipid and protein oxidation. Zakrys, Hogan, O'Sullivan, Allen, & Kerry (2008) investigated the effects of oxygen concentration (0%, 10%, 20%, 50%, and 80%) on the quality of MAP beef steaks and pointed out 50% O_2 samples were more acceptable to panelists. Bao, Puolanne, & Ertbjerg (2016) showed that premature browning of cooked beef patties occur at a relative low oxygen concentration of 20%. In addition, the mechanisms for oxygen-induced meat quality

changes in high oxygen MAP have been studied. Evidence of formation of protein cross-linking through disulfide bonding for myosin heavy chain has been shown (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Other than disulfide bond, dityrosine bridges or amide bonds between amino groups of lysine and carbonyls also have been suggested to contribute to the formation of cross-links for the protein oxidation in meat (Estevez, 2011; Feng, Li, Ullah, Hackman, Chen, & Zhou, 2015). Cross-linking rather than reduced proteolysis appear to be the main factor which induce the toughening of meat (Bao and Ertbjerg, 2015) .

Several studies have thus focused on the influence of oxygen in MAP on the sensory evaluation, drip loss, tenderness, and the physical and chemical changes which are involved in the lipid and protein oxidation in meat. However, knowledge is lacking on the influence of oxygen concentration in MAP on one of the most important functional properties of muscle protein after cooking, the gel properties. In comminuted meat products, such as hamburgers, myofibrillar protein (MP) is extracted during cooking and form a gel created by a dense protein network (Tornberg, 2005). Muscle protein gelation is thereby contributing to the textural quality of comminuted fresh meat after being cooked by the consumers and the MP are the main gelling substances (Xiong, Blanchard, Ooizumi, & Ma, 2010). Protein oxidation has been recognized to influence the heat-induced gelation properties (Liu & Xiong, 2000). Myofibrils oxidized with $FeCl_3/H_2O_2$/ascorbate exhibited a decrease in thermal stability and gel-formation ability (Liu, Xiong, & Butterfield, 2000). However, these studies were performed in protein

model systems and, therefore, the effect of oxygen in MAP on MP gel properties is still not known.

The objective of the present study was to investigate the effect of different oxygen concentrations (0, 20, 40, 60 and 80% O_2 together with 20% CO_2) on MP gel properties. The MP were isolated from minced beef after 8 days refrigerated storage in the modified atmospheres. Water-holding capacity, gel strength and rheological properties of heat-induced MP gels were evaluated.

2. Material and methods

2.1 Preparation of samples

2.1.1 Raw materials and packaging

Two batches of beef shoulder (*Triceps brachii*) were obtained from a commercial slaughterhouse in Finland. For each batch, around 2.5 kg of *triceps brachii* muscles from two different beef carcasses were used. The muscles were excised at 48 h postmortem, vacuum packaged and transported to the university laboratory. The muscle pH was 5.58 ± 0.04 upon arrival. After removing the external fat and connective tissue, meat was sliced and then minced by a meat grinder fitted with a 4.5 mm cutting disk. Approximately 200 g of minced meat was placed in plastic trays (16.0 cm × 10.5 cm × 2.0 cm) and two trays were prepared for each packaging system. The ratio of headspace to meat volume was about 1:1. Trays were heat-sealed in different oxygen concentrations (0, 20, 40, 60 and 80%) using film based on polyamide/polyethylene (CO 80 HFP, Wipak, Nastola, Finland) with an oxygen transmission rate around 4 cm^3

m^{-2} $(24\ h)^{-1}$. All packages were balanced with N_2 and contained 20% CO_2 to inhibit the microbial growth. MAP was carried out using food grade gasses with a Multivac D-8941 (Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany) equipped with a gas controller Witt-Gasetechnik D-5810 (Witt-Gasetechnik GmbH & Co KG., Witten, Germany). The gas atmosphere (% O_2 and % CO_2) in the packages was controlled by Checkmate 9900 O_2/CO_2 (Dansensor, Ringsted, Denmark). All packages were stored in a 3 ± 1 °C walk-in cooler for 8 days. Tubular fluorescent lamps (OSRAML 36W-76 G13 NATURA, Osram, Munich, Germany) positioned approximately 50 cm above packages were used to give light of approximately 700 lux for 12 hours every day. The 0 day storage samples (48 h postmortem) were sampled from the batch of minced meat before packaging and storage.

After storage, gas atmosphere (% O_2 and % CO_2) in each package was checked and no major change was observed. The minced beef samples were then vacuum-packaged and stored at -80 °C until analysis.

2.1.2 Preparation of MP

Frozen minced meat was thawed at 5 °C overnight. The extraction of MP was conducted as described by Liu, Ruusunen, Puolanne, and Ertbjerg (2014) with some modification. Minced meat (200 g) was homogenized by IKA Ultra-Turrax T25 homogenizer (Labortechnik, Staufen, Germany) at 13,500 rpm for 2 min in 7 volumes of cold rigor buffer (75 mM KCl, 10 mM potassium phosphate, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0). After centrifugation at 10,000 g for 10 min, the pellet was washed twice

by the same process in rigor buffer. The final MP pellet was re-suspended in 15 mM potassium phosphate buffer (pH 6.0) containing 0.1 M KCl and diluted to 80 mg/mL. The protein concentration of the MP pellets was determined by the RC DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

2.1.3 Preparation of MP gels

The obtained MP solutions (80 mg/mL) were used for the preparation of all the heat induced MP gels. The MP gels obtained after cooking were strong enough to be self-supportive. Three different sizes of gels were made to suit the needs for the different modes and devices for the measurements. For cooking loss and water holding capacity tests, 3.0 ± 0.2 g of MP solutions were heated in glass test tubes (15 mm inner diameter). For compression tests, 12 g of MP solutions were heated in plastic test tubes (28 mm inner diameter). For rheological measurements, a metal household muffin pan with 12 holes (45 mm inner diameter at the bottom) was used to make gels. Ten gram of MP solutions was placed in each hole. Before heating, the samples in the test tubes were centrifuged at 200 g for 3 min and the muffin pan was shaken several times in order to get rid of air bubbles in the MP solutions. Thermal-induced gels were prepared by cooking in a water bath at 80 °C for 1 hour followed by chilling on ice.

2.2 Cooking loss

After overnight storage on ice, the chilled gel was taken out of the glass tube, wiped dry with a filter paper and weighed. Cooking loss was measured as weight loss

during cooking and expressed as a percentage of initial MP solution weight (3 g):

$$Cooking\ \ loss\ \ (\%) = \frac{W_0 - W_1}{W_0}\ \times\ 100\ \%$$

where W_0 and W_1 represent the weight (g) of the original MP solution before cooking and the weight of the gel after cooking, respectively.

2.3 Water holding capacity (WHC)

Gels were placed in tubes and centrifuged at a low speed (1000 g for 10 min) at 5 °C (Jin, Kim, Kim, Jeong, Choi, & Hur, 2007). The centrifugation loss was measured as the weight loss during centrifugation and expressed as a percentage of initial gel weight:

$$Centrifugation\ \ loss\ \ (\%) = \frac{W_1 - W_2}{W_1}\ \times\ 100\ \%$$

where W_2 represent the weight (g) of the gel after centrifugation.

Total water loss was measured as the total weight loss during the cooking and centrifugation process and expressed as a percentage of the initial MP solution weight W_0:

$$Total\ \ water\ \ loss\ \ (\%) = \frac{W_0 - W_2}{W_0}\ \times\ 100\ \%$$

2.4 Compression tests

A compression test of the MP gels was performed at ambient temperature with a TA-XT2 Texture Analyser (Stable Micro Systems, UK) according to Kong, Tashiro, & Ogawa (2001). One compression cycle of 90% was used to measure hardness, defined as the maximum force (g) required to achieve the given deformation of the gels by a

cylinder probe P36 (36 mm diameter of the pressing surface). The trigger force was 10 g, and pre-test speed, test speed and post-test speed was 1.5 mm/s.

2.5 Rheological measurements

To maintain stability during the test, gels in the muffin pan for rheological measurements were cut by a cylindrical aluminum slicer (35 mm diameter) into cylinders of 35 mm diameter × 4 mm thickness. An oscillatory rheometer (Haake RheoStress 600, Thermo Electron, Germany) equipped with a 35-mm parallel plate-plate system (222-1266) and data acquisition software (Haake RheoWin) was used.

2.5.1 Stress sweep tests

The linear viscoelastic region of the MP gel samples were measured by running stress sweeps from 0.1 to 2000 Pa at a constant frequency of 1 Hz at 20 °C. G' and G'' were determined as functions of stress. Critical shear stress (σ_c) was calculated from the stress sweep curves as the point at which the complex modulus (G*) deviates more than 10 % from a constant G* (plateau) value (Mezger, 2006). G' is the elastic modulus (or storage modulus), a measure of the energy stored and recovered per oscillation; and G'' is the viscous modulus (or loss modulus), a measure of the energy dissipated and lost as heat per oscillation. G* is the complex modulus $G^* = \sqrt{(G')^2 + (G'')^2}$ (Rao, 2014; Tunick, 2011).

2.5.2 Frequency sweep tests

Frequency sweeps were conducted over the range 0.1–50 Hz at 1 Pa (within the linear viscoelastic region). G' and G'' were determined as functions of frequency.

2.5.3 Creep and recovery tests

An instantaneous stress σ_0 of 10 Pa for all the gel samples was applied for 300 s. After the stress was released, the recovery was observed during 600 s. The creep and recovery results can be described in terms of the sheer compliance function, $J(t) = \gamma(t) / \sigma_0$. J_0 (instantaneous compliance) is the limiting value of the J (t)-function at the very beginning of the creep test (when t=0). J_{MAX} is the maximum deformation corresponding to the compliance value at 300 s in the creep analysis. For $t \rightarrow \infty$, J (t) is equal to J_∞. All these data were obtained by the data acquisition software (Haake Rheo Win).

2.6 Statistical analysis

For each batch, two trays were packaged for each gas combination. Each package resulted in three samples for compression tests and rheological measurements and four samples for determination of water holding capacity. Data was analyzed using the general linear model procedures of SPSS Statistics 17.0. Package atmosphere was included as fixed factor and package batches as random factor. Tukey HSD test was used to identify significant differences at $P < 0.05$.

3. Results

3.1 Compression test

Table 1 shows the influence of oxygen concentration in MAP on compression test values of MP gels. The maximum force of MP gels of 12.7 kg was obtained from day 0 samples.

Table 1. Maximum compression force, cooking loss, centrifugation loss and total water loss of myofibrillar protein gels from minced beef before storage (day 0) and after refrigerated storage for 8 days in modified atmosphere with different oxygen concentrations.

	Day 0	0%	20%	40%	60%	80%	SEM [A]
Compression force (Kg)	12.7^{c}	5.3^{a}	11.4^{b}	11.2^{b}	11.1^{b}	12.2^{c}	0.2
Cooking loss (%)	53.9^{c}	48.5^{ab}	48.2^{a}	49.3^{ab}	49.6^{ab}	50.0^{b}	0.5
Centrifugation loss (%)	6.4^{a}	7.7^{ab}	9.4^{c}	8.8^{bc}	9.1^{b}	8.7^{bc}	0.5
Total water loss (%)	56.8^{c}	50.7^{a}	52.3^{b}	53.2^{b}	53.4^{b}	53.4^{b}	0.5

Data of compression force are expressed as the mean and SEM (n=12)
Data of cooking loss, centrifugation loss, and total water loss are expressed as the mean and SEM (n=16)
[a-c] Means without a common letter differ ($P < 0.05$)
[A] Standard error of the mean

After 8 days storage, the gel hardness decreased ($P < 0.05$) in 0, 20, 40 and 60% oxygen. Compared to gels from meat packaged in oxygen, the hardness of MP gels prepared from beef packaged in 0% oxygen was lower ($P < 0.001$) whereas the highest hardness value of the gels was found after storage in 80% oxygen. The hardness of MP gels prepared from minced beef after storage in 20, 40 and 60% oxygen did not differ

($P > 0.05$).

3.2 Cooking loss

MP gels from day 0 minced beef had higher cooking loss compared to the aged samples ($P < 0.05$) (Table 1). However, cooking loss of the aged samples were similar and only differed ($P < 0.05$) in the comparison between gels prepared from meat packaged in 20 and 80% oxygen with the highest cooking loss of the latter.

3.3 WHC

Centrifugation loss of gels prepared from meat stored between 0% and 40-80% oxygen did not differ (Table 1). Storage in 20% oxygen atmosphere resulted in significant higher centrifugation loss of MP gel compared to 0% oxygen ($P < 0.05$). However, the total water loss of MP gels, calculated from the difference in weight before cooking and after centrifugation, differed between packaging with or without oxygen. Compared to 0% oxygen packaged meat, the total water loss of MP gels prepared from beef packaged in 20-80% oxygen was higher ($P < 0.001$). About 52-53% of total water was lost for the gels prepared from packages with the presence of oxygen.

3.4 Rheological properties

3.4.1 Stress sweep

Typical curves obtained for MP gel stress sweep is shown in Fig. 1-A.

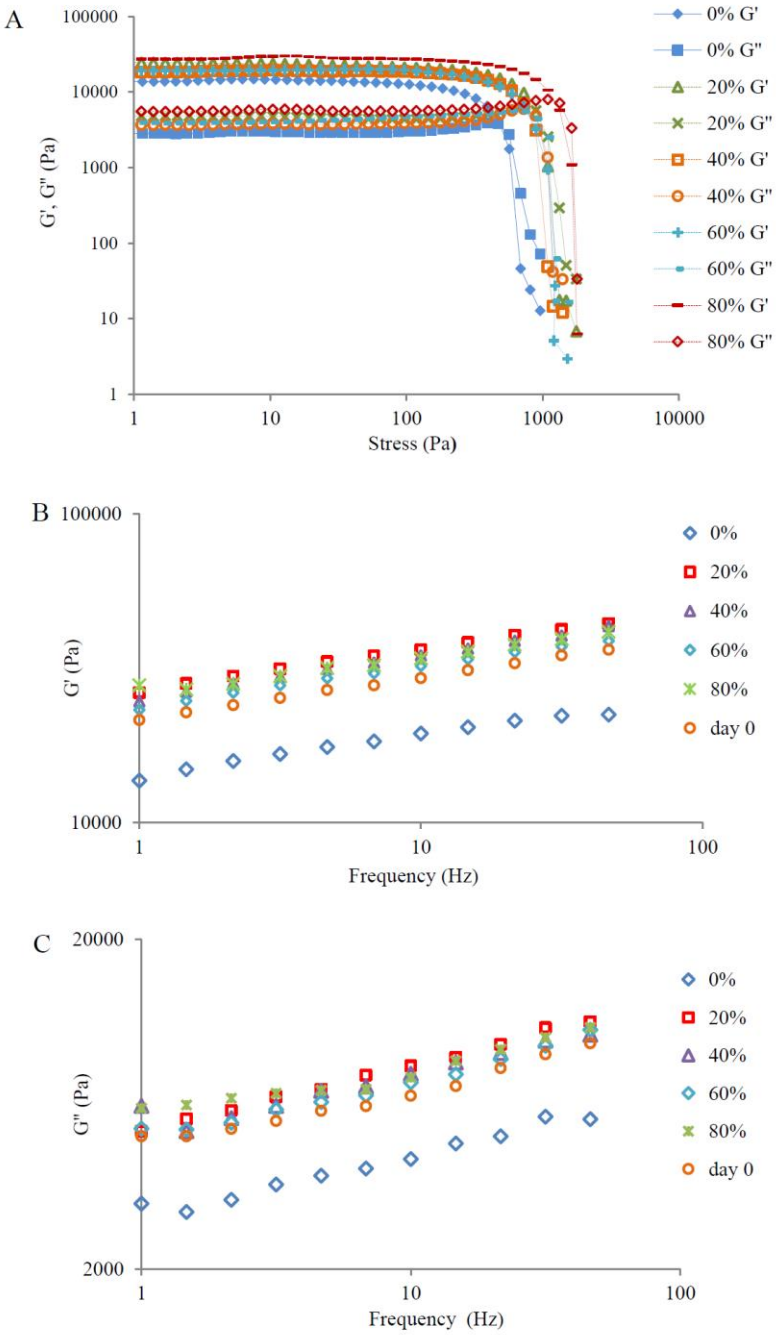


Fig. 1 Stress sweep and frequency sweep curves of myofibrillar protein gels from minced beef stored refrigerated for 8 days in modified atmosphere with different oxygen concentrations. A: Stress sweep at the frequency of 1 Hz; B: Frequency dependence of storage modulus (G') at the stress of 1 Pa; C: Frequency dependence of loss modulus (G'') at the stress of 1 Pa. Data are expressed as the mean (n=12).

The stress range over which both G' and G'' are independent of the applied stress amplitude is defined as the linear viscoelastic region (Steen, Fraeye, De Mey, Goemaere, Paelinck, & Foubert, 2013). The limiting value of the linear viscoelastic region in terms of the shear stress is the yield stress or critical shear stress (σ_c) (Mezger, 2006; Steen et al, 2013). The σ_c therefore indicates the onset of the non-linear region where the system's structure starts to deform under applied stress. A wider linear viscoelastic region, thus a larger σ_c, indicates the system stability to better resist the external stress (Gamonpilas, Pongjaruvat, Fuongfuchat, Methacanon, Seetapan, & Thamjedsada, 2011). MP gels from the 0% and 20% oxygen MAP minced beef had a shorter linear viscoelastic region (Fig. 1-A) and lower values of σ_c (Table 2) compared to gels from 40%, 60% and 80% oxygen MAP. These results indicated that gels from minced beef in low oxygen or oxygen-free MAP had lower stability and required lower stress to cause structure deformation. On the contrary, MP gels from minced beef exposed to higher oxygen concentrations had the highest σ_c and thus had higher stability.

3.4.2 Frequency sweep

Fig. 1-B and Fig. 1-C illustrates the influence of frequency on the G' and G'' in the linear viscoelastic region of MP gels. All the MP gels had a solid-like behavior with G' exceeding G'' over the entire frequency range. Both G' and G'' of the MP gels showed a weaker dependence on frequency and they increased with frequency. G' and G'' of gels prepared from minced beef stored in the presence of oxygen were higher than

without oxygen, indicating that storage of beef in oxygen results in gels with increased elasticity and viscosity. G' and G'' values obtained at 1 Hz are shown in Table 2. MP gels from minced beef stored with oxygen had significant higher G' values ($P < 0.05$).

Table 2. Critical shear stress σ_c, G' (1 Hz) and G'' (1 Hz) of myofibrillar protein gels from minced beef stored refrigerated for 8 days in modified atmosphere with different oxygen concentrations.

	Oxygen					
Trait	**0%**	**20%**	**40%**	**60%**	**80%**	**SEM [B]**
Critical shear stress σ_c (Pa)	70 [a]	160 [ab]	190 [b]	180 [b]	190 [b]	30
G' (Pa)[A]×10⁴	1.4[a]	2.6[b]	2.5[b]	2.3[b]	2.8[b]	0.3
G'' (Pa)[A]×10³	3.5[a]	6.3[b]	6.2[b]	5.3[ab]	6.1[b]	0.8

Data are expressed as the mean and SEM (n=12)
[A] Means of G' and G'' from frequency sweep at 1 Hz
[B] Standard error of the mean
[a-b] Means without a common letter differ ($P < 0.05$)

3.4.3 Creep and recovery

Creep–recovery curves for MP gels as affected by different oxygen concentrations in MAP are presented in Fig. 2. The MP gels from minced beef in 0% oxygen exhibited higher $J(t)$ values during creep and recovery. J_0, J_{MAX}, and J_∞ values of MP gels from 80% oxygen were significant higher compared to MP gels from minced beef stored in 0% oxygen, reflecting greater elasticity and less fluidity of the former gel (Table 3). However, no significant difference was observed in the interval of 20 - 80% oxygen in MAP.

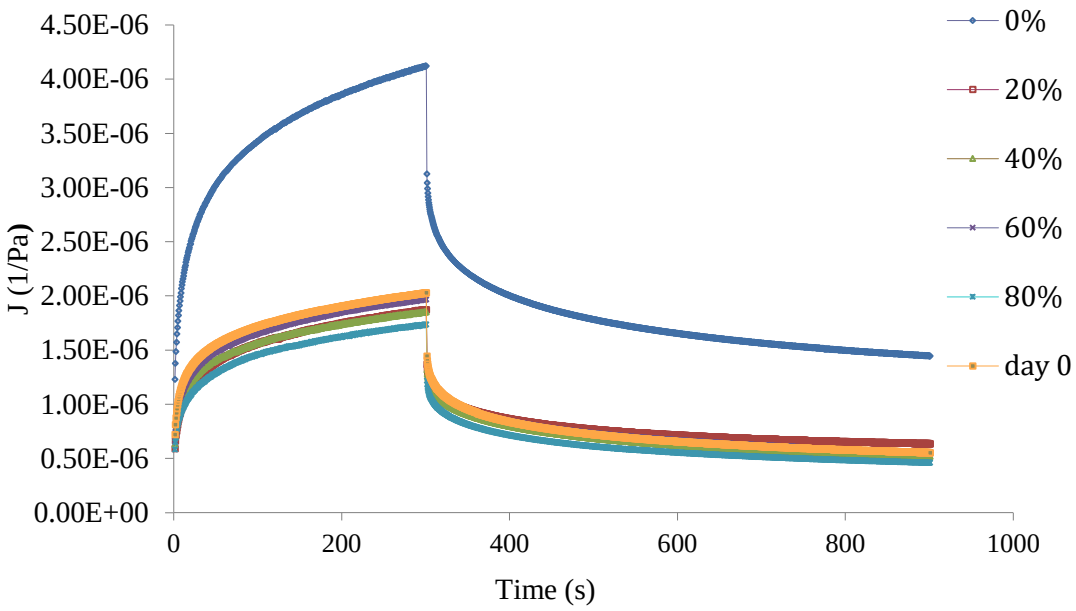


Fig. 2 Creep and recovery curves of myofibrillar protein gels from minced beef stored refrigerated for 8 days in modified atmosphere with different oxygen concentrations. Data are expressed as the mean (n=12).

Table 3. J_0, J_{MAX} and J_∞ obtained from the creep and recovery curves of myofibrillar protein gels from minced beef before storage (day 0) and after refrigerated storage for 8 days in modified atmosphere with different oxygen concentrations.

Recovery parameters	Packages						
	Day 0	0%	20%	40%	60%	80%	SEM[A]
$J_0\times10^{-4}$ (Pa^{-1})	0.6[a]	1.0[b]	0.5[a]	0.6[a]	0.6[a]	0.5[a]	0.1
J_{MAX} $\times10^{-4}$ (Pa^{-1})	2.0[a]	4.3[b]	1.9[a]	1.9[a]	2.0[a]	1.7[a]	0.3
$J_\infty\times10^{-4}$ (Pa^{-1})	0.6[a]	1.5[b]	0.6[a]	0.7[a]	0.5[a]	0.6[a]	0.2

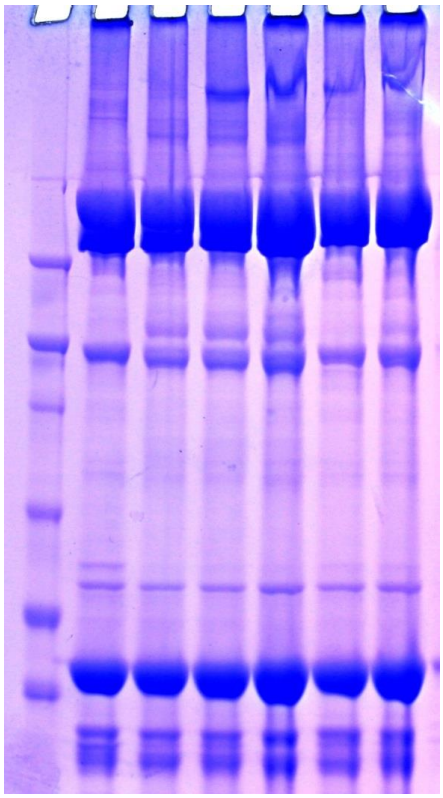
Data are expressed as the mean and SEM (n=12)

[a-c] Means without a common letter differ ($P < 0.05$)

[A] Standard error of the mean

4. Discussion

The results of the current study provide new insights into how the gel properties of myofibrillar protein after cooking are influenced by exposing meat to oxygen during refrigerated storage. Higher gel strength was observed when the gels were prepared from minced beef packaged and stored in the presence of oxygen. High oxygen in the atmosphere (80% oxygen) increased the hardness of MP gels compared to lower oxygen concentrations (20%-60% oxygen). Interestingly, the gels were much weaker when prepared from meat stored in 0% oxygen. Also the rheological properties were markedly influenced by a relative low oxygen concentration of 20% - similar to the oxygen level in air. Packaging of minced beef in MAP systems containing oxygen will thus have an impact on basic properties influencing the meat quality. This information may prove to be useful not only to the producers of minced beef but also for the consumers. Xiong et al. (2010) found that the hardness of gels tended to increase when MP was exposed to oxidizing conditions with H_2O_2 in an iron-catalyzed oxidizing system. It has been shown that intramolecular cross-linking occurs through disulfide bonds when MP is incubated in different model oxidation systems (Ooizumi & Xiong, 2004; Ooizumi & Xiong, 2006; Xiong et al., 2010). Furthermore, formation of cross-linked myosin heavy chain in fresh meat is affected by the oxygen level in MAP (Bao & Ertbjerg, 2015; Lund et al., 2007). In agreement, we observed a high molecular protein band in non-reducing SDS-PAGE and it was referred as cross-linked myosin heavy chain (CL-MHC) (supplementary material).



Supplementary Figure. Representative SDS-PAGE gel of myofibril protein of minced beef stored in modified atmosphere with different oxygen concentrations, run without reducing agent. MHC: myosin heavy chain; CL-MHC: cross-linked myosin heavy chain

The CL-MHC band was seen in all samples packaged with oxygen and appeared already at 20% oxygen. Such cross-links will increase protein-protein interactions and may thus contribute to the increase in G' (elasticity) of the gel network, which previously has been related to an increase in gel strength (Zhou, Zhao, Su, Cui, and Sun, 2014). However, the hardness of gels tended to decline when MP was exposed to a strong oxidizing environment. It was speculated that excessive cross-links of MP before heating would generate large protein aggregates, which might hinder ordered interactions of reactive functional groups and, hence, inhibit formation of a fine gel network (Liu et al., 2000; Xiong et al., 2010; Zhou et al., 2014).

Comparison of the five packaging atmospheres (Table 1) showed that cooking loss

of MP gels was only little affected by the presence of oxygen in the packages. The pronounced higher gel strength due to oxygen in MAP can therefore not be accounted for by a higher water loss during cooking. Higher cooking loss has previously been found in steaks stored in high O_2 and CO_2 MAP compared to vacuum skin packaging steaks, but CO_2 was considered to be the main cause for the increased cooking loss, rather than O_2 (Clausen et al., 2009; Sorheim, Ofstad, & Lea, 2004). An increased total water loss (calculated from the difference in weight before cooking and after centrifugation) was found for MP gels from meat stored in MAP with presence of oxygen (Table 1). It was indicated that oxygen packaging weakened water-binding potential of MP upon cooking. Though the mechanism of water binding in oxidized MP during heating is not yet understood, several previous studies have provided insights into the mechanism of WHC in fresh meat affected by protein oxidation. According to these studies, the loss of water-binding capability is one of the main consequences observed in oxidized meat and it was supposed to be caused by reduced proteolytic activity of tenderizing enzymes and cross-linking of MP (Kim et al., 2010; Lund et al., 2007). Similarly, Bertram, Kristensen, Østdal, Baron, Young, and Andersen (2007) reported that the oxidation reduced the ability of myofibrillar protein to retain water and illustrated the effect of cross-linking on water-holding. In a study by Liu, Xiong, and Chen (2010), expansion of extracellular spaces were induced by oxidation in salt-marinated muscle which reduced the capability of myofibrils to withhold absorbed water. Moreover, the water-binding capacity could also be explained by changes in the net charge of proteins or in protein surface hydrophobicity (Puolanne & Halonen, 2010).

Li, Li, Wang, Zhang, Sun, Wang, & Xie (2014) reported that the surface hydrophobicity of MP increased with increasing H_2O_2 concentrations. It was speculated that more nonpolar amino acids was exposed after cooking which may have reduced the interactions of protein and water.

Heat-induced gelation of the salt-soluble MP leads to the formation of a three-dimensional network which exhibits both viscous and elastic properties (Ikeuchi, Tanji, Kim, & Suzuki, 1992). The viscoelastic behavior of MP gels investigated by rheological measurement have shown that viscoelastic gels responded with G' ≥ G'', indicating a solid-like behavior of the systems (Ikeuchi et al., 1992; Wu, Yuan, Chen, Liu, Ye, & Hu, 2015). Our results are in agreement with these findings. From the viscoelastic parameters σ_c, G' and G'', obtained from the stress and frequency sweep, it is clear that oxygen in MAP resulted in stronger gels with more elastic and viscous characteristics. These results were consistent with the compression test and also in agreement with previous findings that protein oxidation could promote elasticity of MP gels (Cao & Xiong, 2015; Xiong et al., 2010). The creep-recovery test may provide useful information relating to the long-term properties of the gel network structure. J_0 is a coefficient characterizing the elastic deformability of a material and is related to those bonds of structural units that are stretched elastically when the stress is applied (Martínez, Nieto, Castro, Salvatori, & Alzamora, 2007; Mezger, 2006). Lower values of J_0 were observed with the presence of oxygen in the atmosphere during storage (Table 3), suggesting that more elastic bonds were formed in the gels when prepared from meat stored in presence of oxygen. Protein cross-linking has been previously

suggested as an important consequence of packaging meat under oxygen (Bao & Ertbjerg, 2015; Lund et al., 2007, Zakrys-Waliwander et al., 2012). Thus, it can be speculated that the elastic bonds are identical to the protein cross-links that are formed during the storage in oxygen in MAP which gives additional strength to the gels that are formed in the heating process of the MP as illustrated in Fig. 3.

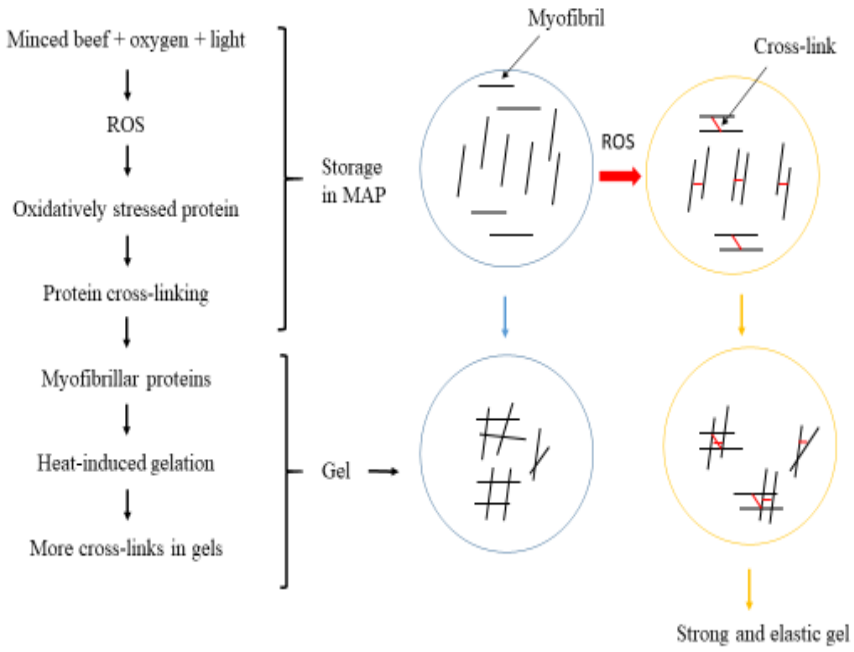


Fig. 3 Hypothesis for influence of oxygen in packaging on formation of elastic bonds in myofibrillar protein gels. ROS: Reactive oxygen species.

This suggestion is supported by Bao and Ertbjerg (2015) and Kim et al. (2010) reporting that cross-links contains myosin and thus may be formed between myosin and myosin, or myosin and titin molecules. The creep-recovery curve defines how compliant a sample is: the higher the compliance the easier the samples can be deformed by a given stress (Schramm, 1994). Gels prepared from meat stored without

oxygen showed the highest J_{MAX} value (Fig. 2 and Table 3) indicating more fluidity of the gels. The irreversible compliance (J_∞) indicates permanent deformation. Deman and Beers (1987) reported that an increase in permanent deformation during recovery indicates the collapsed gel structure and irreversibly broken elastic bonds. Gels prepared from meat stored in presence of oxygen were much more firm and hard (Table 1), as also can be seen from their lower J_∞ values (Fig. 2 and Table 3).

5. Conclusions

In the present study, minced beef was packaged and stored in five different oxygen concentrations. The oxygen level had a significant impact on the heat-induced MP gel properties. Gel strength and rheological properties of MP gels were shown to differ significantly between packaging with or without oxygen. Generally, storage with presence of oxygen in MAP resulted in stronger and more elastic MP gels with reduced water-binding potential. These effects were observed already at a relative low oxygen concentration of 20%. The oxygen concentration in the range 20 – 80% did not affect the rheological properties of the MP gels. The increased gel strength due to oxygen may have been caused by the formation of cross-links during MAP storage.

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