The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology 38(2):32-42

ORIGINAL RESEARCH PAPER

RHEOLOGICAL BEHAVIOR OF PORK *BICEPS FEMORIS* MUSCLE INFLUENCED BY INJECTION-TUMBLING PROCESS AND BRINE TYPE

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Received on 5th April 2014
Revised on 14th July 2014

The effect of tumbling time (1-9 h), injection rate (20, 30, 40, and 50 %) and k-carrageenan addition (0, 0.25, and 0.5 %) on the rheological characteristics of pork *Biceps femoris* muscle were assessed. The results of the creep-recovery tests were analyzed using Burger's equation. Increasing tumbling time up to 9 h along with injection rate also increased compliance values and decreased viscosity. K-carrageenan addition showed the occurrence of a more gel-like structure of the brine-meat system, causing further increase of the compliance and strain values. Samples injected with brine were more elastic compared to those containing k-carrageenan. A longer mechanical treatment provided a softer like matrix. Mathematical modeling of creep-compliance data showed a decreasing tendency for viscosity values with k-carrageenan addition. Discrete retarded elastic compliance values increased when adding k-carrageenan to meat-brine system. Addition of k-carrageenan did not affect the equilibrium compliance values.

Keywords: creep, compliance, viscosity, Burger's model, k-carrageenan, tumbling time

Introduction

The meat-brine system can be considered a particular solid in rheology. From a colloidal point of view, the muscular tissue can be thought of as a system of protein gel mix and a colloidal protein solution. Salt diffusion into the muscular tissue modifies its structure and properties; the texture becomes more gel-like, the water is better retained and the system starts behaving like a viscoelastic material. Knowing the rheological characteristics of solid food ingredients can be very useful for identifying potential industrial applications (Myhan *et al.* 2012).

The viscoelastic behavior of a matrix can be investigated by performing quasi-static and dynamic tests. Creep-recovery and stress-relaxation tests are most typical for quasi-static tests. Chattong *et al.* (2007) showed the dynamic oscillatory measurements to be unsuitable for accurate prediction of products' textural changes and recommended creep tests for this kind of investigation. The creep tests consist of applying a constant stress to the sample and the resulting strain is measured as a function of time (Del Nobile *et al*. 2007).

Pork ham muscles are often used for specialties treated by injection and tumbling. It was observed that mechanical treatment, together with hydrocolloid addition, highly affects product texture and water holding capacity (Ivanovic *et al*. 2002, Lachowicz *et al*. 2003, Pietrasik & Shand 2005, Patrascu *et al*. 2011).
Most of the existing studies are focused on the textural characteristics of whole muscle products, after thermal treatment, whereas the rheological profile during the technological processing before cooking is not covered. Furthermore, the emulsified minced meat products are mostly subjected to textural and rheological testing in comparison with whole meat products. Lachowicz *et al.* (2003) and Żochowska-Kujawska *et al*. (2007) studied the effect of massaging time, together with muscle type, on the rheological characteristics of pork and wild boar meat.
Polysaccharides are successfully used in a wide number of minced and whole meat products in order to improve texture and water holding ability (Kumar & Sharma, 2004). In ham like products κ-carrageenan and alginate are widely and successfully used for restructured meat production (Sun, 2009). The success of carrageenan is due to the low viscosity when dispersed in brine, hydration during heat treatment and jellifying during cooling (Pietrasik & Jarmoluk, 2003). K-carrageenan is an anionic sulphated polysaccharide widely used in food industry as a gelling, thickening and stabilizing agent (Thaiudom & Goff, 2003). It was stated to form thermo-reversible gels in aqueous solution and in presence of cations (Musampa *et al*. 2007). Moreover, Warrand, (2006) reported health benefits of hydrocolloids presence in food products (including carrageenans).
The present study was aimed to investigate the rheological behavior of pork *Biceps femoris* muscle processed by injection with different types and ratios of brine and tumbled for different time intervals. Moreover, the influence of k-carrageenan addition on structure changes occurring during tumbling was investigated and the importance of rheological testing, especially quasi-static ones, on determining the effect of a technological process on muscle structure was highlighted.

Materials and methods

Raw materials

Biceps femoris muscles obtained from both sides of pork carcass (24 h after slaughter) were purchased from a local distributor within a period of two months. Muscles weight varied between 2400 and 2600 g. After purchasing, the meat samples were immediately processed at 4 °C. Any seen fat or connective tissue was removed and muscles were cut in cuboids of approximately 100 g.

Injection and tumbling process

The sample injection was performed manually using a single needle syringe, parallel to muscle fiber distribution in both sides of a cut, so that brine could be uniformly distributed in the entire sample. The brine used for injection was prepared and stored at 4 °C, as described by Patrașcu *et al.* (2013), and consisted of 1.8 % salt, 0.3 % sodium tripolyphosphate, 0.015 % sodium nitrite, 0.3 % sugar and 0-0.5 % k-carrageenan (CaragenanCeamgel M9191, SUPREMIA GRUP, Romania).

The experiment was carried out by varying three factors, summarizing a total of 108 samples per replicate, as following:

- Four injection rates were used: 20, 30, 40 and 50 %;
- Three different levels of k-carrageenan were introduced in the brine, such as to get the following ratios after meat injection: 0 kg k-carrageenan/100 kg meat (brine without any k-carrageenan addition), 0.25 kg k-carrageenan/100 kg meat, and 0.5 kg k-carrageenan/100 kg meat. Carrageenan quantities were added to every injection rate, so that in the end 12 batches resulted. Additive quantities in brine differed every time, so that, after injection, the desired quantity resulted in the final sample. Utilized formula and additive quantities in brine were previously reported in Patrascu *et al*. (2013).
- Variation of tumbling time from one to nine hours.

For the technological section at least three replicates were considered.

The tumbling process was conducted in a small capacity tumbler (ReveoMarivac, USA) using a vacuum of 0.85 bar with a drum speed of 14 rpm. Samples were tumbled intermittently (20 min on, 10 min of), at 4 °C up to 9 h, summarizing a total of 5040 rotations (560 rotations per hour). After the tumbling process, raw samples (uncooked) were subjected to rheological testing.

Rheological measurements

After each hour of tumbling, a piece of meat was removed from the drum, and 2 cm from the external parts of the samples were removed. Afterward a slice of 2 mm was cut perpendicular to muscular fiber distribution (measured in three points with a digital caliper). A circled sample with a diameter of 40 mm was then cut from the center of the slice, using a circular drift. The rheological characterization of the samples was carried out by performing creep-recovery tests using a stress-controlled rheometer (AR2000ex, TA Instruments, Ltd). The temperature was set at 20 °C using a Peltier temperature control system. The room temperature during tests can be explained by Núñez-Santiago *et al*. (2011) who reported temperature of a k-carrageenan solution to be very important for its gel structure conformation, lower temperatures like 9 °C determining the existence of helices with no aggregation and hence the lack of capacity to form self-supporting gels. The procedure was conducted using parallel plate geometry with a 40 mm diameter, and a gap of 2000 μm. For the creep step, a constant stress of 30 Pa was applied for 300 s. A stress-sweep test was preliminary performed to ensure that the creep tests are carried out in the linear viscoelastic domain. For the recovery step, the stress was set at 0 Pa, allowing the sample to recover for a period of 600 s. The obtained data were mathematically modeled using the Rheology Advantage Data Analysis Program (TA, New Castle, DE) by applying an equation which combines Voigt's and Burger's models:

$$J(t)= J_0+sum\{J_k[1-exp(-t/\lambda_{ret})]\}+ t/\eta_0 \qquad (1)$$

where J_0(=1/G_0) is the instantaneous and fully recoverable elastic compliance (Pa^{-1}), J_k defines the retarded (delayed) compliance from Kelvin-Voigt model and can be represented as $J_1+J_2+J_3+...+J_e$ were J_1=1/G_1, (Pa^{-1}) together with the equilibrium compliance J_e=1/G+t/η, after Barnes (2000), λ_{ret} (=η_0/G_1) is the retardation time from Kelvin-Voigt model (s), η_0 is the Newtonian viscosity (Pa×s), and *t* is time (s) as described by Chattong & Apichartsrangkoon (2009) and Sun & Hayakawa (2002).

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel Software with application of Anova Single Factor and Regression. Each rheological experiment was carried out in duplicate and the results were reported as mean values (three technological replicates × two rheological replicates). The Fisher's least significant difference (LSD) test ($p<0.05$) was used to determine differences between treatment means with the Statgraphics Centurion XVI.I software.

Results and discussion

Rheological profile of injected and tumbled samples

Rheological measurements showed that the variation of the tumbling time significantly ($p<0.05$) influenced the creep-recovery evolution by increasing the resulting strain. All samples presented creep and recovery phenomena (Figure 1). For a better visualization of the results, the final strain values recorded after 300 s of applied stress were presented in column figures.

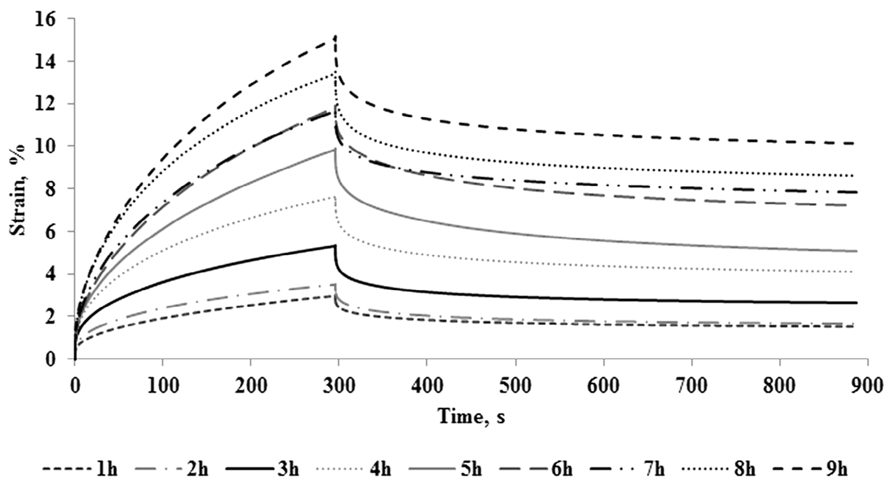

Figure 1. Effect of tumbling time on creep and recovery curves (Results taken from data obtained for samples containing 0.25% k-carrageenan, 20% injection rate)

When using an injection rate of 20% (Figure 2), the elastic behavior of the samples changed gradually after each hour of tumbling, longer mechanical treatment resulting in softer matrices. For the meat samples injected with 20% simple brine, the maximum strain values recorded after 300 s of applied stress increased linearly from 1.76 %, after one hour of tumbling, to 4.15 %, after nine hours of mechanical treatment.

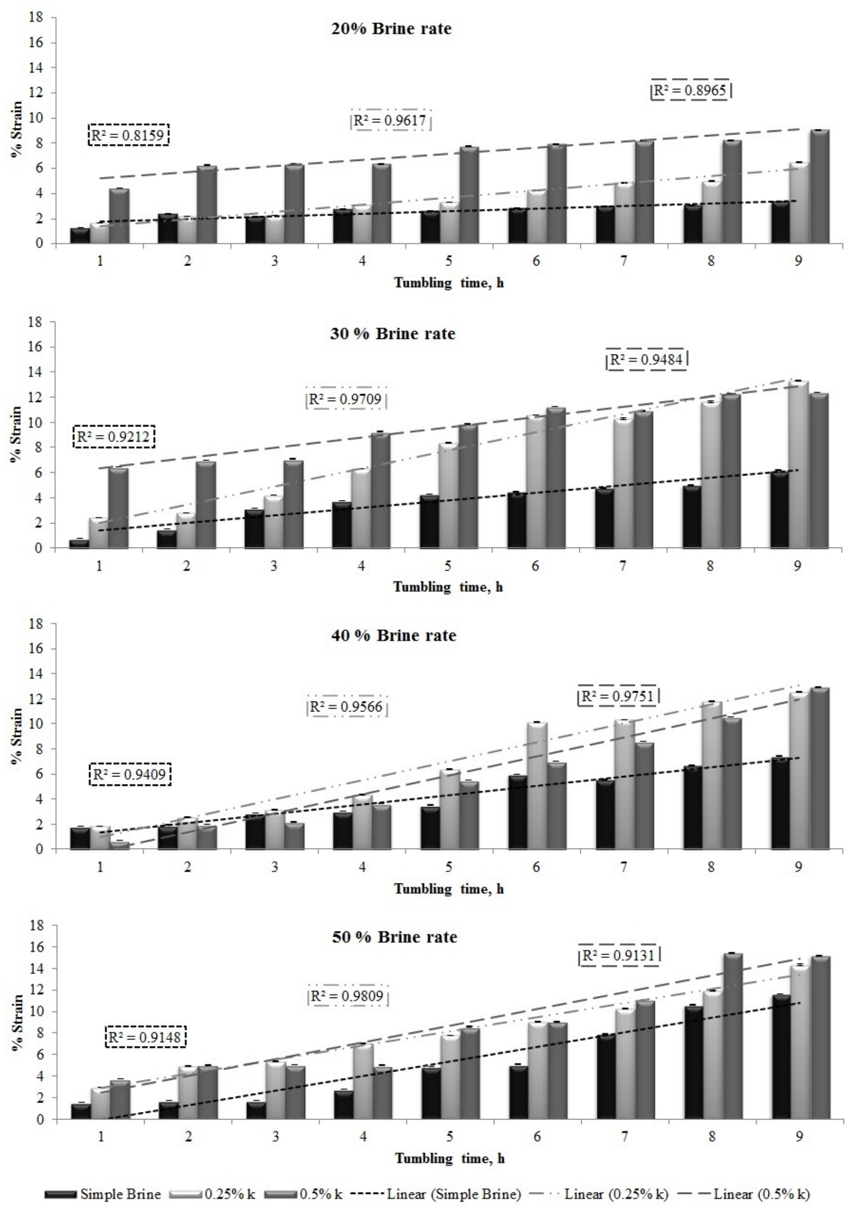

Figure 2. Strain values recorded after 300 s of creep for analyzed samples depending on tumbling time, k-carrageenan and brine rate. 0.25%k stands for samples containing 0.25kg of k-carrageenan/100 kg meat and 0.5% stands for samples containing 0.5kg of k-carrageenan/100 kg meat

A similar trend was observed in case of the samples injected with brine supplemented with k-carrageenan at two different levels, when significantly higher compliance and strain values were recorded each hour during the entire tumbling period ($p<0.05$). In all cases a linear distribution in time of the strain values was obtained (Figure 2).

Further increase of the injection rate up to 50% resulted in curves with higher values for strain and compliance. The strain values measured at the end of the creep period are comparatively presented in Figure 2. In this regard, it was observed that, at the end of every hour of tumbling, depending on the brine rate injected into the sample and level of k-carrageenan addition, the strain values gradually increased in almost all cases.

Comparisons were also made between strain values recorded for each hour of tumbling for all four injection rates used. It was observed that, after 300 s of applied stress until the fifth hour of tumbling, the strain data were not linearly distributed, but significantly different ($p<0.05$). Taking into account these results, we might consider that, for the same injection rate, small and medium tumbling periods do not necessarily influence the elastic behavior of the muscle. A linear distribution of stress data was however recorded at the end of creep, starting from the sixth hour of tumbling when comparing the data for injected samples with simple brine in four levels. K-carrageenan addition in low concentrations (0.25 and 0.5 %) also determined a linear increase of strain data (for different injection rates and same hour of tumbling) at the end of the tumbling process, beginning with the seventh hour. Lachowicz *et al.* (2003) reported that the elasticity of *Biceps femoris* muscle decreased by about 30 % between six and 12 hours of effective massaging. Low levels of carrageenans (0.5 %) were reported to be sufficient to generate palatable binding in restructured pork. The increase of the carrageenan concentration up to 1.5÷2 % does not affect the water holding capacity of restructured pork (Hong *et al*. 2008).

Burger's model for analyzing rheological profile of the samples

Appling Burger's mathematical model on creep curves can provide six viscoelastic parameters, as described previously in equation 1. In the present work we focused on four rheological parameters of Burger's model, namely J_1, G_1, η_0 and J_e, which showed to have a significant statistical relevancy.

Table 1 illustrates that both analyzed compliances (J_1 and J_e) increased with increasing the tumbling time for samples injected with simple brine. Since compliance is the reciprocal of the elastic modulus, lower compliances values denoted a greater rigidity of gels. These results are in agreement with our previous observations (Patraşcu et al. 2011), confirming that a prolonged tumbling time softens meat texture. The Newtonian viscosity η_0 displayed a decrease with a longer mechanical treatment, revealing a less rigid matrix.

Another important factor that influenced viscosity values was the injection rate. Lower viscosity values were obtained for the samples with higher brine percentages. K-carrageenan addition at two different concentrations, 0.25 and 0.5 %, resulted in higher values for J_1, and surprisingly lower values for J_e compared to samples with no added hydrocolloids (Table 2 and Table 3).

Table 1. Creep compliance parameters determined with Burger's model for creep curves of samples injected with simple brine in different proportions, after each hour of tumbling process

Sample	Tumbling time, h	$J_1\times10^{-4}$(1/Pa)				G_1(Pa)				$\eta_0\times10^5$ (Pa×s)				$J_e\times10^{-4}$ (1/Pa)			
		Injection rate, %				Injection rate, %				Injection rate, %				Injection rate, %			
		20	30	40	50	20*	30	40*	50*	20*	30	40	50	20	30	40	50
Simple Brine	1	1.85	1.22	2.57^{a}	2.26^{a}	5383^{a}	8182	3883^{a}	4422^{a}	13.13^{a}	22.43	8.45^{a}	11.05	3.69^{a}	2.46^{a}	4.87^{a}	4.08^{a}
	2	3.61^{a}	2.44	2.80^{ab}	2.39^{ab}	2762^{a}	4084	3572	4179^{b}	7.60^{ab}	13.44^{a}	8.77^{a}	10.03^{a}	6.21^{b}	4.07^{a}	5.67^{ab}	4.67^{ab}
	3	3.64^{ab}	6.82^{a}	3.86^{abc}	2.49^{ab}	2745^{a}	1465^{a}	2589	4016^{c}	6.82^{a}	11.93a	5.64^{b}	9.71^{a}	6.23^{b}	10.8^{b}	7.09^{bc}	4.63^{ab}
	4	4.42^{c}	5.93	5.26^{cd}	4.22	2259^{a}	1686^{a}	1900^{a}	2370^{a}	7.41^{ac}	5.56^{b}	7.09	7.06	7.49^{c}	9.70^{bc}	8.39^{c}	7.81
	5	3.73^{abc}	7.09^{ab}	5.31^{cd}	7.23^{c}	2680^{a}	1409^{ab}	1881^{a}	1382^{a}	6.83a	4.84^{bc}	5.39^{b}	3.74^{b}	7.28^{cd}	10.82^{bcd}	9.97	12.21^{c}
	6	4.18^{abcd}	7.71^{bc}	10.68^{e}	7.80^{c}	2392^{a}	1296^{abc}	935^{a}	1282^{a}	6.17^{a}	5.54^{bcd}	3.83^{c}	3.81^{b}	7.59^{cde}	12.84^{bde}	16.96	12.94^{c}
	7	4.85^{cde}	7.42^{abcd}	8.28	10.82	2057^{a}	1346^{abcd}	1208^{a}	924^{ab}	7.09^{a}	3.85^{bcde}	3.29^{cd}	1.98^{c}	8.52	11.38^{b-f}	14.84	15.28
	8	4.94^{cef}	7.12^{abcd}	9.95^{ef}	17.74	2020^{a}	1404^{abcd}	1005^{a}	564^{abc}	5.1^{a}	3.26^{b-f}	2.85^{cde}	1.94^{cd}	9.77	11.34^{b-f}	19.39^{d}	25.94
	9	4.50^{cdef}	10.19	11.46^{ef}	19.72	2220^{a}	981	872^{a}	507^{abc}	4.61^{abc}	3.67^{b-f}	2.67^{cde}	1.68^{cd}	7.47^{cde}	16.68	20.54^{d}	27.84
	P value	$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}>0.05$; $p_{rows}<0.05$			

Means with same superscript within same column do not differ significantly ($p>0.05$).

*Means with same superscript within same column differ significantly ($p\leq0.05$).

Table 2. Creep compliance parameters determined with Burger's model for creep curves of samples injected with 0.25kg of k-carrageenan/ 100 kg meat in different proportions, after each hour of tumbling process

Sample	Tumbling time, h	$J_1\times10^{-4}$(1/Pa)				G_1 (Pa)				$\eta_0\times10^5$ (Pa×s)				$J_e\times10^{-4}$ (1/Pa)			
		Injection rate, %				Injection rate, %				Injection rate, %				Injection rate, %			
		20	30	40	50	20	30	40	50	20	30	40	50*	20*	30	40	50*
0.25% k-carrageenan	1	3.37^{a}	3.00	2.88	2.21	2967^{a}	3332	3470	4513	6.41^{a}	6.48^{a}	8.35	2.05^{a}	0.65^{a}	0.54^{a}	0.45^{a}	2.53^{a}
	2	3.65^{ab}	4.20	4.82^{a}	5.27	2736^{a}	2380	2073^{a}	1896	10.44	6.79^{a}	11.60	2.84^{ab}	0.69^{b}	0.74^{ab}	0.84^{ab}	0.82^{ab}
	3	3.22^{ab}	5.93	4.84^{a}	8.57^{a}	3097^{a}	1685	2063^{a}	1167^{a}	7.08^{ab}	3.52^{b}	5.69^{a}	3.18^{ac}	0.58^{c}	0.96^{ab}	0.82^{abc}	0.13^{ab}
	4	4.36^{c}	10.22	6.41	8.75^{a}	2292^{b}	978^{a}	1559	1142^{ab}	7.81^{b}	3.28^{b}	4.00^{ab}	2.28^{d}	0.94^{d}	1.66^{c}	1.04^{bcd}	0.14^{ab}
	5	4.99^{c}	11.46	8.58	9.70^{a}	2001^{b}	872^{a}	1165	1031^{ab}	5.65^{ac}	1.95^{c}	2.60^{bc}	2.03^{c}	0.92^{e}	1.79^{c}	1.47^{d}	0.16^{ab}
	6	6.68^{d}	18.22^{a}	14.67	13.88	1497^{c}	548^{b}	681^{b}	720^{c}	4.51^{cd}	2.06^{cd}	1.85^{cd}	2.01^{c}	1.18^{c}	2.61^{d}	2.43^{e}	0.22^{ab}
	7	7.85^{e}	18.09^{a}	23.28^{a}	12.04	1274^{cd}	552^{bc}	429^{bc}	831^{c}	4.27^{d}	2.26^{cde}	2.94^{b-e}	1.53^{bc}	1.37^{abc}	2.67^{de}	3.10	0.21^{ab}
	8	7.24^{de}	20.90	22.77^{a}	19.63	1381^{cd}	478^{bcd}	439^{bcd}	509^{d}	3.03^{e}	1.86^{c-f}	2.39^{b-f}	1.70^{bc}	1.21^{ac}	2.93^{def}	3.54	0.27^{ab}
	9	10.48	23.30	18.65	17.59	954^{d}	429^{bcd}	536^{bcd}	569^{d}	2.73^{e}	1.50^{c-f}	1.38^{c-f}	1.05^{bcd}	1.51^{a-e}	3.12^{ef}	2.65^{e}	0.25^{ab}
	P value	$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}>0.05$; $p_{rows}<0.05$				$p_{columns}<0.05$; $p_{rows}<0.05$				$p_{col}<0.05$; $p_{rows}>0.05$			

Means with same superscript within same column do not differ significantly (p>0.05).
*Means with same superscript within same column differ significantly (p≤0.05)

Table 3. Creep compliance parameters determined with Burger's model for creep curves of samples injected with 0.5kg of k-carrageenan/100 kg meat in different proportions, after each hour of tumbling process

Sample	Tumbling time, h	J_1×10^{-4}(1/Pa)				G_1(Pa)				η_0×10^{5} (Pa×s)				J_e×10^{-4} (1/Pa)			
		Injection rate, %				Injection rate, %				Injection rate, %				Injection rate, %			
		20	30	40	50	20	30	40	50	20*	30*	40	50	20**	30*	40	50
0.5% k-carrageenan	1	12.05	9.59^{a}	1.26^{a}	4.90^{a}	829^{a}	1042	7929	2039	19.95^{a}	2.81^{a}	25.61	4.13^{a}	1.95	1.42^{a}	0.26^{a}	0.74^{a}
	2	9.68^{a}	11.44^{b}	3.00^{ab}	7.12^{ab}	1032^{b}	874^{a}	3330^{a}	1404^{a}	2.88^{a}	3.01^{b}	9.31^{a}	3.27^{b}	1.45	1.69^{b}	0.51^{ab}	1.13^{ab}
	3	8.98^{a}	11.02^{ab}	3.11^{abc}	7.28^{abc}	1113	907^{a}	3207^{a}	1372^{ab}	2.74^{a}	2.40	8.14^{ab}	3.31^{bc}	1.46	1.55^{c}	0.63^{abc}	1.15^{abc}
	4	9.96^{a}	13.46^{c}	5.02^{abcd}	7.80^{bc}	1003^{b}	742	1989	1282^{ab}	3.03^{a}	1.93^{b}	4.54^{c}	3.71^{abc}	1.63	2.04^{d}	0.91^{abcd}	1.32^{abcd}
	5	12.57	16.26^{d}	8.39^{bcde}	13.33^{d}	795^{a}	615^{b}	1191^{b}	750^{c}	2.33^{a}	2.22	3.52^{cd}	2.16^{d}	1.83	2.52^{abc}	1.53^{de}	1.93^{de}
	6	14.11^{b}	17.66^{de}	9.47^{def}	15.40^{d}	708^{c}	566^{bc}	1055^{bc}	649^{cd}	2.77^{a}	1.73^{ab}	2.55^{cde}	2.41^{de}	2.04	2.56^{abc}	1.88^{ef}	2.31^{ef}
	7	14.17^{bc}	15.10^{cd}	23.97^{g}	18.41	706^{cd}	662^{b}	417^{d}	543^{d}	2.63^{a}	1.66^{ab}	8.81^{ab}	1.63^{def}	2.06	2.61^{abc}	3.64^{g}	2.58^{f}
	8	14.15^{bcd}	20.80	14.12^{ef}	30.35^{e}	706^{cde}	481^{d}	708^{bcde}	329^{e}	2.77^{a}	1.72^{ab}	1.65^{def}	1.60^{defg}	2.22	3.00^{abcd}	2.43^{fgh}	3.88^{g}
	9	14.80^{bcd}	18.66^{e}	20.57^{g}	28.79^{e}	675^{cde}	536^{cd}	486^{cde}	347^{e}	2.13^{a}	1.57^{ab}	1.53^{def}	1.47^{dfg}	2.13	2.97^{abcd}	3.05^{h}	3.84^{g}
	P value	$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}<0.05$; $p_{rows}>0.05$				$p_{col}>0.05$; $p_{rows}<0.05$				$p_{col}>0.05$; $p_{rows}<0.05$			

Means with same superscript within same column do not differ significantly ($p>0.05$).
*Means with same superscript within same column differ significantly ($p\leq0.05$).
**Means within same column did not present any differences.

The equilibrium compliance (J_e) was stated by Barnes (2000) to be, along with viscosity (η), the most important data to collect for many applications, J_e being a measure of elasticity at short times and η a measure of steady-state deformation at long times. This could mean that, at short stressing time, meat samples containing k-carrageenan tumbled for a certain period will respond with a harder structure which will soften eventually. Samples injected with simple brine showed an opposite behavior (Table 1). They presented a more tender structure at shorter times and a more rigid one at long-time behavior.

When considering the delayed elastic response (J_1), significant differences ($p<0.05$) were recorded between samples injected with simple brine and those with added k-carrageenan, at low injection rates (20 and 30%). No important differences among samples containing 0.25 % and 0.5 % of k-carrageenan were recorded ($p>0.05$) in terms of J_1 values, regardless of the level of brine injected.

The viscosity of samples containing k-carrageenan was significantly ($p<0.05$) lower when compared with samples injected with simple brine. Increasing the k-carrageenan level from 0.25 % to 0.5 % did not provide important statistical differences in terms of viscosity values ($p>0.05$).

Conclusions

Increasing the tumbling time up to nine hours, together with the injection rate (20, 30, 40, and 50 %) resulted in samples with softer gel structure. The addition of 0.25 % and 0.5 % of k-carrageenan had as a result higher values for J_1 compared with samples injected with simple brine. The values of the equilibrium compliance (J_e) were higher with respect to elastic compliance (J_1) in case of simple brine injection. When adding k-carrageenan to the meat-brine system, the resulted J_e data were lower than those recorded for J_1. Using different concentrations of k-carrageenan did not lead to significant differences between the creep compliance parameters recorded during experiment. Viscosity values were lower when using brine with k-carrageenan for injection.

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