STUDIA UBB CHEMIA, LIX, 1, 2014 (p. 13-21)
(RECOMMENDED CITATION)

RHEOLOGICAL AND PHYSICAL CHARACTERISTICS OF MINCED *BICEPS FEMORIS* MUSCLE IN DIFFERENT BRINING SYSTEMS

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ABSTRACT. The effect of brine rate (20, 30, 40 and 50 %) and k-carrageenan addition (0, 0.25 and 0.5 %) on rheological and physical characteristics of pork *Biceps femoris* muscle was investigated. Minced muscle was mixed with different types of brine so that a total of 12 samples resulted in order to obtain meat restructured products without fat addition. Water holding capacity of admixtures was determined. After cooking process samples in the form of meat restructured products were subjected to textural analysis determining samples' hardness. Rheological tests were carried out by applying oscillatory dynamic tests and behavior of G', G'' and δ angle was characterized. Results showed that water holding capacity, represented by expressible moisture (EM, %), significantly increased ($p<0.05$) with k-carrageenan addition for samples with 20 and 30% of added brine. Samples' hardness was also significantly influenced ($p<0.05$) by k-carrageenan addition with exception of samples with 40 and 50% of added brine. Rheological data showed a higher G' versus G'' in all cases when subjected to frequency sweep and temperature ramp tests. For every percent of added k-carrageenan G' values had a decreasing tendency in most of the cases when more brine was added to the system.

Keywords: *Hardness, water holding capacity, viscoelastic moduli, k-carrageenan.*

INTRODUCTION

Meat restructured products are wide spread on the food market and the majority of meat available nowadays is processed to some extent [1]. For economic benefits and for the fact that consumers judge quality based on tenderness, products made from chopped meat have various types of lipids added, from animal or vegetable sources. On the other hand consumers demand healthier products with low fat content [2]. Reducing fat content

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without affecting water holding capacity and texture can be achieved by using fat replacers such as hydrocolloids, vegetable and connective-tissue proteins [3].

Carrageenans are widely used in the food industry because of its water binding, thickening and gelling properties [4-6], with *kappa* form being the most common. More, Warrand [7] reported health benefits of hydrocolloids presence in food products (including carrageenans).

Rheological tests in food industry are important because of the possibility to characterize raw material before processing, intermediary product before manufacturing but also the final product itself [8].

The objective of this study was to investigate the effect of k-carrageenan addition and brine rate on water holding capacity; textural and rheological characteristics of minced *Biceps femoris* muscle in order to obtain a meat restructured product with no added fat.

RESULTS AND DISCUSSIONS

Physical characteristics

Results showed in figure 1 present the effect of k-carrageenan addition and brine rate on samples water holding capacity. Adding k-carrageenan to *Biceps femoris* muscle–brine system significantly ($p<0.05$) decreased quantity of brine lost during centrifugation at little brine rates used.

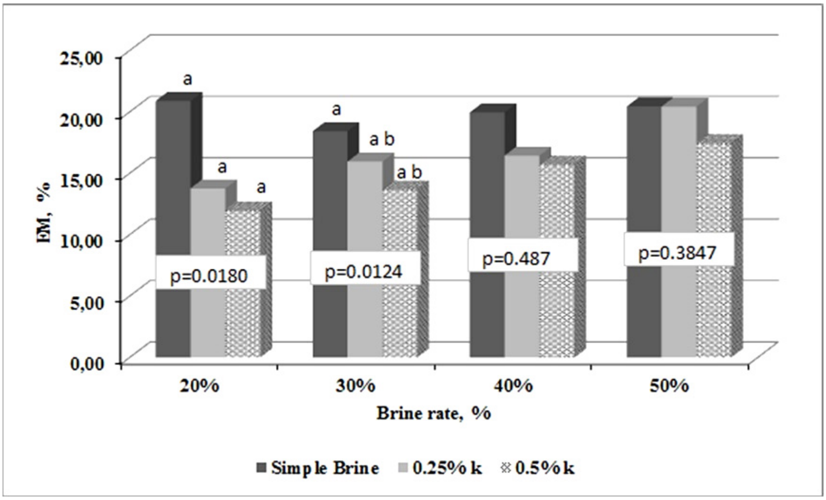

Figure 1. Expressible moisture of samples treated with different types of brine added in four rates. K-stand for k-carrageenan. Values marked with same letter within same block of columns differ significantly ($p<0.05$).

Contrary, when 40 and further 50 % of brine was added to the system the effect of k-carrageenan addition in low quantities decreased, values being insignificantly different ($p>0.05$). More, increasing brine intake resulted in a linear increase in expressible moisture values for samples were k-carrageenan was added ($R^2=0.905$ for samples with 0.25 % of added k-carrageenan and $R^2=0.998$ for samples with 0.5 % of added k-carrageenan). Verbeken, et al. [5] reported that increasing carrageenan concentration led to an increase in water holding capacity (WHC) of meat extracts. An improvement in WHC of meat emulsified systems/sausages with different concentrations of added k-carrageenan was also reported by Ayadi, et al. [9] and Patrașcu, et al. [10].

When speaking of samples' texture, results presented in figure 2 showed tendencies similar to those encountered for expressible moisture. Therefore, hardness significantly decreased ($p<0.05$) with k-carrageenan addition for samples mixed with 20 and 30 % of brine. Further increasing brine rate led to a smoothing in hardness's values. Analyzing hardness for every type of brine used in the survey when increasing brine rates from 20 to 50 % denoted linear regression in all cases.

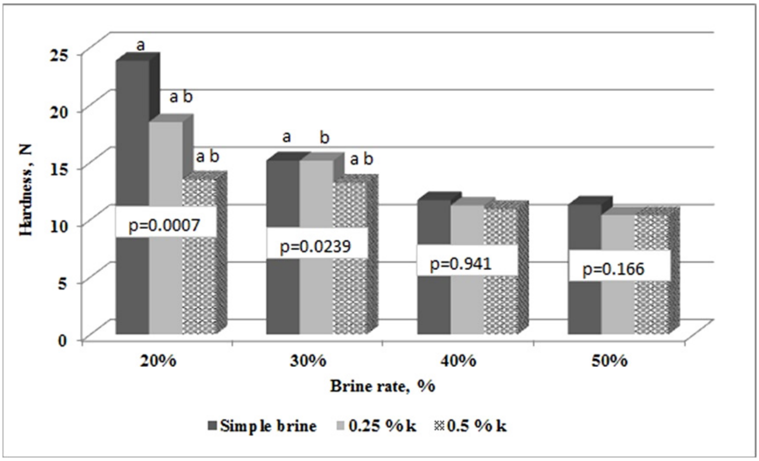

Figure 2. Hardness for samples treated with different types of brine added in four rates. K - stand for k-carrageenan. Values marked with same letter within same block of columns differ significantly ($p<0.05$).

Thus, for samples with simple brine in the system, increasing brine rate showed a linear decrease in hardness with $R^2=0.828$, for samples with 0.25 % of added k-carrageenan $R^2=0.946$, and for samples with 0.5% of added k-carrageenan $R^2=0.902$. A slightly decreasing effect of k-carrageenan addition on hardness was reported by Alexa, et al. [11], & Patrașcu, et al. [10]. Cierach, et al. [*3*] reported that sausages containing carrageenan, independently of the amount of the addition were characterized by significantly higher values for hardness. It seems that k-carrageenan presence in products containing any amount of fat strengthens the final product. Fat being softer than the muscle itself, it is expected for k-carrageenan to soften texture of any meat product with no added fat, as reported by Patrașcu, et al. [12], when addition of k-carrageenan to ham like products determined a decrease of product's hardness. The influence of fat level on the texture of sausage has been previously reported by Hensley & Hand [13], and Candogan & Kolsarici [14] who noted that hardness was correlated with fat content.

Rheological data analyses

When speaking of food products with hard texture (named solids in rheology), there can be performed creep tests over a range of time and oscillatory tests over a range of frequency. So that the short times will correspond to high frequencies and long times will relate to low frequencies [15]. The data presented in figure 3 (A, B, C), show admixtures' rheological behavior during frequency sweep test. It can be seen that for every type of brine used, increasing brine rate led to a decrease in G' values. However, for the entire experiment, G' values were higher than those of G'', including samples in solid-like materials. When looking at G' curves for samples with similar brine level and different k-carrageenan concentration there seem to be no effect of hydrocolloid addition. But, at a lower frequency, the data showed different tendencies. For samples with 20 % of added brine G' values recorded at 0.1 Hz, decreased with increasing k-carrageenan concentration from 2160 Pa to 1392 Pa (R^2=0.966). For samples with 30 % of added brine at 0.1 Hz G' values decreased from 1343 Pa to 843 Pa (R^2=0.895). For samples with 40 % of added brine G' values decreased from 1434 Pa to 807 Pa (R^2=0.842) and for 50 % of added brine G' values decreased from 940 Pa to 697 Pa (R^2=0.981) confirming that k-carrageenan addition softened admixtures structure. Delta values recorded during frequency sweep test, presented significant differences with k-carrageenan addition (Table 1) increasing from ~20˚ for simple brine to ~30˚ for samples with 0.5 % of k-carrageenan. Delta values below 45˚ enclose the mixtures in solid-like materials.

Table 1. Effect of brine rate and k-carrageenan addition on delta values (δ˚) during frequency sweep test

Sample type	Injection rate			
	20 %	**30 %**	**40 %**	**50 %**
Simple Brine	$20.66{\pm}1.2^{a}$	$21.26{\pm}1.51^{a}$	$20.49{\pm}1.12^{a}$	$21.73{\pm}0.99^{a}$
0.25% k-carrageenan	$19.85{\pm}1.68^{b}$	$29.94{\pm}2.9^{a}$	$31.61{\pm}2.47^{a}$	$34.04{\pm}0.68^{a}$
0.5% k-carrageenan	$27.79{\pm}2.6^{ab}$	$32.06{\pm}1.83^{a}$	$33.4{\pm}1.35^{a}$	$34.05{\pm}0.17^{a}$
p value	*0.0453*	*0.0314*	*0.0094*	*0.0006*

Values represent means ± Standard Deviation
Means with same superscript within same column differ significantly ($p<0.05$)

Temperature ramp test highlighted the moment of protein denaturation which took place at ~50÷60 °C, as seen in figure 3 (D, E, F). After that moment G' curve showed a sudden growing tendency until the final test temperature of 72 °C. Rheological tests with rising temperature are often utilized for observing material behavior during heat treatment, being possible determination of proteins' nature preventing destruction of the system [16, 17]. All samples began to form a gel like structure as seen from viscoelastic moduli curves at about 40 °C, similar to findings of Westphalen, et al. [18]. This tendency continued till ~ 60 °C. The inflexion points are associated with myosin denaturation [19]. The mechanism through myosin form a gel is thought to involve the aggregation of the heavy meromyosin (HMM), which is the head of the protein, and the formation of a network amongst the LMM, or tail portions of the protein [20]. The exact temperature of these transitions was observed from G' data recorded at temperatures indicated to be the inflection points (41, 50 and 57 °C) as reported by Westphalen, et al. [18]. Elasticity of paste meat products continued to develop after gelling point. This corresponds to reinforcement of the gel network [21].

Delta curves during temperature ramp test showed tendencies similar to those of viscoelastic moduli, presenting significantly different values ($p<0.05$) between temperature range of 4 ÷ 50.7 °C and 51 ÷ 72 °C, as seen in table 2. With exception of samples with 20% of added brine in other cases (samples with 30, 40 and 50 % brine) k-carrageenan addition significantly increased delta values ($p<0.05$).

Table 2. Effect of brine rate and k-carrageenan addition on delta values (δ˚) during temperature ramp test, before and after protein denaturation

Injection rate	Temperature range, ˚C	Simple Brine	0.25 % k-carrageenan	0.5 % k-carrageenan	*p value*
20 %	4 ÷ 50.7	19.34±1.36^{a}	19.46±1.51^{b}	26.96±3.36^{ab}	*0.0686*
	51 ÷ 72	15.31±0.85^{a}	15.39±0.84^{b}	19.64±1.91^{ab}	*0.0713*
30 %	4 ÷ 50.7	19.7±1.4^{a}	29.58±2.85^{a}	31.79±1.88^{a}	*0.0209*
	51 ÷ 72	15.44±0.83^{a}	21.41±1.68^{a}	22.7±1.39^{a}	*0.0239*
40 %	4 ÷ 50.7	19.38±0.93^{a}	29.38±2.46^{a}	30.32±2.16^{a}	*0.0198*
	51 ÷ 72	15.47±0.60^{a}	21.13±1.55^{a}	21.48±1.29^{a}	*0.0265*
50 %	4 ÷ 50.7	20.12±0.85^{a}	30.71±0.84^{a}	31.13±0.71^{a}	*0.0014*
	51 ÷ 72	15.78±0.57^{a}	21.36±0.60^{a}	22.07±0.55^{a}	*0.0029*

Values represent means ± Standard Deviation
Means with same superscript within same row differ significantly ($p<0.05$)

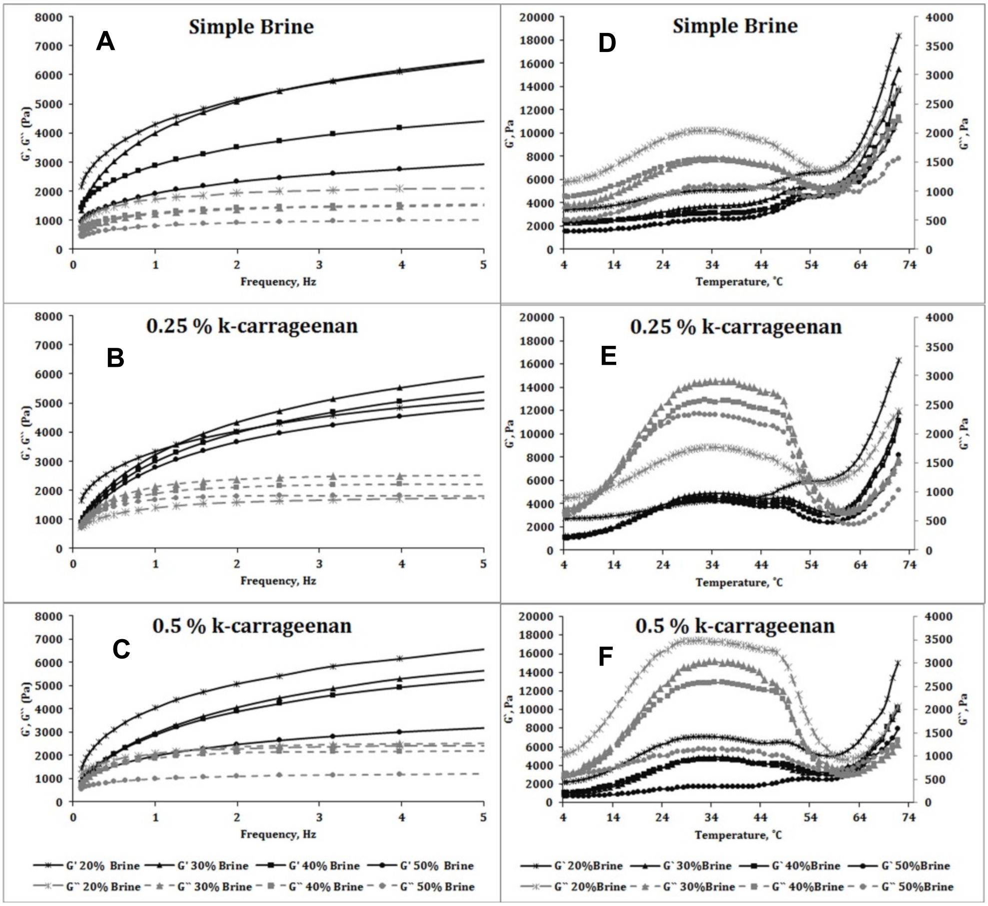

Figure 3. Samples' rheological behavior during frequency sweep (A, B, C) and temperature ramp (D, E, F) tests.

CONCLUSIONS

K-carrageenan addition to minced meat - brine system improved water holding capacity and texture of samples from *Biceps femoris* pork muscle, by decreasing hardness's values, when small amount of brine is desired (20 or 30 % of added brine). However, in order to add more brine to the system there are needed higher quantities of k-carrageenan in order to improve water retention. Rheological analyses showed softer structure for samples mixed with more brine. Also, a higher quantity of k-carrageenan led to significantly higher values for delta angle ($p<0.05$) both for frequency sweep test and for temperature ramp test. Results showed that products from minced/restructured meat with proper binding characteristics and texture can be produced without fat addition.

EXPERIMENTAL SECTION

Raw material

Biceps femoris muscles obtained from one side of pork carcass (24 h after slaughter) were purchased from a local distributor (pH=5.78 ± 0.05; total lipids: 2.51 ± 0.12 g/100 g). Meat, a total of 7 kg (3 muscles) per batch, was processed immediately after collecting in refrigeration conditions at a temperature of +4 ˚C. Any seen fat or conjunctive tissue was removed. The meat was chopped through Philips HR7625/70/AC home chopper, for two minutes at second speed, obtaining a homogenous batter. Obtained mass was separated into 12 samples (500 g per sample).

Mixture formation and processing

The study was projected with variation of two factors:

a. Four brine rates used – 20, 30, 40 and 50 %.

b. Three types of brine added – simple brine with no added hydrocolloid, brine containing 0.25 kg of k-carrageenan/100 kg meat and brine containing 0.5 kg of k-carrageenan/100 kg meat.

Brine used for injection contained sodium chloride, sodium nitrite, sodium tripolyphosphate, sugar, water and k-carrageenan when needed. The ingredients were added in different proportions, depending on the type of brine used and the percentage of added brine so that in finished product the following quantities could be found: 1.8 % salt; 0.3 % sodium tripolyphosphate; 0.015 % sodium nitrite; 0.3 % sugar and 0.25 % or 0.5 % of k-carrageenan as described by Patrașcu, et al. [12]. Brine temperature was kept at +4 °C. Samples were subjected to rheological analyses immediately after forming the mixture, allowing a resting time of 30 min at room temperature. In order to perform textural analysis, mixture was stuffed in cylindrical impermeable casings with 2 cm in diameter, and pasteurized in a water bath (MEMMERT, WNB-45, Germany) gradually increasing water temperature with ~1.5 °C per min from 20 °C ± 2 °C to 75 °C until the internal temperature of the sample reached 72 °C, measured with a thermocouple. After heat treatment, the samples were cooled down in running water to about 20 °C.

Expressible moisture

Meat samples of known weight (3.0 ± 0.5 g) were placed in centrifuge tubes fitted with thimbles of filter paper and centrifuged (Refrigerated Centrifuge TGL-16M) at 2400 rpm for 20 min at 4 °C. Expressible moisture was calculated as the percentage of moisture lost during centrifugation as described by Pietrasik & Shand [22].

$$\%,EM = (initial\ weight - final\ weight)/(initial\ weight) \times 100 \qquad (1)$$

Textural analyzing

The textural characteristics of samples were analyzed using a TA.XT. *Plus* Texture Analyzer. The technical parameters of the apparatus were: Compression Test Mode; Test Speed of 1.5 mm/s. Maximum force recorded during the test was reported as *hardness (N)*.

Rheological analyses

Samples were assessed rheologically by oscillatory tests using a control–stress rheometer (AR2000ex, TA Instruments, Ltd). The temperature was set at 20 °C using a Peltier temperature control system. The procedure was conducted using con - plate geometry with an angle of 2°, 40 mm diameter and a gap of 2000 µm was used. Samples' viscoelastic domain was determined by a strain sweep test (frequency set to 1 Hz). Dynamic viscoelastic characterization was performed by a frequency sweep test increasing the oscillations frequency from 0 to 5 Hz, at an imposed strain of 0.5%. Temperature ramp step was performed from 4 to 72 °C (increased with 5 °C/min), changing the sample between tests, at an imposed strain of 0.5 % and oscillations' frequency of 1 Hz.

Statistical analyses

Statistical analyses were carried out using Microsoft Excel Software with application of Anova Single Factor (α=0.05), and Regression tests. Each experiment was carried out in duplicate and the results were reported as mean values. The Fisher's least significant difference (LSD) test was used to determine differences between treatment means with Statgraphics Centurion XVI.I software.

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