POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

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Pol. J. Food Nutr. Sci.
2010, Vol. 60, No. 1, pp. 51-55

SELECTED PROPERTIES OF I-Z-I BAND PROTEINS OF SONICATED BEEF

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Key words: proteins, sonication, I-Z-I band, -SH groups, ORP

The objective of the reported study was to determine the effect of meat sonication within up to 2 h after slaughter with low-frequency ultrasounds (25 kHz) and at medium vibration intensity (2 W/cm^2) on selected properties of myofibrilar proteins of the I-Z-I band isolated from ageing beef. Proteins were isolated directly after sonication as well as after 24, 48 and 72 h of storage at a temperature of +4°C. The isolated protein fraction was determined for: myofibrillar fragmentation index (MFI), content of reactive sulphydryl groups, redox potential, surface hydrophobicity, and proteins capability for fat emulsification.

Based on the results of analyses of the isolated proteins of the I-Z-I band, no significant effect of post-slaughter sonication immediately after the slaughter was noted on the content of reactive sulphydryl groups, area of hydrophobic surface, myofibrillar fragmentation index nor redox potential. The results obtained point to a significant decrease in the capability of I-Z-I band proteins isolated from sonicated meat for fat emulsification.

The research needs to be continued and extended at a molecular level in order to determine which of the myofibrillar proteins are the most susceptible to the action of ultrasounds, and to identify the mechanism of their action on biologically-complex protein systems in meat.

INTRODUCTION

Meat quality may be defined in a variety of ways, a variety of compounds may as well serve as its indices. Advance in biological sciences indicates that proteins constituting the structures of meat and – by being a constituent of enzymes – participating in metabolic processes may also be used as indicators of meat quality. From the practical point of view, quality plays a significant role in the case of meat intended for culinary purposes [Pospiech *et al.,* 2007]. During ripening, meat tissue is becoming meat that is characterised by desired tenderness and tastiness. The mechanism of changes proceeding throughout that process is highly complex and not fully elucidated. At the first stage of post-slaughter changes, there occurs the stage of *rigor mortis.* This is also the stage of interaction of myofibrillar structures. Its course and duration exert a significant impact on changes in tenderness. At the second state of the transformation, the structures are becoming more loosened and the myofibrills are subject to fragmentation into smaller structural units [Stadnik & Dolatowski, 2007]. By inducing fragmentation of myofibrills [Ahn *et al.,* 2003; Pospiech & Grześ, 1997] expressed numerically as the myofibrillar fragmentation index (MFI), the structural changes in muscle fibrills evoke a desirable effect on meat tenderness. Reasons of those changes have, however, not been elucidated explicitly yet. Some researchers speak of the involvement of lysosomal and calpain degradation pathway [Koohmaraie, 1996; Pospiech *et al.,* 2003], whilst others [Takahashi, 1996] claim that the transformations of structures are induced by non-enzymatic degradation of a protein system in a cell.

Muscle proteins, especially those of myofibrills, determine sensorical and physicochemical properties of food [Marcone, 2000]. They participate in protein-protein, protein-water and protein-fat interactions. The functional properties of proteins are due to interactions that result, among other things, from their structure, including: a degree of hydrophobicity and hydrophilicity, elasticity of molecules, surface charge, composition and sequence of amino acids and characteristics of the environment, including: temperature, ionic strength, acidity as well as interactions between proteins and other food constituents. Though it is common knowledge that the abovementioned characteristics of proteins exert a direct effect on their functional properties, the knowledge of exactly which physical properties and which interactions between them play a key role in expressing particular property is still insufficient [Marcone, 2000].

Ample investigations have confirmed the possibility of extensive application of ultrasounds in food analysis and processing. In meat treated with ultrasounds analyses have demonstrated organoleptic changes, including the improvement of tenderness and juiciness [Babick *et al.,* 2000; Dolatowski, 1999; Dolatowski *et al.,* 2007; Got *et al.,* 1999; Lyng & Allen, 1997; Lyng *et al.,* 1998; Mason *et al.,* 1996]. The ultrasounds have been reported to alter the integrity of muscle cells. They have been shown to induce degradation of lysosomal membranes, thus releasing catepsins and sarcoplasms,

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and consequently accelerating decomposition of proteins and intracellular components, including endoplasmic reticulum. In addition, they have been demonstrated to increase the rate and effectiveness of enzymatic reactions in beef, poultry meat and mutton [Pohlman *et al.,* 1997].

The objective of the reported study was to determine the effect of beef meat sonication immediately after slaughter with low-frequency ultrasounds (25 kHz) and at medium vibration intensity (2 W/cm^2) on selected properties of myofibrillar proteins of the I-Z-I band.

MATERIALS AND METHODS

Material

Analyses were conducted on *semimembranosus* muscle (*m. semimembranosus*) of 18 months old heifers of the Black-and-White Lowland breed, with the body weight 450-500 kg. The animals were slaughtered at a meat processing plant with a slaughter house owned by J. and B. Królik in Kalinówka near Lublin, Poland. Raw material free of quality defects, trimmed immediately after slaughter was divided into 2 parts (weight of *ca.* 0.5 kg, thickness of 4-5 cm), thus a control sample (C) and a sample intended for treatment of ultrasound waves with a frequency of 25 kHz and intensity of 2 W/cm^2 (sample S) were obtained. The exposure time in the ultrasound field was 2 min (one minute at each side of the sample). The muscle was placed on the surface of a transducer so that the ultrasound wavers were directed across the fibres. Each of the samples was divided into 4 portions that were next stored under chilling conditions (4°C). Next, 2, 24, 48 and 72 h after slaughter, one portion of meat was taken out from each sample and subjected to I-Z-I band proteins isolation according to the procedure described below.

Isolation of I-Z-I band proteins

Proteins of the I-Z-I band were isolated following the method described by Ahn *et al.* [2003]. Meat comminuted by means of a meat grinder (ø 3 mm) was homogenized three times (13,500/min, 1 min) with an aqueous solution (1:4, 1°C) containing (per 1 L): 0.1 mol KCl, 5 mmol EDTA, 1 mmol DTT, 1 mmol NaN_3 and 10 mmol Tris-maleate buffer. After each homogenization, the mixture was centrifuged (14,000×*g*, 10 min). The precipitate obtained after the last centrifugation was homogenized twice with an aqueous solution (1:6, 1°C) containing (per 1 L): 0.6 mol KCl, 10 mmol $Na_4P_2O_7$, 1 mmol $MgCl_2$, and 0.1 mol phosphate buffer, and then centrifuged again at 14,000×*g* for 10 min. After the second centrifugation, the resultant precipitate was suspended in the above-mentioned solution (1:6) and extracted (18 h, 4°C).

Total content of –SH reactive groups (R-SH)

The total content of –SH reactive groups (R-SH) in protein was determined with spectrophotometric method by Ellman [1959] which consisted in colorimetric determination of the number of reduced thiol groups (-SH) in the form of conjugates with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid). The product of reaction has a yellow colour and displays the maximum of absorbance at a wavelength of λ=417 nm.

Determination of hydrophobic surface

The hydrophobic surface of a myofibrillar protein solution (0.5%) was determined according to the method by Lieske & Konrad [1994]. The method is based on the binding of a surface-active compound Tween 80 (polyoxyethylene(20) sorbitan monooleate) by hydrophobic fragments of protein and quantitative determination of complexation capability of the dye tested (Bio – Rad). During analysis, there occurs the phenomenon of blocking the hydrophobic surfaces of protein with molecules of the surface-active compound, as a result of which they lose their capability to bind the dye. The absorbance value of protein solutions with and without the addition of the surface-active compound was measured against 0.1 mol phosphate buffer (pH 6.2) at a wavelength of λ=595 nm, with the use of a Nicolet Evolution 300 spectrometer (Thermo Electron Corporation).

Myofibrillar fragmentation index (MFI) [Olson *et al.,* 1976]

The determination of MFI consisted in measuring the absorbance of a 0.2% suspension of myofibrillar proteins (λ=540 nm) and magnifying the resultant value by 200.

Oxido-redox potential (ORP)

The oxido-redox potential (ORP) [John *et al.,* 2005] was measured in a 0.3% solution of proteins by means of a potentiometer equipped in a platinum electrode. The result obtained was converted into the value of the redox potential against a standard hydrogen electrode Eh. To this end, the known value of the potential of the reference electrode (E=211 mV, 20°C) was added to the measured value of redox potential.

Capability of proteins for fat emulsification

The capability of proteins to emulsify fat was determined using the method by Imm & Regenstein [1997]. To this end, one portion of a 2% solution of proteins was homogenised (10,000/min, 1 min) with two portions of plant oil (v/v). The cooled emulsion (4°C) was centrifuged (10,000×g, 15 min). After centrifugation the 3 following fractions were obtained: a solution containing dissolved proteins, non-emulsified oil, and emulsion. The content of proteins was determined in the first solution. The difference between protein content of the solution subjected to emulsification and that obtained after centrifugation enabled calculating the content of protein in the emulsion. In turn, the difference between the quantity of oil used for the assay and that obtained after centrifugation enabled calculating the quantity of oil bound in the emulsion, referring the result to 1 g of protein.

Protein content

The content of proteins was assayed with the biuret method. In the basic medium peptide bonds of protein react with copper ions, forming a colour complex. The intensity of coloration is directly proportional to the number of peptide bonds and, thus, to the concentration of protein. A Cormay diagnostic kit for total protein determination was used in this assay. Absorbance (A) of the resultant color was measured at a wavelength of 456 nm. The concentration of protein in the solution was computed based on the following formula:

Concentration of total protein = (A sample/A standard) × concentration of the standard.

The protein solution obtained as a result of isolation was dissolved with an aqueous solution containing (per 1 L): 0.6 mol KCl, 10 mmol $Na_4P_2O_7$, 1 mmol $MgCl_2$, and 0.1 mol phosphate buffer to the concentration required for particular assays. The content of proteins was determined with the biuret method.

Statistical analysis

Analyses were conducted on meat originating from 4 animals, each measurement was done in triplicate. Mean values and standard deviations were computed wit the use of Microsoft Office Excel. The significance of differences between the mean values was analysed at a significance level of $\alpha \geq 0.05$.

RESULTS AND DISCUSSION

Over the successive stages of meat ripening, there occur phenomena of dissociation, partial denaturation and disruption of disulphide bonds. Transformations of structure and weakening of protein-protein bonds result in the exposure of reactive functional groups of protein being inaccessible at the early stage of ripening. Previous investigations [Dolatowski & Twarda, 2004; Latoch & Dolatowski, 2006; Latoch, 2007] demonstrated the effect of meat sonication on the course of the ripening process and exposure of the reactive functional groups of protein.

In analysing the results obtained, it was found that I-Z-I band proteins of the sonicated sample (S) were characterised by an insignificantly higher content of reactive groups R-SH as compared to proteins of the control group (C). In turn, in analysing the dynamics of changes in the content of reactive groups -SH in the samples during chill storage (Table 1), the lowest content of the groups was determined in proteins isolated from meat immediately after slaughter (27-28 μmol/g protein), whereas the highest one in proteins isolated after 72 h (35.6 μmol/g protein in sample S and 41 μmol/g protein in sample C). The differences were, however, not statistically significant. The upward tendency in the content of R-SH during storage was also confirmed by other authors [Farouk *et al*., 2003].

Surface hydrophobicity (SH) is a characteristic of proteins which determines their biological and functional properties. It determines protein-protein and protein-water interactions. Konieczny [2001, 2002] demonstrated a strong correlation between the functional properties of proteins and their hydrophobicity. Changes in the size of the hydrophobic surface, likewise the above-discussed changes in the content of reactive R-SH groups, are determined by conformational changes of proteins undergoing upon physical actions, *e.g.* the action of ultrasounds, technological process or environmental factors (pH and ionic strength). Analyses made in the study over 72-h storage of meat demonstrated (Table 1) that the size of the hydrophobic surface of the I-Z-I band proteins in sample S was insignificantly smaller than in sample C. Only the proteins isolated 48 h after the slaughter from the control sample were characterised by a significantly higher value of SH. Still, the value of SH noted for proteins isolated in the first 24 h of analyses was at the same level for both samples (35%). In the period of after-slaughter changes as well as under the influence of ultrasound waves, functional groups of proteins may be blocked by other groups and thus, the number of hydrophobic groups is likely to change [Stadnik & Dolatowski, 2007]. This may also be due to interactions proceeding in the internal structures of proteins [Latoch, 2007]. The differences in the size of the hydrophobic surface between the control (C) and the sonicated (S) sample result from differentiated inhibition of oxidation of the –SH groups [Badyal *et al.,* 2001] and from transformation of the already existing disulphide bonds into the non-oxidized form. The removal of those bonds enables development of proteins structure [Krusemark *et al*., 2008] and revealing hydrophobic residues of aromatic amino acids [Wang, 2005].

During meat ripening, the major structural changes of muscle fibres occur on the Z line or in the vicinity of that line and are accompanied by its degradation, which in turn

TABLE 1. Content of reactive –SH groups, surface hydrophobicity, myofibrillar fragmentation index, oxido-reductive potential and capability to emulsify fat in I-Z-I band proteins isolated from beef during 72-h chilling storage.

Parameter	Sample	Time since after slaughter (h)			
		2	24	48	72
Content of reactive –SH groups (μmol/g protein)	S	26.98±8.50	32.81±10.22	30.02±9.60	35.57±9.30
	C	28.29±7.40	35.07±10.71	28.72±8.00	40.98±11.60
Surface hydrophobicity (%)	S	35.90±11.50	30.80^{Aa}±9.73	26.00±7.20	29.60±9.40
	C	35.40±9.50	49.30^{Aa}±11.10	28.80^{a}±8.70	36.60±10.30
Myofibrillar fragmentation index	S	127.98^{b}±17.87	134.20±21.98	153.00^{b}±18.10	143.03±13.87
	C	144.50±16.47	129.38±17.70	145.95±16.60	158.58±13.54
Oxido-reductive potential (mV)	S	358.90±35.60	357.90±25.16	359.80±30.36	360.80±37.17
	C	344.40±26.33	356.20±23.25	357.10±32.12	367.20±34.38
Protein capability for fat emulsification (g oil/g protein)	S	142.97^{Bcd}±33.20	15.70^{Ccd}±0.50	201.09^{Dc}±64.12	204.08^{Ed}±66.11
	C	326.45^{Be}±42.30	203.00^{Cef}±40.90	384.32^{Df}±57.55	74.40^{Eef}±29.31

Results are presented as mean value±standard deviation; mean values denoted with the same capital letters in columns and small letters in rows are statistically significantly different ($\alpha \geq 0.05$).

induces fragmentation of myofibrills [Ahn *et al.,* 2003; Pospiech & Grześ, 1997] expressed numerically in the form of the myofibrillar fragmentation index (MFI). In the reported study, an insignificant increase of MFI was observed along with the successive time of meat storage (Table 1). Those observations confirm previous findings [Hopkins *et al.,* 2000]. Meat sonication immediately after slaughter has been shown to change the rate and degree of degradation of muscle fibrils [Dolatowski *et al.,* 2007; Dolatowski & Twarda 2004; Dolatowski, 1999; Stadnik & Dolatowski, 2007]. The value of MFI noted 2 h and 72 h after slaughter in the sonicated sample was found to be insignificantly lower as compared to the control sample (Table 1).

Assays of the redox potential (ORP) of the I-Z-I band proteins did not demonstrate any significant differences in the particular measurement periods between the control (C) and the sonicated (S) sample (Table 1). In contrast, a negligible, statistically insignificant upward tendency of ORP was reported during chill storage of meat, *i.e.* from 344 mV in the first 24 h to 367 mV after 72 h of analyses.

Determinations of proteins capability to emulsify fat – being an indicator of the protein-fat interaction – demonstrated that in the first 72 h of analyses the proteins of the control sample were characterised by a significantly higher capability to emulsify fat than the proteins of the sonicated group (Table 1).

SUMMARY

Previous investigations addressing the effect of meat treatment with ultrasounds on properties and structure of total myofibrillar proteins have demonstrated that this treatment was changing protein systems and conformation of proteins, thus determining their functional properties [Dolatowski, 1999; Dolatowski *et al*., 2007; Got *et al*., 1999; Latoch, 2007; Lyng & Allen 1997; Lyng *et al*., 1998; Stadnik & Dolatowski, 2007]. The action of ultrasounds of low frequency and medium intensity of oscillation on meat tissue evoked a variety of secondary phenomena, including: temperature increase, polymerization or depolymerization, as well as acceleration of oxidation and reduction reactions. All those phenomena affect the content of available R-SH groups, surface hydrophobicity and redox potential of proteins. Most of those changes enable more effective exploitation of the properties of protein myofibrils in processing and reduced use of functional additives.

The presented study did not demonstrate any significant effect of post-slaughter sonication of meat with ultrasounds of low frequency (25 kHz) and medium intensity of oscillation (2 W/cm^2) immediately after slaughter on: the content of reactive sulphydryl groups, area of the hydrophobic surface, myofibrillar fragmentation index nor redox potential of the isolated myofibrilliar proteins of the I-Z-I band. The results obtained point to a significant decrease in the capability of I-Z-I band proteins isolated from sonicated meat for fat emulsification.

The research needs to be continued and extended at a molecular level in order to determine which of the myofibrillar proteins are the most susceptible to the action of ultrasounds, and to identify the mechanism of their action on biologically-complex protein systems in meat.

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Received January 2009. Revision received April and accepted May 2009.