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The effect of high temperature on collagen solubility and tenderness of roasted beef

Der Einfluss hoher Temperaturen auf die Kollagenlöslichkeit und die Zartheit von Rinderbraten

Monika Modzelewska-Kapituła

Summary

The aim of the present study was to investigate the changes in collagen concentration and solubility, tenderness and other sensory attributes in *semimembranosus* muscles roasted to an internal temperature of 80°C and 90°C. The temperature increase caused an increase in the concentration and proportion of water-soluble collagen. There was also a trend towards an increase in the remaining collagen fraction concentration, however no significant differences between meat heated to 80 °C and 90 °C in terms of the content of total, acid- and total soluble and insoluble collagen were noted. Higher cooking loss, lower juiciness and acceptability of colour were noted for the roasts cooked to 90 °C. No effect of the internal temperature increase on shear force values and sensory tenderness of roasts were noted. It can be concluded that the increase in temperature from 80 °C to 90 °C increases water-soluble collagen content and has no adverse effect on tenderness, taste, aroma and overall liking of beef roasts prepared from *semimembranosus* muscle.

Keywords: bovine meat, connective tissue, shear force, tenderness, cooking

Zusammenfassung

Ziel der Arbeit war die Überprüfung des Einflusses der Endpunkttemperaturen von 80 °C und 90 °C auf Kollagenkonzentrationen und Löslichkeiten sowie auf die Zartheit und andere sensorische Parameter des *M. semimembranosus*. Die Temperaturerhöhung hatte eine Erhöhung der Konzentration und des Anteils des wasserlöslichen Kollagens zur Folge. Bei den anderen Kollagenfraktionen wurden ähnliche Tendenzen beobachtet. Es konnten allerdings keine deutlichen Unterschiede in den Gesamtkollagengehalten, der säurelöslichen Kollagenfraktion sowie des löslichen und unlöslichen Gesamtkollagens ermittelt werden. Fleisch welches auf 90 °C erwärmt wurde zeigte im Vergleich zu dem auf 80 °C erhitztem Fleisch höhere Kochverluste, eine geringere Saftigkeit und geringere Farb-Akzeptanz. Die Temperaturerhöhung hatte dagegen keinen Einfluss auf die Scherkraft und Zartheit des Bratens. Es kann also geschlussfolgert werden, dass eine Erhöhung der Gartemperatur von 80 °C auf 90 °C den Anteil des wasserlöslichen Kollagens erhöht und keine negativen Einflüsse auf Zartheit, Geschmack, Aroma und Gesamteindruck des Bratens aus dem *M. semimembranosus* hat.

Schlüsselwörter: Rindfleisch, Bindegewebe, Scherkraft, Zartheit, Braten

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Introduction

Beef quality depends on many factors, such as animal breed, sex, age, diet, pre- and post-slaughter handling, storage conditions, muscle type and its chemical composition. Although these factors have been studied since decades, still the role of some of them remains not fully recognized. One of the aforementioned factors is the concentration and solubility of collagen. Collagen is the main constituent of connective tissue (1.5 % to 10 % of muscle dry weight) (Lepetit, 2008), which surrounds single muscle fibres *(endomysium),* muscle fibre bundles *(perimysium)* and whole muscles *(epimysium)* (Purslow, 2002). Some reports have shown the influence of collagen on beef tenderness measured as Warner-Bratzler Shear Force (WBSF). According to Chriki et al. (2013) in raw *semitendinosus* muscle from young bulls carcasses, the WBSF values were positively correlated with total and insoluble collagen amount, however the relationship was not noted in the muscles obtained from cows carcasses. Thermal treatment affects the nature of connective tissue in meat (Purslow, 2005), and that could explain the observations of Li et al. (2010) and Girard et al. (2012), who found that total collagen content was not correlated with WBSF of *semitendinosus* and *gluteus medius* muscles subjected to a thermal treatment. Collagen content and its solubility might also affect the sensory quality of beef, although conflicting results have been reported (Jeremiah et al., 2003; Chriki et al., 2013; Dubost et al., 2013) as well. Many studies concerning the changes of collagen solubility and the relationship between collagen and tenderness were conducted on steaks (Li et al., 2010; Dubost et al., 2013) or small meat cubes (Vasanthi et al., 2007) cooked in water-bath in hermetically sealed plastic bags, whereas there is a lack of reports in which meat was roasted similarly as in home practice. The most frequently meat heating was conducted only to achieve 70°C in the centre of meat element (Jeremiah et al., 2003; Dubost et al. 2013). Li et al. (2010) studied the dynamical changes in intramuscular connective tissue and muscle fibre during heating of beef *semitendinosus* muscle heated to an internal temperature of 40, 50, 55, 60, 65, 70, 75, 80, 85 and 90 °C, however they reported only concentration of total and insoluble collagen and collagen solubility (%). Thus, there is a lack of information if any changes in the content or the proportion of water-soluble, acid-soluble and insoluble collagen occur in the higher temperatures. In beef steak preparation, six categories of doneness according to internal temperature are used: very rare (55 °C), rare (60 °C), medium rare (63 °C), medium (71 °C), well done (77 °C), and very well done (82 °C) (AMSA, 1995, as cited by Li et al., 2010). In the case of roasts, which are prepared as very well done, the meat must be heated to an internal temperature that ensures that no differences in colour between the central and peripheral parts of a roast cross-section will occur; otherwise, the consumer will regard a roast as undercooked. Moreover, in home practice, there is a difficulty in exact internal meat temperature monitoring, which may resulted in heating meat to a temperature higher than 82 °C. Taking it into account in the present study meat was heated to 80 °C and 90 °C.

Semimembranosus (SM) muscle is one of the round muscles, and is considered as one of the underutilized muscles of beef carcasses (Rubio et al., 2007), however as shown in our previous study it could be used for roast preparation (Modzelewska-Kapituła et al., 2012). SM contains low fat and low total collagen (Jeremiah et al., 2003), in which water soluble collagen accounts for only approx. 20 % (Modzelewska-Kapituła and Nogalski, 2014). According to my knowledge, there is a lack of reports concerning the changes in collagen content and solubility in roasted SM muscle in the conditions similar to those used in home practice and their influence on quality of the roast. In our previous study we found that an increase in internal temperature, from 75 ºC to 95 °C, did not influence collagen concentration in roasted SM muscle. However, the changes in collagen solubility were not studied. An internal temperature of 95 °C negatively influenced the juiciness and WBSF values of muscle heated in dry air (Modzelewska-Kapituła et al., 2012), and thus, in the present study, the beef was heated to 80 °C and 90 °C. The aim of the study was to investigate the influence of high internal temperature on collagen concentration and solubility, cooking loss, WBSF values and eating quality of beef *semimembranosus* muscle.

Materials and Methods

Material

The material for the study comprised of *semimembranosus* (SEM) muscles (n = 27). The muscle was removed from carcass (n = 27) of young (approx. 600 days old) crossbred (Polish Holstein Friesian x Limousine, Polish Holstein Friesian x Charolaise, Polish Holstein Friesian x Hereford, nine from each group) bulls 96 h *post mortem.* No electric stimulation of the carcasses was applied. The muscles were delivered to the laboratory of Meat Technology and Chemistry Department in isothermal containers at approx. 6 ºC. The delivery lasted approx. 1 h. The next day (fifth day *post mortem*) the muscles were trimmed of all external connective tissue *(epimysium),* weighed and divided in two parts (approx. 1 kg). One part of the same muscle was heated to obtain 80 ºC in the centre of the muscle, whereas the other to 90 ºC. The muscles were placed in a cool convectional-steam oven (Küppersbusch CPE 110, Küppersbusch Großküchentechnik GmbH, Gelsenkirchen, Germany) and subjected to thermal treatment at 180 ºC in dry air. The internal temperature of the muscles was continuously controlled using a thermometer (connected to the oven) which was placed in the central part of each muscle. After thermal treatment, the muscles were cooled to room temperature (approx. 20 ºC, approx. three hours) and then in a refrigerator to 3 ± 2 ºC (for approx. twelve hours). Cooking loss, collagen profile, Warner-Bratzler shear force and the eating quality of the cooked meat were determined. The sampling protocol was as follows. Firstly, the samples for sensory assessment were cut from both ends of the cooled muscles (fragments approx. 4 cm long). From the remaining cooked meat the crusted cooked surface was removed using a knife. The samples for shear force determination were cut. The remaining cooked meat was ground along with the crusted cooked surface cut earlier, and samples for collagen profile analyses were taken.

Cooking loss

Cooking loss (evaporative and drip) was calculated as the ratio of the difference between the meat weight before and after cooking to the meat weight before cooking and was expressed in percentage.

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Collagen content

The total collagen content in raw and cooked meat was determined according to Reich (1970). The water-soluble collagen content was extracted according to the method of Hill (1966) with modifications, whereas the insoluble collagen content was analysed according to the Blomfield and Farrar (1964) method with modifications (Modzelewska-Kapituła et al., 2015). The amount of acetic acid-soluble collagen was calculated as the difference between the total collagen and the sum of water-soluble and insoluble collagen contents, whereas total soluble collagen was calculated as the sum of water and acetic acid-soluble collagen contents. The proportion of each collagen fraction (%) was calculated for total collagen content.

Warner-Bratzler shear force

The Warner-Bratzler shear force (WBSF) values (N) were measured using an Instron 5965 (Instron, Norwood, MA, USA) equipped with a shear blade. Samples being cylindrical cores (1.27 cm diameter, approx. 40 mm long) were cut using cork borer out in the direction of muscle fibres. The shear blade (V-shaped, with a triangular aperture of 60º) was applied perpendicularly to the direction of the fibres at a crosshead speed of 2 mm/s (Walsh et al., 2010). The test was done at room temperature (about 18 ºC). The measured data were evaluated using Bluehill 3 software (Instron, Norwood, MA, USA.). Five samples were cut from each muscle.

Sensory analysis

Sensory analyses were conducted using samples cut from cooked muscles. From both ends of the cooled muscles a fragment approx. 4 cm long was cut and sliced using a slicing machine (Ma-Ga S 712p, Automatic Slicing Machine, Bydgoskie Zakłady Maszyn Gastronomicznych, Bydgoszcz, Poland) into 2 mm thick slices. The temperature of the samples subjected to evaluation was approx. 10 ºC. Expert sensory assessment was conducted by five panellists experienced in sensory evaluation of meat products and familiar with the current product. A comparison between meat samples was conducted according to the PN-ISO 4121:1998 Polish Standard (Polish Standard PN-ISO 4121, 1998). A scale of 1–9 was used in the sensory evaluation. Panellists scored each sample for tenderness (1, extremely tough; 9, extremely tender), juiciness (1, extremely dry; 9, extremely juicy), taste (1, extremely bland, atypical; 9, extremely intense, typical), color acceptability (1, not acceptable, too dark or too red; 9, extremely acceptable), aroma (1, extremely bland, atypical; 9,extremely intense, typical) and overall liking (1, not acceptable; 9, extremely acceptable). Samples were randomly distributed on white plates for evaluation. Water and bread were provided for cleansing the palate.

Data analysis

To compare WBSF, cooking loss and collagen content values, a variance analysis (in the case of normal distribution of the data) or non-parametric Mann-Whitney test (when the assumption of normal distribution data was not fulfilled) were used. The variance analysis was conducted for cooking loss and WBSF values, whereas the collagen concentration and proportion and eating quality results were analysed using the non-parametric test. The significance of the differences was reported at $P < 0.01$ and 0.05. All calculations were performed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA).

Results and discussion

The increase in the final internal temperature caused an increase in cooking loss, which was expected (Tab. 1). The increase in cooking loss with the temperature was also noted in our earlier study (Modzelewska-Kapituła et al., 2012) and by others (García-Segovia et al., 2007; Vasanthi et al., 2007; Alfaia et al., 2010). Cooking loss is caused by denaturation of meat proteins, which take place during heating, and demonstrates in release of extra- and intracellular water (Honikel, 2004). An increase in cooking time to obtain a higher internal temperature in the roast resulted in higher water release and higher cooking loss.

The increase in the final internal temperature from 80 ºC to 90 ºC did not increase WBSF values of the SM roasts (Tab. 1). Similar results were reported by Li et al. (2010), found no significant differences in WBSF of *semitendinosus* muscles cooked to 80 ºC and 90 ºC in plastic bags in waterbath. Generally, three temperature intervals might be distinguished in respect to WBSF increase rate during cooking: 40–65 ºC, 65–75 ºC and 75–90 ºC, in which no significant changes, significant increase at a faster rate and slow increase in WBSF are noted, respectively (Li et al. 2010). At the temperatures from 75 to 90 °C, the increase in WBSF is caused by shrinkage of myofibrillar components, however it is depressed by the changes in the collagen fibers, which began to granulate, which results in increase in crispness of *perimysium* (Li et al. 2010). The results of the present study might suggest that in meat heated to the temperature ranged from 80 ºC to 90 ºC, the increase in WBSF caused by the changes in myofibrillar proteins was compensated by the increase in crispness of connective tissue and therefore no differences in WBSF were noted.

Roast beef, specially sliced in thin slices (as was done in the study), are an alternative to sausages and are consumed cold with bread. This method of roast consumption is quite popular in Poland. The increase in the internal meat temperature from 80ºC to 90ºC had no effect on tenderness, taste, aroma and overall liking, whereas decreased juiciness and colour acceptability of meat (Tab. 1). The decrease in juiciness might be explained by higher water loss (cooking loss) in the samples heated to 90 ºC. The colour acceptability of muscles heated to 90 ºC received a lower score than those heated to 80 ºC, due to the much darker colour of the

TABLE 1: *Cooking loss, Warner-Bratzler shear force (WBSF) values and eating quality of semimembranosus muscles heated in dry air to the different final temperatures (mean values ± standard deviation).*

Attribute	Final internal temperature		Significance
	80 °C (n = 27)	90 °C (n = 27)	(*P* – value)
Cooking loss (%)	42.9 ± 2.5	47.1 ± 2.2	0.000
WBSF (N)	51.3 ± 14.9	50.5 ± 15.1	NS
Eating quality (pts.)			
Tenderness	6.2 ± 1.4	6.5 ± 1.6	0.052 (NS)
Juiciness	5.9 ± 1.4	5.5 ± 1.7	0.044
Taste	7.7 ± 1.2	7.7 ± 1.1	0.606 (NS)
Aroma	8.0 ± 1.1	7.9 ± 1.1	0.299 (NS)
Color	8.0 ± 0.8	7.6 ± 1.0	0.000
Overall liking	6.6 ± 1.1	6.6 ± 1.1	0.880 (NS)

Eating quality – scores given from 1 to 9; NS – not statistically significant

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peripheral part of the muscle cross-section compared to its centre. This dark brown colour was caused probably by denaturation of meat proteins, myoglobin and Maillard reactions. A negative influence of higher internal temperature on SM juiciness heated in dry air was also noted in the previous study (Modzelewska-Kapituła et al., 2012).

The increase in the internal temperature of SM roasts caused the increase ($P < 0.05$) in concentration and proportion of water-soluble collagen (Tab. 2). There was also a trend towards an increase in the remaining collagen fraction concentration, however no significant differences between meat heated to 80 ºC and 90 ºC in terms of the content of total, acid- and total soluble and insoluble collagen were noted. The trend might be explained by the loss of water during cooking and, as a result, increased concentration of collagen in meat samples. Results obtained indicated also that the heat denatured collagen molecules remained inside the meat samples and were not evacuated from the meat with cooking losses. The results corroborate with the findings of Combes et al. (2003), who reported the lack of differences in total collagen content in rabbit meat heated to 80 ºC and 90 ºC. Different results were reported for cooked beef by Li et al. (2010), who noted a significant increase ($P < 0.05$) in total and insoluble collagen content (% of wet weight) in *semitendinosus* muscle heated to 80 ºC and 90 ºC and no differences in collagen solubility (%) and Vasanthi et al. (2007) who found that the higher temperature of water bath was and longer cooking time the collagen concentration in meat was lower and collagen solubility was higher. According to Purslow (2014), there is a proportion of collagen molecules in meat that are easily heat solubilized after short periods of cooking and a proportion of collagen that is heat insoluble during cooking. However, this insoluble collagen may be increasingly solubilized during prolonged cooking at intermediate temperatures (e. g. sous vide cooking) or during high-temperature-long-cooking-time thermal processing (e. g. stewing) (Purslow, 2014). The results of the present study indicate that an increase in temperature from 80 ºC to 90 ºC, during roasting is not sufficient to solubilize the so-called "insoluble" collagen, however produces changes in collagen solubility such as increase in the content and proportion of water-soluble collagen.

TABLE 2: *Collagen concentration in semimembranosus muscles heated in dry air to the different final temperatures (mean values ± standard deviation).*

Attribute	Final internal temperature		Significance
	80 °C (n = 27)	90 °C (n = 27)	(*P* – value)
Collagen concentration (mg/100 g wet tissue)			
Total collagen	1276.0 ± 587.7	1383.4 ± 692.0	0.270 (NS)
Water-soluble	289.0 ± 162.5	357.2 ± 184.6	0.000
Acid-soluble	762.4 ± 484.3	787.6 ± 520.6	0.955 (NS)
Total soluble	1051.4 ± 521.7	1144.9 ± 617.3	0.316 (NS)
Insoluble	224.6 ± 95.9	238.5 ± 117.6	0.590 (NS)
Collagen (% of total)			
Water-soluble	24.6 ± 13.1	27.6 ± 13.1	0.027
Acid-soluble	56.6 ± 17.9	53.8 ± 16.6	0.069 (NS)
Total soluble	81.2 ± 6.7	81.5 ± 8.2	0.366 (NS)
Insoluble	18.8 ± 6.7	18.5 ± 8.2	0.366 (NS)

NS – not statistically significant

Conclusion

The results of the present study indicated that an increase in the internal temperature of *semimembranosus* muscle from 80 ºC to 90 ºC during roasting, increased concentration and proportion of water-soluble collagen. The increase in the temperature did not have an adverse effect on tenderness, taste, aroma and overall liking of the roasts, whereas it increased cooking loss and decreased juiciness and colour acceptability. Generally, it can be concluded that overheating the muscle to 90 ºC in home practice, did not decrease its eating quality compared to 80 ºC.

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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