QUANTITATIVE ASSESSMENT OF MEAT TISSUE STRUCTURAL DAMAGES PROMOTED BY MECHANICAL ACTION IN TUMBLING

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Abstract – In meat brining-massaging salt homogenization, protein extraction and texture of cooked products partly depend on meat tissue structural modifications. Our aim was to quantify these damages by automated image analysis of many sirius red stained histological images from pork *Semimembranosus* muscles submitted to various massaging conditions (number and rate of deformations). Muscles were massaged in brine (5 or 13 %) using a new laboratory tumbling simulator that enable to control and characterize the mechanical treatment. In samples taken from massaged muscles, zones where fibres could not be automatically separated as in the reference samples were considered as damaged and the percentage of damaged area (Pda) in the images was calculated. The Pda was equal to about 40% in the middle of the muscle at the end of a mechanical treatment that was equivalent to usual massaging conditions in an large industrial tumbler. The cumulated deformation energy (Et) which was assessed from measured force *versus* strain curves during massaging was varied from 35 to 1500 J. Pda varied linearly with Log(Et) and stronger in muscle centre than on the periphery.

Key Words – microstructure, degradation, massaging, strain energy.

I. INTRODUCTION

Tumbling is performed before cooking in baffled rotating drums that have diameters ranging from 0.5 to 2 m. The mechanical energy which is transmitted to meat pieces due to falling and striking against the baffles leads to meat deformation. Studies in pilot or industrial tumblers show from microscopic observations that tumbling modifies meat tissue structure and affect final product texture [1, 2, 3, 4] but no quantitative data are available to relate these modifications to the mechanical action.

Our aim was to quantify the structural damages by automated image analysis of many sirius red stained histological images from pork *Semimembranosus* muscles submitted to various controlled massaging conditions using a laboratory tumbling simulator [5] that enable to characterize the mechanical action undergone by meat pieces.

II. MATERIALS AND METHODS

Muscles and chemical analysis

A batch of 16 *Semimembranosus* (SM) pork muscles with ultimate pH equal to 5.6 ± 0.15 was used. The muscles were trimmed of their connective tissue envelope, vacuum-packed and stored at -18°C until use. They were thawed overnight at 4°C before massaging. NaCl and water contents were measured using a chloride analyzer (Sherwood, MHII-926) and by oven drying at 104°C for 24 h, respectively.

Controlled brining-massaging

Massaging was controlled using a new lab-scale tumbling simulator [5] that can reproduce mechanical treatments occurring in real-world tumblers: hundreds of rapid deformations (controlled compression orthogonally to muscle main length and lateral free extension) are applied to one muscle to simulate the successive falls promoted by tumbler drum rotation. The maximum compression rate (Cr) is controlled and the dissipated strain energy of each deformation (E) is calculated from force *versus* strain recordings. The muscles were rotated at 8 rpm in the simulator tank to vary muscle position from compression to compression and to ensure brine deposition at muscle surface. The brine was a NaCl solution at concentration Csalt; the amount of brine in the simulator tank was equal to muscle weight.

Height brining-massaging conditions were tested by varying Csalt (5 % or 13 %), Cr (10 % or 30 %), and the number of compressions (S= short, 350 or L= long, 2500). All trials were performed at 4°C and repeated 3 times; two repetitions were used for structural analysis.

Histological images

A reference sample was cut before massaging of each muscle. After brining-massaging 3 samples (2 × 1 ×0.5 cm) were taken from a transverse slice 2 cm in thickness cut in the middle of the muscle. They were located close to muscle surface (S), at muscle centre (M) and at an intermediate position (I). Three samples were cut from an adjacent slice for water and NaCl content measurements.

Histological samples were frozen immediately after collection in cooled isopentane (−160 °C), chilled with liquid nitrogen (−196 °C), and stored at −80 °C until subsequent analyses. Thin transverse sections (10 μm thick, orthogonal to meat fibres) were sliced using a cryostat (Cryo-star HM 560 Microm international GmbH, Germany), mounted on glass slides and air-dried (20 °C). Connective tissue (*perimysium* and *endomysium*) was revealed by sirius red staining specific for collagen.

Observations were performed using an Olympus BX61 (Olympus-Europa GmbH, Hamburg, Germany) transmission microscope coupled to a digital acquisition kit (digital camera Olympus DP71 and Cell F software, Olympus-Europa GmbH, Hamburg, Germany). Twelve images, representing about 1 200 muscle fibres in total, were acquired for each muscle section in order to obtain a representative sampling.

Image analysis

An algorithm was elaborated to automatically separate the fibers in the images of the reference samples using the open source Image J software (V 1.43 U). The main steps were: homogenization of brightness by comparison to a blank image, splitting the RGB image into a greyscale image containing the green component, thresholding based on a mean filter and a series of dilation/erosion operations to smooth objects and remove isolated black pixels. The zones where fibers could not be automatically separated in images from massaged samples were considered as damaged.

III. RESULTS AND DISCUSSION

Whatever the brining-massaging conditions water contents in M and I samples never differed significantly than that in reference samples and the NaCl contents were always below 0.3 %. This means that structural changes in these samples were only due to mechanical action while those in samples S were also dependent on NaCl content (Table 1).

Table 1 shows the measured characteristics of the mechanical action undergone by the muscles. Two very different massaging intensities were obtained according to the mean compression rate (Cr); note that standard deviations are very high due to differences in muscle shapes:

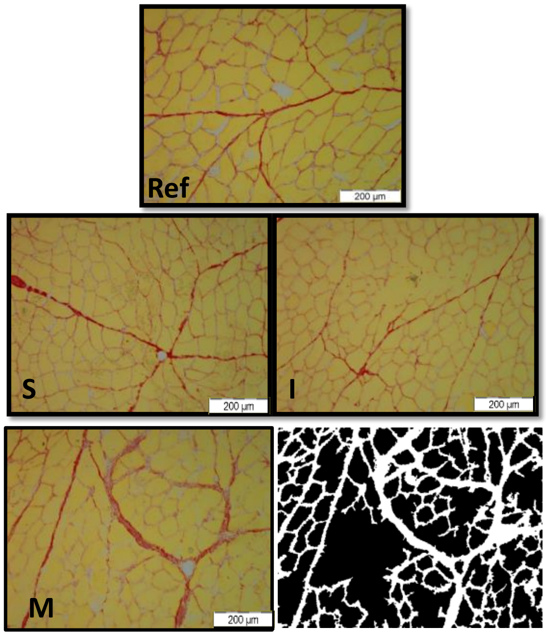
(1) at Cr 10 %, which simulated massaging in a small pilot tumbler, the maximum reaction force was about 40 N and the deformation energy was around 100 mJ,

(2) at Cr 30 %, which simulated massaging in a very large industrial tumbler, the maximum reaction force was between 150 and 240 N and the deformation energy was 5 to 6 times higher than previously.

Table 1: Brining-massaging conditions, average and standard-deviation of mechanical action characteristics (Fmax and deformation energy) and average and standard-deviation of water and salt contents in sample S.

Csalt (g NaCl/100 g)	Cr %	Duration	Fmax (N)	Energy (mJ)	Water content %	NaCl content %
13	10	S	37 ± 10	96 ± 13	75,0 ± 0,0	2,1 ± 0,4
		L	40 ± 1	96 ± 2	72,3 ± 0,6	4,3 ± 0,6
	30	S	153 ± 66	485 ± 197	74,7 ± 1,5	2,4 ± 0,3
		L	238 ± 59	676 ± 159	74,7 ± 1,2	5,8 ± 0,1
5	10	S	48 ± 9	119 ± 27	74,0 ± 1,0	0,7 ± 0,2
		L	42 ± 9	89 ± 28	75,3 ± 1,2	1,5 ± 0,1
	30	C	189 ± 76	610 ± 282	75,0 ± 2,0	0,9 ± 0,1
		S	160 ± 57	598 ± 159	75,3 ± 3,2	1,8 ± 0,2

Figure 1: Examples of sirius red stained histological images and of the automated image treatment. (Ref) Reference sample; (S, I and M) samples taken at 3 locations (Surface, Intermediate and Middle) in the muscle after 2 500 deformations at Cr 30% using brine at Csalt 13%; (T) result of the treatment of image M.



In the sirius red stained image of a reference sample in Figure 1 the meat fibres (coloured in yellow) are well delimited by *endomysium* (coloured in red). In the images from the massaged samples the red lines are less clear at many locations and sometimes disappear, indicating weakening or degradation of *endomysium*. The black and white treated image (T) of image M reveals these damaged areas which appear as black zones also delimited by a white edge but much larger than the fibres. The treatment algorithm was calibrated so that the number of 'separated black elements' (fibres and part of fibres along image edges) was correct for the reference samples. Consequently the extracellular spaces were thickened and the measured areas of the separated elements were lower than fibres cross section areas.

Figure 2 shows typical histograms related to the 4 samples of a brining-massaging trial. The number of separated elements that had an area higher than 10 000 µm^2 was negligible for the reference sample; all the measured areas corresponded to undamaged fibres. In contrast a bin with element areas higher than 15 000 µm^2 clearly appeared for the massaged samples, especially for M samples. These elements were defined as the damaged zones and the percentage area covered by these zones in the 12 images was calculated (Pda).

Figure 2: Histograms of separated element areas from samples taken in a muscle after 2 500 deformations at Cr 30% using brine at Csalt 13%.

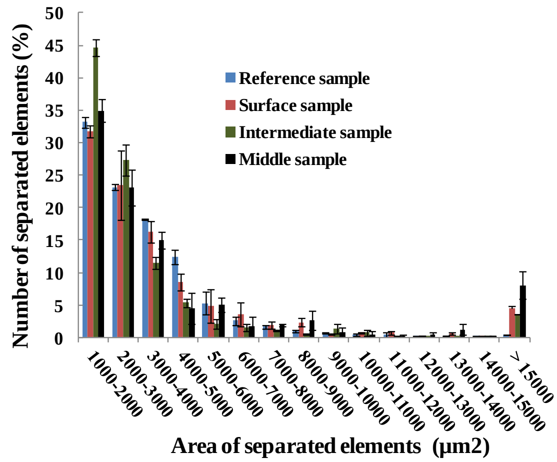
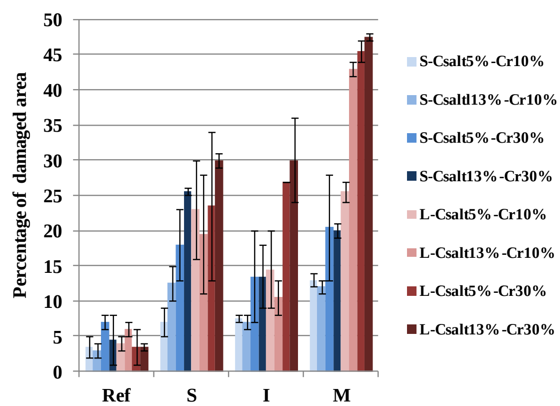
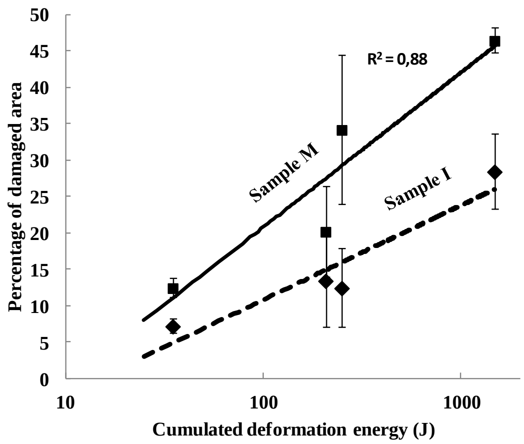


Figure 3: Comparison of the percentages of damaged area in samples S, I and M according to brining-massaging conditions. Mean, min and max of 2 repetitions.



The 8 brining-massaging conditions are compared in Figure 3. The mean Pda was between 1 and 6 % in the reference images; this do not correspond to damaged zones but to measurement errors. It is difficult to conclude for the S sample since there was probably interaction between mechanical action and presence of NaCl. However the Pda of the S samples was higher or equal to that of the I samples for the same massaging conditions. In addition, for the short trials (350 compressions) the higher the salt content (Table 1) the higher the Pda.

Figure 4: Relationship between the cumulated deformation energy during massaging and the average structural degradation in samples I and M. Bars show the standard deviation of 4 repetitions.



Since only mechanical action can explain structural modifications in samples I and M, the trials with the two Csalt levels were grouped together and the mean Pda of 4 repetitions was plotted against the cumulated deformation energy. This quantity is the product of the mean deformation energy (Table 1) by the number of deformations; it was approximately equivalent for either 350 deformations at Cr 30 % or 2 500 deformations at Cr 10 %. Figure 4 shows that Pda varies linearly with Log(Pda) and that structural damages are more pronounced in muscle middle than on the periphery. If this relationship was confirmed by new trials for other values of cumulated energy (i.e. 100 J and 700 J) it would provide a mean to transpose observations between tumblers of different sizes that promote different deformation rates.

IV. CONCLUSION

Our trials with the laboratory tumbling simulator were able simulate massaging conditions in tumblers of different sizes.

An automated treatment of sirius red stained histological images revealed structural damaged zones. The percentage area covered by these zones was put in relation with muscle cumulated deformation energy.

It would be interesting to observe the damaged zones by electronic microscopy to determined the exact nature of the structural damages.

ACKNOWLEDGEMENTS

This work was partly supported by a grant from “Conseil Régional d’Auvergne”.

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