

## STUDIES OF SOME HISTOLOGICAL CHANGES OF BUFFALO MEAT DURING AUTOLYSIS

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### INTRODUCTION

Factors affecting the histological changes of muscular tissues of farm animals are nowadays very attractive for the majority of workers in the field of meat production and technology. Three types of histological changes in muscular fibers contemporaneous with the observed stages during meat storage are differentiated. The relation between meat tenderness and the degree of the contraction of muscular fibers was best explained by Sakalov (1965), who showed that the degree of meat tenderness or meat hardness at any time of autolysis depends on the relation between the number of contracted and non contracted fibers at the end of the same period of autolysis.

The length of sarcomeres or the thickness of fibres are used as a parameter to determine the degree of fiber contraction (Hiner 1953; Samasodova 1955; Abdelbaki, 1957; Mux ley 1957; Sakalov and El Dashlouty, 1963; Tinikov and Markaiev, 1966; El Dashlouty and El Ashry 1957).

Later on El Dashlouty et al. (in press) suggested the Factor of

tenderness =  $\frac{\text{sarcomere length in microns}}{\text{fiber diameter in microns}}$  as a tool for expressing the degree of fiber contraction and hence the degree of meat tenderness. An advantage of using the Factor of tenderness is that the amount of changes in both the sarcomere length and fiber diameter are taken in consideration.

Many investigators tried to use the results of microscopic studies to indicate pure technological purpose (meat hardness).

Different workers studied the histological changes in meat during autolysis. These studies were carried out on cow meat (Salaviev, 1962; Tinikov and Markaiev, 1966), sheep meat (Sakalov and El Dashlouty 1963) and on camel meat (Abdel baki, 1957, El Dashlouty and Saied, 1969). As far as the authors are aware no studies of the histological changes on the muscular tissues of buffalo meat are recorded. Hence the present work is directed to study the histological changes of buffalo meat during autolysis and to correlate these autolytic changes with those obtained on the meat of different species of farm animals (sheep and camel meat).

An evaluation of the different methods used for measuring the degree of muscular contraction and their meat tenderness lies within the scope of the present study.

### MATERIALS AND METHODS

Three Egyptian buffalos males 2 years old of about 400 kilograms live weight were slaughtered. 30 samples of Biceps femoris and longissimus dorsi were taken immediately after slaughtering, 24, 48, 72, and 192 hours of storage at 4°C. The fixation was held in 10 % neutral formalin solution. Samples were dehydrated and blocked in collosion. Section of 2 - 5 microns in thickness were prepared and stained using the

+ The stage of freshness, the stage of rigor mortis and the stage of tenderisation.

A Hematoxylin method and the method of Van-Geison. (Diord Kisly, 1962). At the end of the same periods of autolysis samples of meat were taken to examine its plasticity as an indication for the meat tenderness using the method of Gran and Hamm (Valvinskaia, 1958).

## RESULTS AND DISCUSSION :

The microscopic investigations of the studied muscles revealed that the general trend of histological changes in the muscular and connective tissues of buffalo meat is similar but with different amounts to the previously recorded in other farm animals. Immediately after slaughtering muscular fibers are observed to be straight or slightly wavy. Very narrow spaces between fibers were noticed. By the attack of rigor mortis muscular fibers showed more waviness and the spaces became wider.

At the end of tenderisation fiber breaks are observed. Muscular fibers tend to be straight and the spacing between them became narrow.

TABLE 1

The Sarcomere length and the fiber diameter of the longissimus dorsi and Biceps femoris of the buffalo during autolysis (in microns).

Animal	periods of auto- lysis hours after slaughter	Biceps femoris		Longissimus dorsi	
		Fiber diameter	sarcomere	fiber diameter	sarcomere length
I Animal	0	43.75-16.25	1.49-1.79	32.5-20.00	1.92-2.09
	24	52.50-37.50	1.00-1.56	46.25-25.00	1.79-2.09
	48	70.00-42.50	1.00-1.19	41.25-25.00	1.56-1.62
	72	47.50-30.00	1.39-1.79	35.00-27.50	1.79-2.09
	192	45.00-22.50	1.49-1.67	35.00-15.00	1.85-2.09
II Animal	0	45.00-15.00	1.67-1.92	32.50-17.50	1.56-2.00
	24	62.50-22.50	1.56-1.79	43.75-17.50	1.79-1.92
	48	67.50-52.50	1.04-1.09	62.50-25.00	1.56-1.79
	72	65.00-22.50	1.56-1.79	32.50-17.50	1.56-2.00
	192	55.00-13.75	1.25-1.85	32.50-17.50	1.79-2.09
III Animal	0	37.50-10.00	1.56-2.00	42.50-10.00	1.67-2.50
	24	50.00-42.50	1.25-1.56	50.00-20.00	1.56-1.92
	48	58.75-50.00	1.32	52.50-35.00	1.19-1.49
	72	45.00-12.50	1.56-1.67	30.00-15.00	1.72-1.85
	192	37.50-10.00	1.79-2.00	30.00-15.00	1.72-2.09

The data presented in table shows that with the attack of rigor mortis the muscular fibers are contracted, later on these fibers relax. Fiber contraction and relaxation accompanied by a decrease and an increase in meat tenderness (Table 2).

At late periods of autolysis ( 5-8 days) the improvement of meat tenderness which is due to proteolysis may be histologically indicated by the appearance of certain type of fiber breaks (breaks with granular matter)

TABLE 2  
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The plasticity of muscular tissue<sub>2</sub> of buffalo  
during autolysis ( in cm )  
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Periods of auto- lysis in	Biceps Femoris			Longissimus dorsi		
	Animal I	Animal II	Animal III	Animal I	Animal II	Animal III
0	2.77	2.97	3.60	3.80	3.60	
24	2.13	2.12	2.30	3.06	2.25	
48	1.72	1.84	2.08	2.05	1.68	
72	1.90	1.94	2.21	2.50	1.86	
192	2.13	2.4	2.82	2.33	2.40	

Differences in sarcomere length and fiber diameter were noticed in the different muscles of different animals.

Also there was a difference in the depth of the histological changes recorded on the different muscles of the same animal. These may be due to the differences in the exerted work (activity) during the animals life.

It may also be due to the difference in chemical composition such as amounts of stored glycogen, ATP and creatine phosphate at the time of slaughtering (Laviev, 1966). This difference in the chemical composition of the different muscles at the attack of rigor mortis, its intensity, its disappearance, and the degree of meat tenderness (Table 2).

In the present study the factor of tenderness (El Dashouty et al. 1966) was calculated and the results are shown in Table 3.

TABLE 3  
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The Factor of tenderness of buffalo  
muscles at different periods of  
autolysis  
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Period of auto- lysis	Biceps Femoris			Longissimus dorsi		
	Animal I	Animal II	Animal III	Animal I	Animal II	Animal III
0	0.0546	0.0598	0.0756	0.0758	0.0714	0.0756
24	0.0285	0.0250	0.0304	0.0545	0.0606	0.0456

48	0.0195	0.0178	0.0242	0.0480	0.0228	0.0446
72	0.0410	0.0394	0.0562	0.0621	0.0714	0.0795
192	0.0460	0.0460	0.0750	0.0789	0.0776	0.0848

The study of sarcomere lengths reveals that the longissimus dorsi is more tender than the Biceps femoris (Table 1). The same conclusion may be obtained from the study of the factor of tenderness (Table 3). Comparing the fiber thickness of both muscles the same observation is valid with some exceptions in few periods of autolysis (Table 1).

The authors believe that these exceptions due to the fact "The number of fibres is not similar in different fibres and the fiber diameter in later periods of autolysis do not show complete Reliable Results".

These changes in fiber thickness can be used to give a general idea about the trend of change due to autolysis. But they do not show properly the slight changes in the degree of contraction or tenderness.

The ranges in Sarcomere length (Table 1) is in agreement with the changes in plasticity (Table 2), however some exceptions are observed, such as in the case of animal No. 1 in Longissimus dorsi muscle after 2 and 5 days of storage. The disagreement in the range of the sarcomere length with the plasticity is observed only in the maximum range or in the minimum range, but in the case of fiber diameter measurements, the disagreement is observed with the whole ranges. Whence the changes in the ranges of the sarcomere length is a more reliable indication of the fiber contraction or meat hardness than the changes in the fiber diameter.

In view of the histological changes the autolysis of buffalo meat can be divided into different stages:

The stage of freshness: the muscular fibers have a wide sarcomeres. (The sarcomere length of Longissimus dorsi fluctuated from 1.56 to 2.09 microns, and those of the biceps femoris muscle ranged from 1.49 to 2.00 microns.

The stage of rigor mortis: after 24 hours of storage at 4° C. The sarcomere became very narrow, its length ranged from 1.119 to 1.786 and from 1.42 to 1.316 for the L.D. and B.F. respectively.

The stage of tenderisation the sarcomere length varied from 1.724 to 2.093 and from 1.25 to 2.00 micron for L.D. and B.F. correspondingly.

The sarcomer length shown in Table 1 is presented as maximum and minimum, because of the fact that the same fiber exhibit different sarcomer length. The average of the sarcomer length shown in Table 4 gives a less proper idea about autolysis.

Sarcomer length and fiber diameter depend also on the orientation and place of the section measured. The sarcomer length in different parts of the wavy fiber is different (Fig. 1). On the convex side the fiber is longer than those on the concave





Fig.1



Fig.2

TABLE 4  
Average sarcomere length and fiber  
diameter during autolysis

Animal	Periods of autolysis in hours	Biceps femoris		Longissimus dorsi	
		fiber diameter	sarcomere length	fiber diameter	sarcomere length
I	0	30.60	1.639	26.50	2.008
	24	45.00	1.282	35.63	1.940
	48	56.25	1.450	33.13	1.594
	72	38.75	1.588	31.25	1.940
	192	33.75	1.579	25.00	1.970
II	0	30.00	1.795	25.00	1.784
	24	42.50	1.675	30.63	1.855
	48	60.00	1.065	43.75	1.639
	72	41.25	1.625	25.00	1.784
	192	34.38	1.536	25.00	1.909
III	0	23.75	1.782	26.25	2.084
	24	46.25	1.407	35.00	1.743
	48	54.38	1.316	28.75	1.341
	72	28.75	1.615	22.50	1.788
	192	23.75	1.784	22.50	1.909

In order to obtain more accurate and comprehensive idea about the changes in sarcomere length it is recommended to carry out measurements along a profile passing through the central parts of the fiber.

The study of the relation between the sarcomere length and the fiber thickness of the muscular tissues at the different periods of autolysis revealed that the sarcomere length is inversely proportional with the fiber diameter (Table 5 and Fig. 11). The rate of the increase in the fiber diameter in the case of short sarcomere lengths is greater than that in longer ones. It is worthy mentioning that the viscosity of muscles, the texture of connective tissues before and after rigor mortis affect the rate of change in the fiber thicknesses and sarcomere length. The effect of the nods of contraction must not be also overlooked. Their appearance causes a decrease in the thicknesses of the neuhiouring muscular fibers.

The above mentioned factors are conditioned by the time of autolysis, so the time of autolysis is of great importance in the study of a relation such as that between the sarcomere length and fiber thickness.

TABLE 5

The average sarcomere length and fiber thickness of the muscular fibers of buffalo at different periods of autolysis ( in microns )

Average fiber thickness	Average sarcomere length
25.00	2.083
27.50	1.923
30.75	1.786
32.50	1.667
35.00	1.563
38.25	1.471
40.00	1.389
42.50	1.316
48.75	1.250

If we compared the measurements of the average sarcomere length, the average fiber thickness and the factors of tenderness with plasticity at the three stages of autolysis it can be easily observed that the most suitable and accurate indicator for meat tenderness is the factor of tenderness. The disagreements between the factor of tenderness and plasticity are observed only in 11% of the studied cases, however, 25 % of studied cases shown disagreement between plasticity and fiber thickness or sarcomere length.

The sarcomere length and fiber diameter are satisfactory as an indicator of rough changes in meat tenderness and in autolysis. The three histological parameters are good indicators of the top of rigor mortis.

Using the precentage of the factor of tenderness, one can easily compare the velocity of autolysis in different animals ( Table 6).

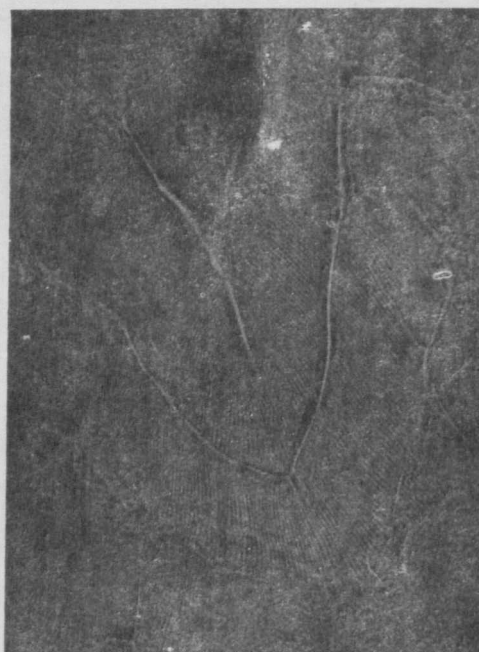


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The sarcomere length and fiber diameter are satisfactory as an indicator of rough changes in meat tenderness and in autolysis. The three histological parameters are good indicators of the top of rigor mortis.

Using the percentage of the factor of tenderness, one can easily compare the velocity of autolysis in different animals ( Table 6).



TABLE 6  
The factor of tenderness of the longissimus  
dorsi of buffalo, camel and sheep during  
autolysis

The studies factor	The Factor of tenderness			The percentage of Factor of tenderness		
The species	Buffalo	Camel	Sheep	Buffalo	Camel	Sheep
The temperature of storage	4°C	4°C	0°C	4°C	4°C	0°C
Immediately after slaughtering	0.074	0.064	0.098	100	100	100
After 24 hours	0.055	0.018	0.055	75	27	56
" 48 "	0.039	0.023	0.068	53	35	69
" 3-5 days	0.071	0.029	0.070	96	45	77
" 8 "	0.080	0.057	0.095	109	58	97

<sup>+</sup> El Dashlouty and Said (on press)

<sup>++</sup> El Ashry and El Dashlouty, 1967

The data presented in Table 3 reveals that rigor mortis attack the Biceps femoris muscle before the longissimus dorsi muscle. The rigor mortis vanishes from the longissimus dorsi before Biceps femoris. The autolytic changes were very intensive in Biceps femoris as compared by Longissimus dorsi.

The percentage of the factor of tenderness (Table 6) showed that the rigor mortis in buffalo meat is in generally slower than in sheep and camel meat. The rigor mortis disappears in buffalo meat after a shorter time than the times needed in the case sheep and camel meat.

The figures of the factor of tenderness (table 6) showed that buffalo meat has a medium position between the camel and sheep meat. This is in full agreement with general observations of the consumers about the tenderness of these different sp.

## CONCLUSIONS

The fiber wavyiness greatly affects the length of sarcomere. The sarcomeres on the concave side of the wave are observed at the central parts of the wavy fibres.

The viscosity of the muscular tissues, the existence of the nods of contraction and the texture of connective tissues are different before and after rigor mortis. All these local conditions greatly affect the length of sarcomeres and fiber diameters.

The fiber measurements do not give a perfect indication about the changes in meat tenderness, since the humber of miofiber varies widely in every fiber.

Using the factor of tenderness it was found that sheep meat is more tender than buffalo meat, camel meat is characterized by the lowest degree of tenderness.

Rigor mortis attacks the Biceps femoris before the longissimus dorsi and vanishes away from the Biceps femoris after the longissimus dorsi. It is characterized to be more intense in the case of the Biceps femoris muscle compared with the longissimus dorsi muscle.

Although the rigor mortis attacks buffalo muscles slower than sheep and camel muscles. It takes a shorter time to vanish away from the muscles of buffalo.

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