Animal Production Research Institute,
Agriculture Reasearch Center,
Dokki, Giza, Egypt.

HISTOLOGICAL CHANGES IN CHICKEN AFTER FREEZING AT - 20°C AND - 40°C

(With 3 Fig. & One Table)

By

M.N. EL-GHAZALI; S.I. EL-SYIAD*
and Sh. M. FATHI**

(Received at 28/9/1994)

التغيرات الهستولوجيه فى عضلات الدجاج بعد التجميد على درجة - ٢٠°م ، - ٤٠°م

محمد نجاتى الغزالى ، سامى الصياد
شوكت فتحى

أجريت هذه الدراسه على عضلة الصدر الكبرى Pectoralis Superficials وعضلة الفخذ الكبرى Glutaeus Superficials وعضلة الدبوس Gastrocnemius من دجاج Cobb عمر ٨ أسابيع لدراسة التغيرات فى القياسات والتراكيب الهستولوجيه للذبائح الطازجه والمجمده على درجتى - ٢٠ م ، - ٤٠ م . وقد وجد أن قطر الليفه العضليه فى عضلة الصدر كانت أقل منها فى كل من عضلة الفخذ وعضلة الدبوس . ومن ناحية أخرى وجد أن قطر الالياف العضليه فى كل من عضلات الصدر والفخذ والدبوس يقل بينما طول الساركومير يزيد مع انخفاض درجة الحراره ، حيث قطر الليفه العضليه يقل وطول الساركوميتر يزيد عند درجة - ٤٠ م عنها عند درجة - ٢٠ م .

وقد أوضحت الدراسه أيضاً أن كل من معامل الطراوه Tenderness يزيد عند درجة - ٤٠ م عن - ٢٠ م بينما معامل الصلابه Hardness يقل عند - ٤٠ م . أيضاً التغيرات الهستولوجيه تكون أكبر فى عضلات الدجاج المجمد عند - ٤٠ م عنها فى المجمد عند - ٢٠ م ، حيث لوحظ أن العقد والتشققات والتموجات والتكسير فى الالياف العضليه وكذا المسافات بين الحزم العضليه تزيد فى العضلات المجمده عند - ٤٠ م عنها فى العضلات المجمده عند - ٢٠ م . أيضاً حجم الحزم العضليه وكذلك الالياف الكولاجينيه تقل فى العضلات المجمده عند - ٤٠ م عنها فى العضلات المجمده عند - ٢٠ م .

---

*: Food Science and Technology Dept. Fac. of Agriculture, Assiut University, Egypt.

**: Food Hygiene Dept. Fac. of Vet. Med., Assiut Univ., Egypt.

SUMMARY

Fiber diameters of breast *(Pectoralis Superficials)*, thigh *(Glutaeus superficials)* and drumstick *(Gastrocnemius)* muscles of cobb chicken *(8 weeks age)* were shorted with decreasing the temperature, whereas, they were shorted at -40 °C more than at-20 °C, while, sarcomere lengths were increased after freezing of chicken muscles under investigation at -40 °C more than at -20 °C. Concerning hardness factor (H.F.), it was decreased, while tendreness factor (T.F.) was increased after freezing at -40 °C more than at -20 °C . Histological changes, including nodes, internodes, fissures, waves, splitting within fiber, kinks, kracks and spaces between bundles were increased in freezing method at-40 °C more than at -20 °C. On the other hand, size and shape of bundles and collagenous fibers were decreased in freezing the examined muscle samples at -40 °C rather than at -20 °C.

Keyword: Histological, chicken, muscles, freezing

INTRODUCTION

The cold is the simplest method for preservation af food. Efficient refrigeration can preserve meat in a condition approaching its natural state for periods adequate for commercial requirements, its appearance, weight and flavour are little altered, and no substance is added to the meat nor any extracted.

On freezing, ice crystals are formed in the tissue. Their size, shape and position, extra-or intracellular, all depened on rate of freezing and whether or not the flesh is frozen pre-or post-rigor. Formation of intracellular ice crystals by quick freezing leaves the overall appearance of the tissue intact, but microscopic examination of individual cells shows needle-like spaces, the dimensions of which vary with the rate of cooling. Formation of extracellular ice crystals by slow freezing gives rise to varying amounts of deformation of the overall tissue structure, depending on the rate of cooling *(VAUGHAN, 1979)*. Quick freezing of meat has made rapid strides and is applied to lambs, calves, pigs, poultry and fish, where the temperature may be reduced to as low as -46 °C *(GRACEY and COLLINS, 1992)*.

Also cystal formation and the possibility of cellular

rupture as produced by a wide range of freezing temperatures have been studied by *KOONZ and RAMSBOTTOM (1939)* and *HINER and HANKINS (1947)*; *LOWE (1948)* showed that sections from muscle of freshly slaughtered chickens are characterized by poorly differentiated fibers with fairly distinct longitudinal striations. The fibers are usually wavy, but may be straight. Also rigor nodes are found with onest of rigor. However, *LUYET (1964)* stated that slow freezing at approximatly -17 °C produces only intercellular or interfiber freezing. The ice crystals are large and few in number and tend to compress the fibers and connective tissue strands into ridges or bandlike formations. Faster Freezing at-40 °C produces ice crystals within the fibers, whereas very fast freezing at-150 °C produces microscopic ice crystals. *WELBOURN et al (1968)* reported a progressive shortening of sarcomere lengths in breast and thigh turkey muscles with decreasing temperature, where their lengths were shorter for breast than for thigh muscles. Also, *HAY et al (1973)* observed that leg chicken muscle in situ sarcomeres showed increase in length than breast muscle.On the other side, *TRZISZKA et al (1981)* and *(1984)* found that histological changes were greater in duck carcases frozen without cooling than cooled and frozen carcases. Also, changes of muscles were greater in thigh and drumstick than breast.Weakening of z-line of breast muscle and lengthening of sarcomeres of leg muscles of chicken were observed as the result of aging *(PIMENTEL et al .,1975)*. However,*KOPEC et al.,(1985)* reported that degradation of frozen duck fibers is considered as a result of advanced post-mortem processes and most probably caused by freezing and thawing

EL-GHAZALI (1989) observed that contraction nodes, fiber kinks, fissures, interfibrillar space between bundles, Zig-Zag, s waves twists, cracks and breaks were increased in breast, thigh and drumstick of both pekin and sudani ducks after freezing at-40 °C rather than in fresh muscles. Also,muscle fiber diameters in ducks muscles were decreased during frozen storage, while sarcomere lengths were increased.

PANDEY et al.,(1989) pointed out that fresh broiler carcases had tougher breast and leg muscles than carcases left at 5 °C for 8 or 24 h., where tenderness and water holding capacity were highest after 24 h. Also, more sarcoplasmic and myofibrillar proteins were extracted pre-and post-rigor (15 min. and 24 h. after slaughter). They suggested that broiler carcases should be aged for at least 8 h. before freezing.

The increase in fiber diameter and decrease in sarcomere length were considered an important changes in lowering the meat tenderness and consequently affect on the meat quality as

reported by several authors, i.e. (*HERRING* et al.,*(1964), (1965a)* and *(1965b), ASGHAR (1969),LOCKER* and *DAINES, (1976a),(1976b)* and *LOCKER and LEET,1976]. ALSO HAMMADI (1980)* and *EL-GHAZALI (1989)*, who illustrated that there is correlation between fiber diameter and sarcomere length with tenderness and hardness of meat in lamb and duck carcases, respectively. Therefore, the present investigation was planned to study the effect of freezing on the histological changes in chicken muscles and its correlation with tenderness and hardness of the meat.

MATERIALS AND METHODS

Cobb chickens (8 weeks age) were obtained from Cairo-Aswan poultry company. The chickens under investigation were fed on ration having 18% protein and fasted for 12 hours before slaughtering. The carcasses were forzen at-20 °C in a blast-freezing tunnel for 15 hours and at-40 °C using multiple plate contact freezey (Micom, made in Japan) for 90 minutes. in Misr-Aswan company plant. Cubic specimens (2x2x2 mm) were taken from concrete place of breast *(Pectoralis Superficials)*, thigh muscle *(Glutaeus superficials)* and drumstick muscle *(Gastrocnemius)* as described by *NICKEL* et al., *(1977)*. The specimens were obtained from either fresh and frozen carcasses at both -20 °C and-40 °C and fixed in 10% neutral formalin. The absolute ethanol and xylol technique was used for dehydrating and clearing them. After infiltration with paraffin, the tissues were embedded in clean paraffin and cut 10 microns thick with a Rotary Microtome *(British American Optical Co. Ltd.)*. Tissues were stained on the slides applying haematoxylin and eosin stain for general histological structure and Van Geison, s stain for collagenous fibers *(DRURY* and *WALLINGTON,1980)*. Slides were investigated and photographed using light microscope (Carl-Zeiss Jena-made in Germany).

The muscle fiber diameter and sarcomere length of different muscles for each chicken carcass either fresh and frozen samples at-20 °C and-40 °C were measured and calculated. Hardness factor (H.F.) and tenderness factor (T.F.) were recorded as histological indications to the texture of meat *(HAMMADI* et al.,*1980)*

RESULTS

The obtained results were illustrated in Table(1) and figures (1),(2) and (3).

DISCUSSION

The quality of meat is retained better at lower temperatures. Meat that has been quick-frozen to a temperature of-40 °C by comparison with meat frozen to a temperature -20 °C has a lighter and more desirable colour in the frozen state. Also, the meat quick-frozen at the lower temperature tends to retain its juices better and is firmer after thawing *(LIBBY, 1975).*

I. Histological Measurements:

Poultry, like meats in general, is tough if it is cooked before or while the meat is still in rigor. Poultry goes into and out of rigor more rapidly than other meats.

The measurements of fiber diameter (F.D.) as well as the length of sarcomere (S.L.) of fresh and frozen breast *(Pectoralis Superficials),* thigh *(Glutaeus Superficials)* and drumstick *(Gastrocnemius)* muscles are presented in Table (1). Also, the ratios between sarcomere length/fiber diameter (S.L./F.D.) and fiber diameter/sarcomere length (F.D./S.L.) could be considered as an index to the tenderness and hardness factors of the chicken muscles.

Fiber diameter of breast muscle recorded 27.42 μ 18.42 u and 10.51 u in fresh, frozen at-20 °C and-40 °C chickens, respectively. Also, fiber diameter of thigh muscle was 40.50 μ while it was 22.50 u and 15.27 u after freezing at -20 °C and -40 °C, respectively. The same changes were found in drumstick muscle, whereas it recorded 52.27 μ in fresh and 37.50 μ and 30.50 μ after freezing at-20 °C and-40 °C, respectively. The fiber diameter in breast muscle of chicken was shorter than those in thigh and drumstick muscles, also fiber diameters of breast, thigh and drumstick of chicken muscles were shortned with decreasing temperature, where as they were shortned at-40 °C than at-20 °C. The obtained results are in agreement with those reported by *EL-GHAZALI (1989).*

On the other hand, it was observed in Table (1) also that sarcomere length in breast muscle of fresh chicken was shorter than those in thigh and drumstick muscles (1.16, 1.28 and 1.40 U., respectively). Moreover, the obtained results revealed that sarcomere lengths were increased during freezing treatment of fresh chicken, where they were increased after freezing at-40 °C than at-20 °C. Whereas they were 1.76, 1.80 and 1.88 U after freezing at-20 °C and 1.95, 2.24 and 2.24 U after freezing at-40 °C in breast, thigh and drumstick of chicken muscles, respectively. These results coincide with the findings outlined by *WELBOURN* et al., *(1968)* and *EL-GHAZALI (1989).* It was noticed from the results illustrated in Table (1) that there is

an inverse relationship between sarcomere length and fiber diameter. As fiber diameter decreased while the sarcomere length increased.

The important changes, which have relevance to meat quality, was the S.L./F.D. value. The increase of this value indicated an increase in the tenderness of the muscles. The present results outlined in Table (1) revealed that hardness factor decreased and tenderness factor increased after freezing at-40°C than at-20°C. This trend of results is also in accordance with the results reported by *HERRING* et al., *(1964), (1965 a)and (1965 b), LOCKER* and *DAINES (1976 a)* and *(1976 b), LOCKER* and *LEET (1976); HAMMADI (1980) and EL-GHAZALI (1989).*

I.I. Histological Changes:

The changes in histological structure of breast *(Pectoralis Superficials),* thigh *(Glutaeus superficals)* and drumstick muscles *(Gastrocnemius)* of fresh chicken and after freezing at-20°C and at-40°C were shown in figures (1), (2) and (3).

Longitudinal sections for muscle fibers of breast, thigh and drumstick muscles of fresh chicken were poorly differentiated with distinct longitudinal striation and no transverse breaks [Fig.1 (left)]. Also, it was observed some longitudinal splitting within fiber [Fig.1 no. 1,2 & 3 left]. The interfibrillar spaces in drumstick muscle were more distinguishable [Fig. 1 no. 3 left] than in breast and thigh muscles [fig. 1 no. 1 & 2 left]. These results are in agreement with those reported by *LOWE (1948) and EL-GHAZALI (1989).*

The transverse sections for muscle fibers of breast, thigh and drumstick muscles of fresh chicken as well as the size and shape of muscle fiber bundles and the amount and distribution of connective tissues are shown in fig. 1(left). The appearance of spaces between fiber bundles and muscle fiber has reflected the different degrees of shrinkage. Also, it appeared that interfibrillar spaces between bundles were small in fresh muscles, separation between these bundles became larger after freezing treatment (Fig. 2 and 3 right). In addition, spaces between bundles were larger in drumstick muscle than in breast and thigh muscles [Fig. 1 no. 3 (right)]. wide separation between fiber bundles could be noticed after freezing treatment than in fresh chickens (Fig. 1 & 2). It was shown that the fiber bundles had variable shape and size in fresh muscles when compared with frozen muscles. On the other hand, a large amount of collagenous fibers observed in perimysium of thigh and drumstick muscles than in breast muscle in fresh case, and after freezing treatment decreased in all muscles (Fig. 1 right). These results are in agreement with those pointed out

by *EL-GHAZALI (1989)*.

Fig. 2 (left) showed longitudinal sections for muscle fibers of breast, thigh and drumstick muscles from chicken immediately after freezing process at-20°C. It appeared that the fibers were straight & slightly wavy [Fig. 2 no. 3-a (left)], Some fibers were straight and others contained nodes and stretched area, some craks and breaks also appeared after freezing process [Fig. 2 no. 1-a and 2-a (left)]. However, Waves, Zig-Zag, kinks and twists were characterized especially in thigh and drumstick muscles than breast muscle. The obtained results coincide with those reported by *KOONZ and RAMSBATTOM (1939); HINER and HANKINS (1947); LUYET (1964) and EL-GHAZALI (1989).*

In Fig. (2) right, it was noticed that cross-striation extremly disappeared and large interfibrillar spaces appeared in all cases. Also, widly separation between fiber bundles and few thin collagenous fibers were observed between those bundles. Similar findings were reported by *KOONZ and RAMSBOTTOM (1939); KOPEC et al.; (1985) and EL-GHAZALI (1989).*

Nodes and internodes were noticed also in examined chicken muscles (Fig. 3, left). Some fibers were split by fissures and increase in frequency of fiber breaks and interfibriallar spaces were observed. Histological changes were greater in thigh and drumstick muscles than in breast muscles after freezing at-40°C. In Fig. 3 (left), it was observed that increase the spaces between bundles and the fiber bundles were separated from one to another by approximately equal spaces. Also, small few thin collagenous fibers were noticed. These results are in accord with the findings recorded by *KOONZ AND RAMSBOTTOM (1939), TRZISZKA et al., (1981) & (1984), KOPEC et al., (1985) and EL-GHAZALI (1989).*

Generally, histological changes were greater in chicken muscles after freezing process at-40°C than at-20°C. Whereas, nodes, internodes, fissures, waves, splitting within fiber, kinks, kracks and spaces between bundles were increased in freezing process at-40°C than at-20°C, size and shape of bundles and collagnous fibers were decreased in freezing muscles process at-40°C than at-20°C. These changes might be due to differentiation of degree of temperature, time of freezing and size and shape of ice crystal formations between the two freezing processes. The denaturation of proteins was also continued. However, the determined denaturated protein in this study increased with the decrease of freezing temperature

REFERENCES

Asghar, A. (1969): Biophysical, biochemical and microstructure aspects of Lambs muscle, grown under different nutritional States, with particular reference to meat quality. Ph.D. Thesis, University of New England, Armidale, N.S.W., Australia.

Drury, R.A.B. and Wallington, E.A. (1980): Carleton's Histological technigue. Fifth Edition, Oxbord, New York, Toronto, Oxford University.

El-Ghazali, M.N. (1989): Technological and histological studies on duck meat. ph. D. Thesis, Faculty of Agriculture, El-Minia university, Egypt.

Gracey, J.F. and Collins, D.S. (1992): Meat hygiene, Ninth Ed., Bailliere Tindall, 24 - 28 Oval Road, London NW1 7DX.

Hammadi, K.A., Dessouki, T.A. and El-Dashlouty, A. (1980): the microscopic structure of different cuts from Egyptian lamb carcass. El-Minia J. of Agricultural Research and Development 5,3.

Hay, J.D., Currie, R.W., and Wolfe, F.H. (1973): Effect of Post. morten aging on chicken muscle fibrils. J.Food Sci. 38, 981.

Herring, H.K., Cassens, R.G., and Briskey, E.J. (1964): Sarcomere length of free and restrained bovine muscle after thaw rigor contracture. Biodynamica. 9, 257.

Herring, H.K., Cassens, R.G. and Briskey, E.J. (1965a): Sarcomere length of free and restrained bovine muscle at low temperature as related to tenderness. J. Sci. Food Agric. 16, 379.

Herring, H.K., Cassens, R.G. and Briskey, E.J. (1965b): Further studies on bovine muscle tenderness as influenced by carcass position, Sarcomere length and fiber diameter. Food Res. 30, 1049.

Hiner, R.L. and Hankins, O.G. (1947): Temperature of freezing affects tenderness of beef. Food Ind. 19, 1078.

Koonz, C.H. and Ramsbotton, J.W. (1939): A method for studing the histological stucture of frozen products I. Pouftry. Food Res. 4, 117.

Kopec, W., Smolinska, T. and Trziszka, T. (1985): Post.morten metabolism and ultrastructural changes in frozen duck tissues: Proteolytic Changes. Arch. Geflugelk. 49, 168.

Libby, J.A. (1975): Meat hygiene, Fourth Ed. Lea and Febiger, philadelphia.

Locker, R.H. and Daines, G.J. (1976a): Transverse anisotrophy in beef muscle. J. Sci. Food Agric. 27, 186.

Locker, R.H. and Daines, G.J. (1976b): Tenderness in relation to the temperature of rigor onest in cold shortened beef. J. Sci. Food Agric. 27, 193.

Locker, R.H. and Leet, N.G. (1976): Histology of highly stretched beef muscle. J. ultrastuct. Res. 55, 157.

Lowe, B. (1948): Factors affecting the palatabillity of poultry with emphasis on histological Post. mortem changes. Adv. Food Res. 1, 203.

Luyet, B.J. (1964): Effects of freezing on muscle tissue. Proc. 17th. Reciprocal Meat Conference.

Nickel, R., Schummer, A. and Seiberle, E. (1977): Anatomy of domestic birds. Verlaypauf Parey, Berlin, Hamburg.

Pandey, N.K.; Anand, S.K.; Mahaputra, C.M. and Verma, S.S. (1989): Physico-chemical and microbial changes in dressed chicken during post morten aging. Indian J. of Poultry Science, 24: 1, 50-55.

Pimentel, D.; W. Dritschilo; J. Krummel and J. Kutzman (1975): Energy and land constrains in food protein production. Science 190: 754 - 761.

Trziszka, T., Smolinska, T., and Kopec, W. (1981): Biochemical Processes and ultrastrucural changes in muscle tissue of ducks. Proceeding of the European Meeting of Meat Research workers, No. 27, Vol. I.A: 37, 137. (C.F. FSTA, 1983, Vol. 15, No. 9 S1387).

Triziszka, T., Smolinska, T., Czarma, Z. and Kopec, W. (1984): Post. mortem metabolism and ultrastructural changes in frozen duck tissues: Ultramicroscopic Studies. Arch. Geflugelk. 48 (3), 93. (C.F. FSTA, 1985, Vol. 17 No. 3 S 144).

Vaughan, J.G. (1979): Food Microscopy. First Ed. Academic Press Inc. (London) LTD. 24-28 Oval Road, London NW 1.

Welbourn, J.L., Harrington, R.S. and Stadelman, W.J. (1968): Relation ships among shear values, sarcomere lengths and cooling procedures in turkeys. J. Food Sci., 33, 450.

Table (1): Histological measurements of fresh and frozen chicken muscles at -20 °C and - 40 °C.

Muscle Parameters	Treatments								
	Pectoralis superficials			Glutaeus Superficials			Gastrocnemius		
	Fresh	20 ◦C.	-40 ◦C	Fresh	-20 ◦C	-40 ◦C	Fresh	-20◦C	-40 ◦C
Fiber diameter (U)	27.42	18.42	10.51	40.50	22.50	15.27	52.20	37.50	30.50
Sarcomere length (U)	1.16	1.76	1.95	1.28	1.80	2.24	1.40	1.88	2.24
Tenderness factor[1]	0.04	0.10	0.19	0.03	0.08	0.15	0.03	0.05	0.07
Hardness factor[2]	23.64	10.47	5.39	31.64	12.50	6.82	37.29	19.95	13.62

1. Tenderness factor = Sacomere length / Fiber diameter ratio.
2. Hardness factor = Fiber diameter / Sacomere length ratio.

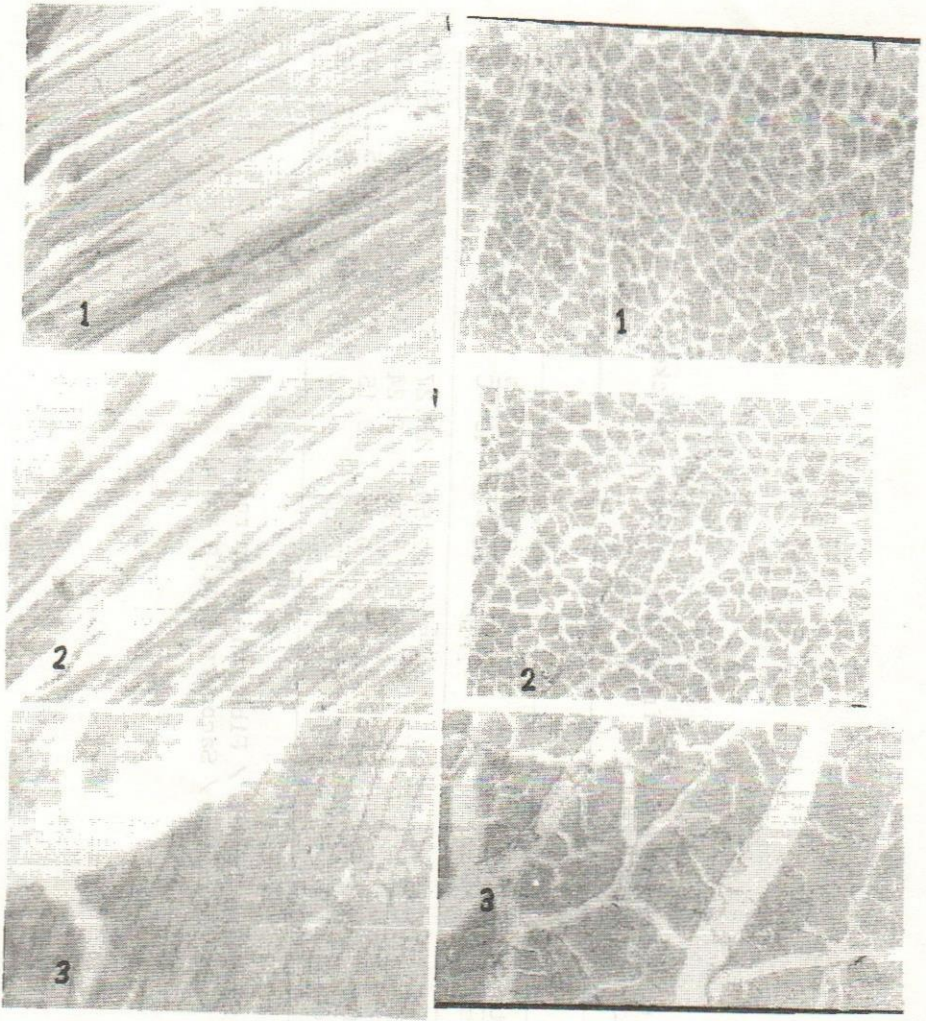

FIGURE (1): Longitudimal sections (left) and transverse sections (Right) of muscle fibers from fresh chicken. X 100.

1. Breast (Pectoratis superficials).
2. Thigh (Glutaeus superficials).
3. Drumstick (Gastrocnemius).

NOTE: (L.S) Straightening of fibers, improved Differentiation erentiation, some longitudinal splitting within fibers (1,2,3, Left).

(T.S) Collagen strands between bundles (*Bright red*), long narrow muscle fiber bundles, shape and size of bundles, spaces between bundles (1,2,3, Right).

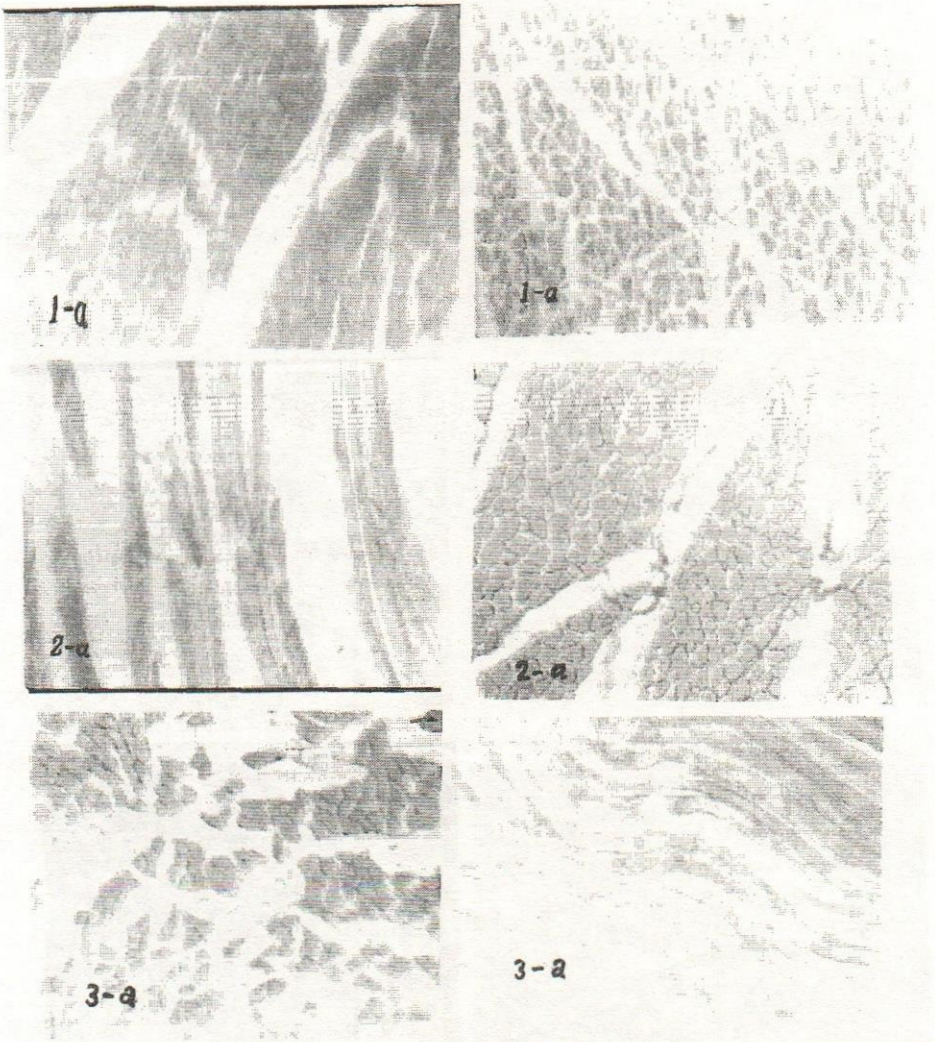

FIGURE (2): Longitudinal sections *(Left)* and transverse sections *(Right)* of muscle fiber from chicken after freezing at - 20° C.
1. Breast (Pectoralis superficialis).
2. Thigh (Glutaeus superficialis).
3. Drumstick (Gastrocnemius).
a) After freezing at -20 °C.

NOTE: (L.S), Disappearamce of longitudinal splitling within fibers, fissures, and macroscopic waves. (1,2,3, Left).
(T.S), Bundles tend to widely separated, spaces between bundles and size of bundles (1,2,3, Right).

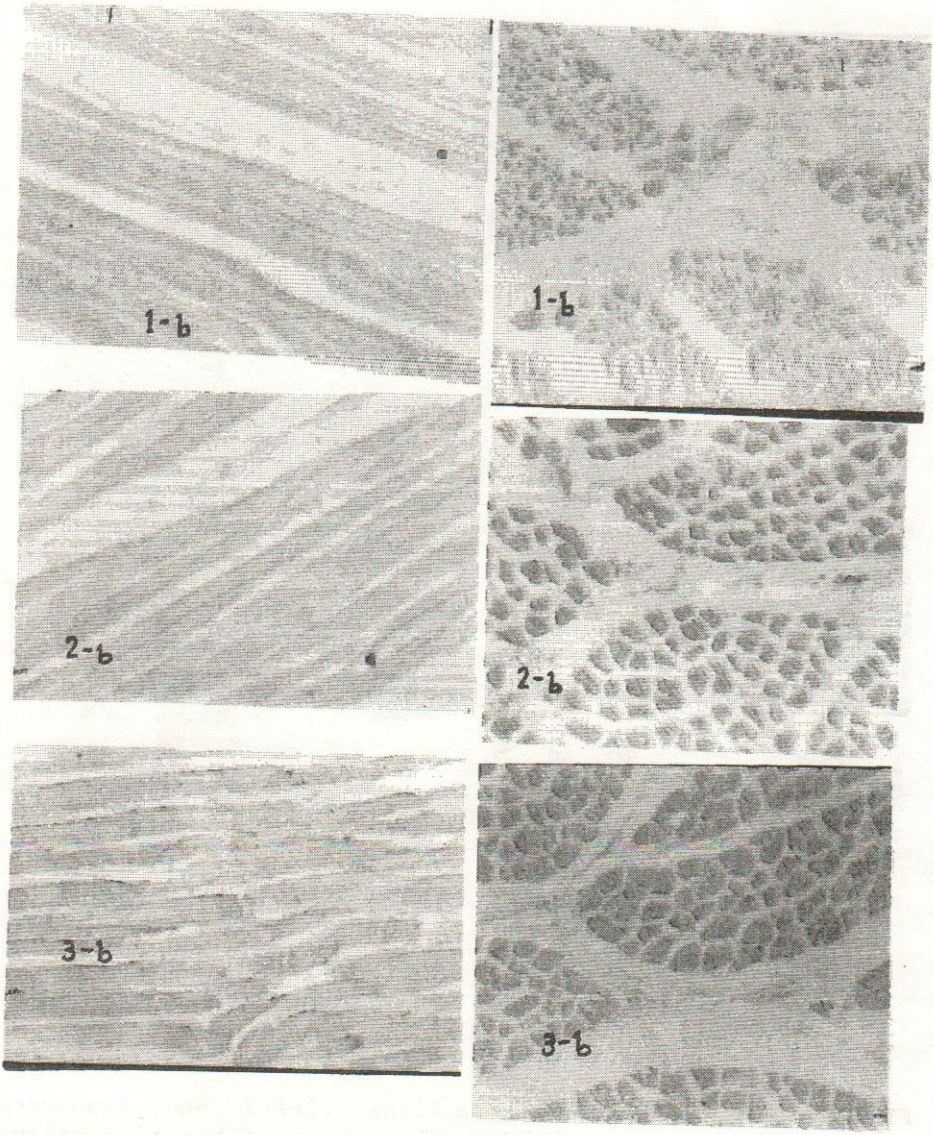

FIGURE (3): Longitudinal sections *(Left)* and Transverse sections *(Right)* of muscle fiber from chicken after feezing at -40 °C.
1. Breast (Pectoralis superficialis)
2. Thigh (Glutaeus superficialis)
3. Drumstick (Gastrocnemius)
b) After freezing at -40 °C.

NOTE: (L.S), Nodes, kinks and fissures (1,2,3, left)
(T.S), Widely separation between bundles and large spaces between bundles (1,2,3 Right).