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Effect of Age and Periods of Rigor Mortis on Quality of Sarcoplasmic Proteins Separation from Meat's Protein by Using Electrophoresis Technique

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Abstract
We were separated sarcoplasmic proteins from beef meats on 12 and 28 months ,mutton meats on 6 and 12 months and chicken meats on 25 and 40 days on rigor mortis stage and after rigor mortis stage by using electrophoresis technique. The results showed the following: Sarcoplasmic proteins differ on all types of meats below number of bands separation from it's as differ of age of animal and stage of rigor mortis ,On beef meat proteins showed presence of 10 and 8 bands on 12 and 28 months on stage of rigor-post mortis the bands separation from sarcoplasmic, On the mutton meat we separation 10 bands of sarcoplasmic proteins on 6 and 12 months on stage of rigor-post mortis, We found 11 bands of sarcoplasmic proteinson on chicken meats on 25 and 40 days respectively.The band of phosphorylase b (PHb) and phosphorylase b kinase (PHbK) enzymes showed as one big pale band,while phosphor glucomutase (PGM), pyruvate kinase (PK), phosphoglucose isomerase (PGI), enolase (EN), creatine kinase (CK), phosphoglycerate kinase (PGAK), aldolase (ALD), glyceraldehyde phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH), phosphoglycerate mutase(PGAM), triosephosphate isomerase (TPI) and myoglobin (Mb) showed as thin and pale bands.
Keywords: Beef meat , mutton meat, chicken meat, meat's protein, sarcoplasmic proteins, electrophoresis, rigor mortis

1. Introduction

Meat to be divided on two kinds (red and white) so that meats are' An excellent source of high biological value protein, vitamin B12, niacin, vitamin B6, iron, zinc and phosphorus. A source of long-chain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and possibly also vitamin D. Mostly low in fat and sodium . Sources of a range of endogenous antioxidants and other bioactive substances including taurine, carnitine, carnosine, ubiquinone, glutathione and creatine' (Williams , 2007),or we can defines meat as'the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include eggs, or foetuses'. Meat can be described as an edible dressed portion derived from the skeletal muscle of healthy livestock or domesticated farm animals and must be free from offals. Sources of meat include livestock such as cow, sheep, goat, pig and poultry, which are domesticated. In some cultures, various types of animals like reptiles, avian and amphibians may become important delicacies such as in a specialized occasion-chieftaincy. The major changes in meat have to do with flavor, texture and microbial quality while in seafood flavour and odour problems are important physiological changes (Adegoke and Falade, 2005).

Muscle conversion to meat is controled by complex interactions of biochemical processes that take place during post- mortem storage of the carcass (Brewer et al., 2008). Although their influence on the final texture and tenderness of the meat is still not clear, it is well documented that fragmentation of myofibrils takes place during post-mortem storage of meat and that the extent of this fragmentation is related to the tenderness of aged meat (Morgan *et al*., 1991).

Skeletal muscle unit was the muscle fiber. Among many components of muscle fiber, protein was the one of important. Muscle proteins are categorized as sarcoplasmic, myofibrillar, and stromal proteins based on their solubility. Sarcoplasmic proteins are extracted in aqueous solution with low ionic strength (0.15 or less). Myofibrillar proteins are extracted by salt solutions and require higher ionic strength, called salt-soluble proteins. Stromal proteins included proteins of connective tissues, which are very fibrous and insoluble (Aberle *et al.,* 2001). Sarcoplasmic proteins from about 25-30% of totale protein content and are mostly water soleble glycolytic enzymes and pigments (Hassan and Javed , 2000).

Electrophoretic technique can be used to identify meat proteins of various animal species. Its based on the separation of proteins in the electric field following their extraction from the muscle tissue and later placed on special media (Montowska and Pospiech, 2007).

2.0 Materials and methods

2.1 Row materials:

2.1.1 Beef meat:

We buying beef meat which taken from thigh muscle from Al-Ashar's butchery on Basrah city, whereas we slaughtering there and the slaughtering time 6 O'cloce on morning, age of caws were about (12 and 28) months , the skin removed and slaughtered cleaned on butchery after that cutting for different muscular portions after slaughtering and cleaning the weight of carcass which was on 12 months 125kg, but the carcass which was on 28 months 220kg , we taken samples of meats after slaughtering (rigar mortis stage)and after passing temporal period to assess about 20 hour after slaughtering (stage of rigor-post mortis), all samples placed on plastic container and getting to meats technological laboratory- Collage of Agricultur /University of Basrah .

2.1.2 Mutton meat:

We buying mutton meat which taken from thigh muscle from Al-ashar's butchery on Basrah governor, whereas we slaughtering there and the slaughtering time 6 O'cloce on morning, the sheep were about (12 and 12) months , the skin removed and slaughtered cleaned on butchery after that cutting for different muscular portion , after slaughtering and cleaning the weight of carcass which was on 6 months 9-11kg, but the carcass which was on 12 months 25kg , we taken samples of meats after slaughtering (rigar mortis stage)and after passing temporal period to assess about 20 hour after slaughtering (stage of rigor-post mortis), all samples placed on plastic container and getting to meats technological laboratory- Collage of Agricultur /University of Basrah .

2.1.3 Chicken meats:

This study to perform on chicken meats which taken from thigh and breast muscle ,after slaughtering chicken on meats technological laboratory,the chicken meats on 25 and 40 days ,the samples of meats taken after slaughtering (rigar mortis stage) and after passing temporal period to assess about 5 hour after slaughtering (stage of rigor-post mortis), the skin removed and cleaned, after cleaning the weight of chicken in 25 days was 850 g and weight of chicken in 40 days was 1200 g, hence that cutting and thigh and breast muscle were taken and mincing by electricity meat chopper and mixing together.

2.2 Methods:

2.2.1 Separation muscular proteins(sarcoplasm)from meat:

Sarcoplasm proteins separated from three kinds of meats following Al-azawy (1996) method and modified method of Huda *et al*.(1994).

2.2.2 Division separated sarcoplasm proteins from (beef , mutton and chicken meat)by Electrophoretic technique and usage polyacrylamide gel:

Electrophoretic technique on polyacrelamide gel with absenced photocopier facter following Laemmli(1970)which clarify by Garfin(1990)on division sarcoplasm proteins with some modification,and this experiment doing in genetics engineering laboratory- Collage of Agricultur /University of Basrah .

3.0 Results and discussion

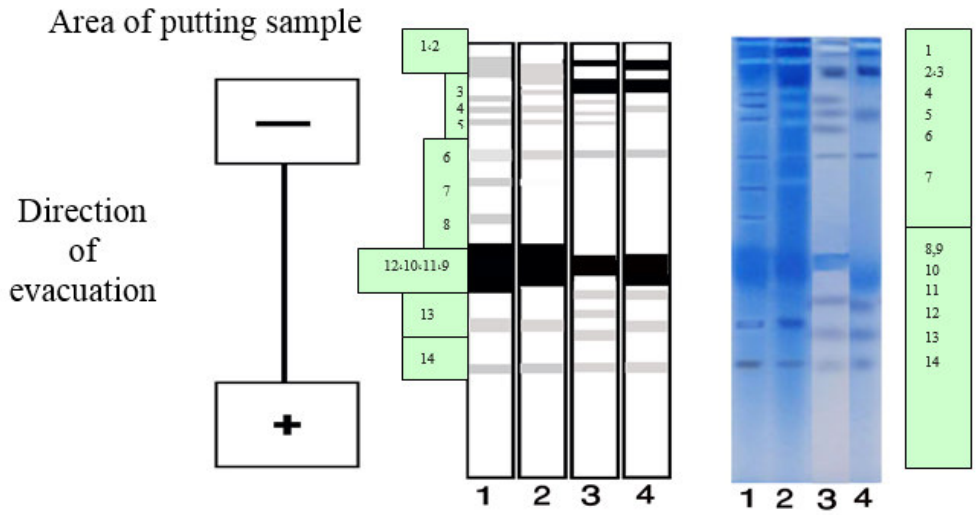
3.1 Electrophoretic technique of sarcoplasm proteins:

3.1.1 Electrophoretic technique of sarcoplasm proteins for beef meats:

The figure(1) clarify results electrophoretic technique of sarcoplasm proteins for beef meats on (rigor- mortis and rigor-post mortis stage) and for caws on age 12 and 28 months , which appeared 8,10 bands on rigor- mortis and rigor-post mortis stage on 12 months and 8,11 bands on rigor- mortis and rigor-post mortis stage on 28 months,also differened on thickness on gel area and molecular weight ranging between heavy and light.

This proteins bands include all main sarcoplasm proteins which dissolved in distilled water besides different kinds of proteses such as cathpsenes and proteins resisting heating treatmen and pyruvalbomenes with low molecular weighting.

on rigor- mortis, we observed pale band but great plased on top of gel and On beef meats at 12 months consisting in phosphorylase b (PHb) and phosphorylase b kinase (PHbK) enzymes merged together, following by three proteins bands pale and thin placed on equal space approximatly they were phosphor glucomutase (PGM), pyruvate kinase (PK) and phosphoglucose isomerase (PGI) enzymes consecutively, after that three pale and thin bands of proteins appeared close together they were enolase (EN), creatine kinase (CK) and phosphoglycerate kinase (PGAK) enzymes consecutively. on the middle completely there was vary thick and wide band including aldolase (ALD), glyceraldehyde phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH) and phosphoglycerate mutase (PGAM) enzymes merged together, on the lower of gel appeared tow pale bands they were triosephosphate isomerase (TPI) enzymes and myoglobin (Mb).



1- Sarcoplasm proteins for beef meats on age 12 months at rigor- mortis stage.
2-Sarcoplasm proteins for beef meats on age 12 months at post rigor- mortis stage.
3- Sarcoplasm proteins for beef meats on age 28 months at rigor- mortis stage.
4-Sarcoplasm proteins for beef meats on age 28 months at post rigor- mortis stage.

Figure (1): Electrophoretic technique of sarcoplasm proteins for beef meats

1- phosphorylase b (PHb),2- phosphorylase b kinase (PHbK),3- phosphor glucomutase (PGM),4- pyruvate kinase(PK),5- phospholucose isomerase (PGI),6 - enolase (EN),7- creatine kinase (CK),8- phosphoglycerate kinase (PGAK),9- aldolase (ALD),10- glyceraldehyde phosphate dehydrogenase (GAPDH),11- dehydrogenase (LDH),12- phosphogly cerate mutase (PGAM), 13- triosephosphate isomerase (TPI),14- myoglobin (Mb)

As to the age12 months in rigor-post mortis stage, we observed great and pale band on the top of gelconsisting in phosphorylase b (PHb) and phosphorylase b kinase (PHbK) enzymes merged together, following by three proteins bands pale and thin placed on equal space approximation they were phosphor glucomutase (PGM), pyruvate kinase(PK) and phospholucose isomerase (PGI) enzymes consecutively, after that pale and thin band caming it was enolase (EN) enzame, but creatine kinase (CK) and phosphoglycerate kinase (PGAK) enzymes were disappeared from gel, this may be reverted to decomposition to peptides with vary smoll molecular weights fall down with pafer solution through pores of gel, on the middle completely there was vary thick and wide band including aldolase (ALD) ,glyceraldehyde phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH) and phosphogly cerate mutase (PGAM) enzymes merged together, and we watched tow pale bands on the lower of gel they were triosephosphate isomerase (TPI) enzymes and myoglobin (Mb). with respect to sarcoplasm proteins of beef meat at 28 months on rigor- mortis, we observed great and thin band on the top of gel including phosphorylase b (PHb) enzyme, following by great and wide band they were phosphorylase b kinase (PHbK) and phosphor glucomutase (PGM) enzymes together, after that we found three pale and thin proteins bands placed on equal space approximation they were pyruvate kinase(PK) ,phospholucose isomerase (PGI) and enolase (EN) enzymes consecutively merged together, but creatine kinase (CK) enzyme was disappeared from gel, also phosphoglycerate kinase (PGAK) enzyme appeared as pale and thin band, on the middle of gel there was vary heavy and wide band including aldolase (ALD) and glyceraldehyde phosphate dehydrogenase (GAPDH), following by four pale and thin bands plased on the lower of gel they were lactate dehydrogenase (LDH)) and phosphogly cerate mutase (PGAM), triosephosphate isomerase (TPI) enzymes and myoglobin (Mb).

On rigor - post mortis, we observed heavy and thin band plased on top of gel it was phosphorylase b (PHb) enzyme, following by thick and wide band it was phosphorylase b kinase (PHbK) and phosphor glucomutase (PGM) enzymes together, after that we found pale and thin proteins band it was pyruvate kinase(PK) ,but phospholucose isomerase (PGI) ,enolase (EN) and creatine kinase (CK) enzyme were disappeared from gel, this may be reverted to decomposition to peptides with vary smoll molecular weights fall down with pafer solution through pores of gel, also phosphoglycerate kinase (PGAK) enzyme appeared as pale and thin band,afer that on the middle of gel the aldolase (ALD), glyceraldehyde phosphate dehydrogenase

(GAPDH), and lactate dehydrogenase (LDH)) merged together on one vary thick and wide band, following by three pale and thin proteins bands plased on the lower of gel they were phosphogly cerate mutase (PGAM), triosephosphate isomerase (TPI) enzymes and myoglobin (Mb) consecutively.

It was noticeable here that the number of proteins bands separated from thigh muscle of caws were converging when the caw on 12 and 28 months of age, this similarity confirmed nonexistence clear difference on this kind of proteins according to difference of age and rigor- mortis stage, while sarcoplasm proteins were not effected with rigor- mortis stage.

Zade *et al*.,(1999) mentionede to that the sarcoplasm proteins separated from meats of animals (caws and buffalo) by SDS-PAGE method including 5-10 and 2-10 bands consecutively,these bands differented on thickness and molecular weight. Its clarify that difference on the number of proteins bands was return to difference kinds of animals, and samples not taken from the same carcass muscle, these results similarity with results of present study.

This results also agreed with studying by Bowker *et al.*,(2007) which separated sarcoplasm proteins by electrophoretic SDS-PAGE method on polyacrylamide gel from caw flank meat not aging and aging for 5 and 8 days after slaughtering, they mentioned that the aging effected on composition of sarcoplasm proteins vary clearly, and increase on tenderness was happened as a results of proteolysis, and the number of bands was 14 with 10-151 Kd ,they were (PGAK) phosphoglycerate kinase, (PGI) phosphoglucose isomerase, (EN) enolase, (PHbK) phosphorylase b kinase,(CK) creatine kinase, (TPI) triosephosphate isomerase, (GAPDH) glyceraldehyde phosphate dehydrogenase, (LDH) lactate dehydrogenase,(ALD) aldolase, (Mb) myoglobin.

Electrophoretic technique of sarcoplasm proteins for mutton meats: 3.1.2

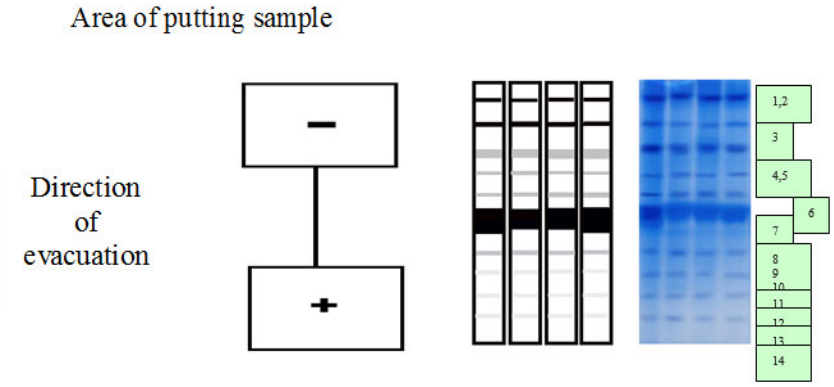
Figure (2) clarified results of electrophoretic technique of sarcoplasm proteins for mutton meats on rigor- mortis and rigor-post mortis stage and for sheep on age 6 and 12 months .These results found 10 proteins bands on rigor- mortis and rigor-post mortis stage at age 6 months ,also they were difference on strength of thickness on gel area and molecular weighing ranging among heavy and light long the beginning and ending of gel.

This proteins bands include all main sarcoplasm proteins which dissolved on distilled water besides different kinds of proteses such as cathpsenes and proteins resisting heating treatmen and pyruvalbomenes with low molecular weighting.

The figure mentioning to appearance heavy and wide band on the top of gel including phosphorylase b (PHb) and phosphorylase b kinase (PHbK) enzymes merged together, following by pale and wide band it was phosphor glucomutase (PGM) enzyme and under this band there was thick and wide band they were pyruvate kinase (PK) and phosphoglucose isomerase (PGI) enzymes consecutively,after that appered tow pale and thin bands including enolase (EN) and creatine kinase (CK) enzymes merged together, but also in the middle of gel there was great and thick band including phosphoglycerate kinase (PGAK), aldolase (ALD) and glyceraldehyde phosphate dehydrogenase (GAPDH)enzames , following by pale and thin band it was lactate dehydrogenase (LDH), also there were three pale and thin bands placed on equal space approximately they were phosphogly cerate mutase (PGAM), triosephosphate isomerase (TPI) enzymes and myoglobin (Mb) consecutively.

It was noticed here that the number of proteins bands separated from thigh muscle of mutton were converging when the caw on 12 and 28 months of age, this similarity confirmed nonexistence clear difference on this kind of proteins according to difference of age and rigor- mortis stage, while sarcoplasm proteins were not effected with rigor- mortis stage.

Kang *et al*., (2007) found 13,11 and 11 proteins bands when they separated sarcoplasm proteins from horse muscles before ,through and after rigor- mortis stage respectively. These bands to be distinguished by thickness ,long gel and havey bands stay at middle of gel, Main bands on top and down of gel was A protein, some actinin , myoglobin and phosphorylase enzame, This study was agre with results of present study.



1- Sarcoplasm proteins for mutton meats on age 6 months at rigor- mortis stage.
2-Sarcoplasm proteins for mutton meats on age 6 months at post rigor- mortis stage.
3- Sarcoplasm proteins for mutton meats on age 12 months at rigor- mortis stage.
4-Sarcoplasm proteins for mutton meats on age 12 months at post rigor- mortis stage.

Figure (2): Electrophoretic technique of sarcoplasm proteins for mutton meats

1- phosphorylase b (PHb),2- phosphorylase b kinase (PHbK),3- phosphor glucomutase (PGM),4- pyruvate kinase(PK),5- phospholucose isomerase (PGI),6 - enolase (EN),7- creatine kinase (CK),8- phosphoglycerate kinase (PGAK),9- aldolase (ALD),10- glyceraldehyde phosphate dehydrogenase (GAPDH),11- dehydrogenase (LDH),12- phosphogly cerate mutase (PGAM), 13- triosephosphate isomerase (TPI),14- myoglobin (Mb)

3.1.3 Electrophoretic technique of sarcoplasm proteins for chicken meats:

Figure (3) clarify results of electrophoretic technique of sarcoplasm proteins form thigh and breast muscle of chicken meats on (rigor- mortis and rigor-post mortis stage) and for chicken on age 25 and 40 days ,these results found 11 proteins bands of chicken meats at 25 and 40 days on rigor- mortis and rigor-post mortis stage ,also they were difference on strength of thickness and distribution on gel area and molecular weighing ranging among heavy and light long the beginning and ending of gel.

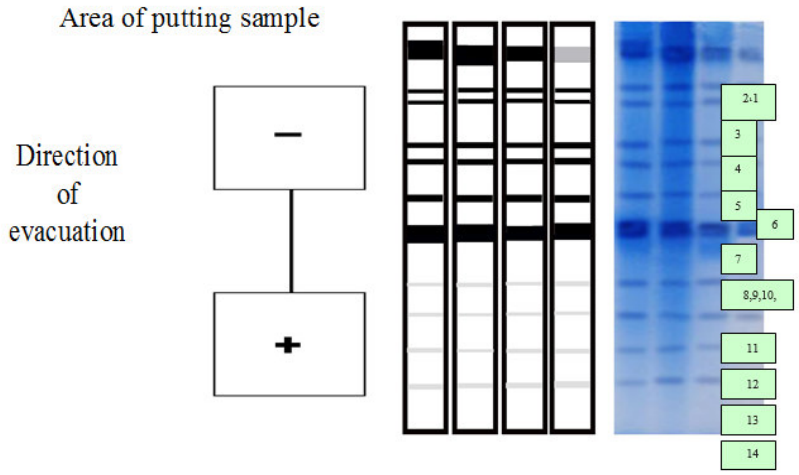
This proteins bands include all main sarcoplasm proteins which dissolved on distilled water besides different kinds of proteses such as cathpsenes and proteins resisting heating treatmen and pyruvalbomenes with low molecular weighting.

It was notice here that the number of proteins bands separated from thigh and breast muscle of chicken meats were converging when the chicken on 25 and 40 days of age.This similarity confirmed nonexistence clear difference on this kind of proteins according to difference of age and rigor- mortis stage, also the figure found havey and great band on age 25 day on rigor- mortis and rigor-post mortis stage and also on age 40 day on rigor- mortis stage,and pale and wide band for chicken on 40 days at rigor-post mortis stage on top of gel including phosphorylase b (PHb) and phosphorylase b kinase (PHbK) enzymes consecutively, following by tow great and thin bands they were phosphor glucomutase (PGM) and pyruvate kinase (PK) enzymes, and ,after that appered tow great and thin bands including enolase (EN) and creatine kinase (CK) enzymes merged together, but also on the little rise of gels middle there was great and thick band including phosphoglycerate kinase (PGAK),but on the middle there was great and wide band including phosphoglucose isomerase (PGI) enzymes consecutively, aldolase (ALD) and glyceraldehyde phosphate dehydrogenase (GAPDH)enzames , following by four pale and thin band placed on equal space approximately they and on same level they were lactate dehydrogenase (LDH), also phosphogly cerate mutase (PGAM), triosephosphate isomerase (TPI) enzymes and myoglobin (Mb) consecutively.

Babji and kee (1994) carried out study about using electrophoretic SDS-PAGE technique to separate sarcoplasm proteins form red chicken meats at age 18 months and white broiler at age 2 months, then they found that the number of proteins bands separated from row meat and minced meat which washing by cold water for one time was 9 , 9 bands for chicken and 11,7 bands for broiler consecutively,after that they washed once more and for three times and then separated proteins to obtain 7,7,6 bands for chicken meats while obtained 11,7,7 bands for broiler meat consecutively,the number of proteins bands which obtained in this study was less than the number of bands in present studying.

Despite the fact that sarcoplasmic proteins probably do not play a role in determining tenderness, the data from the current study suggest that changes in sarcoplasmic protein profiles may reflect increased

proteolysis and might be useful as potential markers for tenderness development.



1- Sarcoplasm proteins for chicken meats on age 25 days at rigor- mortis stage.
2-Sarcoplasm proteins for chicken meats on age 25 days at post rigor- mortis stage.
3- Sarcoplasm proteins for chicken meats on age 40 days at rigor- mortis stage.
4-Sarcoplasm proteins for chicken meats on age 40 days at post rigor- mortis stage.

Figure (3): Electrophoretic technique of sarcoplasm proteins for chicken meats

1- phosphorylase b (PHb),2- phosphorylase b kinase (PHbK),3- phosphor glucomutase (PGM),4- pyruvate kinase(PK),5- phospholucose isomerase (PGI),6 - enolase (EN),7- creatine kinase (CK),8- phosphoglycerate kinase (PGAK),9- aldolase (ALD),10- glyceraldehyde phosphate dehydrogenase (GAPDH),11- dehydrogenase (LDH),12- phosphogly cerate mutase (PGAM), 13- triosephosphate isomerase (TPI),14- myoglobin (Mb)

4.0 Conclusions

In conclusion, difference number of proteins bands were noticed when separation sarcoplasm proteins by using electrophoresis technique following kinds of proteins ,kinds of meats rigor- mortis stage and age of animals. whereas the number of proteins bands ranging from 8 -11.

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