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Research Note

Effect of Freeze Thaw Cycles on Myofibrillar Proteins of Chevon

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Semitendinosus, Semimembranosus and *Biceps femoris* muscles from goat carcass were collected aseptically, packed in LDPE bags and chilled for 24hrs. Later, chevon was subjected to frozen storage (-18±2°C). One set of meat samples were frozen and thawed in refrigeration temperature (4±1^{0}C),whereas, the second and third sets of samples were frozen and thawed at room temperature (37±2^{0}C) and in hot water (40±2^{0}C), respectively. The freeze thaw cycles were repeated for 3 times.At every freeze thaw cycle, myofibrillar proteins were isolated and analyzed by SDS-PAGE. Current study analysed the detailed pattern of myofibrillar proteins of fresh chevon. Also, from the study, it is evident that low molecular weight bands (Polypeptides) increased with increasing number of freeze thaw cycles that could be indication of proteolysis. Also, hot water and room temperature thawing influenced the proteolysis in 3rd freeze thaw cycle due to sudden exposure to high temperature.

Keywords : *Chevon, Freeze thaw cycles, Myofibrillar Protein, Troponin, Actin*

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Freezing is one of the classical preservation methods of meat and meat products. It is the commonest, economically feasible conservative which is employed during transportation and storage of meat for several months without adding or removing any constituent. Freezing is considered as an excellent means of maintaining sensory quality of meat to acceptable level and this fact support the industry as well as consumer to buy frozen meat without concern of changes in eating quality (Xia *et al*. 2012). Nevertheless, thawing is the most important and crucial step in meat processing. The dramatic expansion of frozen food market has paved the need for improved thawing methods as the current methods are mostly slow and results in undesirable changes in meat quality (Anderson and Singh 2006). It is well known that improper thawing method contributes to deterioration in the form of thawing loss, increasing protein denaturation and microbial load.

In shops, home, restaurant or in meat industry, meat or meat products may reasonably be expected to undergo multiple freeze thaw cycles which cause damage to cell membrane and organelles as well as muscle structure due to repeated melting and reformation of ice crystals. It has been traditionally thought that protein denaturation could result during freezing due to an increased intracellular ionic strength following the migration of water to the extracellular spaces. Myosin was the protein most affected by freezing and slow freezing causes more pronounced protein denaturation than rapid freezing. This mechanism has been refuted by several authors (Jayathilakan 2014). Moreover, functional properties of myofibrillar proteins (emulsification and gelation) decreased significantly when muscles were subjected to different freeze thaw cycle (Xia *et al*. 2009; 2010). Benjakul *et al*. (2003) found that freezing and frozen storage caused a marked decreased in Ca_2+- ATPase activity and an increase in Mg_2+-EGTA-ATPase activity, which translates into denaturation of myosin and the troponin-tropomyosin complex.

In this context, the present study was conducted with the objectives of assessing the myofibrillar proteins bands pattern of fresh chevon and the effect of freeze thaw cycles on myofibrillar proteins of chevon.

The muscles comprising of *Semitendinosus, Semimembranosus* and *Biceps femoris* from goat carcass were collected aseptically, packed in LDPE (100 guage) bags and chilled for 24hrs. Later, subjected to frozen storage (-18±2°C). The frozen chevon samples were thawed at every 5th day by one of the thawing conditions viz., refrigeration temperature (4±1°C), hot water (40±1°C) and room temperature (35±2°C). The freeze thaw cycles were repeated for 3 times. At every freeze thaw cycle, myofibrillar proteins were extracted and analyzed by SDS-PAGE.

Isolation of Myofibrillar Proteins: Myofibrillar proteins were isolated according to procedure of Jeong *et al.* (2011). Thereafter, protein concentration was estimated as per Lowry *et al.* (1951).

SDS-PAGE: The method of Laemmli *et al.* (1970) was followed for subjecting sample to SDS-PAGE. Visualization of bands

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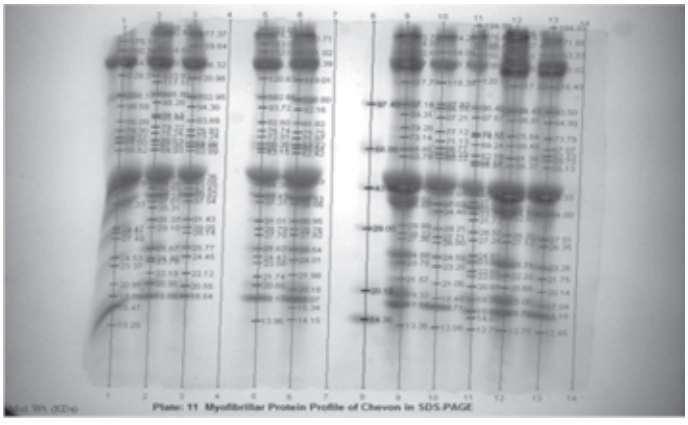
(Bright blue) against transparent background was read by Gel Doc system (Bio Rad, US). Further, the analysis of protein profile was done by employing quality one software (Bio Rad, US).

The results of SDS-PAGE analysis of fresh and frozen thawed chevon are presented in Table 1 and Figure 1. It was evident that fresh chevon (Table 1, Lane 1) revealed myofibrillar proteins wherein, 22 bands appeared bearing molecular weights ranging from 13.290 to 175.138 kDa. The detailed band pattern could be grouped as 13.290 to 16.474 kDa; 23.373 to 29.465 kDa; 66.817 to 79.301 kDa; 86.086 to 108.176 kDa; 129.310 and 144.438 kDa; 160.761 and 175.138 kDa. Apart from these, individual bands of 18.887 kDa, 20.907 kDa, 37.329 kDa, 46.152 kDa were also noticed. The band of 20.907 kDa as observed in the fresh sample could be the representative of light chain of myosin (Claeys *et al*. 1995; Warriss 2000), whereas 46.152 kDa as actin, 66.816 kDa as tropomyosin and 79.301 kDa as troponin. Similar observations were also reported by Warriss (2000), where the worker mentioned light chain of myosin bearing molecular mass between 17-22 kDa, actin bearing 42 kDa, tropomyosin as 66 kDa and troponin at 80 kDa. The heavy chain of myosin however reported to bear molecular weight of 220 kDa were not apparent in our investigation which can be attributed to variation in the composition of gel as the demonstration of higher polypeptide are reported at 8% (Claeys *et al*. 1995, Nagaraj *et al*. 2005) whereas, present study employed 12.5% gel for separation of proteins.

Troponin assumed as bearing molecular weight 79.301 kDa (Table 1 and Lane 1) was found to get degraded to around 76 kDa in day5 (Lane 4 & 5, Figure 1), 75.751 kDa (Lane 6) and 75.636 kDa in day 10 (Lane 9); and 77.118 kDa and 76.147 kDa in day 15 (Lane 10 and11). The protein bearing molecular weight of 66 kDa as assumed representing tropomyosin was found to get degraded in all storage and thawing conditions. At 5 days the degraded product could be noticed as 64.929 kDa (Lane 2& 11), 63.890 kDa (Lane 3) and 63.148 kDa (Lane 4) (all 5 Days); 65.810 (Lane 6), 61.345 kDa (Lane 12) and 62.726kDa (Lane 14) (all Days), 63.227 kDa (Lane 9), 62.199 KDa (Lane 10) and 65.810 kDa (Lane 11) (all 15 Days). As reported by Claeys *et al.* (1995) troponin C might have been represented by polypeptide at 37.329 kDa (Lane 1) which was found persisting more or less at the same position during whole trial period. Observations or appearance of polypeptide at around 30 kDa (i.e. 31.867 kDa, 31.434 and 31.008 kDa in lane 2, 3 and 5 respectively-5days), 30.953 kDa 10 days (Lane 6) and 31.730 kDa (Lane 10) for 15 days is in accordance with Nagaraj *et al.* (2005) who reported appearance of 30 kDa band as a proteolytic product of troponin T from myofibrillar protein in *Biceps femoris* of chevon. Apart from these, the band in the range of 28 to 32 kDa as observed during storage conditions were corroborated with Wheeler and Koohmaraie (1994). Hot water and room temperature thawing influenced the proteolysis in 3rd freeze thaw cycle severely due to sudden exposure to high temperature, leading to disintegration of muscles.

The results were further in agreement with Kye *et al.* (1988), Xia *et al.* (2009), Jeong *et al.* (2011) who also recorded increase in low molecular weight bands with an increase of freeze thaw cycles. On contrary Kiran *et al.* (2013) observed no changes in band intensity of myofibrillar proteins between control and blade tenderized spent hen meat sample.

Figure 1: Freeze thaw cycles myofibrillar protein profile of chevon in SDS-PAGE



Current study analyzed the detailed pattern of myofibrillar proteins of fresh chevon. The freeze thaw cycles increased proteolysis as number of low molecular weight bands (polypeptide) was increased. Proteolysis was more evident in hot water thawed chevon samples when compared to samples thawed at refrigerator temperature and room temperature.

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Table 1:Myofibrillar protein profile of chevon in SDS PAGE

Lane No. →	1	2	3	5	6	8	9	10	11	12	13
====	Fresh	1st FT	1st FT	1st FT	3rd FT		2nd FT	3rd FT	3rd FT	2nd FT	2nd FT
Mol Wt. (kDa) ↓											
1								194.987		194.973	
2		188.63		185.664				174.26	176.67	186.879	
3	175.138	180.49	177.369	176.771	170.71		173.725		166.65	179.755	171.658
4	160.761			163.07					160.653	167.49	
5		154.635	159.642	152.033	151.01		152.6	154.09	150.785	151.714	153.33
6	144.438	139.164	136.318	136.375	137.391		137.873	137.70	138.078	136.455	135.016
7	129.310	123.92	120.983	120.626	119.024				120.783		
8		177.153			100.598		117.770	118.93		117.221	116.396
9	108.176	105.805	102.945	102.656	92.165			97.93			
10	98.59	98.227	94.297	93.715		97.4	97.13	94.56	95.397	94.493	93.497
11	86.086	88.134			81.819		89.305	87.21	87.666	86.811	84.99
12		85.993	83.689	82.602							
13	79.301	79.316	76.928	76.739	75.751		79.280	77.12	76.147	75.736	
14	74.367	75.244	73.239	72.808	71.875		73.144	71.13	74.555		73.788
15	71.504	70.632	68.997	68.355	67.720				69.241	69.486	
16	66.816	67.954	66.852	66.709	65.810	66.0		66.77			67.074
17		64.929	63.890	63.148	62.416		65.810	63.23	62.194	61.345	62.726
18					46.034		60.786	60.22	58.189	56.37	59.728
19	46.152	48.479	48.356	47.809		43	43.468	44.18	43.035		55.129
20		44.965	45.05	37. 315	38.43		38.58	41.03	40.71	40.646	40.325
21		42.677	42.42		36.66		36.65	37.02	37.91	36.226	33.997
22		39.593	39.82						34.48	35.87	34.404
23	37.329	37.613								33.938	32.804
24		35.310							31.73		
25		31.367	31.43	31.01	30.95						
26	29.465	29.10	29.05	28.79	28.76	29.0	29.21	28.569	28.248		
27	28.510		28.14	27.78	27.82		28.22		27.235		
28	27.492	25.670	25.77	25.62	25.54		27.36	27.71		27.119	27.512
29	24.533	24.299	24.45	24.43	24.01		24.88	26.80	24.87		26.350
30	23.373	23.783		23.58			23.78	24.59	23.937	23.710	23.260
31		22.193	22.12	21.74	21.99		21.57	23.28	22.656	22.223	
32	20.907	20.909	20.55	20.68	20.18	20.1	21.06	22.688	20.680	20.142	
33							19.32		20.688	20.680	20.142
34	18.887	18.660	18.64	18.17	18.07		17.13	18.48	18.260	18.061	
35								17.043			
36											
37	16.474			16.34	16.71		15.879	15.699		15.177	
38	13.290			13.96	14.15	14.30	13.36	13.08	14.631		
39									12.748	12.614	12.65
Total	**22**	**29**	**25**	**26**	**25**	**6**	**22**	**25**	**31**	**24**	**21**

↑ **Marker**

REFERENCES

Anderson BA, Singh RP (2006) Modeling the thawing of frozen foods using air impingement technology. Inter J Ref 29:294-304

Benjakul S, Visessanguan W, Thongkaew C, Tanaka M (2003) Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. Food Res Inter 36:787-795

Claeys E, Uytterhaegen L, Buts B, Demeyer D (1995) Quantification of beef myofibrillar proteins by SDS-PAGE. Meat Sci 39:177-193

Jayathilakan K (2014) Impact of freezing and thawing on the quality of meat. In: Compendium, National Symposium on Sustainable Meat Production for Nutritional Security and Consumer Well-being: Challenges and Strategies. Indian Meat Science Association, Hyderabad. pp 152-153

Jeong JY, Kim GD, Yang HS, Joo ST (2011) Effect of freeze-thaw cycles on physicochemical properties and color stability of beef *Semi membranous* muscle. Food Res Inter 44: 3222-3228

Kiran M, Reddy KS, Reddy KK, Rao TM, Naveena BM (2013) Effect of blade tenderization on physicochemical and

ultrastructural properties of spent hen meat. J Meat Sci 9(1):16-20

Kye HW, Nip WK, Moy JH (1988) Changes of myofibrillar proteins and texture in fresh water prawns, *Macrobrachium rosenbergii*, during iced storage. Marine Fishery Review 50(1):53-56

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 227: 680-685

Lowry OH, Rosenbrough NJ, Farr AL, Randall RL (1951) Protein measurement with the folin phenol reagent. J Bio Chem 193: 165-75

Nagaraj NS, Anilkumar KR, Santhanam K (2005) Post mortem changes in myofibrillar proteins of goat skeletal muscles. J Food Biochem 29:152-170

Warriss PD (2000) Assessing eating quality. In : Meat Science-An Introductory Text, 2nd (eds), CAB International, pp 194

Wheeler TL, Koohmaraie M (1994) Prerigor and postrigor changes in tenderness of ovine longissimus muscle. J Anim Sci 72:1232-1238

Xia X, Baohua K, Qian LK, Liu J (2009) Physicochemical change and protein oxidation porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci 3(2): 239-245

Xia X, Kong B, Xiong Y, Ren Y (2010) Decreased gelling and emulsifying properties of myofibrillar protein from repeatedly frozen-thawed porcine longissimus muscle are due to protein denaturation and susceptibility to aggregation. Meat Sci 85:481-486

Xia X, Baohua K, Liu J, Diao X, Qian L (2012) Influence of different thawing method on physicochemical changes and protein oxidation of porcine longissimus muscle. LWT-Food Sci Tech 46:280-286