Effect of Plant Proteolytic Enzyme on the Physico-chemical Properties and Lipid Profile of Meat from Culled, Desi and Broiler Chicken

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ABSTRACT : Proteolytic enzymes are used for meat tenderization, an important process with regard to consumer preference. The proteolytic enzyme, IVRIN was isolated from the plant *Cucumis pubescens W* and its effect on physico-chemical properties and lipid profile of thigh and breast muscle of culled, desi and broiler birds was studied. Fifty-gram meat was treated with IVRIN containing 32.5 mg enzyme protein at 60°C for 20 min. The pH of IVRIN treated meat was decreased significantly ($p<0.01$) and the effect was more pronounced in breast than thigh muscle. The water holding capacity (WHC) was increased significantly ($p<0.01$) in broiler as compared to desi and culled bird, and in breast compared to thigh muscle. IVRIN failed to produce any impact on muscle fiber diameter (MFD). The MFD of desi was significantly higher ($p<0.01$) than broiler and culled birds. The total lipid concentration in thigh and breast muscle of desi was lower ($p<0.01$) than broiler and culled birds, latter being similar in this respect. The cholesterol content was lower ($p<0.01$) in breast than thigh muscle, in broiler than desi and culled and in IVRIN treated than untreated meat samples. The phospholipid concentration was unaffected by IVRIN. Broiler and culled birds exhibited more phospholipid content than desi birds. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 6 : 884-888)***

Key Words : Proteolytic Enzyme, Meat, Physico-chemical Properties, Lipid Profile, Desi and Culled Birds, Broilers

INTRODUCTION

The quality of meat for commercial purpose and acceptability depend on the amount and nature of biochemical constituents. Besides the physico-chemical properties, the postmortem biochemical changes transforming muscle into meat also plays important role in determining the quality of meat. The broiler industry is gaining rapid momentum and consumers usually do not prefer old or culled birds for table use because of its poor quality due to lack of tenderness. Proteolytic enzymes either endogenous or exogenous were found to be very useful for tenderization and improvement of meat quality to consumers enhancing market value of the meat. The tenderzation of meat depends mainly upon nature and extent of connective tissue of muscle. Hence proteolytic enzymes (viz. papain, ficin etc) are used widely for articificial tenderization purpose (Sahoo and Panda, 1984). The proteolytic pattern of papain, ficin and IVRIN revealed that IVRIN results in more profound proteolytic effect as compared to papain or ficin (Yadava, 1982). The present communication reports the physico-chemical properties and lipid status in meat of broiler, desi and culled birds upon the *In vitro* action of IVRIN.

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MATERIALS AND METHODS

Birds

The experimental birds were 63 weeks culled birds and 6 weeks broilers of White Leghorn breed that were procured from Ranchi Veterinary College farm. Non-descript desi birds of 32 weeks were brought from local market. The weight of the birds was between 1-1.6 Kg. These 36 birds of three groups (viz. culled, desi and broiler) were randomly divided into two subgroups; control and treatment each containing 6 birds.

Isolation of IVRIN

The plant proteolytic enzyme, IVRIN was isolated from unripe fresh fruits of *Cucumis pubescens W*, as described by Yadava (1982). The isolated enzyme was lyophilized in TBP centrifuges lyopholizer Model FD-6000 and was preserved for the experiment.

Treatment of IVRIN

The meat in the experimental groups was treated with enzyme IVRIN using the method of Herring et al. (1967). The 250 ml incubation medium was prepared that was consisting of 50 g breast or thigh whole meat each from different groups, enzyme protein 32.5 mg, 200 ml of phosphate buffer pH 7.0 and 50 ml 0.9% NaCl. The mixture was incubated at 60°C for 20 minutes in a water bath. The meat obtained was considered as enzyme treated (experimental) and fresh (control) meat. The control was incubated as above consisting of all ingredients except enzyme. The different parameters were studied with or without enzyme treated meat samples.

Table 1. pH, water holding capacity (WHC) and fiber diameter of thigh and breast muscle of culled, desi and broiler chicken in control and proteolytic enzyme treated groups.

	Group	Control			Treatment			Control & treatment (pooled)	Anova significance between		
		Thigh muscle	Breast muscle	Pooled	Thigh muscle	Breast muscle	Pooled		Treatment	Muscle	Groups
pH	Culled(6)	6.05^{e}±0.02	5.95^{de}±0.04	6.00±0.03	5.76^{b}±0.02	5.63^{a}±0.02	5.70±0.02	5.85^{A}±0.08	**	**	NS
	Desi(6)	5.33^{bc}±0.03	5.91^{cd}±0.03	5.86±0.03	5.83^{bc}±0.04	5.63^{a}±0.02	5.773±0.04	5.80^{A}±0.02			
	Broiler(6)	6.00^{de}±0.04	5.95^{de}±0.04	5.98±0.02	5.80^{b}±0.03	5.61^{a}±0.02	5.71±0.03	5.84^{A}±0.038			
	Overall		5.95^{X}±0.03			5.71^{Y}±0.04					
WHC (ml/100g wet meat)	Culled(6)	6.73^{b}±0.13	7.02^{b}±0.14	6.58±0.11	5.19^{b}±0.04	6.33^{a}±0.03	5.76±0.17	6.32^{A}±0.40	**	**	**
	Desi(6)	11.94^{f}±0.08	11.83^{f}±0.08	11.90±0.06	8.58^{de}±0.20	10.41^{c}±0.15	9.50±0.30	10.69^{B}±0.78			
	Broiler(6)	13.57^{g}±0.79	13.57^{g}±0.30	13.63±0.41	9.62^{ef}±0.22	11.33^{d}±0.25	10.48±0.30	12.06^{C}±0.98			
	Overall		10.80^{X}±2.02			8.58^{Y}±1.44					
Muscle fiber diameter (μm)	Culled(6)	34.75^{c}±1.31	27.90^{cd}±1.67	31.32±1.45	33.82^{c}±0.43	29.52^{cd}±1.42	31.67±0.96	31.49^{A}±1.65	NS	**	**
	Desi(6)	65.42^{a}±3.78	43.11^{b}±7.03	54.27±3.85	66.54^{a}±3.21	43.84^{b}±1.23	55.19±3.80	54.73^{B}±6.50			
	Broiler(6)	34.16^{c}±1.02	25.14^{d}±1.15	30.15±1.41	33.82^{c}±0.43	29.46^{cd}±1.41	31.64±0.96	30.90^{A}±1.91			
	Overall		38.58^{X}±5.90			39.50^{X}±5.82					

a,b,c,d,e,f or A, B, X, Y, Means bearing same superscript did not differ significantly.
Figures in parenthesis are number of observation, * $p<0.05$, ** $p<0.01$, NS-Nonsignificant

Physico-chemical and biochemical measurements and statistical analysis

pH was measured immediately after slaughter using pH meter. Water holding capacity (WHC) was determined by using centrifugation technique of Hamm (1960) as modified by Wardlow et al. (1973). Muscle fiber diameter was measured as per method of Hammond (1932). Lipid of the meat was isolated with the method of Folch et al. (1957). The total lipid content was estimated with the charring procedure of Marsh and Weinstein (1966), while total phospholipid and cholesterol content were estimated by the method of Wagner et al. (1962) and Zlatkis et al. (1953), respectively. Analysis of variance was performed (Snedecor and Cochran, 1968) in which main effects for groups (culled, desi, broiler), muscle and IVRIN treatment were tested.

RESULTS AND DISCUSSION

Data pertaining to the effect of proteolytic enzyme on physico-chemical properties of poultry meat is presented in Table 1. The pH of meat plays important role in maintaining the quality of meat. The toughness of meat occurs even at low pH. It was observed that the fall in pH of breast muscle was more rapid as compared to thigh muscle in treatment group. Khan and Nakamura (1970) reported an ultimate pH near 5.7 as desirable for maintaining quality of poultry breast meat. This value is similar to the present observation after IVRIN treatment in breast muscles of broiler, desi and culled birds suggesting IVRIN as a very good tendarizer. However, pH in thigh muscle was higher than breast muscle though difference was not significant. Rao and Reddy (1990) reported significantly high pH value in thigh muscle than breast muscle. IVRIN treatment lowered overall pH and water holding capacity (WHC). A similar effect of papain on chick meat was noted by Singh and Bhatia (1988). The WHC of meat was highest in broiler followed by desi and culled bird. No effect of age on WHC in case of White Leghorn cockerels (Panday and Shyam Sunder, 1990) and no effect of papain on WHC of breast and thigh muscle of culled White Leghorn birds (Singh and Bhatia, 1988) has been found earlier. However, Goll et al. (1977) observed that connective tissue protein had direct effect on WHC besides structural changes in muscle protein during post mortem storage and presence of charged aminoacids in these protein. These reports support the present findings on low WHC in culled (aged) birds as compared to broilers, an effect dependent upon connective tissue content. The comparison between muscle type did not show differences in control groups but significantly increased WHC was observed in breast muscle as compared to thigh muscle after treatment. The high WHC shows effect of enzyme which resulted in hydrolysis of protein contents into charged amino acid that differ in the thigh muscle (Lawrie, 1968).

The comparison between breeds in relations to muscle fiber diameter (MFD) showed differences. However, no differences due to age in the some breed were seen as observed in case of broiler and culled. Some workers reported no difference in MFD due to sex and muscle type in case of rabbit (Nath and Narayan Rao, 1983), while differences occurred due to age and plain of nutrition (Joubert, 1956), However, present result agrees with the finding of Hammond (1932) and Joubert (1956), who reported in case of pig that MFD differ from one muscle to another, between species, breed and sex as reported. Muscle fibre diameter were found high in case of thigh muscle as compared to breast muscle in the same group which might be due to type of muscles and exercise (Dumont, 1978).

Table 2. Lipid profile of thigh and breast muscle of culled, desi and broiler chicken in control and proteolytic enzyme treated groups

	Group	Control			Treatment			Control & treatment	Anova significance between		
		Thigh muscle	Breast muscle	pooled	Thigh muscle	Breast muscle	pooled	pooled	Treatment	Muscle	Groups
Total lipid (mg/g wet mass)	Culled(6)	119.63^{b} ±6.36	123.83^{b} ±3.50	121.73 ±3.52	109.37^{b} ±5.57	112.26^{b} ±3.80	110.81 ±3.25	116.27^{B} ±3.05	NS	NS	**
	Desi(6)	56.70^{a} ±7.08	57.06^{a} ±5.17	56.88 ±4.19	48.29^{a} ±6.91	45.00^{a} ±5.68	46.65 ±4.29	51.76^{A} ±3.03			
	Broiler(6)	113.07^{b} ±7.10	113.49^{b} ±7.94	113.28 ±5.08	102.62^{b} ±0.41	103.37^{b} ±7.29	102.99 ±4.82	108.14^{B} ±2.97			
	Overall	97.30 ±12.89			86.82 ±12.80						
Total Cholesterol (mg/g wet mass)	Culled(6)	6.09^{f} ±0.82	2.68^{abcd} ±0.20	4.39 ±0.65	4.94^{ef} ±0.80	2.22^{abc} ±0.10	3.58 ±0.57	3.98^{A} ±0.92	*	**	**
	Desi(6)	4.50^{def} ±0.86	4.03^{cde} ±0.58	4.27 ±0.50	3.61^{bcde} ±0.78	3.31^{abcde} ±0.44	3.46 ±0.43	3.81^{A} ±0.30			
	Broiler(6)	3.87^{cde} ±0.36	1.71^{ab} ±0.21	2.79 ±0.38	3.07^{abcde} ±0.29	52^{a} ±0.19	2.29 ±0.66	2.54^{B} ±0.56			
	Overall	3.81^{x} ±0.62			3.11^{y} ±0.48						
Phospholipid (mg/g wet weight)	Culled(6)	43.54^{cd} ±2.57	46.56^{cd} ±3.68	45.05 ±2.19	39.62^{bc} ±2.52	41.35^{bcd} ±0.41	40.48 ±2.09	42.75^{B} ±1.48	NS	NS	**
	Desi(6)	27.29^{ab} ±4.50	27.66^{ab} ±3.39	27.48 ±3.95	23.89^{a} ±4.35	23.13^{a} ±3.09	23.15 ±3.72	25.49^{A} ±1.16			
	Broiler(6)	46.88^{cd} ±5.50	54.58^{d} ±4.14	50.72 ±3.48	42.51^{cd} ±0.57	50.31^{cd} ±4.14	46.41 ±3.51	48.57^{B} ±2.56			
	Overall	41.09 ±4.55			36.80 ±4.46						

a,b,c,d,e,f or A, B, x, y, Means bearing same superscript did not differ significantly

Figures in parenthesis are number of observation, * $p<0.05$, ** $p<0.01$, NS-Nonsignificant

However, muscle fibre diameter was observed exceptionally high in case of thigh muscle of desi, which might be due to breed, type of muscle and exercise (Goldspink, 1962; Dumont, 1978). The effect of enzyme treatment did not show differences in the MFD of control of the respective muscle in their respective groups on incubation at 60°C. This is in agreement with Price and Schweigert (1971) who reported no significant changes in the diameter of *Longissimis dorsi* muscle before and after cooking.

The data pertaining to total lipid, phospholipid and cholesterol of thigh and breast muscle of broiler, desi and culled birds upon the impact of IVRIN are presented in Table 2. No significant differences between muscle and also between culled and broilers were observed. However, Sharma et al. (1982a) reported low total lipid in case of broiler as compared to our present investigation. Such variation might be due to breeds as reported by Yeats (1965) and Kesari et al. (1990) in case of squabs and pigeon. The desi breed contained low total lipid as compared to White Leghorn broiler and culled birds which might be due to nature of physical activity performed by these birds under free range system, plane of nutrition in comparison to deep litter system of broiler (Price and Schweigert, 1971; Yeats, 1965). The difference between total lipid content between muscle was nonsignificant, which is supported by Kesri et al. (1990). The treatment of enzyme did not influence the lipid content indicating no effect of the enzyme on hydrolysis of the lipid. However, the pattern showed slightly low content of total lipid in treated group irrespective of species and muscle type which appeared to be due to the effect of cooking or incubation at 60°C in which loss of the lipid had taken place, especially of the phospholipids. Such loss has been reported by Lee and Dawson (1976) and Sharma et al. (1982a).

The differences between cholesterol content in different groups were evident indicating effect of age and breed in present investigation which is similar to the observations of Price and Schweigert (1971). The variation of total cholesterol between muscles was significant especially in case of broiler and culled birds. However, no differences were observed in desi either in case of control or treatment. The thigh muscle in White Leghorn (broiler or culled) and desi contain more total cholesterol as compared to breast muscle. This agrees with reports of Marion and Woodruff (1965) and Sharma et al. (1982b). The culled bird showed highest cholesterol content in case of thigh muscle, which might be influenced by age (Price and Schweigert, 1971). The treatment however showed slight variation ($p<0.05$) but

overall pattern showed low cholesterol content of treated group of poultry indicating loss of cholesterol during incubation. Sharma et al. (1982b) found no significant decrease in cholesterol after cooking, while Lobanov et al. (1958) noted significant decrease in cholesterol content of chicken muscle due to cooking as a result of alteration of different protein-cholesterol complexes due to denaturation of protein. Accordingly to Freely et al. (1972) also cooking or incubation decreased the cholesterol content in turkey. More reduction in cholesterol in treated group indicates probable facilitative action of proteolytic enzyme in breaking down protein cholesterol complex.

Significant difference between groups showing low phospholipid content in case of desi as compared to broiler or culled group was observed. Such observation has been confirmed in previous findings (Lea, 1962) that reported effect of species, age and type of muscle on the composition of phospholipids. The influence of age has also been reported by Keshri et al. (1990) in case of squabs and pigeon. The comparison between muscle did not show significant difference but the breast muscle appeared to have slightly higher content of phospholipid than thigh muscle, which was supported by Sharma et al. (1982a). They reported high phospholipid content in breast muscle than thigh muscle in case of broiler birds. The effect of treatment did not influence the phospholipid content of the muscle though it revealed decrease phospholipid content in both breast and thigh muscle irrespective of groups due to incubation or cooking (Sharma et al., 1982a, Lee and Dawson, 1976). The loss of phospholipid on incubation or cooking could be due to the hydrolysis of fatty acids by phospolipases which was not getting inactivated even at 100°C for 30 min (Lee and Dawson, 1976).

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