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BIOCHEMICAL AND QUALITY CHANGES IN POST-RIGOR OVINE MUSCLE AFTER THAWING AT LOW AND HIGH TEMPERATURE*

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A study was made of the effects of freezing followed by thawing at low (8°C) and high (25°C) temperature upon some physical and chemical properties of the post-rigor ovine *longissimus dorsi* muscle. Measurements of total nitrogen, protein nitrogen (PN), non-protein nitrogen (NPN), acidic and basic groups, moisture content, pH value, refractive index and tenderness were made on the muscle and its exudate squeezed out under defined conditions. A new refractometric method for the determination of protein alterations has been evaluated.

Tenderness of the cooked meat was linearlyrrelated to PN, % PN/TN, and % NPN/PN of the muscle, but was not related to the refractive index of the muscle exudate.

The relation of various nitrogenous components of the exudate was plotted against tenderness. There was no relationship between ultimate pH value and moisture content of muscle or muscle exudate. Cooked meat tenderness increased with increase of acidic and basic groups on the muscle proteins and moisture content of the muscle up to a certain level and thereafter the trend was reversed. Possible explanations for various changes in muscle components after freezing and thawing and their relation with tenderness have been discussed.

Following slaughter, a series of changes take place in the physical and chemical properties of muscle. Some are independent of outside agencies and others may be due to microorganisms on the surface or within the carcase of the animal, nearly all can be slowed down or almost checked by lowering the temperature of the tissue.[1] Hence the changes affected in muscle tissue by chilling, freezing and thawing are of great economic importance.

Freezing alone has no demonstrable effect upon the colour, flavour, odour or juiciness of meat as judged after cooking[2] but it does affect the raw muscular tissue due to the formation of hard ice crystals and the concentration of mineral constituents which gradually damage the proteins and irreversibly alter them.

When thawed the frozen meat has a tendency to exude a viscous reddish brown fluid known as drip, the quantity of which is affected by the method of thawing, as also is tenderness.[3] The presence of drip and, to a lesser extent, the colour of the meat serve to identify frozen from unfrozen tissue.

Frozen meat cooked after thawing for 48 hours is slightly less tender than its chilled counterpart.[4] Miller and May[5] concluded that it is not the rate of freezing that significantly affects tenderness in chicken meat but the temperature of storage after freezing. Cook and Langsworth[6] found that ovine *longissimus dorsi* muscle frozen pre-rigor and thawed for 24 hr at temperature up to 40°C was significantly more tender than unfrozen muscle. Furthermore, the meat thawed at 5°C was signicantly more tender than meat thawed at 10, 15 or 20°C.

The refractometeric properties of the components of meats have been used by a number of workers as indices of its characteristics. Thus, Weirbicki *et al.*[7,8] used the refractive index of extracted proteins of beef as a measure of and as a guide to the tenderness of meat. For determination of anatomical differences in the fat of beef *longissimus dorsi*, Cook, Bray and Weckel[9] made use of the refractometric properties of fat. The use of refractive index for the evaluation of protein denaturation in cod was suggested and used by Elerian.[10,11] A similar photometric technique of cell fragility measurement was developed by Love and Mackay[12] to estimate the extent of protein denaturation in cold stored cod muscle. No such technique has been used for measuring protein denaturation in ovine muscle exudate obtained by squeezing under defined conditions.

This study was undertaken to ascertain the effects of thawing ovine muscle, at low and high temperature, on changes in protein and some other quality characteristics. A new refractometric method was evaluated for the estimation of protein denaturation and its possible relation with meat tenderness.

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Experimental

Longissimus dorsi muscles from nine sheep of unknown history and nutrition were purchased from a retail shop approximately 3 days after slaughter. Only the left side of the loin was used. Visual fat and connective tissues were removed as completely as possible and then each muscle was divided into three equal parts; two parts were placed in a freezer at −15°C for one week while the third portion was used immediately. After one week the two frozen parts were removed from the freezer and thawed at either 8°C or 25°C for 24 hr.

Hence the three muscle treatments were: (1) post-rigor non-frozen (2) post-rigor frozen, thawed at 8°C and (3) post-rigor frozen, thawed at 25°C. With the exception of the tenderness studies, all determinations were done on well-mixed minced muscle.

Analytical Methods.—All forms of nitrogen were estimated using the micro-Kjeldahl method. Protein nitrogen was calculated by difference after estimating non-protein nitrogen by the TCA-precipitation method using about 10 g of muscle.

Moisture content was determined by oven drying for 48 hours at 110°C from 0.2–0.5 g of muscle in pre-dried thimbles. In the case of muscle exudate, clean 10 ml glass tubes were used.

Meat tenderness was measured with a Warner–Bratzler shear, following cooking to an internal temperature of 82°C in boiling water, as measured by a thermocouple. Two cores of 1 cm diameter were used to determine shear force at five different positions along the length of the fibres.

pH measurements were made with a Radiometer pH meter with a glass electrode, at room temperature (20–23°C), with the electrode standardised against a phosphate buffer of pH 6.86.

Acidic and basic groups of muscle and muscle exudate proteins were determined by the method of Frankel-Conart and Cooper[13] as modified by Hamm and Deathrage,[14] in the following manner. The coarse mince was passed through a La Tapple mincer before use, and 0.1 g of muscle or 0.1 ml of the muscle exudate were used for the charges determination. The following formula was used to calculate charged groups:

$$\text{Acidic or basic groups per } 10^4 \text{ g protein} = \frac{\text{Valence of dye} \times 10 \times \text{mg of dye bound}}{\text{Mol wt of dye} \times \text{mg of protein used}}$$

Optical density was read in a 4-ml cell and the dilutions of the dye solutions were adjusted accordingly.

The refractive index of the muscle exudate was determined using Abbe's Refractometer Model G maintained at 25°C. The muscle exudate was obtained by squeezing about 30 g of muscle mince in an Apex Hydraulic Press Type MIR at 1500 lb/in² for 2 min. The meat was wrapped in a strong polyester cloth and then pressed between two Perspex slabs.

All determinations were done in duplicate except the Kjeldahl nitrogens which were estimated in triplicate. The refractive index of each exudate sample was taken many times and no variation was observed in the readings. Standard curves of 0.1% orange G and 0.2% safranine dyes were made from 0–10 mg/l for the determination of acidic and basic groups.

Results

Tables 1 and 2 show the analysis of variance, for some properties of muscle and exudate respectively. Freeze-treatment significantly affected the ultimate pH value of the muscle, tenderness of the cooked meat, PN of the muscle, acidic and

TABLE 1.—ANALYSIS OF VARIANCE FOR SOME PHYSICAL AND CHEMICAL PROPERTIES OF POST-RIGOR NONFROZEN AND FROZEN MUSCLE AFTER THAWING AT LOW AND HIGH TEMPERATURES.

Source of variation	Degrees of freedom	Mean squares							
		pH value	Shear value	Protein nitrogen	% PN/TN	% NPN/PN	% Moisture	Acid groups protein	Basic groups protein
Animals	8	0.0039**	9.8183**	15.0750**	17.9750**	32.0759**	7.642^{ns}	2.8458**	11.2515**
Freezing and thawing treatments	2	0.0092**	6.8878**	18.4078**	8.4044^{ns}	16.1348^{ns}	6.9099^{ns}	1.7344*	9.1781**
Error	16	0.0008	0.2411	1.9328	3.9119	6.8036	4.1146	0.3419	0.1527
Total	26								

**P>0.01; *P>0.1; ns: Nonsignificant.

TABLE 2.—ANALYSIS OF VARIANCE FOR SOME PHYSICAL AND CHEMICAL PROPERTIES OF POSTRIGOR NONFROZEN AND FROZEN MUSCLE EXUDATE AFTER THAWING AT LOW AND HIGH TEMPERATURES.

Source of variation	Degrees of freedom	Mean squares								
		pH value	R_f value	Total solids	TN	PN	% PN/TN	% NPN/PN	% Moisture	Acidic group proteins	Basic group proteins
Animals	8	0.0053**	837.3148ns	1.1856*	13.3879**	7.1742**	13.2256ns	90.2020ns	1.2001ns	18.0590**	4.9900**
Freezing and thawing treatments	2	0.0083**	75.7037ns	0.4559ns	4.3159**	6.5100*	340.8192**	1619.9359**	5.7781**	17.2892**	21.2800**
Error	16	0.0007	351.4109	0.3463	0.3830	0.8108	21.3863	97.3201	0.7469	0.8476	1.1250
Total	26										

**P>0.01; *P>0.1; ns: Nonsignificant.

basic groups on muscle protein. In the case of muscle exudate pH value, TN, PN, %PN/TN, %NPN/PN, moisture content, acidic, and basic groups on the exudate proteins were, significantly influenced by the freeze-treatment.

Tables 3 and 4 show the mean values of different muscle and exudate properties respectively, for the non-frozen and frozen-thawed samples. The results are the means of nine post-rigor sheep loins. Duncan's multiple range test was applied to test the significance of the means. The ultimate pH value and PN decreased, meat tenderness, acidic and basic groups on the muscle proteins increased, on thawing of the frozen muscle. The frozen muscle was less tender and of low ultimate pH value if thawing was carried out at low temperature (8°C). The temperature of thawing, used in the present study, did not affect significantly, the amount of PN of the muscle, acidic and basic groups of the muscle proteins. The process of thawing increased the ultimate pH value, PN, %PN/TN and acidic groups and decreased the TN, %NPN/PN, the moisture content and the basic groups on exudate proteins (Table 4). The temperature of thawing significantly influenced the ultimate pH, TN, % moisture of the muscle and basic groups of the exudate proteins. The freeze-treatment and thawing (at 8°C or 25°C) did not change the refractive index of the muscle exudate.

Table 5(a) and 5(b) show the average increase or decrease, in the properties of the muscle and its exudate respectively, after thawing at low and high temperature as compared with non-frozen sample. Following the treatment of thawing at two temperatures, the relative increase in the acidic and basic groups on muscle proteins was remarkable. Table 6 gives the correlation coefficients between some physical and chemical properties of the muscle and its exudate.

Discussion

Effect of Freezing and Thawing on the Properties of the Meat.—In the present study, the order of meat tenderness was non-frozen < thawed at 8°C < thawed at 25°C. It clearly indicated that, provided the risk of bacterial growth could be avoided, the thawing of the post-rigor ovine muscle at room temperature should result in more tender meat than thawing at chill temperatures. The effect of freezing and thawing in enhancing the tenderness of pre-rigor ovine muscle[6] and post-rogor beef[15] is already known. However, Howard[4] claimed the frozen beef slightly less tender than its chilled counterpart when the meat was cooked after thawing. Similarly, Brow[16] indicated that

oil-coated steaks were less tender when defrosted at room temperature than those defrosted in a refrigerator. The difference between the present study and that of Howard and Brow may be due to the time after slaughter when the freezing was affected and the oil coating of steaks respectively.

The non-frozen muscle had higher and exudate had a lower ultimate pH value than their respective frozen counterparts. The same was shown also by Cook and Langsworth[17] using pre-rigor ovine muscle. Hamm and Deathrage[14] attributed the rise in pH value of beef, on its incubation at high temperature, to the loss of acidic groups of proteins. Table 5(a) shows increase in the charged groups of both kinds on thawing of the frozen muscle. The increase in the acidic groups, after thawing at 25° C was half (0.4) of the increase at 8°C (0.8). But the increase in basic groups of muscle proteins was almost similar (1.7 and 2.0, Table 5(a)) at the two temperatures. These alterations, in the charged groups on proteins, appeared to cause pH changes.

It was interesting to note that the tendency towards increase or decrease of acidic or basic groups on muscle or exudate proteins, was less pronounced, after thawing at high temperature, compared with that of low. Anglemeir, Weir-

TABLE 3.—THE MEAN VALUES OF VARIOUS PHYSICAL AND CHEMICAL PROPERTIES OF POST-RIGOR NONFROZEN AND FROZEN MUSCLE AFTER THAWING AT LOW AND HIGH TEMPERATURES.

Treatment	Ultimate pH	Shear value	PN[2] (mg/g)	% PN/TN[2]	% NPN/PN[2]	% Moisture	Acidic groups[1]	Basic groups[1]
Post-rigor nonfrozen	5.66[c]	7.7[c]	28.1[b]	87.1[a]	14.9[a]	73.9[a]	3.9[a]	7.2[b]
Thawed at 8°C	5.58[a]	6.7[b]	26.2[a]	86.9[a]	15.2[a]	75.3[a]	4.7[b]	9.2[a]
Thawed at 25°C	5.63[b]	6.0[a]	25.4[a]	85.3[a]	17.4[a]	73.6[a]	4.3[b]	8.9[a]

All means within a treatment followed by same upper case subscript not significantly different.
1=Acidic and basic groups per 10^4 g of proteins.
2=On wet weight basis.

TABLE 4.—THE MEAN VALUES OF VARIOUS PHYSICAL AND CHEMICAL PROPERTIES OF POST-RIGOR NONFROZEN AND FROZEN MUSCLE EXUDATE AFTER THAWING AT LOW AND HIGH TEMPERATURES.

Treatment	Ultimate pH	*Rf* value	Total solids	TN (mg/ml)	PN (mg/ml)	% PN/TN	% NPN/TN	% Moisture	Acidic groups	Basic groups[1]
Post-rigor nonfrozen	5.58[a]	1.3581[a]	16.5[a]	20.1[b]	13.1[a]	65.1[a]	54.6[b]	88.1[b]	9.8[a]	14.7[c]
Thawed at 8°C	5.61[b]	1.3574[a]	16.0[a]	18.7[a]	14.0[b]	74.2[b]	34.8[a]	86.5[a]	12.5[b]	11.6[a]
Thawed at 25°C	5.64[c]	1.3579[a]	16.4[b]	19.6[b]	14.8[b]	76.8[b]	29.1[a]	87.4[b]	11.8[b]	13.3[b]

All means within the treatment followed by same upper case subscript not significantly different.
1=Acidic and basic groups per 10^4g of proteins.

TABLE 5(a).—ALTERATIONS IN SOME PHYSICAL AND CHEMICAL PARAMETERS OF MUSCLE AFTER FREEZING AND THAWING AT LOW AND HIGH TEMPERATURES.

Difference between	pH value	Shear force	PN (mg/g)	% PN/TN	% NPN/PN	% Moisture	Acidic groups	Basic groups
Nonfrozen and thawed at 8°C	−0.08	−1.0	−1.9	−0.2	+0.3	+1.4	+0.8	+2.0
Nonfrozen and thawed at 25°C	−0.03	−1.7	−2.7	−1.8	+2.5	−0.3	+0.4	+1.7

TABLE 5(b).—ALTERATIONS IN SOME PHYSICAL AND CHEMICAL PARAMETERS OF MUSCLE EXUDATE AFTER FREEZING AND THAWING AT LOW AND HIGH TEMPERATURES.

Difference between	pH value	*Rf* value	TN (mg/ml)	PN (mg/ml)	% PN/TN	% NPN/PN	% Moisture	Acidic groups	Basic groups
Nonfrozen and thawed at 8°C	+0.03	−0.0007	−1.4	+0.9	+9.1	−19.8	−1.6	+2.7	−3.1
Nonfrozen and thawed at 25°C	+0.06	−0.0002	−0.5	+1.7	+11.7	−25.5	−0.7	+2.0	−1.4

TABLE 6.—CORRELATION COEFFICIENTS BETWEEN SOME PHYSICAL AND CHEMICAL PROPERTIES OF MUSCLE AND ITS EXUDATE AT INDIVIDUAL AND COMBINED TREATMENTS.

Treatment	Shear value (M)				Moisture (M)		Moisture (E)		R_f value (E)		Ultimate pH value (E)	
	PN (M)	% PN/TN (M)	% NPN/PN (M)	pH (M)	pH (E)	Acidic groups (M)	Basic Groups (M)	Acidic groups (E)	Basic groups (E)	Shear value (M)	PN (E)	Moisture (M)	Moisture (E)
Fresh (non-frozen)	+0.08^{ns}	0.00	0.00	+0.24^{ns}	−0.07^{ns}	+0.61^{ns}	+0.96**	+0.22^{ns}	−0.11^{ns}	+0.35^{ns}	+0.47^{ns}	−0.25^{ns}	−0.37^{ns}
Thawed at 8°C	+0.49^{ns}	+0.93**	−0.92**	−0.11^{ns}	−0.30^{ns}	+0.84**	+0.49^{ns}	+0.79*	+0.34^{ns}	−0.39^{ns}	−0.23^{ns}	−0.61^{ns}	+0.43^{ns}
Thawed at °C	+0.40^{ns}	+0.72*	−0.69*	+0.26^{ns}	−0.03^{ns}	−0.39^{ns}	−0.11^{ns}	+0.34^{ns}	+0.37^{ns}	−0.15^{ns}	+0.13^{ns}	+0.38^{ns}	−0.19^{ns}
Combined	+0.39*	+0.58**	−0.57**	+0.19^{ns}	−0.28^{ns}	+0.54**	+0.49**	+0.15**	+0.51**	−0.15^{ns}	−0.08^{ns}	0.07^{ns}	−0.34^{ns}	−0.12^{ns}

M = Muscle; E = Exudate; *P > 0.05; **P > 0.01, ns: Nonsignificant.

bicki and Deathrage[18] did not observe similar changes when comparing charged groups after cooking beef at 160°F, with that of leaving it for 72 hours at 38°F. The absence of freezing and thawing phenomenon in their study may be the probable cause of disagreement with the present observation.

The PN was less in the frozen-thawed muscle than in the non-frozen but the reverse was true for the PN of the exudate (Tables 3 and 4). Since freezing and thawing of muscle tissue increases the activity of its enzymes, increased rate of proteolysis seemed to be the cause of lower PN in the muscles, under these conditions. The smaller protein molecules, made available by the proteolysis in the muscle, could be readily squeezed out, to increase PN of the exudate. As the amount of PN in the exudate, after thawing the muscle at 8°C or at 25°C, did not differ significantly; it appeared that significantly higher TN in the exudate at higher thawing temperatures, resulted from more NPN fraction coming into it. The temperature of thawing did not affect significantly the %PN/TN and %NPN/PN of the muscle or its exudate.

Correlations

Meat Tenderness and Various Kinds of Muscle Nitrogen.—Table 5(a) and Figs. 1 and 2 show the correlation coefficients and graphic relationships between some physical and chemical parameters of the muscle and its exudate. The cooked meat tenderness showed a correlation coefficient of +0.39 (P 0.05) with PN, of +0.58 (P 0.01) with %PN/TN and of −0.57 (P 0.01) with % NPN/PN, of the muscle.

The strong belief, that the connective tissue plays a major role in meat toughness,[19] has slowly changed towards the realisation that it is the contraction state of the muscle protein filaments, during rigor[20,21] and ion-protein relationship after rigor[22] which affect tenderness of meat. The correlation coefficients mentioned above and the linear relationship of shear value with different protein components of the muscle in Fig. 1 emphasised the changed criterion. The almost equal but opposite correlation coefficients of %PN/TN and %NPN/PN with shear value suggested constant amounts of PN and NPN at a certain level of TN. Some earlier workers have already shown it to be true for pig[23] human[24] and chicken[25] muscles.

Figure 2 shows the graphic relationship between shear value and PN, %PN/TN and %NPN/PN of the exudate. None showed a linear relation-

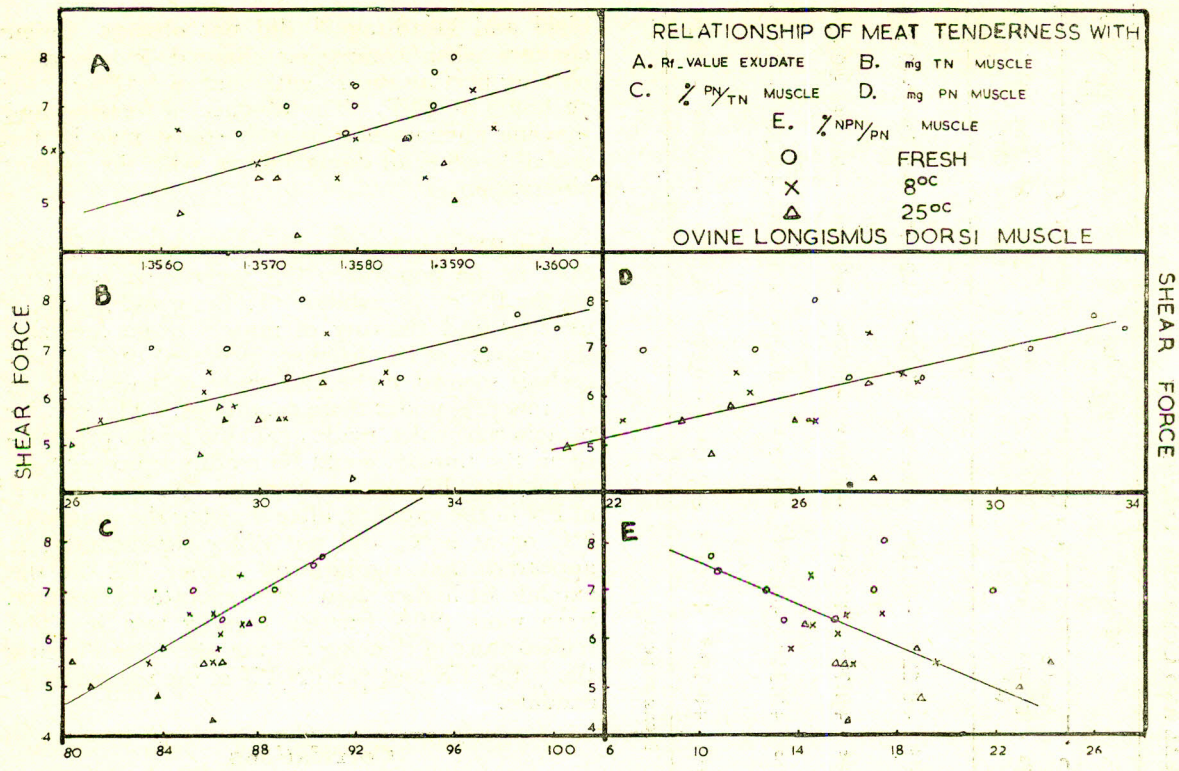

Fig. 1.—Linear relationships of some muscle and exudate components with cooked meat tenderness.

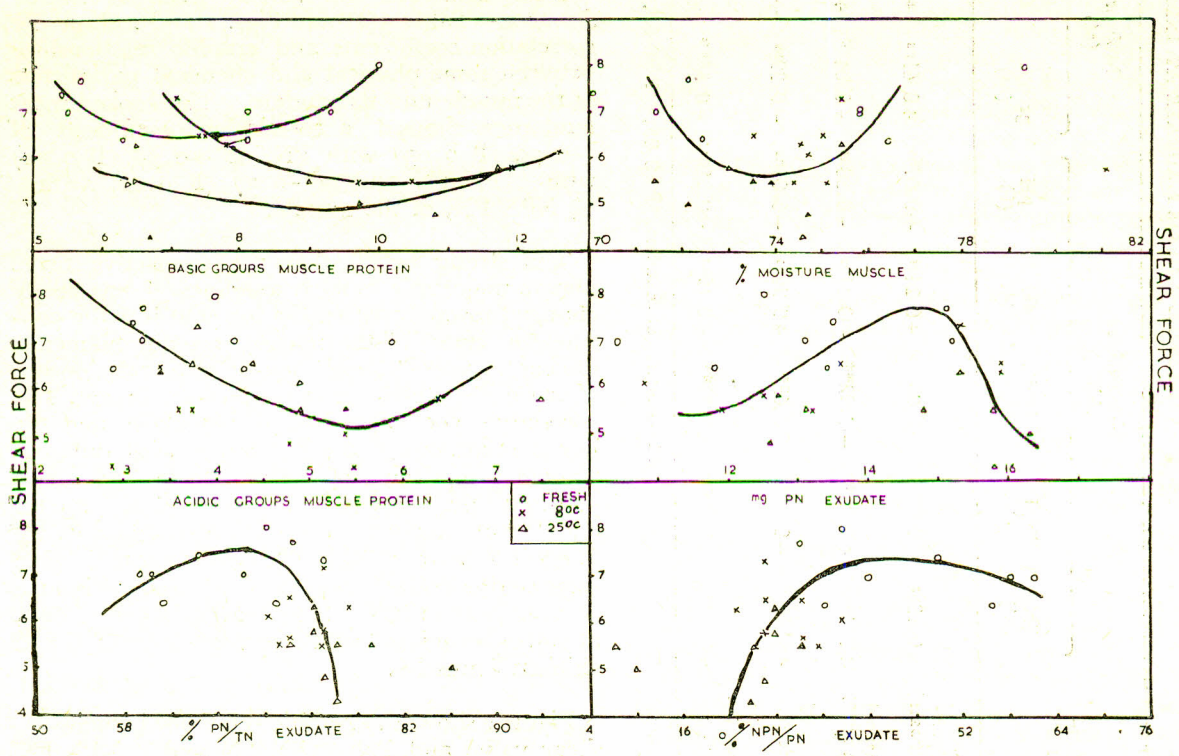

Fig. 2.—Non-linear relationships of some muscle and exudate components with cooked meat tenderness.

ship. In all these curves the cooked meat first became tough up to a certain value of PN, %PN/TN or %NPN/PN and then became tender on further increase of these values. The typical pattern of the curves suggested that PN up to the transitional value of 14.75 mg/ml might have a different origin in the muscle cell from the PN above this value. It may be safe to suggest, therefore, that the PN up to the former value came from sarcoplasma, having poor relationship with meat tenderness, and the PN above this value was contributed by contractile proteins, after the establishment of physiochemical changes, on thawing of the frozen muscle. The well-known contribution of contractile proteins towards the functional properties of meat, strengthens the above explanation.

Meat Tenderness and Charged Groups.—Meat tenderness increased with increasing charged groups of the muscle proteins up to a certain value and decreased again like its relationship with the muscle moisture (Fig. 2). Significant correlation coefficients of %0.54 (P 0.01) and +0.49 (P 0.01) were computed between the moisture content, the acidic groups and basic groups, on muscle proteins respectively. The similarity in relationship of meat tenderness with charged groups and moisture content appeared to suggest that increased meat hydration, due to increase in number of charged groups, may account for the more tender meat. The reason for tougher meat, when moisture content of the muscle was above 74%, or the charged groups above their transition value, was not known (Fig. 2).

pH, Moisture and Tenderness of Meat.—There was found no relationship between pH value and moisture content or pH value and meat tenderness. This may be due partly to the fact that pH values only above 5.8 could cause, increase in water holding capacity of meat[26] and also that tenderness of meat was not related to pH between 5.5–5.6.[27] Although McLouchlin[28] reported a positive relationship between pH value and moisture content of pig *longissimus dorsi* muscle, he suggested a further examination of his findings, using different muscles of the pig as well as muscles from other species.

Evaluation of a Refractometric Method for Denaturation of Protein in Ovine Muscle.—The correlation analysis did not give significant relationship, between refractive index of the exudate and PN or TN of the muscle or exudate. This observation indicated explicitly that the prediction of protein quality of the ovine muscle, through the optical properties of its exudate, is not possible. Weirbicki *et al.* in 1954 reported a direct, and in 1956 an indirect significant relationship, between R_f value and mg/ml of a protein extract, obtained by using Kcitrate buffer of pH 5.6 and ionic strength 0.48. The different physiochemical nature of the exudate, used for R_f measurement in the present study, from that of Weirbicki *et al.*[7,8] may be the cause of disagreement amongst the two.

The refractive index of a liquid decreases with increase of temperature and the number of molecular species per unit volume. Table 2 shows that freezing and thawing decreased the refractive index of the post-rigor ovine muscle exudate which suggested increased proteolysis but surprisingly enough, the decrease in R_f value was more at 8°C as compared with the thawing temperature of 25°C. The reason for this could not be traced. In Fig. 1 the scatter diagram of the R_f value, versus tenderness of the cooked meat, is shown. The finding of non-significant correlation coefficient between R_f value and tenderness (Table 6) was in agreement with that of Weirbicki *et al.*[8] with beef; although in an earlier study, these workers reported a positive correlation coefficient of 0.565 (P 0.01) between R_f value of a protein solution and the tenderness score of meat.

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