CHARACTERIZATION OF HEIFER BEEF COLOUR RELATED TO NATURAL ONSET OF ESTRUS AND ESTRUS ONSET CAUSED BY WITHDRAWAL OF MELENGESTEROL ACETATE

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SUMMARY

Natural estrus (E) and anestrus (A) at slaughter were compared to E and A caused by feeding or removing melengesterol acetate (MGA) from the diet of 12 beef heifers. Munsell hue of the longissimus at the twelfth rib was higher (7.50 R) for A heifers 25 hr after ribbing at 4°C than for E (1.65 R), indicating that A muscle changed from red-purple toward yellow-red during the 25-hr exposure to air. Likewise, A Munsell value rose 0.257 to 3.667 during the same period, indicating the development of lighter colour. Munsell chroma for the three natural E heifers was higher (7.133) than for all other heifers (4.144), indicating a more intense colour. MGA muscle had lower pH readings at 1/2, 2, 3 and 16-hr postmortem. Beef from E heifers tended to be darker, but pH drop in beef from MGA heifers proceeded more rapidly and modified the tendency toward darker colour meat.

INTRODUCTION

Colour is probably the single greatest appearance factor that determines whether or not a meat cut will be purchased (MacKinney et al. 1966). Marketing channels discriminate against dark cutting beef. Meat packers in the Midwest United States continue to experience monetary losses from dark cutters.

Work by O'Brien (1969) and many field trials (Lauderdale 1983) have shown MGA feeding to have no effect on beef colour, while Hawkins et al. (1972) found in some cases a higher incidence of dark cutting heifers that were fed MGA. Personal communications with Illinois and South Dakota meat packers in 1988 indicate heifers coming through a terminal market are the main source of dark cutters. All the aforementioned reports were based on subjective evaluations of colour by a United States Department of Agriculture (USDA) grader.

The objective of this study was to evaluate the effects of estrus caused by MGA removal prior to slaughter vs. natural estrus on muscle colour by using more definitive colour measures and monitoring postmortem muscle glycolysis. A secondary objective was to determine if beef palatability was affected by E and A.

EXPERIMENTAL METHODS

Twelve Angus x Hereford heifers were fed 0.4 mg of MGA/head/day during the final 116 days of a finishing period. Heifers were slaughtered in replicates of four on three separate days. Each replicate consisted of two heifers showing visual signs of estrus (E): one in natural estrus, the other in estrus by virtue of having been taken off MGA 48 hours previously (United States Food and Drug Administration regulations); and two heifers not showing visual signs of estrus (A): one naturally in anestrus, the other held out of estrus by MGA feeding. Muscle pH was measured (Pearson 1973) at slaughter, 1/2 hour, hourly to six hours at 8, 12, 16 and 24 hr postmortem, when the internal temperature of each carcass reached 4°C and 24 hr later. Muscle samples for glycogen and myoglobin determinations taken at slaughter, when the carcass reached 4°C and 24 hr later were frozen in liquid nitrogen immediately upon removal from the carcass for later analysis.

A 2.54-cm rib steak was removed from each carcass when the carcass reached 4°C and allowed to "bloom" at room temperature (20°C) for 60 minutes before colour readings were taken with a Photovolt Reflection Meter. Hue, value and chroma were calculated from Munsell tristimulus colour notations (Romans et al. 1965). Expressible juice was determined on this steak using the technique described by Merkel (1971). Following initial colour determinations, rib steaks were covered with an oxygen permeable polyvinyl chloride film (conventional rib eye cover) and stored at 2°C for an additional 24 hr before reflectance readings were repeated. Glycogen was determined as described by Montgomery (1957). Myoglobin and haemoglobin were determined using a carbon-monoxide conversion procedure (Romans et al. 1965). After carcasses were aged 7 days, rib steaks were removed for taste panel palatability and Warner-Bratzler shear tests. Steaks were broiled to 66°C internal temperature in a 232°C electric broiler for both tests. USDA yield and quality grades were determined.

RESULTS

Quality grades did not differ between treatments, the mean being near USDA average Choice. All carcasses were fat USDA Yield Grade 4's, averaging 3.15 ± 1.07 cm fat thickness over the rib eye.

Munsell hue of the longissimus at the twelfth rib did not differ among treatments 1 hr after reaching 4°C, but 24 hr later A muscle showed a higher hue reading, indicating that the colour had changed from red-purple toward yellow-red during the 24-hr holding period (table 1). Munsell value rose 0.257 to 3.667 in A muscle during this

Table 1--Effect of melengesterol acetate and estrus on beef color

	Estrus		Anestrus	
Hue, 1 hr after 4°C	1.83R		2.88R	
Hue, 25 hr after 4°C*	1.65R		7.50R	
Value, 1 hr after 4°C	3.503		3.410	
Value, 25 hr after 4°C	3.440		3.667	
Value change during 24 hr*	-.063		.257	
	MGA	No MGA	MGA	No MGA
Chroma, 1 hr after 4°C	3.793	4.310	4.987	5.140
Chroma, 25 hr after 4°C*	4.150	7.133	4.280	4.003

*P<.10.

Table 2--Effect of melengesterol acetate and estrus on glycolysis

	Estrus		Anestrus	
Glycogen at slaughter, mg/g fresh muscle	9.80		12.82	
Glycogen at 4° C, mg/g fresh muscle*	1.15		3.07	
pH drop, slaughter to 4° C*	1.24		1.44	
Postmortem hr to 4° C*	36.1		29.5	
	MGA	No MGA	MGA	No MGA
pH 2 hr postmortem**	6.32	6.69	6.54	6.57
pH 3 hr postmortem*	6.10	6.42	6.49	6.36
	MGA		No MGA	
pH 1/2 hr postmortem*	6.76		6.88	
pH 2 hr postmortem***	6.43		6.63	
pH 16 hr postmortem*	5.41		5.50	

* P<.10.
** P<.05.
*** P<.01.

same period, while E muscle value decreased 0.063, indicating a lighter colour developed in A muscle during the 24-hr holding period (Table 1).

There was no significant MGA effect on hue and value. However, chroma (colour intensity) was higher in natural E muscle only after holding 24 hr (Table 1). Thus, E muscle showed a tendency toward darkness after holding for 24 hr, but only the natural E muscle gained in colour intensity.

Expressible juice and myoglobin and haemoglobin content did not vary among treatments and times sampled, averaging 67.6 ±6.5% and 4 mg/g of fresh tissue, respectively, for all muscle. E muscle at slaughter and following glycolysis during cooling to 4°C contained less glycogen (table 2). Logically, pH drop was less during postmortem cooling in E muscle (Table 2). E carcasses required 6.6 additional hr to reach internal temperature of 4°C (table 2). MGA hastened glycolysis in E muscle early in the cooling period (2 and 3 hr postmortem, Table 2). The MGA glycolytic effect as exemplified by pH drop was significant at 1/2, 2 and 16 hr postmortem in statistically pooled E and A muscle (table 2). In all muscle, pH did not differ at slaughter (6.8 ± .10) nor at 4oC (5.5 ±.18).

Taste panel palatability and Warner-Bratzler shear did not vary among treatments. On a 9-point scale (9 preferred) all muscle averaged 6.2 ± .9 for tenderness, 6.3 ± .9 for flavour, 6.2 ± 1.1 for juiciness and 7.5 ± .6 for overall acceptability. Warner-Bratzler shears averaged 6.8 ± 1.2 kg.

DISCUSSION

That the dark cutting condition occurs most frequently in heifers that are in estrus at the time of slaughter is a postulation made by many in the industry. Lean colour is routinely evaluated subjectively by USDA meat graders. Depending on the degree to which this characteristic is developed, the final grade of carcasses which otherwise would quality for the Prime, Choice or Select grade may be reduced as much as one full grade (Romans et al. 1985). This study was thus designed to objectively measure muscle colour and the biochemical reactions involved in muscle colour development. Only two carcasses from the twelve heifers in this study showed visual evidence of being dark. Both were from E heifers, one by virtue of receiving MGA, the other in natural estrus.

The objective colour measures indicated that E heifers tended to have darker meat, but this tendency toward darkness was modified in those heifers that had been fed MGA. This trend was substantiated by glycogen levels in muscle and rate of pH drop. Lauderdale (1983) stated that MGA has glucocorticoid activity. Lehninger (1986) stated "cortisol, the most important of the glucocorticoids promotes gluconeogenesis from amino acids and glycogen deposition in the liver, increases blood glucose and decreases peripheral utilization of glucose." It is possible that the glucocorticoid activity of MGA provided the basis for the moderation of dark colour development in E heifers at slaughter. The level of biochemical activity in the E carcasses could be responsible for their slower cooling rate.

CONCLUSIONS

E heifers tended to yield darker colored muscle as measured objectively. The development of darker muscle colour was attenuated by the feeding of MGA. No detectable differences in palatability among all twelve heifers in the study existed.

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INFLUENCE OF VACUUM AND MODIFIED ATMOSPHERE PACKAGING ON QUALITY OF VENISON LOINS

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SUMMARY

A study were conducted at the Invermay Agricultural Centre to evaluate the use of vacuum and modified atmosphere packaging using 100% carbon dioxide on the quality of venison loins. Loins were packaged in either vacuum packages (VP) or modified atmosphere packaging (100% CO_2) using containment films of ultra-high barrier plastic (CO_2-UHB) or a aluminium foil laminant (CO_2-Foil). Surface colour of loins exposed to the modified atmosphere discoloured as the oxygen content of the headspace increased especially in the CO_2-UHB treatments. pH values were higher ($P < 0.05$) in VP loins than in those packaged in CO_2. Sensory traits of loins were unaffected by packaging but decreased as meat was stored from 12 to 18 weeks. Total anaerobic and lactic acid forming bacteria counts increased as meat was stored up to 18 weeks. Packaging venison loins in CO_2 had no apparent advantage over vacuum packaging.

INTRODUCTION

World wide preference seems to regard chilled meats as being superior to frozen. This has forced meat processors in exporting countries to utilize innovative methods capable of preserving meat quality to allow for the assembly, transport and distribution of the products. Oxygen-impermeable, heat shrinkable films have been successfully used for several years for vacuum packaging beef and lamb for up to 8-12 weeks (Gill 1987), but have several limitations including the fact that high pH meat cannot be vacuum packaged. Recent advances in packaging systems utilizing low storage temperatures (-1°C) and high concentrations (100%) of CO_2 has resulted in the extension of shelf life of chilled lamb beyond 18 and up to 23 weeks (Gill 1986). Although high concentrations of CO_2 have been found to result in the discoloration of fresh meat (Brooks 1933), discoloration using 100% CO_2 modified atmospheres can be reduced or eliminated by first packaging the meat in permeable films before exposure to the gas. The objectives of this study were to measure changes in chilled venison quality (changes in pH, surface discoloration, microbial quality, and sensory characteristics) when stored for extended periods of time (up to 18 weeks) when the meat is either vacuum packaged of stored in a modified atmosphere (100% CO_2) within bags made of an ultra-high barrier film (CO_2-UHB) or aluminium foil laminated film (CO_2-Foil).

EXPERIMENTAL METHODS

Slaughter and packaging.

Thirty red deer stags carcasses were electrically stimulated (45 v, 1.2 amps, 90 sec) and stored at 4°C overnight. Boneless loins were removed 24 hr postmortem, vacuum packaged and sent to Invermay Agricultural Centre. Pairs of loin muscles were cut into six, 18 cm portions and allotted to 3 packaging treatments according to a predetermined scheme to assess variability among animals; vacuum packaged (VP); packaged in 100% CO_2 using the UHB outer film (CO_2-UHB); packaged in 100% CO_2 using CO_2-Foil. A pair of loin portions were packaged individually in Cryovac (W.R. Grace, Porirua, New Zealand) barrier bags (ethylene/vinyl acetate copolymer-polyvinylidene chloride laminate; OTR = 30-40 ml m^{-2} 24 hr^{-1} atm^{-1} at 25°C and 75% RH) to 722 mm Hg and heat shrunk by dipping in a 90°C water bath for 2-3 seconds. Paired loin portions (one from each side) were kept together as a unit and will be denoted as such throughout this paper. Modified atmosphere packages were prepared by first packaging two loin portions in Cryovac E-bags (OTR = 517 ml m^{-2} 24 hr^{-1} atm^{-1} at 0°C and 75% RH, CO_2TR = 7223 ml m^{-2} 24 hr^{-1} atm^{-1} at 27°C and 75% RH) to 722 mm Hg. Paired loin portions were placed inside either the specified outer bag and evacuating the bag twice before filling with 1 L CO_2/kg and heat sealing. Meat was stored at -1°C and sampled after 6, 12 and 18 weeks.

Quality assessment.

The pH of each loin was determined by taking duplicate readings using an Orion spear-tip combination electrode (91-63) and a portable pH meter 24 hr postmortem prior and also from each loin portion (in duplicate) after each designated storage period. Residual oxygen content (expressed as percent) was determined in the modified atmosphere packages by using a Servomex oxygen meter. A composite sample of venison (10 g total) was taken from the two loin portions in each package unit after storage to enumerate lactic acid bacteria (Rogosa media), total aerobes (Plate Count Agar) and total anaerobes (Plate Count Agar) were used (Messer et al. 1984). All enumerations were performed in duplicate. Microbiological counts were reported as $\log_{10}$ CFU/gram. Surface discoloration of loins upon opening was determined by a 3 member evaluation panel using a 5 point colour scale (5 = bright fresh red venison colour, 4 = bright red venison colour, 3 = slightly dark or brown, 2 = moderately dark or brown, 1 = extremely dark or brown) and a 3 point colour acceptability scale (3 = purchase with no reservation, 2 = purchase with reservation, 1 = would not purchase).

Statistical methods.

Experimental units in this study consisted of 2 paired loin portions from each animal which were either packaged individually (VP) or in pairs within a modified gas package (CO_2-UHB or CO_2-Foil). Data were analyzed by analysis of variance. Statistical significance was assessed at the 5% level throughout.

RESULTS AND DISCUSSION

Surface discoloration.

Loins packaged in either VP or CO_2-Foil exhibited higher ($P < 0.05$) surface discoloration scores than those packaged in CO_2-UHB (Table 1). Surface discoloration scores did not change markedly when loins were stored from 12 to 18 weeks. In addition, higher oxygen concentrations were present in CO_2-UHB packages than in CO_2-Foil after 12 and 18 weeks storage resulting in 1.0% and 0.62% oxygen for CO_2-UHB and CO_2-Foil, respectively after 18 weeks. Oxygen concentrations as low as 4 mm Hg (0.5% at atmospheric pressure) have

Table 1. Surface colour and residual oxygen content as affected by packaging method and storage time

	Packaging method			
	Vacuum	CO_2-UHB	CO_2-Foil	SED
DISCOLOURATION[1]				
Week 12	4.3	2.8	4.0	0.13*
Week 18	4.0	2.1	4.3	
RESIDUAL OXYGEN				
Week 6	—	0.3	0.2	0.13*
Week 12	—	0.5	0.2	
Week 18	—	1.0	0.6	

*(P<0.05)

[1] Scored using a 5 point scale (5 = bright fresh red venison colour, 4 = bright red venison colour, 3 = slightly dark or brown, 2 = moderately dark or brown, 1 = extremely dark or brown)

Table 2. Effect of method of packaging and length of storage on the number of bacteria recovered from venison loins (values in brackets are the ranges for each treatment).

Bacteria	Method of packaging		
	Vacuum	CO_2-UHB	CO_2-Foil
		(Log_{10} CFU/g)	
ANAEROBES			
Week			
6	2.45 (1.23-3.76)	1.15 (0-2.08)	1.33 (0-3.23)
12	3.89 (1.66-6.00)	3.62 (2.46-6.00)	3.04 (1.15-5.43)
18	5.22 (3.82-6.59)	4.12 (2.18-591)	2.45 (0-4.70)
SED	0.529*		
LACTIC ACID BACTERIA			
Week			
6	0.45 (0-2.20)	0 (0)	0.47 (0-2.98)
12	2.65 (0-5.86)	2.10 (0-6.00)	1.65 (0-3.18)
18	4.06 (0-6.51)	3.04 (0-4.91)	1.34 (0-4.04)
SED	0.742*		

Table 3. Means of sensory traits of venison loins affected by storage time.

Trait	Week of storage			
	6	12	18	SED
Aroma	5.36	5.29	4.82	0.11*
Texture	5.86	5.64	5.39	0.16*ª
Flavour	5.29	4.99	4.31	0.15*
Juciness	4.67	4.44	4.62	0.32
Acceptability	5.01	4.77	4.30	0.17*

* Significant (P<0.05)
ª Significant packaging method X storage time interaction

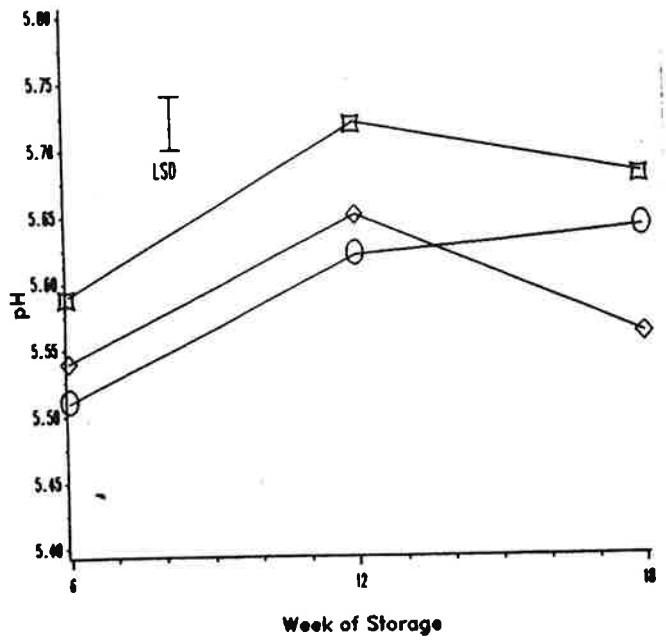

Fig 1. Effect of storage time on pH of venison loins packaged by various methods: Vacuum, ¤; CO_2-UHB, o; CO_2-Foil, ◇.

been found to accelerate the discoloration of meat by increasing the oxidation of myoglobin to form metmyoglobin (Giddings 1977). Although residual oxygen was scavenged to immeasurably low levels three days after packaging (data not presented), residual oxygen levels increased in the CO_2-UHB packs due to ingress through the outer UHB film to levels which were able to accelerate discoloration on the loin surfaces.

pH.

Loins packaged in modified atmosphere packages exhibited lower pH values ($P < 0.05$) than loins which were vacuum packaged except after 18 weeks storage (Fig.1). The same trends have been found in other studies and has been attributed to the absorption of dissolved CO_2 into the meat fluids and the subsequent formation of carbonic acid (Huffman 1974). Lower pH values can result in increased meat discoloration, but in this study, surface discoloration seems more a consequence of oxygen ingress than changes in pH since only venison packaged in the UHB films was discoloured significantly.

Colour stability.

Discoloration scores of loin steaks decreased after 6 weeks of storage from average scores of approximately 4.7 (bright, fresh red) to scores as low as 1.8 after 5 days of display in air under simulated retail conditions (Fig. 2). Trends were similar for each packaging

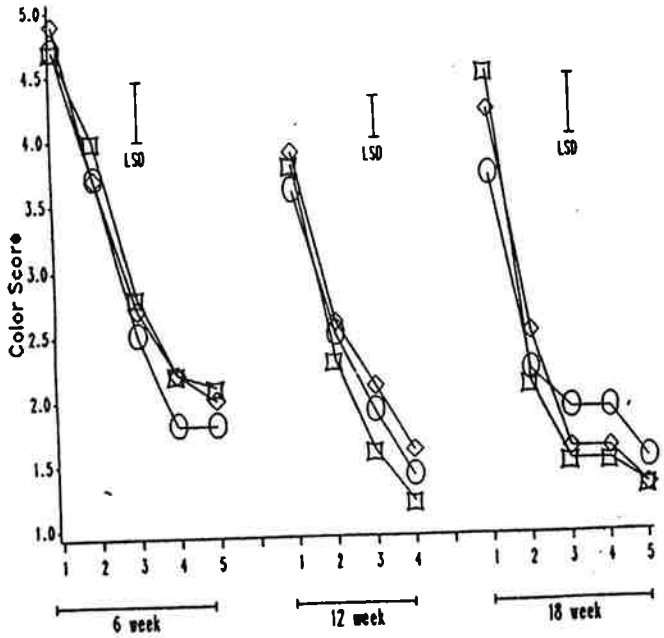

Fig 2. Mean panel discoloration scores of venison loins. Vacuum, ¤; CO_2-UHB, o; CO_2-Foil, ◇.
5=Bright-red venison color; 1=Extremely dark or brown

treatment except CO_2-UHB exhibited lower scores from the third day of display onwards. Trends for steaks stored for 12 weeks were similar except that steaks from VP loins had lower scores after 2 or more days of display than either modified atmosphere treatments. After 18 weeks storage, steaks from CO_2-UHB had lower initial colour scores, but deteriorated less in colour than those from VP or CO_2-Foil. Loin steaks stored for 6 weeks reached the acceptable discoloration limit after 3 days of display, but the time of acceptable display was reduced to 2 days after 12 weeks storage and to 1.5 days after 18 weeks. Decreasing periods of acceptable display has been attributed to decreased effectiveness of intracellular reducing systems as the meat is held for extended periods (Bevilacqua and Zaritzky 1986).

Microbial evaluation.

Recoverable anaerobic bacteria increased up to 2.5 times during the storage period of 6 to 18 weeks after packaging (Table 2). Increases in recoverable lactic acid forming bacteria followed the same trends. Significant effects of packaging method were not found until 18 weeks of storage where meat packed in CO_2-Foil had lower counts ($P < 0.05$). Reasons for the lower levels of lactic acid bacteria recovered after 18 weeks from the CO_2-Foil are not readily apparent. Both the low storage temperatures and high concentrations of CO_2 presented conditions suitable for the proliferation of lactic acid forming bacteria which have been shown to dominate the flora of meat packaged in modified atmospheres with high CO_2 concentrations. The growth of these types of bacteria can be tolerated and contribute little to spoilage in meat destined for extended storage (Gill 1986).

Sensory traits.

Scores for sensory traits were not affected by packaging method but were affected ($P < 0.05$) by storage time (Table 3). Sensory traits of loins (aroma, texture, flavour, and overall acceptability) were similar after 6 and 12 weeks, but were lower when loins were stored to 18 weeks. Juiciness scores did not differ. This indicates the maximum shelf life of chilled venison lies somewhere between 12 and 18 weeks since scores below 5 indicate panelists began to dislike the product.

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SHRINKAGE AND MUSCLE QUALITY IN SPRAY-CHILLED BEEF CARCASSES

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SUMMARY

Four experiments were conducted to examine the effects of an intermittent water spray during cooling on beef carcass yield and muscle quality. Experiments 1, 2 and 3 used 4, 8 and 12 hour periods of an intermittent water spray (4 cycles per hour with a spray duration of 60 seconds) applied to shrouded carcass sides compared to control sides with no spray applied. Experiment 4 used the same procedure as experiment 2 except that unshrouded sides were used. Side weight shrinkage at 24 hours following slaughter was significantly reduced ($P < .01$) by 0.48, 0.69, 0.89 and 1.43% in experiments 1-4 for spray-chilled compared to control sides. Most measures of muscle quality were similar for treated or control sides, except that fat colour became lighter with a longer period of spray-chilling. Drip losses and colour changes were similar for meat displayed in retail packages for 4 days irrespective of treatment. It was concluded that spray-chilling was effective in reducing carcass cooler shrinkage and had no obvious detrimental effects on muscle quality.

INTRODUCTION

Typical weight losses in beef carcasses over the first 24 hours post slaughter range from 0.75-2.0% (Kastner 1981) in North America and from 1.2-1.7% in the U.K. (Collett and Gigiel 1986). Allen et al. (1987) reported that carcass shrinkage over 24 h was reduced from 1.46 to 0.32% when unshrouded carcasses were spray-chilled for 8 h (32 cycles of 90 seconds duration every 15 min.), but purge (drip) was marginally increased (0.26%) in vacuum packaged inside rounds.

The objectives of this study were to: 1. Determine shrinkage losses in sides of shrouded and unshrouded beef spray-chilled for various times during cooling; 2. Evaluate the possible influence of spray-chilling on muscle quality, carcass and primal cut shrinkage during storage and subsequent retail display.

EXPERIMENTAL METHODS

Four separate experiments were conducted over a period of 5 months. Experiment 1 used 19 shrouded carcasses and alternate sides were placed in one of two coolers with identical conditions (temp. 1°C, air velocity 0.5 m/s), to have equal numbers of left and right sides within each treatment. Carcass sides were spray chilled with water (average temperature 12°C) for a period of 4h (4 cycles/h; 60s duration) and held in the cooler for another 20h, so that total chilling time amounted to 24h. Carcass sides placed in the second cooler were air chilled only for 24h and served as controls. Shrouds were removed at the end of the spray-chilling period. Experiments 2 and 3 followed the same procedures as described for experiment 1 except that the duration of the spray-chilling treatment was increased to 8 and 12h, respectively. Experiment 4 examined the effect of 8h of spray-chilling compared to control sides without the use of a shroud.

Half the treatment and control sides (where permitted by the design) were fabricated about 30h post-slaughter into the major primal cuts and two subprimal cuts (boneless rib - IMPS 112; inside round - IMPS 168) were vacuum packaged in high oxygen barrier film bags (oxygen transmission rate of 10 cc/m^2/24h at 37.8°C and 90% RH) and held at 2°C for 6 days. The whole sides remained in a holding cooler for 6 days, and the same subprimals removed. Two rib steaks 28.5 mm thick were removed from each rib (from side or vacuum package) and placed in separate polystyrene trays overwrapped with an oxygen permeable wrap, and held at 2°C for 4 days to simulate retail display. Two roasts weighing approximately 1 kg, were prepared from the inside round and packaged and held in the same way as described for the rib steaks. Meat colour for the LD and overall fat colour were measured at the cut surface of the LD using a Minolta Chroma Meter II (Minolta Camera Company, Meter Division, Ramsey, NJ., USA) at 24h post-slaughter. On day 11 post-slaughter a 28.5 mm rib steak from each side was cooked to an internal temperature of 80°C in a microwave oven and held overnight at 2°C. After equilibration to room temperature (20°C), two cores of 19mm were removed and sheared using the Ottawa Texture Measuring System (Canners Machinery, Simcoe, Ontario, Canada) equipped with a Warner-Bratzler cell. Data collected in this study were analyzed using analysis of variance procedures.

Table 1. Effects of spray-chilling on carcass shrinkage and drip in vacuum packaged cuts

	Time of Spray-Chilling, h							
	0	4	0	8	0	12	0+	8+
Side wt. kg	126.6	126.6	137.6	137.2	142.1	141.8	129.7	129.7
Shrinkage %:								
End of spray	.83[a]	.09[b]	.79[a]	.18[b]	.90[a]	.26[b]	1.26[a]	.72[b]
To 24h	1.63[a]	1.15[b]	1.29[a]	.60[b]	1.29[a]	.40[b]	1.78[a]	.35[b]
To 6 days	3.53	3.24	2.60	2.26	2.66[a]	2.19[b]	4.16[a]	3.22[b]
Drip %:								
Rib,6 days	.38	.41	.31	.36	.34	.36	.40	.41
Round,6 days	1.30	1.28	1.02	1.18	1.05[a]	.88[b]	.92	.91

+ Unshrouded sides [ab] Means within a spray chill treatment are significantly different ($P<0.01$)

Table 2. Effects of spray-chilling on muscle quality and carcass fat thickness

	Time of spray chilling, h							
	0	4	0	8	0	12	0+	8+
Meat colour								
% Y	8.59	8.54	7.92	7.67	8.39	8.19	8.19	7.58
Fat colour								
% Y	39.9	41.7	39.3a	45.8b	42.1a	50.6b	34.4a	40.0b
Shear, kg	6.9	6.5	7.7	7.3	7.0	6.9	7.3	8.4
Fat, mm	4.1	4.7	5.2	5.4	6.1	6.5	5.4	5.6

+ Unshrouded sides. [ab] Means within a spray chill treatment are significantly different ($P<0.01$)

RESULTS

Spray chilling significantly reduced carcass shrinkage at the end of the spraying period and this result continued through to 24h post-slaughter (Table 1). After 6 days of cooler ageing total shrinkage in carcass weight was similar for sides spray-chilled for 4 and 8h, respectively. However, sides that were spray-chilled for 12h and unshrouded sides spray-chilled for 8h had significantly lower carcass weight shrinkages to 6 days than control sides. Drip or purge losses over 6 days in vacuum packaged ribs and inside rounds tended to be similar whether the sides were spray-chilled or not (Table 1).

Muscle colour was not influenced by the spray-chilling treatments although it should be noted that there was a trend for the LD from spray-chilled sides to have slightly lower C.I.E. Y values than those from control sides (Table 2). Fat from spray-chilled sides where the spraying period was 8h or more had significantly higher Y values than control sides. Muscle shear values and carcass fat thickness were not influenced by spray-chilling.

Weight losses, % drip and colour changes of rib steaks and round roasts held in retail packages for 4 days were largely unrelated to spray-chilling.

DISCUSSION

Spray-chilling was found to have a highly significant effect on carcass shrinkage, particularly in the first 24h following slaughter. Carcass shrinkage at 24h was proportionally related to the length of the spray-chilling treatment, so that shrinkage was reduced by 0.48-0.89% as the spray-chilling period was increased from 4 to 12h. Allen et al. (1987) reported a shrinkage saving of 1.14% in unshrouded carcasses sprayed for 8h, which is similar to our results. The 6 day shrinkage data reveal that spray-chilling becomes a less effective method to conserve weight loss over a period of time. Drip losses in cuts that had been vacuum packaged did not appear to be influenced by spray-chilling. Allen et al. (1987) essentially found similar results except for inside rounds where purge or drip was increased by 0.26%, after 15 days of storage. Hamby et al. (1987) reported that spray-chilling (2 cycles of 30 secs. /h for 12h) had no effect on purge in various cuts after 28 days of vacuum packaged storage. Loin muscle colour at 24h post-slaughter was not influenced by spray-chilling, although luminosity (Y) always tended to be lower in treated compared to control sides. Allen et al. (1987) also found that spray-chilling had no effect on loin muscle colour. Fat colour in sides spray-chilled for 8 and 12h was whiter than control sides based on objective measurements of colour. This difference was visually apparent and has not been previously reported in the literature. Retail cuts held for 4 days generally tended to have similar weight losses and colour changes which were independent of treatment.

CONCLUSIONS

This study has demonstrated that spray-chilling can substantially reduce carcass shrinkage particularly during the 24h period that elapses following slaughter. The longer the spray-chilling period, the greater are the potential savings in shrinkage. However, some caution should be used in interpreting these results. Jones et al.(1988) found that spray-chilling pig carcasses reduced shrinkage over 24h by 2.4%, but that the majority of this saving (60%) was in reduced subcutaneous fat weight loss during cooling. If a similar mechanism exists in beef carcasses then the spray-chilling process is mainly conserving evaporative weight losses in subcutaneous fat. Although the savings in shrinkage attributed to spray-chilling are substantial over 24h post-slaughter, much of this saving in weight loss may well disappear in additional fat trim which has a low value compared to meat in typical vacuum packaging operations. On the other hand, slaughter plants merchandizing whole carcasses within 1 day of slaughter would realize large benefits by utilizing the spray-chilling process.

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A COMPARISON OF MEAT PROPERTIES OF YOUNG CATTLE WITH LOW OR ADEQUATE LIVER COPPER CONCENTRATIONS

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SUMMARY

The mechanical properties of cooked samples of *M. pectoralis profundus* from young cattle (carcass weight 184 kg, age 297 days) with low (LCu), 8 mg/kg of dry matter, or adequate (ACu) 132 mg/kg, liver copper concentrations were measured. There were no significant differences in Warner-Bratzler peak force, initial yield force, or peak force minus initial yield force values due to liver copper status. Liver copper status did not affect the values of parameters from tensile (adhesion) force-deformation measurements, considered to reflect intramuscular connective tissue strength. There were no differential changes in values of parameters from Warner-Bratzler and adhesion measurements with increased cooking (60°C for 1 hr, 80°C for 1 hr, 80°C for 5 hr) between samples from the LCu and ACu groups.

It was concluded that low copper status, of the order of that of the LCu animals, had no effect on the contribution of intramuscular connective tissue to meat toughness.

INTRODUCTION

Copper deficiency can produce defects in the connective tissue of animals. Clinically, the deficiency retards skeletal growth and bone development and weakens vascular connective tissue. Carnes (1968) considered that the weakness of vascular connective tissue was the result of defects in elastin and, possibly, the molecular structure of collagen. Chou, Savage and O'Dell (1969) showed that in the collagen of tendons of copper deficient chicks the ratio of to chains was increased three-fold.

The common effect of copper deficiency on cross-linking in collagen and elastin results from a reduced activity of tissue monoamine oxidase (Carnes 1968; Chou, Savage and O'Dell 1969; Dutson 1976). Reduced cross-linking in collagen and elastin, weakens connective tissue, and increases collagen solubility.

No one appears to have tested the effects of copper deficiency on the mechanical properties of cooked beef. In the experiment described below the mechanical properties of cooked samples of muscle, with a relatively high collagen content, from young cattle with either a low, c. 8 mg of copper per kg of liver dry matter, or an adequate, c. 132 mg of copper per kg of liver dry matter, are compared.

MATERIALS AND METHODS

Animals

The 24 animals, steers and heifers, were born, and raised together, on a farm which had a history of copper deficiency. Two groups, LCu and ACu, each of twelve animals, were compared. The animals in the LCu group received a single subcutaneous injection of 400 mg of copper glycinate containing 120 mg of elemental copper (Glaxo Australia Ltd.) at birth. Animals in the ACu group received a total of 10 such injections at intervals of 4-6 weeks.

The animals were slaughtered at a commercial meatworks. The caudate lobe of the liver was removed

TABLE 1

MEAN (±S.E.) WARNER BRATZLER INITIAL YIELD (IY), PEAK FORCE (PF), AND PEAK FORCE MINUS INITIAL YIELD (PF-IY) VALUES (KG) OF SAMPLES OF M.PECTORALIS PROFUNDUS, FROM YOUNG CATTLE WITH LOW (LCu) OR ADEQUATE (ACu) LIVER COPPER CONCENTRATIONS

Group			Low Copper	Adequate Copper
Cooking Temp(°C)	Conditions Time(hr)	Parameter		
60	1	PF	4.24±0.24	4.27±0.22
60	1	IY	1.91±0.10	1.87±0.09
60	1	PF-IY	2.33±0.25	2.40±0.20
80	1	PF	4.47±0.19	4.62±0.14
80	1	IY	3.17±0.14	3.47±0.13
80	1	PF-IY	1.30±0.18	1.15±0.13
80	5	PF	2.87±0.14	2.86±0.12
80	5	IY	2.63±0.57	2.65±0.12
80	5	PF-IY	0.24±0.04	0.21±0.01

TABLE 2

MEAN (±S.E.) ADHESION MEASUREMENTS OF SAMPLES OF THE M.PECTORALIS PROFUNDUS OF YOUNG CATTLE WITH LOW (LCu) OR ADEQUATE (ACu) LIVER COPPER CONCENTRATIONS

Group			Low Copper	Adequate Copper
Cooking Temp(°C)	Condition Time(hr)	Parameter		
60	1	A	0.55±0.06	0.57±0.06
80	1	IY force	0.60±0.02	0.53±0.04
80	5	(kg)	0.61±0.02	0.49±0.04
60	1	B	0.53±0.08	0.51±0.04
80	1	IY distance	0.45±0.04	0.38±0.04
80	5	(cm)	0.39±0.08	0.28±0.04
60	1	C	1.15±0.08	1.17±0.07
80	1	Peak force	1.62±0.10	1.75±0.08
80	5	(kg)	1.35±0.10	1.32±0.07
60	1	D	1.44±0.1	1.44±0.1
80	1	Final yield	2.24±0.2	2.28±0.1
80	5	distance(cm)	2.12±0.2	2.24±0.2
60	1	E	146±14	140±10
80	1	Work done	323±43	344±36
80	5	(arbitrary units)	362±83	355±57

for the determination of dry matter and liver copper concentration (Bingley and Anderson 1972). Carcasses were chilled for 18 hr before they were boned. The brisket (= *M. pectoralis superficialis* and *M. pectoralis profundus* PP) was removed at this time, frozen, transported, to the Meat Research Laboratory, and stored. When required, the briskets were thawed at 5°C overnight and the PP dissected from the thawed briskets.

Three samples (approximately 150 g each) were cut from each PP muscle. They were assigned in a randomised manner to one of three cooking treatments. Samples were cooked in polyethylene bags totally immersed in water in a water bath either at 60°C for 1 hr, or 80°C for 1 hr or 80°C for 5 hr. After cooking, samples were cooled, dried with paper towels and stored, overnight at 1°C, before mechanical evaluations were performed.

Sub-samples were cut from each cooked sample for Warner-Bratzler shear and adhesion measurements. Initial yield (IY) and peak shear force (PF) values were determined from Warner-Bratzler force-deformation curves (Bouton and Harris 1972a).

Adhesion measurements were carried out as previously described (Bouton and Harris 1972a). The following parameters were quantified from the adhesion force-deformation curves (Bouton, Harris and Shorthose 1975):-

(A) initial yield force (kg), taken at the first major inflexion of the curve; (B) initial yield distance(cm), the distance from the first registration of force to the critical yield; (C) peak force(kg), the maximum force registered; (D) final yield distance (cm), the distance from the first registration of force to the distance at which the sample broke; (E) work done, was the area under the force-deformation curve.

RESULTS

Mean liver copper concentrations (p.p.m. of dry matter) were 7.8 0.1 and 131.5 8.1 for the LCu and ACu groups. Neither mean age at slaughter (298 v 297 days) nor mean carcass weight (183.3 v 185.1 kg) differed between the LCu and ACu groups, respectively.

The means and standard errors of the parameters determined from the Warner-Bratzler shear force-deformation curves are given in Table 1. Means and standard errors of parameters determined from adhesion force-deformation curves are given in Table 2.

There were no significant differences in any of the parameters measured between the two groups of animals. There were significant differences between the three cooking regimes. Warner Bratzler IY and PF values were significantly affected by the cooking regime. PF-IY values decreased significantly with the increase in cooking intensity from 60C for 1 hr to 80C for 5 hr.

Similarly, the cooking regime used influenced the values of the parameters determined from the force-deformation curves of adhesion measurements. Final yield distance and the work done both increased as cooking temperature was increased from 60°C to 80°C. Peak force values (C) were least in samples cooked for 1 hr at 60°C and greatest for samples cooked for 1 hr at 80°C. Although increased cooking decreased Warner Bratzler PF-IY values there were no differential effects of copper status on these decreases.

DISCUSSION

The *M.pectoralis profundus* was used in this experiment as it has a relatively high connective tissue content (Ramsbottom and Stradine 1948), is restrained from shortening postmortem, and has a sarcomere length of about 2.8 m (Bouton, Harris and Shorthose 1975).

PF-IY values and initial yield force values (A) and peak force values (C), in the adhesion measurements, are all considered to be indices of connective tissue strength in cooked meats; values for this parameter increase with animal age, are not affected when meat is aged, and are reduced with increases in cooking intensity (Bouton, Harris and Shorthose 1975).

If the copper deficiency in LCu animals was sufficient to reduce connective tissue strength then PF-IY values from Warner-Bratzler shear force measurements and A & C values would have been expected to have decreased.

Also the weakening effect of increasingly extreme cooking conditions would have been expected to have been greater on the values of the above parameters for samples from LCu animals.

It could be concluded either that copper deficiency has no effect on the contribution of intramuscular connective tissue to meat toughness or that the deficiency in the LCu group was not sufficiently severe to affect collagen metabolism.

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CHANGES IN THE STRUCTURAL PROTEINS AND THE SHEAR VALUES OF MUSCLE AS A RESULT OF PROLONGED HIGH PRESSURE TREATMENT

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SUMMARY

Beef semimembranosus muscle samples, in rigor either stretched or contracted, were heat treated at 30°C or 60°C for up to 24 h with and without the application of pressure (150 MPa). Treatment effects on Warner-Bratzler shear force values of samples, their SDS gel electrophoresis patterns, and their appearances under the scanning (SEM) and transmission (TEM) electron microscopes, were examined.

Compared to heat treatment alone, 30°C heat plus pressure treatment of contracted samples resulted in a steady moderate decrease in shear values as treatment time increased. At 60°C with pressure for 4 or 24 h a large decrease to small shear values occurred. A presumed pressure-accelerated enzymatic breakdown of myosin was inferred from an examination of SDS gel patterns. Myosin breakdown also appeared likely from the disruption of thick filaments seen under the TEM. However, from a consideration of the available evidence, it is thought that the presumed enzymatic mechanism may not be the sole mechanism involved in pressure-heat tenderisation.

Evidence of connective tissue disruption seen in the scanning electron micrographs of stretched muscle samples treated at 60°C for 24 h is consistent with a decrease in the shear values of these samples.

Because pressure treatment at 60°C rapidly destroys the influence of the myofibrillar component on toughness, but reduces that of the connective tissues relatively very slowly, if at all, the treatment can be used to estimate the influence of each of these components on the toughness of meat.

INTRODUCTION

At this Laboratory the effects of high pressure treatment on muscle have been studied to explore the possible technological usefulness of such treatments, as well as to increase the understanding of muscle when used as a food (Bouton et al. 1977; Macfarlane 1973; 1974; 1985; Macfarlane and McKenzie 1976). In connection with the latter application, a pressure-heat treatment has been developed which has been interpreted as destroying the influence of the myofibrillar component on toughness but having little or no effect on that of the connective tissues (Bouton et al. 1977). Such a treatment can be used to estimate the contribution of the myofibrillar proteins and the connective tissues to cooked meat toughness.

To further explore the mechanisms involved in pressure-heat treatment, meat has been pressure treated

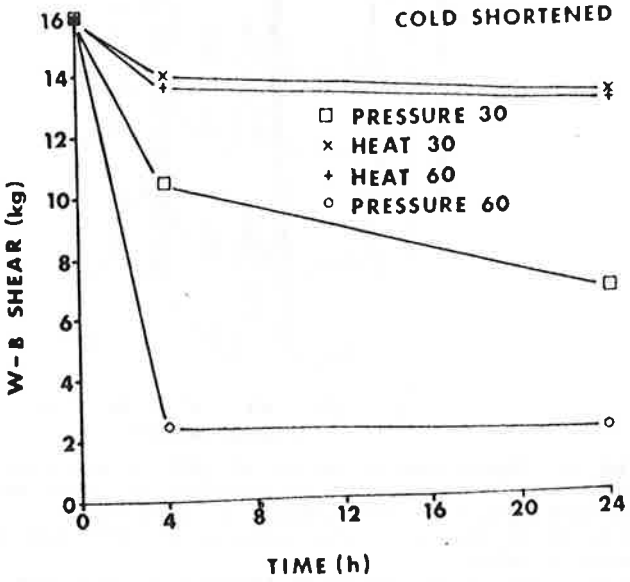

Fig 1: Warner-Bratzler shear force values for samples of contracted (cold shortened) muscle that were cooked at 80°C for 1 h after application of the treatment as indicated for the times shown.
Least significant difference (P = 0.05): 4.0 kg.

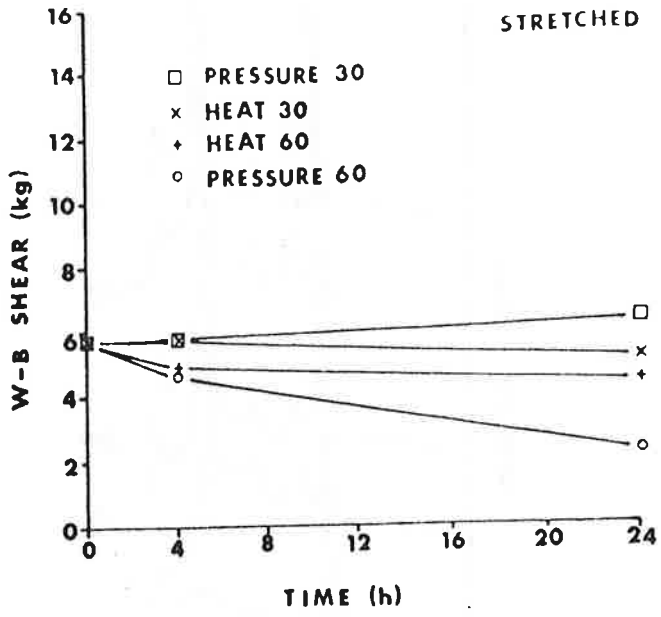

Fig 2: Warner-Bratzler shear force values for samples of stretched muscle that were cooked at 80°C for 1 h after application of the treatments as indicated for the times shown.
Least significant difference (P = 0.05): 2.4 kg.

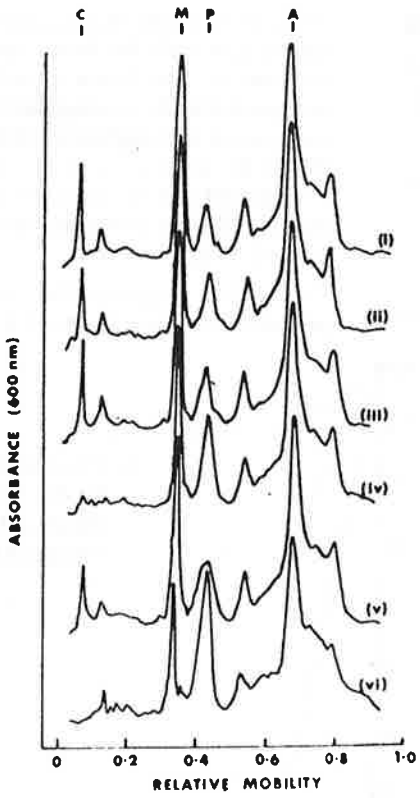

Fig 3: Densitometer scans of SDS gels (2.6% acrylamide) obtained from extracts of samples of beef semitendinosus muscles that had been treated as follows:
(i), (iii) and (v): heat treated at 30°C for 1, 4 or 24 h respectively.
(ii), (iv), (vi): pressure treated at 30°C for 1, 4 or 24 h respectively.
Peak identification: A-actin; C-connectin; M-myosin; P-145000 MW component(s).

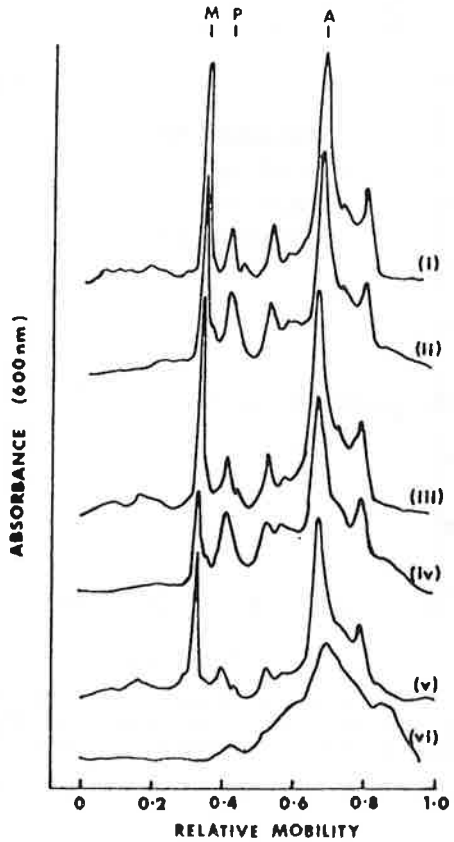

Fig 4: As for Fig 3, except treatment temperature was 60°C.

at 30° and 60°C and the effects on Warner-Bratzler peak shear values, SDS gel electrophoresis patterns, and ultrastructure investigated.

EXPERIMENTAL METHODS

From one side of each of three beef carcasses the semimembranosus muscle was excised within one hour of slaughter, placed in a polyethylene bag and immersed in iced water for two days to allow it to cold shorten and enter rigor. The other side of each of the carcasses was aitchbone hung ('tenderstretched' - to maintain the semimembranosus muscle in a stretched condition during onset of rigor), and placed in a chiller. The semimembranosus muscle of each of these sides was boned out approximately three days post-slaughter. The shortened and the stretched muscles were then cut into samples approximately 7 cm (in the direction of the fibres) x 7 x 4 cm which were individually sealed in polyethylene bags. The samples were stored frozen at -20°C until required for treatment, when they were placed in a chiller at +2°C for 24 h to thaw.

Pressure treatment was at 150 MPa and was carried out as described by Macfarlane and McKenzie (1986) except the pressure vessel had a chamber 11.4 cm in diameter and 35.6 cm in length.

SDS gel electrophoresis and Warner-Bratzler shear measurements were carried out as described by King et al. (1981) and Bouton et al. (1975) respectively.

Analysis of variance was used to test for significance between treatment effects.

Transmission electron microscopy and scanning electron microscopy of samples taken directly after heat or heat-under-pressure treatments, i.e. without subsequent cooking, followed the procedures described by Rowe (1978, 1984).

RESULTS AND DISCUSSION

Warner-Bratzler shear values

W.B. peak shear values for contracted muscle samples are shown in Fig 1, and for stretched muscle in Fig 2. Contracted muscle treated at 30°C produced results consistent with those reported by Macfarlane and McKenzie (1986). Thus shear values for the heat only treated samples changed little even after treatment for 24 h, but those for pressure-treated samples showed a significant decrease. However, this decrease was not as great as that achieved

by pressure treatment at 60°C, when it can be seen that both 4 and 24 h treatments produced low shear values. Shear values for the non-pressure-treated 60°C samples were similar to those for the 30°C samples.

Untreated samples of stretched muscle (Fig 2) gave much lower shear values than did the corresponding contracted samples, reflecting the low influence of the myofibrillar proteins on the toughness of the stretched samples. Compared to the shear values for the untreated samples, only those for the 60°C, 24 h pressure treatments were significantly decreased. It appeared likely that this decrease was in the connective tissue component of toughness as the myofibrillar influence in stretched muscle is already low.

SDS gel electrophoresis

No pronounced differences were apparent between the SDS gel electrophoresis patterns from the contracted and the stretched muscle samples, and only the results obtained from stretched muscle are illustrated here.

For samples treated at 30°C, it can be seen (Fig 3) that with increase in duration of treatment, in the pressurised samples the peak for myosin decreased while that at approximately M.W. 145000 (peak P) increased, so that by 24 h, the latter peak was the most pronounced. Therefore it appears that pressure treatment results in myosin breakdown giving a fragment(s) of approximately 145000 M.W. In the 24 h pressure-treated samples, compared to the non-pressure-treated samples there appeared to be an increase in the components giving rise to the hump on the low molecular weight side of the actin peak. This hump possibly was due to other fragments of myosin associated with the presumed cleavage that gives rise to the fragment(s) of peak P. It can also be seen that pressure treatment accelerated the breakdown of connectin (peak C).

Pressure treatment at 60°C for 1 or 4 h appeared to result in an increase in the size of peak P. However at 4 h there was a large decrease in the size of the peak for myosin, and in addition to the breakdown of myosin, this reduction might be due to heat treatment reducing its dye-binding ability. That the dye-binding ability of myosin is reduced by heat treatment at 60°C appears likely from consideration of the results for heat treatment at 24 h. Thus from Fig 4 it is evident that the size of the peak for myosin was greatly reduced but from Fig 1 it can be seen that there was little reduction in the high shear values for the samples of contracted muscle. From the latter observation it is inferred that the myosin molecules of thick filaments were largely intact, a view substantiated by the TEM appearance of myosin. However staining of myosin appears to be little affected by heat and pressure-heat treatments for 1 h (Fig 4). The latter pressure-heat treatment virtually eliminates the myofibrillar influence on toughness, but does not result in as great an apparent yield of peak P as is achieved in 30°C pressure treatments that have much smaller effects on toughness. Therefore it appears likely that another mechanism is involved in pressure-tenderisation.

Ultrastructure

Structural effects brought about by heat/pressure treatments can be categorised either as connective tissue or myofibrillar effects. There is a somewhat surprising difference in the effect of pressure treatment at 60°C for 24 h between the stretched and the contracted samples. The perimysial collagen component is almost totally removed (Fig 5A). This is consistent with the finding reported above that 60°C 24 h pressure treatment reduced the shear values of stretched samples. All the other treatments examined in this experiment produced no obvious dramatic degradation of the perimysial connective tissue, see, eg. Figs 5 B, C and D.

In stretched samples pressure treatment at 30°C even for 1 h produced changes in the A band of the sarcomeres such that they are apparently more fragile. This fragility apparently results from a weakening in the region of the pseudo-H zone. Fig 6A shows the A bands of a 30°C 1 h pressure treated sample with breaks at the pseudo-H zone (double arrows). These fractured A bands are not seen in the heat treated only samples. This weakening of the A band in the pseudo-H zone appears to be linked with a loss of M-line material from this region, eg. Fig 6B shows a 30°C, 24 h pressure treated sample. However the myosin rod portions of the thick filaments are still *in situ* and can be seen spanning the pseudo-H zone.

At 60°C pressure treatment appears to be structurally more destructive, even after treatment for only 1 h. Figs 6 C and D show the 1 h and 4 h 60°C pressure treatment samples. There is evidence of extensive A-band fragility, apparently to a greater extent than in the samples pressure treated at 30°C. Double arrows, Figs 6 C and D, indicate breaks at the pseudo-H zone. Just about every A band is involved. In addition the actin filaments of the I band appear less substantial. TEM confirms this greater, pseudo-H zone fragility (Fig 6E). There appears to be no myosin filaments crossing the normal region for the pseudo-H zone, leaving this region apparently cleared. The remainder of the myosin filaments appear fragmented.

In contracted samples the pressure-heat treatments have less obvious structural effects. The extensive overlap of actin and myosin filaments in shortened sarcomeres mask some of the features which would otherwise be visible, particularly in SEM micrographs. Heat treatment without pressure at either 60°C or 30°C has very little visible effect on the myofibrils (eg. Fig 7A). With pressure, however, as in the stretched samples the thick filaments of the A bands are progressively weakened. The fragility, as interpreted by an increased tendency to break at the pseudo-H zone, becomes progressively more common with longer time and higher temperature, eg. Figs 7 B, C and D.

In samples subjected to the 30°C 24 h pressure treatment the rod portions of myosin molecules remain in the pseudo H zone (Fig 6B). However pressure treatment at 60°C for 1 h clears this region (Fig 6C). If this clearance indicated advanced enzymatic action that resulted in the pressure-tenderising effect and that also produced peak P, then a larger peak would be expected from the 60° than the 30°C treatment. That this expectation is not the case is a further indication that the enzymic action responsible for the production of peak P is not primarily responsible for pressure tenderisation at 60°C.

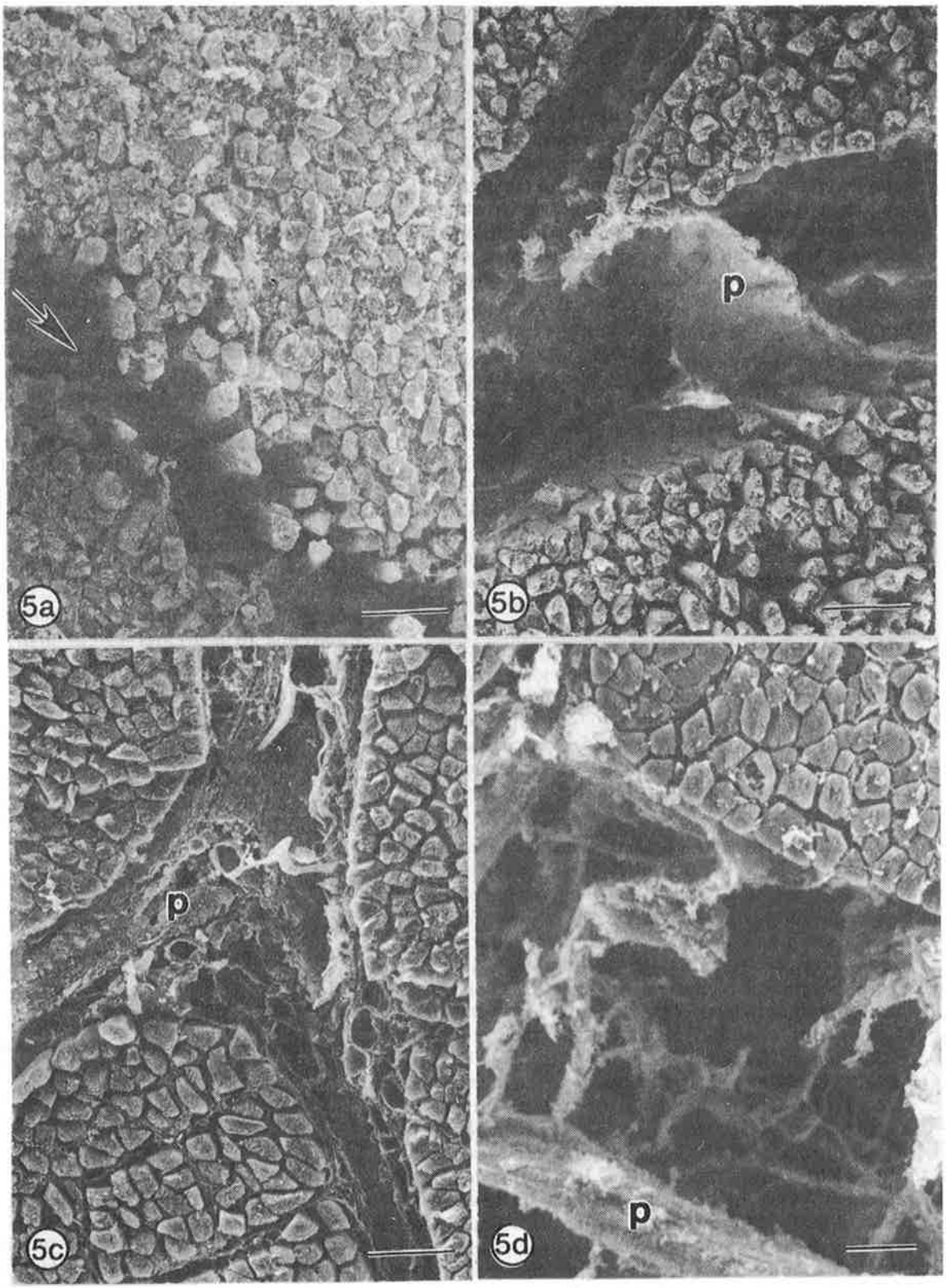

Fig 5: SEM micrographs of transversely cut bovine semimembranosus muscle. A, stretched sample, pressure, 60°C for 24 h. Arrow indicates region between two muscle fibre bundles where perimysial tissue would be expected. B, stretched sample, 60°C for 24 h. C, stretched sample, pressure, 30°C for 24 h. D, cold shortened sample, pressure, 60°C for 24 h. P = perimysium, bars = 100 μm.

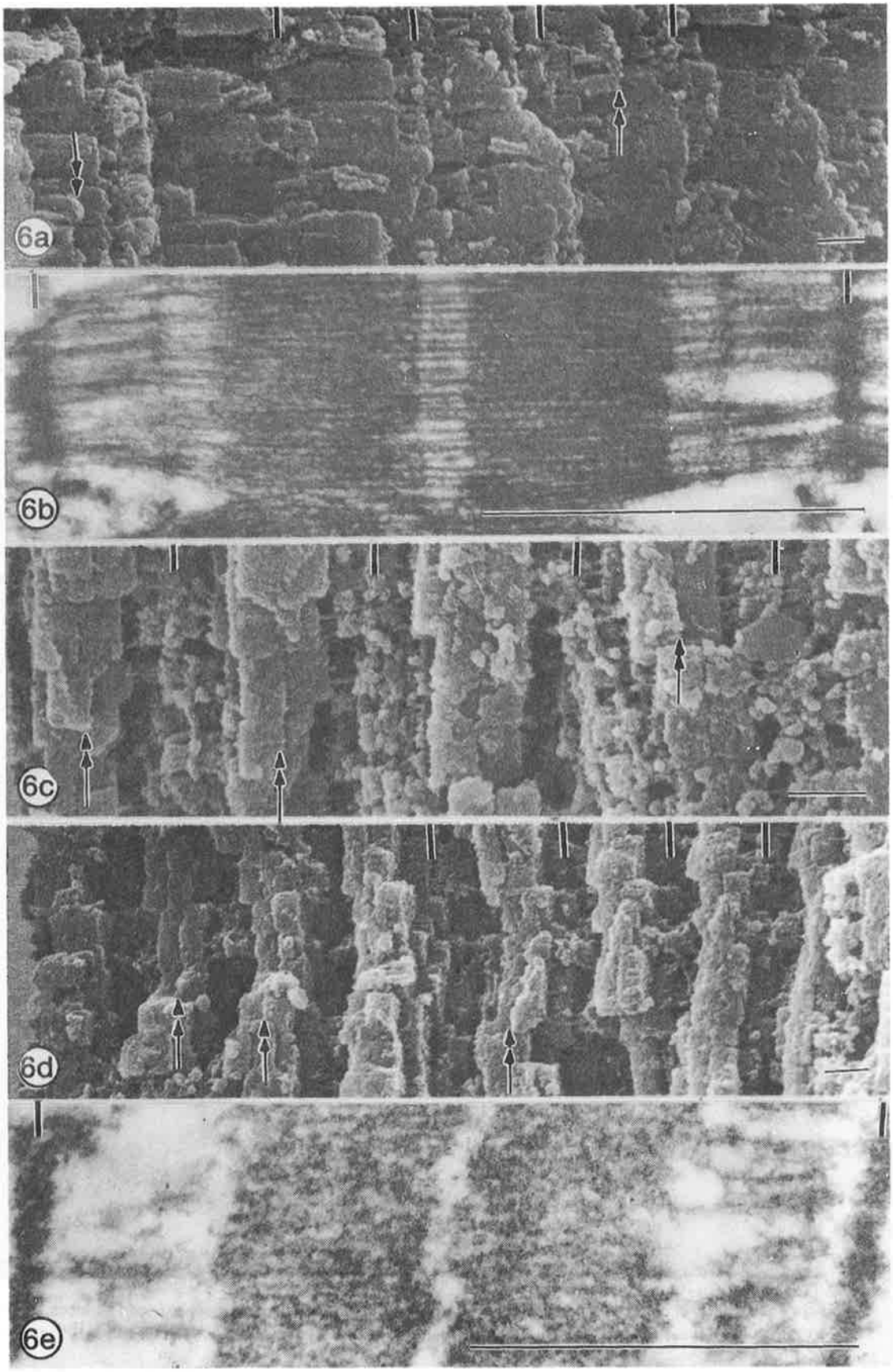

Fig 6: SEM and TEM views of longitudinally exposed myofibrils of stretched bovine semimembranosus muscle. A, SEM sample, pressure, 30°C for 1 h. B, TEM sample, pressure, 30°C for 24 h. C, SEM, pressure, 60°C for 1 h. D, SEM pressure, 60°C for 4 h. E, TEM, pressure, 60°C for 24 h. Vertical bars at tops of micrographs indicate the sarcomere repeat patterns. Double arrows indicate regions of breaks in pseudo - H zone of A bands. Bars = 1 μm.

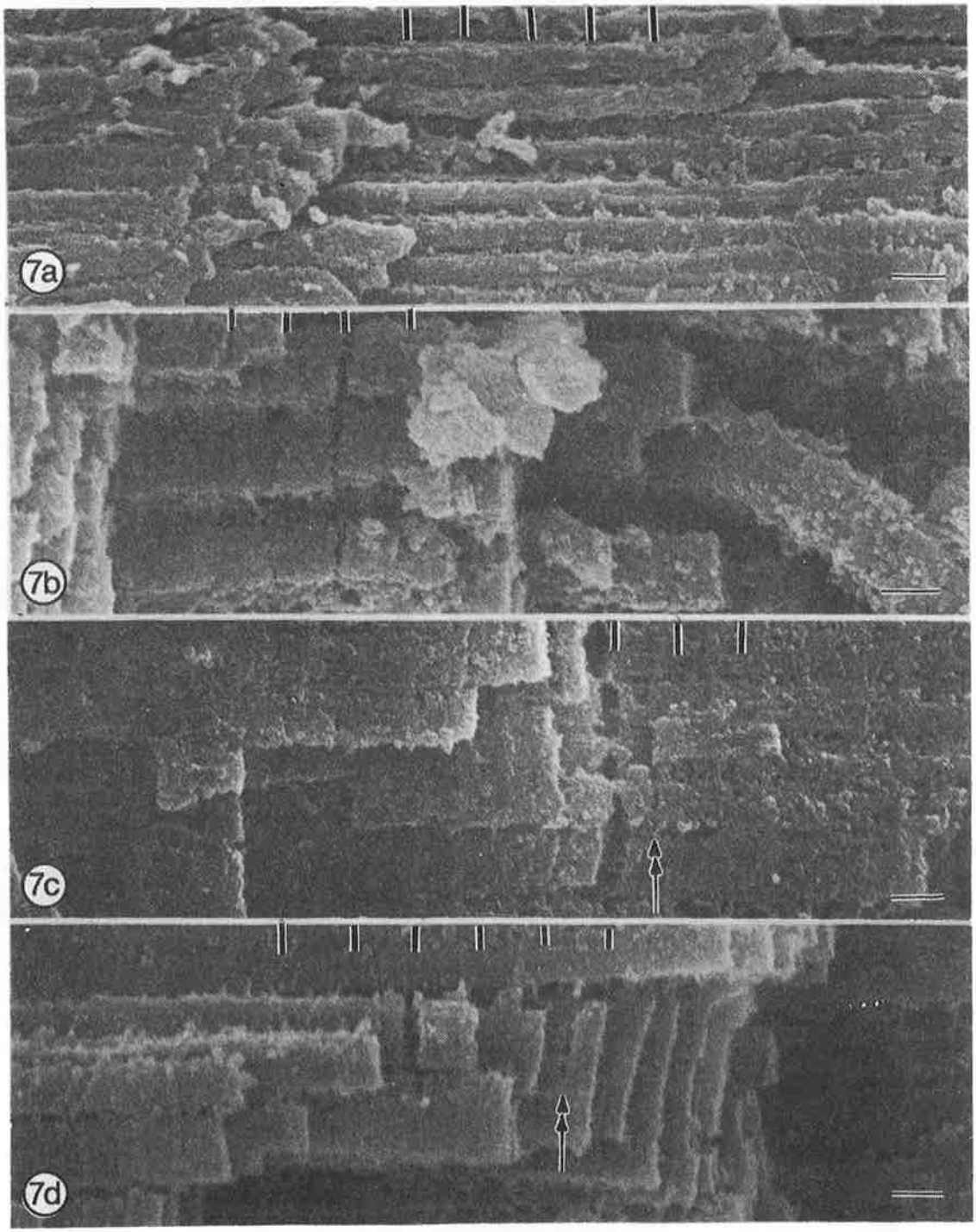

Fig 7: SEM views of cold shortened samples showing the myofibrils in longitudinally exposed surfaces. A, pressure, 60°C for 1 h. B, pressure, 60°C for 4 h. C, pressure, 60°C for 24 h. Vertical bars and arrows as Fig 6. Bar = 1 μm.

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THE PRACTICAL SIGNIFICANCE OF HIGH TEMPERATURE CONDITIONING FOR THE TENDERNESS OF HOT BONED BEEF AND VEAL

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SUMMARY

The effect of high temperature conditioning of shear force and sarcomere length of electrically stimulated, hot boned beef and veal longissimus muscle was investigated. There was no difference between conditioned and non-conditioned longissimus primals. As compared with electrically stimulated, cold boned beef, hot boned beef loins were similar in tenderness. It is questionable whether time of boning of stimulated veal carcasses affects tenderness. Our results may have been influenced by the occurrence of cold-toughening in cold boned sides that were used as controls. Further experiments should include whole carcasses as controls.

INTRODUCTION

Since it was shown that blast-chilling systems for the refrigeration of lamb, beef and veal carcasses might be associated with cold shortening, several processing techniques have been advocated to prevent or minimize this phenomenon. Both electrical stimulation and high temperature conditioning have been advocated as effective means to counteract cold-induced toughening.

Electrical stimulation, through accelerating the onset of rigor mortis, prevents cold shortening (Carse 1973; Davey et al. 1976) and it appears that, in addition, a so-called 'extra-tenderising effect' may exist (Vanderkerckhove and Demeijer 1978; Smulders et al 1986) brought about by other mechanisms (Dutson et al. 1980). Hence electrical stimulation has been introduced successfully in accelerated processing systems where beef and veal are hot boned within the first few hours post mortem. It was reported that it promises similar tenderness scores for hot boned - as for (non-stimulated) cold boned meat (Smulders et al. 1988). Model-experiments with small cuts further suggest that under conditions of extremely rapid chilling, electrical stimulation makes hot boned beef as tender as cold boned (Smulders et al. 1981). It was shown that, in addition to electrical stimulation, high temperature conditioning may further reduce the shear forces of small hot boned cuts (Smulders et al. 1984).

It remains unclear whether this effect is still observable when larger primals are (delay-) chilled under practical conditions.

Purpose of the present study was, therefore, to investigate the practical significance of high temperature conditioning for stimulated, hot boned, beef and veal primals by comparing their tenderness of the latter with that of stimulated cold boned counterparts.

MATERIAL AND METHODS

Three experiments were conducted, involving beef (experiment I) and veal (experiments II and III).

Experiment I: Eight cows of the Meuse Rhine IJssel (MRIJ) breed were stimulated electrically within 5 min post mortem (85V, 14Hz, 30s). Within 1 h post mortem the entire righthand longissimus muscle was hot boned. Immediately after excision the muscles were divided in two, and vacuum-packaged. One part was held at 15°C for 5 h (conditioning) and subsequently stored at 1 ± 1°C. The other part was stored at 1 ± 1°C immediately after boning. Using thermocouples the internal temperature of longissimus muscle of both treatment groups was monitored until 15 h post mortem. Immediately after grading (approximately 30 min post mortem) the lefthand carcass-sides were subjected to blast-chilling for 1 h and subsequently stored overnight at 1 ± 1°C. At approximately 24 h post mortem the lefthand side longissimus muscles were cold boned, divided in two parts, vacuum-packaged and stored at 1°C. After twelve days of storage, meat was unpacked and sampled for assessment of sarcomere length and shear force.

Experiment II involved 8 Dutch Friesian calves of approximately 22 weeks old. Experimental procedure was essentially the same as in experiment I, except for the applied electrical stimulation for which a high voltage;

Table 1 The effect of a 5 h/15°C conditioning period on the shear force (1A) and sarcomere length (1B) of hot boned beef and veal loins as compared with cold boned controls (n = 8 unless indicated (V) where n = 16)

1A: Shear force (N cm^{-2})

	Hot boned			Cold boned
	Conditioning	No conditioning	Pooled	
beef exp. I	4.59	4.52	4.56 (V)	4.85 (V)
veal exp. II	2.55^{a*}	2.53^{a}	2.54^{a} (V)	3.66^{b} (V)
veal exp. III	3.14^{ab}	3.45^{a}	3.29^{b} (V)	2.68^{a} (V)

1B: Sarcomere length (µm)

	Hot boned			Cold boned
	Conditioning	No conditioning	Pooled	
beef exp. I	1.88	1.72	1.80	1.66
veal exp. II	nd**	nd	nd	nd
veal exp. III	1.82^{ab}	1.77^{b}	1.80^{b} (V)	1.93^{a} (V)

* Figures with different superscript are different ($p < .05$)

** nd = not determined

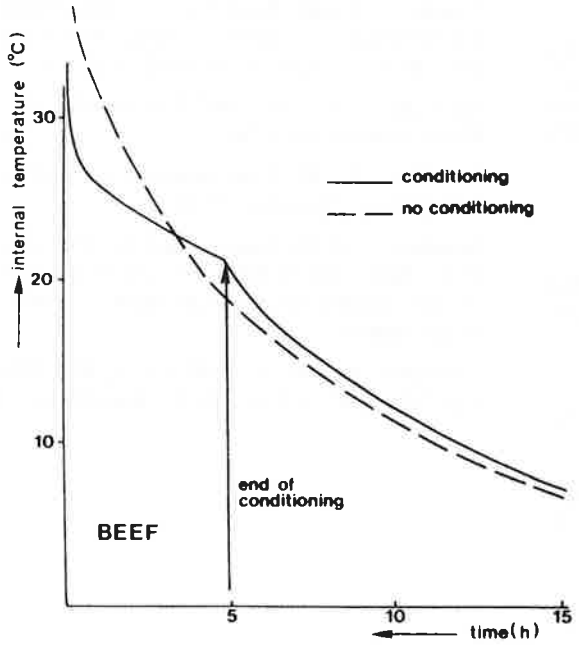

Figure 1. The effect of a 5 h/15°C conditioning period on the temperature fall of hot boned beef loin.

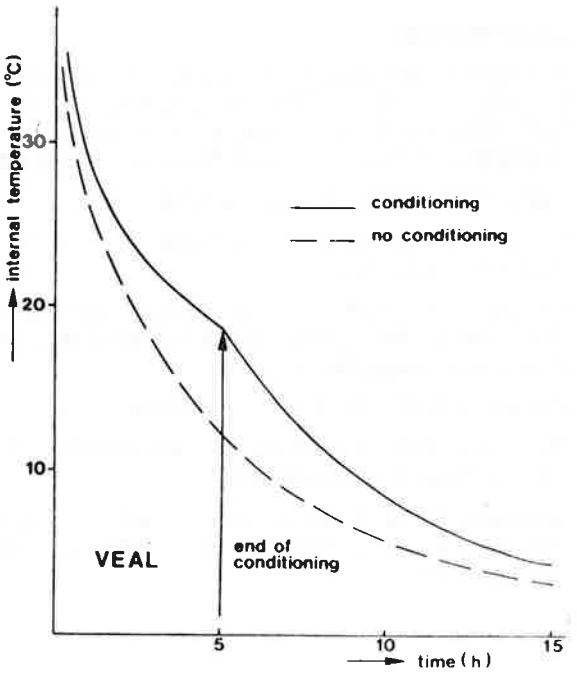

Figure 2. The effect of 5 h/15°C conditioning period on the temperature fall of hot boned veal loin.

low voltage system (3000V, 0.83Hz, 1.5ms pulsewidth/35V, 14Hz) was applied (Smulders and Eikelenboom 1985).

Experiment III: Sixteen Dutch Friesian calves of 22 weeks old were electrically stimulated as in experiment II. Eight calves were hot boned and 8 calves were cold boned. Half of the hot boned longissimus muscles was subjected to chilling after 5 h/15°C conditioning, the remaining muscles were chilled immediately at 1 ± 1°C.

Sarcomere lengths measurements were performed according to the procedure described by Koolmees et al. (1986).

Shear force was assessed on samples of approximately 3 cm thick. Samples were heated in polyethylene bags in a water bath for 50 min at 75°C whereafter they were chilled in running tap water for 40 min (Boccard et al. 1981). Using a mechanically driven borer, cylinders of 1 cm^2, excised from the cooked samples, where subjected to Instron-Warner Bratzler shear force measurements.

Significance of differences were assessed by Student t-tests (paired, where appropriate).

RESULTS AND DISCUSSIONS

Table 1A and 1B illustrate that both in hot boned beef and veal, conditioning has no significant effect on either sarcomere length or shear force. These results fail to support earlier observations which indicated a significant effect of conditioning on sarcomere length and shear force of small beef cuts (Smulders et al. 1987). Clearly, chops rapidly respond to external temperature whereas the primals used in the present experiment exhibit a comparatively slow internal temperature fall (Figures 1 and 2). Even with immediate chilling it takes 3-4 h to achieve internal temperatures of 15°C.

The results of experiments I and II show that there are no differences between shear forces of hot and cold boned meat. In experiment III shear forces of cold boned veal were significantly lower than those of hot boned veal. This is probably due to the fast chilling of carcass-sides as opposed to whole carcasses where cooling efficiency of longissimus muscles is much lower. Thus, it cannot be excluded that cold shortening and/or cold toughening (a change in tenderness without concomitant sarcomere length changes; Dutson 1983) has occurred in cold boned sides. Such a phenomenon would mask the effects of stimulation and conditioning. As it is common practice in the veal industry to chill whole carcasses these should constitute the controls in future experiments. However, between-carcass differences, which may be considerable, may have greatly influenced results. More research is necessary to establish if tenderness of veal is adversely affected by hot boning.

CONCLUSIONS

It appears that in hot boning beef, there is little need for delayed chilling. Electrically stimulated, hot boned beef loins are similar in tenderness as electrically stimulated cold boned counterparts.

It is questionable whether time of boning of stimulated veal carcasses affects tenderness.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mr. B. van de Haar for technical assistance. Thanks are also due to Erkro B.V. at Apeldoorn, Wolff Vlees B.V. at Twello, Aklbert Heijn at Zaandam and The Netherlands Commodity Board for Livestock and Meat at Rijswijk for supporting this study.

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THE DEVELOPMENT OF A TECHNOLOGY OF BONELESS READY-TO-COOK MEATS FROM HOT BEEF

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The aim of the present study was to develop a cost-effective industrial technology of natural ready-to-cook meats from hot beef.

As the object of the study served 400-420 kg beef carcasses of 13-month-old animals (raised at a commercial feeding complex), as well as M.longissimus dorsi dissected from these carcasses.

The expediency of hot carcass cutting and vertical deboning using the installation Я4-ФАФ has been experimentally proved.

The advantages of ready-to-cook meats chilling at -1 to +1°C just after cutting and packing within 2-2.5 hr post mortem are shown.

On the basis of physico-chemical, microstructural and organoleptical indices cold-storage time up to 15 days at -1 to +1°C and up to 7 days at 4 to 6°C was recommended.

Technological process for natural boneless read-to-cook meats from hot beef has been suggested and tested under commercial conditions.

One of the ways of eliminating the problem of reducing meat shrinkage during chilling, storage and transportation is to process hot meat.

The aim of the present study is to utilise hot beef for boneless ready-to-cook meats production and the development of a cost-effective technology on this basis.

The research was conducted on the 400-420 kg beef carcasses of 13-month-old animals, raised at a commercial feeding complex.

Sides were hot-boned vertically in an installation Я4-ФАФ in compliance with the approved technological specification.

Tests were made on the longissimus dorsi muscles.

Test samples (versions 1, 2 3; see below) were dissected from hot muscles, controls - from muscles, chilled traditionally.

L.dorsi was divided into 200-600 g pieces and were used for tests.

Muscle sampling followed a strict succession, starting from the blade of the side.

At predetermined intervals the samples having the same number were selected from each test version. They were weighed to the nearest 1 g, vacuum-packed using "Multivac", chilled for 2-2.25 hrs down to the internal meat temperature at least +4°C and stored at -1 to +1°C for 10 days.

Version 1: - hot beef samples were weighed, vacuum-packed, immediately chilled and stored; version 2 - hot beef samples were weighted, vacuum-packed, aged at 22-26°C for 6 hours, chilled and stored; version 3 - a hot side was aged at 22-26°C for 6 hours, l.dorsi was excised, cut into samples, which were weighed, vacuum-packed, chilled and stored; version 4 (control) - a side was chilled in a 0 to 4°C cooler for 24 hours. Then the longissimus dorsi muscle was excised, cut into samples, which were weighed, vacuum-packed and stored.

A test with preliminary ageing of hot beef before chilling was necessary to determine ageing effect on the qualities of packed ready-to-cook meats.

pH, waterbinding capacity, drip loss, shear force were measured; samples were studied microbiologically, histologically and organoleptically.

The regularity of sampling was as follows: the initial hot meat; samples after 1, 3, 5, 7 and 10 days of storage at -1 to 1°C.

The results of a comparative study of the selected test versions showed that waterholding capacity (WHC) in the first version is significantly higher than in the third one ($tp>t_{Table} = 2.09$, $\alpha = 0.05$, f=19).

The orientation of changes in WHC is similar for all the test and control samples both during the first and the following days of storage.

Changes in drip loss during storage are shown in Figure 2. It was established that the least drip loss was observed on the 3rd, 5th, 7th and 10th day of storage in case of test samples of version 1 and that it was 1.5-2 times as lower as compared to the controls (version 4) and to the test samples (version 2 and 3).

Drip loss in version 1 is significantly lower than that of other versions ($tp>_{Table} =3.18$, $\alpha = 0.05$, f=3).

The data on the pH change during packed meat storage indicated that most intensively pH falls in all the samples during the first day of storage (6.25-5.8). Within the following two days pH continues to drop slightly, on the fifth day it becomes somewhat higher and then remains at the same level (5.8).

The pH in the first version is significantly higher than those in versions 2, 3, 4 ($tp>t_{Table}=2.77$; $\alpha =0.05$; f=4). The data obtained coincide with the earlier results which indicated that the muscle protein condition of packed meat changed against the background of a somewhat retarded alterations of hydrogen ions concentration. Obviously, under low partial oxygen pressure some special environment in the package influences the protein system of chilled meat due to predominant growth of lactobacilli.

Organoleptical scores (9-point scale) of cooked meat samples (variants 1, 2, 3) after 10-day storage are practically the same (6.52; 6.57; 6.55), being slightly higher (7.17) in case of controls (version 4).

The difference in organoleptical scores of cooked meat is statistically insignificant ($tp<t_{Table}=3.18$; $\alpha =0.05$; f=3).

Organoleptically, the broth prepared from the sample of version 2 is evaluated significantly higher (7.16) than that of

version 3 (6.30); samples of version 1 and 4 do not differ from each other ($tp<t_{Table}=4.3$; $\alpha=0.05$; f=2).

The analysis of the shear force data showed that after 10-day storage there was no significant difference between cooked meat of versions 1, 2 and 3, 4 ($tp<t_{Table}=3.18$; $\alpha=0.05$; f=3).

It is necessary to note, that the shear force of the samples of versions 1 and 2 is relatively higher than that of versions 3 and 4 (Table).

Version	x	s	v	m
1	2.81	1.11	39.50	0.55
2	2.11	0.91	43.51	0.45
3	1.51	0.72	47.95	0.36
4	1.19	0.28	23.75	0.14

Microbiological tests showed the absence of microorganisms in hot beef and low bacterial contamination in packed meat after 10 days of storage ($2.8 \cdot 10^2$ up to $4.5 \cdot 10^2$cells per cm_2).

As for the total bacterial count, there was no pronounced difference between meat samples of versions 1, 2, 3, 4; they were microbiologically safe.

On the basis of histological examination it was established that ageing of vacuum-packed meat after 3-5-day cold storage proceeded at different rates: more intensively in meat pre-aged in sides before cutting and packing at plus temperatures (sample of version 3), less intensively in controls (cutting and packing of chilled meat - sample 4) and even more slowly in hot packed meat (sample of versions 1). These differences are levelled by the 7th day of storage.

Out of all versions studied, version 1 is preferred, which means hot meat packing followed by immediate chilling and storage.

To establish optimum storage time of beef boneless ready-to-cook products prepared from hot meat, temperature conditions and storage time were investigated.

Storage and sale periods of boneless vacuum-packed meat were determined.

On the basis of physico-chemical, microstructural and organoleptical results cold storage life was determined to constitute up to 15 days at -1 to 1°C and 7 days at 4 to 6°C.

The performed complex research made it possible to substantiate a new technology of boneless ready-to-cook products from hot beef, this technology reducing losses, improving their quality and cold storage life.

Table

Version	$\bar{x}$	s	v	m
I	2.8I	I.II	39.50	0.55
2	2.II	0.9I	43.5I	0.45
3	I.5I	0.72	47.95	0.36
4	I.I9	0.28	23.75	0.I4

Microbiological tests showed the absence of microorganisms in hot beef and low bacterial contamination in

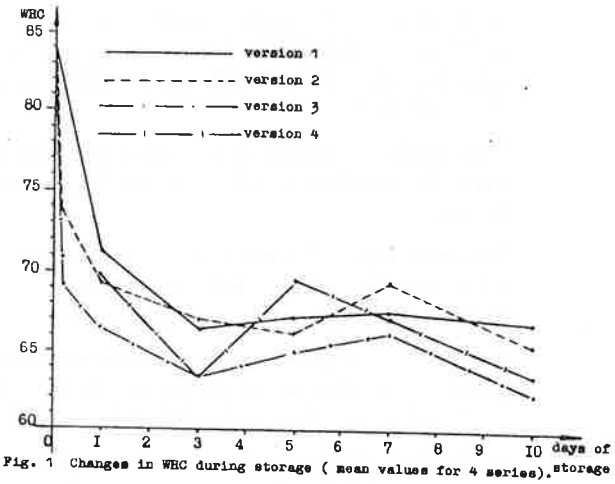

Fig. 1 Changes in WHC during storage (mean values for 4 series).

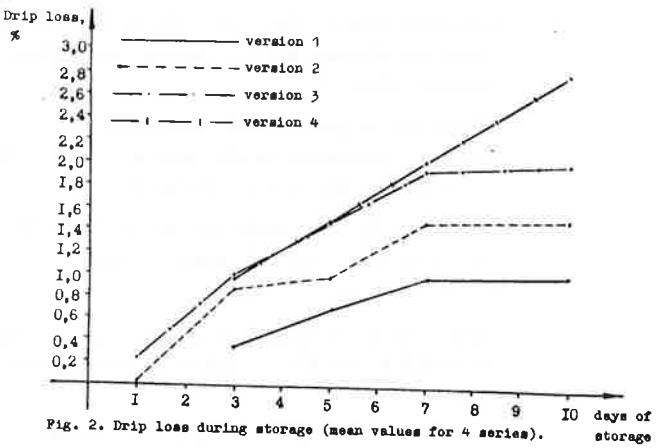

Fig. 2. Drip loss during storage (mean values for 4 series).

AUTOLYTICAL CHANGES OF HOT VACUUM-PACKED MEAT DURING CHILLING AND STORAGE

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The structuro- mechanical characteristics, changes in the ATP content, pH-value and muscle tissue microstructure as related to the chilling rate of hot-cut and packed boneless meat have been studied.

The dependance of the indices under study characterising the autolytic process has been experimentally proved.

The effect of ATP content and pH on the elastic deformation during the longitudinal stretching of muscle fibres in the process of vacuum-packed hot meat chilling has been determined.

With a similar pattern of autolysis, some specificity of this process during chilling and cold-storage of hot-cut and packed meat has been established.

Scientific papers in this and foreign countries have been devoted to the study of processes, characterising the quality of hot-cut packed meat. However, at present no optimum chilling conditions for hot boneless meat, as well as no complex research of autolytic process are available; the published data on the changes of some quality characteristics of packed hot meat are not sufficient to serve the basis of new technological process.

Survey and analysis of the available scientific literature have determined the direction of our experiments, viz., a study into the autolysis of hot vacuum-packed meat during chilling and cold storage.

The object of the study was meat from 18-month-old beef animals of the high finish belonging to the Red Steppe breed. Experiments were conducted on the M. longissimus dorsi, M. triceps scapuli and coxofemoral group of muscles. In every test both sides of the carcass were used; the left side serving as control, the right as a test one. Before cutting control sides were chilled with a one-step method according to the conventional conditions (at -1 up to 2°C, for 24 hours).

48 hours post mortem control sides were separated into cuts, the muscles were selected, portioned and packed. Muscles from test sides were dissected 1 hour post mortem, then they were portioned and packed similarly to controls.

Packing into thermoformable polyethylene-polyamide film was performed under vacuum (the pressure inside the package 13·2 kPa).

3 procedures of test samples chilling at -1 to 2°C down to the centre temperature of the average 4°C were tested: at the mean rate of average-volume temperature fall (Vav) 2·5°C/h; at V av = 1·0°C/h with packed samples pre-ageing time at 16°C for 12-14 hours. Pre-ageing and temperature were chosen on the basis of the analysis of temperature curves derived from a full bifactorial experiment (8, 16, 24 hours; 10, 12 to 14°C) with account for pH at meat temperature of 10°C (Honikel et al., 1983).

To judge on the intensity and pattern of fibre contraction in boneless hot-packed meat at the initial stage of autolysis in relation to the chilling rate, semitendinosus was studied, 1, 3, 6, 9,12, 15, 24, 48 hours post mortem by such characteristics as microstructure, pH, ATP content, the value of the plastic and elastic meat deformation of meat due to the load along muscle fibres according to a modified Locker method (Locker and Wild, 1982). The autolytical process and microbial growth during 28-day cold storage (at -1 to 2°C) of test samples were evaluated by the chosen characteristics as compared to controls.

A complex research of the processing characteristics of meat at the initial stage of autolysis made it possible to establish some specificity of rigor mortis dynamics and resolution as effected by the rate of average-volume temperature fall in packed hot meat. It was found that the test samples chilled at the mean rate of average-volume temperature fall of 2·5°C/hr are characterised with a prolonged rigor-mortis process, which deeply affects physico-chemical and biochemical changes in the tissue, and that the development of processes accompanying rigor mortis resolution is retarded.

At 3-6 hours post-mortem muscle fibres are greatly contracted, this lowering the elastic tensile deformation of samples. However, contrary to the post mortem contractions, the samples may be stretched again by applying more force. In this case the loss of elasticity by the samples typical of the rigor mortis is regained at 9-12 hours post mortem when ATP content constitutes 30% of the initial one (Shishkina and Kanevskaya, 1986). At 48 hours post mortem the muscle tissue of these samples is still in the rigor state, which is indicated by low waterbinding capacity and plasticity (Shishkina and Kanevskaya, 1985), as well as the impossibility of recording the plastic deformation of samples due to their rapid destruction under high loads.

Within this period the elastic deformation of samples during stretching is not increased yet which would be indicative of the rigor development in meat (see Figure). The table shows the strain causing plastic deformation of vacuum-packed meat samples, $6^{x}\ 10^{3}$N per m^{2} of sample conventional sectioned area.

Some retarding observed in the changes of physico-chemical and biochemical characteristics of such meat may be explained with a more intensive cold-shortening within the range of 15-10°C, the negative effects of which are eliminated in the process of further meat ageing in the package at -1 to 2°C. This is proved with statistically insignificant differences in the quality characteristics of control and test hot beef samples chilled in the package at V av = 2·5°C/hr, starting from the 14th or 21st day of storage.

In test samples, chilled with a one-step method at V ac = 1·0/hr without and with pre-ageing, rigor mortis is smoother and shorter and its resolution is faster.

Lower elastic deformation characteristic of rigor appears after 3-6 hours at 18°C and does not depend on the stretching force.

Microstructural changes of the test samples chilled at V av = 1·0°C/hours show that ageing proceeds more intensively than in controls, packed after chilling in sides. By the second day the initial stage of ageing effected by proteolytic enzymes is recorded, while in controls transversal slit-like breakage of muscle fibres occurs only at some spots. By the 7th day the second stage of ageing is observed in the deep of

test samples, while only first stage becomes obvious in controls.

Only by the 21st day of storage at -1 to 2°C these differences are levelled.

Microbiological results show that by the end of cold storage (28 days) the total microbial load in all the samples did not exceed $10^6/cm^2$ (the admitted sanitary standard for packed meat has been met); in all the cases lactobacilli prevailed, constituting 70-80% of the total load by the 21-28th day of storage.

Thus, the conducted study made it possible to reveal the effect of average volume temperature rate lowering on the physico-chemical, structuro-mechanical and biochemical processes of hot-packed meat.

It was established that improved technological properties of the test samples are achieved due to lower average chilling rate in packages from 2·5°C down to 1·0°C/hr.

Our investigations of the 3 versions of samples chilling rendered it possible to recommend one-stage chilling of packed hot meat at the mean rate of average-volume temperature fall 1.0°C/hour, while maintaining the technological properties of ready-to-cook meats.

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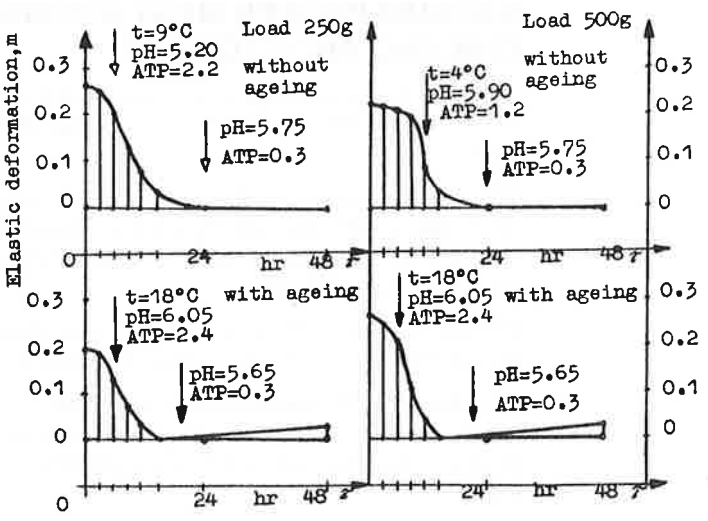

Fig. Elastic deformation resulting from stretching vacuum-packed hot meat during chilling.

↓ - the beginning and development of post mortem contraction

↓ - "cold-shortening"

Table

Hours post mortem, hr	Rate		
	2.5°C/hr	I.0°C/hr	control
I	0.8 ± 0.05	0.8 ± 0.05	0.8 ± 0.05
I6	I.I ± 0.08*	I.0 ± 0.07	-
48	I.0 ± 0.II*	0.6 ± 0.I0	0.9 ± 0.09
I68	0.7 ± 0.09	0.4 ± 0.08	0.6 ± 0.07
336	0.3 ± 0.05	0.2 ± 0.07	0.4 ± 0.I0
504	0.3 ± 0.07	0.I ± 0.05	0.3 ± 0.08

* Samples destruction under stretching

MEMBRANAL LIPID PEROXIDATION IN RELATION TO MEAT STABILITY

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SUMMARY

Studies were designed to evaluate the effect of dietary oils and/or α-tocopherol supplementation on the oxidative stability of membranal lipids in broiler and pig muscles, and on the oxidative stability of broiler and pork products during storage. Results of these investigations indicated that: (a) the consumption of the polar lipids of the membranes were influenced by dietary oil and that changes in the fatty acid composition of the membranal lipids of broilers were reflected in their susceptibility to metmyoglobin/hydrogen peroxide initiated peroxidation, and (b) membrane-bound α-tocopherol stabilizes the membranal lipids of both broiler and pork muscles, and this reduced the extent of lipid oxidation occurring in broiler meat and pork during refrigerated and frozen storage. These observations support the hypothesis that lipid peroxidation in raw meat is initiated in the membrane-bound lipids and that stabilization of the membranal lipids, either through alteration of the fatty acid composition or by incorporation of -tocopherol into them, has positive influence on the oxidative stability of muscle foods during storage.

INTRODUCTION

Lipid peroxidation is one of the major causes of deterioration in the quality of meat and meat products, particularly during frozen storage. Oxidative deterioration can directly affect many quality characteristics such as colour, flavour, texture, nutritive value and safety (Pearson et al. 1983). Membrane-bound lipids associated wit the muscle cell wall, the mitochondria and the sarcoplasmic reticulum are especially susceptible to oxidation because of their high contents of polyunsaturated fatty acids (Pearson et al. 1977). This study is based on the hypothesis that membrane-bound lipids in muscle foods are the central point in the development of oxidative rancidity in fresh meat products during storage. Thus, stabilization of the membrane lipids should influence the stability of meat products. The major objectives of this study were: (1) to determine the effects of dietary oils and -tocopherol supplementation on the oxidative stability of membranal lipids in dark and white meat of broilers, and (2) to study the influence od dietary α-tocopherol and oxidized oil supplements on the stability of pig membranal lipids toward metmyoglobin/ hydrogen peroxide-initiated peroxidation and on the oxidative stability of pork products during storage.

EXPERIMENTAL METHODS

Broiler study: One hundred and seventy five one-day old White Mountain chicks were randomly divided into five groups. Each group was given standard broiler feed supplemented (5.5%) with vegetable oils of varying degrees of unsaturation: coconut oil, olive oil, linseed oil and partially hydrogenated soybean oil (HSBO). A fifth diet containing HSBO and α-tocopherol (100mg/kg feed) was used. The broilers were raised for seven weeks and then slaughtered. Lipids (neutral and polar) were extracted from the muscle samples using the dry column method of Marmer and Maxwell (1981), while fatty acid composition was established by GC analysis of the fatty acid methyl esters. The TBA method of Tarladgis et al. (1964) was used to monitor lipid oxidation in the meat samples during storage.

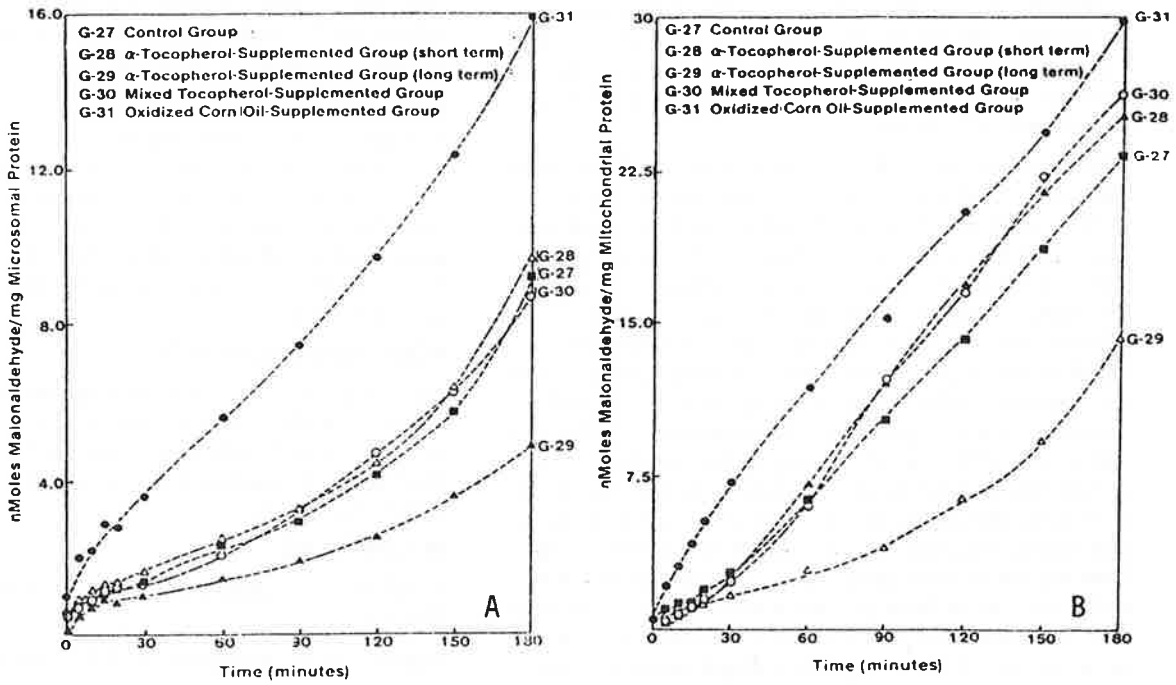

Figure 1. Metmyoglobin/hydrogen peroxide-initiated peroxidation in microsmal (A) and mitochondrial (B) lipids from pigs fed various diets

Isolation of microsomal and mitochondrial subcellular fractions of the white and dark muscles was achieved by differential centrifugation (Schenkman and Cinti 1978). The lability of the isolated fractions to peroxidation was determined using the metmyoglobin-hydrogen peroxide assay of Harel and Kanner (1985).

Pig study: Thirty cross-bred pigs (approximately three months old, equal numbers of barrows and gilts) were alloted at random into five groups of six pigs and fed the following diets: (1) control diet; (2) control diet + added α-tocopherol (200 mg/kg feed) for the last four weeks of the feeding trial; (3) control diet + added α-tocopherol (200 mg/kg feed) for the duration of the feeding trial (10 weeks), (4) control diet + natural mixed tocopherols (200 mg/kg feed) for ten weeks and (5) a diet containing oxidized corn oil to produce a meal having a peroxide value of 9 meq/kg feed. The semitendinosus muscles from the pigs were used to study the extent of peroxidation of membrane-bound lipids (microsomes and mitochondria) when subjected to the non-enzymic (metmyoglobin/ hydrogen peroxide) lipid peroxidation assay of Harel and Kaner (1985). The oxidative stability of various pork products (pork chops, restructured pork roasts) from the pigs fed the various diets was evaluated using the TBA procedure described previously.

RESULTS AND DISCUSSION

Results of the broiler experiments indicated that the composition of the polar lipids of subcellular membranes was influenced by dietary oil and that the changes in the fatty acid composition of the microsomal and mitochondrial lipids were reflected in their susceptibility to metmyoglobin/hydrogen peroxide initiated peroxidation. The polar lipids of the microsomes isolated fr the broilers fed linseed oil contained much higher levels of pentane ($C_{20:5}$) and hexane ($C_{22:6}$) fatty acids compared to the other broiler groups (Table 1). This indicates that the high amounts of linolenate in the diet led to the biosynthesis and deposition of these fatty acids in the microsomes. Another noticeable feature was the higher content of saturated fatty acids ($C_{12:0}$) and $C_{14:0}$) in the polar lipids of the broilers fed coconut oil. Similar fatty acid trends were observed for the mitochondrial fractions.

When the metmyoglobin/ hydrogen peroxide initiator was added to the isolated microsome preparations, the microsomal lipids from the broilers fed linseed oil were more rapidly oxidized than those fr the other groups. This rapid rate of oxidation can be explained, in part, by the higher content of the polyunsaturated fatty acids. Microsomes isolated from the α-tocopherol - supplemented group had lower TBARS numbers than the respective HSBO control group, which reflected the presence of α-tocopherol in the membranes. This was substantiated by gas chromatographic analysis. Dark muscle microsomal lipids peroxidized fasted than lipids from white muscle microsomes, an observation similar to that reported earlier by Harel and Kanner (1985). Meat from the linseed oil group, as expected, was much less stable than meat from the other groups, when stored at 4°C for 9 days and at -20°C for 6 months. In contrast, meat from the α-tocopherol-supplemented broilers exhibited the highest oxidative stability among the five treatments. Trends in meat stability generally followed those established for the subcellular membranes, as reported above.

The rates of metmyoglobin/ hydrogen peroxide-initiated peroxidation of microsomes and mitochondria isolated from the semitendinosus red muscles of pigs receiving diets supplemented with tocopherols (groups 28, 29 and 30) are shown in Figure 1. These data suggest that membranal lipids from the pigs receiving the α-tocopherol supplement for ten weeks (group 29) were much more stable to oxidation than those from the control pigs (group 27), whereas short term α-tocopherol supplementation was less effective. In contrast, long term supplementation with mixed tocopherols (group 30) made little difference to the stability of the microsomes and mitochondria in semitendinosus red muscle. The rates of metmyoglobin/ hydrogen peroxide-initiated lipid peroxidation in the subcellular fractions isolated from the pigs fed the oxidized oil (group 31) were much higher than those from the control pigs (group 27). This suggests that oxidized fat in the diet becomes a source of free radicals which can destabilize the lipids in subcellular membranes. Several trends were established for the mitochondrial fractions.

When pork chops were stored at 4°C in the dark and under fluorescent light, products from pigs fed -tocopherol-enriched diets were more stable than those from the control pigs (Table 2). These data support the hypothesis that incorporation of antioxidants into the membrane lipids well influence the stability of meat products, particularly during refrigerated and frozen storage of uncooked meat products. Similar trends for restructured pork roasts were observed (Table 3). However, TBHQ, when added to the control pork samples, was more effective in reducing lipid peroxidation than the membrane-bound antioxidants. Similarly, nitrite, when added during the processing of restructured pork roasts from pigs fed the oxidized oil diet, was effective in reducing lipid peroxidation during refrigerated storage. Similar trends were observed when the pork roasts were stored at -20°C for six months.

CONCLUSIONS

Results of this study support the theory that lipid peroxidation in raw meat is initiated in the membrane-bound lipids. Stabilization of these lipids, either by alteration of the fatty acid composition or by the incorporation of α-tocopherol into them through diet, has a very positive influence on the oxidative stability of meat during storage.

ACKNOWLEDGEMENTS

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Table 1. Effect of dietary oils on the fatty acid composition of the polar lipids of microsome fractions isolated from dark muscles of broilers

Fatty[a] Acid	Dietary Treatment				
	Coconut Oil	Olive Oil	Linseed Oil	HSBO +α-Tocopherol	HSBO
Saturated	36.7	23.6	24.9	25.2	24.5
Monoene	25.5	28.1	22.8	19.8	22.6
Diene	18.8	14.8	22.6	21.6	21.2
Triene	3.7	5.0	4.8	4.4	3.4
Tetraene	11.3	12.6	5.2	16.3	12.0
Pentaene	1.3	2.3	3.9	2.8	1.9
Hexaene	0.1	1.2	5.5	1.5	1.1

[a] Fatty acid data represent the mean of three analysis of a composite meat sample from 35 broilers.

Table 2. Effect of tocopherol-supplementation and oxidized oil in pig diets on the stability of pork chops during refrigerated storage as measured by the TBA procedure

Treatment	Days of storage at 4°C							
	Fluorescent light				Dark			
	0	3	6	9	0	3	6	9
Control	0.09[a,b]	0.48	0.82	2.76	0.09	0.54	0.77	0.47
Short-term α-tocopherol	0.08	0.18	0.70	0.96	0.08	0.35	0.28	0.33
Long-term α-tocopherol	0.09	0.15	0.51	0.84	0.09	0.36	0.27	0.24
Mixed tocopherols	0.09	0.26	0.60	0.88	0.09	0.37	0.62	0.39
Oxidized oil	0.09	1.4	4.34	5.46	0.09	1.22	3.66	4.15

[a] TBARS number, expressed mg malonaldehyde/kg of meat product
[b] TBARS numbers are based on triplicate analyses of two composite samples obtained on grinding three pork chops per treatment

Table 3. Effect of tocopherol-supplementation and oxidized oil in pig diets on the stability of restructured pork roasts during refrigerated storage, as measured by the TBA procedure

Treatment	TBARS number [a,b]			
Day	0	3	8	15
Control	0.17	0.59	1.42	2.20
Control + TBHQ[c]	0.18	0.18	0.25	0.40
Short-term -tocopherol	0.16	0.25	0.38	1.00
Long-term -tocopherol	0.10	0.22	0.28	0.87
Long-term mixed tocopherols	0.21	0.31	0.88	1.55
Oxidized oil diet	0.32	1.37	2.55	3.23
Oxidized oil + nitrite during processing[d]	0.12	0.27	0.36	0.60

[a] TBARS number, expressed as mg malonaldehyde/kg of meat product
[b] TBARS numbers are based on triplicate analyses of two restructured pork roasts per treatment
[c] TBHQ, 0.02% based on fat content
[d] Nitrite, 156 mg/kg

EFFECTS OF ELECTRICAL STIMULATION ON POST-MORTEM CHANGES IN MYOFIBRILLAR AND SARCOPLASMIC PROTEINS OF BEEF

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SUMMARY

Sixteen Holstein steers and cows were electrically stimulated at low voltage (40 V and 13.8 Hz) for 0, 30, 60 or 90 sec. Myofibrillar and sarcoplasmic proteins from *biceps femoris* stored at lflOC for 21 days were analyzed by SDS-PAGE and HPLC. The degradation of troponin T and production of 30 K component were observed in myofibrillar proteins of electrical stimulation (ES) muscles for 60 and 90 sec at the early stage (0-3 days). On the other hand, in the case of Holstein cows,the degradation of troponin T and production of 30 K component were observed in control and ES muscles at 14 days. However, there were a few bands between tropomyosin and 30 K component in ES muscles. Although sarcoplasmic proteins were not changed in the SDS-PAGE patterns until 7 days, the degradation ot sarcoplasmic proteins was observed at 0 day on the HPLC pattern in ES muscles.

INTRODUCTION

Many studies have been carried out on the changes of myofibrillar proteins during post-mortem storage, and these alterations have been related to the development in meat tenderness. Electrical stimulations (ES) has been recognized as a means of improving tenderness (Carse 1973; Salm et al. 1983). ES induces a rapid exhaustion of ATP and reduction in muscle pH, with a concomitant increase in muscle lactate and fall in glycogen. So this process hastens the time of rigor mortis and avoidance of cold shortening, because the meat achieves rigor before entry to a refrigerator or freezer (Bendall 1980). ES also causes disruption of the myofibrillar structure (Takahashi et al. 1987) and increases the activity of proteolytic enzymes (Dutson et al. 1980). Other muscle proteolytic enzymes could be involved in the conditioning process, as well the calcium-activated neutral protease (Ca ANP; Olson et al. 1977) and the lysosomal proteases (Ouali et al. 1987). There is also the possibility of a cooperative mechanism between Ca ANP and lysosomal proteases (Penny and Ferguson-Pryce 1979; Ouali and Valin 1981). Although Ca ANPs that have activity against myofibrillar proteins function around neutral pH, the pH of carcass rapidly falls bellow pH 6.0 after ES. So it is assumed that the activity of this protease is restricted in ES meat around ultimate pH. Therefore, the possibility for increased tenderness of ES meat could be due to the increased rate of enzymes release from the lysosomes into the rapidly acidifying environment within the muscle fiber and to the greater activity of these enzymes at low pH. Other merits of ES are the bright red color appearance of the meat and improvement in palatability etc. Our purpose is to confirm the proteolysis of myofibrillar and sarcoplasmic proteins in ES muscles from Holstein steers and cows under Japanese fattening conditions.

MATERIALS AND METHODS

Experiment I:

Eight Holstein steers, 18 month-old and 700 Kg liveweight, were slaughtered. ES of low voltage (40V, 13.8 Hz) was carried out on pair of steer for 30, 60 and 90 sec, respectively within 5 min after slaughter. Two steers were used as controls. At 3hr post-mortem, samples of *biceps femoris* muscles were obtained and divided into 6 portions of approximately 400 g. Each muscle was packed into a Cryovac bag and stored at 1 ± 1°C within 3 or 4hr after slaughter. Muscles were then analyzed at 0, 3, 5, 7, 14 and 21 days.

Experiment II:

Eight Holstein cows, 6-7 year-old and 750-800 Kg liveweight, were slaughtered. Low voltage ES was carried out for 60 sec for 5 cows within 5 min after slaughter. Three cows were used as controls. Muscle preparation and storage were the same as in Experiment I. After storage, the bags were opened, and 10 g of muscles was homogenized with 30 ml of rigor buffer in an ice water bath, centrifuged at 1°C, 11,000 g for 20 min. The supernatants were filtered through Toyo No.5c filter paper and then passed through a 0.2 μm membrane filter. This was used as sarcoplasmic proteins for the sample of SDS polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC). Myofibrils were prepared from the centrifugation of the rigor buffer homogenate, repeating the homogenization with rigor buffer and centrifugation. SDS-PAGE of the myofibrillar and sarcoplasmic proteins was performed according to the method of Laemmli (1970) and a 50 μg sample of protein was loaded into each slot. For HPLC a model CCP-8000 system (Toso Co.) was used. In this study the column of TSK-G3OOOSW (d.7x60 cm) and G2OOOSW (0.7X60 cm) were connected. The elution buffer was 0.25 M phosphate pH 6.5 and pumped at a flow rate of 0.5 ml/min. Sample injection was at a constant volume of 10 μl, and proteins were detected by absorbance at 280 nm.

RESULTS

SDS-PAGE patterns of myofibrillar proteins from Holstein steers are showed in Fig.1. The degradation of troponin T and the concomitant appearance of a 30 K component in ES muscles were observed at an earlier stage compared with the control. This finding was most apparent in ES 60 and 90 sec muscles. During conditioning of meat from these two groups, troponin T

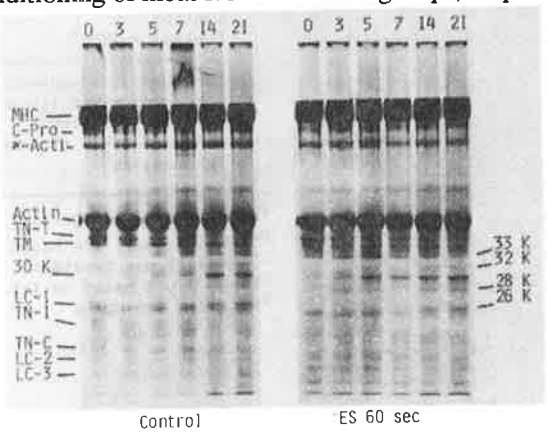

Fig. 1. SDS-PAGE patterns of myofibrillar proteins from Holstein steers. The numbers of 0 to 21 gives the storage time in days at 1°C after slaughter. Electrophoresis was performed in a 11% polyacrylamide slab gel.

was degraded by 5 days and the 30 K component had appeared at 0 and 3 days, respectively. Besides new bands (33 K component) had appeared below the tropomyosin band. In the case of Holstein cows, troponin T was degraded and the 30 K component had appeared at 14 days in control. In the case of the 33 K component, this had appeared at 0 day in ES 60 sec muscles and at 14 days in control. Tropomyosin was slightly degraded both in control and ES muscles by 21 days (Fig.2). Other changes found in the myofibrillar proteins patterns during post-mortem storage were the appearance of 26, 28 and 32 K components and these bands were most noticeable in ES muscles. SDS-PAGE patterns of sarcoplasmic proteins from Holstein steers and cows showed the degradation of 33, 36 and 44 K components in control and

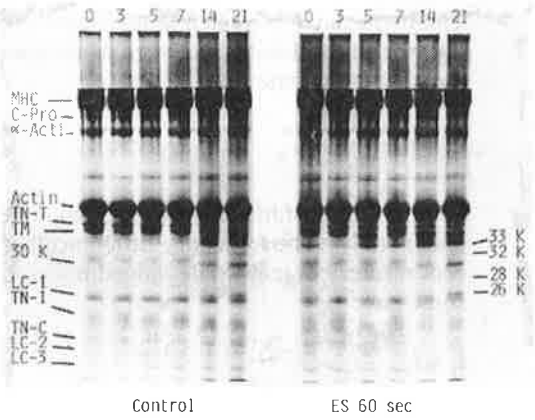

Fig. 2. SDS-PAGE patterns of myofibrillar proteins from Holstein cows. The numbers and electrophoretic conditions are the same as in Fig.1.

ES muscles at 7 and 14 days, although there were some changes to be seen in the HPLC patterns at 0 day.

From the analysis of sarcoplasmic proteins using HPLC, we can observe changes in the peaks of proteins, peptides and amino acids. After ES treatment, some protein components were degraded as in the peaks of RT-61 and 65, and new peaks appeared as at RT-58, 70 and 77. The peak of RT-81 was mainly myoglobin and became larger after ES treatment. The peaks from RT-93 to 103 were peptides and amino acids, and some of these peaks were degraded or replaced by new peaks (RT-103; Fig. 3). From these facts it was found that ES caused the degradation of sarcoplasmic proteins and made some peptides and amino acid concerning the palatability.

DISCUSSION

Temperature and pH are well established as important factors in post-mortem muscle tenderization (Yates et al. 1983), In the environment of high temperature and low pH, it is assumed that these are favorable condition for acidic proteases such as cathepsins B and L, because ES treatment causes a rapid fall in muscle pH and a more rapid release of lysosomal enzymes (Moeller et al. 1976 and 1977; Dutson et al. 1980). The apparent changes of myofibrillar proteins in ES muscles are the production of the components of 26, 28, 30, and 33 K. Ducastaing et al. (1985) also reports the appearance of 30 and 32 K components at 4hr post-mortem after ES treatment. Cathepsin B and L degrade myosin and other myofibrillar proteins (Noda et al. 1981; Mikami et al. 1988). However,

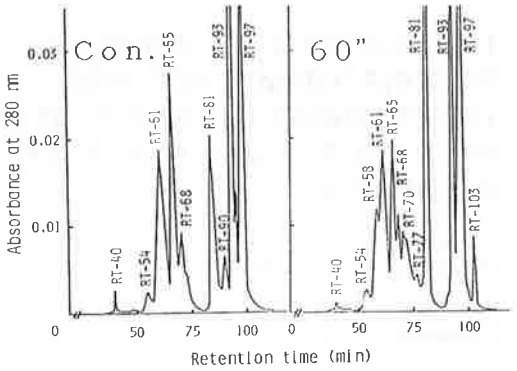

Fig. 3. HPLC chromatogram of sarcoplasmic proteins from Holstein steer. Column, TSK-G3000SW G2000SW; buffer, 0.25 M phosphate pH 6.5; flow rate, 0.5 ml/min; sample, sarcoplasmic proteins at 0 day after slaughter; Con, control; 60", ES 60 sec.

degradation of myosin, α−actinin and actin was not clearly identified from the SDS-PAGE patterns. The main reason was probably due to the low temperature (1±1°C) of conditioning in this experiment. However, this temperature is normal employed in meat storage. We can not certain thous which proteases had contributed to conditioning in ES meats, although myofibrillar and sarcoplasmic proteins were degraded faster in ES muscles compared with control.

CONCLUSION

Myofibrillar proteins from Holstein steers were degraded and produced several bands in ES muscles on SDS-PAGE patterns. However, the degradation of myofibrillar proteins from Holstein cows was slower than that of steers in control and ES muscles. It was found from the HPLC patterns that sarcoplasmic proteins were degraded and produced several new peaks of these fragments at 0 day in ES muscles.

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DEVELOPMENT OF AN ULTRASONIC RESONATOR METHOD FOR DETERMINING THE CONTENT OF PROTEIN, FAT AND MOISTURE IN MEAT PRODUCTS

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The acoustic parameters of muscle tissue as effected with protein, fat and moisture levels were studied. Changes in the ultra-sound propagation rate and absorptivity were investigated relative to the level of protein, fat and moisture in muscle and fat homogenates and in minced meat. Muscle structure was found to influence little the acoustic parameters, which changes depended basically on the moisture in the former. Analytical and correlation relationships of the acoustic characteristics of muscle homogenates to their protein, fat and moisture contents are derived. Ultra-sound propagation rate in homogenates of different fatness degrees was measured as a function of temperature.

The data obtained allowed to develop a resonator method for determining protein, fat, moisture and mineral salts percentages in beef and pork homogenates. It may be useful for express-analysis of the quality of meat and meat products and for developing automated systems of monitoring technological production lines of least-cost products.

The acoustic parameters of meat as influenced with its basic components, viz., protein, fat and moisture levels, were studied. Changes in the ultra-sound propagation rate and absorptivity were investigated relative to the level of protein, fat and moisture in muscle and fat homogenates and in minced meat. Analytical and correlation relationships of the acoustic characteristics of muscle homogenates to their protein, fat and moisture contents are derived. Ultra-sound propagation rate in homogenates of different fatness was measured as a function of temperature. The data obtained allowed to develop a resonator method for determining protein, fat, moisture and mineral salts percentages in beef and pork homogenates. It may be useful for express-analyses of the quality of meat and meat products and for developing automated systems of monitoring technological production lines of least-cost products.

The application of ultra-sonic methods is based on the relation of the acoustic parameters of biological tissues to their composition, structure and condition. In studying ultra-sound propagation in biological objects, of interest are the rate, absorption, dissipation, reflection and refraction of resilient waves. Recently researchers concentrated on the use of ultra-sound to study the composition and properties of biological tissues, and there is certain progress in this field. In this respect, the establishment of a possible application of ultra-sound to determine meats composition is of scientific and practical importance.

The purpose of the reported experiments was to develop an express-method for determining protein, fat and moisture in the quality evaluation of meat and meat products; the method is based on the changes in ultra-sound propagation rate and absorptivity in meat homogenates.

The effect of the basic components of uncured raw meat (fat, protein, moisture) upon acoustic parameters was studied with a resonator method for measuring ultra-sound rate (U), it being based on measuring the frequency and width of the resonance peaks of an acoustic resonator. The latter is the space between two high-parallel pieso-tranducers filled with the medium under study.

Resonance peaks are determined with the appearance of a standing wave in the cell at the frequences, for which a whole number of semi-waves is covered by the length (1) between the pieso-transducers, i.e.

$$1 = n\frac{\lambda}{2} = \frac{U}{2f}$$

The frequency f_n of each n-th resonance is determined with the permanent distance between pieso-tranducers and with the ultra-sound rate

$$f_n = n\frac{U}{2l}$$

i.e. f_n is a linear function of ultra-sound rate in the medium under study. The frequency difference between two adjacent resonances in a single-valued linear function of ultrasonic rate:

$$f_1 = f_{n+1} - f_n = \frac{1}{2l}$$

The absolute value of ultrasonic rate in liquids can be derived from Eq. (3) starting from the measured frequency difference of two adjacent resonances.

It is convenient to estimate relative changes in ultrasonic rate ($\Delta U/U$) from the frequency of one of the chosen resonance peaks by the formula:

$$\frac{\Delta f_n}{f_n} = \frac{\Delta U}{U}$$

The resonance peak width δf_n is determined with the total power loss in the acoustic resonator due to ultra-sound absorption with a liquid, to partial reflection of an acoustic wave at the liquid-piesotransducer interface, etc. At low amplitudes of an acoustic wave this loss may be regarded as the additive one; then, the following expression is true:

$$\frac{\delta f_n}{f_n} = \frac{(\alpha\lambda)}{\Pi} + B$$

where α is sound absorptivity in a liquid, λ is wave length, B is a constant characterizing power losses in the acoustic cell, which are not due to sound absorption in a liquid; B is a function of frequency, depends on the design of a given resonator and can be found by measuring δf_n of the reference liquid having a known absorptivity value. Thus, ultra-sound propagation rate is proportional to the frequency δf_n, and absorptivity - to the width δf_n of the n-th resonance peak of an amplitude-frequency characteristics.

Eqs 3-5 allow to calculate ultra-sound absorptivity and rate in meat homogenates starting from the measured parameters of the resonance peaks of the cell. On this basis a model laboratory analyzer has been developed.

As test objects served uncured beef and pork. Samples were disintegrated in a meat grinder, a test portion m_{tp} was placed into the cup of a homogenizer and diluted with distilled water; the amount of added water was measured. The test portion was dispersed in an ultrasonic disintegrator for 4 min followed with comminution for 3 min since after dispersion there were some fibres left. Acoustic measurements of thus prepared homogenates were taken in the model laboratory analyzer in the frequency range from 2.5 to 3.0 MHz at 20 and 40°C.

A relation of acoustic characteristics to homogenate composition was found by measuring the acoustic parameters. Ultrasonic rate, absorption, temperature dependences of ultrasonic rate and absorption have different sensitivities to the level of meat components (fat, protein) in homogenates, and it enabled the determination of their composition by the measured values of the parameters.

In the simplest case, if one substance is dissolved in water and if the parameter to be measured (e.g., ultrasonic rate) is linearly related to the concentration "c" of the substance, e.g., of protein (c_{pr}), then, for a diluted solution

$$U = U_o + a_1 c_{pr} \quad (6)$$

where U_o is the value of a given property of pure water; a_1 is the coefficient of the relation between concentration c_{pr} and property U (to be determined experimentally). If U_o and a_1 are known, c_{pr} can be calculated from the measured U.

When there are several solutes in a mixture,

$$U = U_o + a_1 c_{pr} + a_2 c_{fat} + a_3 c_{water} \quad (7)$$

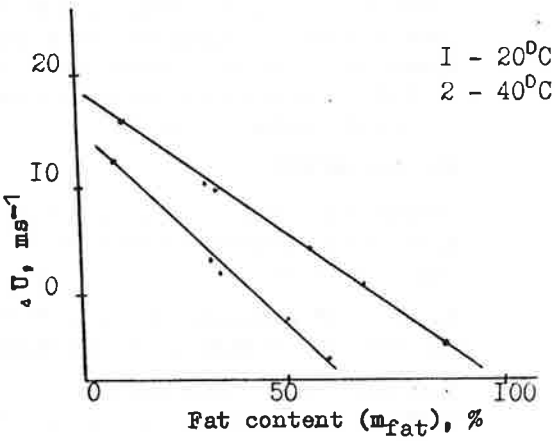
Then, to determine the concentration of c_{pr}, c_{fat} and c_{water}, it is necessary to measure several different physical properties (f, α, U, etc.), which would differ in their sensitivity to the concentration of meat components. The problem is then reduced to solving a system of linear equations:

$$\begin{aligned} U &= U_o + a_1 c_{pr} + a_2 c_{fat} + a_3 c_{water} \\ \alpha &= \alpha_o + b_1 c_{pr} + b_2 c_{fat} + b_3 c_{water} \quad (8) \\ f &= f_o + c_1 c_{pr} + c_2 c_{fat} + c_3 c_{water} \end{aligned}$$

On the basis of the experimental results on the acoustic characteristics of raw meat homogenates, data are obtained on the independence of ultrasonic rate of the structure of raw meat (of meat integrity). This coincides with the earlier conclusion (Gorelov et al., 1984) that the acoustic rate in liquid and semi-liquid media is completely related to the molecular composition of a medium and does not practically depend on the type of the cellular and tissue structure.

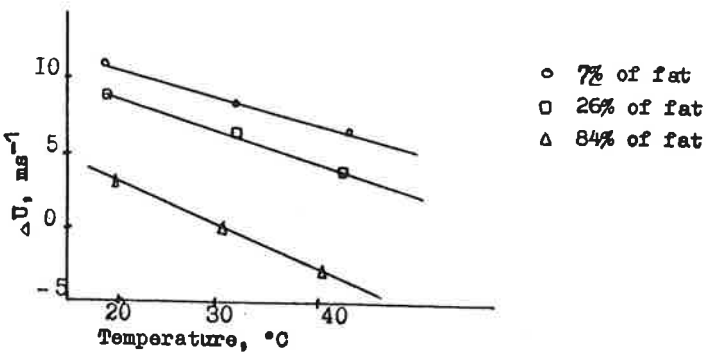
Muscle tissue contains, on the average, 76% of water, 1.9% of lipids, 21% of protein (Krylova & Lyaskovskaya, 1968). The main component, protein (without account for water) constitutes 86% of the dry solids of the tissue: consequently, changes in the acoustic parameters of muscle homogenates are primarily due to the content of protein. Fatty tissue contains, on the average, 7% of water, 91% of fat and 2% of protein; thus changes in the acoustic parameters of fatty tissue homogenates are basically due to the content of fat.

In the experiments on meat homogenates of different fat levels it was found that with a higher fat level ultra-sound absorption is linearly increasing and the acoustic rate is linearly decreasing (Fig. 1).



This agrees with the data available in literature on the relationship among these characteristics and lipid levels in biological tissues and liquids.

Fig. 2 shows ultra-sound rate in homogenates of different fatness as a function of temperature.



The rate of ultra-sound decreases practically linearly with a temperature rise, the decrease being steeper in case of higher fat contents in samples. If the values of the temperature dependence slope are extrapolated to the pure fat component and to the pure protein component, the slope for fat is one order higher than that for protein. Therefore, the slope of the temperature dependence is also a sensitive characteristic to determine fat-to-protein ratio. Contents of salts and organic low molecular components in the muscle tissue is about 3.5% (1% of salts and 2.5% of other compounds), i.e. 5 times as less as compared to protein content. Their total contribution to the rate of ultra-sound is by 4 times lower than that of protein (it was established experimentally by means of

dialysis separation of low-molecular compounds from muscle homogenates). On the basis of the above-said and earlier results (Lyrtchikov et al., 1986; Solntseva et al., 1986), one may state the lawfulness of the application of the additive scheme to calculate the acoustic parameters of meat homogenates. The data obtained allow to use the resonator method to estimate protein, fat and moisture percentages in raw muscles and minced meat.

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INFLUENCE OF ELECTRICAL STIMULATION ON THE QUALITY OF CHILLED AND FROZEN READY-TO-EAT MEAT STEAKS

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The paper presented data on the impact of electric current after 10 min of slaughtering (voltage 220V, 36V, frequency 50 Hz) on the quality of cooled and frozen meat.

It was demonstrated that efficacy of the electrostimulation depended on the quality of raw meat and post-mortem changes.

With regard to the electrostimulation of the fresh meat it was shown that its quality could be organoleptically assessed by 1,5 grades. In comparison, fresh meat which was kept under 12°C during 16 hours was evaluated by 0,15 grades.

The author concluded, in particular, that low voltage stimulation (36V) would permit to optimize the deboning procedure of fresh meat as well as its better quality during and after of refrigeration storage.

The intensification of refrigerated treatment of ready to cook meat steaks manufactured from hot-boned beef carcasses has been dictated by technological necessity, technical feasibility and economical considerations.

It is known that low temperature air causes cold shortening of muscle fibres and results in the increase of toughness of meat and loss of meat juice during storage.

Electrical stimulation is widely used abroad to improve the quality of meat while keeping the rapidity of refrigerated treatment.

Research has been carried out by the authors to study the influence of electrical current of different parameters (voltage of one half-period current 220 V and 36 V, frequency 50 Hz, duration of the influence -120 s) on the quality of meat boneless steaks, prepared from raw materials of different stage of autolysis.

Electrical stimulation of beef carcasses was carried out at a bleeding station with the use of a pilot plant, developed in VNIKTIkholodprom. The time between stunning and beginning of electrical stimulation was 8-10 min. The carcasses with hides on after electrical stunning and cutting of the heads came on a moving conveyor to the tunnel, where the electrical stimulation plant was situated. In the tunnel the carcass with its neck part touched the lower electrode of the installation and was subjected to the effect of electrical current. The hanging rail served as a second electrode. The carcasses not subjected to the influence of electrical current were used as controls. The object of the investigations was the cattle chosen by the method of similarity (sex, breed, age, fatness, weight) from the same farm, where they were held under similar conditions of feeding and management.

The Longissimus dorsi and neck muscles without the surface fat and connective tissue were used for sampling for carrying out physical and chemical investigations.

The influence of electrical stimulation and holdng of meat on organoleptical indices was also determined in the investigations.

The experiments were carried out according to the following procedures:

1 procedure - dressing of sides in a hot condition with the excision of boneless steaks and their subsequent refrigeration in air at -3°C, air velocity 1.5 m/s during 4 hours.

2 procedure - chilling of sides at -3°C during 16 hours; dressing and boning of sides with the excision of boneless steaks.

3 procedure - precooling of sides at -15°C, air velocity 0.8 m/s during 2 hours; dressing of sides with the excision of boneless steaks and their additional cooling at -3°C, air velocity 1.5 m/s during 3 hours.

4 procedure - holding of sides at +12°C during 16 hours; dressing and boning for boneless steaks with their subsequent subfreezing at -30°C, air velocity 2.5 m/s during 10 min.

5 procedure - dressing of hot sides with the excision of boneless steaks, 10 and 40 min after slaughter with their subsequent freezing at -30°C and air velocity 1.5 m/s during 3 hours and contact thawing in water of 18°C during 1.3 hours up to the temperature of the muscle depth 0-4°C.

All the steaks were vacuum packed in plastic film and stored (except the procedure 5) at -1°C during 3 days.

The quality of stimulated and nonstimulated meat was evaluated according to the following indices:

- pH of meat - potentiometrically
- amount of drip of boneless steaks - on net weight difference
- easiness of separation of meat from bones during deboning - according to the opinion of deboners
- degree of contraction of muscle tissue - on sample length difference prior to freezing, after freezing and defrosting
- sensory evaluation of the appearance of raw and cooked meat according to a 9-score quality scale/procedure of VNIIMP/.

Tables 1-3 show the results of the investigations of electrical stimulation and different methods of refrigerated treatment on physico-chemical and organoleptical quality indices of steaks.

The analysis of the investigations (Table 1) shows, that pH was 0.5 - 0.6 units lower, if electrical stimulation was carried out prior to boning, as compared to boning without electrical stimulation, which is in agreement with results of (Eikelenboom et al., 1981, Schreuder et al., 1982, Verbeke et al., 1986).

TABLE 1: Change of meat pH in dependence of electrical current voltage

pH			
Voltage 220V		Voltage 36V	
Nonstimulated meat after	Meat after electrical stimulation	Nonstimulated meat	Meat after electrical stimulation
6.67 ± 0.15	$= 6.08 \pm 0.21$	$= 8.66 \pm 0.16$	$+ 6.11 \pm 0.24$

The comparative evaluation (Table 1) of the influence of electrical stimulation on pH change at different voltages show, that the difference in the degree of decrease of active acidity of medium (pH) in experimental and control carcasses is confident ($P < 0.001$) irrespective of the voltage, while differences in pH value between experimental carcasses at different voltages are not confident ($P < 0.05$), though the carcasses muscles contraction is more evident visually at 220 V.

Taking into account the data obtained and safety factors, further investigations on the determination of influence of electrical stimulation on quality indices of meat were carried out at voltage of 36 V.

Investigations on the influence of electrical stimulation on drip losses of vacuumed packaged steaks, obtained from hot-boned meat, showed, that in experimental samples the average drip losses were 0.42% and in control ones - 0.82% while drip losses during thawing (procedure 5) were 3.35% in control samples and 1.42% - in the experimental ones. These data are in agreement with the experimental results obtained by Kotula A.B. and Berry V.V. (1981) during electrical stimulation of hot sides with the voltage 250 - 400V.

TABLE 2: Influence of electrical stimulation on degree of contraction of neck muscles, excised from the carcasses at different time after slaughter

Samples	Initial length of muscles, cm	Length of muscles after thawing, cm	Degree of muscle contraction, %
Time of excision of muscles - 10-15 min after slaughter			
Control	24.0	6.0	75.0
Control	21.0	6.0	71.4
Experiment	23.0	17.0	26.0
Experiment	19.5	13.0	31.0
Time of excision of muscles - 35-40 min after slaughter			
Control	25.0	14.0	44.7
Control	22.0	13.5	38.6
Experiment	21.3	18.5	18.1

From Table 2 it can be seen that electrical stimulation exerts a significant influence on the process of thaw-rigor during defrostation of meat, frozen in a hot condition.

Thus, the muscle contraction during thawing of control samples was 71.4 - 75%, and of the experimental ones - 26-41%. It was found that the degree of contraction of muscle tissue of control and experimental samples depends on post-mortem period and stage of autolysis.

The size of contraction of muscles isolated from the carcasses after removal of the hides (35-40 min) is 1.8-2 less than that of the muscles, isolated at the bleeding stage (10-15 min).

The difference in the degree of contraction of muscles as related to the time of isolation from the carcasses is possibly associated with the decreases of pH of the control and experimental samples, which is indicated in the paper of Currie R.V. and Wolfe F.H. (1979) having established the relationship between pH of meat and the amount of meat contraction as a percentage.

Sensory evaluation of quality of steaks, obtained according to the technological procedures 1-4 (Table 3) shows that the effect of electrical stimulation on organoleptical indices of steaks depends to a large extent on the initial state of meat and post-mortem changes.

TABLE 3: Organoleptical evaluation of steaks quality in relation to the refrigerated treatment of meat

Technological procedure	Organoleptical score	
	non-stimulated samples	stimulated samples
1	5.5	7.0
2	5.6	5.8
3	4.7	6.3
4	7.15	7.3

Thus, the difference in the scores of control and experimental samples for hot meat is 1.5, and for meat samples held during 16 hours at 12°C is only 0.15 of the score.

The comparison of quality attributes of steaks, manufactured according to procedures 2 and 3, confirms the fact of a significant influence of electrical stimulation when using rapid refrigeration of meat.

THE EFFECT OF GLYCOLYSIS ON THE BIOLOGICAL VALUE OF DUCK MEAT

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SUMMARY

It has been found that there is a positive correlation between the protein attacking by digestive tract enzymes in vitro and the biological value by 120 h glycolysis of duck meat (grade I and II). The rate and the degree of protein digestibility depends on the stage of meat ageing.

Autolytic processes taking place in poultry meat after slaughtering influence the meat quality and in many cases predetermine its food and biological value. It has been found that the highest protein hydrolysis degree resulting from the action of digestive enzymes is reached by the end of 120 h glycolysis in the first-grade duck meat was well as in the second-grade one. The relatively slow digestion of the second-grade duck meat is very likely due to the large proportion of connective tissues and to the structural difficulties of the interaction between enzymes and proteins. The integral score value for duck meat of the first and second grades has been shown to be 8.99 and 9.04% of answering the balanced nutrition formula, respectively. According to the present-day biochemical concepts (Solovjev 1966, Chemikoiv 1975, Pokrovsky 1975, Dujic 1978, Ugolev 1986) it is quite obvious that the proteolysis during meat glycolysis occurs as an "explosive" breakdown of protein molecules with the immediate formation of the final reaction products. Thus it may be assumed that the biological value index of duck meat changes during glycolysis as a result of structural changes in protein molecules themselves and their interaction with other components, the protein digestion rate and degree being dependent on the stage of meat maturing.

The present-day concepts of biological and nutritive value of foods are based on the principles of balanced and adequate nutrition theories (Prokrovsky and Ertanov 1965, Pokrovsky 1975), Chemikov 1975, Ugolev 1986, Rogov et al 1987). Autolytic processes taking place in chilled poultry meat during storage predetermine its biological value.

In this connection it should be noted that the biological value reflects the quality of the product's protein components connected both with the protein digestion and with the balancing degree of its amino acids composition (Ugolev 1986).

Despite the increasing interest in the poultry meat processing there is limited information on the changes in protein biological value during glycolysis and digestion.

The objective of this paper is to study the food and biological values of duck meat during maturing, the rate and the degree of its digestibility in vitro.

The carcasses of the first and second-grade ducks obtained after commercial slaughtering and initial treatment were investigated. The carcasses packed under vacuum in Saran film (Povidan) were stored for 5 days at 275-277K in the cold store. The total content of moisture, protein, fat ash and pH were determined by the conventional techniques (Zhuravskaya et al. 1985). The amino acid composition of the samples was studied in a model AM-881 automatic amino acid analyser (Chech.). To obtain the full characteristics of the biological value of duck meat protein digestibility has been determined by the technique using proteolytic enzymes of the digestive tract (Pokrovsky and Ertanov 1965). The protein digestibility degree was estimated by the tryosine amount stored during hydrolysis by measuring the optical density of the dialysate at 280 nM in a CF-16A spectrophotometer. The obtained data were subjected to the mathematical statistics.

The experimental data show that the chemical composition and nutritive value of duck meat differ in the content of protein, fat and moisture depending upon the poultry grade (Table 1).

Chemical Composition of duck meat (% per 100 g product)

indexes	carcasses of ducks	
	grade I	grade II
total moisture	45,6±0,5	56,5±0,4
total protein	15,8±0,9	17,3±0,5
fat content	38,0±0,45	24,2±0,5
ash	0,6±0,15	0,9±0,1
protein/fat ratio	0,42±0,03	0,71±0,04
caloricity, Kj	1695±16	1201±20

In order to obtain fuller characteristics of the biological value of duck meat proteins amino acid scores were estimated (Table 2) by the method suggested by the international organisation FAO/WHO (Pokrovsky 1975).

Integral score method gives the possibility to reveal all advantages and disadvantages of food products (Pokrovsky 1975). The estimations have shown that the integral score value for duck meat of the first and second grades are 8.99 and 9.0% of answering the balanced nutrition formula, respectively.

Amino acid score of duck meat (% per 100 g product)

Amino acids	duck meat	
	grade I	grade II
isoleucine	I,05	I,15
leucine	I,14	I,18
lysine	I,50	I,29
methionine + cystine	0,88	0,91
phenylalanine + tyrosine	I,16	I,20
threonine	I,12	I,09
tryptophane	I,16	I,21
valine	0.98	I.0I

The results of the investigations on the rate and the degree of the duck meat (of both grades) protein digestibility in vitro during maturing show that the amount of hydrolysis products depends on the glycolysis rate and nature in muscular tissue (Fig. 1, 2). The first-grade duck meat showed the highest hydrolysis degree of proteins under the influence of digestive enzymes by the end of 120 h glycolysis (Fig.1, curve 1-5). The relatively slow digestion of the second-grade duck meat is probably associated with a great deal of connective tissue and with the structural difficulties of contacting between enzyme and proteins owing to the shift of the meat pH to the isoelectric point of muscular proteins. It is also associated with the change of their ionisation degree and with the increase of the interaction between molecules.

One may assume that the conformation changes in raw materials influence the character of meat protein changes during ageing. The results in the formation of complexes which are more stable to the action of digestive enzymes. The duck meat proteins have different stability degrees to the action of proteinases depending upon the poultry grade. Different stability degrees are also due to hydration levels of muscular proteins (Solovjev 1966, Dujic 1978).

The increase of protein stability to the action of digestive enzymes during rigor mortis may be caused by the formation of specific links which are not destroyed by these enzymes and by the decrease of the peptide bonds accessibility due to the aggregating protein molecules (Solovjev 1966). The increase of protein attacking bez enzymes during meat ageing may be explained by their change resulting from the pH increase and the weakening of actin-myosin link. Apparently the proteolysis during meat maturing occurs as an "explosive" protein molecule breakdown with the immediate formation of the reaction final products according to the modern biochemical concepts (Solovjev 1966, Pokrovsky 1975, Chernikov 1975, Dujic 1978, Ugolev 1986).

Thus, it may be assumed that the duck meat biological value index changes during ageing as a result of the protein molecule structure changes and their interaction with other components.

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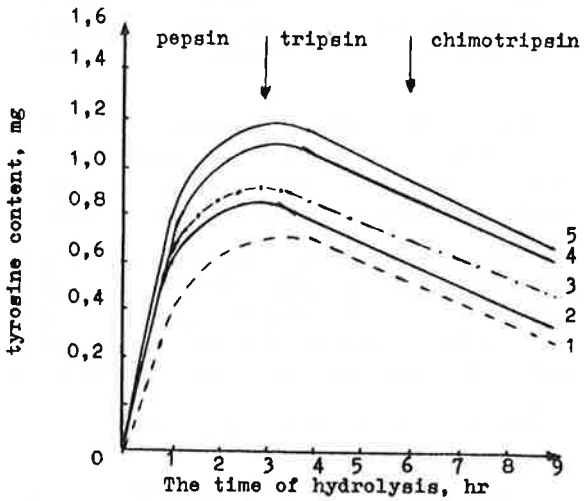

Fig.1. Attacking rate of the first-grade duck meat proteins during maturing
1 - 24hr; 2 - 48hr; 3 - 72hr; 4 - 96hr; 5 - 120hr.

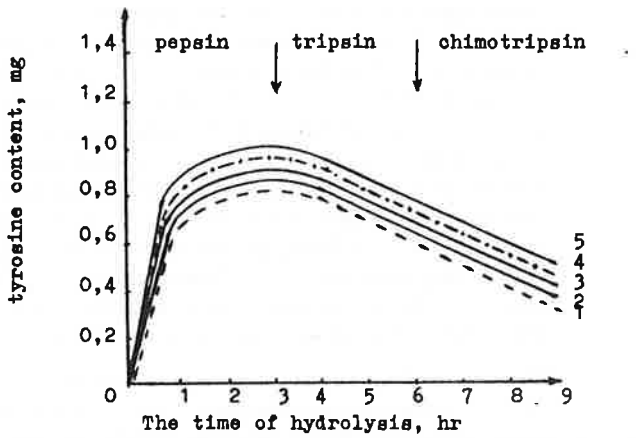

Fig.2. Attacking rate of the second-grade duck meat proteins during maturing
1 - 24hr; 2 - 48hr; 3 - 72hr; 4 - 96hr; 5 - 120 hr.

ANTAGONISM IN DIFFERENT CARCASS AND MEAT QUALITY TRAITS OF CATTLE

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SUMMARY

The antagonistic relations between and within the traits of growth, muscling, carcass and meat quality of -the dual-purpose cattle "Deutsches Fleckvieh" were investigated by calculating the correlation coefficients. 120 young bulls from progeny testing for meat performance were examined. Beyond the antagonistic relations between musculature and fat only few significant antagonistic relations were found. The improvement of the fattening yield leads to an increase of the fatty tissue; this relation is antagonistic by sense. With increasing daily gain also the drip loss increases. If the muscling increases the pH drops quicker and the drip loss rises. These relations however are not statistically significant, Distinct antagonistic relations exist between the lean meat proportion in the carcass and the fat, but also to meat quality characteristics as marbling, colour, tenderness and flavour. The pH value indicates antagonistic relations to tenderness, juiciness and flavour.

INTRODUCTION

Growth and muscling are the most important factors of selection in beef production; other characteristics are usually not directly considered. The relations between the preferred characteristics of selection and other traits of the slaughter value are not always positively correlated, partly even antagonistic. Correlation coefficients hIve been calculated in order to see if there are negative correlations and also to find those positive correlations which have a negative effect on the carcass and meat quality. Correlated were:

- traits of growth with traits of the carcass and meat quality
- traits of muscling with traits of the carcass and meat quality
- gross tissue composition of the carcass (lean, fat, bone) with traits of the meat quality
- different traits of meat quality.

MATERIAL AND METHODS

One hundred and twenty young bulls (10 sires with 12 offsprings each) of the dual purpose breed "Deutsches Fleckvieh" (Simmental) out of the progeny test were used. Feeding was equal for all animals (maize, silage ad lib., 2 kg hay and average 2 kg concentrate mainly as protein supplement). Slaughtering took place at an age of 500 days. Table 1 shows the "basis" parameters of the test material. All traits diverge distinctly in spite of the same age of slaughter and the same feeding. The animals were slaughtered under standardized conditions. Since the stable was located near the test slaughterhouse, no

Table 1: Means, standard deviations and range of "basis" parameters of the experiment (young bulls - German Fleckvieh - slaughter age 500 days) n = 118

Traits		$\bar{x}$	s	range
Live weight	kg	613,4	45,5	482 - 737
Hot carcase weight	kg	350,5	27,6	252 - 417
Dressing	%	58,7	1,3	54,1 - 61,7
Gain	g/d	1211,3	98,6	932 - 1468
Net gain	g/d	703,5	54,9	506 - 843
Kidney/pelvic fat	%	3,2	0,7	1,7 - 6,4

Table 2: Antagonism between fattening yield and carcase and meat quality traits (correlation coefficients)

Carcase quality			Meat quality		
Daily gain					
Bone (carcase)	%	-0,59**	Drip loss	%	+0,26*
Pistola	%	-0,11	Shear force	kg	-0,18
Lean/fat	:1	-0,05	Heating loss	%	-0,23
Fat (carcase)	%	+0,36**			
Fat class		+0,31*			
Fat (best ribs)	%	+0,23			
Live weight					
Bone (carcase)	%	-0,64***	Drip loss	%	+0,24
Pistola	%	-0,08	Shear force	kg	-0,20
Lean/fat	:1	-0,11	Heating loss	%	-0,23
Fat (carcase)	%	+0,45***			
Fat class		+0,37**			
Fat (best ribs)	%	+0,26*			

Significance: *** $p \leq 0,001$, ** $p \leq 0,01$, * $p \leq 0,1$

Table 3: Antagonism between muscling and carcase and meat quality traits (correlation coefficients)

Carcase quality			Meat quality		
Fleshiness (EUROP)					
Bone (carcase)	%	-0,33**	pH 6 hrs p.m.		-0,11
Fat (carcase)	%	+0,14	pH 22 hrs p.m.		-0,21
Fat class		+0,16	Drip loss	%	+0,24
Fat (best ribs)	%	+0,13	Shear force	kg	-0,30*
M.long.dorsi area					
Fat (carcase)	%	-0,18	pH 6 hrs p.m.		-0,15
Fat class		-0,13	pH 22 hrs p.m.		-0,17
Fat (best ribs)	%	-0,26*			

Significance: ** $p \leq 0,01$, * $p \leq 0,1$

Table 4: Antagonism between carcase quality traits and meat quality traits (correlation coefficients)

Carcase composition					
	Lean in carcase			Fat in carcase	
Fat (carcase)	%	-0,87***	Lean (carcase)	%	-0,87***
Fat (best ribs)	%	-0,53**	pH 3 hrs	p.m.	-0,22
Fat (M.long.d.)	%	-0,24*	pH 6 hrs	p.m.	-0,27*
Marbling score		-0,37**	Shear force	kg	-0,23*
Lightness (Hunter L)		-0,25*			
Panel score					
Tenderness		-0,11			
Juiciness		-0,14			
Flavor		-0,21			
Shear force	kg	+0,14			

Significance: *** $p \leq 0{,}001$, ** $p \leq 0{,}01$, * $p \leq 0{,}1$

Table 5: Antagonism within meat quality traits (correlation coefficients)

Meat quality					
	pH 6 h p.m.			pH 48 h p.m.	
Panel score					
Tenderness		-0,24*	Lightness (L*)		-0,24*
Juiciness		-0,18	Panel score		
Flavor		-0,23	Tenderness		-0,20
Shear force	kg	+0,20	Flavor		-0,29*
			Drip loss	%	-0,34**
			Heating loss	%	-0,22
			Shear force	kg	+0,21

Significance: ** $p \leq 0{,}01$, * $p \leq 0{,}1$

lorry transport was necessary. Many different parameters were recorded during the investigation in order to determine potential antagonistic relations. The growth was determined by recording of the daily gain, net gain, final fattening weight and carcass weight.

The conformation was judged by subjective evaluation of the profiles of round, back and shoulder, the determination of the fleshiness (European Classification System, EUROP), various measurements at the round (round circumference, spiral round measure, round width and round depth) as well as the determination of the loin-eye-area between 8 th and 9 th thoracic vertebra.

The proportion of primal cuts (pistola) was used for the determination of the carcass quality- Besides that the proportion of the lean, fat and bone of the carcass was estimated. In the prediction equation the tissue proportions of the thin flank, the half carcass weight, the kidney and pelvic fat and the weight of the four legs were included.

The meat quality evaluation was carried out mainly according to the proposed methods of the ECE working group in the beef research program (Boccard et al. 1981). It comprised besides the sensory examination the determination of the pH-drop after slaughter (pH 45 min, 3, 6, 22 and 48 h p.m.), the determination of the concentration of the metabolites of the postmortem glycolysis (glycogen, lactate, ATP, creatine phosphate) (Fischer and Augustini 1977), the colour (Hornsey 1956) and physical colour measurements (Hunter L*, a*, b*), the fat content of the *M.longissimus dorsi* and of the entire best ribs (ISO 1973), the marbling, the shear force (Warner-Bratzler) and the drip loss (weight loss between 48 hours after slaughter and the 9 th day) (Kim et al. 1985). All examinations were carried out at the *M.longissimus dorsi* and the best ribs (8 th / 9 th - 11 th / 12 th thoracic vertebra).

Sensoric evaluation took place after 13 days of aging at +2°C with low vacuum. A 2 cm thick slice from the best ribs (9 th / 10 th thoracic vertebra) was heated in a plategrill until 70°C (approximately 7 min). Only the *M.longissimus dorsi* was used for tasting. The test was carried out by a 6 member panel which scored the sample for tenderness, juiciness and flavour according a 6 point scale, where "6" is very desired and "1" not desired. The remaining part of the *M.longissimus dorsi* was used for shear force measurements. 6 cylindrical samples (∅ 1.25 cm) were cut parallel to the muscle fiber and the shear force was calculated on the basis of these measurements. Heating loss was recorded by weighing the samples before and after grilling.

RESULTS AND DISCUSSION

Various numbers of traits have been investigated which can be summarized under growth, muscling, carcass and meat quality.

Antagonistic relations between traits of the growth and traits of carcass and meat quality

There are predominately correlations with positive signs. Among the traits of the carcass quality only the proportion of primal cuts (pistola) and the meat/fat ratio in the carcass are negatively correlated to the criteria of the fattening yield (table 2). Antagonistic, but economical desired, is the negative correlation between growth and bone proportion of the carcass. Although the fat criteria are positively correlated with the fattening yield they have to be considered as antagonistic in case of the carcass composition, since increasing daily gain leads to an increase of the fatty tissue proportion in the carcass. The drip loss was in all samples very low (2.27 ± 0.6 %; range: 0.98 - 4.21), but positively correlated with the criteria of the fattening yield. The positive correlation in this case has to be considered negatively because higher daily gain show higher drip losses. In reverse are the negative correlations between fattening yield and shear force to be considered as positive since heavier carcasses show at least in tendency a better tenderness as far as the animals are slaughtered at the same age, which was the case in the present test. The negative correlations between fattening yield and grill loss have to be valued as positive since with increasing weight the grill loss is decreasing. The correlation coefficients of the pH-values -measured at different times after slaughter - and of the metabolites of the postmortem glycolysis to the fattening yield were not different from zero.

Antagonistic relations between the muscling and traits of the carcass and meat quality

The correlation coefficients between traits of muscling and carcass and meat quality traits are generally very low (table 3). It exists the tendency that the proportion of fatty tissue increases in the carcass at the improvement of muscling, if the muscling is subjectively scored. But if one takes the loin-eye-area as criterion for the muscling it shows antagonistic relations with the degree of fatness, i.e. the fatness is getting lower at increasing loin-eye-area. This can be valued positively in the view of the carcass quality. The muscling also shows low but negative correlations to the pH-value. There is a tendency to a faster pH-drop and a higher drip loss with increasing muscling.

Antagonistic relations between traits of the carcass composition and meat quality traits

The most important components of the carcass are the proportion of lean meat and the proportion of fatty tissue. There is a distinct antagonism between both proportions (table 4). Above that correlates the lean meat proportion negatively with some traits of the meat quality. At increasing proportion of lean is the meat getting darker and the sensory characteristics are scored inferior. Also the positive correlation between musculature and shear force has to be valued negatively by sense. The fatty tissue proportion correlates negatively with the shear force, a hint that with an increasing fat proportion the tenderness is getting better.

Antagonistic relations between different traits of meat quality

Between the pH-values 6 and 48 h p.m. and some quality traits a tendency to antagonistic relation is found. As well the 6 h p.m. pH-values as the final pH-values correlate negatively with the sensory traits of tenderness and flavour. The positive correlation with the shear force confirms the sensory judging of the tenderness (table 5). The negative correlations of the final pH-value to the colour and also to the drip and grill loss can not be attributed to DFD meat. The average final pH-value was 5.45 ± 0.05 (range: 5.34 - 5.59).

CONCLUSIONS

The results presented demonstrate some antagonistic relations. Clearly antagonistic are the relations between some carcass composition traits e.g. lean meat and fat proportion as well as between lean meat proportion in the carcass and some meat quality traits as marbling, tenderness and flavour. Between the fattening traits and muscling on the one hand and fat, drip loss and pH on the other hand, antagonistic relations were also found, but they are only in few cases significant. The samples were taken from a relatively limited material, which is therefore not representative for the breed "Fleckvieh", but the existence of antagonistic relations is as least a signal for an unilateral selection. It would be necessary to carry out a comprehensive analysis in order to counteract in time to prevent such developments as we know from pig breeding.

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AN INITIAL EVALUATION OF THE TAIL MEAT OF FARMED, FRESHWATER, JOHNSTON RIVER (CROCODYLUS JOHNSTONII) AND SALT-WATER (CROCODYLUS POROSUS) CROCODILES

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SUMMARY

Tail meat from farmed, juvenile, salt-water (SW) and sub-adult, freshwater (FW) crocodiles produced after "hot" - butchering and varied, but rapid, cooling, was found to be of an acceptable bacteriological and organoleptic standard. However, the limited data indicate that "hot" butchering and very rapid cooling may induce muscle shortening and suggest that these procedures should be investigated in larger numbers of animals slaughtered commercially.

INTRODUCTION

The sale of crocodile meat is a necessary adjunct to the sale of skins from crocodile farms, particularly in the case of freshwater (FW) crocodiles whose skins are less valuable. In this paper preliminary evaluations of the tail meat of two crocodile of each species are described.

Animals, capture, transport and slaughter

Tail meat was obtained from two, sub-adult, male, freshwater crocodiles (bodylengths 1.22 and 1.47 m) 4.4 and 5.4 years old and two, juvenile, one male and one female, salt-water (SW) crocodiles (bodylengths 1.55 and 1.65 m), 3.2 and 5 years old. On the crocodile farm they were subdued (jaws bound and placed in an hessian sack) after periods of intense struggle lasting less than 5 minutes, transported to the slaughter facility (20 minutes), and kept in shade until slaughter. Ambient temperature was 31°C and deep cloacal temperature 31.0 ± 0.21°C (S.D.). Animals were shot through the frontal part of the brain and their spinal cord severed; they were not exsanguinated. Immediately after slaughter the carcasses were transferred to an air-conditioned room, 24.9°C and skinned. About 30 min later each carcass was skinned, cut into portions (trunk, legs and tail) and, sequentially, put into a chest freezer to freeze. Rates of cooling, thus, differed with order of slaughter. Times for muscles to cool to 10°C were determined from logarithmic plots of recorded muscle temperatures.

Meat evaluation

The four tails, wrapped in polyethylene bags, were thawed for 49 hr at 5-6°C, and sampled for bacteriological evaluation. Four, 5 cm^2, core samples were taken, symmetrically, around the base of each tail and blended in 180 ml of 0.1% peptone water (PW). Samples were plated on Tryptone Yeast Extract Soya Glucose Agar (TYSG) with, or without, NaCl; 0.25 ml of a 30% solution of NaCl was spread over the agar surface before plating. Plates were incubated aerobically at 25°C for 4 days before Total Counts (TC) were made. The tails were weighed, the flesh (muscle and fat) separated from the bones and the bones weighed. The *Mm. ischiocaudalis* (IS) and *iliocaudalis* (IL) were dissected and weighed. The ultimate pH of both muscles was measured at 20°C and samples taken for sarcomere length measurement using a laser-diffraction method. Each muscle of each pair of IS muscles was weighed, one muscle was cooked at 60°C and the other at 80°C, tightly wrapped in polyethylene bags, in water baths, for an hour. The

TABLE 1: Total aerobic plate counts on TYSG, with or without 0.5% NaCl, (3 days at 25°C) of core samples taken from the base (proximal) of the tail

Tag No.	Species	Count (cm^{-2}) TYSG	Count (cm^{-2}) TYSG+0.5% NaCl
1	FW	4.59×10^4	3.5×10^4
3	FW	2.65×10^4	2.42×10^4
2	SW	3.8×10^4	1.92×10^5
4	SW	7.2×10^3	3.5×10^4

TABLE 2: Some objective measurements of tenderness of the cooked, 60°C or 80°C for 1 hr, striated muscle of the tail (IS)

Tag No.	Species	WBIY shear values(kg)		WB PF values(kg)		Compression values		Percent cooking loss	
		60°C	80°C	60°C	80°C	60°C	80°C	60°C	80°C
1	FW	2.1	3.3	2.5	3.6	1.0	1.3	23.5	38.1
3	FW	2.9	4.5	3.7	4.9	1.2	1.7	22.5	36.8
2	SW	2.7	4.5	3.2	4.8	1.4	1.8	21.5	37.9
4	SW	2.8	5.0	3.0	5.1	1.6	1.8	21.5	38.4

TABLE 3: Some characteristics of the segmented muscle of the tail (IL)

Tag No.	Species	Ultimate pH	Sarcomere length(m)	Juiciness	Flavour	Texture	Overall accept.
1	FW	5.62	2.23	4.74	3.87	3.00	2.63
3	FW	5.81	2.00	4.74	3.54	3.16	3.06
Specific Mean				4.74[a]	3.71[a]	3.08[a]	2.85[a]
2	SW	5.54	3.24	5.78	3.66	4.22	3.96
4	SW	5.70	2.09	5.14	3.81	3.83	3.63
Specific Mean				5.46[a]	3.74[a]	4.04[b]	3.80[b]
Overall Mean				5.01	3.72	3.55	3.32

[a] Specific means with different superscripts differ significantly, $P<0.05$. (v.juicy, v.good, v.tender, v.good = 0 to v.dry, v.poor, etc. = 10)

samples were dried, reweighed after cooking to determine cooking losses, and used for objective measurements, after they had cooled overnight at 1°C. Strips, 1 cm^2 in cross-section, were cut for Warner-Bratzler shear measurements and slabs, 1 cm thick, for Instron Compression (IC) measurements.

The IL muscle was used for subjective assessments. Steakettes, 2 cm thick, were baked in a forced-draught oven, at 230°C for 20 min. Duplicate samples, hot, were presented to a 10-member taste panel. They scored their opinions of the juiciness, flavour, texture and overall (organoleptic) acceptability. The divided scales were identified at the extremes (eg. very juicy and very dry).

RESULTS AND DISCUSSION

The mean proportions of bone in the tails of the two species were 19.1 (FW) and 13.6% (SW). The total plate counts are shown in Table 1.

Total plate counts were acceptably low. Recovery of bacteria from tails of SW crocodiles was increased when NaCl was added but this addition did not significantly affect recovery from FW tails. Oblinger et al. (1981) sampled *Alligator mississipiensis* meat and skins, on Plate Count Agar, at 20 or 35°C and found lower counts than those on the IC muscle.

The ultimate pH values of the striated IS muscles were 5.55 and 5.62 (FW) and 5.47 and 5.49 (SW) and the, corresponding, sarcomere lengths 2.01 and 2.29 (FW) and 1.79 and 2.51 μm (SW). Other objective measurements are given in Table 2.

The two IS muscles (regardless of species) of the two carcasses which cooled faster had shorter sarcomere lengths and there appeared to be an inverse relationship between the sarcomere length of the IS and IL muscles. This may have resulted from cold-induced shortening in the IS muscles distorting the tail and stretching the IC muscles and indicates that in a commercial situation the time of butchering of the flayed carcass and the cooling rate could have a significant effect on eating quality. The ultimate pH values of the striated, IS, tail muscles were less than those of the segmented, IL, muscles (Tables 2 and 3). Cooking losses at either of the cooking temperatures were similar to those of beef and sheep muscles. The Warner-Bratzler initial yield (IY) and peak force (PF) values and IC measurements indicated that the IS muscles were "tender" to "acceptably tender".

The taste panel (Table 3) found no specific difference in the juiciness, or flavour, of IL muscles but considered that this muscle of FW animals was more tender and more acceptable than that of the SW crocodiles. Overall, the panel found this meat to be tender, relatively bland, and of average juiciness. These data indicate that crocodile meat of an acceptable bacteriological and organoleptic status can be produced from farmed FW (sub-adult) and SW (juvenile) crocodiles. They also indicate that time of butchering (relative to time of slaughter) and cooling rates of carcasses or butchered cuts may influence tenderness.

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ESTIMATION OF CHANGES IN BEEF MUSCULAR TISSUE AT DIFFERENT PROCESSING AND STORAGE CONDITIONS.

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The object of the study is to estimate changes in muscular tissue during its storage in the refrigerated, sub-refrigerated and frozen state with preliminary processing and without it.

The samples removed from half-tendon beef tissue along the fibres were selected as the objects of the investigation. Changes in meat were estimated by the reaction of pH medium, elasticity modulus E. and the value of moisture removal B, all these values being determined by electom-etric, quasistatic and pressing methods using the devices KP-2, pH-121 and ID-1.

The investigations showed that the application of variable temperature conditions of refrigerating, electric stimulation and mechanical tenderisation prevents from shrinkage of muscular tissue caused by refrigerating and accelerates mechano-chemical processes in beef during its subsequent storage in the refrigerated state 1.5 - 3.0 times, in the subrefrigerated state - 4 - 8 times in the frozen state - 16 - 100 times. Some ideas of the essence of mechano-chemical changes in muscular tissue are formulated, their basis being the mechanism of formation and propagation of solutions in myosin filaments.

The results of the study of changes in beef during its storage in the refrigerated, subrefrigerated and frozen state with the preliminary processing and without it are presented. Changes in beef were estimated by the reaction of pH medium, elasticity modules and the value of moisture removal. The investigations showed that the application of variable temperature conditions of refrigerating, electric stimulation and mechanical tenderisation prevents from shrinkage of muscular tissue caused by refrigerating and accelerates mechano-chemical processes in beef during its subsequent storage in the refrigerated state 1.5 - 3.0 times, in the subrefrigerated state - 4-8 times, in the frozen state - 16-100 times. Some ideas of the essence of mechano-chemical changes in muscular tissue are formulated, their basis being the mechanism of formation and propagation of salitons in myosin filaments.

INTRODUCTION

Intensification of refrigerated meat processing results in some cases in deterioration of its quality due to muscular tissue shrinkage caused by cold. This phenomenon and the ways to prevent it are still studied insufficiently. It is essential to carry out extensive research concerning the influence of different factors on changes in meat. The object of the study is to estimate changes in muscular tissue during its storage in the refrigerated, subrefrigerated and frozen state with preliminary processing and without it.

PROCEDURE

The samples removed from half-tendon beef tissue along the fibres were selected as the objects of the investigation. Changes in meat were estimated by the reaction of pH medium, elasticity modules E and the value of moisture removal B, all these values being determined by electrom-etric, quasistatic and pressing methods using the devices KP-2, pH-121 and ID-1 (1984). The technological process-ing and storage of muscular tissue were performed under the following conditions.

Condition 1. Refrigerating at temperature 0°C and air velocity 2 m/s up to 4°C, storage at 0°C.

Condition 1a. Refrigerating at 0°C and air velocity 2 m/s up to 12°C in the surface layer, holding at 12°C for 18-20 h. Recooling and storage under condition 1.

Condition 1b. Electric stimulation at voltage 150 V, frequencyχ = 25 Hz during 70-100 s, recooling and storage under condition 1.

Condition 1c. Mechanical tenderisation with power 7 x 10^2 j/kg. Recooling and storage under condition 1.

Condition 2. Subfreezing muscular tissue at temperature -28°C, air velocity V=2 m/s up to t = -2°-3°C in the surface layer, storage at 7t = -2°C.

Conditions 2a, 2b, 2c. Technological processing of meat under conditions 1a, 1b, 1c respectively. Freezing and storage under conditions 3. Electric stimulation was carried out by means of an impulse stimulator, mechanical tenderi-sation was performed with the use of destructor IA-2, refrigerated processing and storage was done in chambers Grünland, Frigera, P - 10.

RESULTS

The results of the investigation of changes in values of elasticity modules E, the factor of the reaction of pH medium and the value of moisture removal B for muscular tissue during its storage in the refrigerated, subrefrigerated and frozen after the preliminary processing with the use of variable temperature refrigerating conditions (1a, 2a, 3a), electric stimulation (1b, 2b, 3b) and mechanical tenderisa-tion (1c, 2c, 3c) are presented in Fig.1-9.

DISCUSSION

The results of the study show that the change of physico-chemical properties of muscular tissue has a phase character. (1985). The shelf of meat being increased, values E and B increase in the initial period at all conditions and later decrease. The change of pH has an opposite tendency. While under conditions 1, 2 and 3 stiffening of meat (maxi-mum values of E and B and minimum value of pH) is observed respectively on the second day, the eighth - ninth day and in the fourth month, with the use of conditions 1a, 1b, 1 c stiffening occurs on the first day, under conditions 2a, 2b, 2c - on the first-second day, under conditions 3a, 3b, 3c - on the first-sixth day. The use of the preliminary process-ing prevents from cold shrinkage of muscular tissue. The investigations showed that the application of variable tem-perature refrigerating conditions, electric stimulation and mechanical tenderisation promotes acceleration of mechano-chemical processes in meat during its subsequent storage in the refrigerated state 1.5 - 3 times, in the subrefrig-erated state - 4-8 times, and in the frozen state - 16-100 times.

The data obtained were applied in the development of meat processing methods maintained by two author's certificates (1983, 1985). Of particular interest is the study of the reasons causing changes in meat. Processing from the

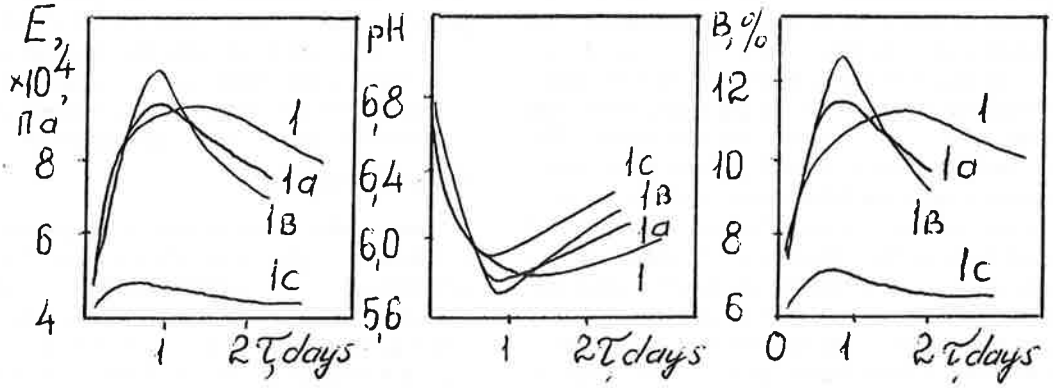

Fig.1 Fig.2 Fig.3

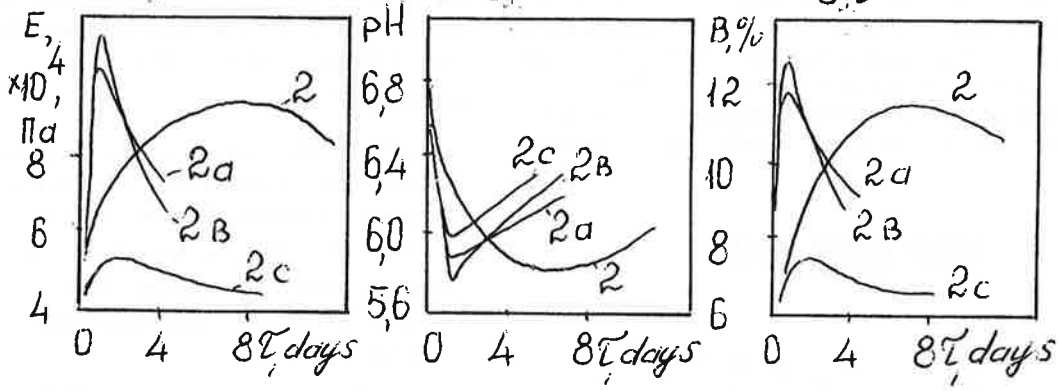

Fig.4 Fig.5 Fig.6

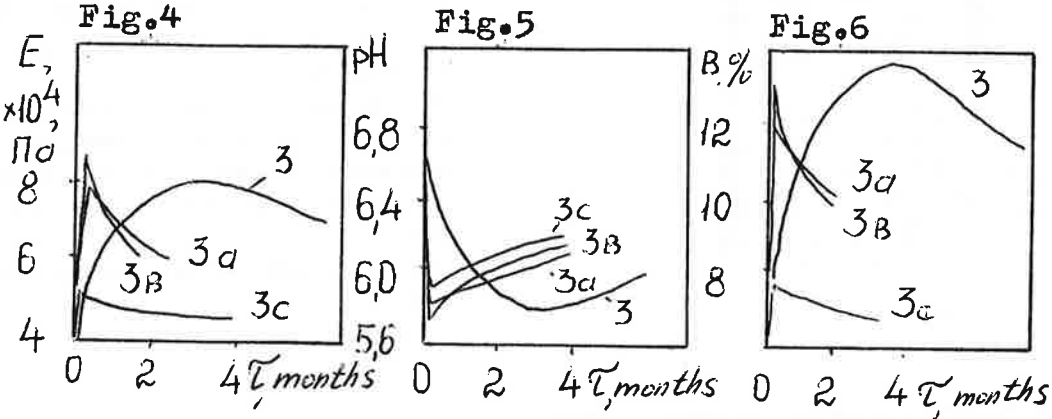

Fig.7 Fig.8 Fig.9

Fig. 1-9. The effect of the preliminary processing conditions on the change of beef muscular tissue properties during the storage in the refrigerated state (fig.1 - 3) subrefrigerated (fig. 4-6) and frozen state (fig. 7 - 9).

investigations carried out by us (1983) and the results obtained by a number of research workers (1978, 1980, 1982) the structural changes in muscular tissue can be account as follows (1985). Ceasing of an animal's life causes depolarisation of membranes in tissue systems containing ions and enzymes. Membrane depolarisation of end tanks of sacroplasmic reticulum causes the release of Ca^{2+} ions from them which results in hydrolysis of ATP molecules combined with myosin. In long spiral parts of myosin molecules the released energy gives rise to salitons. The latter are quasiparticles stable in nature (bound state of inner peptide excitation and local deformation) which more from molecule heads toward their ends located in the area of sarcomere centre (Fig.10). Movement of saliton along a protein molecule is accompanied by its local bending and increasing the diameter of a filament's pait formed by a beam of myosin molecules. The movement of the "swollen" part of the filament from its end toward the centre results in the displacement of actin protein filaments toward the centre of a sarcomere. An analogous phenomenon occurs on the other end of a myosin filament. The counter movement of actin filaments results in the reduction of the length of each sarcomere and thus in that of muscular tissue. Meat turns into the state of stiffening which is gradually followed by weakening. Electrolytes in myofibrillar space affect the penetrability of lysosome membranes, catepsins and other enzymes are released. The effect of enzymes on proteins results in the change of their physico-chemical properties increase of solubility, hydration and disturbance of saliton formation mechanism. All these processes develop in the proper period of time. The use of variable temperature refrigerating conditions, electric stimulation and mechanical tenderisation results in the change of penetrability of systems containing ions and enzymes and promotes acceleration of mechano-chemical processes in muscular tissue.

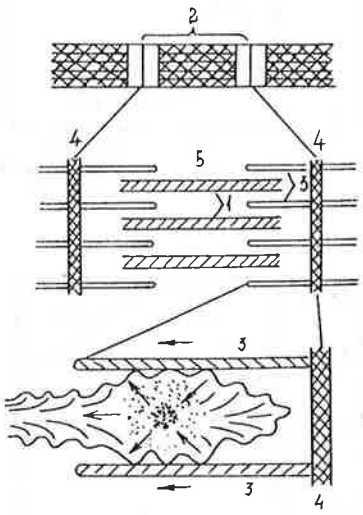

Fig. 10. Diaram illustrating the displacement of actine-filaments relative to miosin filaments due to the displacement of salitons inside miosine filaments: 1 - miosin filaments; 2 - sarcomere; 3 - actine filaments: 4 - cross-sectional membranes; 5 - the centre of miosin filaments.

CONCLUSIONS

It is shown that the use of variable temperature refrigerating conditions, electric stimulation and mechanical tenderisation prevents from cold fibre shrinkage and promotes acceleration of mechano-chemical processes in meat during its subsequent storage in the refrigerated state 1.5 - 3.0 times, in the subrefrigerated state - 4 - 8 times and in the frozen state - 16 - 100 times. The ideas of the essence of mechano-chemical changes in muscular tissue have been formulated. The mechanism of formation and propagation of salitons in myosin filaments serves as their basis.

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ELECTROSTIMULATION OF MEAT OF DIFFERENT PATTERNS OF AUTOLYSIS

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Literature on electrostimulation effects on the proteolytic activity in muscles is limited and contradictory. The authors studies the influence of ES on the catheptic activity (as exemplified with cathepsin D) in beef muscles of different autolysis patterns. Normal (NOR), DFD and PSE l. dorsi muscles dissected from carcasses within 3.6 x 10^3s post slaughter were tested. The left muscles were electrostimulated, the right ones served as controls. Samples were packed and stored at 277 K for 72 h. The free activity was recorded before ES and after 0, 24, 48, 72 h. The results indicate that ES of the NOR meat causes initially and increased (3-fold) activity, it remaining twice as high and 1.5 times as high as compared to controls after 24 and 48 h storage, respectively. At 72 h the enzymic activity decreases, while it reaches the peak value in the control samples. Similar relationships are derived for DFD and PSE meat, though the proteolysis extent in such meat is much lower. With account for the fact that DFD meat constitutes a high percentage in the total beef production volume, one should selectively decide on meat electrostimulation.

The catheptic activity (as exemplified with cathepsin D) in the extracts of normal (NOR), DFD and PSE beef muscles after electrical stimulation (ES) and in the course of autolysis (2, 24, 48, 72 h) was evaluated quantitatively. The results indicate that ES of the NOR meat causes initially a 3-fold increase of the activity; it remains twice as high and 1.5 times as high as compared to controls after 24 and 48 h, respectively. At 72 h the enzymic activity decreases while it reaches the peak value in controls by the same period. Similar relationships are derived for DFD and PSE meat, though the extent of proteolysis in this meat is much lower. DFD meat constitutes a high percentage in the total beef production, therefore one should selectively decide on meat stimulation.

INTRODUCTION

Certain effects upon the muscle tissue of animals during or after slaughter can retard or accelerate enzymes released from lososomes and thus influence the intensity of autolytic processes. The results of determinations of the total, free and lisosome-bound activity of cathepsin C and ß-glucuronidase (Melo et al. 1974; Dutson et al. 1980) showed that ES contributed to a faster release of enzymes from their limiting structures to cytoplasm. A comparison of the histochemical determination of acid phosphates activity and the assay of the free activity of cathepsin D in beef muscles with the normal autolysis (NOR) after ES (Gorshkova et al. 1986) evidence activation of lisosomal proteases. At the same time Wu et al. (1985) found no effect of ES on the lysosomal enzymic activity. Dutson et al. (1982) and Fjelkner-Modig and Ruderus (1983) recorded no significant advantages of ES-treated DFD meat (muscle colour, ultimate pH, strength and sensory qualities) as compared to non-ES meat. There was no data on the influence of meat ES in case of different patterns of autolysis (NOR, DFD, PSE) upon the activity of tissue proteases. The reported here experiments were performed to elucidate the expediency of electrical stimulation of DFD meat.

EXPERIMENTAL METHODS

As test objects served NOR, DFD and PSE l. dorsi muscles dissected from beef sides within 2.7 x 10^3s. The right muscles were control The left (test) muscles were stimulated (220 V, 1 Hz, pulse duration 0.4 s, pulse period-to-pulse duration ratio 0.6 s the total ES time 3 x 10^2s). Samples were packed and stored at 277 K for 73 h. The free activity of cathepsin D was determined in control and test samples in the course of autolysis at 2, 24, 48 and 7 h. The lisosomal fraction was isolated from muscle according to Stagni and De Bernard (1968). The free activity of cathepsin D was found according to Caldwell and Grosjean (1971) with some modifications. Protein concentration in the headspace liquid was measured according to a modified method by Whitaker and Granum (1980).

RESULTS

The Figure illustrates that the activity of cathepsins in stimulated NOR meat increased by 3-fold as compared to non-stimulated samples. During autolysis the activity of tissue proteases continues to grow and becomes twice and 1.5 times as high 24 and 48 h later (respectively) as compared to control samples. A similar relation was observed in case of ES and DFD and PSE meat, though changes in the enzymic activity here are less pronounced. Thus, after stimulation of PSE meat the catheptic activity grew by 1.5 times and of DFD meat by about 1.3 times. 24 h post slaughter the catheptic activity in stimulated PSE meat remains 1.3 times higher as compared to controls, whereas in case of DFD meat it is practically similar both in test and control samples.

From the Figure it is also clear that 24 h post slaughter the catheptic activity in stimulated PSE and DFD meat starts to decrease. For control PSE and DFD samples activity reduction becomes noticeable 48 h post slaughter.

DISCUSSION

The problem of ES influence upon changes in meat has long been discussed. There are however very few publications (Dutson et al. 1982; Fjelkner-Modig and Ruderus. 1983) which deal with the problem from the viewpoint of variations in pHs of the meat supplied. It is known that DFD meat constitute a high percentage in beef production (Tatulov, 1984; Warriss, 1984). Our results demonstrated that ES has an effect on the catheptic activity of meat with different patterns of autolysis. After stimulation of NOR meat its level is however much higher than in PSE and DFD meat. Such a difference in the results is due, first of all, to different initial levels of the enzymic proteolytic activity in hot NOR, DFD and PSE beef muscles prior to ES. Gorshkova et al. (1987) showed that a high level of tissue proteases in DFD and PSE meat results from the destabilisation of lisosomal membranes and from a higher release of enzymes from lisosomes. Therefore, electrical treatment of such muscle tissue had no significant effect on the muscle tissue as was observed in NOR beef. Of great importance is, obviously, the course of glycolytic processes, which is known to differ considerably in NOR, DFD and PSE meat. Thus, Pezacki (1980) thinks disturbances in the processes of glycogenoly-

sis in animals as a result of stress causes changes in metabolic dynamic equilibrium, it, in its turn, influencing the activity of tissue enzymes during meat autolysis. This may be the reason of the fast reduction in the activity of enzymes both in stimulated and non-stimulated DFD and PSE meat.

CONCLUSIONS

The data obtained allow to believe that different effects of ES upon NOR, DFD and PSE meat requires a selective approach to deciding on the necessity and expediency of raw meat electrical stimulation.

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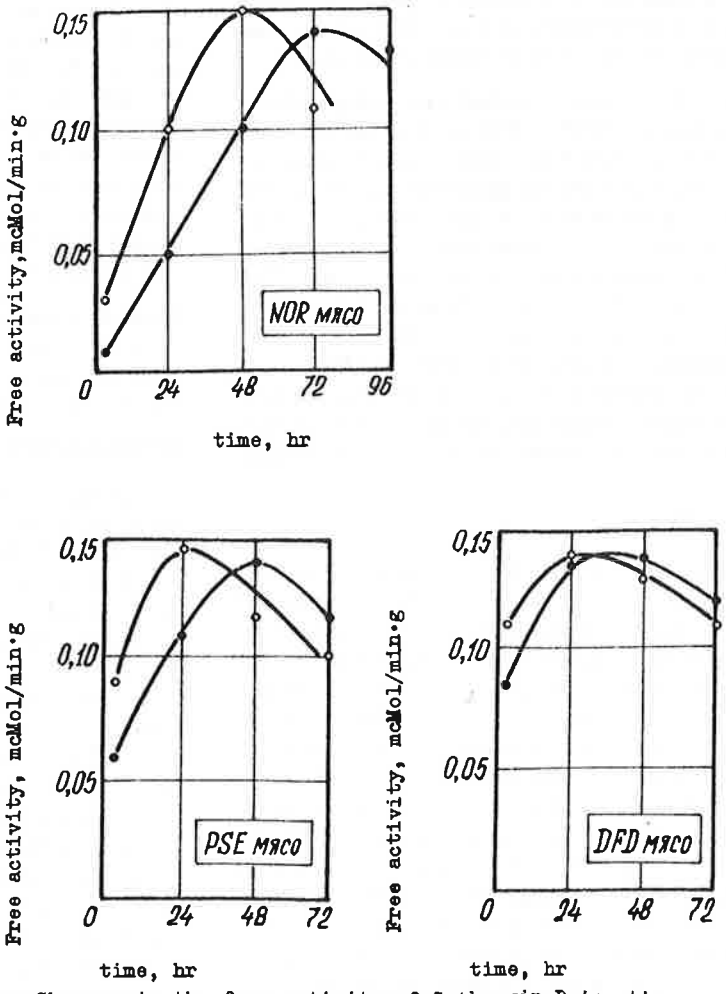

Fig. Changes in the free activity of Cathepsin D in stimulated (o) and non-stimualted (•) NOR, DFD and PSE beef in the course of autolysis

MULTIELEMENT CONTENT OF RAW AND COOKED BEEF AS DETERMINED BY NEUTRON ACTIVATION ANALYSIS

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SUMMARY

Neutron activation converts a stable isotope into a radioactive nuclide which emits gamma and x-radiation with characteristic energies and half lives. Neutron activation analysis as used to determine twelve elements in beef fat and muscle showed good agreement on muscle content of aluminium, bromine, chlorine, calcium, iodine, sodium, titanium and vanadium with those in the literature. Elemental content ot subcutaneous fat was reduced by roasting while most muscle elemental content increased under these conditions. Chlorine in the raw, subcutaneous fat was highly correlated ($P < 0.01$) with the Armour tenderometer. Thus, determination of chlorine by a portable non-selective electrode in a beef cooler is suggested as a rapid method to predict beef tenderness.

INTRODUCTION

Neutron Activation Analysis (NAA) is a technique in which a stable isotope, when immersed in a flux of neutrons, undergoes a nuclear transformation producing a radioactive nuclide which emits gamma and x-radiation with characteristic energies and half lives Lyon (1964). Activation analysis was defined by Kamen (1951) as a nuclear reaction analogous to a chemical reaction. Furr et al. (1975) reported that animal tissues (liver, kidney muscle and spleen of guinea pigs) were analyzed for 39 elements using NAA. Of the 39 elements they reported, twelve: aluminium (al), bromine (br), calcium (ca), chloride (cl), copper (cu), iodine (I), potassium (k), magnesium (mg), sodium (na), tin (sn), titanium (ti) and vanadium (v) are the subject of this paper.

OBJECTIVES

The objectives were: 1. To determine the usefulness of NAA as a method for determination of elemental content of beef muscle and fat. 2. To determine the elemental loss/gain encountered in cooking- 3. To relate elemental content of beef to its' tenderness.

MATERIALS AND METHODS

Twenty-eight USDA choice carcasses were divided into four groups based on Armour Tenderometer readings, Hansen (1 9 72) as follows : 5.0-6.2 Kg ; 6.3-7.1 Kg; 7.2-8.1 Kg; and 8.2-10.0 Kg. A wholesale rib removed from each carcass was divided into three sections: 6-7-8 ribs for cooking, 9-10-11 ribs for proximate analysis, and 12th rib to obtain the raw cores (1.27 cm dia) for tenderness as described by Bratzler and Smith (1963) and Kramer et al. (1951). The remainder of the 12th rib was used raw for NAA analysis. The 6-7-8 ribs were roasted to an internal temperature of 76°C. The cooked *longissimus* muscle over the eighth rib was sliced 3.81 cm thick and cores 1.27 cm in diameter were removed for machine tenderness values. The remainder was used for NAA analysis. A drip sample was obtained from the roasting pan on removal from the oven. A cooked fat sample was excised from the roast over the eighth fib. The samples were activated as specified by Furr (1971). Standards for al, br, ca, cl, cu, i, k, mg, na, s, sn, ti, and v were run as a reference. A lithium- germanium detector was used to read the gamma-ray emissions. The data were fed to a magnetic tape recorder which read into an IBM 360 computer. The data were analyzed by a computer program designed to print out the element concentration in ppm as described by Roscoe and Furr (1976).

RESULTS AND DISCUSSION

Elemental concentrations in muscle from guinea pigs, Furr et al. (1975); and bovine, Westing (1978) are compared to values obtained in this study, Table 1, Generally, there is good agreement on the muscle content of al, br, cl, ca, i, na, ti and vanadium. The levels of k, mg, and sn were all higher for bovine muscle in this study than those reported by Westing (1978) or those in the guinea pig, Furr et al. (1975). However, the effect of varied elemental levels in the rations of animals was not a part of this study whereas those previously mentioned were designed to study this effect. Roscoe and Furr (1976)

Table 1. Comparisons of element concentration of moisture-free muscle from guinea pigs and bovine.

Element	Guinea Pigs		Bovine			
	Furr, et al. 1975		Westing, 1978		Kelly & Williford 1988	
	ppm	± S.E.	ppm	± S.E.	ppm	± S.E.
Aluminum++	19.6	1.1	8.67	.96	13.4	1.18
Bromine	6.0	2.1	2.47	.19	5.6	0.73
Chlorine	---	---	1103.0	52.78	1239.17	73.68
Calcium	287.0	58.0	99.35	10.29	310.00	28.99
Copper	---	---	45.07	1.89	63.55	0.40
Iodine	---	---	0.14	.09	0.42	0.18
Potassium	10250.0	763.0	1172.7	464.9	15267.62	628.95
Magnesium	832.0	33.0	622.6	24.1	1580.41	85.02
Sodium	1334.0	174.0	1219.0	51.2	1329.69	65.55
Sulfur	12520.0	939.0	---	---	17292.97	1784.73
Tin	<0.5	---	.357	.18	14.48	1.84
Titanium	8.8	1.7	1.86	.37	8.93	1.17
Vandadium	0.05	---	0.12	.0031	.041	0.007

++ Reported as Al + Si in Furr et al 1975.

Table 2. Gains and losses of elements in fat and muscle during cooking

Element	Fat		Muscle	
	% loss	% gain	% loss	% gain
Aluminum	14.5			18.5
Bromine	28.5			43.8
Chlorine	13.1			21.7
Calcium	19.5			1.3
Copper		5.9		61.2
Iodine	50.0		---	---
Potassium		108.4	8.3	
Magnesium		39.1		1.4
Sodium	10.5			9.3
Sulfur	24.6			45.0
Tin	19.7			6.7
Tinanium	22.1			10.8
Vanadium	33.3			41.7

published results obtained by the Neutron Activation laboratory at Virginia and compared them to National Bureau of Standards certified values for bovine liver (NBS-SRM 1577) as reported by Becker (1976). Three bovine liver samples were analyzed from heifers and steers by Westing (1978) who reported that levels of br and mg were within the ranges of those certified. These values have not been established for beef muscle.

Table 2 shows the gains and losses of elements in beef fat and muscle as a result of roasting. Generally, the elements in fat were reduced by roasting. These losses ranged from 10.5% of the na to 50 percent for the i. Only cu, k, and mg increased in fat as a result of roasting. Concentration in the fat when roasted is partially explained by the drip loss.

The back fat liquifies during roasting and bastes the lean portion of the roast where some of the cl ions may be bound. The increase in cl concentration in the cooked lean demonstrates this possibility. In muscle, the pattern observed with fat was reversed. With the exception of k, which was reduced by 8. 3 percent on roasting and i having no change, all of the elements increased as a result of cooking. Percent increases in cu, s, and br were 61. 2, 45.0, 43.8, respectively. Copper is the only element studied which has a recommended dietary allowance by the National Academy of Science (1980). Although pronounced CU deficiencies in the free-living population of the U.S. are very rare, dietary surveys have shown that average intakes far below the recommendation are quite common (Mertz 1981). Since this work reports an increase in the cu content as a result of roasting, bovine muscle may well be recommended as a source of dietary copper. Vanadium content of bovine muscle was also increased in this study by 41.7 percent. Mertz (1981) states "that ranges for the 'new' trace elements, va, ni, si and ar are unknown."

Table 3. Correlations between element content of beef tissues and tenderness.

Element and sample	Armour Tenderometer	Warner-Bratzler	Allo Kramer
Aluminum			
Raw fat	---	---	-.349
Cooked fat	+	---	+
Raw Lean	0.448*	0.252	-.200
Cooked lean	0.387**	---	+
Drip	0.512	+	0.234
Chlorine			
Raw fat	-.612**	-.234	---
Cooked fat	-.452*	---	---
Raw Lean	-.281*	+	---
Cooked lean	-.463*	-.273	-.257
Drip	-.392	+	+
Calcium			
Raw fat	---	---	-.212
Cooked fat	-.310	---	---
Raw Lean	-.230	---	-.210
Cooked lean	---	-.218	-.213
Drip	-0.622**	+	+
Sodium			
Raw fat	-.580**	-.216	---
Cooked fat	-.455**	---	+
Raw Lean	---	---	-.214
Cooked lean	-.442*	-.205	-.276
Drip	-.357	---	---

* = P<.05 ** = P<.01

The correlations of elemental concentration to tenderness of beef as determined by the Armour tenderometer, Hansen (1972) ; the shear device as reported by Bratzler and Smith (1963); and the shear press of Kramer et al. (1951) are presented in Table 3. Chlorine in the raw samples taken from the subcutaneous fat over the longissimus muscle was highly correlated ($P < 0.01$) with the Armour tenderometer measurements. This negative correlation ($r = -.612$) indicates that as the tenderometer readings decrease i.e., the meat becomes more tender, the concentration of total cl in the raw fat increases. The correlations between cl in other tissues i.e., cooked fat, raw lean, cooked lean and the drip are all significant ($P < 0.05$) but not of the magnitude of the raw fat vs. the tenderometer. The highly significant ($P < 0.01$) correlations (data not shown) between concentration of cl in the raw fat and lean, $r = 0.69$, and the cooked fat and lean, $r = 0.67$ show that the content of this element in the fat is highly related to muscle level. This high relationship between cl concentration of the fat and tenderometer value suggests the possibility of taking a back fat sample from the carcass and, based on its cl content, predicting tenderness, or the cl concentration of the fat could be determined rapidly in the cooler by a portable ion-selective electrode. The correlations between the cl concentration and tenderness as measured by the Warner-Bratzler and the Allo-Kramer were not significant. This may be due to using the Armour tenderometer values for the selection of carcasses in this study. As with cl, the tenderometer was found to be significantly correlated with the na content of both raw ($r = -.580$) and cooked ($r = -.455$) fat. The implications concerning nas' decrease in cooked fat and increase in cooked lean are similar to those discussed for cl. Both elements were found in fat and lean but approximately 30 percent more na was lost in the drip (9.99 ppm) than chlorine (7.56 ppm). The strong affinity of the two elements for each other is indicated by the high correlation ($r = 0.97$) between chlorine and sodium in the raw fat. The effect of salt concentration on muscle tenderness has been widely studied and researchers agree that increased salt concentrations in meat increases tenderness, (Deatherage 1963; Wierbicki et al. 1957). A positive correlation ($P < 0.05$) was found between the tenderometer measurements and the al content of raw and cooked lean, $r = 0.45$ and 0.39, respectively. The ca content of the drip was highly negatively correlated ($P < 0.01$) with the tenderometer. None of the other elements determined were highly correlated to tenderness in this study.

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HEAT-INDUCED GELATION OF ACTOMYOSIN BY PRESSURE TREATMENT

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When myosin, which plays an important role in binding capacity and water holding capacity of meat products, is given pressure treatment, gel is formed at low salt concentration around 0.2 M, where myosin usually does not form gel. Myosin exists in meat in the form of actomyosin at the time it is used for processing. Therefore, the effect of pressure treatment on heat-induced gelation of actomyosin was investigated in this study.

Actomyosin was prepared from porcine longissimus muscle by using the method of Szent-Györgye. The prepared samples were adjusted the required pH value (5.0-9.0), the protein concentration (20 mg/g or 30 mg/g) and the salt concentration (KCl; 0.05-1.0 M). The samples were then filled up into plastic containers and pressured up to 150 MPa for 10 min at 0°C. After pressure treatment, the samples in containers; along with non-pressure treated control samples, were heated at 70°C for 30 min, then the gel strength and the work done value were measured. The gel network of actomyosin was observed by scanning electron microscope.

Pressure treatment of actomyosin samples was most effective at pH 6.0, where actomyosin formed gel with fairly high water holding capacity at low salt concentration. The effect of pressure treatment was most remarkable when the pressure employed was raised to 100 MPa in low salt concentration of 0.25 M KCl, and to 150 MPa in high salt concentration of 0.7 M KCl. Such effect continued for at least 15 days. In conclusion, the heat gelling ability of actomyosin was clearly improved by pressure treatment.

STRUCTURAL CHANGES IN BEEF MUSCLE PROTEINS DURING HEATING

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Conformational changes in the muscle proteins of uncured beef having pH 6.6 and 5.4 were studied with the protein intrinsic fluorescence method in the process of meat ageing for 3 to 96 hours.

It was found that during heating

- conformational changes in beef muscle proteins occur in several steps characterised with structural transitions of denaturation or coagulation type;
- the basic differences in the pattern and temperature ranges of structural rearrangements of beef myofibrillar proteins are observed at 50-9°C, the ageing time and meat pH being of great importance;
- irrespective of pH, within the first hours post slaughter beef is characterised with the development of coagulation at quite low temperatures (60-70°C) in the structure of its muscle proteins;
- the denaturation loosening of the structure of meat myofibrillar proteins post rigor (pH 6.6) occurs at the temperatures up to about 70°C, whereas in PSE beef (pH 5.4) denaturation takes place only up to about 60°C.

The quality and yields of the finished products are known to be greatly related to the state of the structure of meat myofibrillar proteins. Technological processing, heating, for example, effects considerably the pattern and extent of the conformational changes in meat proteins. The knowledge available is insufficient, especially with account for large variations in the raw meat materials delivered for processing (Solovyov, 1966; Sokolov, 1970; Acton and Dick, 1984; Honikel & Kim, 1986).

During meat ageing post slaughter considerable biochemical and structural changes occur in muscles, which determine the water-hold capacity (WHC), tenderness and juiciness of both raw and heated meat, the ageing time being a very important factor (Davey & Graafuis, 1976; Hamm, 1982; Lee and Schön, 1985; Hecht, 1987).

Meat pH after slaughter and the course of its changing during ageing have a marked influence upon meat quality (Monin & Selklier, 1985; Honikel & Kim, 1986; Hofmann, 1987).

Knowing how the structure of muscle proteins changes in the process of heating due to these factors, one can regulate the heat treatment of different raw meats in such a way as to ensure stable manufacture of high-quality products.

The purpose of the present work was to study changes in the conformation of beef myofibrillar proteins during heating, meat having different pH-values and being taken at different intervals post animals slaughter.

Studies were carried out on beef l. dorsi muscles with pH 6.6 and 5.4. Samples were assessed at 3, 24, 48, 72 and 96 h post mortem. The 24-96 hour samples were polyethylene-packed and stored at 4±1.5°C.

Structural changes of muscle proteins were studied with the protein intrinsic fluorescence method in the process of heating, as described by Oreshkin et al. (1985). The experiment was repeated four times, muscles of similar type and pH were chosen for parallel tests. Data spread was ±1 nm and ±2°C.

Fig. 1 shows the relationships of the main fluorescence parameters of the actomyosin proteins (Permyakov et al. 1986), viz., those of the maximum location ($\bar{\lambda}$) and fluorescence yield (S) to beef temperature (pH 6.6) during 3-96 h ageing post mortem. Curves are of a quite complicated and ambiguous nature depending on ageing time. Thus, 3 h post slaughter two pronounced shifts of the maximum of the fluorescence spectrum toward the long-wave region are obvious in the meat: at 50-62°C and at 67-75°C. It indicates that within these temperature ranges there occur the denaturation twisting of protein chains and their structure loosening. At 62-67°C and at above 75°C the fluorescence spectrum maximum shifts towards the short-wave region, it evidencing the occurrence of an opposite process at these temperatures, viz., that of inordinate approaching and coalescence of protein chains, i.e. coagulation. Considerable changes in the fluorescence yield within the above temperature ranges show that such conformational alterations are of a general character, not localised at certain spot of the structural arrangement of the proteins of the actomyosin complex.

24 h post mortem changes in the conformation of the muscle proteins of such beef during heating occur in a different way. Now denaturation loosening lasts up to 68-70°C and at 75-83°C, coagulation taking place at 68(70)-75°C. From the 48th hour post mortem and through the 96th hour considerable changes in proteins conformation during heating are taking place as two rearrangements: denaturation at 55-70°C and coagulation at 70-80°C.

The behaviour of muscle proteins during heating of beef having a lower pH-value (5.4) (it is considered as PSE-beef) differs greatly from the one described above. Thus, in case of ageing for 3, 24, 72 and 96 h denaturation transitions in beef proteins at 50-62°C and 68-78°C are evident, while at 60-70 and above 75°C coagulation transitions are observed. And it is only 48 h post mortem that one denaturation transition at 50-68°C and one coagulation transition at above 75°C occur in such meat. A comparison of changes in the muscle protein structure during heating of normal and PSE beef demonstrates that in both cases within the early post mortem period these changes are similar, their important feature being coagulation at sufficiently low temperatures (60-70°C). However, the similarity disappears one day post slaughter. Denaturation is developing up to 70°C in the protein structure of normal meat and only up to 58-60°C in PSE beef, coagulation following denaturation at 60-70°C being extensive and, obviously, covering a greater part of the structure of myofibrillar proteins and filaments if judged by an abrupt fall of the fluorescence yield. Though, at higher temperatures (68-75°C) denaturation loosening of the protein structure in PSE beef re-appears; the development of extensive coagulation compacting of the structure at comparatively low temperatures (60-70°C) is however extremely undesirable during meat heat treatment.

CONCLUSIONS

In the process of uncured beef heating the following changes in its myofibrillar proteins take place:

- within the first hours post mortem at 50-90°C two rearrangements in protein conformation occur which are accompanied with the denaturation loosening of protein structure: at 50-62°C and at 68-75°C; as well as two rearrangements of the opposite, coagulation character: at 62-68 (70°C) and at above 75°C. These changes are observed both in normal (pH 6.6) and PSE beef (pH 5.4). It is necessary to emphasise that at such ageing time coagulation proceeds at comparatively low temperatures (60-70°C), this determining, probably, toughness of the finished products prepared from beef treated within the first hours after slaughter (Hamm, 1982);

- at 48-96 hours post mortem the denaturation process in the proteins of normal beef 9pH 6.6) occurs up to 70°C, further heating causes coagulation rearrangements in the protein structure;

- in PSE beef (pH 5.4) low temperature coagulation at 60-70°C proceeds practically through the 4th day of ageing.

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CHANGES IN THE PROTEOLYTIC ACTIVITY OF LISOSOMAL ENZYMES IN PORK MUSCLES UNDER ELECTRICAL AND MECHANICAL EFFECTS

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SUMMARY

The catheptic activity in pork cured under electrical and mechanical effects was studied biochemically and histochemically. Experiments were made on pork l.dorsi muscles after 2.7 x 10^3 s after slaughter. The right muscles were electrically treated (electrostimulated), injected and massaged in a vacuum mixer. The left muscles were first injected, then electrically treated (electromassaged) and vacuum-massaged. It was established that electrostimulation and electromassaging cause an increase in the catheptic activity. Histochemical studies of hot meat indicated a low activity of the enzymes, it being reflected in the diffusion distribution of the acid phosphatase. Electrostimulation and electromassaging result in the destructive changes of muscle fibres and in the release of big amounts of the enzyme. After vacuum-mechanical treatment a high activity of the acid phosphatase was found. The purposeful application of electrical and mechanical effects to meat activates tissue enzymes and, thus, allows to intensify the process of ageing and curing.

INTRODUCTION

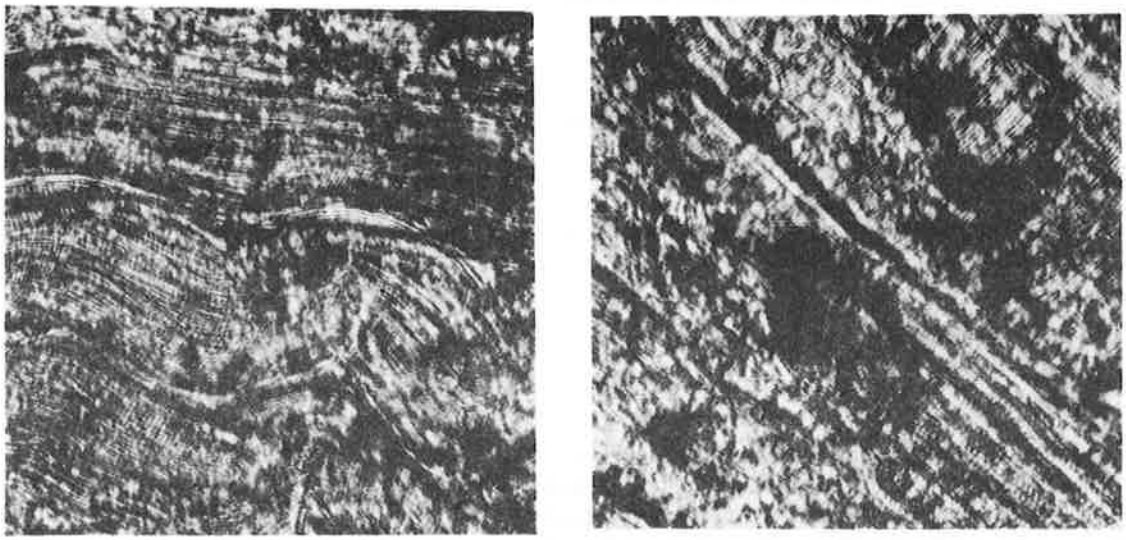
After an animal dies, various enzymic systems are activated in the tissues, which results in meat consistency improvement, in the accumulation of flavour compounds, in a higher digestibility (Bolshakov et al., 1986). Studies of muscle fibres histostructure at different stages of meat autolysis indicated the start of decomposition processes which are connected, according to Dutson et al. (1980) with tissue proteolytic enzymes. In the manufacture of cured meat products raw meat materials are treated in different ways to improve their consistency, to impart a specific flavour, to reduce losses and production process. Undoubtedly, enzymatic processes are of importance for ham and smoked meats technology. Recently, electrostimulation, electromassaging and mechanical massaging of cured meat are finding wider application, they effect the enzymic systems and shortening meat ageing and curing time. In this respect, we performed biochemical and histochemical studies into the catheptic activity of pork muscle tissue.

PROCEDURE

Tests were made on l.dorsi muscles of 9-10 month-old Kemerovo pigs. Hot muscles of the right sides were electrostimulated with the current of commercial frequency (220 Volts, pulse duration 0.4 s, pulse period-to-pulse duration ratio 0.6 s, the total time 3.10^3s) 2.7 x 10^3 a post mortem. Then they were injected with a brine having the density of 1,100 kg/m^3 and consisting of 14% NaCl, 0.075% $NaNO_2$, 1% sugar (at the injection level 10% of the meat weight) and massaged in a vacuum-mixer at 0.35 rps and 283-285 K, at the residual pressure of 29.4 kPa for 7.2 x 10^3 s. The left muscles were injected with the same brine, electromassaged for 3 x 10^2 s and mechanically treated under vacuum for 7.2 x 10^3 s. After every treatment the catheptic activity was biochemically determined according to the earlier described procedure (Kudryashov et al., 1987), and the histochemical pattern of the activity distribution of acid phosphatase, a marker of lisosomal hydrolases, was examined according to Gomori (1950).

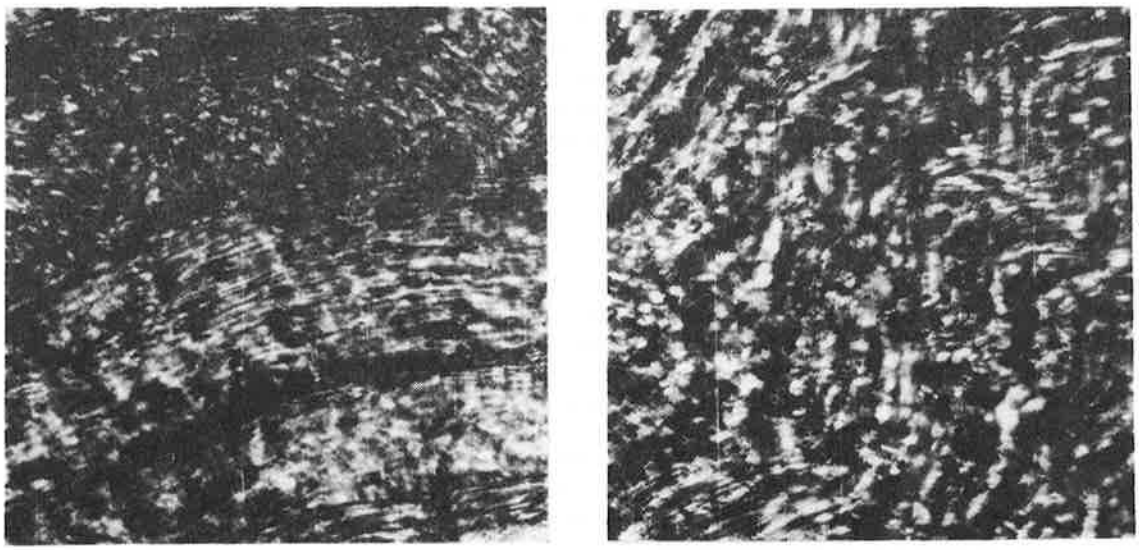
RESULTS

The biochemical results obtained show that the catheptic activity in hot muscles is weak and equal to 0.22-0.28 mcMoles of tyrosine per gram of muscle. Electrostimulation and electromassaging of cured muscles result in a higher activity of proteases (by 2.7 and 2.1 times, respectively). The following vacuum-mechanical treatment of the left and right muscles caused an increase in the proteolytic activity of tissue enzymes. The comparison of biochemical and histochemical results demonstrated the increased activity of tissue proteases during electrical and mechanical treatments. Histostructural data show that just after slaughter the location of muscle fibres in pork is rectilinear, acurragated and folded nature of fibres is evident with some differences in thickness (Fig. 1a). There are few contraction knots, cross striations are well pronounced, sarcolemma is tightly bound with sarcoplasm. The fibres are, mainly, close to each other. The lisosomal enzyme is distributed through the muscle fibre sarcoplasm diffusely and as individual aggregations of dark brown grains. At sarcolemma damaged spots there are found bigger aggregations of enzymes. Electrostimulation results in the destructive changes of muscle fibres as moderate damages, deformation, the occurrence of spaces inbetween fibres (Fig. 1b). Cross striations are not typical of all the fibres. Many fibres are swollen, connective tissue interlayers are loosened. Large amounts of the enzyme are observed between fibres, among protein grains and in the connective tissue interlayers. Acid phosphatase distribution in muscle fibre sarcoplasm is more pronounced in test samples. Injection of electrostimulated meat with brine followed with vacuum massaging causes a considerable extent of destruction of muscle fibres and multiple cross-breakages (Fig. 2a). The fibres are swollen, not clearly defined, cross striations are poorly seen. The location of muscle fibres is rectilinear, wavy or zigzag-like, slits among them are filled with a grained protein mass. The histological pattern resembles a monolith structural link of muscle tissue elements. An appreciable amount of the enzyme present is evenly distributed as small blocks throughout the fibres. In the damaged myofibrils practically no enzyme is found. When studying the influence of electroeffects upon pre-injected hot muscles, it was established that the fibres are moderately swollen and have different configurations (rectilinear, wavy or zigzag-like) (Fig. 2b). There are spaces, slits and microcracks among the fibres. In many places the muscle fibres are deformed and destructed. It was shown histochemically that, in case of cured meat electromassaging, a diffuse distribution of the enzyme without clear local



a b

Fig 1. Acid phosphatase distribution in hot pork muscles (a), after electrostimulation (b) (X 500)



a b

Fig.2. Acid phosphatase distribution in pork muscles after electrostimulation and vacuum-mechanical treatment (a), after electromassaging (b) (X 500)

aggregations is seen in the cracks and among the fibres. Noticeable is the enzyme in the grained protein mass and connective tissue interlayers. The activity of the acid phosphatase is higher than in the hot non-treated meat. The character of the microstructural changes of muscle tissue, mechanically treated under vacuum after massaging, is similar to that of vacuum-massaged electrostimulated muscles (Fig. 3). No cross-striations are seen. The enzyme is distributed throughout the fibre sarcoplasm mainly in a diffuse way, though there are some big local aggregations.

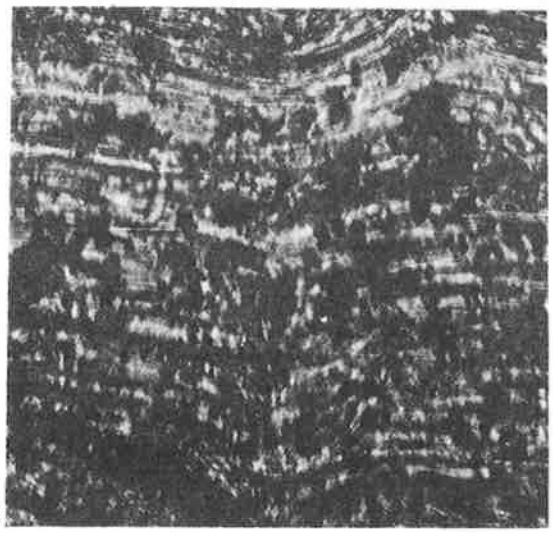


Fig. 3. Acid phosphatase distribution in pork muscles after electromassaging and vacuum-mechanical treatment (X 500)

DISCUSSIONS

The recorded low catheptic activity in hot pork muscle indicate a retarded course of autolytic processes at the initial stage, it being confirmed with the data by Pavlovsky and Simbiryova (1974). An increase in the enzymic proteolytic activity after electrical stimulation and vacuum-mechanical treatment implies destructive processes in the tissues at the lisosomal level. We derived similar data when studying the nature of the catheptic activity in beef (Bolshakov et al., 1986). Dutson et al. (1980) studies the effect of electrostimulation on the activity of lisosomal enzymes in mutton muscles and observed its growth due to the decomposition of the membranes surrounding enzymes. Histological and histochemical analyses demonstrated that electrical and mechanical effects result in enzyme release and localization at the points where muscle tissue elements are damaged.

CONCLUSIONS

On the basis of the performed biochemical and histochemical studies it may be stated that electrical treatment of uncured and cured pork causes a growing free activity of lisosomal enzymes due to their release from the surrounding membranes. The following vacuum massaging contributes to a uniform distribution of the enzymic activity by the structure of fibres, it enabling improved consistency of the raw meat and, therefore, of the finished product. A higher activity of proteases in pork muscle as a result of the application of intensive treatments renders it possible to shorten cured meat ageing duration and to ensure high processing qualities of the raw meats and a high quality of the finished products.

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MEAT PIGMENT DETERMINATION BY A SIMPLE AND NON-TOXIC ALKALINE HAEMATIN METHOD - AN ALTERNATIVE TO THE HORNSEY AND THE CYANOMETMYOGLOBIN METHODS

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SUMMARY

A method is described for the determination of total pigment content in porcine meat by conversion to haematin (ferriprotoporphyrin hydroxide), and using the non-ionic detergent Triton® X-100 to increase the absorptivity. The method is presented as an alternative to the well-known Hornsey and cyanometmyoglobin methods. The alkaline haematin method requires a single reagent, sodium hydroxide and the detergent Triton X-100. This combination is probably less poisonous and more stable than the reagents used in the Hornsey and the cyanometmyoglobin methods. Reference solutions of alkaline haematin can be prepared easily.

INTRODUCTION

Meat colour is an important factor of meat quality. When discussing meat quality in pigs, the term PSE (Pale, Soft, Exudative) or watery meat is applied. The pale colour of the porcine Longissimus dorsi (LD) muscle for example, can be due to either or a combination of two causes. Firstly, there is PSE meat where the muscle proteins have become denatured due to rapid glycolysis post mortem while the body temperature is still high. The denatured proteins reflect more light than in normal meat and the surface looks pale. Secondly, the meat can look pale because of a low pigment content. When measuring meat colour with optical instruments, where a pigment influence can be suspected from the wavelength used, the pigment effect needs to be quantified.

The Hornsey haematin chloride method (Hornsey 1956) is the most widely used spectrophotometric procedure for the determination of total pigment in meat (Bünnig and Hamm 1970; Warriss 1979; Pikul et al. 1982; Dransfield et al. 1985; Monin and Sellier 1985). Another common method for pigment determination is the cyanometmyoglobin method (Drabkin et al. 1950; Ginger et al. 1954; Warriss 1976). The principle of these two methods, and their advantages and disadvantages are discussed later in this paper.

The alkaline haematin method described here is based on a method using alkaline haematin detergent for the determination of haemoglobin in blood, as developed by Zander et al. in 1984 (see also Wolf et al. 1984 and O'Halloran 1987). The reagent is a solution of the non-ionic detergent Triton X-100 dissolved in sodium hydroxide.

No references to the use of alkaline haematin for the purpose of analysing haematin in meat have been found in the literature. An alkaline haematin method to determine total haematin concentration was therefore developed.

MATERIALS AND METHODS

Extraction buffer. A phosphate buffer of low ionic strength was used for extraction (0.05 M K_2HPO_4/KH_2PO_4, pH 7.4, ionic strength 130 mM; 4 parts 0.1 M K_2HPO_4, 1 part 0.1 M KH_2PO_4 and 5 parts distilled water). This solution must be stored at about 4°C. The chemicals used were of pro analysi grade and obtained from Merck, Darmstadt, FRG.

Detergent solution. The detergent used was an aqueous solution of 10 % Triton® X-100 in distilled water. This solution can be stored at room temperature and stays stable for over 2 years (Wolf et al. 1984). Triton X-100 for scintillation techniques analytical grade was used and obtained from Merck, Darmstadt, FRG.

Standard haematin solution and standard curve. To evaluate the haematin concentration in the meat samples, a haematin standard curve was prepared by using haematin chloride dissolved in a solution of extraction buffer, detergent solution and sodium hydroxide in the same proportions as in the final meat extract. As a control, haematin chloride was also solved in filtered meat extract, also with detergent solution and sodium hydroxide in the same proportions. Haematin chloride was used instead of haematin, because of its better solubility in alkaline solutions and greater purity (Zander, personal communication 1988). The standard series should cover the range of concentrations that can be of interest. The haemin standard was obtained from Serva, Heidelberg, FRG.

Sample preparation. The muscle used was *M. longissimus dorsi* (LD) from slaughter pigs. The samples were taken from the last rib and backwards 24 hours post mortem and kept at -20°C. Samples were thawed overnight in a refrigerator. Muscle samples were freed from fat and connective tissue and minced twice using a mincer with plate hole size 3 mm (Electrolux Assistent, Electrolux Svenska Försäljnings AB, Stockholm, Sweden).

Pigment determination. Samples (5 g) of minced meat were weighed into centrifuge tubes, and 50 ml chilled (4°C) extraction buffer added to each. The samples were then homogenized (Ultra Turrax, Janke and Kunkel GmbH, Staufen, FRG) for 10 sec, the centrifuge tubes being immersed in ice-water before and after the procedure. The detergent solution was added (2.5 ml) and the tubes were covered and stored overnight at 4°C. The following day the samples were stirred using a wooden spatula and filtered at room temperature, using Whatman no. 42 filter paper. About 30 min after starting the filtration, 4 ml of the filtrate was mixed with 200 µl detergent solution. Finally 250 µl 5.0 M sodium hydroxide was added. The absorbance was read after 5 min (directly or within 12 hours according to O'Halloran 1987) at 575 and 700 nm using a Gilford 300 N spectrophotometer (Gilford Instruments Lab. Inc., Oberlin, Ohio). The haematin concentrations were calculated by using the regression equation from the standard curve (1) and expressed as ppm haematin per gram fresh meat.

$$ppm = \text{dilution factor} \times (96.261 \times (A_{575} - A_{700}) - 0.075) \quad (1)$$

RESULTS

The absorption spectra of the alkaline haematin method, with and without the detergent solution and sodium

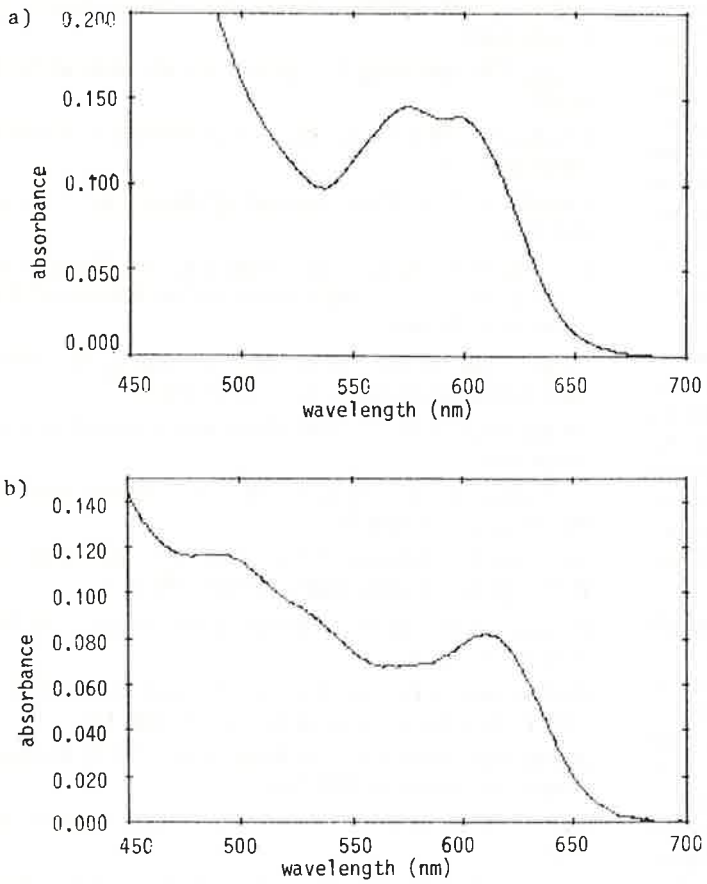

Fig. 1. Comparison of the spectra of alkaline heamatin, a) with and b) without the detergent solution for haemin

hydroxide in the range 450 to 700 nm for haematin chloride, are shown in Fig. 1. The same spectrum was also obtained irrespective of if the pigment was from whale or horse myoglobin or porcine haemoglobin. This was not the case when Triton X-100 was omitted. Due to turbidity of the solutions, the absorbance increased when not using the detergent solution or sodium hydroxide, or when using neither of these reagents, which gave invalid results (not shown).

Linearity and standard curve. The linearity of the interval of normal haematin concentration in porcine LD (range 15.0 to 50.0 ppm) was checked by constructing a standard curve. This showed that the Lambert-Beer law was followed within the range of normal haematin concentrations. When only using the absorbance at 575 nm, the standard curve made with the filtered meat extract showed lower regression coefficient compared with the standard curve without meat extract. It thus seems to be a non-specific absorption from the meat. To correct for that the absorbance at 700 nm was subtracted, yielding approximately the same regression coefficient both with and without meat extract.

DISCUSSION

Alkaline haematin generated by means of the haemoglobin method of Zander et al. (1984) shows a distinct absorption peak at 575 nm, and a shoulder around 600 nm. We have obtained the same results when using pure haematin chloride, whale and horse myoglobin, and porcine haemoglobin. According to Zander et al., all sorts of non-ionic detergents interact with haemoglobin in this way. In the original alkaline haematin method developed for blood, no pre-extraction step was used. With the high pH (13) of the reagent solution, problems arose with denatured muscle proteins when used directly on minced meat. As the pigment protein, myoglobin, is a sarcoplasmic protein, it can be readily extracted from meat by using a chilled buffer of low ionic strength (.2 M) (Helander 1957; Reynafarje 1963), e.g. a phosphate buffer. It should be pointed out, that the pH value will influence the absorbance level. Due to the great buffering capacity of porcine muscle (Warriss 1979), it is of great importance that pH is the same in the meat samples and in the solution used for the standard curve. The non-specific absorption obtained from the meat extract might be due to unclear solutions not detected by the human eye. We corrected for that contribution by subtracting the absorbance at 700 nm, i.e. where no absorption is obtained from the haematin (see Fig. 1).

The polyether-derived non-ionic detergent Triton® X-100 was used here as it interacts with haematin, causing the development of a specific absorption peak. As described by Helenius and Simons (1975), this detergent does not usually denature proteins, and it appears to be very inefficient in stopping protein-protein interaction. Most proteins preserve their quaternary structure in the presence of high concentrations of non-ionic detergents. However, examples of dissociation are also known. For example, haemoglobin is partially dissociated into subunits by Triton X-100. At the same time, the detergent also increases the solubility of proteins and lipids, in the same way as deoxycholate (bile salt) does (Kirkpatrick et al. 1974; Clarke 1975; Hearing et al. 1976; Schubert et al. 1983).

Sodium hydroxide was used to oxidize the iron of the myoglobin prostetic haem (Fe^{II}) group to haematin (Fe^{III}). It also reduced the turbidity and a clear extract was obtained. Plasma proteins, such as serum albumins and serum globulins, as well as lipids, are much more soluble in alkaline than in acid solutions (Wu 1922; Klein 1934; Clegg and King 1942; Ponder 1942; Bell et al. 1965).

In the Hornsey method for pigment determination, the haematin (Merck Index 1983) of myoglobin is extracted with a solution of 2-propanon (acetone) and water. The haematin is converted to haemin (Merck Index 1983) by adding concentrated hydrochloric acid. This derivate has absorption maxima at 512 and 640 (Lewis1954; Hornsey 1956; MacDougall and Disney 1967). The advantages of the Hornsey method are its rapidity and simplicity. However, its disadvantages are chiefly that 2-propanon easily evaporates, which will make the spectrophotometric values uncertain and will cause environmental problems for the analyst. Moreover, it is flammable, it may defat the skin and it can also act as an anaesthetic.

The factor 680, used to convert the absorbance reading to ppm haematin in meat (Hornsey 1956), is based on a too low water content of 65-70 % in porcine muscle. Other authors (Callow 1938; Poel 1949; Reynafarje 1963; Warriss 1976; Asghar and Pearson 1980; Lawrie 1985) have reported that the water content in meat is

approximately 75 %. This great difference may be due to the fact that the porcine meat used by Hornsey (1956) had a higher content of adipose tissue. The factor 680 is also based on a millimolar absorptivity coefficient of 4.80, which is calculated from a 50 μM haemin solution. In porcine muscle, the reported haematin concentrations (based on myoglobin content) vary between 8.4 and 115.6 ppm (for ref. see Pikul et al 1982). The coefficient seems, however, to be based only on a 50 μM haemin solution, with no dilution, i.e. no standard curve within the concentration of interest for pig muscle. The linearity of the method is thus not proven. An additional source of error not pointed out by Hornsey is the temperature in the acidic acetone solution. As already shown by Lewis (1954), the solution has to be chilled in order to precipitate the proteins before filtering the extract. As shown by Bünning and Hamm (1970), also the fat content of especially pig muscle can cause an increase in absorbance and thus an overestimation of the pigment concentration. The authors suggested defatting of the muscle and triple extractions to get valid results.

In the cyanometmyoglobin method, all haem compounds are converted to cyanmetmyoglobin by different cyanides. These derivates have absorption maxima at 540 and 545 nm (Drabkin et al. 1950; Ginger et al. 1954; Warriss 1976). The great disadvantage with this method is that the reagents used contain cyanides, which are very toxic substances. Zander et al. (1984) has summarised the disadvantages of this method as follows: (1) when preparing the reagent, the analyst is handling a highly dangerous poison, and this may be a drawback for routine use, when large amounts of reagent are required; (2) the reaction solution is light labile; (3) standardization of the method is based on purified cyanhaemoglobin solutions, whose quality is controlled only indirectly by spectrophotometry; (4) the reaction times of the different haemoglobin species and derivatives differ markedly.

In conclusion, the alkaline haematin method described here has certain advantages, even though it is more time-consuming than the Hornsey and cyanometmyoglobin methods. It is probably possible to further simplify the method, but due to lack of time this could not be attempted in this investigation.

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