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SECTION

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Elektrophysical Studies on Fresh and Frozen Meat Differentiation.Introduction

Meat can be preserved by refrigeration above the freezing point or by storage below the freezing point, but the results and effectiveness of cold preservation in the meat industry are very different. Therefore an understanding of differentiation between fresh or refrigerated and frozen meat by laboratory techniques is of the greatest importance.

According to the above consideration, different efforts have been made to differentiate the fresh and refrigerated meat samples inducing specific norms, eg. Grau and Hamm (1956), Hamm and Körmendy (1966), Turmel (1957), Moreno-Calvo, Fuster and Garcia-Matamoros (1967).

The present work aims to obtain an electrophysical differentiation of refrigerated and frozen meat samples, based on physical and electrical properties of press juice, since new data and experiences in this field are rather lacking.

Material and methods

Meat of beef cattle of two-years age from the Madrid slaughter-house were taken for our study and transported to our Centro with in 24 hours after slaughtering. The experimental meat was maintained as normally carried out commercially at 0°C to minimize the protein denaturation and the bacterial growth.

The 300-gram specimens were prepared from the superior muscular pieces of the animals for slaughter (sirloin, rump, middle-rib, fore-rib and steakmeat) and packaged in polyethylene liners for the tunnel or room freezing and in aluminium foil in the case of immersion in dry ice mixture.

The following lots of meat specimens were arranged:

Lot 1 : Chilled meat at the temperature of 0°C, air velocity of 2 m/s (394 ft/min) and 85 + 90 per cent relative humidity with subsequent storage.

Lot 2 : A - Frozen meat in blast tunnel freezer at -40°C and 5 m/s air velocity (984 ft/min)

B - Frozen meat by immersion in dry ice mixture (96 % C₂H₅OH plus carbon dioxide) at the temperatures of

- B₁ Freezing temperature of -20°C

- B₂ Freezing temperature of -40°C

- B₃ Freezing temperature of -70°C

Lot 3 : - Cold room freezing

C - Frozen meat at the temperature of -10°C without air blasting.

D - Frozen meat at the temperature of -20°C without air blasting.

- Blast tunnel freezing

E - Frozen meat at the temperature of -30°C and 4,5 m/s air velocity (945 ft/min)

F - Frozen meat at the temperature of -40°C and 8 m/s air velocity (1574 ft/min)

Meat juice preparation

The press juices were obtained by means of an hydraulic mechanism acting on the meat specimens at a pressure of 100 kg/cm^2 (97 atmospheres) under the same circumstances in all samples.

Time-temperature curves

The temperature registrations were performed by using conventional thermocouples giving the time-temperature curves for beef specimens during the process of freezing.

The mean temperature differences Δt between the meat and the cold or freezing ambient was obtained from the formula:

$$\Delta t = \frac{1}{Z} \int_0^Z (t_a - t_f) e^{-Kz} dz$$

in which Z is the time (hours)

t_a is the initial temperature of meat specimen

t_f is the freezing temperature of air

K is the cooling coefficient.

Free water estimation

This test was performed by using the Grau and Hamm technique (1956).

Separation of sarcoplasmatic meat proteins by paper electrophoresis

A Durrum-type paper electrophoresis cell was used, with electrical generator and curves integrator (Spinco Beckman). The paper strips were Schleicher-Schüll $3 \times 30 \text{ cm}^2$. The normal electrophoretic patterns were performed by using phosphate buffer pH 7,6 0,1 M specific electrical conductivity of 15,97 mS/cm, 0,677 ionic strenght, 125 Volts continuous current and 4,2 V/cm of electrical field.

The electrostatic charge densities of all the paper

electrophoretic fractions of proteins were computed as average net charge V , taking each percent of each protein function (c), its distance in cm to starting point (d), the global time t in seconds and l being equal to 30 cm:

$$\bar{V} = \frac{\lambda V t}{l} \sum_u \frac{c}{d}$$

The numerical values obtained by using our formula must be multiplied by 10^{-5} to obtain the global result expressed in C/ml (coulombs per cm^3) as mean valence of all the meat protein electrophoregrams.

The value in all the electrophoretic patterns of this work (experimental value) for the coefficient

$$\frac{8}{7 \lambda k} 5^{-\eta}$$

was 43,12 (for 125 V and 18 hours of electrophoresis).

Specific electrical conductivity

This previously reported technique (Moreno-Calvo and Garcia-Matamoros, 1966, Garcia-Matamoros and Moreno-Calvo, 1967) was used.

Electro-dynamic conductivity

This technique was used by applying the new device proposed by us for the milk (Moreno-Calvo and Garcia-Matamoros, 1967) as applied in this case to the meat press juice. The meat press juice must be conveniently diluted with distilled water to obtain a proper value of this electro-dynamic conductivity. The best dilution to determine the electro-dynamic conductivity was 10 ml of meat press juice plus 20 ml of distilled water.

The integral values were computed by using the trapezoid rule of integration between the starting point and

the first maximum obtained

$$\int_1^n W ds = 2.48 \times 10^{-3} \left(\frac{W_1 + W_n}{2} + \sum_{i=2}^{n-1} W_i \right)$$

The factor 2.48×10^{-3} results from converting the Watts-seconds into Watt-hours. The Watts were read each 10 seconds.

Results and Discussion

The technological aspects concerning the thermic measurements (time-temperature curves) are included in the Gigs. A to F and Table I. By examining the parameters of these time-temperature curves (different Δt and K) when various refrigerating conditions were employed, the effects can be studied more rigorously in considering the absolute values of these parameters. In addition the Δt values are interrelated very closely with the freezing process (air velocity, freezing temperature) and with the freezing time in the graphical form previously expressed.

It should be pointed out that before freezing all the Δt values are rising as a function of the freezing temperature. The Δt computations measured in depth are greater than on the surface, which may be due to the fact that the external surface tends to have a greater thermal flow. In considering the cooling coefficients before freezing (K values), the global velocity of cooling is greater on the surface of the meat specimen as compared with those in the depths.

On the other hand, after the freezing process essentially the same relative phenomena can be observed (but of a less absolute value) when considering the Δt computations. However, the contrary is observed in considering the K values, since the cooling velocity is greater in the depth as compared with the ones of on external surfaces.

The results concerning the free water estimation are given in the Table II. As can be seen in this table the first effect is fairly obvious: an increase of free water takes place in all the samples and lots as a result of the cold treatment. However this effect cannot be interpreted as very significant to control the meat quality under the industrial conditions.

The electrophoretic patterns of the sarcoplasm proteins are represented in both figures G and H, which show the possibility of differentiation between a fresh meat specimen and the other frozen. It is clear from these figures of sarcoplasm electrophoresis that in the fresh meat there are a polydisperse condition with only two or three anionic fractions and only one cationic (initial value), nevertheless in the electropherogram corresponding to a refrigerated storage or to a frozen meat, four to five anionic fractions and one to two cationic ones are observed. It may be also observed that the lower the temperature the less are the concentrations of the different proteinic fractions.

Concerning the identification of the aforesaid proteinic fractions it is believed (Weber and Meyer 1933, Kronman and coll. 1960) that these proteinic fractions of sarcoplasm may be interpreted as myogen and perhaps some other globulinic fraction.

The paper electrophoretic measurements are given in the Table III and the results are expressed in Coulombs per ml as average net charge or mean valence of all the electrophoretic ions, i.e. of the sarcoplasmatic proteins of meat muscle. In favour of this determination of the global valence the following reasons can be inferred : easy interpretation of the results, rapidity of calculations and it is not necessary to identify the protein, as would be necessary in the conventional paper electrophoresis.

After different tests we have observed that the determination of the mean valencies of electrophoretic proteins in the meat muscle is a good possibility to differentiate the fresh from the refrigerated meat specimen as also the frozen meat, since the mean valence of the fresh meat is higher during the refrigerated storage and the mean valence of the frozen meat tends on the contrary to fall off (negative increase) diminishing in fact, which is really of great significance.

The values of the specific electrical conductivity of meat press juice as shown in Table IV are extremely critical to be considered as quality test of meat or to find a proper correlation between the refrigeration technique and the fresh or frozen condition of meat specimen.

In Table V, the values of the electrodynamic conductivity of meat press juice are shown. These figures are not very significant to differentiate the intrinsic quality of a meat specimen and its relations to a refrigerated or frozen meat state remain of the utmost complexity, nevertheless more studies are necessary in this field.

From all the results obtained in this work it seems that, for the moment, the electrophoretic studies is the best method for the differentiation of fresh meat from the refrigerated and frozen ones, since under the influence of the low temperatures the myogen decrease in sarcoplasm, other proteinic fractions increasing at the same time. This same phenomenon was observed early in this laboratory.

The facts that the electrical and electro-dynamic conductivities of the meat press juice decrease very often as consequence of cold treatment, as also the mean valencies of electrophoretic proteins, would signify a certain rate of myogen polydispersity as a distinguishable effect of low temperatures on the sarcoplasmatic proteins of treated meats.

Conclusions

- The t values are very closely interrelated with the freezing process (air velocity, freezing temperatures) and with the freezing time. The t measured in depth are greater than in the surface of a meat specimen.
- Under the influence of low temperatures, the electrophoretic patterns of meat specimen showed an increase in the polydisperse conditions of their sarcoplasmatic proteins.
- The lower the temperature of a meat specimen, the less are the concentrations of the different proteinic fractions, individually taken.
- From the behaviour of the electrophoretic patterns and from the computations of the mean valencies, it may be inferred whether one meat specimen was fresh or was frozen, then the increases of mean valencies are positive in chilled meat and negative in all the frozen samples of meat.
- The specific electrical and electro-dynamic conductivities of the meat press juice prove to be not very significant to differentiate the intrinsic quality of a meat specimen. Their relations to a refrigerated or frozen meat state remain of the utmost complexity.
- The myogen polydispersity increases as an effect of low temperatures on the sarcoplasmatic proteins, which would mean a decrease in myogen concentration.

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S U M M A R Y

ELECTROPHYSICAL STUDIES ON FRESH AND FROZEN MEAT DIFFERENTIATION.

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An understanding of differentiation between fresh or refrigerated and frozen meat by laboratory techniques is one of greatest importance.

The present work aims to obtain an electrophysical differentiation of refrigerated and frozen meat samples, based on physical and electrical properties of meat press juice, since new data and experiences in this important field are rather lacking.

In this research the following methods were applied:

- Chilling and freezing of meat;
- t and K determinations;
- Free water estimation;
- Paper electrophoresis for the separation of meat proteins;
- Average net electrostatic charge of sarcoplasmatic proteins;
- Specific electrical conductivity of meat press juices;
- Electrodynamic conductivity of meat press juices.

The results are shown in eight figures and five tables, from which after discussion the following conclusions may be reached:

- The t values are very closely interrelated with the freezing process (air velocity, freezing temperature) and with the freezing time. The t measured in depth are greater than in the surface of a meat specimen.
- Under the influence of low temperatures, the electropho-

retic patterns of meat specimen showed an increase in the polydisperse condition of their sarcoplasmatic proteins.

- The lower the temperature of a meat specimen, the less are the concentrations of the different proteinic fractions, individually taken.
- From the behaviour of the electrophoretic patterns and from the computations of the mean valencies, it may be inferred whether one meat specimen was fresh or was frozen, then the increases of mean valencies are positive in chilled meat and negative in all the frozen samples of meat.
- The specific electrical and electrodynamic conductivities of the meat press juices prove to be not very significant to differentiate the intrinsic quality of a meat specimen. Their relations to a refrigerated or frozen meat state remain of the utmost complexity.
- The myogen polydispersity increases as an effect of low temperatures on the sarcoplasmatic proteins of meat specimen which would mean a decrease in myogen concentration.

Δt and K

TABLE I

| Lots | BEFORE FREEZING | | AFTER FREEZING | | | |
|----------------|-----------------|------|----------------|------|------------|------|
| | Δt | K | Δt | K | Δt | K |
| A | Deep | 40.5 | 0.14 | | 11.8 | 2.48 |
| | Surface | 39.9 | 0.30 | | 11.3 | 1.79 |
| B ₁ | Deep | | | 19.6 | 2.04 | |
| B ₂ | Deep | | | 40.4 | 1.56 | |
| B ₃ | Deep | | | 72.6 | 2.79 | |
| C | Deep | 13.8 | 0.27 | | 3.1 | 0.22 |
| | Surface | 13.1 | 0.31 | | 2.9 | 0.21 |
| D | Deep | 23.1 | 0.48 | | 5.7 | 0.35 |
| | Surface | 15.5 | 1.42 | | 2.8 | 0.33 |
| E | Deep | 29.9 | 0.18 | | 12.7 | 0.71 |
| | Surface | 28.9 | 0.21 | | 10.6 | 0.66 |
| F | Deep | 42.4 | 0.49 | | 13.9 | 3.46 |
| | Surface | 41.2 | 1.02 | | 11.7 | 3.07 |

FREE WATER

TABLE II

| Lots | Temperature °C | Air Velocity m/s | INITIAL VALUES (Fresh meat) | MEAT VALUES AFTER THAWING | INCREASE % | STORAGE DAYS | | |
|----------------|-------------------|------------------------|--------------------------------------|------------------------------|---------------------------|--------------|---------|---------|
| | | | | | | 2 | 5 | 7 |
| 1 | 0 | 2 | 30.6 | | a. - b. 7.1 c. 55.2 | a. 26.7 | b. 32.8 | c. 47.5 |
| A | -40 | 5 | 33.1 | 43.7 | 32 | | | |
| B ₁ | -20 | - | 33.1 | 41.4 | 25 | | | |
| B ₂ | -40 | - | 33.1 | 35.7 | 7.8 | | | |
| B ₃ | -70 | - | 33.1 | 40.2 | 21.4 | | | |
| C | -10 | - | 30.6 | 34.1 | 11.4 | | | |
| D | -20 | - | 30.6 | 32.3 | 5.5 | | | |
| E | -30 | 4.5 | 30.6 | 39.3 | 28.4 | | | |
| F | -40 | 8 | 30.6 | 38.6 | 26.1 | | | |

MEAN VALENCE OF ELECTROPHORETIC PATTERNS C/ml

TABLE III

| Lots | Temperature °C | Air Velocity m/s | Initial values (fresh meat) | Meat va- lues (after thawing) | Increase % | Storage days | | |
|----------------|-------------------|------------------------|--------------------------------------|--|------------------------------|--------------|----------|----------|
| | | | | | | 2 | 5 | 7 |
| 1 | 0 | 2' | 6.516 | | a. 1.5 b. 23.5 c. 28.2 | a. 6.611 | b. 8.049 | c. 8.356 |
| A | -40 | 5 | 12.643 | 9.208 | -27.2 | | | |
| B ₁ | -20 | - | 12.643 | 10.937 | -13.5 | | | |
| B ₂ | -40 | - | 12.643 | 9.249 | -26.8 | | | |
| B ₃ | -70 | - | 12.643 | 8.906 | -29.6 | | | |
| C | -10 | - | 6.516 | 3.534 | -45.8 | | | |
| D | -20 | - | 6.516 | 4.299 | -34.0 | | | |
| E | -30 | 4.5 | 6.516 | 6.127 | - 6.0 | | | |
| F | -40 | 8 | 6.516 | 5.504 | -15.5 | | | |

C/ml (i.e. Coulombs per cm³)

SPECIFIC ELECTRICAL CONDUCTIVITY (mS/cm)

TABLE IV

| Lots | Temperature °C | Air velocity m/s | Initial values (fresh meat) | Meat values (after thawing) | Increase % | Storage days | | |
|----------------|-------------------|------------------------|--------------------------------------|--------------------------------------|-------------------------------|--------------|----------|----------|
| | | | | | | 2 | 5 | 7 |
| 1 | 0 | 2 | 12.99 | | a. -4.7 b. -9.4 c. -7.3 | a. 12.37 | b. 11.76 | c. 12.03 |
| A | -40 | 5 | 12.94 | 12.05 | -6.8 | | | |
| B ₁ | -20 | - | 12.94 | 11.57 | -10.5 | | | |
| B ₂ | -40 | - | 12.94 | 12.85 | -0.6 | | | |
| B ₃ | -70 | - | 12.94 | 11.52 | -10.9 | | | |
| C | -10 | - | 12.99 | 11.12 | -14.3 | | | |
| D | -20 | - | 12.99 | 11.81 | -9.0 | | | |
| E | -30 | 4.5 | 12.99 | 12.04 | -7.3 | | | |
| F | -40 | 8 | 12.99 | 11.69 | -10.0 | | | |

$$1 \text{ mS/cm} = 10^{-3} \text{ Ohm}^{-1} \text{ cm}^{-1}$$

ELECTRODYNAMIC CONDUCTIVITY (Wh) - INTEGRAL VALUES BETWEEN START AND MAXIMUM TIME
 MEAT PRESS JUICE DILUTED WITH DOUBLE VOLUME OF DISTILLED WATER

TABLE V

| Lots | Temperature °C | Air velocity m/s | Initial values | Meat values (after thawing) | Increase % | Storage days | | |
|----------------|-------------------|---------------------|----------------|--------------------------------|----------------------------------|--------------|---------|---------|
| | | | | | | 2 | 5 | 7 |
| 1 | 0 | 2 | 1.35 | | a. -14.8 b. -21.5 c. -39.3 | a. 1.15 | b. 1.06 | c. 0.82 |
| A | -40 | 5 | 1.21 | 1.12 | - 7.4 | | | |
| B ₁ | -20 | - | 1.21 | 0.89 | -26.5 | | | |
| B ₂ | -40 | - | 1.21 | 0.94 | -22.3 | | | |
| B ₃ | -70 | - | 1.21 | 1.00 | -17.4 | | | |
| C | -10 | - | 1.35 | 1.19 | -11.8 | | | |
| D | -20 | - | 1.35 | 0.98 | -27.4 | | | |
| E | -30 | 4.5 | 1.35 | 1.07 | -20.7 | | | |
| F | -40 | 8 | 1.35 | 1.23 | - 8.9 | | | |

1 Wh = 367 Kgm

Fig. A

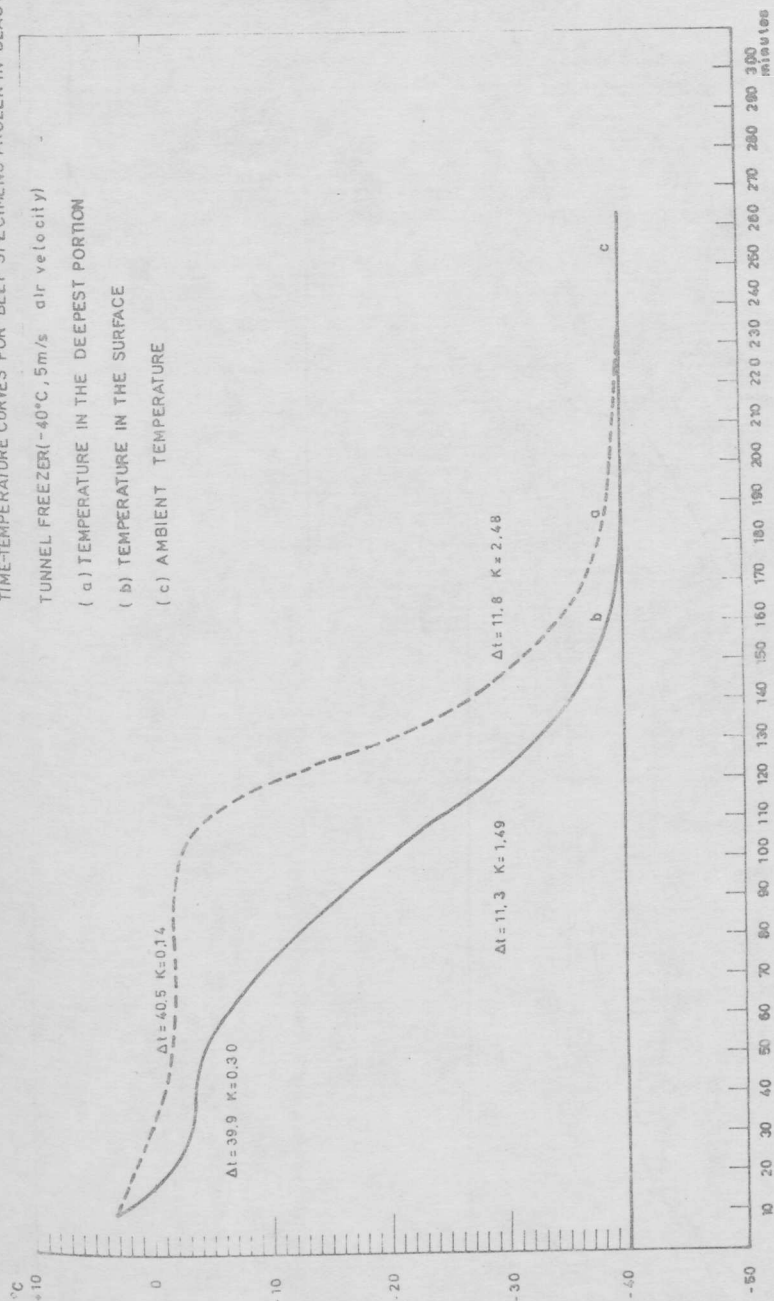
TIME-TEMPERATURE CURVES FOR BEEF SPECIMENS FROZEN IN BLAST

TUNNEL FREEZER(-40°C, 5m/s air velocity)

(a) TEMPERATURE IN THE DEEPEST PORTION

(b) TEMPERATURE IN THE SURFACE

(c) AMBIENT TEMPERATURE



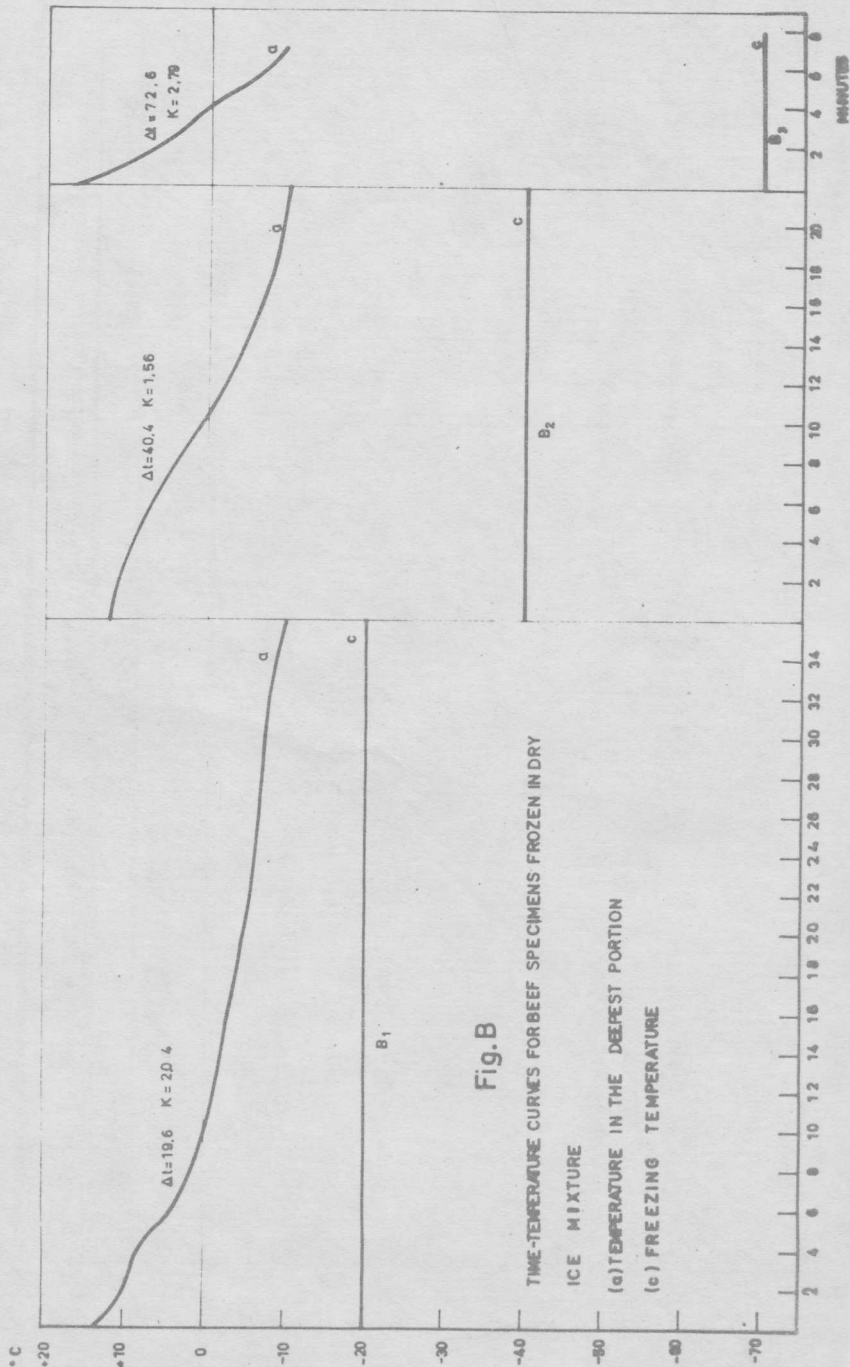


Fig.B

TIME-TEMPERATURE CURVES FOR BEEF SPECIMENS FROZEN IN DRY

ICE MIXTURE

(a) TEMPERATURE IN THE DEEPEST PORTION

(b) FREEZING TEMPERATURE

(c) TEMPERATURE IN THE DEEPEST PORTION

Fig. C

TIME-TEMPERATURE CURVES FOR BEEF SPECIMENS FROZEN

AT -10 C° WITHOUT AIR BLASTING

(a) DEEPEST PORTION TEMPERATURE

(b) SURFACE TEMPERATURE

(c) FREEZING TEMPERATURE

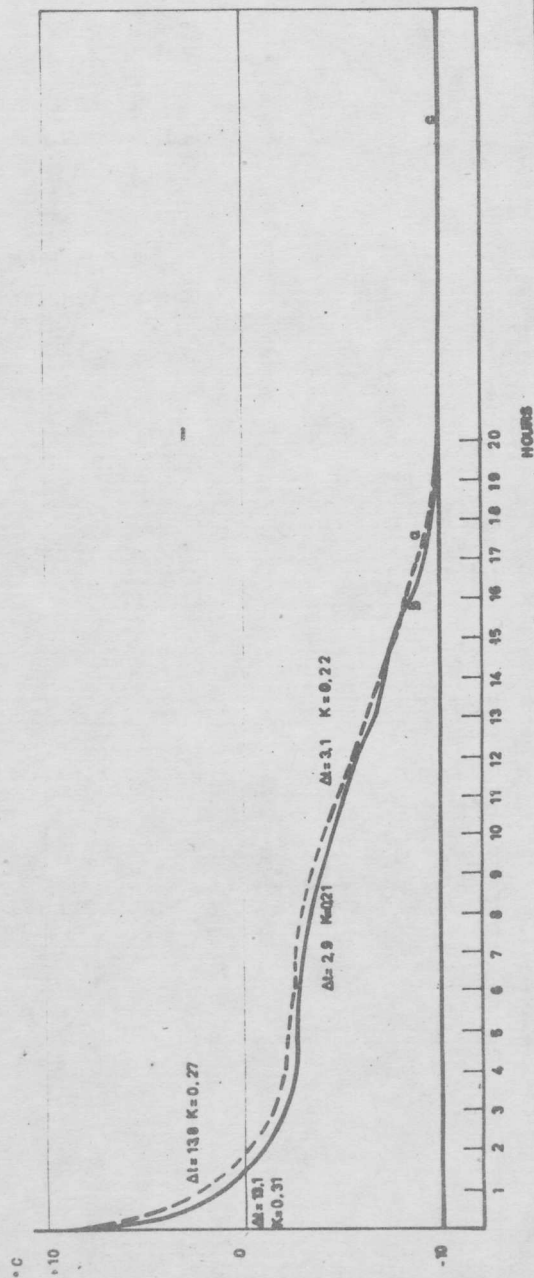


Fig. D

TIME-TEMPERATURE CURVES FOR BEEF
SPECIMENS FROZEN AT -20°C WITHOUT AIR
BLASTING.

(a) DEEPEST PORTION TEMPERATURE

(b) SURFACE TEMPERATURE

(c) FREEZING TEMPERATURE

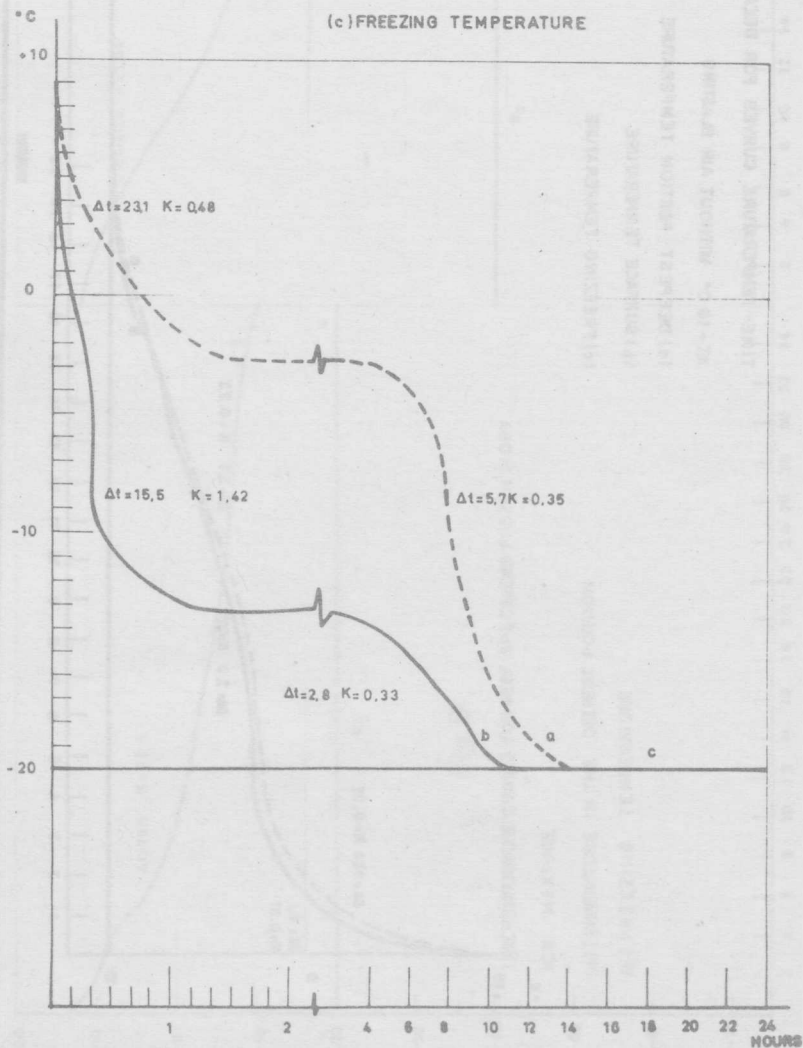


Fig. E

TIME-TEMPERATURE CURVES FOR BEEF SPECIMENS
FROZEN IN BLAST TUNNEL FREEZER-30°C.

4.5 m/s AIR VELOCITY

(a) TEMPERATURE IN THE DEEPEST PORTION

(b) TEMPERATURE IN THE SURFACE

(c) AMBIENT TEMPERATURE

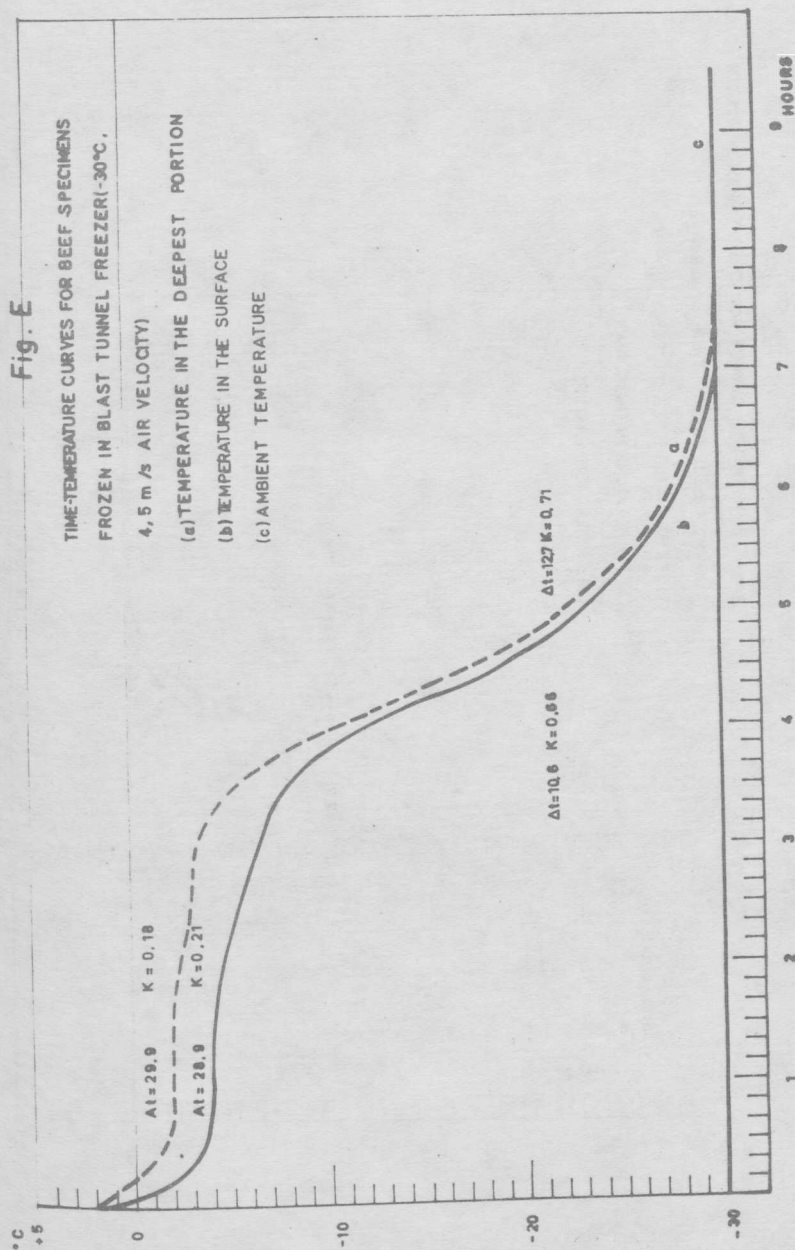


Fig. F

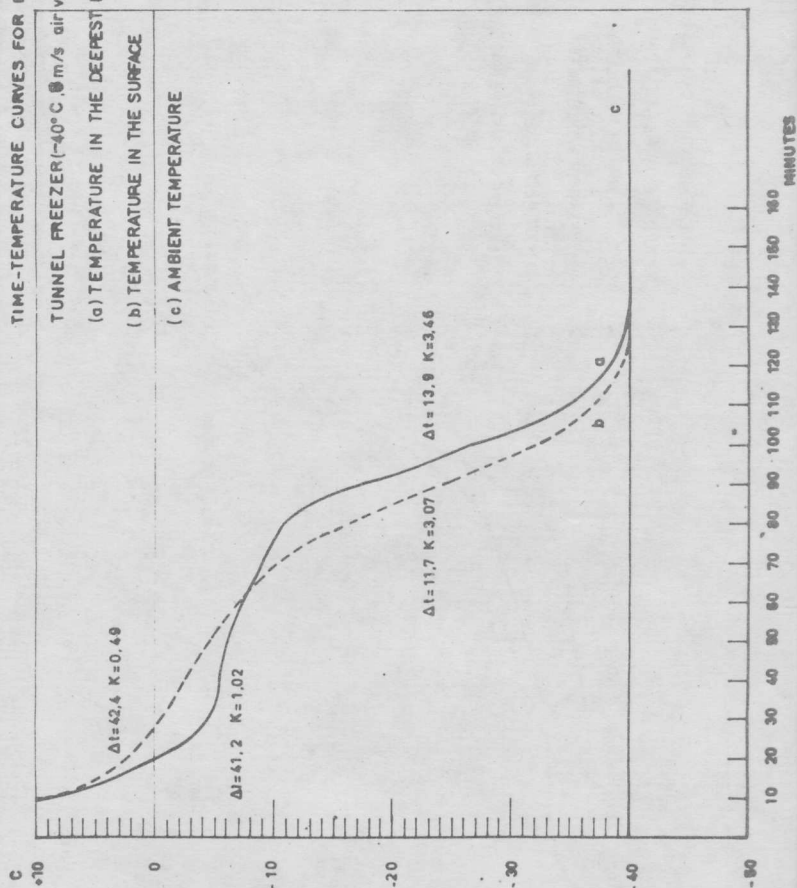
TIME-TEMPERATURE CURVES FOR BEEF SPECIMENS FROZEN IN BLAST

TUNNEL FREEZER (-40°C , 0.8 m/s air velocity)

(a) TEMPERATURE IN THE DEEPEST PORTION

(b) TEMPERATURE IN THE SURFACE

(c) AMBIENT TEMPERATURE



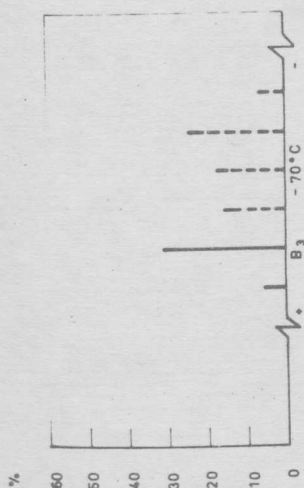


Fig. G

ELECTROPHORETIC PATTERNS OF MEAT PROTEINS (SARCOPLASM)
REPRESENTING THE POLYDISPERSE CONDITION AND THE RELATIVE
CONCENTRATIONS (%)

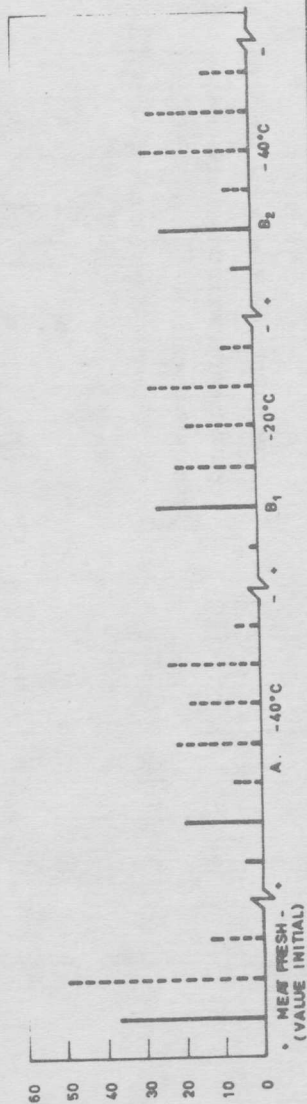
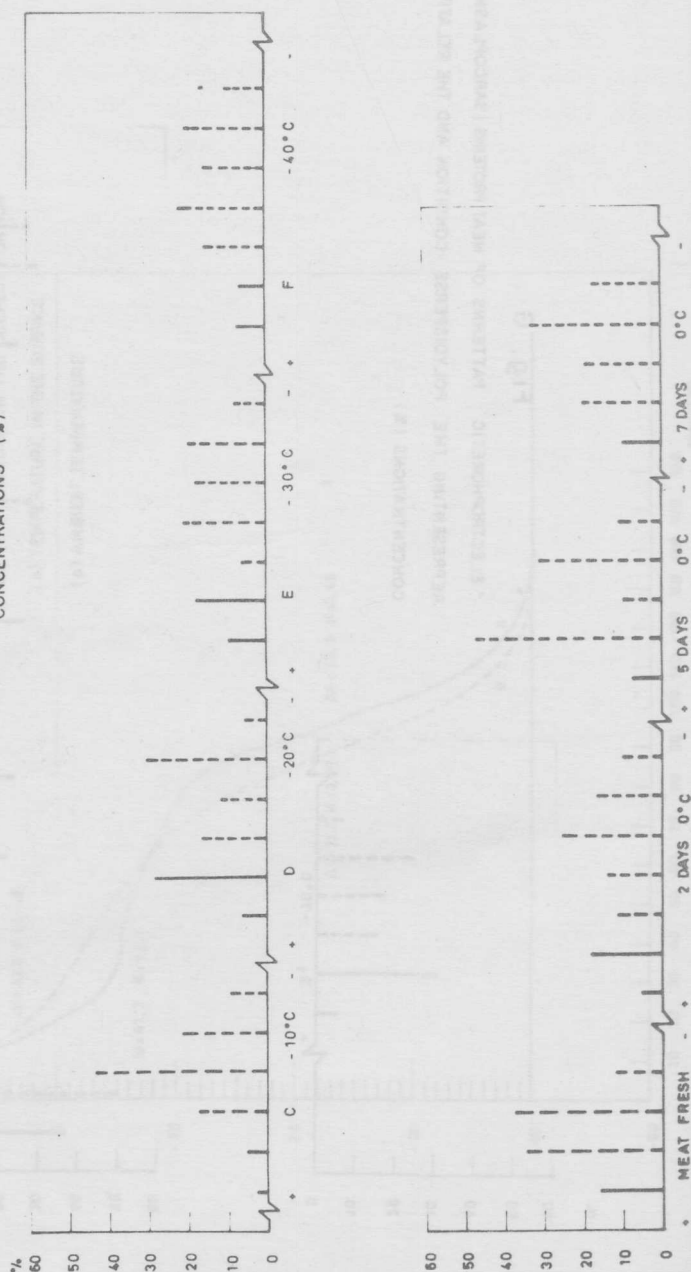


Fig. H

ELECTROPHORETIC PATTERNS OF MEAT PROTEINS (SARCOPLASM)
REPRESENTING THE POLYDISPERSE CONDITION AND THE RELATIVE
CONCENTRATIONS (%)



The publishers of the "Proceedings of the 14th European Meeting of Meat Research Workers" wish to appologize to the authors of "The Effect of Road Transportation and Lairage Treatment on Pig Muscle" page 394, Messrs. R.J. Elliott and R.J. Patton, and to the kind rearder, for ommiting to enclose the attached diagramms in the Froceedings.

Thank you

The Editor

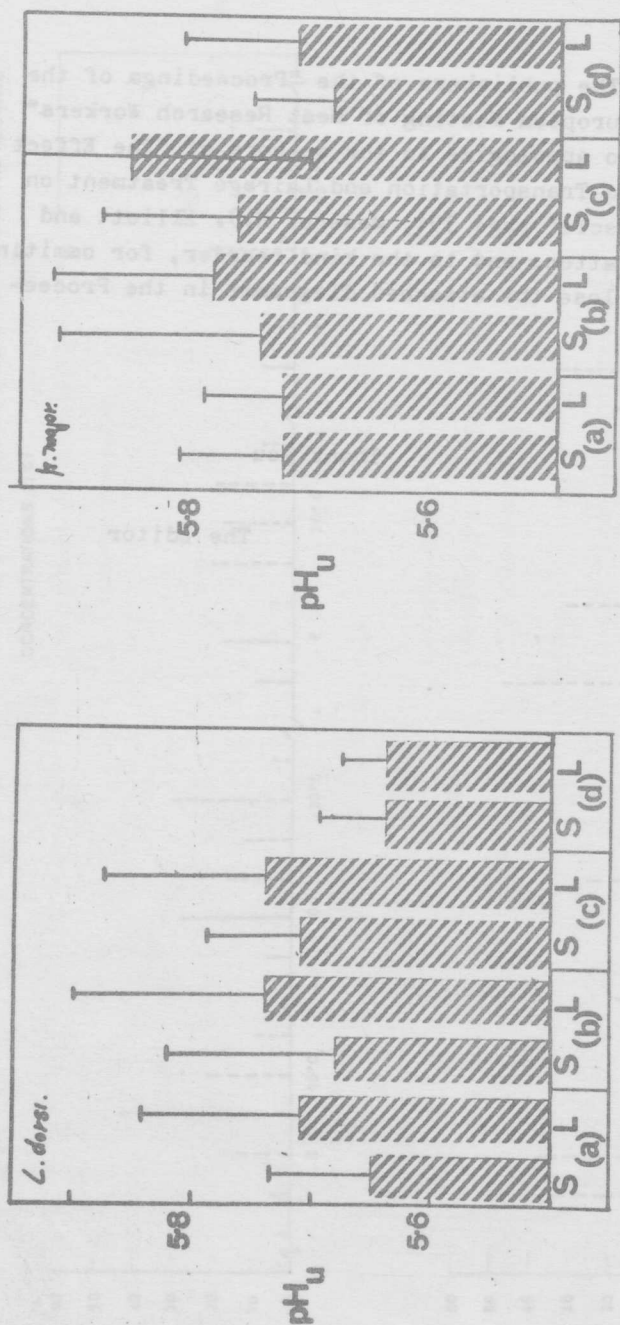


Fig. 1. The effect of transport distance (S = 20 miles, L = 100 miles) and lairage treatment [(a) killed on arrival; (b) held 2 hr in lairage; (c) held overnight, no food; (d) held overnight, fed 2 lbs sugar] on the mean ultimate pH of the *L. dorsalis* and *P. major*. Standard deviations are also given.

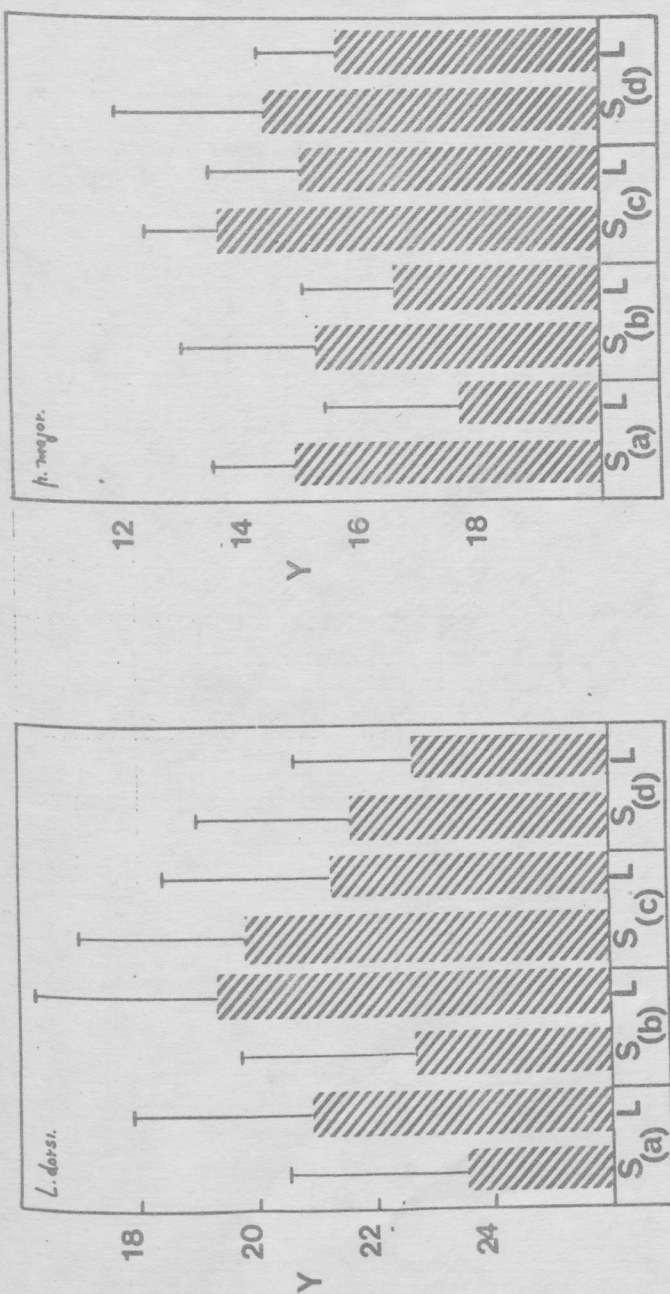


Fig. 2. The effect of transport distance (S = 20 miles, L = 100 miles) and lairage treatment [(a) killed on arrival; (b) held 2 hr in lairage; (c) held overnight, no food; (d) held overnight, fed 2 lbs sugar] on the mean Y (brightness) values of the *L. dorsa* and *P. major*. Standard deviations are also given.

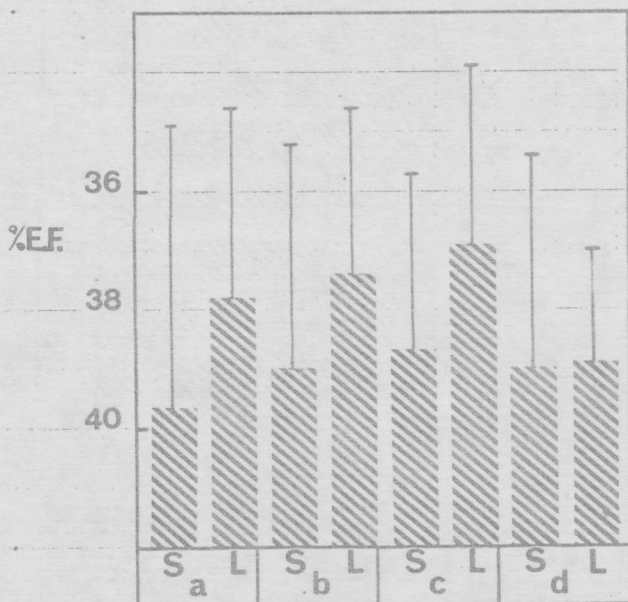


Fig. 3. The effect of transport distance (S = 20 miles, L = 100 miles) and lairage treatment [(a) killed on arrival; (b) held 2 hr in lairage; (c) held overnight, no food; (d) held overnight, fed 2 lbs sugar] on the mean expressible fluid value (E.F.) of the *l. dorsis*. Standard deviations are also given.

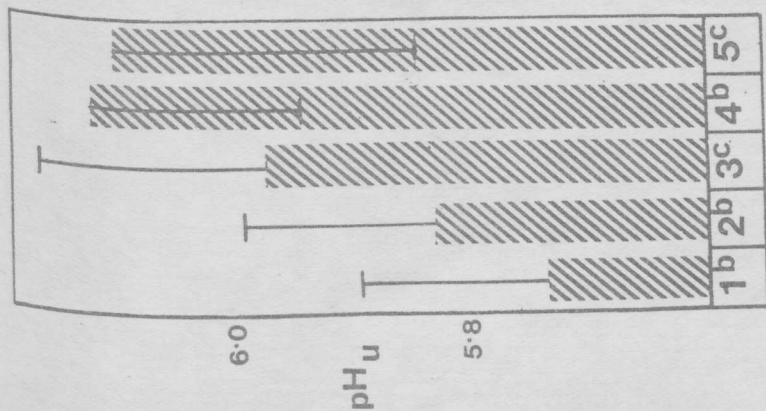


Fig. 4. The effect of type of transport on the mean pH and standard deviation of the *L. dorsi* of pigs transported 100 miles [(b) held 2 hr before slaughter; (c) held overnight without food before slaughter; 1, short lorry (Expt. 1); 2, short lorry; 3, long lorry; 4 & 5, double-decker lorry].

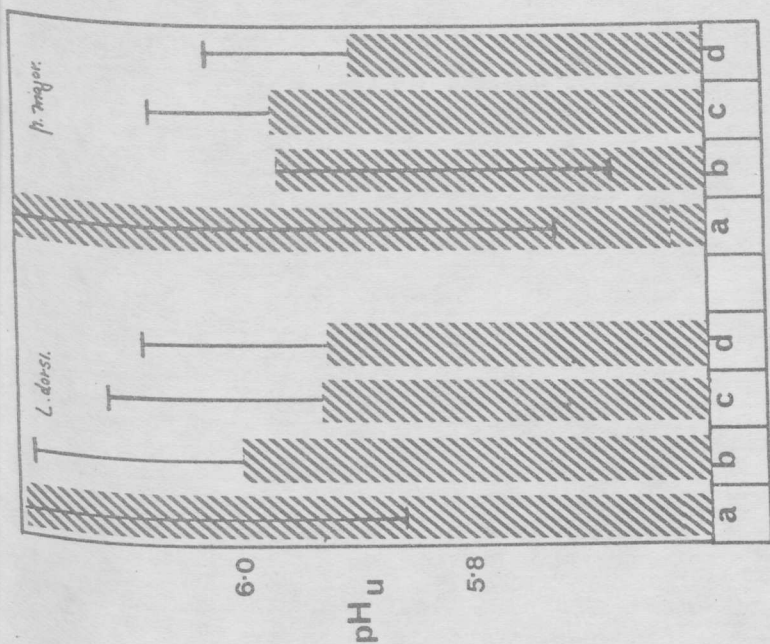


Fig. 5. The effect of lairage treatment [(a) killed on arrival; (b) held 2 hr in lairage; (c) held overnight, no food; (d) held overnight, fed 2 lbs sugar] on the mean ultimate pH and standard deviation of *L. dorsi* and *P. major* of 40 pigs brought 20 miles in short lorry to factory B, mixed with another 40 pigs and transported 100 miles in a double-decker lorry to factory C.