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Calorimetric properties of lamb and other meats

A. K. FLEMING

Summary. A description is given of an automatic adiabatic calorimeter which was used to obtain enthalpy–temperature data for lamb brains and kidneys, calf veal and five selected lamb cuts. Water content of the cuts correlated well with fat content and with 'latent heats' of freezing. Calculations indicated 11% of the total water content of low fat meat was not frozen at −20°F and there appeared to be a tendency for this non-frozen portion to increase with meat of higher fat content.

Introduction

In New Zealand, the cooling and freezing of lamb and mutton carcasses constitute one of the most important unit operations in the preparation of meat for export. It is important, therefore, to understand the physical factors which govern heat transfer rates in cooling and freezing, and a knowledge of the thermal properties of meat is essential for this purpose. Engineering aspects of cooling and freezing have a useful end in themselves in that freezing equipment can only be rationally designed with a thorough understanding of factors influencing freezing rates. However, other benefits may accrue from a knowledge of heat transfer rates within meat since these can have profound effects on meat quality (Marsh & Leet, 1966; Marsh, Woodhams & Leet, 1968). If the engineer can predict temperature changes resulting from various heat-transfer rates at the surface of a carcass, then the biochemist and microbiologist are more favourably placed to specify environmental conditions aimed at improving product quality.

Calorimetric studies can provide information not only on enthalpy (or specific heat) but also on the proportion of water remaining unfrozen in muscle as the temperature is progressively lowered below 32°F. It may be in the non-frozen phase that slow reactions occur which affect meat quality. Love (1966) discussed the mechanism of ice crystal growth in muscle tissue and the importance of water frozen out. Calorimetric methods for determining frozen water content appear to offer advantages in accuracy over other techniques employing dilatometry, flotation and histology, although calorimetry involves more complex apparatus. Plank (1925), Watzinger (1949) and Riedel (1956a, 1957, 1961) obtained results using calorimetric techniques on fish,

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beef and other foods, and the findings of Moran (1930) using dilatometry showed little difference between beef, pork and mutton. Calorimetric studies on muscle tissue have also been made by Jason & Long (1955) and Charm & Moody (1966). However, no results have been presented yet for lamb meat.

An adiabatic calorimeter was used in the present work. This technique is relatively slow compared with other calorimetric methods such as unsteady-state heat penetration into slabs and 'method-of-mixtures.' On the other hand these lack the flexibility and control inherent in the adiabatic system which is particularly suitable for studying heat capacities during phase changes associated with freezing.

Experimental

Apparatus

Heat capacities of samples were required over a wide temperature range, including the 'latent heat' of freezing region. It was not practical to freeze the sample calorimetrically by subtracting known quantities of heat from it, so the reverse technique of thawing was employed. Known pulses of electrical energy were injected into a heater in the calorimeter at intervals of 20–90 min, depending on the sample characteristics. Temperatures, initially about −40°F, were recorded as the system reached equilibrium. The long equilibrium time necessary after each heat increment required an adiabatic system to reduce any heat loss error to a minimum. For convenience, the operation of the calorimeter was automatically controlled, since each test took some 15–22 hr to complete.

Fig. 1 shows a schematic diagram of the calorimeter and Plate 1 a photograph of

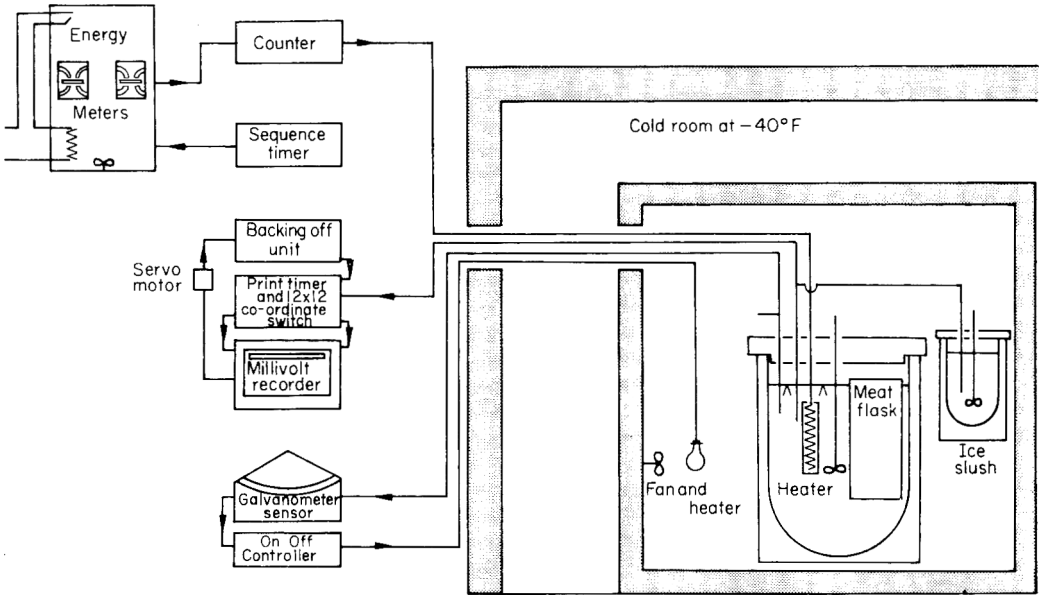

FIG. 1. Schematic diagram of calorimeter.

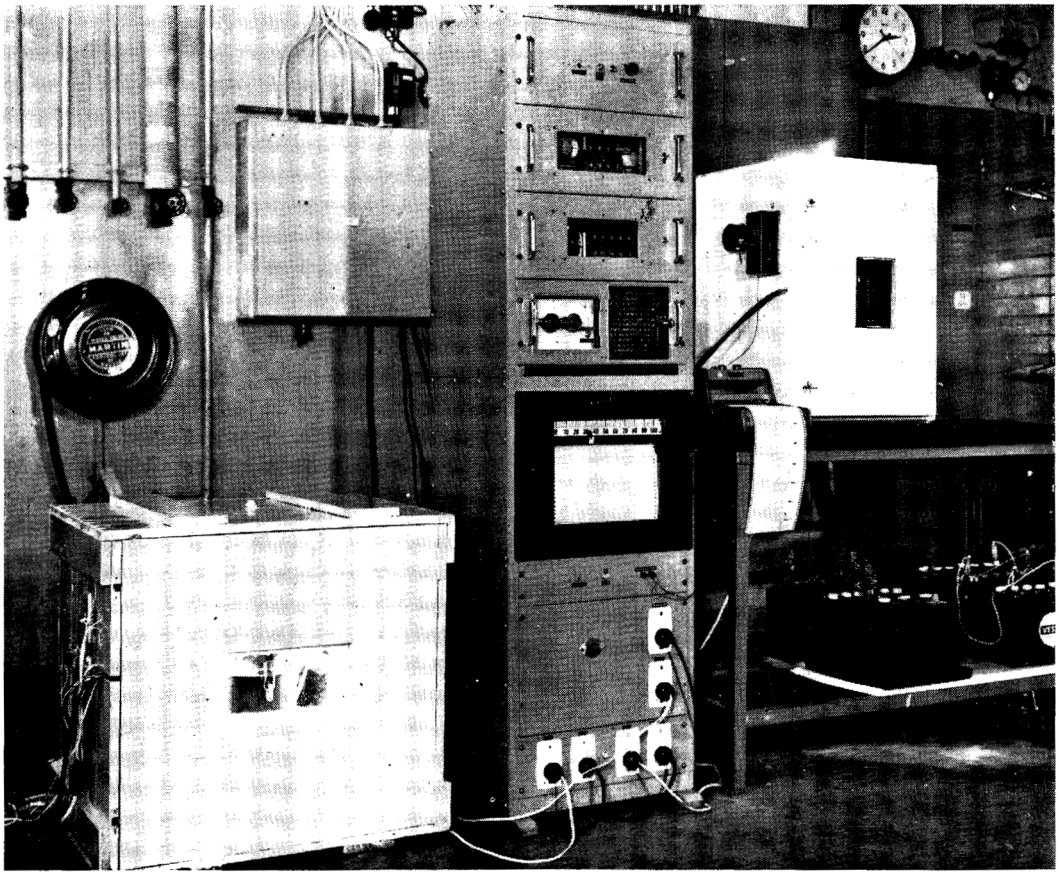

PLATE 1 Calorimeter box (outside cold room) and associated controls.

(*Facing p.* 200)

the apparatus. A 4·5-litre silvered-glass vacuum flask contained the calorimetric fluid, a 150-W immersion heater, thermocouple sensors, stirrer and the sample for analysis in a chrome-plated copper flask of annular shape, 0·7 in. wide. This container gave the maximum sample volume possible in the cylindrical vacuum flask, consistent with a reasonably quick thermal equilibration time for the sample and the calorimetric fluid.

The adiabatic environment was an insulated box with three 150-W light bulbs for heaters, a fan, stirrer motor, adiabatic thermocouple sensors and stirred ice-water reference bath. The calorimeter box was situated in a cold room at −40°F, the lowest temperature at which calorimetric measurements were begun. The recorder and automatic controls were situated adjacent to the cold room.

(a) *Sequence controller.* This unit allowed energy to be delivered to the calorimeter for a specified period at regular intervals. A synchronous motor (2 rev/hr) connected through a variable gear train rotated three cams, each of which operated a single-pole change-over microswitch. Each cam was of the split-plate type, and had a profile giving a 1:1 mark/space ratio. By rotating one plate relative to the other, any mark/space ratio could be obtained. The microswitches energized the main supply to the energy meters, calorimeter immersion heater, the transistorized counting circuit and an alarm circuit which operated if a fault occurred in the counting circuit. This last system automatically disconnected the power supply to the calorimeter to prevent large unmetered amounts of energy from passing into the apparatus.

(b) *Energy meters and counter.* Two eddy-disc Watt-hour meters were mounted in a temperature-controlled (± 1°F) cabinet. Each was standardized to ± 0·05% at the 150-W load which was to be metered. The amount of energy delivered to the calorimeter was determined by counting the number of revolutions made by the aluminium disc of one of the meters, the other meter being kept only as a check. The gear train register of the Watt-meter was removed and replaced by a small photo-conductive cell mounted beneath the rotating disc. Two anti-creep holes in the disc allowed light to fall on the cell once every half revolution. An electronic detector sensed the change in photo-cell resistance and pulsed a uniselector which was pre-set to count from 1 to 22 disc revolutions (2·26–49·69 Btu). When the desired heat input had been injected, the supply voltage to the heater was automatically disconnected and the uniselector homed to its initial position.

(c) *Temperature sensing and recording.* Initial calorimetric tests used a calibrated thermistor with a Callendar–Griffiths bridge and a sensitive nano-ammeter as a null detector. This system was satisfactory but did not lend itself to automatic recording.

Hence single and multiple thermocouples were used, their outputs being recorded on a modified twelve-point 2·5-mV potentiometric recorder.

The principal temperature sensor was a 24-junction thermopile, made from 30 s.w.g. copper–constantan thermocouple wire calibrated to ± 0·25°F over the temperature range −40° to 104°F. The twelve pairs of junctions were linked between the fluid in the calorimeter flask and ice bath reference, giving a sensitivity of 0·01°F on

the recorder, corresponding to 25 μV. Calibration tests indicated an accuracy of ± 0·05°F for the differential temperature increments. Additional thermocouples monitored temperatures within the calorimeter and the surrounding air, and the temperature difference between the meat sample and the calorimeter fluid.

Although the 24-junction thermopile increased the sensitivity of the temperature measurements, it limited the span of the 2·5-mV recorder to about only 10°F. The temperature range involved in the experiments was from −40° to +104°F, giving a thermopile output of −17·5 to +17·5 mV. To meet this extended range, up to seven precise backing-off voltages were automatically applied to the recorder input. The potentials, in steps of 2·5 mV, were obtained from precision resistors supplied from a zener-diode stabilized power supply. The required backing-off potential was selected by means of a reversible motor-driven range switch controlled by two microswitches, one at each end of the recorder print carriage. The range switch was driven either forwards or backwards, depending on which microswitch was operated and also upon the measured temperature. With this technique, the recorder was able to follow the sharp temperature rise during each heat pulse and the subsequent fall to a steady value. The extended range facility provided an effective increase in chart width of fifteen times (or more if required). It was used on only one recorder point, while another point monitored a fraction of the total backing-off potential in order to locate the total thermopile voltage.

(d) *Adiabatic temperature control.* Temperature differences between the calorimeter flask and the surrounding air were detected by a six-junction thermopile connected to a 24-Ohm spot galvanometer. Once the test was started, the calorimeter temperature rose above that of the surrounding cold store (−40°F). The tendency for the air temperature in the box to fall was corrected by switching on heaters. This was achieved by a photoconductive cell sensing the position of the galvanometer light spot. Threshold changes in the cell resistance were detected and amplified to operate a relay supplying mains current to the air heaters. Manual modulation of the heater current through a 'triac' balanced the on–off automatic control to suit the increasing heat load as the calorimeter warmed. The maximum sensitivity of this arrangement (0·001°F) was far better than that required (0·1°F). However, some unaccountable drift problems were sometimes encountered leading to non-adiabatic conditions. Within limits, it was possible to calculate the non-adiabatic heat loss or gain and correct the observed temperature increment.

(e) *Calorimetric fluid.* Specific requirements limited the choice of a calorimetric fluid, in which the various calorimeter elements were immersed, to only a few possibilities. These requirements were: liquid over the range −40° to +110°F, non-inflammable, non-corrosive, stable, a low viscosity for good heat transfer characteristics, easily purified, a known specific heat and a low thermal capacity on a volume basis. Chlorinated hydrocarbons fulfilled most of these requirements, and of these, trichloroethylene (TCE) was chosen as being readily available and having one of

the lowest volume thermal capacities (specific heat C, 0·22 Btu/lb°F, specific gravity 1·47). Its freezing point was −99°F, boiling point 189°F and viscosity at −40°F 12 lb/ft-hr (5 cP).

Specific heat data for TCE were available from a number of sources. The *Handbook of Chemistry and Physics* (1963) gave C=0·223 Btu/lb°F at 68°F, and the *International Critical Tables* (1929) gave C=0·223 Btu/lb°F at 68°F with a tolerance of ± 0·4%. McGovern (1943) presented extrapolated values over a temperature range of −22°F (C=0·223 Btu/lb°F) to 176°F (C=0·243 Btu/lb°F). The value at 68°F (0·225 Btu/lb°F) was slightly higher than the 0·223 ± 0·001 Btu/lb°F given by the *International Critical Tables* (1929). The specific heat data shown in Fig. 2 were used, i.e. from 0·216 Btu/lb°F at −40°F to 0·226 Btu/lb°F at 105°F.

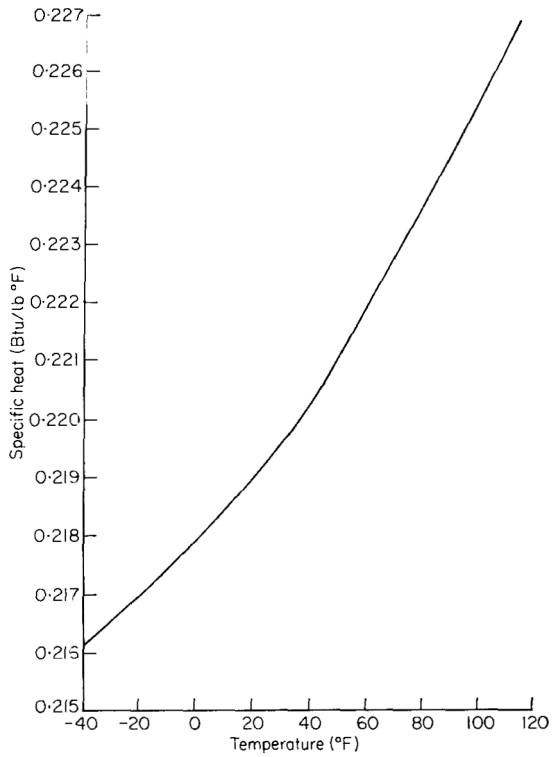

FIG. 2. Specific heat of trichloroethylene.

Method

Ten right sides of lamb, encompassing the more common New Zealand export grades and weights, were each split into five standard cuts (Fig. 3): full leg, short

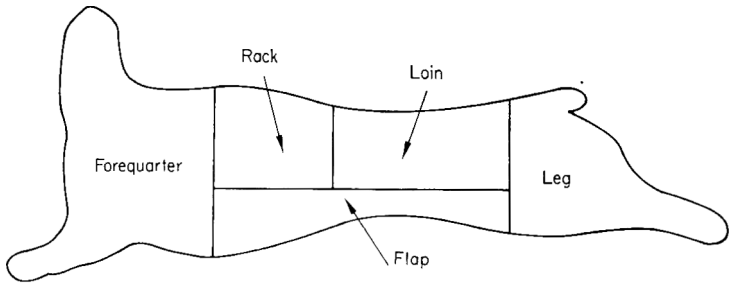

FIG. 3. Carcass cuts used in calorimetric tests.

loin (from pin-bone to thirteenth rib inclusive), rack (sixth to twelfth ribs inclusive), short forequarter and flap. Carcass groupings and details are given in Table 1. In addition, calorimetric tests were made on lamb brains and kidneys, and calf veal.

TABLE 1. Carcass details and analyses

Carcass No.	Breed	Grade	Dressed carcass weight (lb)	Water (%)	Fat (%)	Dry fat-free residue (%)
1	Romney × Romney	Prime 2	31·7	50·3	29·8	19·9
2	Dorset Down × Romney	Prime 8	43·5	52·0	28·2	19·8
3	Romney × Romney	YM	30·2	55·1	23·4	21·5
4	Romney × Romney	Prime 2	37·3	52·7	26·9	20·4
5	Suffolk × Romney	Prime 4	50·0	48·1	33·0	18·9
6	Southdown × Romney	D	26·0	53·7	28·1	18·2
7	Suffolk × Romney	Prime Down D	35·0	55·2	25·7	19·1
8	Romney × Romney	YL	25·8	56·6	20·5	22·9
9	Southdown × Romney	Down D	23·5	57·9	21·2	20·9
10	Romney × Romney	Alpha	20·3	62·2	13·8	24·0

In each test, the whole lamb cut, including bone, was minced through a perforated plate (0·15-in. diameter holes) and a weighed sample of up to 1·5 lb placed in the copper calorimeter flask. After precooling to about −40°F overnight, the sample was placed in the calorimeter with 8 lb of TCE in the main flask. When the system reached thermal equilibrium, heat inputs were injected at about 40-min intervals. The test was stopped when the calorimeter temperature reached about 100°F.

To correlate the enthalpy–temperature data for the various lamb cuts, fat and moisture contents were assessed by standard oven drying and soxhlet petroleum ether extraction (Association of Official Agricultural Chemists, 1960). In general, three fat and moisture determinations were made for each cut. By weighting the analyses of

each cut in proportion to its weight, the average composition of the whole side was found. As a check, analyses were also available for the complete opposite left-hand side. Results for the two sides agreed to within 1%.

Analysis of results

A computer program was developed to translate the recorded millivolt readings directly to temperature–enthalpy curves. In this program, a third degree Lagrange interpolation formula (Mickley, Sherwood & Reid, 1957) was used to convert all millivolt recordings to temperatures. To avoid computation errors, the equation was developed to an accuracy order greater than the significance of the readings:

$$T = 32{\cdot}0106 + 46{\cdot}7949E - 1{\cdot}4845E^2 + 0{\cdot}1153E^3$$

where T = temperature (°F),

E = e.m.f. developed by a copper–constantan thermocouple, reference junction 32°F (mV).

Compared with smoothed values in extended significance thermocouple tables (Adams & Davisson, 1965) the maximum deviation was ±0·018°F over the range −45° to +113°F (Fig. 4).

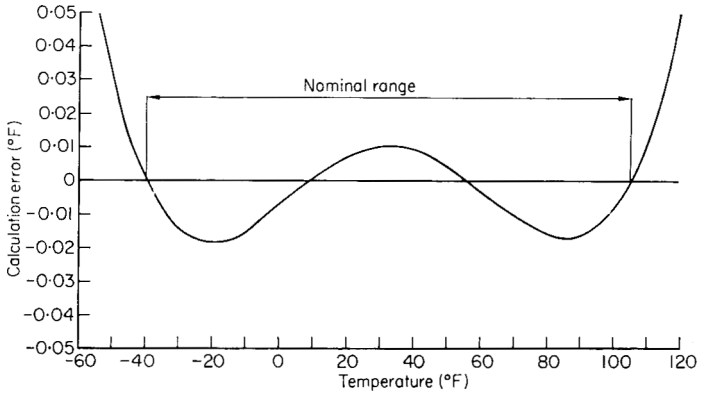

FIG. 4. Error in thermocouple e.m.f. – temperature equation.

Since the specific heat of TCE was temperature-dependent, a quadratic equation was used:

$$C_t = 0{\cdot}21782 + 5{\cdot}28 \times 10^{-5}\, T + 2{\cdot}4 \times 10^{-7}\, T^2$$

where C_t = specific heat of TCE (Btu/lb°F),

T = temperature (°F).

The maximum deviation of this equation from the line shown in Fig. 2 was ±0·0003 Btu/lb°F.

Under ideal (adiabatic) conditions, thermal leakage between the calorimeter flask and its surroundings was zero; but in some tests, slight heat gains or losses were apparent.

These were indicated by finite linear slopes in the final portion of the temperature–time graph. This is illustrated in Fig. 5 where slopes of about 0·005–0·015°F/min were

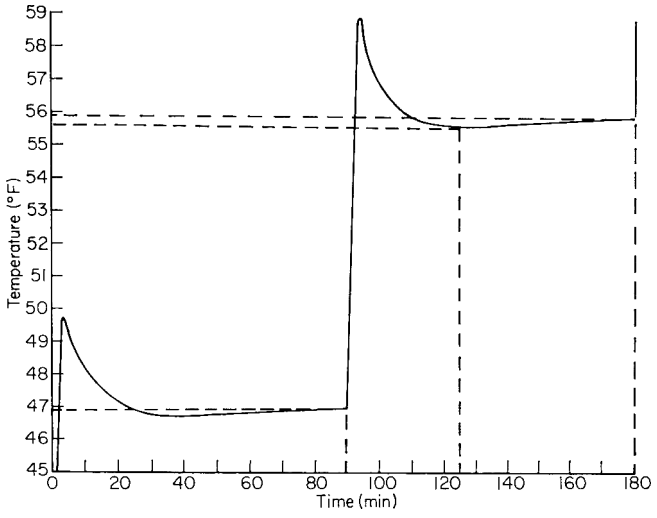

FIG. 5. Calorimeter temperature record showing non-adiabatic conditions.

encountered in a test. With reference to Fig. 5, the calorimeter temperature rose sharply after heating from 46·96°F, then fell exponentially from the peak value to 55·66°F. The temperature then rose slowly over a period of 55 min to reach a final value of 55·93°F before the next increment. The uncorrected temperature rise was, therefore, 8·97°F. The linear slope in the temperature–time graph over the final portion of each increment indicated that the heat gain was constant and consequently must have been present throughout the whole period of 90 min. On this basis, the corrected temperature rise was:

$$8{\cdot}97 - (55{\cdot}93 - 55{\cdot}66) \times 90/55 = 8{\cdot}51\,°\text{F}$$

In this case, the heat increment injected into the calorimeter was 31·62 Btu and the non-adiabatic heat gain responsible for the additional 0·45°F rise was 1·68 Btu.

Calculation of meat enthalpy

The total heat change of the meat (Q_m Btu) was calculated from:

$$Q_m = Q - Q_f - Q_t$$

where Q=heat injected into the calorimeter,

Q_f=heat capacity change of calorimeter flask,

Q_t=heat capacity change of TCE.

For each heat increment, the enthalpy change (Btu/lb) and mean specific heat of the meat (Btu/lb°F) can be calculated knowing the weight of the meat and the

temperature increase, respectively. With adequate calibration of the apparatus and with temperatures accurate to 0·1°F, the expected error in enthalpy and specific heat was about ±1%.

Calculation of frozen water content

If the total enthalpy difference ($\triangle H$) between 32°F and a lower temperature (t) is known, together with the fractional water content of the sample (W), and the average specific heat of non-aqueous matter in the sample (C_{na}), then the proportion of water frozen out as ice (Z) can be calculated from:

$$H = WZL + (32-t)\,[W(1-Z)\,C_w + WZC_i + (1-W)C_{na}]$$

where C_w = mean specific heat of unfrozen water at $(t+32)/2$°F,
C_i = mean specific heat of ice at $(t+32)/2$°F,
L = latent heat of fusion of ice.

The terms in the equation represent, respectively, the heat absorbed by ice formation, unfrozen water, frozen water and dry matter. Putting $L = 143{\cdot}4$ Btu/lb and $C_w = 1{\cdot}01$ Btu/lb°F for temperatures below freezing point:

$$Z = \frac{H - (32-t)\,[1{\cdot}01\,W + C_{na}\,(1-W)]}{W\,[143{\cdot}4 - (32-t)\,(1{\cdot}01 - C_i)]}$$

In the calculations the non-aqueous matter was divided into two portions: fat and dry protein matter. The mean specific heat of fat between 32°F and −40°F was assumed constant and equal to 0·46 Btu/lb°F, based on work by Riedel (1956b). The specific heat of the dry protein matter was taken as 0·22 Btu/lb°F at a mean temperature of −4°F, increasing to 0·23 Btu/lb°F at 31°F (Charm & Moody, 1966). Thus a composite specific heat figure (0·22–0·41 Btu/lb°F) was obtained dependent on the relative proportions of fat and dry protein matter.

Results

Calibration of calorimeter

Fourteen calorimetric tests were made on the two vacuum flasks containing only TCE. Constant thermal capacities for the two flasks and ancillary equipment were assessed at 0·94 Btu/°F for one flask and 0·81 Btu/°F for the other.

A check on the performance of the calorimeter was made with one pound of distilled water in the sample flask. The average specific heat obtained for the water was 0·99 Btu/lb°F over the range 32–93°F, the latent heat of fusion was 144·1 Btu/lb and the mean specific heat of ice over the range −20° to +32°F was 0·50 Btu/lb°F, (Fig. 8). These figures compare favourably with the accepted values of 1·00 Btu/lb°F, 143·4 Btu/lb and 0·48 Btu/lb°F, respectively (*Handbook of Chemistry and Physics*, 1963).

Correlation of fat and moisture contents

The carcass sides lost an average of 5·7% by weight of water from evaporation and sublimation during cooling, freezing and storage. All references to water contents of the meats refer to the actual moisture content at the time of the test.

Good correlations were obtained for the fat–water interrelationships of the lamb cuts (Fig. 6). The correlation coefficient, r, was 0·941, significant at the 0·01% level,

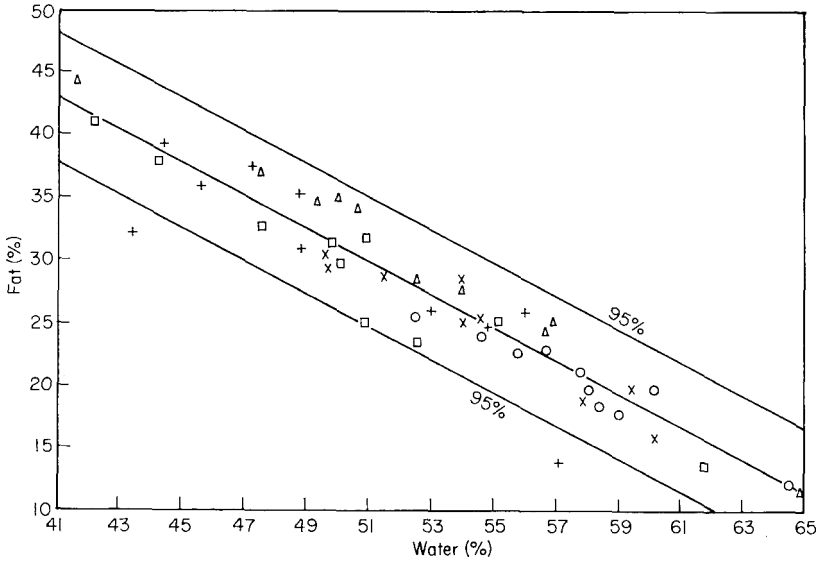

FIG. 6. Correlation of fat and water contents in lamb cuts. ○, Legs; △, loins; □, racks; ×, forequarters; +, flaps.

and the 95% confidence limits gave a fat tolerance of ±5%. Individual correlation coefficients for each group of cuts were: legs 0·95; loins 0·99; racks 0·96; forequarters 0·95; and flaps 0·84. All results were significant at the 0·1% level with the exception of flaps at the 1% level.

Enthalpy–temperature results

The five cuts from each of ten sides of lamb produced a total of fifty enthalpy–temperature curves and additional results were obtained for calf veal, lamb brains and lamb kidneys. Typical values for enthalpy, specific heat and percentage of water frozen are shown in Table 2 for lamb kidneys, calf veal and three cuts of lamb.

Fig. 7 shows the characteristic enthalpy–temperature curve through the experimental

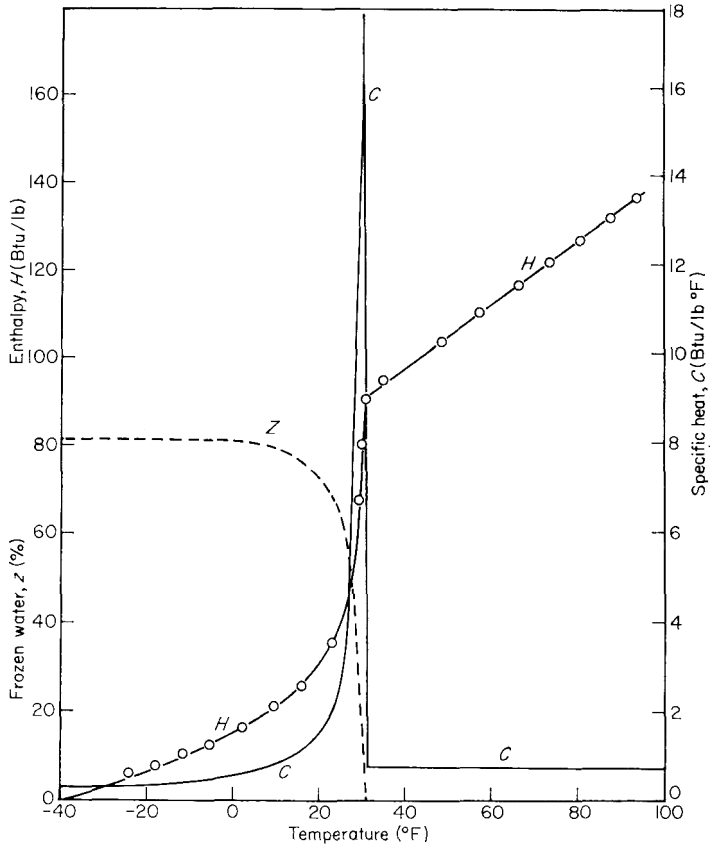

FIG. 7. Typical enthalpy, specific heat and percentage of water frozen out for a lamb cut (52·5% water, 28·4% fat).

points obtained for the moderately fat lamb loin in Table 2. The derived curves relating temperature to specific heat and to percentage of water frozen are also shown. Fig. 8 shows the effect of varying water content on the enthalpy–temperature curves and includes the experimental result for distilled water.

Correlation of latent heat of freezing with water content

Latent heat may be defined as heat change characterized by a change of the substance concerned, for a given pressure, appearing at a constant temperature. According to this definition, the term 'latent heat' cannot strictly be applied to foodstuffs, since the phase change (freezing) is accompanied by a change in temperature (Fig. 7). The specific heat increases to very large values (20–25 Btu/lb°F) at the commencement of freezing but nevertheless remains finite. The total enthalpy change between arbitrary

TABLE 2. Enthalpy (H) Btu/lb, specific heat (C) Btu/lb°F, and % of water frozen out (Z) for various meats

Temperature (°F)	Lamb kidneys 79·8% water 2·9% fat			Calf veal 77·5% water 4·4% fat			Lean lamb loin 64·9% water 11·7% fat			Moderately fat lamb loin 52·5% water 28·4% fat			Fat lamb loin 44·4% water 39·4% fat		
	H	C	Z	H	C	Z	H	C	Z	H	C	Z	H	C	Z
−40	0·0	0·39	89	0·0	0·39	88	0·0	0·31	84	0·0	0·32	(73)	0·0	0·33	(64)
−20	8·1	0·40	89	7·9	0·43	89	6·2	0·36	88	6·4	0·37	79	6·5	0·35	(71)
−10	12·1	0·51	89	12·8	0·51	89	10·6	0·46	88	10·2	0·42	80	10·0	0·36	(74)
0	18·5	0·76	87	17·9	0·61	89	15·6	0·59	87	14·7	0·51	81	13·7	0·43	77
10	27·5	1·1	84	25·1	0·82	86	21·7	0·86	85	20·6	0·72	79	18·7	0·65	76
20	40·5	2·1	76	35·6	1·3	81	33·2	1·6	76	30·0	1·3	73	27·0	1·1	71
24	50·5	4·8	69	41·6	2·0	77	41·1	3·1	71	36·5	2·2	67	32·3	1·8	65
26	63·0	7·2	59	48·0	3·2	72	48·6	5·0	64	41·8	3·3	61	36·8	2·6	60
28	82·0	12·0	43	56·7	7·0	65	65·0	11·0	48	51·2	8·5	50	44·3	4·8	49
30	116·0	50·0	15	91·1	20·0	35	91·0	23·0	21	73·0	14·0	22	62·5	8·0	22
32	135·2	0·98	0	131·7	0·88	0	111·7	0·81	0	91·0	0·70	0	78·0	0·74	0
50	152·8	0·90	—	147·0	0·86	—	125·1	0·81	—	103·8	0·70	—	91·3	0·80	—
70	170·9	0·90	—	161·6	0·86	—	142·2	0·80	—	117·8	0·70	—	108·0	0·80	—
90	(197·0)	(1·31)	—	181·4	0·86	—	158·2	0·81	—	131·8	0·70	—	124·0	0·84	—

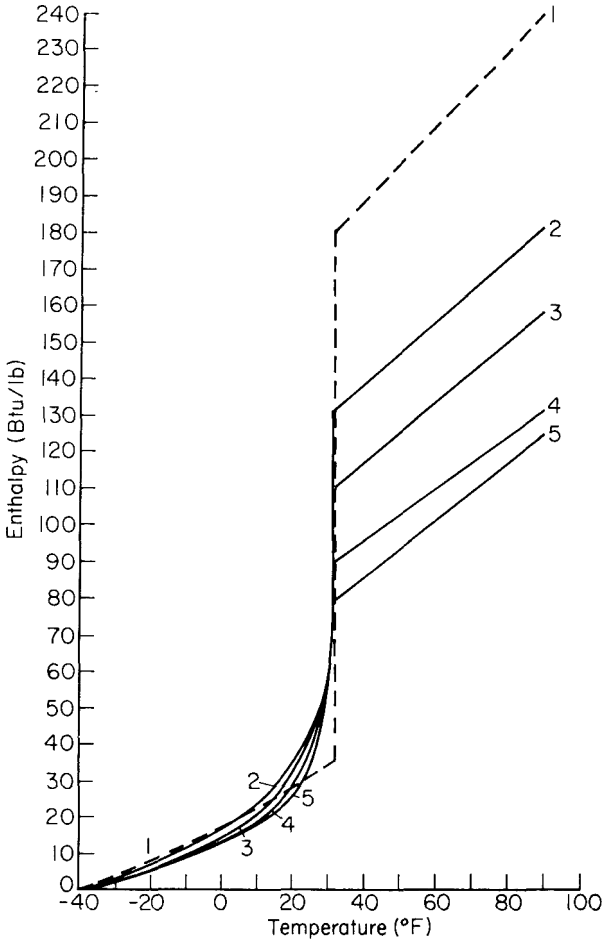

FIG. 8. Experimental enthalpy–temperature curves illustrating the effect of different water contents. 1, water; 2, calf veal 75·5% Water; lean lamb, 64·9%; moderately fat lamb, 52·5%; fat lamb, 44·4%.

temperature limits can be used as a convenient measure of the heat absorbed during freezing. In this work, the arbitrary limits chosen were 32°F and 20°F and the 'latent heat' of freezing (ΔH_{32-20}) defined as the enthalpy change between these two figures. Fig. 7 shows that nearly 90% of the water capable of being frozen at a temperature of −40°F has already frozen at 20°F. In Fig. 9, ΔH_{32-20} values for the lamb cuts are shown plotted against respective water contents. Despite a considerable scattering of the points, a good correlation was obtained, $r=0{\cdot}84$, significant at the 0·1% level. The first degree regression line and 95% confidence limits are shown in the figure.

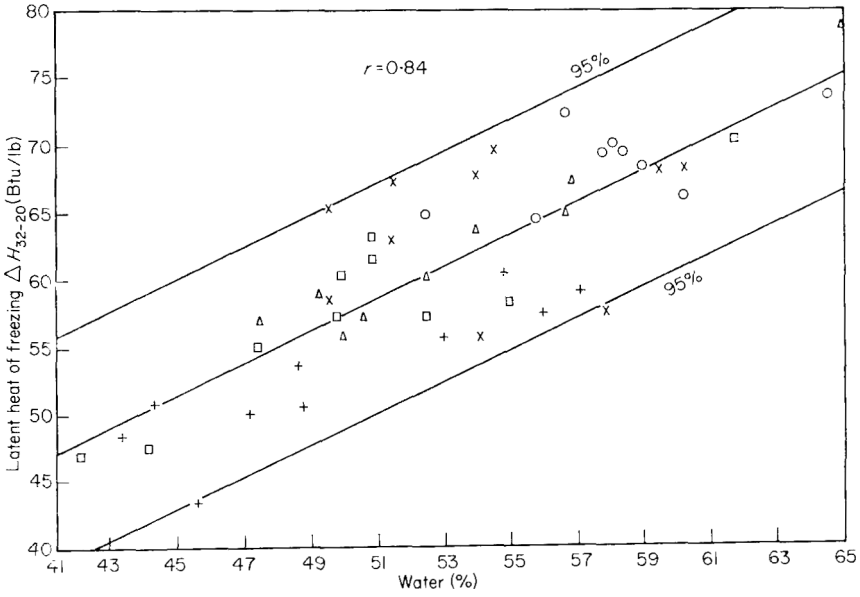

FIG. 9. Correlation of 'latent heat' of freezing with water content in lamb cuts. ○, Legs; △, loins; □, racks; ×, forequarters; +, flaps.

Specific heat above freezing point

Values ranging from about 0·60 to 0·81 Btu/lb°F were obtained for the lamb cuts and there was no significant correlation with moisture or fat contents. The specific heat of fat (Riedel, 1956b) in this region (32– 90°F) was roughly the same as for lean meat with about 60% water content, i.e. 0·75 Btu/lb°F. The mean specific heats obtained for each group of cuts, with corresponding fat and moisture contents, are shown in Table 3.

TABLE 3. Specific heats above freezing point

Cut	Carcass weight (%)	Water (%)	Fat (%)	Specific heat (Btu/lb°F)
Leg	34·5	57·8	20·4	0·76
Loin	12·7	52·3	30·4	0·75
Rack	11·5	50·5	29·2	0·72
Forequarter	34·1	54·3	25·1	0·73
Flap	7·2	49·9	30·2	0·69
Weighted mean, all carcasses	100·0	54·5	25·0	0·74

Discussion and conclusions

The results of this work illustrate the interdependence of water and fat contents, and the 'latent heat' of freezing. Reasonably accurate predictions of these variables can

be made knowing the type and grade of meat which is being processed.

In many previous publications, it is apparent that tables showing latent heats of freezing for foodstuffs have been calculated by multiplying the percentage water content of the food by 143·4 Btu/lb, the accepted value of the latent heat of fusion of water. The latent heat figures derived above differ considerably from the results obtained by use of this simple formula. For example, for the moderately fat lamb loin (Table 2), water content 52·5%, $\Delta H_{32-20}=61{\cdot}0$ Btu/lb, whereas the above relation predicts $143{\cdot}4\times 52{\cdot}5/100=75{\cdot}4$ Btu/lb. However, if the lower temperature limit is extended from 20° to 5°F, the 'latent heat' of freezing and the sensible heat of subcooling (ΔH_{32-5}) increases to 74·0 Btu/lb, which is fortuitously close to that predicted by multiplication of the latent heat of water by the water content. Fig. 10 illustrates

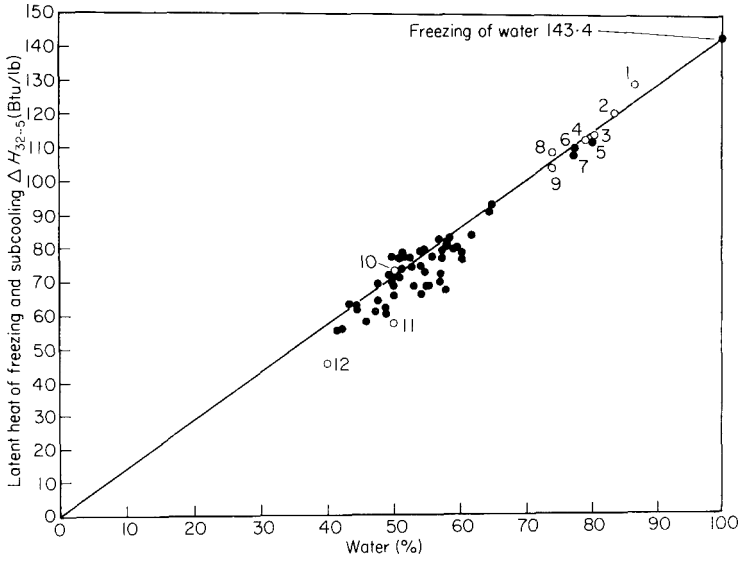

FIG. 10. Correlation of heat absorbed during freezing with water content for various foodstuffs. 1, Egg white; 2, haddock; 3, cod; 4, red fish; 5 kidneys; 6, calf veal; 7, lamb brains; 8, whole egg; 9, beef; 10, egg yolk; 11, potato starch; 12, white bread; ●, lamb cuts.

the total enthalpy change between 32° and 5°F plotted against the water content for all the experimental data obtained here, together with results published by Riede (1956a, 1957, 1959, 1961). The points, with the exception of those for some foods of low moisture content, lie very close to the line connecting the origin with 143·4 Btu/lb for pure water. There is little significance in this relationship, other than that the temperature of about 5°F is near the final temperature to which most foodstuffs are frozen.

Thus the total heat released in freezing the food to say 5°F may be obtained from the simple rule that latent heat is proportional to water content.

Design figures for engineering calculations

The following enthalpy figures should provide a useful guide for calculating heat loads during cooling, freezing and storage. They may be used for a freshly prepared, moderately lean lamb carcass which has lost not more than 2% of its initial weight by water evaporation.

Carcass details: water content, 60%; fat content, 22%; dry protein matter + ash, 18%.

Temperature (°F)	−40	−20	0	10	20	24	26	28	30	32	90
Enthalpy (Btu/lb)	0	7	16	22	32	39	46	59	84	103	148

Above 32°F, the specific heat may be taken as 0·77 Btu/lb°F.

These figures should be used only as a rough guide, since considerable enthalpy variations occur with different fat and water contents, the latter in turn being affected by weight loss after slaughter. For meats of different water content, the figures given in Table 2 or results interpolated from Figs. 8, 9 and 10 should be used.

The proportion of water frozen out

The results presented for the proportion of water frozen out are in reasonable agreement with those published by other workers (Love, 1966). Some of the figures obtained in the low temperature region (+10°F to −40°F) appear to be up to 10% too low compared with Riedel's results (1957) and further work will be necessary at these low temperatures to determine accurately the proportion of water frozen out and the nature of the unfrozen water. Part of the experimental error can be attributed to high initial starting temperatures in the calorimeter, sometimes up to −20°F, instead of the −40°F origin. Also, there may have been insufficient time at −40°F for the ice-water system to equilibrate. Up to 15 days may be necessary for the completion of freezing. Nevertheless, there appears to be a tendency for the proportion of unfrozen water to increase with the fat content, possibly as a result of some fat–water emulsification in the homogenized samples.

Water in tissue which does not freeze is often loosely defined as 'bound' water. This 'bound' water is responsible for latent heats of freezing being lower than expected, particularly for low moisture content foods. Riedel (1957) showed that 1 kg of dry muscle would bind about 0·35 kg of water, equivalent to two molecules of water per molecule of amino acid. Meryman (1966) mentioned the significance of the chemistry of water in organized structures and cells and he emphasized the importance of 'bound' water which obviously plays a vital function in animal tissue, in comparison with free water acting merely as a solvent.

It is obvious that more information is required on the specific heats of the supercooled unfrozen water and the dry protein–fat constituents before greater precision in the results can be obtained.

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The production of pito, a Nigerian fermented beverage

J. A. EKUNDAYO

Summary. The traditional method for preparing pito, a Nigerian fermented beverage, from maize, sorghum or a mixture of both, is described. Pito was prepared from maize in the laboratory and microbiological aspects of the process investigated. The conversion of starch to sugars during steeping and malting appears to be due partly to enzymes in the grain and partly to moulds (*Penicillium* spp., *Rhizopus oryzae* and *Aspergillus* sp.) present on the surface of the grain. The first stage of fermentation of the extract from the malted grain, souring, is dependent upon micro-organisms from the atmosphere and a second stage, after boiling to concentrate, on the addition of a 'starter' from a previous brew. *Candida* sp., *Geotrichum candidum* and *Lactobacillus* sp. appear to be important in both stages, and result in the production of lactic acid and about 3% alcohol. The production of alcohol and organic acids by inoculation of sterile extracts of malted grain by pure cultures of the above organisms was demonstrated.

Introduction

The preparation of fermented beverages from maize, sorghum or millet, or from various mixtures of these cereals, is carried out by villagers in many parts of the world. Such beverages are particularly important in Africa, those produced in East or South Africa being collectively referred to as Kaffir Beer or Bantu Beer. Similar beverages (Van der Walt, 1956) are merissa (Sudan), bouza (Ethiopia) and pombe (East Africa). In the Republic of South Africa the brewing of one form of Kaffir Beer has developed into a major industry in the hands of the authorities, with an output comparable in volume to the South African output of European beers (Novellie, 1963, 1966a,b, 1968); in addition a large amount of home-brewed Kaffir Beer is made in South Africa from commercially malted sorghum.

In spite of the importance of these beverages, serious studies of their production, microbiology and nutritional value are few. The most extensive investigations are those by Novellie and co-workers, who have recently published the nineteenth paper (Dabier & Novellie, 1968) of a series on 'Kaffir corn malting and brewing studies'.

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These, however, are devoted exclusively to various aspects of the industrially produced South African beverage. The production of the traditional Kaffir Beers of South Africa are described by Fox (1938) and those of Nyasaland (now Malawi) by Platt & Webb (1946), Platt (1964) and Williamson (1955). The social and nutritional significance of these beverages is considered by Fox (1938), Fox & Stone (1938), Platt & Webb (1946) and Platt (1955, 1964). Little is known about the microbiology of any of the traditional Kaffir Beers, and very little has been published about any aspect of the West African fermented cereal beverages. One such beverage, pito, is an important food in the Mid-western, West-central, Western, Benue-Plateau and North-central states of Nigeria. The present paper describes the traditional method of preparing this beverage, its production under laboratory conditions, and investigations on the identity and role of the micro-organisms involved.

Experimental methods

Media

The following media were used for the isolation of micro-organisms involved in the fermentation. Their composition is expressed as %, w/v. All media were solidified with 2% Oxoid Agar No. 3.

Glucose peptone agar: peptone, 1·0; D-glucose, 0·5; 10 ml 0·4% (w/v) bromocresol blue; distilled water, pH 6·7–7·0.

Yeast extract agar: yeast extract, 0·3; peptone, 0·5; distilled water, pH 7·2.

Glucose ammonium chloride agar: D-glucose, 1·0; NH_4Cl, 0·1; KH_2PO_4, 0·15; $MgSO_4.7H_2O$, 0·05; distilled water, pH 4·5.

Malt extract agar: malt extract, 2·0; distilled water, pH 5·5.

Cornmeal agar: cornmeal, 1·7; distilled water, pH 5·8–6·2.

Maize extract agar: 50 g germinating maize grains were ground, 250 ml distilled water added and the mixture boiled for 30 min and filtered. The filtrate was made up to a litre with distilled water, pH 5·0.

Maize extract nutrient agar: to 100 ml maize extract prepared as above, D-glucose, 1·0; NH_4Cl, 0·1; KH_2PO_4, 0·15; $MgSO_4.7H_2O$, 0·05, were added, pH 4·5–5·0.

Identification of micro-organisms

The micro-organisms isolated were sent to the Centraalbureau voor Schimmelcultures, Baarn, Netherlands and the Commonwealth Mycological Institute, Kew, Surrey, England for identification. The identification by paper chromatography of the sugars, amino acids and organic acids in pito is described below.

Sugars

The sugars in pito were separated using one way descending paper chromatography. The test sample was centrifuged and 50 ml supernatant were passed through 10-cm

columns of ion exchange resins, Amberlite IR-120 (H^+ cycle) and IRA-400 (OH^- cycle), for cation and anion removal, respectively (Phillips & Pollard, 1953; White & Hess, 1956). The deionized sample was deprotenized by mixing 25 ml with 50 ml 0·3 N $Ba(OH)_2$, followed by 50 ml 5% aqueous $ZnSO_4.7H_2O$ (Somogyi, 1945). Ten microlitres of the treated sample and a 1% (w/v) mixture of the following reference sugars in 70% (w/v) ethanol (xylose, arabinose, fructose, glucose, sucrose, maltose, lactose, melibiose, raffinose and glucuronic acid) were spotted at 2-in. intervals onto Whatman No. 1 papers and developed in n-butanol–acetic acid–water (5 : 1 : 2) for 40 hr. The chromatograms were dried for 30 min at room temperature and drawn through $AgNO_3$ solution (Trevelyan, Procter & Harrison, 1950) to locate the sugar spots. The R_f values of the sugars were determined.

Amino acids

Fifty millilitres of the supernatant were passed through Amberlite IR-120 (H^+ cycle) and the column was washed with water. The amino acids were eluted with 2 N NH_4OH using the ninhydrin test to indicate complete elution (Redfield, 1953). Excess ammonia was removed by concentration *in vacuo* and the residue dissolved in 10 ml water and passed through a column of Amberlite IRA-400 (OH^- cycle). The amino acids were then eluted with concentrated NH_4OH (Clarkson & Kench, 1956) and 10 µl of both the reference and test samples were spotted on Whatman No. 1 papers. The following reference amino acids, 5 mg in 10 ml 10% (v/v) isopropanol were used: aspartic acid, tyrosine, cystine, cystein-hydrochloride, glycine, arginine, threonine, glutamic acid, proline, leucine, isoleucine, serine, α-tryptophan, methionine, asparagine, glutamine, valine and histidine hydrochloride. One way descending paper chromatography using n-butanol–acetic acid–water (12 : 3 : 5) for 14 hr gave good separation and was generally used. The chromatograms were dried for 5 min at 75°C, sprayed with 0·2% ninhydrin and dried for 3 min at 100°C.

Organic acids

One way descending paper chromatography was used. The organic acids were first separated into volatile and non-volatile acids. Fifty millilitres of the supernatant were mixed with an equal volume of acidified $MgSO_4.7H_2O$ solution and distilled in micro-Kjeldahl distillation apparatus. The first 50 ml of the distillate were used for volatile acid determination and the solution in the distillation apparatus for non-volatile acid. The organic acids in each portion were first converted to their ammonium salts by titrating with excess $Ba(OH)_2$ using phenol red as indicator. The barium salts precipitated were evaporated to dryness and the residue dissolved in 1 ml saturated ammonium oxalate to give the ammonium salts of the acids. Ten millilitres of the volatile, non-volatile and the reference acids (0·5% (w/v) formic, acetic, lactic, succinic, buytric, tartaric, citric, malic acid) were spotted onto Whatman No. 1 papers and

developed in n-propanol–ammonia (8 : 2) for 12 hr. The chromatograms were dried at room temperature and sprayed with bromocresol purple (Reid & Lederer, 1951) to locate the spots. The papers were then exposed to an atmosphere of 3% (v/v) ammonia.

Determination of alcohol content of pito

Pito samples of 100 ml were neutralized by titrating against 0·1 N NaOH using phenolphthalein as indicator. The solution was distilled and 50 ml collected. The specific gravity of the distillate was determined using the specific gravity bottle method. The corresponding percentage alcohol by volume in the distillate at room temperature was obtained from the reference table in *Official Methods of Analysis* by the Association of Official Agricultural Chemists (1960).

Results

Traditional process for the preparation of pito

A widespread procedure among the Bini tribe, to which the author belongs, is to wash and soak cereal grains (maize, sorghum or a combination of both) in water for 2 days after which they are malted by leaving for 5 days in baskets lined with moistened banana leaves. The malted grains are ground, mixed with water and cooked. The mash is allowed to cool and filtered through a fine mesh basket. The residue is used as animal feed. The filtrate is then left, usually overnight, until it tastes slightly sour. It is then boiled to concentrate it. A small quantity of the 'starter' (sediment from a previous brew) is added to the cooled concentrate and left overnight. The product is pito, a dark-brown liquid with taste varying from sweet to bitter.

Preparation of pito samples in the laboratory

Pito samples were prepared in the laboratory in a manner similar to the traditional procedure outlined above. Two hundred grams of maize grains were washed and soaked for 2 days in a glass jar, 20 × 20 cm, containing 1000 ml distilled water. The grains were then spread out in a $\frac{3}{8}$-in. square mesh copper basket, $6\frac{1}{4} \times 6\frac{1}{4} \times 6$ in., lined and covered with sterile moistened banana leaves and left for 5 days for germination to occur. The malted grains were rinsed in two changes of distilled water and ground. The mash was extracted by boiling for 30 min in a pressure cooker containing 2 litres of distilled water and filtered by passing through a copper basket. The filtrate was allowed to cool and left overnight in an uncovered glass jar until it tasted slightly sour. This constitutes the first phase of the fermentation. The filtrate was then boiled until the sour taste disappeared and the brew became concentrated. A small quantity of the 'starter' was added and left overnight to bring about the second phase of the fermentation. The product has the usual slightly sweet taste and dark-brown colour of pito.

The micro-organisms involved in pito production

The micro-organisms involved at all stages of pito production were investigated and isolated. Those present on the surface of the cereal grains during steeping and malting were isolated by placing three grains centrally on sterile media in Petri dishes or in Petri dishes lined with moistened sterile filter paper, then covering and incubating for 7 days at room temperature. Pure cultures of the micro-organisms growing from the grains were obtained and identified. *Rhizopus oryzae*, *Aspergillus flavus*, *Penicillium funiculosum*, *Penicillium citrinum*, *Giberella fujikuroi* and *Botryodiplodia theobromae* were isolated from unsterile grains during steeping and malting. No micro-organisms were isolated from grains surface-sterilized by rinsing with 0·5% mercuric chloride and distilled water. All the fungi thus appear to have been on the grains and develop during steeping and malting when conditions are suitable for growth

Preparation of pito involves leaving the hot water extract from malted grains overnight until it tastes slightly sour. It is then heated to concentrate it and the 'starter' added to produce a preparation fit for drinking. The micro-organisms involved in converting the sweet mash extract to sour and those in the 'starter' responsible for the ultimate conversion of the extract to pito were investigated. Samples of the starter were obtained from Lokoja (West-central State), Ikare (Western State) and Ibillo (Mid-western State) in Nigeria. The dilution plate method was used. A 10^{-3} dilution of the appropriate sample was made, 5 ml of each solution was placed in a Petri dish, 15 ml sterile agar medium cooled to about 45°C added, and the dishes rotated to ensure proper mixing. The following agar media were used: malt extract, yeast extract, glucose–peptone, maize extract, cornmeal, maize extract with added nutrients (see 'Experimental methods'). The plates were incubated for 36 hr at room temperature after which the colonies of micro-organisms developing were counted, pure cultures of each type obtained and identified. *Geotrichum candidum*, *Candida* sp. and *Lactobacillus* spp. were frequently isolated and *Aspergillus versicolor*, *Penicillium simplicissimum* and *Penicillium purpurogenum* occurred occasionally.

The role of micro-organisms in the steeping and malting of maize grains

The role of the micro-organisms isolated from maize grains during steeping and malting was investigated by studying the ability of the fungi to hydrolyse starch, since unpublished data by the author have shown that the amount of hexose sugars increases on malting while starch concentration decreases. The fungi were grown on media containing starch as the main carbon source and a clear zone underneath and around the growth of mycelium on these media indicated the amount of starch hydrolysed (Gordon & Mihm, 1959). The results are indicated in Table 1. All the fungi isolated hydrolysed starch. The importance of these fungi in malting was further investigated by malting grains surface-sterilized to prevent fungal growth. It was observed that pito prepared from surface-sterilized maize grains malted for the usual

5 days was of a rather thick, viscous, milky composition (compared to medium-thick cooked starch) and pito of usual characteristics was obtained from surface-sterilized grains only by malting for 9 days. These results show that the fungi isolated from malted grains are saccharifying agents, and assist the malting process.

TABLE 1. The ability of some fungi isolated during malting to hydrolyse starch, as indicated by growth on starch-containing substrates

Fungus	Medium		
	Starch–NH_4Cl agar	Cornmeal agar	Mashed ungerminated maize grain agar
Rhizopus oryzae	+++	++	+++
Botryodiplodia theobromae	++	+	++
Aspergillus flavus	+++	+++	+++
Penicillium funiculosum	+++	+++	+++
Penicillium citrinum	+++	+++	+++

+++, Good growth; ++, average growth; +, poor growth.

The role of micro-organisms in the fermentation of mash extract to pito

The fermentation of mash extract to pito takes place in two stages: the overnight conversion of the mash extract to a sour-tasting liquid by micro-organisms from the atmosphere and the conversion of the concentrated liquid to pito by micro-organisms in the starter. The role of these organisms in the fermentation was investigated. Fifty-millilitre portions of sterile mash extract were inoculated with standard suspensions of the micro-organisms, singly and in all combinations, and the changes after 18 hr in the sugar, amino acid and organic acid composition of the extract were determined qualitatively by paper chromatography. Changes in the alcohol content were determined quantitatively (see 'Experimental methods'). The sugars (raffinose, melibiose, maltose, sucrose, glucose, fructose) and amino acids (cystine, histidine, asparagine, aspartic acid, glutamic acid, alanine, proline, tyrosine, valine and leucine) detectable in mash extract were still detectable after stages 1 and 2 fermentation, but lactic, acetic and traces of formic acid and about 3·0% alcohol had been formed. This shows that organic acids and alcohol are produced during the fermentation. Table 2 shows that alcohol production during pito fermentation is mainly by yeasts.

TABLE 2. The effects of micro-organisms, singly and in combinations, in producing organic acids and alcohol from mash extract during pito fermentation

	Micro-organisms						
	Candida sp.	*Geotrichum candidum*	*Lactobacillus*	*Candida* + *Geotrichum*	*Candida* + *Lactobacillus*	*Geotrichum* + *Lactobacillus*	*Geotrichum* + *Candida* + *Lactobacillus*
Lactic acid	+	+	+	+	+	+	+
Acetic acid	Trace	Trace	+	Trace	+	+	+
Formic acid	—	—	Trace	—	Trace	Trace	Trace
Alcohol (%) in sample	3·0	2·3	1·1	3·8	3·1	2·7	3·9

+, Present; —, absent; trace, present in small quantity.

Discussion

As mentioned in the 'Introduction', beverages are produced by villagers in many parts of the world by fermentation from the grain of maize, millet or sorghum or from mixtures of these cereals. Here the preparation and properties of pito will be discussed in the light of what is known of these similar beverages.

Fermentation can only occur when some of the starch present in the cereal has been converted into sugars. In pito this saccharification is achieved by means of 2 days soaking in water followed by leaving for 5 days to germinate in baskets lined with banana leaves. Similar malting procedures have been described in Kaffir Beer production (Fox, 1938; Platt & Webb, 1946; Williamson, 1955; Platt, 1964). It has been maintained that the saccharification process is due to fungal amylases, mainly produced by *Aspergillus flavus* and *Mucor rouxii* (Platt & Webb, 1946; Platt, 1964). An opposing viewpoint is that fungal activity is unimportant and that the cereal amylases are the effective agent in saccharification (Novellie, 1965, 1966a,b, 1968; Van der Walt, 1956). As Platt investigated traditional methods of making Kaffir Beer from maize in Malawi, and Novellie and co-workers, its industrial production from sorghum in South Africa, their conclusions do not necessarily conflict, as was appreciated by Platt (1965). The present study suggets that in pito production both fungal activity and cereal amylases may be important in bringing about saccharification.

Most forms of Kaffir Beer are prepared from a mixture of malted and unmalted grain, although Fox (1938) mentions that some forms of the beverage are prepared

from malted grain alone. In the present author's experience, only malted grain is employed in pito production.

The first phase of the pito (and of Kaffir Beer) fermentation is souring, lactic acid production by organisms from the atmosphere, mainly lactobacilli, being responsible. The lactobacilli bringing about the souring phase in the industrial production of Kaffir Beer have been studied by Van der Walt (1956); thermophilic species were particularly important. Pure cultures of lactobacilli are not yet employed even in the industrial process, at most a 'starter' from a previous batch being used (Schwartz, 1956; Novellie, 1968). It is the lactic acid produced in the first phase of fermentation which distinguishes Kaffi Beer and related beverages from European beers.

A 'starter' is employed in the second phase of the pito fermentation. Those examined by the author consisted mainly of *Candida* spp. and *Geotrichum candidum* in contrast to the conclusion of Van der Walt (1956) that *Saccharomyces cerveisiae* was the predominant yeast in the alcoholic fermentation stage of the industrial production of Kaffir Beer. The presence of *Candida* spp. was, however, noted by Van der Walt, and a study on one traditional form of Kaffir Beer (Doidge, 1910, cited by Van der Walt, 1956) revealed a varied flora which, as in the present study, lacked sporogenous yeasts. Pure cultures of *S. cerevisiae* are now widely used in the industrial production of Kaffir Beer.

Pito resembles Kaffi Beer in containing lactic acid, sugars, amino acids and about 3% alcohol. Studies on vitamin content have not been undertaken, but pito will presumably resemble Kaffir Beer, which contains abundant thiamin, riboflavin and nicotinic acid (Aucamp *et al.*, 1961) and perhaps significant amounts of ascorbic acid. (Fox & Stone, 1938), although the vitamin content of the industrial product has recently been deteriorating (Novellie, 1968). Like Kaffi Beer, pito deteriorates rapidly on storage, and within about 48 hr of preparation becomes undrinkable.

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The detection and determination of synthetic emulsifiers in foods

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Summary. Various analytical procedures which may be used for the detection and determination of synthetic emulsifiers in foods are reviewed.

Introduction

A wide range of diverse substances are used as emulsifiers in foods. They may be present as some basic ingredient of the product, or they may be added during preparation. Substances in this latter group may either be obtained from natural sources, or be produced synthetically. Although emulsifiers are major food additives, only a limited number of methods have been developed for their separation, identification and determination. The deficiency is primarily due to the difficulties involved. For instance, many emulsifiers are mixtures, the composition of which vary according to the method of preparation. In addition, some foods contain several different emulsifiers in order to obtain the desired hydrophile–lipophile balance.

One previous review has been published (Hibbert, 1968), which was restricted to the consideration of those emulsifiers and stabilizers permitted by law in the United Kingdom. Although the present survey deals with a more extensive range of emulsifiers, it cannot cover the analysis of every emulsifier in every possible food product. However, it is hoped that the examples given will provide the basis for the development of further methods. Analytical details have been kept to a minimum since these are available in the original publications.

General glycerol esters

A method has been described for the separation of mono-, di- and triglycerides in shortenings (Distler & Baur, 1966). The sample is adsorbed on a silica gel column and the tri-, di- and monoglycerides are separated in that order by eluting with benzene, benzene plus 10% ether, and ether, respectively. The fractions are collected separately, the solvent removed and the amounts of mono-, di- and triglycerides determined by weighing. This method is not applicable when other emulsifiers are present. Samples having a high free fatty acid content tend to give high results, since 20% of free fatty

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acid is eluted with both the tri- and monoglyceride fractions and 60% with the diglyceride fraction. These errors can be avoided if the separated fractions are saponified and their glycerol content determined colorimetrically by oxidation to formaldehyde and subsequent reaction with chromotropic acid (Franzke, Heims & Vollgraf, 1967). The technique of separation by elution with increasingly polar solvents can be used to resolve mixtures of polar glycerides, i.e. glycerides containing hydroxy and keto-acids (Evans *et al.*, 1967). Other methods available for the analysis of mono-, di- and triglycerides include molecular distillation (Privett, Blank & Lundberg, 1961) and charring of chromatograms (Privett & Blank, 1962). The chromatographic method depends upon the differences in the volatility of the glycerides which results in spots of different intensities after charring.

A general test for 1-monoglycerides is available (Baur & Distler, 1966). This test depends upon the formation of aldehydes when polyhydric alcohols containing adjacent hydroxyl groups are oxidized with periodic acid. The amount of unused periodic acid can be determined iodometrically by adding potassium iodide and titrating with standard arsenite or thiosulphate solution. Other compounds containing two or more adjacent hydroxyl groups will interfere. The method has been used for determining 1-monoglycerides after extraction from bread and lard (Kuhrt *et al.*, 1952). Alternatively, the glycol aldehyde fatty acid ester formed on periodic oxidation can be converted to the 2,4-dinitrophenylhydrazone, which can be determined colorimetrically (Szonyi & Sparrow, 1964). This method has the advantage that glycerol does not interfere. The chromotropic acid reaction has also been used to determine 1-monoglycerides in ice-cream (Schmidt, 1963) and citrus juice concentrates (Rother, 1964a). The samples of molten ice-cream are first dried over anhydrous sodium sulphate and extracted with ether. The ether is evaporated and the extract then dissolved in chloroform. Direct extraction with chloroform is possible with citrus juice concentrates.

Fatty acid mono- and diglycerides can be analysed by gas chromatography; and methods involving the preparation of methyl esters (Canuti, 1964) and trimethylsilyl ether derivatives (Sahasrabudhe & Legari, 1967) have been reported. Alternatively, monoglycerides can be identified by infrared spectroscopy and counter-current distribution (Kuhrt *et al.*, 1952).

Acetic acid glycerides

The percentage of acetylated monoglycerides in food can be found by determining the fat content of the food followed by a determination of its Reichert–Meissl value (Ma & Morris, 1968).

Citric acid glycerides

A paper chromatographic method has been reported for detecting these esters in sausage preparations at concentrations down to 0·1% (Kroeller, 1964a). Steary

monoglyceridyl citrate can be determined in liquid shortenings by saponification with aqueous sodium hydroxide, the citric acid being determined colorimetrically (Ma & Morris, 1968).

Lactic acid glycerides

Lactic acid analyses can be used as an indirect determination of these esters. They are eluted from silica gel columns with the corresponding mono- and diglyceride fractions (Sahasrabudhe, Legari & McKinley, 1966) which can be saponified, acidified and the lactic acid separated by column chromatography (Buswell, 1964) or liquid–liquid extraction (Stetzler & Andress, 1962). The resolved lactic acid fraction is then titrated with standard alkali. The water-insoluble lactic acid in shortenings can be found colorimetrically (Fett, 1961). Addition of acid after saponification releases the lactic acid which is then degraded to acetaldehyde with concentrated sulphuric acid. Acetaldehyde reacts with *p*-phenylphenol in strong acid solution to produce a purple compound which is used as the basis of the estimation.

Succinic acid glycerides

Succinylated monoglycerides can be extracted from bread and cakes with water and n-propanol. The succinic acid formed on saponification can then be methylated and the resultant dimethylsuccinate determined by gas chromatography (Ma & Morris, 1968).

Tartaric acid glycerides

It is possible to detect these esters in flour by mixing with an equal weight of purified sand and extracting with methylene chloride (Kroeller, 1964a). After purification, the emulsifiers are identified by spraying a paper chromatogram with potassium ferrocyanide. Tartaric acid glycerides can be extracted from margarine with methanol (Kroeller, 1962). The tartaric acid fraction obtained can then be resolved on paper using an n-propanol–ammonium hydroxide solvent, and the spots developed with nile blue or silver nitrate. Paper chromatography has also been used to determine tartaric acid glycerides in emulsified fats (Venturini, 1966). Acetyl-tartaric acid glycerides can be detected in margarine by a similar method to that used for tartaric acid glycerides (Kroeller, 1962). However, in this case an n-butanol–ammonium hydroxide mixture is used as mobile phase.

Polyglycerol esters

These esters can be extracted from shortenings by the use of column chromatography (Sahasrabudhe *et al.*, 1966). Tri-, di- and monoglycerides are eluted from the silica gel as described previously and the polyglycerol esters are then quantitatively eluted

with ethanol. The esters can then be identified by their molecular weights and fatty acid distribution. Polyglycerol esters have also been analysed by gas chromatography using trimethylsilyl ether derivatives (Sahasrabudhe, 1967). Methyl ester formation can likewise be used for their determination (Ma & Morris, 1968). A method for detecting small quantities of polyglycerol esters in edible fats and fat-containing foods has been described (Wurzinger & Gebauer, 1962). The fat is saponified and the fatty acids removed by ether extraction. Most or all the glycerol is evaporated under vacuum and the residue dissolved in ethanol. The presence of dark rings on spraying a paper chromatogram with a mixture of ammonium hydroxide and silver nitrate indicates the presence of polyglycerol.

Propylene glycol esters

A gas chromatographic method for the analysis of these esters has been described (Sahasrabudhe & Legari, 1968). The method was designed for their determination in shortenings containing mono- and diglycerides, the mono- and di-esters of propylene glycol being first separated by the method which is also used to separate different classes of glycerides (Distler & Baur, 1966). The mono-esters are eluted in the diglyceride fraction and the di-esters in the triglyceride fraction.

Sorbitan esters

The properties of the most commonly used sorbitan esters have been reviewed and a simple procedure given for their detection (Kroeller, 1968). Thin-layer chromatography (TLC) using a benzene–methanol solvent is employed and the method can be used for the detection of sorbitan esters in products having a high sugar content such as fondant and nougat.

Several different polyols, all derived from sorbitol, are formed on saponification of these emulsifiers. When the polyols are subjected to paper chromatography using *sec*-butanol saturated with water as the mobile phase, four zones are formed: one corresponding to sorbitol, two to the sorbitol monoanhydrides and a fourth to isosorbide (1,4:3,6-dianhydrosorbitol) (FAO/WHO Expert Committee on Food Additives, 1964). The paper chromatography of purified polyols has been used for the detection of sorbitan esters in some simple baking improvers (Schrepfer & Egle, 1958). In this case the paper chromatograms were sprayed with lead tetra-acetate and the presence of sorbitol, glycerol, polyethylene glycol and polyoxyethylene sorbitol was indicated by white spots on a brown background. The sorbitan esters can be determined from their isosorbide content. The method involves alkaline saponification followed by recovery of the polyols and determination of the isosorbide formed by gas chromatography. This method has been used for the determination of sorbitan monostearate in cake mixes and baked cakes (Wetterau, Olsanski & Smullin, 1964; Ma & Morris,

1968). The emulsifier is extracted with ethanol, subjected to partition chromatography on silica gel, saponified and the resultant isosorbide determined. Alternatively paper chromatography can be used, the 1,4-sorbitan spot being cut out and oxidized to formaldehyde which is then determined colorimetrically with chromotropic acid.

Oxidation of the polyols formed on saponification can also be used to determine sorbitan monostearate in whipped vegetable oil toppings (Ma & Morris, 1968). An emulsion of the whip in water, ethanol and ether is broken with the addition of petroleum ether, and the emulsifier together with the oil is obtained in the ether layer. The triglycerides are then removed by partition chromatography. After saponification, the polyols are purified by nonionic exclusion chromatography and determined by dichromate oxidation, the amount of unused dichromate being determined iodometrically. Periodate oxidation of the polyols has also been used for the assay of sorbitan esters (Gatewood & Graham, 1960), but this method has not been applied to foods.

Polyoxyethylene sorbitan esters and other polyoxyethylene derivatives

A comparatively large number of methods for determining emulsifiers containing polyoxyethylene groups have been reported. Most of these methods are based on a determination of the polyoxyethylene groups and, therefore, will not distinguish between different emulsifiers containing these groups. A method is described for detecting these compounds in fruit juices and lemonade (Kroeller, 1964b). A lengthy extraction procedure is employed followed by chromatography on paper impregnated with dimethylformamide using a chloroform–ethyl acetate–n-heptane mixture as mobile phase. The spots are detected by spraying the chromatogram with an ammonium thiocyanate–cobalt nitrate mixture or Dragendorff reagent. As with sorbitan esters, saponification of the extracted emulsifier and paper chromatographic examination of the polyols obtained has been used. Polyoxyethylene emulsifiers can, for example, be detected in some simple bread improvers (Schrepfer & Egle, 1958). In the case of bread itself, pre-heating the crumbs with hydrochloric acid has proved useful in aiding the extraction. The emulsifiers can then be determined semi-quantitatively via their 'free' polyethylene glycol content (Veitch & Jones, 1962), or saponified and detected by paper chromatography using an n-butanol–acetic acid–water mixture as mobile phase (Boari, 1959).

Many reagents have been used to precipitate polyoxyethylene emulsifiers, including those of Dragendorff (Gallo, 1953), Muntoni (Muntoni, Turi & De Giuli, 1959) and Anselmi (Anselmi, Boniforti & Monacelli, 1959). More recently phosphomolybdic acid has been used and several gravimetric determinations have been reported. Thus polyoxyethylene (20) sorbitan monostearate can be determined in shortening and sugar-type confectionery coatings (Ma & Morris, 1968) and polyoxyethylene (20) sorbitan mono-oleate may be determined in bread, cake mixes, ice-cream (Hall, 1964) and salt (Ma & Morris, 1968). In general, these methods involve saponification

of the extracted emulsifier followed by removal of the fatty acids with petroleum ether. The polyols formed are extracted with chloroform and the solvent evaporated. The residue is then dissolved in water and precipitated with barium phosphomolybdate. This precipitate can be dissolved in concentrated sulphuric acid and determined colorimetrically using stannous chloride and ammonium thiocyanate (Garrison, Harwood & Chapman, 1957). This method has been used for determining polyoxyethylene (20) sorbitan tristearate and polyoxyethylene (20) sorbitan mono-oleate in frozen desserts and ice-cream (Ma & Morris, 1968; Butz & Neobels, 1961).

Silicotungstic acid can also be used to precipitate polyoxyethylene compounds. This reagent is used for the gravimetric determination of polyoxyethylene (20) sorbitan mono-oleate in yeast defoamers (Ma & Morris, 1968) and pickle products (Barcklow, 1967). In the latter case the determination can also be carried out by gas chromatography of the methyl ester of the oleic acid formed on saponification (Ma & Morris, 1968). When present in the raw materials used in non-alcoholic drinks, polyoxyethylene sorbitan esters can be precipitated with heteropoly acids, decomposed with hot concentrated phosphoric acid and the acetaldehyde produced determined colorimetrically with cobalt thiocyanate (Rother, 1964b). Direct weighing of the extracted polyoxyethylene (20) sorbitan mono-oleate is possible with some aqueous liquid vitamin preparations (Ma & Morris, 1968).

Sucrose esters

Although the chemistry of sucrose esters has received some attention, little work has been published on their extraction from foods. It has been reported that they can be extracted from margarine, lemonade and pastry and then resolved on polyamide paper using an n-butanol–n-propanol–water mixture as mobile phase (Kroeller, 1963).

Sodium lauryl sulphate

This compound has mainly been used in egg whites in which it can be determined quantitatively. A protein-free extract is evaporated to dryness and the residue taken up in water. The aqueous solution is then titrated in the presence of chloroform with standard benzethonium chloride solution using bromophenol blue as an indicator (Wiskerchen, 1967). Alternatively the sodium lauryl sulphate can be complexed with azure A and determined colorimetrically (Wiskerchen, 1968). Methods for determining sodium lauryl sulphate in soy sauce have been developed (Narahu *et al.*, 1965; Bando & Kinoshita, 1965) and it has been claimed that as little as 3 ppm can be detected in wine, beer, fruit juice and milk (Renault & Bigot, 1965).

Stearyl lactylates

These emulsifiers co-ordinate very strongly with protein, making difficult any extensive

recovery by simple solvent extraction. Digestion with a mixture of sulphuric acid and phosphoric acid, followed by extraction with benzene, has been used. The benzene can then be evaporated and the stearyl lactylates hydrolysed and determined by way of the liberated lactic acid (Ma & Morris, 1968).

Stearyl tartrate

This compound can be extracted from bread, saponified and the stearyl alcohol obtained then determined semi-quantitatively by TLC (Williams, 1964).

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The lithium contents of some consumable items

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Summary. The atomic absorption technique has been used in the analysis of tobacco ash and a number of common consumable items, namely water, salt, lettuce and potato, for their content of lithium in view of the apparent medical importance of this element.

Introduction

Previous work on the alkali metal contents of various foodstuffs has been largely confined to sodium and potassium, little attention having been given to lithium. The possible significance of the latter element is indicated by its successful use in the treatment of patients during the manic phases of manic-depressive disorders. This has been known for many years (Cade, 1949; Schou, 1959; Wharton & Fieve, 1966) though its exact mode of operation remains obscure. Manic-depressive psychosis is characteristically a disease which manifests itself in recurring episodes, and it has been found that, prior to and in the early stages of manic attacks in many patients, there is a positive sodium balance during which the dietary intake of sodium exceeds its excretion via the urine and faeces (Hullin *et al.*, 1968). In view of the known chemical similarity of lithium and sodium, it is plausible to assume that the alleviation by lithium treatment of the mental symptoms associated with mania might be due to the displacement of the retained sodium by lithium. Such an hypothesis raises the question whether lithium in trace amounts is important for the normal mental functioning of the human organism. If this is indeed the case then the relative abundance of lithium in foodstuffs becomes a matter of some significance.

The quantities of lithium found in soils, however, differ greatly from one region to another and it seems reasonable to suppose that those found in plants grown in these soils will vary also. Indeed, in 1965 Borovik-Romanova determined the lithium content of samples of plants and the soils upon which they grew. This investigator found that the amount of lithium present in plants depended on its abundance in the soil as well as on the type of plant; lithium was absorbed from the soil in varying amounts depending on the soil characteristics. An alteration in the soil lithium content was reflected in the plant lithium content. Hence, vegetable foods might differ according to the part of the country in which they were grown and to their particular species and variety. As far

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as possible, therefore, foodstuffs originating from different parts of the country were examined for lithium content.

Review of literature

Little work has previously been carried out on the determination of lithium in foodstuffs and other consumable items. That which has been undertaken has been chiefly limited to the study of water (Ballczo, 1950; Borovik-Romanova, Korolev & Kutsenko, 1954; Mashiko & Kanroji, 1956) and plant tissue (Robinson, Steinkoenig & Miller, 1917; Ramage, 1930; Bertrand, 1943; Yamagata, 1950; Guelbenzu, 1952; Bertrand, 1952; Gavva, Zin'kovitch & Palyanitsa, 1965). In the latter field of study, for example, Bertrand found in 1952 that, from a determination of lithium in sixty-eight families of higher plants, those poor in lithium (0·47–1·07 ppm) included the *Gramineae* and *Liliaceae*, those rich in lithium (1·8–7·96 ppm) the *Solanaceae*.

Among foods of animal origin, those which have been found to contain lithium include eggs (Press, 1941) and milk (Wright & Papish, 1929; Drea, 1934).

The plants examined by Bertrand (1943) were not confined to edible species, but included two types of tobacco, the lithium contents of which were found to be extraordinarily high. This seemed to support the earlier observations of Tate & Whalley (1940), and in its turn received further corroboration from Yamagata (1950). In addition, Bertrand (1943) found that the green parts of lettuce contained 7·9 ppm of lithium.

Choice of material

In the present investigation, it was decided to examine the lithium content of water of various origins, since most lithium salts are soluble and water from most supplies will have percolated through layers of soil, some of which may have contained lithium. The great importance of water as an item in the diet was another potent reason for beginning with this substance.

Salt also features largely in the diet and, since it is an alkali metal compound occurring naturally, it was reasonable to suppose that other alkali metals, such as lithium, might be found in association with it.

Previous findings by Bertrand (1943) on the lithium content of the green part of lettuce, added to the fact that this salad vegetable is popular in many parts of the world, made it seem likely that some of the human intake of lithium could stem from this source.

The high lithium content of tobacco previously noted (see above) prompted the present authors to investigate this material also. Whilst it may be objected that tobacco can hardly be regarded as a foodstuff, it is nevertheless true to say that the products of its combustion are ingested by the user to a considerable extent. It seemed likely, therefore, that smoking provides another means of ingress of lithium into the body.

Yet another reason for investigating tobacco was its botanical relationship to certain

edible plants such as the potato and the tomato. The former of these is well established as a major item of diet, and its lithium content has also been investigated. That of the tomato will be reported elsewhere.

Choice of method

Methods for determination of lithium in foodstuffs have in the past been limited almost entirely to the use of the spectrograph and the flame photometer. In the present investigation, however, it was decided to apply the technique of atomic absorption for this purpose. The chief reason for this choice was the lack of occurrence of spectral interference occasioned by elements other than lithium. Indeed, the only elements which were thought likely to prove troublesome were calcium and strontium. Even these, however, were found not to interfere. The instrument used throughout this work was the Unicam SP90 Atomic Absorption Spectrophotometer, a propane–air flame being employed.

Methods

Construction of the calibration graphs

It was decided to construct two calibration graphs, one covering the range 0–1 ppm of lithium and the other the range 0–10 ppm. For this purpose a stock solution, to be diluted as appropriate, was made up from a sample of 'specpure' lithium carbonate, which was dissolved in the minimum quantity of hydrochloric acid and made up to the required volume. The lithium content of the resulting solution was checked gravimetrically by means of the triple acetate procedure (Grüttner, Keck & Haubold, 1951).

The following instrument settings were used for the calibration as well as for the subsequent analyses:

Slit width	0·13 mm
Wavelength	670·8 nm
Fuel	225 ml/min
Air	5 l/min
Burner height	0·4 cm below the optical centre

Preparation of samples

(a) *Water.* Samples of tap water were obtained from various parts of the country and prepared for examination as follows:

One litre of the sample was evaporated to dryness, and the residue was dissolved in 2 N hydrochloric acid. This solution was again evaporated to dryness and the resulting solid was dissolved in deionized water. The solution obtained was made up to 25 ml with more deionized water.

One of the samples examined was Harrogate spa water. This was found to have a much higher lithium content than other types of water and was, therefore, not concentrated by the procedure described above.

(b) *Salt.* The preparation of salt samples for analysis was achieved by the following method:

Ten grams of the sample were dissolved in 35 ml of deionized water. In some cases this gave rise to an insoluble residue of magnesium carbonate, which was filtered off. The solution was then transferred to a 50-ml graduated flask and made up to the mark with deionized water.

(c) *Lettuce.* A quantity of lettuce was cut up, spread out on trays and dried in an oven at 100°C. When most of the moisture had been driven off, the lettuce was transferred to beakers, and the drying process continued until constant weight had been attained. The dried material was finely ground with a pestle and mortar, and 10 g were weighed into a silica crucible. This was carefully heated over a bunsen flame until the emission of smoke ceased, and then transferred to a muffle furnace where the ashing was continued at 550°C. When constant weight had been reached the residue was treated with 2 N hydrochloric acid, about 10 ml of this being required to ensure the completion of the reaction. The contents of the crucible were boiled gently for about 10 min. After cooling, the acid was neutralized with a few drops of 0·88 M ammonia and the contents of the crucible were filtered through a Whatman No. 42 filter paper. The crucible and filter were washed with deionized water, and the combined filtrate and washings collected in a 50-ml graduated flask. The contents of this were subsequently made up to volume.

(d) *Tobacco ash.* The samples used consisted of the remnants from tobacco which had been smoked in a cigarette or pipe.

One to 2 g of tobacco ash, accurately weighed, were heated in a silica crucible at 550°C by means of a muffle furnace. When the ashing process had thus been brought to completion and constant weight reached, the residue was treated in the same way as the lettuce ash.

(e) *Potatoes.* The sample of potatoes was peeled and dried in the manner described for lettuce. Twenty grams of the dry material were then ashed and extracted again by the same technique as for lettuce.

All analyses were made at least in duplicate; agreement between replicates was good.

Results

The results are shown in Table 1.

TABLE 1

Commodity	Origin or type	Lithium content (ppm)
Water	Leeds	0·003
	Birmingham	0·001
	Central London	0·007
	Makerfield	0·012
	Southport	0·016
	Hartlepools	0·018
	Harrogate (Spa water)	1·8
Salt	Cerebos (table)	1·0
	Sifta (table)	2·85
	Dripak (table)	2·28
	Saxa (table)	2·21
	Tesco (cooking)	3·42
Lettuce	Hull (outer leaves)	0·5–0·9
	Hull (inner leaves)	0·00
	Leeds (outer leaves)	1·53
	Leeds (inner leaves)	0·47
	Gosport (outer leaves)	2·04
	Gosport (inner leaves)	0·82
	Coventry (outer leaves)	1·25
	Coventry (inner leaves)	0·26
Tobacco ash	Senior Service (cigarette)	49
	Embassy (cigarette)	88
	Golden Virginia (cigarette)	61
	Hamlet (cigar)	23
	Whisky (pipe)	36
	Anfield (pipe)	148
Potatoes	Early Majestics (Leeds)	0·28
	Pentland Dells (Lincolnshire)	0·07
	Red Skins (Middlesbrough)	0·11

Discussion

The fact that lithium was found in almost every sample examined, although sometimes in very small quantities, indicates that such consumable items make a small but steady contribution to the intake of lithium into the body of the consumer. All the tobacco ash samples contained very high concentrations of lithium, so that the inhalation of ash during smoking could provide a further source of this metal.

Naturally, the actual quantity of lithium derived from any given food will depend on the amount of that food which is regularly consumed. Moreover, it is necessary to take into account the different bases on which the figures quoted in Table 1 were

calculated. Thus, the data for lettuce and potatoes were worked out on the dry weights of the commodities, whilst those for salt refer to the sample as received. The same is, of course, true also of water. The lithium content of tobacco might be inferred from the results to be inordinately high, but it must be remembered that the figures quoted are those for the ash and not for the leaves themselves. In the present context, it is, of course, the ash which is significant in determining the amount of lithium ingested by a smoker.

It must be admitted that little is at present known with certainty about the human body's requirements of lithium and it is, therefore, impossible to conclude whether the commodities mentioned above are rich or poor sources of the metal.

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A lipid analysis of the Greater Silver Smelt [*Argentina silus* (Ascanius)] and an evaluation of its potential for food and fish meal production

P. R. MACKIE AND R. HARDY

Summary. Samples of the fish species *Argentina silus* (Ascanius) have been studied for utilization as a food fish and also for the production of fish meal. The flesh was found very acceptable by taste panels, but because of its unsaturated oil content, rancidity would occur during long-term storage unless precautions were taken to reduce this spoilage.

The lipid content and composition of this fish together with the amino acid composition has been measured. The results indicate that fish meal produced from it would be nutritious and have a relatively high oil content, if the fish was not processed as a fatty fish.

Introduction

In view of the over-exploitation of many fish species such as cod, haddock, etc., it is inevitable that there will have to be a diversification of catching effort towards fish that are not at present used for human consumption. One of the species that could be exploited in this manner or used for the production of fish meal is the Greater Silver Smelt.

According to Wood & Raitt (1963, 1968) a commercial fishery for this fish is a distinct possibility in areas to the West of Ireland. In recent years, the above workers have examined in detail the biology and habitat of this fish, but there appears to be no information published on its chemical composition or indeed acceptability as a food fish. It is known that it was used for this purpose in Britain at the turn of the century (Jenkins, 1925), as it is today to a small extent in Europe. It is now used, however, primarily for conversion to fish meal.

The present work was undertaken to provide a fairly complete picture of the lipids, which are of importance with regard to the cold storage life of the fish, and also the storage and nutritional properties of meal produced from it. In addition, a culinary assessment of both the white and smoked flesh was made.

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Materials

Samples of fish were caught off Rockall, North Cape Bank (Norway) and Litlö Bank (Lofoten). Apart from the Rockall fish, which were frozen whole, all the fish used were gutted and then frozen. Size measurement, tasting, smoking and oil content were carried out on fish caught on all grounds. Unless otherwise stated all the other results quoted were obtained from fish caught off Rockall.

Methods

The water content of the fish was determined by Dean and Stark azeotropic distillation and by drying the sample in a vacuum oven at 70°C. Nitrogen determinations were carried out by the macro-Kjeldahl method and the ash content was determined by incineration at 500°C for 2 days. The antioxidant and haem contents were determined by the methods of Hardy & Mackie (1969). Lipids were extracted by the Bligh & Dyer (1959) technique, the water content of the solvent mix being modified to give a final solvent ratio of chloroform–methanol–water (10 : 10 : 9). With the exception of total nitrogen, which was carried out on the flesh only, the analyses were performed on thoroughly minced samples of the whole fish.

For taste assessment four fillets from different fish out of each batch were given a commercial-type smoke cure and these, with a further two fillets, again from different fish, were steamed for 30 min before tasting.

Separation of the major lipid classes

A column of 10 g of silicic acid was packed in chloroform, according to the method of Hanson & Olley (1963), and 100 mg of total lipid extract dissolved in 1 ml of chloroform, which was then applied to the column. The neutral and free fatty acids (FFA) were eluted with 250 ml of chloroform and the phospholipids then eluted with 100 ml of 9 : 1 methanol–chloroform.

The non-phospholipid fraction was further fractionated on an alkaline silicic acid column prepared according to the method of McCarthy & Duthie (1962). The neutral lipids were eluted with 250 ml of diethyl ether and the FFA eluted with 50 ml of 1% formic acid in ether followed by 100 ml of diethyl ether.

The method of Smith, Hardy & Davie (1968) was slightly modified for the completion of the fractionation of the neutral lipids. The resultant elution pattern is shown in Table 1. All fractions were checked for purity by thin layer chromatography (TLC) using the two solvent system devised by Freeman & West (1966). The TLC separation of the various lipid classes is shown in Figs. 1 and 2.

TABLE 1. Elution pattern of neutral lipid separation

Eluting solvent	Volume (ml)	Lipid material eluted
Benzene	20	—
	10	Sterol ester
	20	Sterol ester
	10	Triglyceride
	50	Triglyceride
Benzene–ether–ethanol (80 : 19 : 1)	25	Triglyceride
	25	Diglyceride
	25	Diglyceride
	25	Sterol
	25	Sterol
	25	Sterol
	20	—
Methanol	50	Monoglyceride

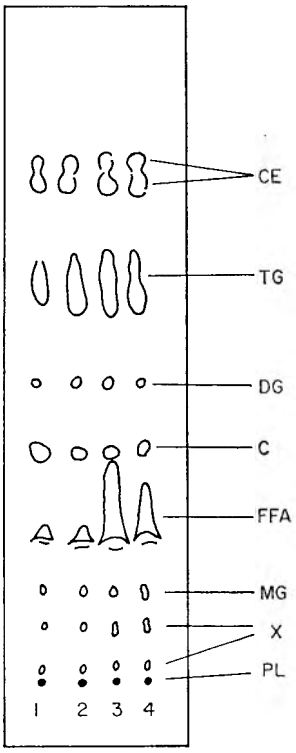

FIG. 1. Chromatogram illustrating the separation of the lipid classes. 1, Total body lipid; 2, flesh; 3, liver; 4, guts. CE, Cholesterolester; TG, triglyceride; DG, diglyceride; C, free cholesterol; FFA, free fatty acid; MG, monoglyceride; X, unidentified; PL, phospholipid.

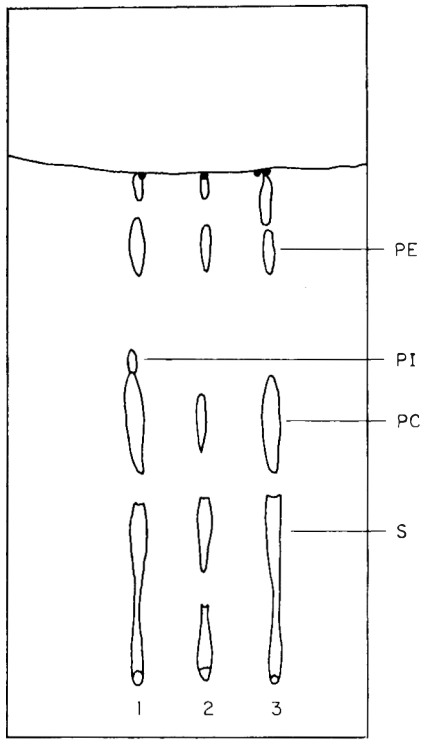

FIG. 2. Chromatogram illustrating the separation of the phospholipids. 1, Flesh; 2, liver; 3, guts. PE, Phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; S, sphingomyelin.

Phospholipid separation

The solvent system proposed by Skipski, Peterson & Barclay (1964) and containing chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2) was used to fractionate the phospholipids on TLC plates. Visualization of the components was achieved by spraying with a solution of potassium dichromate dissolved in sulphuric acid followed by charring the TLC plates with heat. Identification of the components was carried out by comparison of the R_f values with those given in the literature and also by running against known standards. This latter procedure did not work too well with some of the standards. Egg yolk lecithin (Koch-Light & Co., Colnbrook, England), for example, did not give the same R_f value as either that quoted in the literature or the apparent lecithin present in *A. silus*. When mixed with the phospholipids of *A. silus* before and after fractionation, however, the standard lecithin behaved in an identical manner with the purported lecithin from the fish.

A Joyce Chromoscan (Joyce-Lobel & Co., Gateshead, England) was used for the quantization of the separated components on TLC plates and the reproducibility with

different concentrations was ± 10%. Although no attempt was made to ascertain the degree of charring obtained with different phospholipids, runs carried out by adding standards to the phospholipid mixture of the fish indicated that the quantization error was within the ± 10%.

For further analyses the fish phospholipids were fractionated by preparative TLC using the solvent system of Skipski *et al.* (1964) and also by column chromatography according to Hanahan, Dittmer & Warashina (1957). Neither method completely resolved the mixture.

Methylation of lipid fractions for GLC analysis

Each fraction (all containing the antioxidant BHT) was refluxed for 1 hr on a steam bath with 10 ml of 3% sulphuric acid in methanol which contained 0·5% benzene to increase solubility. Twenty millilitres of distilled water was then added and the esters extracted with redistilled 40–60 petroleum ether. Before analysis by GLC the esters were treated by passage down a silicic acid column to remove extraneous material. The conversion yield was greater than 99%.

Gas–liquid chromatography

The apparatus used was a Perkin-Elmer model F11 capillary gas chromatograph. The columns and conditions used for the methyl esters identification are given in Table 2. Quantization was carried out with a Kent Chromalog integrator, the computational error in the calculation of the minor peaks (i.e. components present at a level of approximately 1%) was of the order of ± 10%. Good correlation was obtained

TABLE 2. GLC operating conditions

Packing	Length	Internal diameter	Temperature (°C)	Carrier gas (N_2) flow rate (cm^3/min)	Split ratio
EGSS-X	50 m	0·01	190°	~0·8	~ 50 : 1
Apiezon L	100 m	0·01	225°	~3·1	~100 : 1
1% SE 30 on AW/HDMS treated chromosorb G	3 ft	0·25	250°	65	—

between iodine values calculated from the GLC traces and those determined chemically by Wij's method. The sterols were analysed as their trimethyl silyl derivatives on the 1% SE 30 column. Prior to the analyses of the esters and ethers the columns were calibrated with known standards. As a further check on the esters they were hydrogenated and the resulting product chromatographed on the GLC columns.

TABLE 3. General analysis of *A. silus*

Sample No.	Length (cm)	Weight (g)	Liver weight (as % of total weight)	Gut weight (as % of total weight)	Fillet yield (as % of whole fish)	Oil content (%)	Tocopherol content (μg/100 ml) of total body	Ubiquinone content (μg/100 ml) of total body	Haematin content (as 1 mg/100 ml)	Ash content (%)
1	39·3	678·7	0·9	6·0	36·6	–	–	–	–	–
2	38·2	545·1	1·0	8·4	38·5	–	–	–	–	–
3	35·1	541·2	0·6	5·8	33·9	–	–	–	–	–
4	41·4	683·2	0·6	6·9	35·7	–	–	–	–	–
Mean of 1–4	38·5	612·0	0·8	6·8	36·2	4·5	Trace	0	2·7	2·4

* The gut weight is exclusive of the liver.

Results and discussion

Filleting the fish was made difficult by the presence of large hard scales. The fillets themselves were of good appearance, though long and somewhat narrow, with a pronounced reddish-brown lateral band. All who tasted the fish found it to be most acceptable. The taste was described as being sweetish and resembling whiting (*Gadus merlangus*) or redfish (*Sebastes marinus*). The smoked fillet had a good colour and gloss and was considered very palatable indeed.

TABLE 4. Composition of the oils

	Oil content†	Iodine value	Tri-glycerides*	Di-glycerides*	Mono-gly-cerides*	Phos-pho-lipids*	FFA*	Sterol esters*	Sterols*
Flesh	3·5	114·9	82·1	0·4	0·3	8·6	6·3	1·0	1·3
Liver	6·4	174·4	41·9	4·1	2·4	4·1	36·3	7·4	3·8
Guts	3·9	159·5	54·7	1·2	0·5	8·2	22·9	8·5	4·0

* Expressed as % of oil.
† Expressed as % of wet weight.

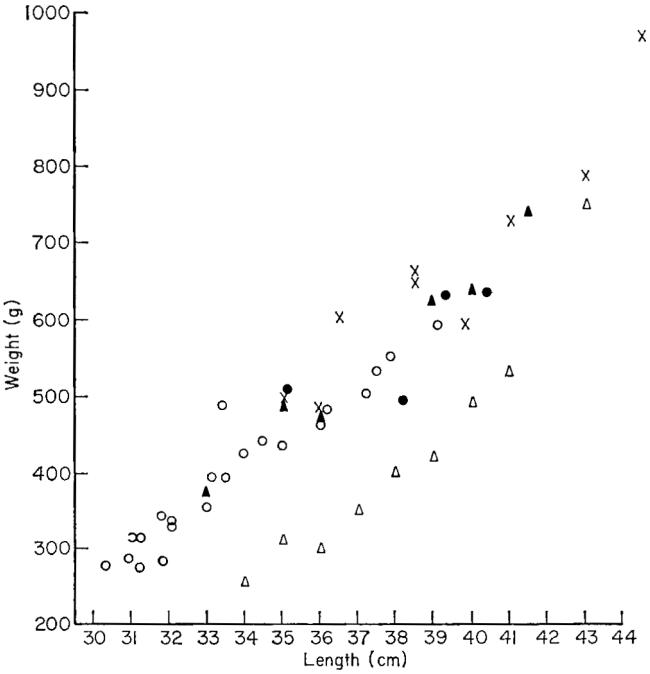

FIG. 3. Length–weight relationship. O, South-east Faroe; ●, Rockall; ▲, Lofoten (Litlo); ×, Norway (North Cape Bank); △, south-west Ireland (Porcupine Bank) (ungutted). The data from Porcupine Bank was taken from Wood & Raitt (1963) by kind permission of the authors.

TABLE 5. Phospholipid composition (expressed as % total phospholipids)

	Flesh	Liver	Guts
Phosphatidylethanolamine	32	24	32
Phosphatidylinositol	11	+	+
Phosphatidylcholine	50	23	44
Sphingomyelin	7	53	25

TABLE 6. Fatty acid composition of flesh oil components

Fatty acid composition	Total oil	Tri-glycerides	Di-glycerides	Mono-glycerides	Phospho-lipids	FFA	Sterol esters
$C_{14:0}$*	7·0	7·5	7·0	6·2	1·0	3·1	7·0
$C_{14:1}$	0·5	0·5	0·5	0·1	0·1	0·1	0·6
$C_{15:0}$	1·0	0·9	1·4	1·0	0·3	0·9	0·9
$C_{16:0}$	14·9	13·0	14·3	18·2	22·7	19·6	15·8
$C_{16:1}$	5·4	5·6	6·2	8·4	2·5	4·1	7·6
$C_{16:2}$	0·9	0·7	1·3	0·2	0·2	0·4	0·9
$C_{16:3}$	0·2	+	+	+	−	+	+
$C_{16:4}$	+	+	0·4	+	+	+	+
$C_{17:0}$	0·6	0·5	0·9	0·2	0·4	0·6	0·3
$C_{18:0}$	4·2	3·6	5·6	4·0	5·9	6·2	2·7
$C_{18:1}$	18·4	18·6	18·7	32·8	12·9	15·4	22·9
$C_{18:2}$	1·4	1·5	2·7	2·1	1·2	2·1	2·2
$C_{18:3}$	0·5	0·9	1·9	1·6	0·8	0·6	1·0
$C_{18:4}$	0·8	1·0	1·8	1·1	0·3	0·3	1·1
$C_{19:0}$	0·3	0·5	0·5	0·1	0·1	0·2	0·3
$C_{20:1}$	11·5	12·6	6·3	5·3	1·7	2·4	10·8
$C_{20:2}$	0·4	0·2	0·3	+	0·2	0·1	1·5
$C_{20:3}$	0·5	1·1	1·3	0·2	2·7	3·4	1·3
$C_{20:4}$	1·0	0·5	0·6	+	0·8	0·5	1·0
$C_{20:5}$	3·0	1·9	4·2	3·6	6·8	8·4	3·9
$C_{22:1}$	19·0	22·1	9·4	5·2	0·6	1·4	10·1
$C_{22:2}$	0·5	0·2	0·7	+	0·2	0·4	0·2
$C_{22:3}$	0·2	0·4	0·3	+	0·3	0·2	1·7
$C_{22:4}$	0·2	−	0·4	+	1·6	1·6	+
$C_{22:5}$	1·0	0·3	0·5	0·5	3·3	1·8	0·7
$C_{22:6}$	4·5	3·6	9·8	5·5	32·3	25·1	2·6
$C_{24:1}$	0·7	0·9	0·4	0·2	0·2	+	0·5
Minor and unidentified components	1·5	1·4	2·6	3·6	0·8	1·1	2·4
Total	100·1	100·0	100·0	100·1	99·9	100·0	100·0

* Shorthand notation for chain length–number of double bonds.

TABLE 7. Fatty acid composition of the liver oil components

Fatty acid composition	Total oil	Tri-glycerides	Di-glycerides	Mono-glycerides	Phospho-lipids	FFA	Sterol esters
$C_{14:0}$	3·5	3·7	5·1	5·8	2·2	3·9	3·3
$C_{14:1}$	0·4	0·2	0·4	0·7	+	0·2	0·1
$C_{15:0}$	0·5	0·6	0·6	1·6	1·2	0·7	0·5
$C_{16:0}$	14·9	12·6	17·2	22·1	16·5	25·2	11·3
$C_{16:1}$	6·1	6·9	8·5	7·4	5·5	5·3	5·2
$C_{16:2}$	0·5	1·1	1·7	1·4	+	0·7	0·8
$C_{16:3}$	+	+	+	+	+	+	+
$C_{16:4}$	+	0·1	+	0·1	+	0·2	+
$C_{17:0}$	0·1	0·4	0·2	0·9	0·9	0·8	0·1
$C_{18:0}$	4·6	2·0	4·0	4·4	8·5	9·1	1·5
$C_{18:1}$	25·1	32·5	19·4	22·1	17·4	15·9	20·2
$C_{18:2}$	0·9	1·9	2·6	1·0	1·9	2·9	1·0
$C_{18:3}$	0·2	0·6	0·2	5·2	1·1	0·3	0·4
$C_{18:4}$	0·2	0·4	0·3	+	+	0·2	0·3
$C_{19:0}$	0·2	0·1	0·2	0·3	0·6	0·3	+
$C_{20:1}$	5·8	10·1	9·1	6·2	3·3	3·6	11·0
$C_{20:2}$	0·3	0·5	0·3	+	+	0·1	0·6
$C_{20:3}$	+	1·5	2·0	0·8	0·8	2·5	1·5
$C_{20:4}$	1·7	1·0	1·0	0·5	0·4	0·3	1·5
$C_{20:5}$	7·2	3·4	6·8	2·4	4·9	8·9	6·1
$C_{22:1}$	5·3	10·0	8·3	6·7	1·9	1·9	14·1
$C_{22:2}$	0·3	+	+	+	+	+	0·6
$C_{22:3}$	1·0	0·3	+	−	+	0·1	6·7
$C_{22:4}$	1·6	0·2	0·4	+	+	0·1	−
$C_{22:5}$	1·7	1·7	0·9	1·3	+	0·4	1·3
$C_{22:6}$	14·3	5·6	8·5	5·2	18·0	14·0	8·1
$C_{24:1}$	1·4	0·5	0·3	1·8	0·4	0·1	0·5
Minor and unidentified components	2·3	2·1	2·0	2·1	14·4*	2·3	3·3
Total	100·1	100·0	100·0	100·0	99·9	100·0	100·0

* Contains 12·7% $C_{24:0}$.

The results of the analyses are given in Tables 3–10 and in Fig. 3. The latter shows a plot of the weight to length relationship of a representative sample of the fish obtained from various grounds. For the sake of completeness and comparison, Wood & Raitt's (1963) figures obtained from the fishing grounds of Porcupine Bank (South West Ireland) have also been included. If the fillet yield quoted in Table 3 is taken into account, it can be seen that on the average, fillets of an acceptable commercial weight (*c.* 200 g) can be obtained from these fish.

TABLE 8. Fatty acid composition of gut oil components

Fatty acid composition	Total oil	Tri-glycerides	Di-glycerides	Mono-glycerides	Phospho-lipids	FFA	Sterol esters
$C_{14:0}$	6·0	8·3	5·6	10·1	4·3	5·1	5·8
$C_{14:1}$	0·6	0·6	0·2	0·3	0·7	0·6	0·7
$C_{15:0}$	0·9	0·8	1·1	1·2	1·3	0·8	1·7
$C_{16:0}$	14·7	18·5	17·0	13·7	20·1	15·9	14·6
$C_{16:1}$	6·8	8·0	8·2	9·2	5·3	6·3	6·0
$C_{16:2}$	0·8	0·6	1·0	+	1·9	1·0	0·5
$C_{16:3}$	0·3	0·3	+	+	+	–	+
$C_{16:4}$	+	+	+	+	+	0·2	+
$C_{17:0}$	0·6	0·5	0·1	0·3	1·0	0·6	0·5
$C_{18:0}$	3·8	3·2	4·2	4·2	7·5	4·9	4·1
$C_{18:1}$	17·9	23·3	20·4	31·1	18·7	17·8	24·6
$C_{18:2}$	1·1	2·2	2·1	6·5	1·8	1·4	1·5
$C_{18:3}$	0·7	1·0	0·8	1·8	0·8	0·4	0·2
$C_{18:4}$	1·1	0·5	0·1	+	+	0·4	1·0
$C_{19:0}$	0·3	0·6	0·1	+	0·7	0·6	+
$C_{20:1}$	8·3	10·0	8·6	4·1	3·3	5·1	8·3
$C_{20:2}$	0·3	0·2	+	+	+	0·2	0·2
$C_{20:3}$	0·3	0·3	0·8	+	1·1	3·6	0·8
$C_{20:4}$	2·0	0·2	0·8	+	0·2	0·4	0·2
$C_{20:5}$	6·4	2·1	4·3	3·8	7·9	11·2	6·8
$C_{22:1}$	12·1	13·8	10·1	5·7	2·2	5·3	10·0
$C_{22:2}$	0·9	+	+	+	+	0·6	0·2
$C_{23:3}$	0·2	+	+	+	+	–	+
$C_{22:4}$	+	+	0·2	+	+	0·5	0·2
$C_{22:5}$	1·7	0·8	1·0	+	+	1·8	1·9
$C_{22:6}$	11·8	2·1	8·7	5·0	17·6	14·2	8·0
$C_{24:1}$	0·3	+	0·1	+	+	0·4	0·2
Minor and unidentified components	2·1	2·1	4·5	3·0	3·5*	1·2	1·9
Total	102·0	100·0	100·0	100·0	99·9	100·3	99·9

* Contains 2·1% $C_{24:0}$

The lipid content of the flesh of the fish varied from 1·9 to 4·1% and because of the small samples available we were unable to correlate the lipid content with sexual maturity of the fish. It was noted that fish caught off Norway tended to have a higher lipid content than fish caught elsewhere but there was a considerable scatter in the results. The distribution of the total lipids and lipid classes from a typical sample is shown in Table 4. Distribution appears to be fairly uniform throughout the fish but it is noteworthy that the phospholipid content is low (291 mg/100 g flesh), whereas,

TABLE 9. Fatty acid composition of the flesh phospholipids*

Fatty acid	Phosphatidyl-choline (a)	Phosphatidyl-ethanolamine (b)	Sphingo-myelin (c)
$C_{14:0}$	2·9	0·8	5·9
$C_{14:1}$	+	+	0·3
$C_{15:0}$	0·5	0·2	1·1
$C_{16:0}$	29·3	9·9	30·9
$C_{16:1}$	2·5	1·1	7·5
$C_{16:2}$	0·2	0·4	1·5
$C_{16:3}$	+	+	+
$C_{16:4}$	0·2	0·1	+
$C_{17:0}$	0·3	0·4	1·0
$C_{18:0}$	1·7	8·1	6·0
$C_{18:1}$	10·4	10·0	22·3
$C_{18:2}$	1·7	1·1	2·1
$C_{18:3}$	1·4	0·8	+
$C_{18:4}$	0·8	0·2	+
$C_{19:0}$	+	0·2	+
$C_{20:1}$	1·3	3·4	1·0
$C_{20:2}$	0·1	0·2	+
$C_{20:3}$	1·2	1·9	0·4
$C_{20:4}$	0·8	1·1	0·4
$C_{20:5}$	12·7	9·1	2·0
$C_{22:1}$	0·3	0·7	0·4
$C_{22:2}$	0·2	—	0·8
$C_{22:3}$	0·5	0·5	—
$C_{22:4}$	+	0·7	+
$C_{22:5}$	1·6	2·8	+
$C_{22:6}$	27·0	45·6	1·0
$C_{24:0}$	—	—	6·3
$C_{24:1}$	1·3	—	—
Minor and unidentified components	1·2	0·6	9·1
Total	100·1	99·9	100·0

* These data were obtained from fish caught in Norwegian waters. (a) Contains 14% phosphatidylethanolamine and 8% sphingomyelin; (b) contains 15% phosphatidylcholine, 5% sphingomyelin and 5% phosphatidylinositol; (c) of 65% purity.

TABLE 10. The amino acid composition of the flesh

g amino acid/ 100 g protein		g amino acid/ 100 g protein	
Arginine	6·83	Valine	6·52
Histidine	2·56	Alanine	7·26
Lysine	15·82	Glycine	6·45
Phenylalanine	4·72	Proline	4·10
Tryosine	3·71	Glutamic acid	19·18
Leucine	8·84	Serine	4·59
Isoleucine	5·56	Threonine	5·63
Methionine	3·74	Aspartic acid	12·13
Cystine	4·21		

Total nitrogen content of the flesh	3·13%
Protein nitrogen content (after 5% TCA extraction)	2·79%

the FFA content especially in the liver and guts is high (2300 and 900 mg/100 g wet tissue, respectively). Enzymatic hydrolysis of the phospholipids would explain the low level of this component. The similarity between the fatty acid composition of the flesh, liver and gut phospholipids and the FFA, as shown in Tables 6, 7 and 8 would lend support to this hypothesis. It is not suggested, however, that all the FFA present were produced from phospholipids.

Analyses of the phospholipids gave the results shown in Table 5. Just how much phospholipolytic activity had changed the ratio of the various components present is not known, but, the relatively high content of sphingomyelin in the gut and liver is somewhat unexpected. This observation may not represent the actual composition in the living fish, for the FFA of sphingomyelin (see Table 9) are less unsaturated than those of the other phospholipids and fish phospholipases may preferentially hydrolyse those lipids with the highest degree of unsaturation, thus leaving the saturated phospholipids relatively intact.

The fatty acid composition of the major lipid classes follow the expected trend with the phospholipid and FFA fractions having higher concentrations of saturated (except $C_{14:0}$) and polyunsaturated members and a lower monoene content than the triglycerides. In the liver phospholipids there is a high concentration of $C_{24:0}$ (12·7%), undoubtedly due to the high proportion of sphingomyelin, which is known to possess a high concentration of C_{24} acids (Lovern, 1955) (see Table 9). In Table 9 the fatty acid composition of the phospholipid is presented only as a guide, as TLC of the fractions on which the analyses were carried out indicated that the fractions were by no means pure.

Only cholesterol could be found in the free and bound sterol fractions. If any other sterol was present it was at a level of less than 0·1%. This result may be contrasted with the work of Hølmer (1967) who found 22-dehydrocholesterol, brassicasterol, 24-methylene-cholesterol and a C_{29} sterol in herring, haddock and whiting oils.

The data presented here suggest that the fish is acceptable as a food fish, but because of its fat content, it would appear necessary for precautions to be taken to minimize autoxidative spoilage changes during long-term preservation such as storage at − 30°C. The fat content of the whole fish studied was too low for it to be processed as a fatty fish in fish meal production. The end product, however, would be a very fatty fish meal (up to 20% fat) and would be prone to oxidative deterioration.

The amino acid content of the fish as shown in Table 10 would indicate that meal produced from the fish would be at least equal to herring meal as a feedingstuff.

Acknowledgment

Thanks are due to Mr A. Youngson of the Fisheries Biochemical Unit of Aberdeen University who performed the amino acid analysis. The work described in this paper was carried out as part of the programme of the Torry Research Station.

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The phenolic compounds of blackcurrant juice and their protective effect on ascorbic acid

III. The mechanism of ascorbic acid oxidation and its inhibition by flavonoids

K. A. HARPER,* A. D. MORTON† AND E. J. ROLFE‡

Summary. The oxidation of ascorbic acid in citrate buffer at pH 2·9 has been investigated with and without added copper ions as catalyst, and the loss of ascorbic acid followed by polarographic analysis. Certain flavonoids occurring in blackcurrant juice were used as antioxidants and their activity, in the absence of added copper ions, was found to decrease in the order, quercetin and dihydroquercetin (equal), kaempferol and rutin. Cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside accelerated the oxidation. In the presence of added copper ions quercetin showed increasing antioxidant activity up to $12{\cdot}5 \times 10^{-5}$ M (7·8 : 1, quercetin–copper ratio) after which the activity decreased rapidly. Anthocyanins were shown to possess slight antioxidant activity in the presence of copper ions. The probable mechanism of ascorbic acid oxidation is outlined, and the mode of action of flavonoids as antioxidants discussed.

Introduction

The inhibition of ascorbic acid oxidation by the flavonoid constituents of blackcurrant juice has been investigated in model systems (Clegg & Morton, 1968) and it was found that the flavonol aglycones gave good protection. The glycosides were less effective and the anthocyanins showed antioxidant activity only in the presence of copper ions. In the absence of added copper ions the anthocyanins accelerated the oxidation.

The protective mechanism of flavonoids is not clearly described in the literature and, indeed, the mechanism of ascorbic acid oxidation itself does not appear to have been fully elucidated. The present study was undertaken to obtain an understanding of these reactions.

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Experimental

Buffer solution

Citric acid (0·15% w/v) was dissolved in deionized water and partially neutralized with sodium hydroxide solution to give pH 2·9.

Ascorbic acid solution

A freshly prepared aqueous solution (2·00% w/v) was used to give a concentration of 1 mM in the buffer solution.

Copper sulphate solution

An aqueous solution of $CuSO_4.5H_2O$ (0·10% w/v) was added to the buffer solution to give a concentration of $1{\cdot}6 \times 10^{-5}$ M (4 ppm).

Pro-oxidants and antioxidants

The flavanols and dihydroquercetin, ethylenadiaminetetracetic acid, disodium salt (EDTA) and copper phthalocyanin were each added as a 1·3% (w/v) solution in ethanol.

The anthocyanins, cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside, were isolated from blackcurrant juice (Morton, 1968) and the purified material used as a 1·3% (w/v) solution in citrate buffer, pH 2·9.

The anthocyanin concentration in solutions was determined by the method of Swain & Hillis (1959).

Polarographic estimation of ascorbic acid

Use was made of the anodic wave of ascorbic acid, $E_{\frac{1}{2}}$ + 0·15 V at pH 2·75 (NCE) (Kolthoff & Lingane, 1952). In the presence of oxygen only the lower portion of the wave could be observed, the upper portion being continuous with the oxygen reduction wave. However, the position of the lower step could be assessed accurately and a quantitative relationship was found between the position and ascorbic acid concentration in the range 1·5 to 10×10^{-4} M.

A Cambridge polarograph was used with a mercury pool as anode. Scanning was confined to the range + 0·1 to − 0·1 V so that a large number of determinations could be accommodated on a single paper chart. A trace was recorded of the oxygenated buffer solution (20 ml), plus catalyst and antioxidant where appropriate, to obtain the step position for zero concentration of ascorbic acid. The ascorbic acid solution (0·175 ml) was then added and polarographic traces recorded at intervals. During the intervening periods oxygen was bubbled through the solution to ensure thorough mixing and saturation with oxygen.

The temperature of the solution was maintained at 37° ± 0·5°C.

Spectra

Quercetin solution was added to citrate buffer to give a 4×10^{-5} M solution. The spectrum was recorded from 220 to 450 mμ on a Unicam SP.700 recording spectrophotometer. Copper sulphate solution was then added to give a concentration of 4×10^{-5} M and the spectrum again recorded.

Estimation of peroxide

The estimation was carried out according to the method of Spanyar, Kevei & Blazovich (1964).

Titanyl chloride reagent was prepared by dissolving 1 g of the salt in 100 ml sulphuric acid (25% v/v). Into a glass-stoppered test tube was pipetted 1 ml peroxide solution (containing up to 1 μg H_2O_2/ml) and 9 ml titanyl chloride reagent added. The tubes were stored in the dark for 4 min and the optical density measured in a 1 cm cell at 405 mμ using a Unicam SP.600 spectrophotometer.

A calibration curve was prepared by diluting hydrogen peroxide with citrate buffer to give a range of concentrations from 0·3 to 1·0 μg/ml, and determining the absorption at 405 mμ after addition of the titanyl chloride reagent as described above.

Thin layer chromatography (TLC)

Glass plates were coated with a thin layer of Kieselgel G and dried at 110°C for 20 min. Samples of the solutions of ascorbic acid undergoing oxidation were applied to the plates together with freshly prepared solutions of ascorbic acid and hydrogen peroxide in citrate buffer. The plates were developed in the solvent system water–ethanol–ethyl acetate (1 : 9 : 2 by volume) (Gänshirt Waldi & Stahl, 1965). The dried plates were sprayed lightly with a solution prepared by mixing 4% (w/v) potassium iodide (10 ml) with glacial acetic acid (20 ml), adding a knife point of zinc powder and filtering. After 5 min the plates were sprayed again with an aqueous starch solution (1% w/v). Peroxides appeared as blue spots on a white background.

Results

The effects of flavonoids upon the oxidation of ascorbic acid in citrate buffer at pH 2·9 without the addition of copper ions are shown in Fig. 1. Quercetin and dihydroquercetin showed identical, and the strongest, antioxidant activity whilst rutin (quercetin-3-rutinoside) was the least effective of the flavonols examined. The anthocyanins, cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside showed equal activity in accelerating the oxidation of ascorbic acid. Changing the concentration, for all the flavonoids, between 4·1 and 16·4 $\times 10^{-5}$ M did not modify the effect.

When copper ions ($1{\cdot}6 \times 10^{-5}$ M) were added to the ascorbic acid solution (Fig. 2) the anthocyanins showed a slight protective effect but were less active than quercetin at one-tenth the concentration. The effect of concentration of antioxidant upon

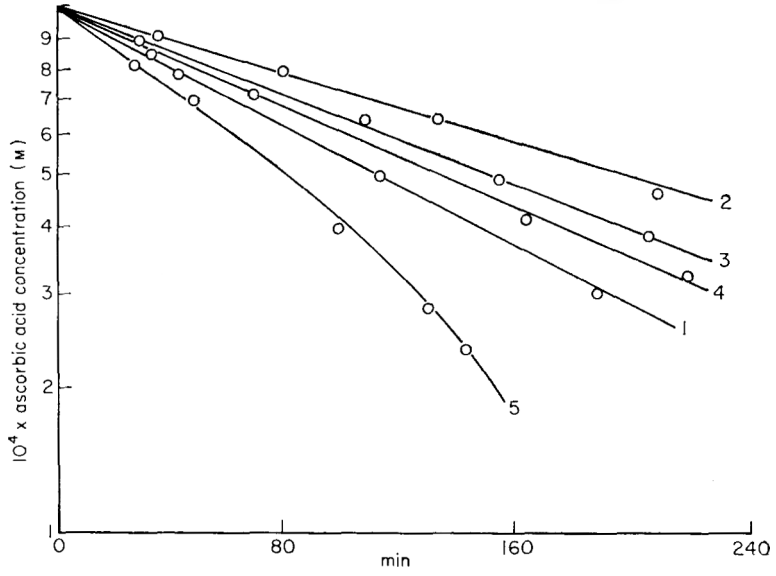


FIG. 1. The effect of flavonoids upon the rate of autoxidation of ascorbic acid. Control (curve 1), quercetin and dihydroquercetin (curve 2), kaempferol (curve 3), rutin (curve 4), cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside (curve 5). Concentration of flavonoids 4·1 to 16·4 $\times$ 10^{-5} M.

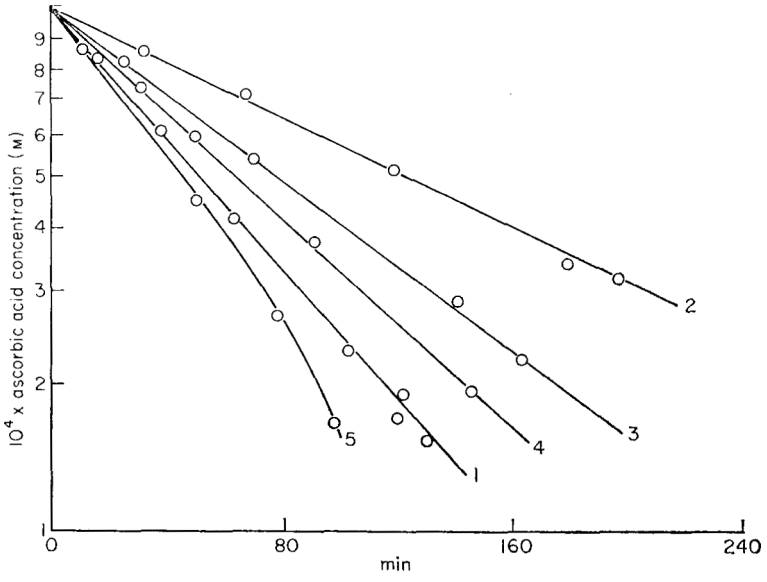


FIG. 2. The effect of additives upon the copper catalysed oxidation of ascorbic acid. Control and EDTA (16·1 $\times$ 10^{-5} M) (curve 1), quercetin (12·5 $\times$ 10^{-5} M) (curve 2), quercetin (4·2 $\times$ 10^{-5} M) (curve 3), cyanidin-3-rhamnoglucoside and delphinidin-3-glucoside (16·4 $\times$ 10^{-5} M) (curve 4), hydrogen peroxide (2·56 $\times$ 10^{-3} M) (curve 5).

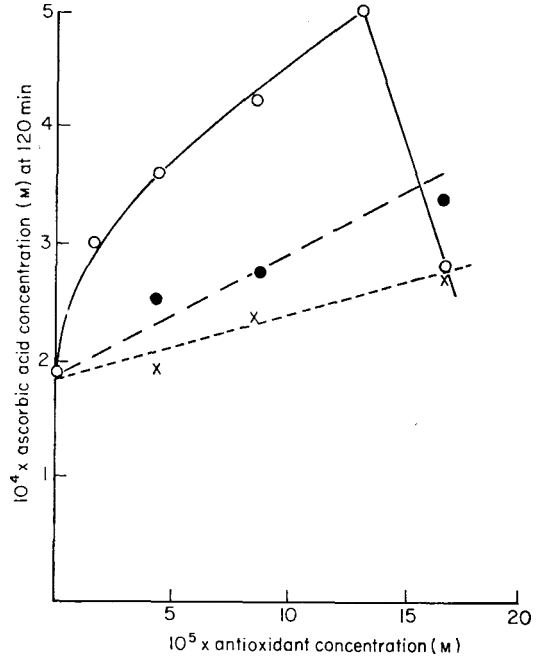


FIG. 3. The influence of antioxidant concentration upon the copper catalysed oxidation of ascorbic acid. ○, Quercetin; ×, cyanidin-3-rhamnoglucoside; ●, cyanidin-3-rhamnoglucoside plus quercetin ($4{\cdot}2 \times 10^{-5}$ M).

ascorbic acid oxidation is presented in Fig. 3. Quercetin showed increasing antioxidant activity with increasing concentration up to a maximum of $12{\cdot}5 \times 10^{-5}$ M above which its effectiveness decreased markedly. The anthocyanins were ineffective at concentrations below 4×10^{-5} M, and gave only slight protection at concentrations above this. Quercetin at a concentration of $4{\cdot}2 \times 10^{-5}$ M was less effective in the presence of added anthocyanins but the net effect remained greater than that for anthocyanins alone.

The spectrum of quercetin in citrate buffer at pH 2·9 showed absorption maxima at 262 and 377 mμ with a point of inflexion at about 300 mμ. An identical absorption spectrum was obtained after the addition of an equimolar concentration of cupric ions, indicating that complexing between the flavone and metal ions had not taken place. A large bathochromic shift in the λ max would be expected if complexing occurred (Jurd, 1962).

The peroxide contents of model systems containing ascorbic acid undergoing oxidation are presented in Table 1. Dihydroquercetin was used as antioxidant in these experiments to avoid interference with the colorimetric estimation of peroxides, due to the absorption of quercetin at 405 mμ. The concentration of total peroxide, expressed as hydrogen peroxide, was similar at identical stages of the oxidation for all the

TABLE 1. The peroxide content of ascorbic acid solutions

Solution	Time (min)	Amount of ascorbic acid oxidized (10^{-1} × m-mole)	Amount of peroxide present as H_2O_2 (10^{-1} × m-mole)
Citrate buffer, pH 2·9	4	0·3	0·5
	28	1·8	0·7
	60	3·4	0·5
	100	5·1	0·4
	140	5·3	0·4
	180	7·3	0·5
Citrate buffer + copper sulphate ($1·6 \times 10^{-5}$ M)	4	0·6	0·9
	20	2·5	0·3
	50	5·2	0·4
	88	7·2	0·5
	120	8·2	0·4
	140	8·7	0·4
Citrate buffer + copper sulphate + dihydroquercetin ($16·4 \times 10^{-5}$ M) (Cu : DHQ = 1 : 10·4)	5	0·4	0·4
	25	1·8	0·6
	60	4·4	0·4
	95	6·4	0·6
	120	7·4	0·5
	155	8·3	0·4
Citrate buffer + dihydroquercetin ($16·4 \times 10^{-5}$ M)	5	0·1	0·1
	30	0·5	0·8
	50	1·0	1·0
	95	2·6	0·7
	140	4·3	0·4
	170	5·3	0·5
	250	7·3	0·5
	300	8·0	0·4

systems investigated, only the rates of the reactions were affected by composition. After the initial rise, the concentration of peroxide quickly fell to a steady low value. It is clear that although there was formation of peroxides they did not persist.

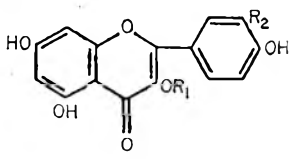
The addition of titanyl chloride reagent during the dissolution of ascorbic acid in water immediately caused the formation of a deep brown colour, indicating the instantaneous formation of peroxides.

Thin layer chromatography of partially oxidized solutions of ascorbic acid showed the presence of peroxide positive material at the origin, distinct from hydrogen peroxide which showed a spot near the solvent front. Freshly prepared ascorbic acid solutions also showed the presence of peroxide positive material, but no hydrogen

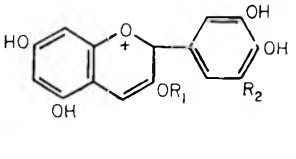
peroxide, indicating that it was this 'base line' material which was detected by the titanyl chloride reagent and would contribute to the estimated peroxides.

Discussion

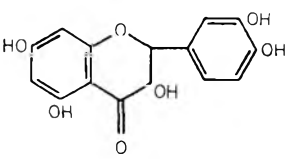
The retarding effect of flavonols, such as quercetin (I, R_1 = H, R_2 = OH), on the



(I)



(II)



(III)

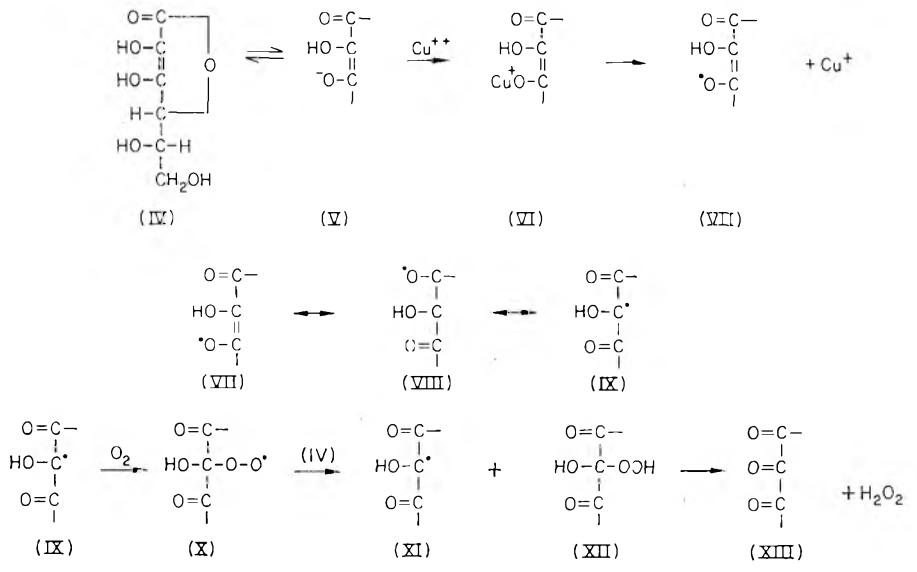
copper catalysed oxidation of ascorbic acid (Fig. 2) has often been ascribed to the ability of such compounds to chelate metal ions, thereby, making them unavailable to act as catalysts (Davidek, 1960; Samaradova-Bianki, 1965). Such a conclusion is feasible, since most inhibitors are capable of forming complexes with copper (Huelin & Stephens, 1948; Butt & Hallaway, 1961) and it can be argued that the inhibiting effect of the flavonol, in the absence of added copper (Fig. 1), is due to complexing with trace metals already present. However, citric acid also forms complexes with copper (Parry & DuBois, 1952) at pH 2·9 but the oxidation proceeds even in the presence of excess citrate ions (7×10^{-3} M) although at a slower rate (Huelin & Stephens, 1948; Timberlake, 1960). Flavonols exerted their inhibiting effect in this medium although EDTA reported as showing strong inhibition of copper catalysed ascorbic acid oxidation (Timberlake, 1960), showed no protective effect (Fig. 2). Furthermore, no spectral shift was observed upon the addition of copper ions to a solution of quercetin in citrate buffer at pH 2·9, and no complex formation between copper and quercetin could be detected using amperometric titration in citrate buffer at this pH (Harper, unpublished results). It follows that some other mechanism must be responsible for the inhibiting effect of flavonoid compounds.

The mechanism of copper catalysed oxidation of ascorbic acid

The oxidation of ascorbic acid (IV) is generally assumed to proceed by a free radical mechanism (Waters, 1964) and free radicals have been detected in solutions of ascorbic acid (Lagercrantz, 1964). Weissberger, LuValle & Thomas, (1943) and Weissberger & LuValle (1944) suggested that in acid solutions, the mono-anion (V) is the active form, thus accounting for the increase in rate of oxidation as pH is raised. Weissberger & LuValle (1944) furthermore suggested that an unstable ascorbic acid–copper complex (VI) forms between the oppositely charged ions, which allows electron transfer to take place and subsequent decomposition liberates Cu^+ and a free radical (VII). It is significant that strongly bound copper (as in the copper–porphyrin com-

plex, copper phthalocyanin) is unable to catalyse the reaction (Fig. 1). Khan & Martell (1967) have recently suggested that a metal–ascorbate–oxygen complex may be involved at this stage. Decomposition of this complex via a number of steps ultimately yielded the free radical (VII) and Cu^{+}.

The radical (VII) would undergo resonance stabilization due to its ability to exist in the various canonical forms (VII) (VIII) (IX), but would add molecular oxygen immediately (Uri, 1961) under the conditions of oxgyen saturation to form a hydroperoxy radical (X). Such hydroperoxy radicals have been observed in oxygenated solutions of ascorbic acid (Norman & Radda, 1962). We have demonstrated the immediate production of 'peroxide' on the dissolution of ascorbic acid in water, and have shown by TLC the presence of 'peroxide' other than hydrogen peroxide in reaction mixtures.



Since 95% of the ascorbic acid is undissociated at pH 2·9 (Weissberger *et al.*, 1943) it is reasonable to expect that the hydroperoxy radical (X) would react with the undissociated acid (IV) to yield, once again, the free radical (IX) and the peroxide of ascorbic acid (XII). Decomposition of the latter would result in the liberation of dehydroascorbic acid (XIII), the intermediate product of ascorbic acid oxidation, and hydrogen peroxide as observed in this work and elsewhere (Silverblatt, Robinson & King, 1943).

The peroxide content of each oxidation system (Table 1) remained constant at about $0{\cdot}5 \times 10^{-4}$ M (expressed as hydrogen peroxide) after an initial maximum, irrespective of the amount of ascorbic acid oxidized. The mechanism discussed requires the liberation of one mole of hydrogen peroxide for every mole of ascorbic acid oxidized

and it is reasonable to suppose that re-oxidation of the cuprous ion absorbs some of the peroxide, i.e.:

$$Cu^{+} + H_2O_2 \rightarrow Cu^{++} + HO^{-} + HO^{\bullet}$$

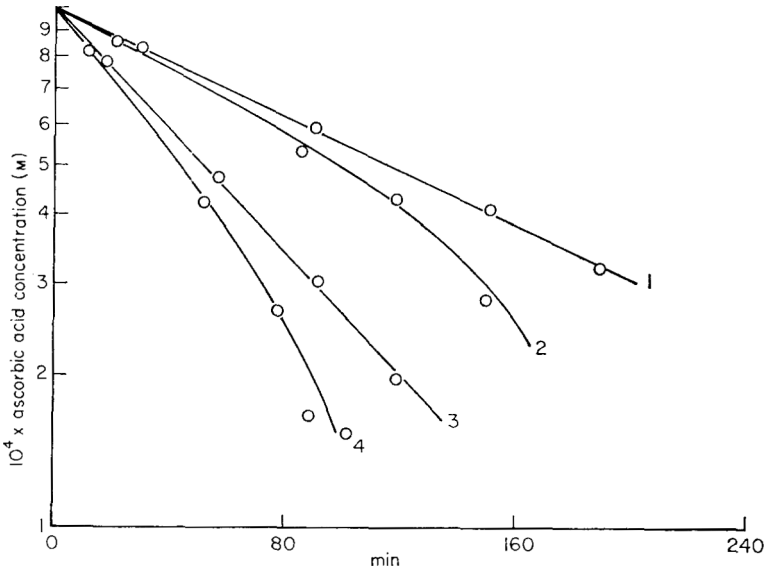


FIG. 4. The effect of hydrogen peroxide upon the oxidation of ascorbic acid. Control (curve 1), hydrogen peroxide (16×10^{-4} M) (curve 2), copper sulphate ($1{\cdot}6 \times 10^{-5}$ M) (curve 3), copper sulphate plus hydrogen peroxide ($2{\cdot}6 \times 10^{-3}$ M) (curve 4).

where the hydroxyl radical is available for further oxidation of ascorbic acid. Since reduction of cupric ions occurs only during initiation of the reaction, obviously this reaction can account for only a minor portion of the hydrogen peroxide produced. But hydrogen peroxide itself can oxidize ascorbic acid, as shown by the addition of hydrogen peroxide to an oxidation system (Fig. 4), and cupric and ferric ions are capable of decomposing hydrogen peroxide with the liberation of more hydroperoxy radicals (Kolthoff & Medalia, 1949; Uri, 1961):

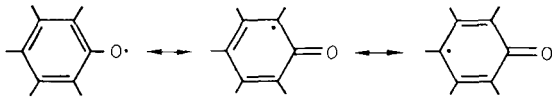
$$Cu^{+} + H_2O_2 \rightarrow Cu^{+} + HO_2^{\bullet} + H^{+}$$

The effect of flavonols

Fig. 1 shows that the flavonols, quercetin, kaempferol, and rutin, and the flavonol dihydroquercetin (III) all inhibited the oxidation of ascorbic acid in the absence of added copper ions, and incubation studies have shown them to be effective anti-

oxidants in the presence of cupric ions (Clegg & Morton, 1968). The flavones are known to be potent inhibitors of the oxidation of oils and fats (Waters, 1964) and it is possible that a similar mechanism operates for the inhibition of ascorbic acid oxidation in an aqueous medium.

In lipid systems the flavones function as free radical acceptors thus breaking the chain reaction of autoxidation. Chain breaking may be accomplished by removal of the free radicals $RO_2^{\cdot}$ and $R^{\cdot}$ but Bolland & ten Have (1947) indicated that inhibitors reacted only with $RO_2^{\cdot}$ radicals, i.e. (X).



(XIV)

Phenols in the chain-breaking reaction lose a hydrogen atom to give a resonance-stabilized free aryloxy radical (XIV) (Waters, 1964). *ortho*-Diphenol and *para*-diphenol are more effective (Uri, 1961) through their ability to undergo full oxidation to the quinone. Substitution with alkyl groups or conjugation with electron releasing groups increases the effect (Scott, 1965).

Flavonols (I, R_1 = H) are structurally well suited to chain-breaking reactions and the effects of hydroxylation pattern and structural features upon their effectiveness as antioxidants for fats and oils have been determined (Letan, 1966a). The order of decreasing effectiveness of the flavonols, quercetin, kaempferol and rutin as antioxidants for ascorbic acid at pH 2·9 (Fig. 1) was in general the same as that for fats and oils. Dihydroquercetin, however, was found to be as effective as quercetin, an unexpected result in view of the fact that the 3,4-dihydroxy grouping is no longer conjugated with the carbonyl group, which results in a decrease in the resonance stabilization of the radical. The α–β unsaturated ketone also is no longer present, a grouping considered to be of major importance for effective antioxidant activity in respect of ethyl linoleate (Heimann & Reiff, 1953). Dihydroquercetin was found to be less effective than quercetin for inhibiting oxidation in fats (Crawford, Sinnhuber & Aft, 1961; Letan, 1966a,b) and in incubation studies was only 86% and 78% as effective in preventing oxidation of ascorbic acid at pH 2·9 in the absence and presence of added copper ions, respectively (Clegg & Morton, 1968). 3-Hydroxy-flavanones are readily oxidized in acid solution in the presence of air (Dean, 1963), but investigations of the spectra of partially oxidized solutions showed that there was no conversion of dihydroquercetin to quercetin. The reason for the high activity of this dihydroflavonol is so far unexplained.

Concentration of flavonol

When copper ions were not added, variations in concentrations from $4{\cdot}1 \times 10^{-5}$ to

$16{\cdot}4 \times 10^{-5}$ M for each of the flavonoids, quercetin, kaempferol, rutin and dihydroquercetin did not alter the amount of ascorbic acid oxidized during four hours (Fig. 1). It is logical to conclude, therefore, that the uncatalysed reaction proceeded so slowly that a small concentration of inhibitor was able to produce a maximum effect, and any increase in concentration conferred no further protection.

In the presence of added copper ions the rate of oxidation of ascorbic acid fell, with increase in quercetin concentration up to $1{\cdot}25 \times 10^{-4}$ M (Fig. 3) but a further increase in concentration up to $1{\cdot}66 \times 10^{-4}$ M caused a reduction in the protective effect to the equivalent of about $0{\cdot}1 \times 10^{-4}$ M quercetin. This phenomenon of optimum concentration of antioxidant is well known (Scott, 1965) and is considered to be due to the prooxidant effect of the inhibitor surpassing its antioxidant effect above a certain concentration.

The effect of anthocyanins

The anthocyanins used in these investigations were those which had been isolated in greatest quantities from blackcurrant juice concentrates (Morton, 1968), i.e. cyanidin-3-rhamnoglucoside (II, R_1 = rutinose, R_2 = H) and delphinidin-3-glucoside (II, R_1 = glucose, R_2 = OH). The behaviour of these two anthocyanins towards ascorbic acid oxidation was identical.

It is apparent from Fig. 3 that in the presence of added copper ions the anthocyanins showed a slight protective effect when present in concentrations above 4×10^{-4} M, thus confirming the results of previous incubation studies (Clegg & Morton, 1968) and of other workers (Hooper & Ayres, 1950; Spanyar *et al.*, 1964). However, the protection afforded by cyanidin-3-rhamnoglucoside is much less than that of the flavonol with the same hydroxylation pattern, quercetin.

Without the addition of copper ions the anthocyanins accelerated the oxidation of ascorbic acid (Fig. 1) as noted previously (Joslyn, 1941; Timberlake, 1960; Clegg & Morton, 1968).

Polarographic investigations have shown (Harper, 1968) that flavylium salts are able to accept electrons at a low negative potential ($-$ 0·3 V, SCE) and possibly, therefore, anthocyanins may initiate the chain reaction in the same way as copper ions. Also as in the case of copper ions the electrostatic charge between the negative ascorbate ion and the positive flavylium ion would bring the sites of high and low electron densities together, thereby, facilitating the reaction.

Loss of ascorbic acid was accompanied by loss of anthocyanins (Fig. 5). This loss may be attributed to oxidation, mainly by hydrogen peroxide (Sondheimer & Kertesz, 1952; Jurd, 1966), but also in part as a result of interference of the anthocyanins in the chain reaction. The loss was increased by the addition of copper ions, and reduced by quercetin. Quercetin was also less effective as an antioxidant for ascorbic acid in the presence of anthocyanins (Fig. 3), although its effectiveness gradually increased as the anthocyanin concentration was raised.

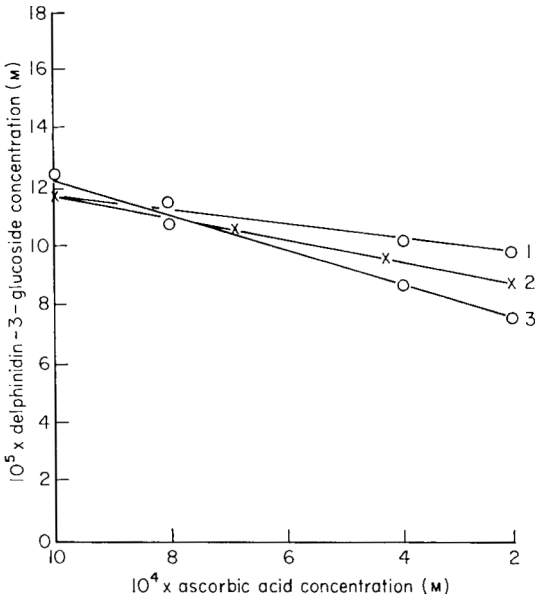


FIG. 5. The effect of ascorbic acid oxidation upon the concentration of delphinidin-3-glucoside in the absence of added copper ions and the presence of quercetin (4×10^{-5} M) (curve 1), in the absence of added copper ions (curve 2), in the presence of copper ions ($1{\cdot}6 \times 10^{-5}$ M) (curve 3).

Conclusions

The marked retention of ascorbic acid in some foods has long been associated with anthocyanin content (Hooper & Ayres, 1950), but here and elsewhere (Spanyar *et al.*, 1964; Clegg & Morton, 1968), it has been shown that the stabilizing factor is not the anthocyanins but other flavonoids which are generally associated with them, i.e. the flavones and flavanones. Their antioxidant activity appears to reside in their ability to act as free radical acceptors, the property which is regarded as their primary function as antioxidants in fats (Waters, 1964). The ability of flavones and flavanones to chelate metal ions (through the carbonyl group at C-4 and the hydroxyl groups at C-3 and C-5, and also with the *o*-dihydroxy grouping sometimes found on the B-ring), although potentially an important secondary function (Letan, 1966b), would not appear to contribute towards their antioxidant activity in fruit juices of low pH. In such a medium the high acidity and large concentrations of sequestering fruit acids, such as citric acid, would prevent complex formation. In citric acid solutions at pH 2·9 no complexing between either quercetin or anthocyanins and cupric ions could be demonstrated.

In considering flavonoids as antioxidants in fruit juices of low pH emphasis should thus be placed upon interference with the chain reactions of autoxidation, i.e. free

radical acceptance, which in phenols is a function of their ability to form stable semiquinones (XIV) and ultimately quinone structures (Uri, 1961).

The remarkable antioxidant activity in lipid systems which has been shown by 3,7,8,2′,5′-pentahydroxyflavone (Uri, 1961) suggests it may be a potent antioxidant in fruit juices as well.

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Protein denaturation and water-holding capacity in pork muscle

I. F. PENNY

Summary. The water-holding capacity of the myofibrils from longissimus dorsi muscles of Large White pigs, selected on the basis of their rate of fall in pH post-mortem, has been compared with the ultimate pH, the protein extractability in M potassium chloride and the adenosine triphosphatase (ATPase) activities. It was found that any loss in water-holding capacity of the myofibrils could be attributed, mainly, to the extent of denaturation, occurring in the myofibrillar protein, as a result of a rapid fall in pH post-mortem. It was also found that the ultimate pH had a small, but significant, influence on the water holding capacity.

Introduction

The condition in pork muscle known as 'white', 'watery' or 'pale soft and exudative (PSE)' muscle has been the subject of recent investigations, which have been reviewed by Briskey (1964) and Bendall & Lawrie (1964). The condition is known to arise in muscles where the post-mortem rate of pH fall is about three times faster than normal, so that the pH of the muscle may fall below 6 before the carcass has had time to cool below 37°C. Some of the proteins in muscle are sensitive to these conditions and readily denature (Bendall & Wismer-Pedersen, 1962). The sarcoplasmic protein, creatine kinase, is particularly sensitive (Scopes, 1964) and the fibrillar proteins myosin and actomyosin are also denatured to a considerable extent (Penny, 1967a,b). It is to this denaturation that the extreme loss of water-holding capacity, characteristic of the PSE condition, can be attributed.

It is possible, however, that in less extreme conditions the water-holding capacity of muscle depends on the extent to which the proteins have been denatured as a result of the rate of pH fall. To examine this, l. dorsi muscles from Large White pigs—a breed which is not very susceptible to the PSE conditions—were selected. The adenosine triphosphatase activity and the extractability of the myofibrils in 1 M KCl were used as indices of denaturation and the results compared with the water-holding capacity of the myofibrils.

Materials and methods

The l. dorso muscles of pigs of the Large White breed were selected on the basis of the pH value of the muscle 90 min after death. This value, which will be referred to as pH_2, gives a measure of the rate of pH fall after death. The pH_2 value was determined with a Radiometer probe electrode inserted between the fifth and sixth ribs

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90 min after death. During this 90-min period the carcasses were held at ambient temperatures which ranged from 4° to 15°C for the months November to April, during which the samples were taken. The muscle temperatures were not recorded but it would be expected that the temperature of the l. dorsi would have fallen to the region of 33–35°C in 90 min under these conditions. After 2 hr post-mortem, the carcasses were transferred to a chill room (3°C) to allow the completion of the rigor process. Muscles with a range of pH_2 values from 5·39 to 6·93 were thus obtained.

Ultimate pH

The ultimate pH 24-hr post-mortem, was measured on a homogenate of 1 g of muscle in 10 ml water.

Preparations of myofibrils

Thirty grams of muscle were homogenized in a top drive blender with 3 volumes of ice cold 0·1 M KCl, 20 mM imidazole–HCl buffer, pH 7·0. The myofibrils were prepared from the homogenate by the method of Perry & Grey (1956).

Protein extractability and ATPase activity

The myofibrils were extracted overnight in M KCl, 30 mM sodium glycerophosphate buffer, pH 6·2, and the amount of extracted protein was determined as previously described (Penny, 1967b).

The Ca^{2+} ATPase activity, at high ionic strength, and the Mg^{2+} ATPase activity, at low ionic strength, were also determined as before (Penny, 1967b).

Water-binding capacity

The water-binding capacity of the myofibrils was measured by the method of Penny, Voyle & Lawrie (1963), in which myofibril suspensions, first equilibrated to pH 7, were centrifuged at 1200 ***g*** for 5 min.

Results and discussion

The results for the ATPase at high ionic strength, the protein extractability in M-KCl and the water binding capacity of the myofibrils have been plotted against the pH_2 values in Fig. 1(a), (b) and (c), respectively. The results obtained for the three parameters show very similar patterns. Between pH_2 5·9 and 6·9, the water-holding capacity, the extractability and the Ca^{2+} ATPase activity were unaffected by the pH_2 value but, when the pH_2 fell below 5·9 the results for all three parameters decreased. From the similarity of the patterns in Fig. 1(a), (b) and (c), it was deduced that the variations in the water-holding capacity were associated with the similar variations in the extractability and ATPase activity. This was confirmed by the calculated linear correlation coefficients in Table 1, which show that all three para-

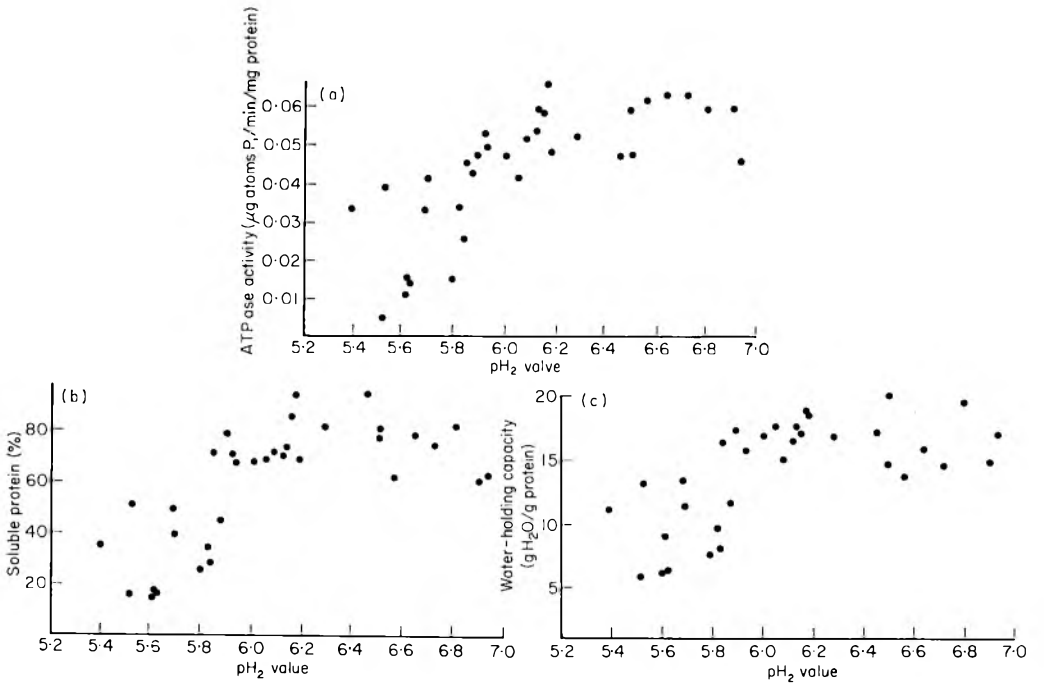

FIG. 1. The results for (a) Ca^{2+} ATPase activity in 1 M KCl; (b) percentage of protein soluble in 1 M KCl, 30 mM sodium glycerophosphate HCl, pH 6·2; and (c) water-holding capacity at pH 7·0 of myofibrils prepared from pork muscles of different pH_2 values.

TABLE 1. The linear coefficients obtained for the relationships between Ca^{2+} ATPase activity and extractability and water binding of myofibrils; the Mg^{2+} ATPase and water binding; extractability and water binding; ultimate pH and water binding

			Correlation coefficients
Ca^{2+} ATPase	*v.*	Extractability	0·878
Ca^{2+} ATPase	*v.*	Water binding	0·812
Mg^{2+} ATPase	*v.*	Water binding	0·913
Extractability	*v.*	Water binding	0·908
Ultimate pH	*v.*	Water binding	0·528

meters were interrelated to a highly significant degree. These results also show that, in addition to the Ca^{2+} ATPase activity, i.e. that due to myosin, the correlation co-

efficient of the Mg^{2+} ATPase acvitity of the intact myofibrils with water-holding capacity was equally significant.

Thus, the water-holding capacity of myofibrils, and hence of the intact meat, is very largely determined by the extent of denaturation of the myofibrillar protein which in these experiments has been measured by protein extractability and ATPase activities. These results agree with, and amplify, those of MacDougall & Disney (1967), who reported that the water-holding capacity and the protein extractability were both related to the rate of pH fall. These authors, however, did not indicate that the pH_2 value is important, only when it falls below a certain value, nor did they show the highly significant relationship between water-holding capacity and denaturation.

The results in Fig. 1(a), (b) and (c), however, show some scatter and it seemed likely that the water-holding capacity was also being influenced by factors other than protein denaturation. The most probable additional factor is the ultimate pH, which has long been established as important in determining the water holding capacity of muscle (Hamm, 1960). As shown in Table 1 a linear correlation coefficient of 0·528 was obtained for water-holding capacity against ultimate pH. A multiple correlation, with water-holding capacity as the dependent variable and protein extractability and ultimate pH as the independent variables was calculated. Table 2 summarizes the contributions made by these factors to the variability of the water-holding capacity.

TABLE 2. The contributions made by protein extractability and ultimate pH to the variability of the water-holding capacity

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-ratio
Regression on protein extractability	507·59	1	507·59	165·9***
Additional regression on ultimate pH	13·65	1	13·65	4·5*
Residual variation	94·90	31	3·06	—
Total variation	616·14	33	—	—

* Significance, $P < 0{\cdot}05$.
*** Significance, $P < 0{\cdot}001$.

Regression on protein extractability accounts for some 82·4% of the total variation in water-holding capacity encountered in this experiment. Regression on ultimate pH accounts for only a further 2·2% leaving 15·4% unexplained. Though the regression on ultimate pH is small, it is still significant, as shown by the corresponding *F*-ratio. The relationships between these characteristics were found to be reasonably

linear and the equation of the best (linear) fit linking water-holding capacity, extractability and ultimate pH was:

$$\text{Water-holding capacity} = 0{\cdot}155 \times \text{extractability} + 3{\cdot}51 \times \text{pH ult.} - 14{\cdot}25.$$

(The significance for extractability was $P < 0{\cdot}01$ and for pH ult. $P < 0{\cdot}05$.) Since the relationships with pH_2 values were obviously non-linear, as illustrated in Fig. 1, no attempt has been made to examine these statistically.

Thus, it can be concluded, that the water-holding capacity of muscle is determined by the amount of protein denaturation which has occurred in the immediate post-mortem period, mainly as a result of the rate of pH fall and, to a lesser extent, by the ultimate pH. The former is more important than the latter, because a high rate of pH fall means that the muscle will become very acid while the temperature is still high; ideal conditions for denaturation (Penny, 1967a,b). The pH_2 value, which is a measure of the rate of pH fall, is, therefore, a good index of the amount of exudation to be expected in a muscle; at values above 6 the amount is likely to be small but as the pH_2 value falls below 5·9 the exudation will become increasingly greater.

Acknowledgments

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Some observations on the histology of cold-shortened muscle

C. A. VOYLE

Summary. Changes in the histology of muscles subject to temperatures just above freezing-point while in the pre-rigor condition are described. Observations are based on the study of sectioned tissue by optical and electron microscopy, and by scanning electron microscopy. The presence of 'active' and 'passive' fibres is noted. These are defined by their contractile state. The appearance of the surface membrane of the active fibres is also described and its influence on the texture of cold-shortened muscle as meat is discussed.

Introduction

The phenomenon of cold-shortening has been widely documented since it was first described in beef muscles by Locker & Hagyard (1963). Many of the observations of the phenomenon have been made on gross tissue, either as entire muscle or strips of tissue (Marsh & Leet, 1966), or alternatively, on preparations of myofibrils from homogenized tissue (Stromer & Goll, 1967a, b). These observations, however, tell us little of the micro-anatomical changes in the cold-shortened muscle; it is for this reason that the present study has been undertaken. Stained sections observed by optical microscopy, and ultra-thin sections examined in the electron microscope reveal a lack of uniformity in the response of a population of fibres to cold-shortening. This difference in response between individual fibres introduces another aspect to the problem of tenderness in cold-shortened muscle.

Material and methods

Neck muscles (m. sternomandibularis) from beef animals were removed within a few minutes of death at the slaughterhouse. On transfer to the laboratory the muscles were clamped at one end, the clamp being attached to a retort stand, and placed in a chill room at $+2°C$. The length of the muscle, measured between two reference marks, was noted and further measurements of length were made at regular intervals until the sample reached constant length. The sample was allowed to remain in the chill

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room for at least 24 hr. Other samples were set up in a similar manner but were held at room temperature (18°C).

Portions of approximately 1 cm^3 were taken from the muscles and were fixed in 5% glutaraldehyde in 0·1 M phosphate buffer at pH 7·2. Fixation was followed by washing in fresh phosphate buffer containing 0·2 M sucrose. It is possible to store blocks of tissue in this medium for extended periods at 1 – 4°C and to use the tissue for both optical and electron microscopy (Sabatini, Miller & Barnett, 1964). The fixed and washed tissue blocks were subsequently embedded in paraffin wax and sectioned at 5 μm. Sections were stained with iron haematoxylin and Van Gieson's picro-acid fuchsin, and examined by optical microscopy. Sections were also cut from fixed tissue on a freezing microtome. By this method small blocks of tissue are quickly frozen by quenching in isopentane cooled in a Dewar flask of liquid nitrogen. The frozen tissue is sectioned on a microtome which is housed in a refrigerated cabinet, the whole apparatus being known as a 'cryotome'. The cut sections are collected onto a cooled cover-glass and may then be stained as required, the finished preparation being mounted onto a microscope slide in the customary manner. Sections prepared in this way were lightly stained with Van Gieson's stain and examined using phase contrast microscopy. Measurements of sarcomere length were made using an eyepiece graticule previously calibrated against a micrometer slide.

Small fragments of tissue approximately 1 mm^3, previously fixed in glutaraldehyde, were post-fixed in 1% osmium tetroxide in 0·1 M phosphate buffer at pH 7·2, dehydrated and embedded in Araldite (Glauert, 1965). Sections were cut on a Cambridge Ultramicrotome, stained with 1% phosphotungstic acid or 1% aqueous uranyl acetate followed by lead citrate, and examined in an A.E.I. EM 6B electron microscope. Some additional tissue samples were prepared for examination by scanning electron microscopy.

Samples of tissue from muscles which had been held at 18°C for 24 hr post-mortem were also examined by the methods described above. These served as controls.

Results

The degree of shortening which occurred in a muscle on cooling depended on the time elapsing between slaughter of the animal and transfer of the muscle to the chill room. The gross muscle length changes observed in these experiments confirmed those reported by Marsh & Leet (1966), the optimal time for maximum shortening at +2°C being in the region of 6 hr.

The most notable information resulting from the microscopical examination of this material was the presence of two main groups of fibres. In general these were:

(a) Fibres which had contracted considerably, having a sarcomere length which was much reduced compared with samples held at rest length at 18°C. Such fibres were quite straight in their configuration and will be referred to as 'actively shortened' fibres.

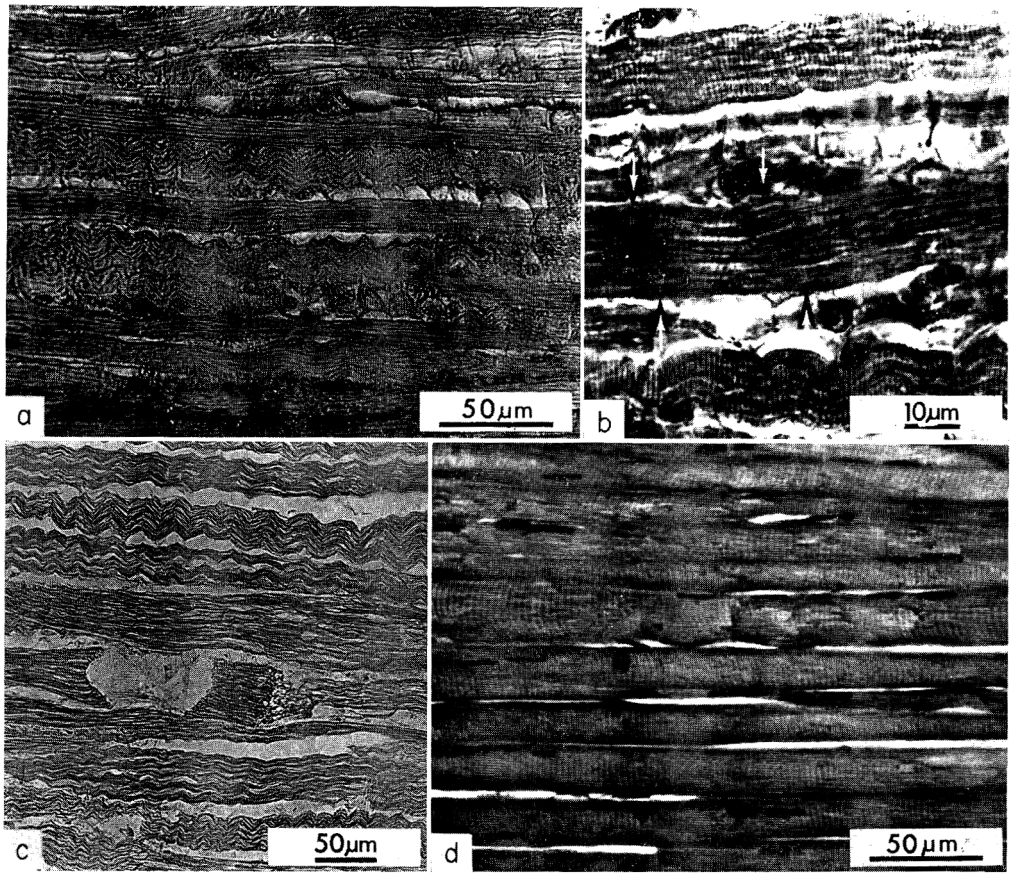

PLATE 1. (a) Section from cold-shortened muscle (m. sternomandibularis) showing actively shortened (straight) fibres, and passively shortened (wavy) fibres. (b) Section through actively shortened fibre from same muscle as (a), showing regions of contraction (arrows) and adjacent regions of stretched sarcomeres. (c) A severely shortened fibre at a point of rupture. The gap is bridged by the plasmalemma. (d) Section from a control muscle held at 18°C for 24 hr.

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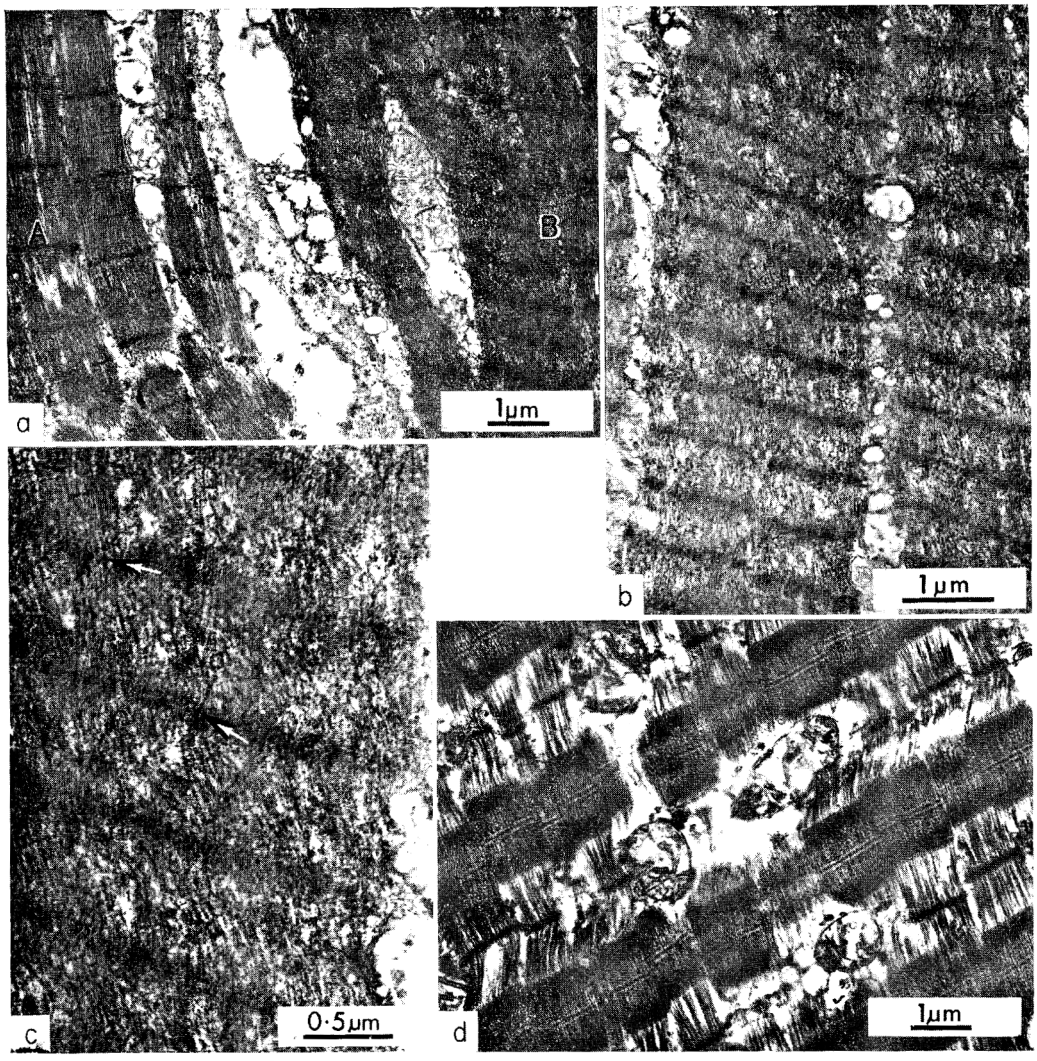

PLATE 2. (a) Electron micrograph of myofibrils in two adjacent fibres, passively shortened (A) and actively shortened (B). Mean sarcomere length in fibre A = 1·57 ± 0·25 μm. Mean sarcomere length in fibre B = 1·10 ± 0·02 μm. (b) Myofibrils in a severely shortened fibre showing 'supercontraction'. Note the disarray of myofilaments. (c) Portion of (b) at higher magnification. Arrows indicate points where myosin filaments have penetrated the Z-discs. (d) Electron micrograph of control muscle showing orderly array of myofilaments and spacing of I-band, A-band and H-zone in a muscle at rest length. Mean sarcomere length = 2·29 ± 0·02 μm.

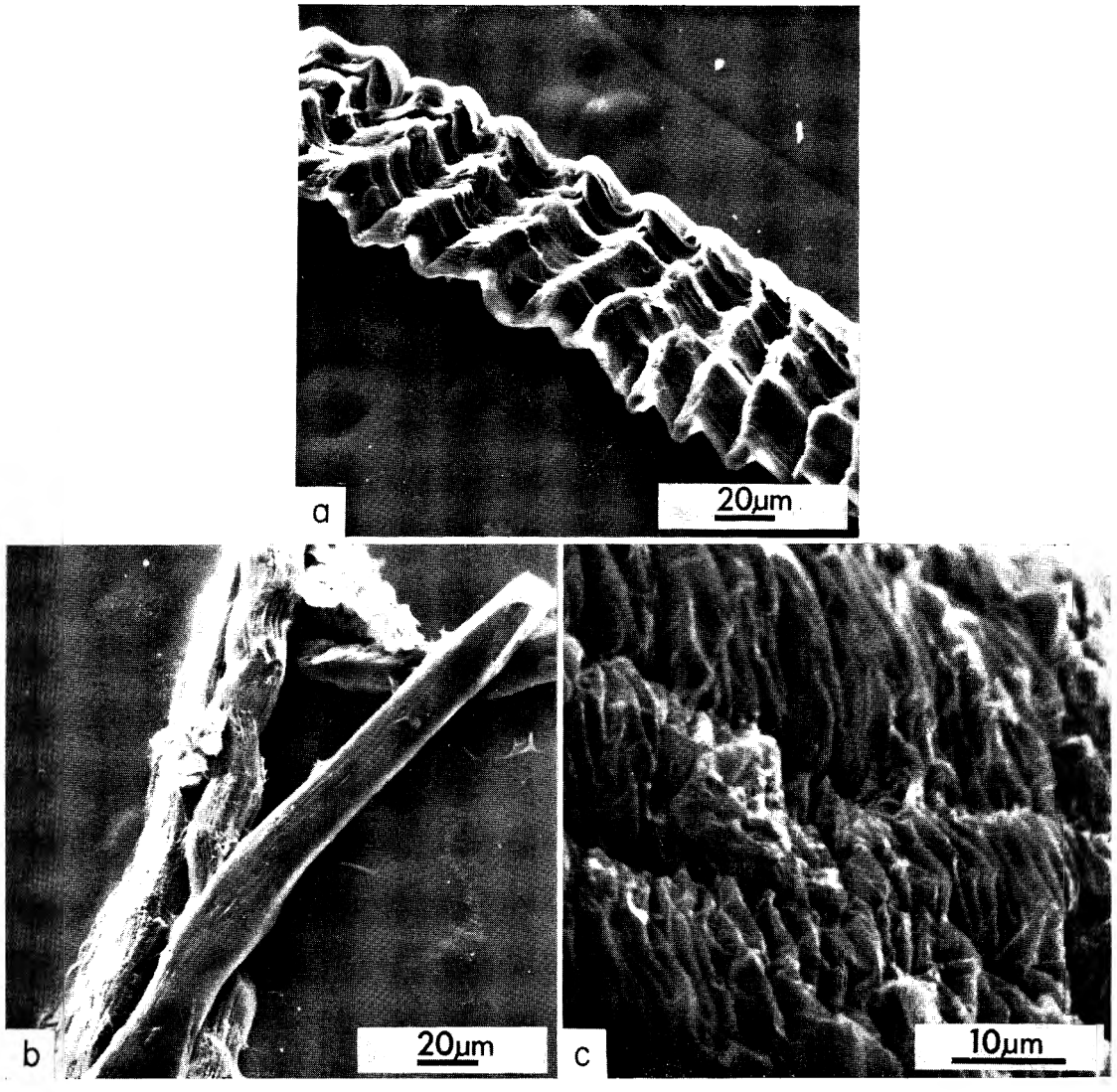

PLATE 3. Scanning electron micrographs. (a) Bundle of passively shortened fibres exhibiting typical wavy form. (b) A teased preparation showing actively and passively shortened fibres. (c) The surface appearance of severely contracted fibres showing folding of plasmalemma.

(b) Fibres in which less contraction had occurred, having a greater sarcomere length than in (a). Due to the constraining influence of the 'actively shortened' fibres and the need to conform to the overall reduction in length of the muscle, these fibres had been pulled up into a wavy form. This group of fibres will be described as 'passively shortened'.

It should be understood that the use of the term 'passive' in this context does not imply complete inactivity. The term is used in a relative sense.

Plate 1(a) shows the two types of fibre in a typical section. Their distribution would seem to be random throughout the tissue. Estimates of the proportions of each type of fibre present indicated that less than half were of the actively shortened type. Frequently it was found that these actively shortened fibres exhibited a variable sarcomere pattern. Zones of excessive contraction alternated with zones where the sarcomeres had been stretched. An example of this variability in sarcomere length in actively shortened fibres is shown in Plate 1(b). Instances were also noted where shortening was so severe that myofibrils were ruptured. The outer membrane of the fibre, however, generally remained intact, as in Plate 1(c). A section from a control muscle, held at 18°C for 24 hr is shown in Plate 1(d). The fibres are uniformly straight and the sarcomeres are evenly distributed.

A situation which is analogous to that shown in Plate 1(b) occurs in a muscle fixed at both ends, as on a carcass, which is exposed to a temperature of +2°C. In some regions of the muscle, marked shortening takes place and gives rise to readily visible swellings or nodes on the surface of the muscle. In adjacent regions, local stretching occurs which may be sufficient to produce constriction of the muscle.

Measurement of sarcomere length showed that the mean value for actively shortened fibres was $1{\cdot}10 \pm 0{\cdot}02$ μm. This compared with a value of $2{\cdot}29 \pm 0{\cdot}02$ μm in samples held at rest length at 18°C for 24 hr. The mean value obtained for passively shortened fibres was $1{\cdot}57 \pm 0{\cdot}25$ μm, showing that these too had undergone some contraction during cooling. It should be pointed out, that the mean sarcomere length for actively shortened fibres quoted above represents only those parts of such fibres where contraction was seen to have occurred, and indicates in some measure how severe this has been. The stretched zones (see Plate 1b) were not included in this observation. Plate 2(a) is an electron micrograph illustrating the difference in sarcomere length in two adjacent fibres of different types. Fibre A clearly has longer sarcomeres than fibre B, but it is also apparent that some contraction has occurred in fibre A, since no H-zone is visible. Electron micrographs also show the severe contraction which has occurred in actively shortened fibres (Plate 2b). It is seen that the I-band has completely disappeared and that the normal orderly array of myofilaments has become considerably disordered. The myosin filaments have undergone compression between the Z-discs, and in some areas have even penetrated these discs, (Plate 2c). This feature has also been observed by Hoyle, McAlear & Selveston (1965) in barnacle muscle, and by Stromer, Goll & Roth (1967) and Stromer & Goll (1967b) in bovine muscle.

This state has been described as 'supercontraction' and is probably akin to the delta state of Ramsey & Street (1940). Stromer *et al.* (1967), in their study of bovine semitendinosus muscle stored at +2°C for 24 hr post-mortem have reported a mean sarcomere length of 1·2 ± 0·02 μm which is in close agreement with our value for the highly contracted zones of actively shortened fibres. Plate 2(d), which is included for comparative purposes, shows myofibrils in section at rest length and illustrates the orderly array of myofilaments normally seen in muscle when prepared for microscopy by the technique described. This muscle was maintained at a temperature of 18°C during rigor.

A bundle of fibres teased out under the dissection microscope and examined by scanning electron microscopy showed clearly the wavy form assumed by fibres which had undergone passive contraction. Plate 3(a) illustrates such fibres 'in perspective' compared with the two-dimensional representation in Plate 1(a). A further preparation is shown in Plate 3(b) illustrating both actively and passively shortened fibres. A group of fibres after active contraction is shown in Plate 3(c), which indicates the folding which occurs in the plasmalemma of active fibres when severe contraction takes place.

Discussion

The phenomenon of cold-shortening, discovered by Locker & Hagyard (1963), has attracted wide attention in the field of meat and muscle research. Besides its intrinsic interest it has a direct bearing on the tenderness of meat. Herring, Cassens & Briskey (1965a, b), Marsh & Leet (1966) and Herring *et al.* (1967) have shown that any shortening which a muscle may undergo during the course of rigor tends to make the resulting meat tough. The most severe increase in toughness becomes apparent when a muscle shortens by 20 – 40% of its rest length.

An even more severe and energetic shortening occurs on thawing out a muscle which has been quickly frozen in the pre-rigor state. This phenomenon is known as thaw-rigor or thaw-contracture. It was suggested some time ago that the release of bound Ca^{++} within the fibres, resulting from the extensive salt flux on thawing (Bendall, 1960), activates the myofibrillar contractile mechanism thus causing severe contraction. The role of Ca^{++} in the contractile muscle and the function of the sarcoplasmic reticulum as a Ca-pump controlling the concentration of this ion has been reviewed by Hasselbach (1964a, b). It must also be said that the presence of ATP is a further essential for such contraction to take place, since this is the source of the energy required for contraction. A detailed account of the mechanism of muscle contraction and relaxation has recently been given by Bendall (1969).

Cold-shortening is in many ways similar to thaw-rigor (Newbold, 1966; Lawrie, 1968), except that it is restricted to certain types of slow 'red' muscles, and, unlike thaw-rigor, will not occur at all in the 'white' musculature of the rabbit (Locker & Hagyard, 1963). Beef and lamb muscles seem to be generally susceptible (Marsh

& Leet, 1966) and the phenomenon has also been observed in this laboratory in 'red' muscles of the pig. Cold-shortening differs from thaw rigor, however, in that the muscle is not subject to freezing. It has been suggested none the less (Bendall, personal communication), that Ca^{++} release is again the direct cause of the phenomenon. With fall in temperature there is a rapid decline in the activity of the Ca-pump and eventually a point may be reached when the gain of Ca^{++} by passive diffusion into the sarcoplasm is faster than the removal of these ions from the sarcoplasm, resulting in a build-up of Ca^{++} to the level at which contraction occurs.

Having briefly outlined what is known of the mechanism of contraction as it occurs in these phenomena, we can now consider the histological changes reported above. Nearly all the fibres shorten to some extent in a pre-rigor beef muscle subjected to temperatures just above freezing-point but the rates vary enormously from fibre to fibre. This gives rise to the observation that the contraction of a minority of active fibres has resulted in the folding of those remaining which are in a passive state (Plates 1a and 3a). A similar effect occurs in a muscle which is cooled while attached to the carcass (Marsh, Woodhams & Leet, 1968). In this situation the tendency is for the more rapidly shortening sarcomeres in the active fibres to pull out the less reactive ones, giving an irregular sarcomere pattern similar to that shown in Plate 1(b). In such circumstances gross nodes of shortening can be observed on the surface of the muscle. Furthermore, it has also been observed that myofibrils within a fibre can be ruptured by the very active shortening of neighbouring sarcomeres, as shown in Plate 1(c). In such cases the more actively shortening fibres have contracted so far that they begin to show the phenomenon described by Sato (1954) in highly shortened frog muscle fibres, that is, fluid begins to be extruded through the plasmalemma. It is interesting to note that at this point, where shortening of more than 40–45% of rest length of the muscle has occurred, the toughness associated with shortening is reversed and the meat becomes more tender again (Marsh & Leet, 1966).

We can now return to the problem of the toughening of meat which is closely associated with shortening of any kind, but particularly with that which results from exposure to temperatures near freezing-point. There is, now, ample evidence in support of the conclusion that a reduction in muscle length gives rise to an increase in toughness. The explanation of this increase solely in terms of change in sarcomere length in the shortened muscle is not, in our view, so readily supported. Such a change in sarcomere length reflects the increased overlap of the interdigitating filaments of actin and myosin as the muscle shortens. The results of Herring *et al.* (1967) suggest that over a range of sarcomere lengths from 3·25 μm down to 2·0 μm there is little increase in toughness. At one extreme of this range the degree of overlap between the actin and myosin filaments is almost nil, while at the other extreme there is nearly maximal overlap of the two types of filaments. These situations represent muscles at 150% of rest length down to 75% of rest length and the results of Herring and his co-workers show that over this range toughness is at a minimum as well as being almost constant.

The most significant increase in toughness occurs, however, over the narrow range between 70% and 60% of muscle rest length. In our observations it is within this range that the most conspicuous change in the sarcomere is the compression of the myosin filaments by the Z-discs in actively shortened fibres, and the mean sarcomere length is $1{\cdot}10 \pm 0{\cdot}02$ μm (Plate 2b and c).

The method used by Herring *et al.* (1967) to measure sarcomere lengths gave a result which was the mean value for a population of twenty-five myofibrils from a homogenized sample. It may be seen from Plates 1(d) and 2(d) of this paper that the sarcomere length in non-shortened muscle is nearly uniform. In shortened muscles, however, as we have shown, there are two populations of fibres and a marked variation in sarcomere lengths, so that although the increased toughness reported by Herring and his co-workers, may be related to a mean sarcomere length of 1·5–2·0 μm, in actual fact, a substantial minority of the fibres have sarcomere lengths considerably below this figure. The relationships between sarcomere length and effective muscle length, and between the ratio of actively shortened to passively shortened fibres and muscle length are still to be demonstrated.

It might be supposed that the contractile state of myofibrils within a muscle fibre will influence the configuration of the outer membrane of the fibre and indeed this would seem to be the case. Recent observations by Rayns, Simpson & Bertaud (1968), using the freeze-fracture method, show that the surfaces of some fibres exhibit creasing while those of other fibres do not. The former were interpreted as representing contracted fibres, the latter being in a relaxed state.

It has been shown that in highly contracted muscles some of the fibres are straight and apparently under tension, although the membranes of such fibres are often severely crumpled, as is shown in Plate 3(c). Other fibres have a wavy form, are not so severely contracted and the membrane is relatively smooth. Muscle which has not been shortened, i.e. has passed through rigor at a temperature around 18°C, has a more uniform alignment of fibres. The differences between these structural configurations will be reflected in the texture of the muscle as meat. Since under tensile or shearing stress the varyingly contracted fibrils and the crumpled or smooth membranes will break at different time intervals during application of the load, the work performed to break the sample, as shown by a stress–strain curve, may be higher for a contracted muscle. It is, therefore, now apparent that the increase in toughness which results from the phenomenon of cold-shortening is due to a combination of physical changes at the fibre level and macro-molecular changes at the level of myofilaments. Further aspects of these changes are being investigated.

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Hydrogen sulphide in cooked chicken meat

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Summary

Precautions necessary for the determination of hydrogen sulphide in chicken meat are outlined and data given for its production during heating and subsequent disappearance on standing.

Introduction

Volatile sulphur compounds (among which hydrogen sulphide, H_2S, is usually present in much the greatest quantity) and carbonylic compounds, particularly aldehydes, are important constituents of the aroma of cooked meat. Minor *et al.* (1965a,b) found that removal of the sulphur compounds from a steam distillate of the volatiles from cooking chicken resulted in an almost complete loss of 'meaty' odour, whereas, removal of the carbonyls caused a loss of 'chickeny' flavour.

H_2S is evolved continuously in the vapours from boiling meat. Kazeniac (1961) showed that the rate of evolution was greater from dark (leg) than from light (breast) chicken meat and that it increased with increasing pH in the range 5–8. Johnson & Vickery (1964) found that when beef, mutton or pork is boiled there is a rapid release of H_2S during the first few minutes, followed by a comparatively steady evolution at a lower rate, which continues for at least 6 hr. Mecchi, Pippen & Lineweaver (1964) boiled individual constituents of chicken muscle in buffer at pH 6·5 and concluded that about 90% of the H_2S derived from cysteine and cystine residues in the protein, with a minor contribution from the cysteine in glutathione. Cysteine in glutathione was shown to be less stable than cysteine plus cystine in protein, but was quantitatively less important as a precursor of H_2S because of its much lower concentration in muscle.

Our own interest lay in determining the amount of H_2S present in freshly cooked meat, by a method that would neither produce more nor, if possible, lose or destroy any of the preformed sulphide during the determination. Previous workers have measured the H_2S content of foods such as cheese or beer by entrainment in inert gas, followed by entrapping in zinc acetate, cadmium hydroxide or bismuth nitrate solution, reaction with *N*,*N*-dimethyl-*p*-phenylenediamine and colorimetric determination as methylene blue, but difficulties were encountered in attempting to apply published procedures to chicken meat.

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Acidification with hydrochloric acid (Brenner, Owades & Golyzniak, 1953; Kristofferson, Gould & Harper, 1959) or phosphoric acid (Lawrence, 1963) was found to liberate H_2S from raw chicken meat, as did the use of temperatures of 80° – 85°C (Brenner *et al.*, 1953; Grill, Patton & Cone, 1966) or even 55°C for 4 hr (McLay, 1967) during gaseous entrainment. Although in McLay's determinations on fish, removal of H_2S at 15°C was so slow that bacterial spoilage commenced before sufficient volatiles could be obtained for analysis, introduction of hydrogen via a sintered bubbler into a rapidly stirred suspension of finely divided chicken meat was found to remove virtually all of the preformed H_2S in 1 or 2 hr at 20°C. This procedure has, therefore, been used to investigate the production of H_2S from chicken meat and its subsequent disappearance on standing.

Materials and methods

Chicken muscle

Breast muscle (pectoral proper and supra coracoid) and leg muscle (those covering the femur, fibula and tibia) were removed from the carcass, finely minced and stored, in 100-g portions in sealed containers over solid carbon dioxide at −60°C. Before use the frozen muscle was usually powdered by grinding with liquid nitrogen in a power mortar, but this was omitted in some of the heating experiments.

Manipulation

Since H_2S produced during heating can easily be lost by volatilization or by oxidation during subsequent handling, 100-g portions of minced or powdered muscle, sealed in air or nitrogen atmospheres in 845 ml (A2½) cans, were heated by immersion in water at the required temperature and immediately cooled in ice-water. After 15 min the can was punctured by a stainless steel needle, projecting through a water reservoir fitted with a rubber collar. Ice-cold deaerated water was, thereby, drawn into the can to absorb gaseous H_2S. The charge was then transferred to the entrainment apparatus.

Gas entrainment

A beaker-shaped reaction vessel, of capacity 600 ml, with ground, flanged top was used, sealed by a silicone rubber 'O' ring and closed by a domed lid carrying a water-sealed stirrer, gas inlet and outlet tubes and thermometer. For runs above 20°C the gas outlet was fitted with a water-cooled condenser. Leakage under pressure was prevented by a plastic collar clamp secured by bolts and wing nuts. The charge, suitably 100 g muscle and 250 ml deaerated water was introduced, and hydrogen, purified by passage through a 'Deoxo' platinum catalyst tube (Engelhard Industries Ltd, Cinderford, Glos.) was passed, at 20 l/hr via a sintered glass bubbler of porosity '0' into the suspension, stirred at 800 rev/min, the temperature being maintained at 20°C by immersion in a water bath. In some runs sodium fluoride (3 g) was added

to inhibit microbial growth, and/or three drops of Antifoam 'A' (Hopkins and Williams) to inhibit foaming.

Absorption

H_2S was absorbed in two 100-ml glass stoppered flasks, in series, each containing 6 ml of the clear bismuth nitrate solution of Koren & Gierlinger (1953). The gas entered the first trap via a glass bubbling tube of 1 mm internal diameter, and passed into the second via a sintered glass bubbler of porosity 2. About 98% of the H_2S collected in the first trap. In some runs a third trap (with sintered glass bubbler) containing 6 ml 3% mercuric cyanide solution was used, to retain mercaptans and a fourth, containing 6 ml 5% mercuric chloride solution, to retain sulphides and disulphides.

Colour Development

After absorption, colour was developed by adding to the liquid in the traps (with bubbler still in position) acid *N*,*N*-diphenyl-*p*-phenylenediamine reagent and ferric chloride–nitric acid (Reissner) solution, as described by McLay (1967), and the amounts of H_2S, mercaptans and sulphides present were deduced by comparison of the observed absorptions at 665, 490 and 500 mμ, respectively, with standard dilutions of the complexed sulphur compound.

Determination of glutathione

Glutathione was determined by the method of Grunert & Phillips (1951) in a metaphosphoric acid extract of the muscle.

Results

Sulphides

McLay (1967) determined organic sulphides in cooked herring, apparently satisfactorily, by the method described above, though Johnson & Vickery (1964) had obtained zero values when the procedure was applied to beef, mutton and pork. We failed to obtain positive results from chicken. Even when appreciable amounts of dimethyl sulphide had been added to the trapping reagent, no colour was obtained.

Recovery experiments

H_2S added (as sodium sulphide) to buffers at pH 5·5 and 7·0 was recovered quantitatively by hydrogen entrainment for 20 and 80 min. Recovery of added H_2S was also virtually quantitative from cooked chicken meat, but was very low from raw muscle.

Effect of acid on the liberation of H_2S from chicken meat

Fresh, raw chicken muscle (pH, after addition of water, 6·15) gave practically no volatile sulphur during the first 20 min purging with hydrogen at 20°C but, thereafter, small but progressively increasing amounts appeared, possibly as a result of denaturation due to frothing. Substitution of hydrochloric or phosphoric acid for part of the water greatly increased the amount of H_2S liberated (Table 1).

TABLE 1. Effect of acidification on the liberation of H_2S* from chicken meat at 20°C

Entrainment time (min)	Without acid	With HCl†	With H_3PO_4‡
20	0	30	19
60	0	32	27
100	1	32	28

* μg S/100 g breast plus leg meat.
† 100 ml 7% HCl/100 g meat.
‡ 100 ml H_3PO_4 (specific gravity 1·75)/100 g meat.

TABLE 2. Effect of heating on the H_2S and mercaptan content of chicken meat*

Heated for 1 hr at	H_2S (μg S/100 g)	Methyl mercaptan (μg S/100 g)
20°C	1	0
50°C	2	0
60°C	8	0
70°C	31	5
100°C	93	12
125°C	510	19

* Breast plus leg meat. Volatile sulphides removed by entrainment in hydrogen for 2 hr at 20°C.

Effect of temperature on the liberation of H_2S

Table 2 shows the amounts of H_2S produced by heating chicken muscle in air for 1 hr at temperatures between 50° and 125°C. H_2S, and the much smaller amount of mercaptans also produced, were measured after entrainment in hydrogen at 20°C for 2 hr.

Whereas with meat pre-heated at 70°C or over more than 92% of the total H_2S

measured was removed in the first hour, with meat pre-heated at 50°C or, in less degree at 60°C, the proportion liberated in the first hour was much smaller. This behaviour probably indicates a destabilising effect of moderate pre-heating temperatures on the H_2S precursors in meat, similar to that previously observed for milk (Lea, 1946).

Some of the H_2S liberated during cooking might be expected to disappear, by reaction with carbonylic or other reactive constituents of the meat, and much higher yields are, in fact, obtained if the H_2S produced by heating is removed continuously as it is produced (Table 3).

Since approximately 205 μg ammonia N/100 g meat was found in breast muscle cooked at 100°C for 1 hr, either in air or in nitrogen, H_2S in the meat would presumably be present partly as ammonium sulphide.

TABLE 3. Comparison of removal of H_2S after heating with continuous removal during heating

Chicken meat	Pre-cook at 100°C		Entrainment in H_2		H_2S produced (μg S/100 g meat)
	Atmosphere	Time (hr)	Temperature (°C)	Time (hr)	
Breast	N_2	1	20	1	60
Breast	–	–	96	1	140
Breast and leg	Air	1	20	1	85
Breast and leg	–	–	96	1	184
Breast and leg	–	–	96	2	342

Glutathione as a possible precursor of H_2S

Glutathione contents of fresh chicken muscle varied considerably, typical values being about 4000 μg glutathione S/100 g for leg and 1300 for breast muscle.

These values decreased rapidly after the muscle had been minced. Such amounts would be much more than sufficient to account for all the H_2S produced on cooking, but, as already indicated, Mechi *et al.* (1964) concluded that only about 10% came from this source and our own observations are not incompatible with this view.

Glutathione was stable under the conditions of the H_2S determintaion, comparatively large additions of glutathione to breast muscle causing only a negligible increase in the apparent H_2S content of the raw meat. Furthermore, addition of gluthathione (1400 μg S/100 g) to breast meat already containing 1000 μg glutathione S/100 g, prior to cooking for 1 hr at 100°C only increased the H_2S content of the cooked meat from 90 to 97 μg S/100 g meat, indicating that most of the H_2S produced was not derived from glutathione. In a further test, H_2S production on cooking was com-

pared for: (a) freshly minced leg muscle containing 3400 μg glutathione S/100 g, and (b) similar muscle after storage at 1°C, in presence of 10 ppm chlortetracycline, for 5 days, during which time the glutathione content decreased to 230 μg S/100 g. H_2S production on cooking for 1 hr at 100°C was for (a) 163 and for (b) 125 μg S/100 g, again indicating that not more than a small proportion of the H_2S could have come from glutathione.

TABLE 4. Volatile sulphides in chicken meat* cooked and stored in air or nitrogen

Atmosphere	Hours after cooking					
	H_2S (μg S/100 g)			Methyl mercaptan (μg S/100 g)		
	0·25 at 20°C	24 at 20°C	72 at 1°C	0·25 at 20°C	24 at 20°C	72 at 1°C
Air	87	12	15	14	12	9
Nitrogen	80	47	54	6	4	5

* Mixed breast and leg meat, cooked for 1 hr at 100°C in air or in nitrogen, cooled immediately and stored in the same atmosphere at the indicated temperature. Volatile sulphides removed by entrainment in hydrogen at 20°C for 2 hr.

Destruction of volatile sulphides in cooked meat on standing

Table 4 gives data for the H_2S and mercaptan contents of chicken meat cooked for 1 hr at 100°C in air or nitrogen and subsequently held for the indicated periods in the same atmosphere before commencement of the determination. Since H_2S disappeared more rapidly in air than in nitrogen it would seem that much, but not all of the loss was oxidative. The same would appear to be true for mercaptan, though the extent of the change was less.

Discussion

Hamm & Hofmann (1965) produced H_2S equivalent to 1770 μg S/100 g protein, i.e. approximately 350 μg S/100 g muscle, by heating beef longissimus dorsi for 30 min at 120°C, and only about 6% less by heating isolated myofibrils. They also showed that, after blocking the –SH groups of the myofibrils by reaction with silver nitrate or with *N*-ethyl-maleimide, heating at 120°C no longer liberated H_2S. These results extend those of Mecchi *et al.* (1964) and prove that the H_2S liberated during the cooking of meat derives very largely from the –SH groups of the structural proteins, and not from SS-groups or methionine.

The amounts of H_2S present in freshly cooked meat, e.g. 0·2 – 1·0 ppm (Table 2) are of the order of 20 – 100 times higher than the odour threshold of H_2S in aqueous solution, and are probably sufficient to influence aroma and flavour directly. Moreover,

even larger quantities of H_2S are liberated during heating, only to react almost immediately to produce compounds some of which may well be more odorous than H_2S itself, so that an indirect contribution to flavour is also possible. Finally, the disappearance of free H_2S from freshly cooked meat on standing could well be a contributory factor to the obvious differences that exist between freshly roasted and reheated 'cold' meat.

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Differential thermal analysis as a control instrument in edible fat hydrogenation

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Summary. The use of differential thermal analysis for following the progressive hydrogenation of ground nut oil with a view to giving an end product of consistent quality is described. The technique appears to be promising as a method for determining the solid fat index at various temperatures and compares favourably with dilatometry in speed, reproducibility of results and the amount of information available from any one analysis.

Introduction

Edible fats are processed within the refinery by neutralization, bleaching and deodorization, none of which has any effect on the final consistency of the product. Interposed between bleaching and deodorization can be the process of hydrogenation (and a second bleaching). This process of hydrogenation has as its primary effect the hardening of the fat, i.e. the proportion of solid to liquid glycerides existing at any specified temperature increases. In the industry this is described in terms of the solid fat index (SFI). The hydrogenation may be varied, both in the degree to which it is taken to completion and in the selectivity of the process, to give a product which is tailored to specific uses in the baking industry. It is essential, therefore, to control the hydrogenation so as to give a reproducible product.

The current industrial practice is to follow the hydrogenation process by the changes in refractive index of the fat, by the slip melting point, by the iodine value or by dilatometry. The dilatometric technique is particularly valuable, as it can be used to give an index of the solid glyceride content at different temperatures, and is being more widely used as the baking trade becomes more sophisticated and lays down more stringent manufacturing specifications. The disadvantages of the technique are twofold. Firstly, the constant which is used to convert the observed isothermal expansion to a solid fat index incorporates an average specific volume for the fats. This inevitably varies for different fats and between the liquid and solid phases, and in consequence there is an inherent error in the calculation; values over 50% SFI do not reflect the true solids content. Secondly, there is a disadvantage in the length of time required to

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perform the full test over the temperature range required. Even with a modified technique where the solids index is estimated at one temperature only, and the assumption is made that the SFI profile follows the normal pattern through this point, it takes at least one hour. Hence, if a test were forthcoming which would yield the same or similar information, namely the melting pattern of the glycerides, and would take less time, considerable cost saving would ensue.

Differential thermal analysis (DTA) is a technique which gives such a melting profile. The experience of Berger & Akehurst (1966) suggested that scanning speeds of up to 5° or 10°C/min were not out of the question and hence, if it were desired to scan a sample from the completely solid state at − 40°C, to the completely liquid at + 70°C, i.e. over a range of 110°C, it was unlikely to take more than 30 min to complete any one run. It appeared, therefore, that it would be valuable to see what information DTA thermograms would give at successive stages of hydrogenation and to assess whether the results could be put on a quantitative basis which would correlate with the results from dilatometry.

Wendlandt (1964) described the development by Speil, Kerr and Kulp of a formula which related the area enclosed by the differential curve to the heat of reaction (either chemical or, as in this case, a physical change of state) and the mass of material. These workers neglected any differences in specific heats between components. Further, if any differences in thermal conductivity and latent heat of fusion of the components are neglected (any differences are relatively small in triglycerides), and the slight deviation from a constant rate of cooling is discounted, then the Speil, Kerr and Kulp formula reduces to:

$$\int_{t_1}^{t_2} \Delta T dt = \text{mg}$$

where m = mass of sample,
ΔT = differential temperature,
dt = time differential between limits t_1 and t_2, and
g = a constant (involving the latent heat of fusion and thermal conductivity of the sample and a factor arising from the structure of the cooling block).

Hence, the area under the thermogram to a temperature plane T, as a proportion of the total area, will give the amount solidified at that temperature. Using this relationship, it should be possible to calculate the 'solid content' of the fats from area measurements of the thermograms.

Experimental

Apparatus

The apparatus used is shown in Fig. 1 and was a modified design of that used by Berger & Akehurst (1966). The aluminium block was supplied with a cartridge heater and a regulating transformer included in the circuit to control the rate of reheating.

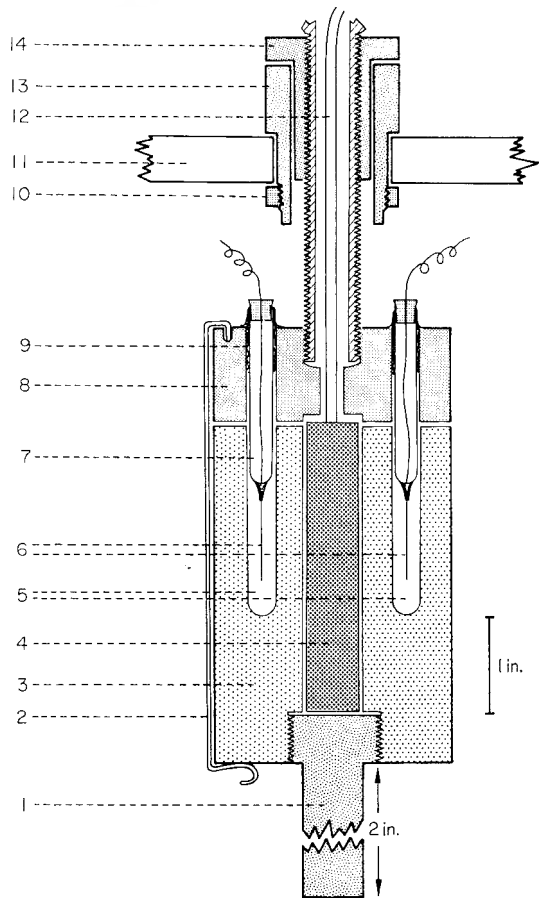

FIG. 1. Diagram of a DTA head assembly. (1) Aluminium tail, (2) one of three wire clips, (3) aluminium block, (4) heater, (5) reference and sample tubes, (6) thermocouples, (7) glass thermocouple holders, (8) nylon head, (9) microcrystalline wax, (10) retaining screw, (11) Perspex, (12) heater wires, (13) keyed bearing, and (14) knurled thumb screw.

The block was of such a size as to permit space for three pairs of cells; this allowed the study of different sample sizes and dimensions. Cooling of the block was through conduction, by means of a narrow cylinder (or 'tail') at the base of the block in contact with the liquid nitrogen. The 'tail' was removable and could be exchanged for different sizes, thereby increasing the range of cooling rates. The whole block and head assembly could be raised or lowered by a screw mechanism and this permitted location of the tail in the liquid nitrogen at the desired level. Dibutyl phthalate was used as the reference material and temperatures were recorded, using a Rikadenki 2 pen recorder, model B241, 1 and 10 mV f.s.d., with a pre-amplifier (model A10, which could increase the sensitivity to a possible 5 μV f.s.d.)

Materials

A series of ground nut oils, kindly supplied by the Peerless Refining Co. Ltd, were examined: a sample of the original groundnut oil (GNO), samples of eight hardened groundnut oils (HGNO 1–8) which had been hydrogenated in the laboratory to different degrees and possessed melting points from 30° to 50°C. Five further samples were provided, obtained during manufacture, HGNO (A) and (B), supplied with and without removal of the nickel catalyst by filtration, and HGNO stearine.

Method

Iodine values, slip melting points and dilatometric assays were determined for all samples.

Preliminary studies with DTA using a cooling cycle were made to determine the optimum cell size, tail size and cooling rate to give well defined thermograms concurrent with a sensible length of time for the test. A standard operating procedure using a cooling cycle was then laid down. The reheating thermogram was also prepared for each sample.

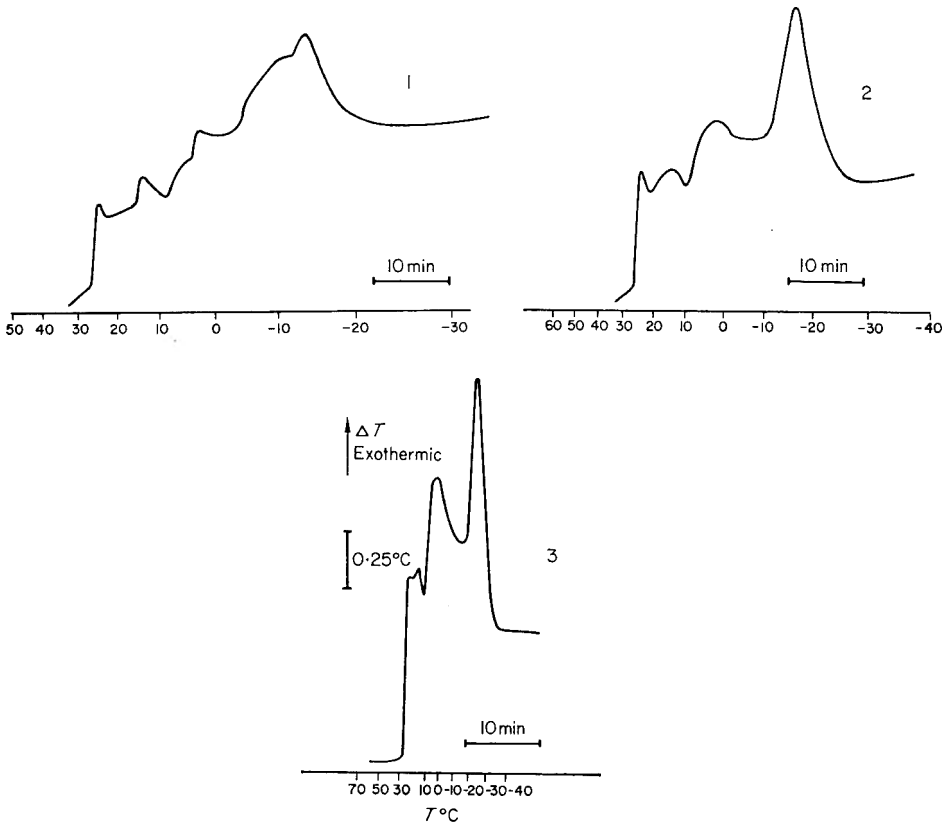

FIG. 2. Thermograms showing effect of cooling rate. (1) Slow cooling, (2) medium cooling, and (3) fast cooling.

TABLE 1. Analysis of oil and fats

	Iodine value	Slip melting point	Dilatation (mm^3/25 g)					
			D.15	D.20	D.25	D.30	D.37	D42
GNO	86·4							
HGNO No. 1	70·8	27·0	204	119	58	13	0	0
No. 2	66·3	31·5	606	387	216	84	0	0
No. 3	60·5	37·0	1413	1173	931	582	136	0
No. 4	54·0	43·0	1725	1655	1533	1262	644	267
No. 5	46·0	45·7	1745	1793	1733	1567	1086	637
No. 6	41·8	49·4	1873	1969	1971	1865	1517	1070
No. 7	33·7	52·2	1850	2013	2066	2035	1865	1547
No. 8	25·6	55·4	1980	2088	2180	2245	2202	2070
HGNO stearine	2·7							
HGNO 30/32°C(A)	68·6	34·2	1162	876	551	239	12	0
HGNO 30/32°C(B)	75·3	30·4	473	310	167	63	0	0

TABLE 2. Comparison of SFI with solids content calculated from DTA thermograms at 15° and 20°C with and without temperature lag correction.

Fat	% solids					
	SFI		From thermograms with no correction		From thermograms with correction for 11°C temperature lag	
	15°C	20°C	15°C	20°C	15°C	20°C
HGNO						
No. 1	8·2	4·8	4·9	0·7	14·9	7·75
No. 2	24·2	15·5	11·1	6·4	24·2	15·8
No. 3	57·5	46·9	35·1	23·0	55·2	47·0
No. 4	69·0	66·2	48·6	36·8	72·5	62·5
No. 5	69·8	71·8	62·6	56·8	75·0	69·0
No. 6	74·9	78·7	72·2	65·0	85·2	80·9
No. 7	74·0	80·5	81·3	74·0	91·1	88·5
No. 8	79·1	83·6	80·5	75·5	94·9	90·8
(A) HGNO filtered	46·5	35·0	14·4	5·8	39·0	30·0
(A) HGNO unfiltered	—	—	14·4	4·3	43·3	29·5
(B) HGNO filtered	18·9	12·4	10·55	5·6	25·0	15·7
(B) HGNO unfiltered	—	—	10·45	5·0	21·9	15·8

Results

The thermograms obtained are presented in Figs. 2–6. The abscissa of the thermograms will be seen to run from + 60° to − 50°C, and thence from − 50° to + 70°C; this is essentially a constant time axis on which has been superimposed the temperatures as taken from the temperature of the reference material. The cooling graph has been vertically inverted for convenience of presentation and is labelled 'exothermic'; the heating curve is presented as observed and labelled 'endothermic'. It will be appreciated that deviation from positive to negative of the base line will necessitate reversal of these labels.

The results of the iodine value, slip melting point and dilatometric determinations are given in Table 1. Table 2 compares the SFI results calculated from dilatometric data and DTA thermograms at temperatures of 15° and 20°C.

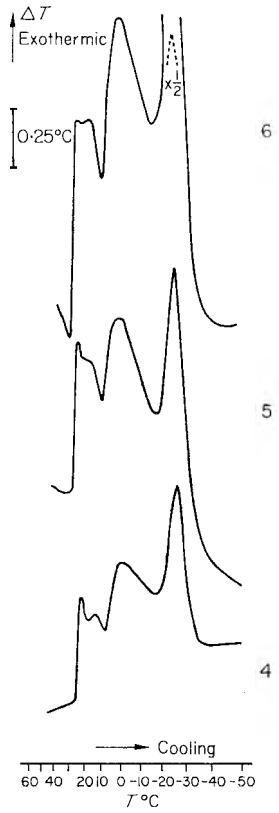

FIG. 3. Thermograms showing effect of cell size. (4) 1·2 ml, (5) 0·6 ml, (6) 0·3 ml.

From these results it was apparent that a temperature lag existed as the majority of values were lower than their respective SFI. However, by using the SFI for HGNO No. 2 as a standard and comparing this with the solids content calculated from the DTA method, the approximate temperature lag was found to be 11°C. Re-evaluation

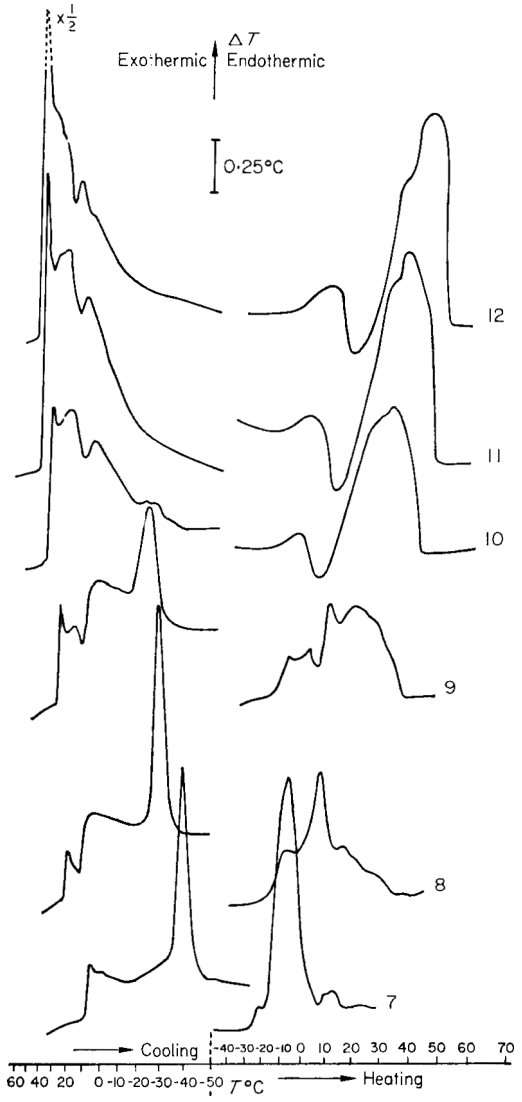

FIG. 4. Thermograms of HGNO series. (7) Soft GNO, (8) HGNO No. 1, (9) HGNO No. 2, (10) HGNO No. 3, (11) HGNO No. 4, (12) HGNO No. 5.

of the solid content incorporating this lag, plus any increment accorded by the temperature differential of the exothermic phase change yielded the results recorded in the right-hand column of Table 2.

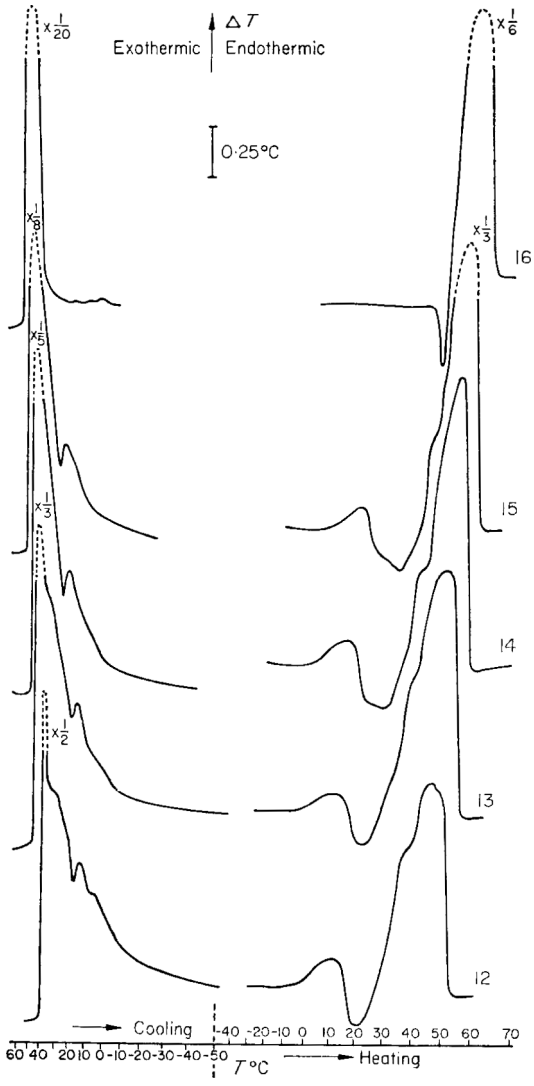

FIG. 5. Thermograms of HGNO series continued. (12) HGNO No. 5, (13) HGNO No. 6, (14) HGNO No. 7, (15) HGNO No. 8, (16) HGNO stearine.

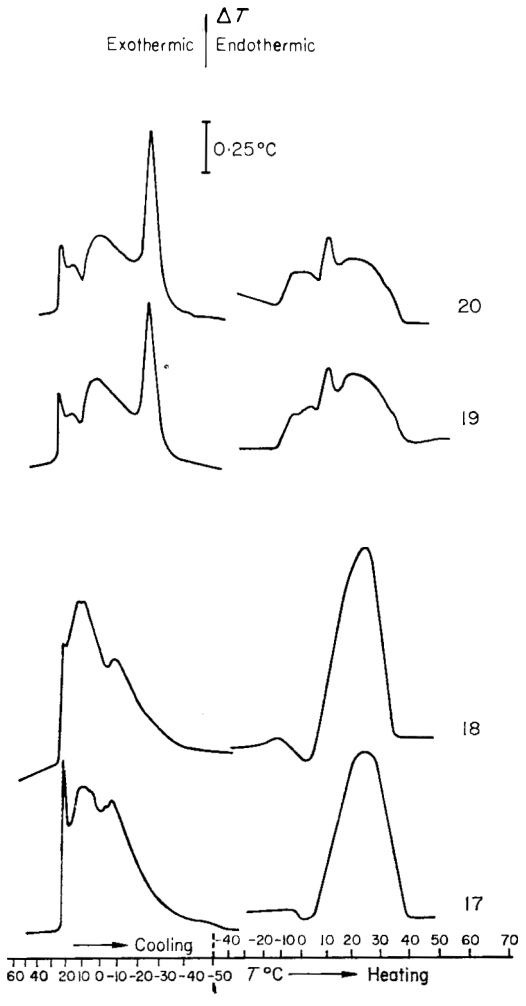

FIG. 6. Thermograms of factory plant hydrogenated GNO. (17) HGNO (A) filtered, (18) HGNO (A) unfiltered, (19) HGNO (B) filtered, (20) HGNO (B) unfiltered.

Discussion

(a) *The development of the standard operating technique*

Variation in cooling rate. HGNO No. 2 was used to show the effect of the cooling rate in thermograms 1, 2 and 3. The slow cooling thermogram No. 1 although having a marked deviation in base line, shows much greater complexity than is evident in the faster cooling thermogram; seven different peaks are found compared with four. The triple complex of peaks at − 5° to − 12°C which it will be seen occurs at a slightly higher temperature than their counterparts at the faster cooling rate, is likely to be due

to the separation of the different tri-unsaturated glycerides; similarly the double peak at 5–0°C shows a separation of the di-unsaturated glycerides. It is obvious, therefore, that a slower cooling rate (and presumably also heating rate) will yield more detailed results, but where speed of operation is a major consideration, this aspect may be overlooked. On the basis of these results it was decided to standardize on the tail of 1·63 cm diameter submerged such that the distance between the surface of the liquid nitrogen and the base of the block was 3·05 cm. All subsequent tests were carried out with this standard condition, but it is important to note that the actual rate of cooling over an entire run was not constant.

Cell size. Thermograms 4–6 show the effect of cell size with a constant height of fat within the cell. The smallest cell gives the most detailed thermogram. It was also established that a use of the smaller cell with a third of the volume (i.e. 0·1 ml) of the same fat gave very similar results but the increased sensitivity required gave a more irregular base line and no advantage could be gained.

(b) *The effect of hydrogenation*

Cooling thermograms of the HGNO series (Nos. 7–16). By studying the laboratory hydrogenated HGNO series an overall pattern of change induced by hydrogenation will be obvious. A more detailed analysis can be made using the fundamental data published by Swern (1964) and Berger & Akehurst (1966), the latter having published thermograms of the individual glycerides from GNO.

It will be noted that with the cooling thermograms the major peak at − 40°C for soft GNO (unhydrogenated) first moves slightly up the temperature scale and then is reduced in height until it completely disappears in HGNO No. 4. This peak in the soft GNO will reflect the solidification of the tri-unsaturated glycerides, including both oleic and linoleic acids. On initial hydrogenation the linoleic acid will preferentially take up hydrogen and hence, the finer peak at − 30°C in HGNO No. 1 will be that for the tri-unsaturated glycerides now principally containing the oleic acid. The wide peak at 0°C for soft GNO will contain the mono and di-unsaturated glycerides. Both of these glycerides will increase in quantity as hydrogenation proceeds and this can be followed with the intermediate two peaks at 0°C and 15°C in HGNO No. 2.

The di-unsaturated glycerides rise to a peak in HGNO No. 4 and subsequently are hydrogenated. The mono-unsaturated glycerides appear to rise in quantity until in HGNO Nos. 5 and 6 the peak merges with the peak of the increasing tri-saturated glycerides. Comparison of HGNO No. 8 with HGNO stearine (thermogram No. 16), shows the di-unsaturated as a negligible quantity in the stearine and the fineness of the peak indicates the mono-unsaturated as completely hydrogenated; this must be so with a stearine with an iodine value of 2·7.

Thermograms 17–20 are those derived from factory plant hydrogenated GNO and consist of two samples. Nos. 17 and 19 are thermograms of unfiltered fats (i.e. the fat

and the catalyst), Nos. 18 and 20 on the other hand have been filtered. It is interesting to note that the dilatometric assay of the filtered samples indicates substantial differences between them, which is surprising when one considers the similarity between the slip melting points and to a lesser degree the iodine values. The difference between thermograms 17 and 18, both emanating from the same sample, is quite marked, the unfiltered sample showing a greater degree of hydrogenation. This can probably be explained by post sampling hydrogenation taking place in the unfiltered sample. On the other hand thermograms 19 and 20 [from sample HGNO (B)] showed almost identical profiles. It appears from these two tests that the catalyst does not interfere with the results and in consequence would save time in analysis if filtration were confirmed as unnecessary.

The profile of HGNO (B) shows it to be intermediate between the HGNO Nos. 1 and 2 which is also supported by the dilatometric results. The profile of HGNO (A), on the other hand, shows similarity to HGNO No. 3 but is displaced some 6–7°C to the cooler end of the thermogram. This is a fascinating finding as it relates to one of the fundamental problems of fat hydrogenation, that is the endeavour to manipulate the hydrogenation process to give a certain dilatometric profile. The use of active catalyst as against used or spent catalyst, low pressure and high temperature all tend to give greater selectivity to the hydrogenation, in that linoleic acid is hydrogenated preferentially to oleic, but these conditions also tend to increase the iso-oleic content and increase the melting temperature thus obviating the advantage gained. Therefore, a compromise has to be maintained and it appears that in HGNO (A) greater selectivity was obtained in the hydrogenation. The evidence is that HGNO (A) shows no peak for the tri-unsaturated glycerides, the iodine value is higher than in HGNO No. 3 and for a similar thermogram profile, HGNO No. 3 has a higher dilatation.

Heating thermograms of the HGNO series. Another major point to be noted is the remarkable difference between the cooling and heating profiles, even taking into account the difference in rate of temperature change. This is exemplified in the thermogram of GNO, the two substantial peaks of the cooling side appear to be merged into one major melting curve with three minor peaks. The profile bears no relation to the glyceride composition of GNO. HGNO No. 3 has lost virtually all its definition in the heating thermogram and gained an exothermic inversion at 8°C. It is evident that analysis of these profiles and peaks is much more difficult, due to the polymorphic changes of the crystalline forms. The heat of crystal transformation, being exothermic, lowers the peak height or produces troughs in the profile; unless such troughs fall below the base line their exothermic nature is not discernible. Thus, as it stands with the present technique the heating thermogram can yield little analytical information. Nevertheless, the progress of hydrogenation can readily be seen from these thermograms and it should be noted that they can be readily reproduced indicating that the degree of polymorphism is constant for this standard mode of operation.

Conclusions

The results show that as a method of control in hydrogenation DTA thermograms permit a good visual appraisal of the changing pattern of glyceride structure. There is evidence too that a degree of selectivity of hydrogenation can be recognized (which is a further problem within the oil refinery).

Comparison of numerical values of percentage solid derived from area measurements of the thermogram against dilatometric values shows that after taking into account the thermal delay within the sample, a satisfactory correlation existed with the HGNO series. With a more precise knowledge of the thermal constants of the glycerides and with a uniform rate of cooling, the technique is capable of more refinement. In comparison with dilatometry, DTA is significantly more rapid in giving results of a similar quality, and overall, the information given by a thermogram is considerably in excess of that given by dilatometry.

Acknowledgments

The authors gladly acknowledge the assistance of the Peerless Refining Co. Ltd in providing the fat samples and financial support.

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J. Fd Technol. (1969) **4**, 303–306.

Book Reviews

Production of Pre-cooked Frozen Foods by Mass Catering. By JOHN L. ROGERS. London: Food Trade Press, 1969. Pp. 271. £3.

In the near future it is very probable that in many mass catering operations pre-cooked frozen food will be used extensively. It has been shown that this method of catering can save labour and kitchen space, and if carefully controlled, can result in better food than hitherto for the consumer in terms of palatability and nutritional value. It is difficult to see how the methods described in this book can improve the food in our mass catering operations. It consists largely of a collection of facts regarding processing equipment and, in collecting this material together, the author does a useful job. But theoretical considerations are ignored and some of the cooking methods described will result in severe palatability and nutritional losses. For example, the liberal use of M.S.G. is recommended in almost everything but dairy products. Workers at Cornell University Hotel School describing the advantages of precooked frozen food, have said 'that no longer will good food be ruined by the addition of such things as monosodium glutamate'. It will be difficult to find the answer in this book to the question of how to improve food quality in our mass catering operations.

G. GLEW

Biodeterioration of Materials. Microbiological and Allied Aspects. Ed. by A. HARRY WALTERS and JOHN J. ELPHICK. Amsterdam: Elsevier, 1968. Pp. x + 740. £12 10s.

'Biodeterioration affects a wide range of materials, goods and structures. Some examples are the attack on wood, paper, textiles and grain by fungi and insects, the blocking and corrosion of pipelines by bacteria, the growth of fungi and algae on concrete and painted surfaces, the fouling of ships by barnacles and molluscs, the destruction of stored products by rodents and the hazards caused to aircraft by birds. It follows, therefore, that biodeterioration is of considerable importance in many industries, especially in developing countries, where biodeterioration hazards can drastically reduce the life of equipment and the availability of foodstuffs.'

This definition, taken from the University of Aston Biodeterioration Information Centre's brochure, helps to explain why so little space is dedicated in this book to the deterioration of foodstuffs—a mere 60 pages out of 740. While no one would wish to impede any new ideas of applied biology, a responsibility rests with its sponsors to ensure that its scope and intention are embodied in the name given to it or that its activities are so organized that the description means what it appears to mean. How-

ever, there are several contributions of a general character of which the food scientist should be aware. Amongst these, one on bacterial taxonomy by Mitchell and Shewan is especially noteworthy and likely to be much quoted by future microbiologists.

E. C. BATE-SMITH

Food Engineering Operations. By J. G. BRENNAN, J. R. BUTTERS, N. D. COWELL and A. E. V. LILLY. Amsterdam: Elsevier, 1969. Pp. 443. £5 10s.

As Professor Rolfe states in his foreword to this book, the food industry 'cannot be supported by processing methods based on art and empiricism'. For it to be supported on a surer base there must be a substantial number of technologists and engineers who can design processes and machinery suited to the complexities of food materials. Specifically written for students and others being trained in this field, this volume sets out to describe the current procedures being used and the theoretical background.

In 443 pages the authors cover preparatory, conversion and preservation operations and give detailed accounts of plant hygiene, water supply/disposal and materials handling methods. The text is clearly arranged and well illustrated, with some excellent schematic drawings. Although much of the basic material is available elsewhere the book has the advantage of giving a generally up to date account of British food equipment and practice. The chapters on pre-processing operations and plant hygiene are particularly good and emphasise the special problems facing the designer of food processing equipment.

A single volume covering such a wide field is almost certain to omit some topics and give no more than a cursory description of others. In this book there is only a minimal consideration of automation of food processing plant (surely a matter of major importance to future technologists and engineers) and no reference to extraction procedures or pumping equipment. The value of the theoretical treatment given is somewhat reduced by the absence of sample calculations. Without these some of the terms used (such as characteristic dimensions and loss factors) will have little significance to the student. Although the information is available elsewhere it would also have been of practical convenience to include some data on the thermal and physical properties of foodstuffs.

Even with these failings there can be little doubt that this book will be useful both to students and those already working in the food industry. It confirms that although empiricism remains, science is replacing art as the basis of processing methods, and this book will itself contribute to this end.

J. LAMB

The Second Book of Food and Nutrition. By WENDY MATTHEWS and DILYS WELLS. Published by Home Economics and Domestic Subjects Review in association with the Flour Advisory Bureau, 1968. Pp. x + 219. £1 1s.

This book is intended for 'O' and 'A' level students. Its six parts cover the history of food in Britain from the time of nomadic tribes down to the present—in ten pages—the principles of nutrition, food preparation, some aspects of food technology, food marketing, and a final section about world food problems. In a short work covering so wide a field it is difficult to do more than set down a simple narrative and, when dealing with scientific matters, describe the facts didactically. Although they have not sufficient elbow room to give the evidence upon which their assertions are based, nor have their readers sufficient knowledge to understand the reasoning upon which the conclusions have been reached, the authors must nevertheless be accurate and their assertions capable of justification should more sophisticated readers wish to verify them. It is a help also in this difficult task, if such a work, while being written simply, is at the same time written in clear unambiguous language.

Because it is in many respects more difficult to teach a subject to people who know little or nothing of it than to more advanced students, it is often said that the professor ought to lecture to the first-year students and leave those in their final year to the junior lecturer. The professor would then, I think, modify such statements as 'amino acids . . . not used to provide energy . . . are converted to a substance called urea' and 'we can estimate the number of calories a food will supply by burning a weighed quantity', neither of which is accurate. He might also explain why 'it is important to start the day with a protein food', particularly if some of his students had been brought up to believe that fifty million Frenchmen, who start their days with a brioche and a cup of coffee, can't be wrong.

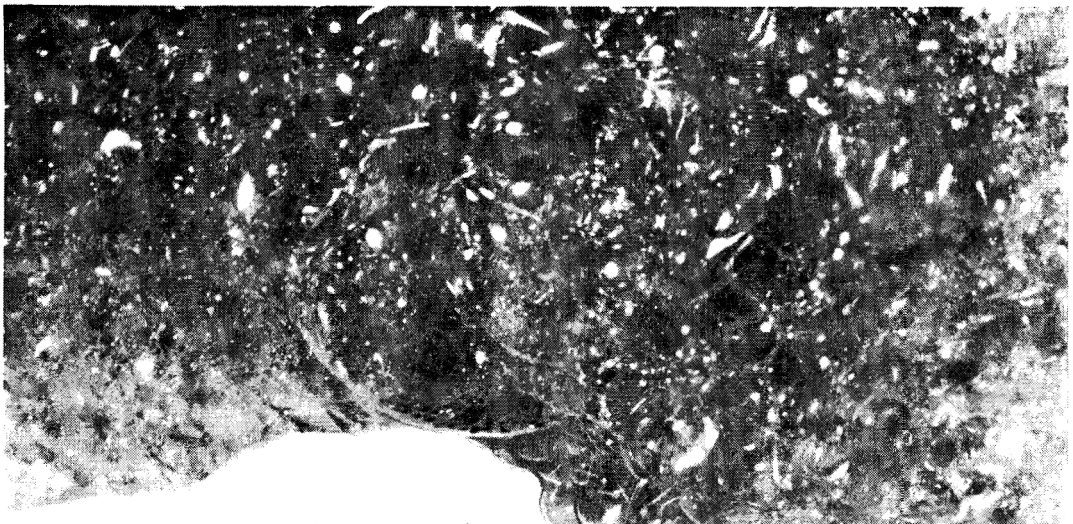
In a book intended for schools, I wondered whether reference to The Colouring Matter in Food Regulations 1957 and to specific revisions thereof was appropriate and whether readers would make very much of the fact that the addition of stearyl tartrate and partial glycerol esters to bread is limited by statute when no one had ever told them what stearyl tartrate is or what partial glycerol esters are. However, the pupils who finish reading *The Second Book of Food and Nutrition* will have touched on a wide range of topics and may at the same time acquire something of the optimism of its authors when, on page 205, they read that 'taboos and habits' (which stop the Indians eating nourishing beef and the Israelis pork chops) 'can easily be overcome by better education'.

MAGNUS PYKE

Books Received

Protein Food Supplements, Food Processing Review No. 3. By ROBERT NOYES. U.S.A.: Noyes Development Corp., 1969. Pp. 412. $35.00.

Snacks and Fried Products, Food Processing Review No. 4. By ALFRED LACHMANN. U.S.A.: Noyes Development Corp., 1969. Pp. 182. $35.00.



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References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s); (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
$(10^{-3}$ g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
$(10^{-6}$ g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
$(10^{-9}$ g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Letters and numbers must be written lightly in pencil. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in capitals with an Arabic number, e.g. TABLE 2. Each table must have a caption in small letters. Vertical lines should not be used.

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