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The softening of frozen meat: Criteria for transportation in insulated containers without refrigeration

D. P. HAUGHEY* AND J. M. MARER†

Summary

Measurements of the effect of temperature on the softness of frozen meat and offal are described. The assessment of softness was made under controlled laboratory conditions, both with a load-deflection penetrometer, and subjectively. The implications for meat quality are also discussed.

The results are used to determine criteria for the maximum allowable temperature for the various meat products at any point in the stow during transportation in an insulated container without refrigeration. It is shown that for frozen beef, lamb or mutton in either carcass, boneless or cut form, the appropriate limit is 25°F (– 4°C). For offals a limit of 23°F (– 5°C) is more satisfactory. These criteria were confirmed by container trials under practical operating conditions in New Zealand.

Introduction

The transport of frozen meat from a freezing works in an exporting country to an overseas buyer constitutes a vital link in the chain of production, processing and marketing. Meat is ultimately sold on the basis of quality as judged by the consumer from its sensory characteristics. Environmental conditions throughout the storage period, which includes transportation, need to be controlled to minimize any deterioration in quality, to maintain an adequately frozen state, and to limit refrigeration loads.

Traditional methods of transport normally require two separate enclosures to act as primary environmental barriers for the product. These are the insulated railway wagon or truck for land transport and the refrigerated hold of the ship for ocean transport. In contrast, the recent development of the container concept enables the complete carriage system to be designed around only one enclosure—the insulated box or container, which is transferred *in toto* between the various freight modes. The frozen cargo thus remains undisturbed within this barrier during the complete transportation period.

Centralized refrigeration systems on the container ship or port terminal can cater economically for product protection in a large number of containers simultaneously. For land transport, where it is only feasible to use separate refrigeration units for each

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container, it is preferable to rely on the container insulation to limit product temperature rise to an acceptable level. Suitable measurements to determine this maximum temperature limit for any point within a container of frozen meat are described below. Temperature–time measurements in container loads under practical operating conditions (Haughey, 1970) can then be used to establish an operational criterion for the maximum total elapsed time without refrigeration.

The softening point of meat is often used to characterize a maximum permissible temperature level. Since meat softening is a progressive phenomenon not rigorously defined by a single temperature, it must be related to the conditions of transportation.

In conventional rail or road transport, the carcasses in the top layer, which is the

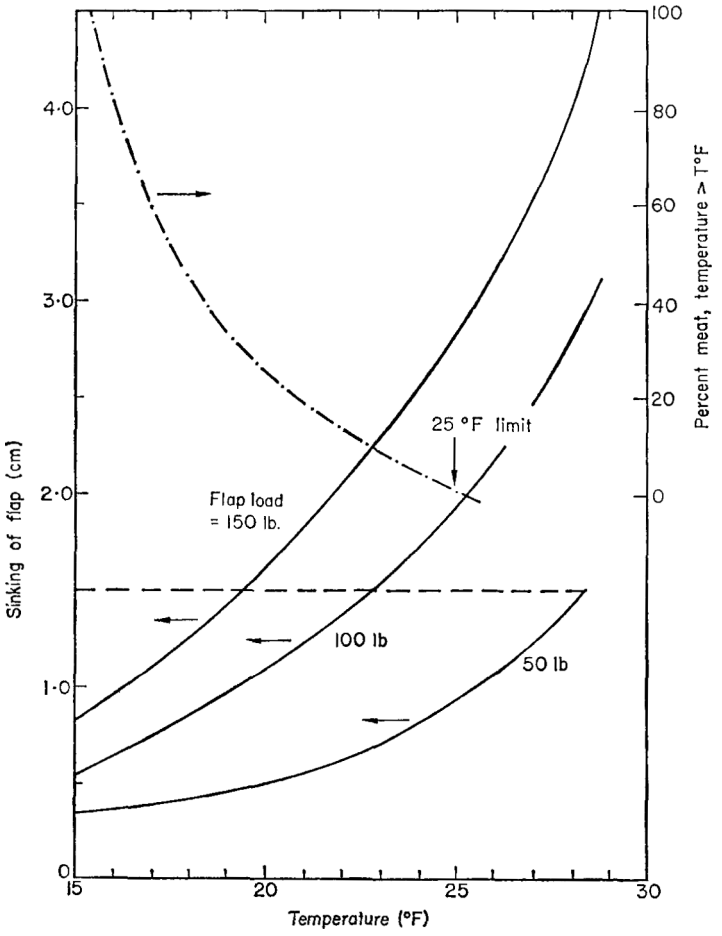

FIG. 1. Effect of temperature and load on the sinking of a carcass flap (- - - - - distortion above this level significant)—adapted from Griffiths *et al.* (1932). Percentage of meat (in a container load of lamb carcasses) with a temperature greater than T°F when the maximum limit of 25°F is reached from an initial store temperature of 10°F.

warmest region of the stow, are further exposed to ambient air temperatures during the loading into the ship where they are liable to be placed at the bottom of a stow some sixty carcasses high. In such cases the thin flap sections can be subjected to a considerable load and grossly crushed. If the body of the meat is sufficiently soft the whole carcass may be distorted and misshapen. Griffiths, Vickery & Holmes (1932) measured the dependence of flap sinking on the load applied to the flap. An adaptation of their results to show directly the effect of temperature is given in Fig. 1. They concluded that the flap temperature should not exceed 18°F. This allows a wide margin of safety and meat is currently accepted by ships in New Zealand at deep meat temperatures of 20–22°F and even higher in some cases. From subjective observations Earle (1963) concluded that 24°F was a suitable upper limit for the transport of frozen meat in insulated railway wagons.

While flap softening is a sensitive test in a narrow temperature range, it is not meaningful with containers where the meat is not subject to rapid temperature rise in ambient air after loading and where the region of maximum temperature level without refrigeration is confined quite distinctly to the constant, unweighted top layer. Fig. 1 shows the percentage of meat below a given temperature in a container stow of lamb carcasses with a maximum temperature of 25°F. This graph was deduced from thermocouple temperature measurements (Haughey, 1970) throughout the container stow during trials simulating inland transport under practical operating conditions in New Zealand. The onset of surface softness on the body of the meat is a realistic limit for the adequately frozen state for containers. No previous measurements of the surface softness of meat at different temperatures appear to have been reported, except for chilled beef at temperatures of 25–32°F (Moran, 1933).

Effect of temperature on meat softening

Experimental procedure

A recirculating wind tunnel provided a controlled environment of low air-speed (50 fpm) and constant air temperature, maintained to ± 0·25°F at any temperature level by a thyristor controller and an electric heater. The temperature fluctuation at the surface of the meat was not detectable and less than 0·1°F. High humidities, to prevent excessive surface desiccation, were obtained by placing blocks of ice close to the heater which was shielded from the meat products. Complete equilibration of the meat to the air temperature set-point required from one to seven days depending on the enthalpy change required (Fig. 5) to reach the new temperature level. Measurements of surface softness were then made for each product using a specially constructed penetrometer (Fig. 2), the design being adapted from a commonly used fruit pressure tester (Haller, 1941) measuring the force required to produce a given depth of penetration. For frozen meat it was found more suitable to measure the depth of penetration

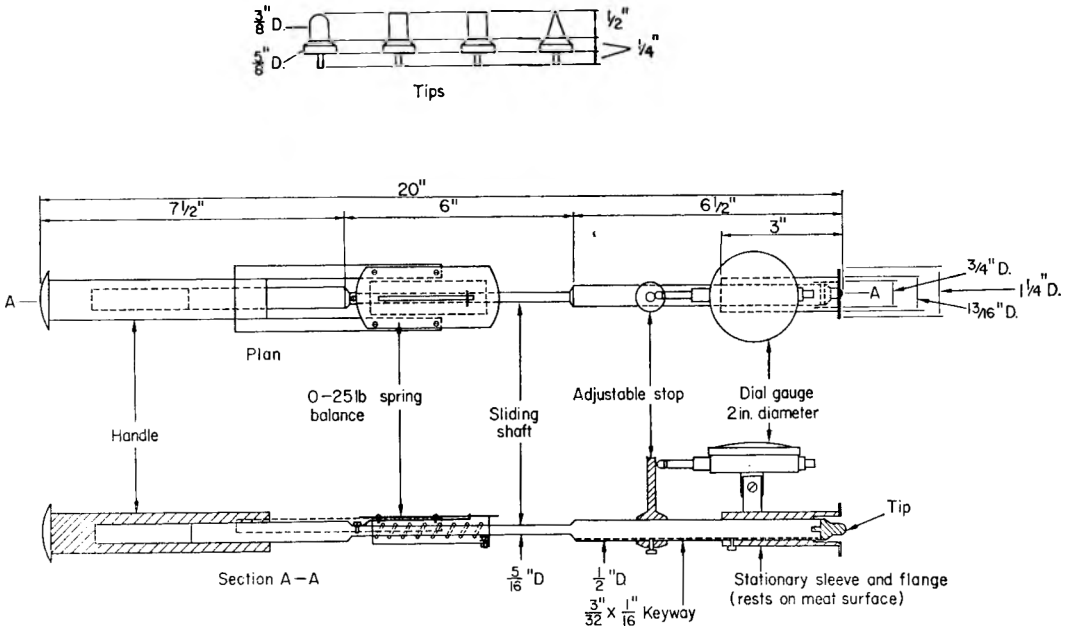

FIG. 2. Load-deflection penetrometer and the various tip shapes used for the measurement of surface softness.

produced by a given force due to the wide range of softness over the temperature range of interest.

This type of instrument was chosen because it could be used on non-flat surfaces and on any size of product. The empirical measurements given by this penetrometer were quite adequate to define the variation of softness with temperature and give a comparison with subjective assessments. For this purpose it was considered unnecessary to use a more sophisticated instrument giving absolute values which could be related to the rheological properties of the product.

A 20 inch × $\frac{5}{16}$ inch diameter steel rod passed through the spring of a 0–25 lb spring balance, being fastened to the spring at one point. The lower end of the rod widened to $\frac{1}{2}$ inch diameter and could slide in a surrounding sleeve 6 inch long attached to the body of the spring balance. A $\frac{3}{8}$ inch diameter hemispherical (or other shape) brass tip screwed into the end of the rod. A projection from the sliding rod deflected the plunger of a micrometer dial gauge attached to the sleeve and read zero when the end of the tip was flush with the annular flange on the end of the sleeve. To take a measurement this flange was placed evenly on the meat surface and pressure increased uniformly on a handle at the upper end of the sliding rod until, after about 5 sec, the required reading in pounds was obtained on the spring balance indicator. The depth of penetration (in thousandths of an inch) was then recorded from the dial gauge to give a measurement of the surface softness.

In order to eliminate any possible effect of anisotropy with depth in a sample, all measurements were made at distributed positions on a complete product module (whole carcass, cut or block) and readings were only taken when the temperature of the module was uniform throughout. There was no variation in reading with the time the load was maintained and each new measurement was made at a previously undeformed part of the product surface.

At each temperature level five to ten penetrometer readings were made at different points on representative surfaces of the various products. Separate sets of readings were made on fat and lean areas where these could be differentiated. The scatter of individual readings was generally less than ± 5%. For most products measurements were made at ascending 5°F temperature intervals in the range 5–20°F and at 1°F intervals for the range 22–30°F. For each temperature level measurements were normally made at 5 lb intervals over the range 5–25 lb, though some of the higher load readings were omitted at the higher temperatures. For some of the offals lower load levels of 1, 2, 4 and 10 lb were used.

The meat products tested were: lamb carcasses (New Zealand grades: PD, P2, P8,

TABLE 1. Effect of tip shape on penetration (⅜ inch diameter tips, boneless beef).

		Penetration (0·001 inch units)				
Pressure, lb		5	10	16	20	25
Temp.	*Tip shape*					
5°F	Cylinder (sharp edges)	2	7	9	12	15
	Cylinder (rounded edges)	3	8	12	17	22
	Hemisphere	—	4	13	18	—
	60° Cone	26	51	86	107	127
28°F	Cylinder (sharp edges)	17	50	110	145	
	Cylinder (rounded edges)	35	107	160	220	
	Hemisphere	69	125	219	—	

YL, YM), lamb cuts (leg, rack), cartoned boneless meat (beef, mutton), offals (lamb brains, kidneys and hearts; sheep lungs, liver and tripe; ox kidneys and spleen).

Results

Several forms of penetrometer tip were tried (cone, cylinder, hemisphere, cylinder with rounded edges) and some comparative readings are shown in Table 1. The cone and cylinder cut the surface fibres of the meat and thus were not considered suitable. The hemisphere and rounded cylinder were comparable with the application of thumb pressure to the surface but the former was preferred since the readings were generally more sensitive to changes in softness. The results shown in Figs. 3 and 4 were obtained using the $\frac{3}{8}$ inch diameter hemispherical tip.

There was no significant difference in results within the different grades of lamb carcasses and the averages of all the carcass measurements are shown in Figs. 3 and 4.

The effect of load on the depth of penetration is shown in Fig. 3 for temperature levels of 20° and 25°F. Penetration increased approximately linearly with applied load for

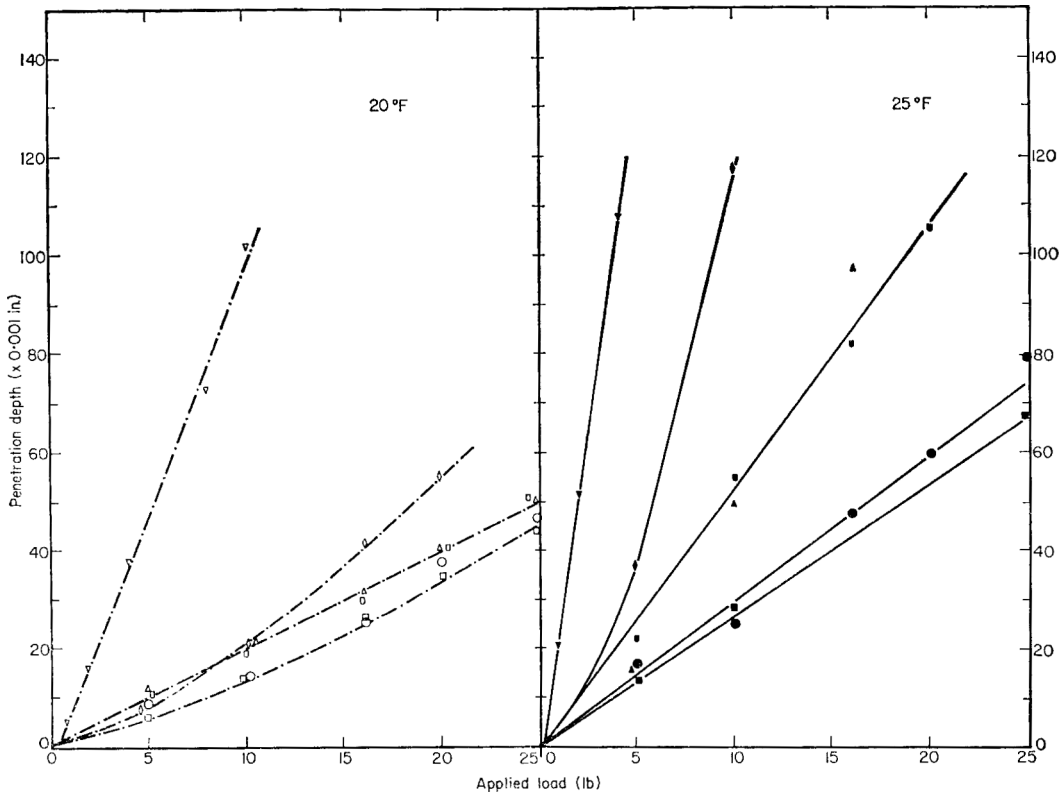

FIG. 3. Effect of applied load on surface penetration depth at 20°F (—·— and open symbols) and 25°F (——— and solid symbols). Lamb carcass □ ■; Lamb hearts ⊃ ●; Lamb brains ▽ ▼; Boneless beef ○ ●; Boneless mutton △ ▲; Ox kidneys ◇ ◆.

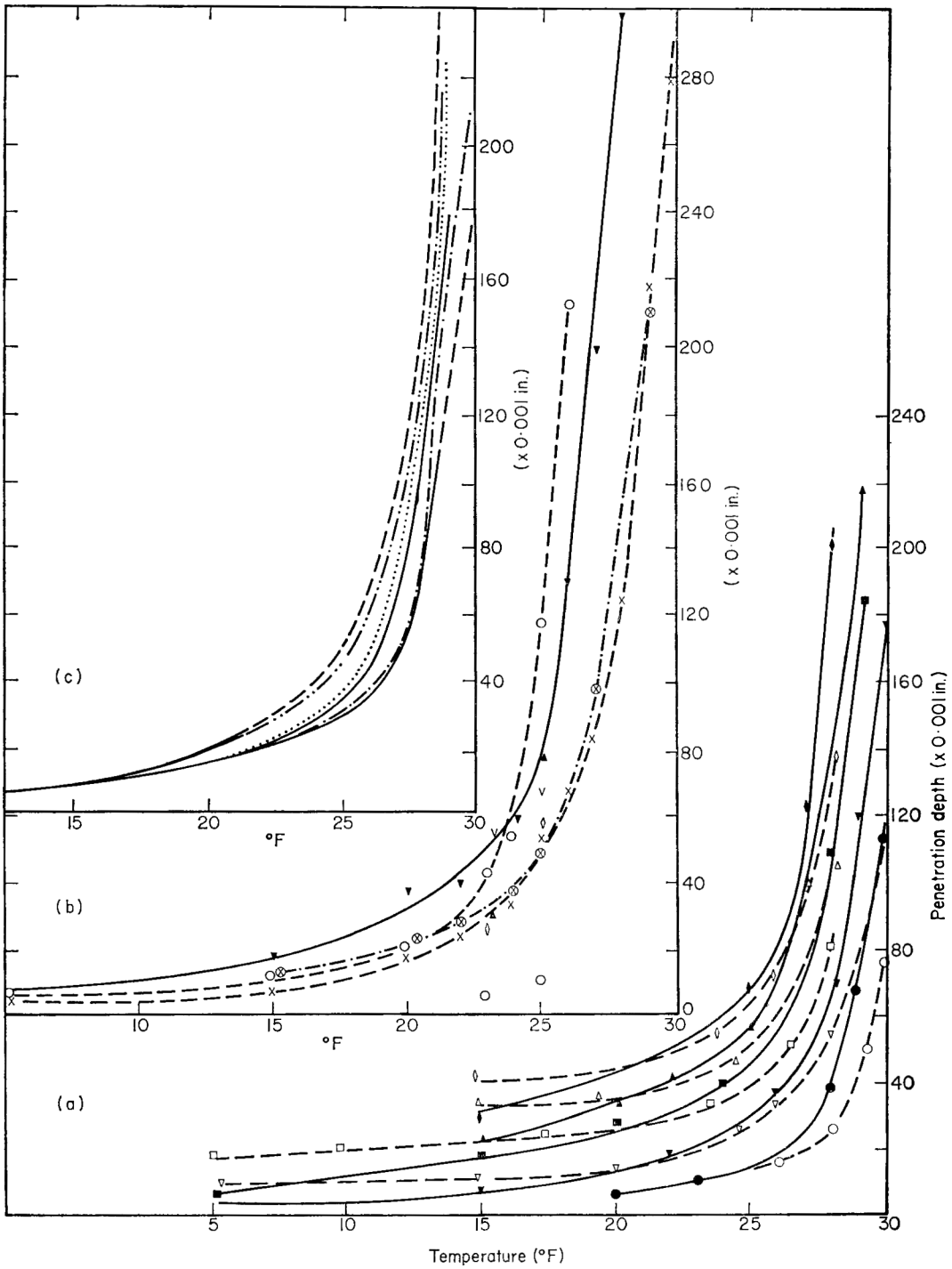

FIG. 4. Effect of temperature on surface penetration depth. (a) Lamb Carcasses: Lean areas (——— and solid symbols) and fat areas (- - - - and open symbols) with applied loads of 5 lb (● ○), 10 lb (▼ ▽), 16 lb (■ □), 20 lb (▲ △), and 25 lb (◆ ◇). (b) Various Offals: 10 lb applied load: lamb hearts (- - × - -), ox kidneys (- - ○ - -), ox spleen (▲), ox tripe (●); 4 lb applied load; lamb kidneys (V), lamb brains (—▼—), sheep liver (◇). 2 lb applied load: sheep lungs (-·-⊗-·-). (c) Lamb, mutton, beef, lamb hearts: 10 lb applied load: Lamb carcass (——— — — —), lamb leg—cut (——) lamb rack—cut (—·—) lamb hearts (—· ·—), boneless beef (. . . . . .), boneless mutton (- - - - -).

most products. The slope of the penetration-load line was dependent on the product and on the temperature level. At 20°F, the lines for lamb carcasses, lamb cuts, boneless beef, boneless mutton, lamb hearts and ox kidneys all had a similar slope except that the kidney line departed from linearity at 15 lb load. The slope for lamb brains was distinctly higher due to the more delicate structure. At 25°F all slopes increased, particularly those for the offals.

Fig. 4(a) shows curves of penetration versus temperature at the different load levels for lamb carcasses. Separate lines are shown for the lean and fat areas of the surface. There was little change in penetration between 5° and 20°F; between 20° and 25°F the penetration began to increase and the rate of increase became very much greater above about 25°F. The temperature at which the change in slope first became significant varied slightly in the range 24–26°F depending on the load applied, the highest temperature level corresponding to the lowest applied load. Since a firm thumb pressure corresponds to about 10 lb load, a temperature of 25°F is seen to provide a suitable limit of the maximum desirable penetration or surface softness (0·03–0·04 in.). At low temperatures the penetration for fat areas was slightly greater than that for lean areas while at the higher temperatures the lean became significantly softer than the fat. The point of cross-over occurred in the range 20–24°F but the difference did not become significant until 26–27°F.

Figs. 4(b) and 4(c) compare the penetration–temperature curves for different products—at an applied load of 10 lb for the products other than some of the offals. The shapes of the curves were similar to those for lamb carcasses and the greatest change in slope again occurred at about 25°F except for some of the offals. The boneless mutton gave a slightly greater penetration than the boneless beef. The behaviour of boneless veal should be comparable.

For offals, differences in structure result in different applied loads being more appropriate for the assessment of softness in some cases: the levels used were 10 lb (lamb hearts, ox kidneys and spleen, sheep tripe); 4 lb (lamb kidney and brains, sheeps liver); and 2 lb (sheep lungs). The greatest change in slope occurred at 24°F for lamb hearts, ox kidneys and spleen, and sheep tripe, and at 23°F for lamb kidneys and brains, sheep liver and lungs.

Subjective assessment

At each temperature level the softening of product surfaces and flap sections was also assessed by subjective means. These consisted of visual inspection, the application of thumb pressure and the movement of carcass flaps by hand pressure. The pressures applied were firm but not severe.

Three groups of meat products were classified from these observations (Table 2) which corresponded closely to the softness measurements. With increasing temperature the meat surfaces became plastic before they could be regarded as soft. Fat-covered areas were observed to be firmer than lean areas above about 26°F.

TABLE 2. Subjective assessments of meat softness at a constant temperature

Temp. (°F)	Lamb carcass flap sections (hand pressure)	Meat and offals Type I (thumb pressure)	Offals Type II (thumb pressure)
20			
21			hard
22			surface plasticity
23	hard		detectable
24	detectable deflection		
25	significant deflection	hard	surface plasticity significant
26	slightly soft and spongy	surface plasticity detectable	
27	quite soft		
28	soft	surface plasticity significant	soft
29		surface soft	
30		soft in depth	

Meat and Offals (Type I). Lamb carcass body sections, lamb cuts; Boneless beef and mutton; Ox spleen and tripe; Ox kidneys and lamb hearts (surface plasticity first detectable at 25°F); Lungs (soft at 28°F).

Offals (Type II). Lamb brains and kidneys, Sheep livers.

Subjective observations of meat surfaces on unloading containers at the conclusion of static and transportation tests (Haughey, 1970) were in complete agreement with the above measurements under controlled laboratory conditions. The only case in which any surface softening was observed was for a number of top-row lamb carcasses from a test continued for an elapsed time of 96 hr, where temperatures of 27–28°F were reached in the top layer. In this case a slight surface softening was evident during exposure to ambient air temperatures in the unloading period. Spongy flaps were observed in top row carcasses from a number of other runs but no flattening or distortion was observed. No surface softening was observed with cargoes of boneless beef, lamb cuts, lamb livers or lamb hearts.

Effect of temperature on meat quality

The main phenomenon likely to affect the quality of frozen meat during the storage and transportation period is the tendency towards recrystallization of the ice crystals in the muscle tissue to form larger crystals which can have quality implications. These

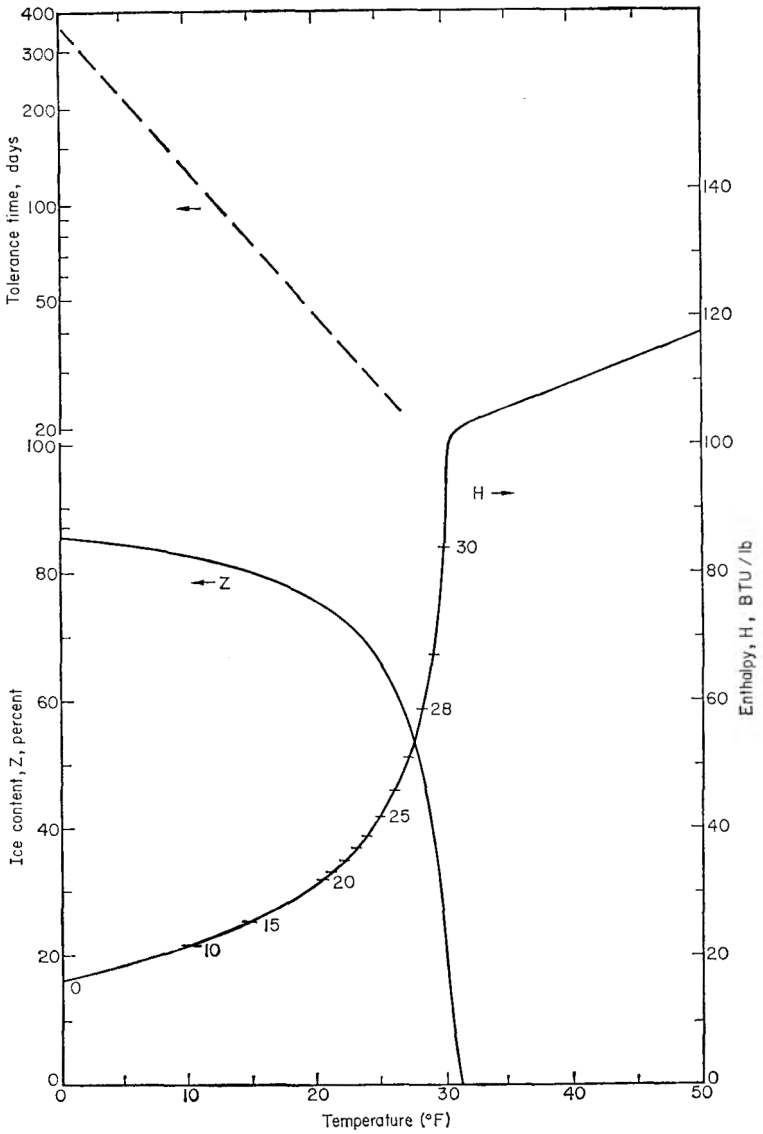

FIG. 5. Effect of temperature on the enthalpy,* ice content* and temperature–time tolerance (time for first detectable quality change) of an average lamb carcass (60% water, 22% fat, 18% protein).

* From Fleming (1969).

could include effects on surface bloom and desiccation, tissue distortion and rupture, the occurrence of significant drip on thawing, and irreversible protein denaturation due to electrolyte concentration changes in the muscle cells. Other deteriorative

changes likely to be accelerated by high transportation temperatures are microbial growth, discolouration and fat rancidity.

Fig. 5 shows the effect of temperature level on the enthalpy and per cent ice content of an average lamb carcass (Fleming, 1969). It is apparent that the major change in these properties due to latent heat effects takes place in the temperature range 25–30°F. At 25°F, 75% of the water content which can be frozen at a store temperature of 10°F is still frozen. Thus a temporary temperature rise to 25°F and subsequent reduction to 10°F of say half the meat in the top layer of lamb carcasses would result in a maximum of 25% recrystallization for 4% of the stow.

The temperature–time tolerance or rate of deteriorative changes producing the first detectable quality change (taste panel assessment) at a given storage temperature is also shown in Fig. 5 as the mean of the lines for beef and pork (International Institute of Refrigeration, 1964). It is normally found that temperature–time influences are cumulative over the entire storage life independently of the sequence so that from Fig. 5, two days at 25°F corresponds to only 2/30 or about 7% of the quality change necessary for this to be detectable.

The unfrozen water content of lamb at 25°F corresponds to a water activity of less than 0·9 at equilibrium (using the data given by Scott (1936) for beef) and microbial growth under these conditions is virtually confined to moulds.

In a number of container rail hauls under practical operating conditions without refrigeration bacteriological samples were taken from lamb carcasses in the stow at the beginning and end of the run. The initial counts (average $1{\cdot}1 \times 10^3/cm^2$) were low, possibly reflecting the difficulty of sampling frozen carcasses. The final counts (averaging $7{\cdot}5 \times 10^3/cm^2$) showed no significant increase over the initial counts although meat temperatutes up to 26°F were reached in some of the sampled carcasses. There were no significant differences in counts for different positions on the carcass, the different positions within the container or total elapsed times of 28 hr and 46 hr. Detailed results are given elsewhere (Haughey, 1970).

Conclusions

1. The laboratory measurements of meat softness and bacteriological quality have been related to the meat temperature, since by means of thermocouples the latter can be measured continuously within the container stow during tests simulating practical operating conditions. Spear temperature measurements taken while unloading a container cannot give a realistic indication of the temperature behaviour during a journey owing to the occurrence of diurnal peaks in the maximum meat temperatures and a rapid rise in meat temperatutes during unloading (Haughey, 1970). To control commercial operations it is therefore necessary to relate maximum temperature limits to a maximum permissible time without refrigeration.

2. A load-deflection penetrometer provides a suitable method for the measurement of the softness of frozen meat and gives results which correspond to subjective assessments.

3. Depth of penetration of frozen meat generally increases linearly with the applied load for the empirical instrument used. At a constant load the surface softness (or ease of penetration) shows relatively little change at temperatures below about 25°F but becomes significant above this temperature.

4. A realistic criterion for the maximum allowable temperature limit for any point within a container load of frozen meat is the onset of surface softness on the body of the meat. Flap softness is not a suitable criterion with lamb carcasses.

5. A temperature of 25°F is an appropriate limit for all meat products except offals for which 23°F is a more satisfactory limit. The experimental evidence shows that these limits contain a reasonable, though not excessive, margin of safety for the maintenance of meat quality and an adequately frozen state in insulated containers for up to a maximum of 48 hr without refrigeration.

Acknowledgments

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Influence of carcass cooling rate on drip loss in pigmeat

A. A. TAYLOR AND S. J. DANT

Summary

Pig sides were cooled to 0°C at two different rates and the amount of drip from joints after storage for two days at 0°C was estimated. Drip occurred according to an anatomical distribution pattern which was unaffected by cooling rate and breed, the greatest loss being from the loin and leg joints. The quicker cooling rate consistently limited the amount of drip, regardless of breed. This was demonstrated using whole carcasses, leg joints and individual muscles. Pigs whose pH fell rapidly after slaughter tended to give joints with high drip loss but, even with these, the effect of quicker cooling was still observed.

Introduction

It is now well understood that, if the rate of lactic acid production in the first few hours after slaughter is such as to produce a low pH in the muscles while the carcass is still warm, the consequence is a muscle tissue which is 'pale, soft and exudative' (Bendall, 1960; Bendall & Lawrie, 1964; Briskey, 1964; Callow, 1958; Hamm, 1960; Krzywicki, 1968; Lawrie, 1966; Wismer-Pedersen, 1958; Wismer-Pedersen & Briskey, 1961). Such muscle loses a high proportion of juice as drip. This abnormally high loss is explained as a result of protein denaturation, the extent of which varies with temperature, pH and time (Penny, 1969), and is by no means an all-or-none phenomenon. It therefore seems conceivable that, with less disparity between acid production and cooling rate, for example with relatively slowly cooled muscle with a normal rate of glycolysis, there might be analogous, if less extreme, changes which would be reflected by a relationship between the time/temperature treatments and the quantities of drip which are normal under such circumstances. The investigation here described was made to test this relationship.

Experimental

Materials and methods

The pigs used in most of the experiments had carcass weights in the range 40–60 Kg. Breeds used were Landrace, Large White, Wessex × Large White and Pietrain, these having a wide range of incidence of drip (Bendall, Cuthbertson & Gatherum, 1966;

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MacDougall & Disney, 1967). Pigs obtained locally were rested at least overnight before slaughter; the Pietrains were hauled 100 miles and held locally for at least a week before being brought into lairage a day before slaughter. All pigs were electrically stunned before conventional slaughter at the Institute.

Chilling

Temperatures during cooling were recorded for all carcasses in the deep leg and, in some, also in the longissimus dorsi muscle. 'Quick cooling' describes the procedure

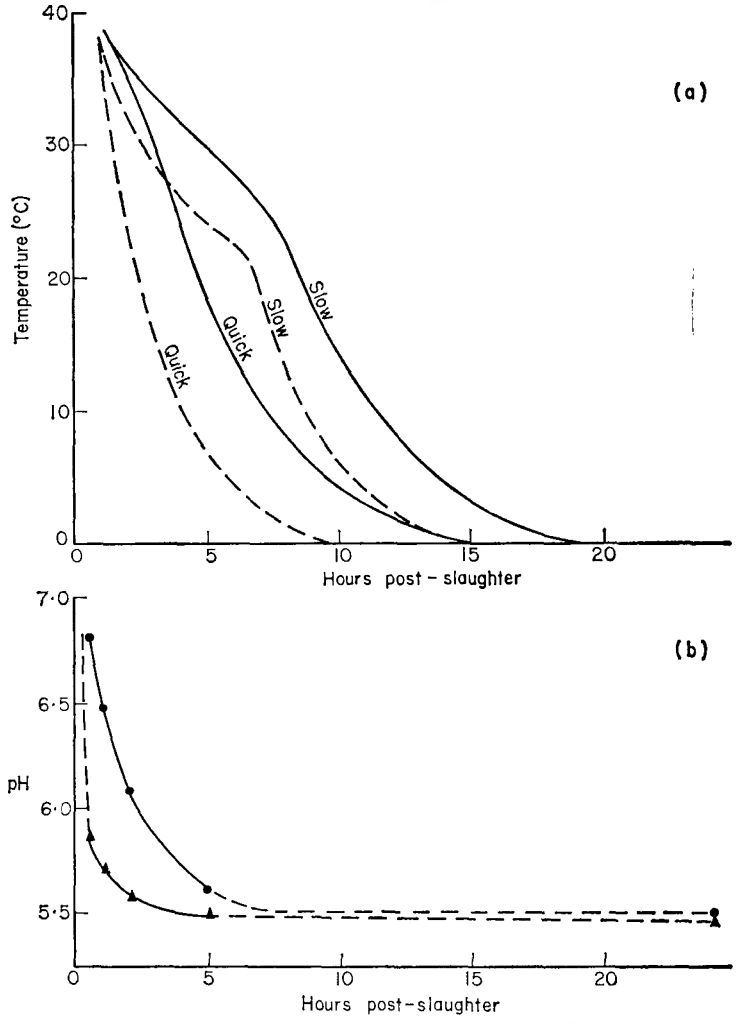

FIG. 1. (a) Temperature in deep leg and l. dorsi of sides cooled quickly and slowly. —— deep leg; - - - - l. dorsi. (b) Typical observed pH curves showing different rates of post-slaughter pH fall. ● typical normal rate (Large White); ▲ typical fast rate (Pietrain).

where the carcass was placed in a chill room at 0°C, in still air, 0·5 hr after slaughter and cooled for a total of 24 hr. In 'slow cooling' the carcass was held for 6 hr at 20°C before going into the same chill room (Fig. 1 (a)).

Where cooling rates were being compared, carcasses were split immediately after slaughter and the paired sides cooled at different rates. Differences in carcass weight were insignificant compared with the differences between rates of cooling. The longissimus dorsi cooled more quickly in a split carcass but the rate in the deep leg was the same in sides and whole carcasses. pH was measured by probe electrode in the l. dorsi at the last rib at 0·5, 1·5, 5 and 24 hr after slaughter. The post-slaughter rate of pH fall was indicated by the pH at 30 min (pH_{30}) in the way that pH at 45 min has been used in similar studies (Wismer-Pedersen, 1958).

Drip estimation

Most methods for estimating water holding capacity and drip are not easily related to practical conditions. In this study an effort was made to measure drip in a way that was more easily applied. Joints or individual muscles were weighed and placed in polythene net which supported the meat without occluding the surface. The meat was then sealed in a loosely fitting polythene bag and hung in a chill room at 0°C. The exudate which accumulated in the bag was weighed at intervals during storage.

Preparation of samples

Most of the drip comes from the cut ends of muscle fibres so that the relationship between cut surface area and volume is very important (Howard, 1956; Ramsbottom & Koonz, 1939). An experiment was carried out using similar portions of longissimus dorsi from twelve pig sides. The muscle was sectioned into slices, 2·5, 1·2 or 0·7 cm thick, with the cut surface area approximately the same in all cases. From Table 1

TABLE 1. Effect of size of piece of pig l. dorsi muscle on drip loss at 0°C. Figures are the means of twelve experiments

Slice thickness (cm)	Weight of slice (g)	Weight of drip per unit area	Weight of drip per unit weight
2·5	90·3	7·1	7·9
1·2	47·0	4·8	10·2
0·7	25·6	3·1	12·1

it can be seen that drip (as % w/w) becomes less dependent on slice thickness and weight as the thickness of slice is increased. It was decided that, in order to relate to conditions met in practice, a minimum thickness of 2·5 cm should be used when cutting to a standard pattern of thirty-one commercially recognizable joints shown in Fig. 2.

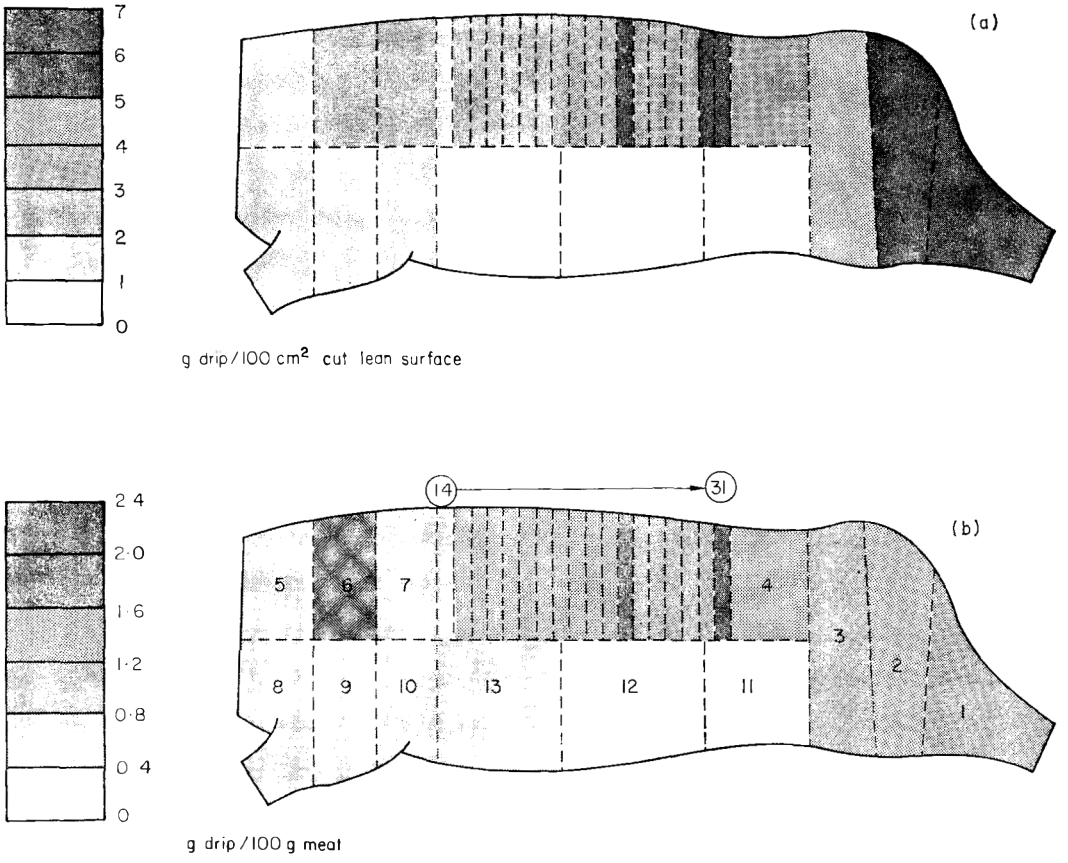

FIG. 2. Distribution of drip in pig carcasses expressed on basis of area of cut lean surface and of weight of joint. Numbers refer to standard jointing system.

Jointing was carried out 24 hr after slaughter, in the chill room at 0°C. In some experiments, the semitendinosus, semimembranosus, adductor and biceps femoris muscles were excised from the leg as quickly as possible at room temperature, 24 hr after slaughter. They were subsequently cut transversely to provide two halves with freshly cut surfaces for drip estimation.

Results and discussion

Rate of formation of drip

Samples from muscles taken from six Pietrains were stored for several days at 0°C after quick and slow cooling and the drip loss measured. Fig. 3 shows the mean rates of drip formation from the slowly cooled muscles. The pattern of loss from the quickly cooled muscles was similar although the amount of drip was less. Much of the drip was lost in the first 2 days of storage, this being consistent with consideration of water holding capacity which passes through a minimum during this period before increasing

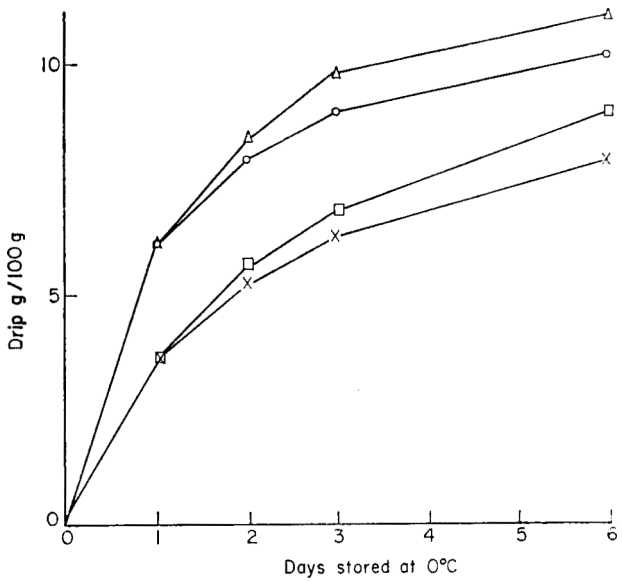

FIG. 3. Rate of formation of drip from leg muscles stored at 0°C. Values plotted are the means from six slowly cooled Pietrain carcasses. △ semimembranosus; O adductor; □ b. femoris; × semitendinosus.

as the muscle ages (Hamm, 1960). In all subsequent experiments drip loss was measured by taking the value after 2 days at 0°C.

Distribution of drip

The drip loss for the different joints taken from twenty-two pig sides showed a significant pattern of distribution (Fig. 2) which was effectively the same for the three breeds used (Large White, Landrace and Pietrain). The rates of cooling did not affect this distribution pattern and sides from the same pig cooled at different rates also gave the same pattern although the total drip was 2·1 times greater in the slowly cooled sides. Joints cut from the posterior part of the loin dripped more than those cut from the anterior, possibly reflecting, to some extent, the variation of pH along the length of the l. dorsi, a major component of the loin (Lawrie, Pomeroy & Cuthbertson, 1963). Struggling during slaughter exercises some muscles more than others and can be a contributory cause of variation in pH among the leg muscles. Moreover, the shape and size of the leg leads to parts of it having slower cooling rates than other parts of the carcass, making it particularly susceptible to protein denaturation during the cooling process.

Commercial implications of the distribution pattern can be appreciated by considering the gross loss as a percentage of joint weight rather than drip per unit area (Fig. 2). When joints were grouped (Table 2) it was seen that 84% of the drip was lost from the

TABLE 2. Loss of drip from joints after 2 days at 0°C. Figures are means of twenty-two sides; for identification of joints see Fig. 2

Joint No.	Weight (kg)	Drip	
		Weight (g)	as % Joint weight
1, 2, 3	5·608	44·9	0·80
4	1·240	13·0	1·05
5, 6, 7	3·752	15·4	0·41
8, 9, 10	3·292	7·6	0·23
11, 12, 13	3·482	2·4	0·07
14–31	5·260	73·3	1·39
Total	22·634	156·6	

commercially valuable chops, chump and leg joints which comprised only 54% of the total weight from the carcass.

Effect of cooling rate

The large variation in drip between carcasses was eliminated in cooling rate experiments by comparing the two sides from individual pigs. All pigs in this experiment were shackled by both legs during slaughter. The leg joint (No 1) was used since the deep muscle was probably the slowest cooling part of the carcass, and was easy to sample. The mean drip values for groups of ten pigs from four breeds are shown in Table 3 and show significant differences ($P < 0{\cdot}001$) in drip losses for the two cooling rates in all four groups. In thirty-eight out of forty paired legs, the drip was less after quick cooling, the mean difference being 41%.

TABLE 3. Drip loss after 2 days at 0°C from leg joints cooled at different rates. Figures for each breed are the means of ten pigs. The standard error based on analysis of variance residual variations for any one mean, slow or quick, is 0·11

Breed	Drip per 100 g	
	Slow	Quick
Landrace	0·47	0·24
Large White	0·73	0·42
Wessex × Large White	0·97	0·61
Pietrain	1·14	0·62

TABLE 4. Drip loss after 2 days at 0°C from four muscles cooled at different rates. The standard errors, based on analysis of variance residual variations for any one of the means in the first four columns were 0·49 (Pietrain); 0·33 (Large White) and for the last column 0·25 (Pietrain); 0·17 (Large White)

		Drip (as % muscle weight)				
	Cooling rate	Semi-tendinosus	Semi-membranosus	Adductor	Biceps femoris	Combined (4 muscles)
Pietrain (13 pigs)	Quick	2·82	4·40	5·52	2·69	3·86
	Slow	3·99	6·47	6·61	4·11	5·30
Large White (6 pigs)	Quick	1·69	2·01	2·92	1·04	1·92
	Slow	1·95	3·50	5·07	2·32	3·21

When the excised leg muscles from two breeds were studied (Table 4), the effect of cooling was again highly significant ($P < 0·001$) in all muscles. The semimembranosus and adductor tended to drip more than the other two muscles although the ultimate pH levels in all cases were not significantly different (between 5·56 and 5·68). However, the adductor probably lies deepest and is adjacent to the semimembranosus so that position and consequent slower cooling rate might be a contributory cause of their excessive drip.

Amount of drip

Although quicker cooling was consistently effective in reducing drip, the actual magnitude of the loss varied with different pigs. The total drip from jointed carcasses ranged from 0·14 to 1·16% carcass weight, and similar wide variation in drip loss was encountered in the cooling experiments. Penny (1969) obtained highly significant correlation between protein denaturation and drip in pigs with pH values at 90 min post-slaughter which indicated normal rates of pH fall. Only when the pH had fallen below 5·9 at 90 min was the meat visibly watery. The corresponding pH_{30} value is 6·1 and in the cooling experiment with leg joints only seven pigs out of a total of forty had pH_{30} below 6·1; higher drip losses were found in these pigs with the low pH_{30} values (Table 5). Minimum drip loss occurred when the quicker cooling rate was associated with slow rate of pH change after death. The majority of these pigs therefore appeared to have normal rates of glycolysis and at no time was there any evidence of what could be termed P.S.E. muscle after these pigs had been cooled slowly. The indication was that the difference in drip loss was caused mainly by the different cooling rates, especially during the initial post-slaughter period.

TABLE 5. Effect of pH_{30} on drip loss (data taken from the cooling experiment using forty pairs of leg joints)

Cooling rate	Mean drip (% joint weight)	
	$pH_{30} > 6{\cdot}1$	$pH_{30} < 6{\cdot}1$
Quick	0·38	0·73
Slow	0·80	0·91

The range of ultimate pH of the carcasses was not great enough in comparison with other variables to show any effect on the amount of drip from different carcasses. The narrow pH range was understandable in a situation where all the animals were well rested before slaughter and their reserves of glycogen likely to be at a normal level. The influence of ultimate pH on the overall amount of drip from a jointed carcass is not readily apparent except where there has been abnormal depletion of glycogen before slaughter (Bouton, Howard & Lawrie, 1957; Penny, 1969).

Breed

When the mean drip values of pigs used in the distribution experiment were examined (Table 6) in terms of cut surface area, for the effect of breed, Large White differed significantly from Landrace and Pietrain. When drip loss was related to weight of

TABLE 6. Effect of breed on drip from all joints (data from Fig. 2). The figures in brackets are the standard errors, based on analysis of variance residual variations, for any one of the means in the preceding column

Breed	Drip after 2 days at 0°C	
	g/100 cm^2 cut lean area	(g/100 g)
Large White	2·51	0·66
Landrace	4·12 (0·16)	1·12 (0·05)
Pietrain	4·22	1·55

joints, however, there was a significant difference between all three breeds, demonstrating the effect of the high proportion of lean in the Pietrain; the ratios of cut lean area to weight of all joints for each breed were (cm^2/g) 0·176 (Large White), 0·198 (Landrace) and 0·234 (Pietrain).

The differences between breeds were also evident in the cooling experiment with leg joints (Table 3). In this case the drip from Landrace was less than from Large White

but, since all pigs of one breed in this experiment comprised a single group from one supplier, they may not have been as representative of breed as the assorted pigs in the distribution experiment. Differences between Large White and Pietrain were observed in the experiment using individual muscles (Table 4). The very fast fall in pH in many of the Pietrains (Fig. 1(b)) was undoubtedly a major cause of the excessive drip found in pigs of this breed (Lister, 1970; MacDougall & Disney, 1967). The mean pH_{30} for Pietrains was 6·04 (range 6·80–5·60) while that for Large Whites was 6·52 (range 6·75–6·35).

To a large extent the potential for drip loss is inherent in the animal at the point of slaughter, having been decided by factors such as breed, diet and pre-slaughter handling, all of which influence post-mortem pH changes. The realization of this potential can, however, be limited by post-slaughter conditions and this investigation has shown that rate of carcass cooling has a direct bearing on drip loss. Commercial joints and individual muscles from quickly cooled pig sides consistently had lower drip losses than their counterparts which had been cooled at a slower rate.

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Effect of cooking on fatty acid composition of beef lipids

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Summary

Paired chuck, rib, round and flank cuts were taken from carcasses of 12 Angus steers (454 kg live weight). Fatty acid composition of subcutaneous, seam (intermuscular), total intramuscular, intramuscular phospholipid and intramuscular neutral lipid fractions was determined on the raw and cooked (70°C) meat. On the basis of the fatty acids analysed (C_{14}, $C_{14:1}$, C_{15}, C_{16}, $C_{16:1}$, C_{17}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:4}$) no significant differences were found between the lipids of the raw and cooked meat. In addition, the drip resulting from the cooked meat was not significantly different in its fatty acid composition from the seam, total intramuscular, or intramuscular neutral fractions.

Introduction

Currently there is concern that the occurrence of atherosclerosis in man may be related, directly or indirectly, to the amount of saturated fat in his diet (Katz, Stamler & Pick, 1958; Enselme, 1962; Stamler, 1958; Crawford, 1969). Although the evidence for this alleged relationship has been challenged (Kummerow, 1967), the high incidence of atherosclerosis has stimulated the need to study the fatty acid composition of various food products.

Previous work with various cuts of pork, beef, lamb, turkey and chicken (Chang & Watts, 1952; Terrell, *et al.*, 1968), variety meats (Siedler *et al.*, 1964), ground pork and beef (Campbell & Turkki, 1967), and freeze dried meats (Giam & Dugan, 1965) have shown that cooking causes little change in the relative fatty acid composition of either the neutral or phospholipid fractions of the extracted lipid. Phospholipids that contain a relatively high concentration of polyunsaturated fatty acids are more liable to oxidation than neutral fat (predominantly triglycerides), especially at elevated temperatures. Therefore, any changes due to oxidation would be expected to occur more readily in the phospholipid fraction.

The purpose of this study was to examine in more detail the effect of cooking on the relative change in fatty acid composition of beef lipid from different muscles and

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different fat depots within a carcass. This would then make it possible to determine if the unsaturated/saturated fatty acid ratio was altered by cooking by either moist or dry methods, as a consequence, for example, of a reduction in the content of poly-unsaturated fatty acids.

Materials and methods

Twelve choice grade Angus steers of similar genetic and nutritional background were slaughtered at a live weight of approximately 454 kg. The carcasses were graded U.S.D.A. choice and had an average cuttability score of 3·9 according to U.S.D.A. specifications (1965).

Four pairs of cuts were taken from each carcass three to seven days post mortem. They included cuts from (1) a 10 cm thick chuck arm; (2) a rib which included the 9th and 10th ribs cut at a length of 15 cm from the ventral edge of the vertebral column; (3) a bottom round cut cranial to the patella and proceeding forward approximately 35 cm; and (4) a flank in which the regular flank steak was tightly rolled starting with the caudal edge. All subcutaneous fat was left intact in the chuck, rib and round. A 3–4 mm layer of intermuscular fat was left on both the inner and outer surfaces of the flank.

Cooking procedure

The cuts from the right side of each carcass were analysed raw, and the paired cuts from the left side were cooked to an internal temperature of 70°C in a 150°C oven. Thermocouple electrodes placed at the centre of each cut were used to monitor the temperature. The roasting pans were equipped with false bottoms to separate the cut from the drippings. The chuck arm was placed in the roasting pan with the ventral surface up, the rib was placed with the cranial surface up, and the round was placed with the subcutaneous fat down. The flank and chuck were cooked with moist heat (150 ml water added to the flank and 400 ml to the chuck) and the rib and round cuts with dry heat. The volume and weight of the drip was measured for each cooked meat and the shrink was determined from the weight of the cooked meat before and after cooking.

Sample preparation

All cuts were dissected into subcutaneous fat, seam fat and muscle. Each component was finely ground, frozen in liquid nitrogen and powdered in a Waring blender. The powdered samples and the drip portion from the cooked meat were stored in poly-ethylene bottles at −20°C until extracted.

A total lipid extract was attained by extracting approximately 35 g of muscle and 10 g of each fat component with 2 : 1 v/v chloroform-methanol as described by Ostrander & Dugan (1961). The volume of the lipid-containing chloroform layer was determined and duplicate aliquots were then evaporated in aluminum pans and weighed to determine the percent lipid in each sample.

A portion of the muscle extract was further separated into a neutral fraction and a phospholipid fraction by the silicic acid procedure of Choudhury & Arnold (1960) as modified by Terrell *et al.*, (1968).

All lipid fractions were transesterified by a modification of the procedure of Sink *et al.*, (1964). Approximately 50–100 mg of triglyceride, 1 ml benzene, 3 g Na_2SO_4 and 10 ml of 2% H_2SO_4/methanol reagent were added to a 15 ml test tube. The tube was capped tightly, mixed on a vortex, and placed in a 60°C water bath for $2\frac{1}{2}$ hr. The tube was cooled to room temperature followed by the addition of 3 ml of redistilled pentane and 2 ml of distilled water. The solution was mixed to allow the methyl esters to be taken up by the pentane layer. The tube was then centrifuged for 2 min at 1000 × gravity. The pentane layer was then pipetted into a glass sample vial, covered with nitrogen, sealed tightly and stored at −20°C until chromatographed. Immediately preceding chromatography, the pentane was evaporated from the sample.

Chromatography

A Beckman GC-2 gas chromatograph with a flame ionization detector was used to characterize and quantify the methyl esters. The column was a 6 ft × $\frac{1}{4}$ in (outside diameter) copper tubing packed with 15% stabilized diethylene glycol succinate on acid washed 80/100 mesh chromosorb w. The column was operated isothermally at 205°C using nitrogen as a carrier with a flow rate of 120 ml/min.

The peaks obtained were identified by comparison with a standard mixture of pure methyl esters. The standard was of known composition and was combined in approximately the same proportions as that found in beef lipids. Equal quantities of standard and unknown methyl ester were used. The weight of a given unknown methyl ester (m.e.) was computed from the peak height by the following formula:

$$\text{weight m.e.} = \frac{(\text{peak height, m.e.})\ (\text{weight, std. m.e.}).}{(\text{peak height, std. m.e.})}$$

The weights of all unknown methyl esters were calculated for each sample and from this data the relative weight percent of each methyl ester was computed (Baumgardt, 1964).

Results and discussion

Physical composition of cuts

The composition of the raw and cooked meat is shown in Table 1 and the composition of the drip is given in Table 2. The rib had the highest percentage of subcutaneous fat and the lowest muscle to fat ratio, whereas the round had the highest muscle to fat ratio. In the round, the subcutaneous and seam fat were similar in amount; however, in the chuck the proportion of seam fat to subcutaneous was about 3 to 1. This is one demonstration of the non-proportionality of fat disposition that exists in the beef carcass.

TABLE 1. Physical composition of raw and cooked meat[a]

	Chuck		Rib		Round		Flank	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Weight (kg)	4·99	3·52	2·88	1·99	4·90	3·90	0·83	0·62
% subcutaneous fat	7·4	7·8	15·8	13·9	9·7	11·3	0·0[c]	0·0[c]
% seam fat	19·0	24·0	22·2	23·1	10·6	12·2	26·0	30·4
% muscle	66·7	59·0	52·4	51·5	79·1	75·9	74·0	69·6
% bone and cartilage	6·9	9·6	9·7	11·5	0·6	0·6	0·0	0·0
% loss in weight due to cooking[b]	28·5		21·5		23·4		28·2	

[a] Each value is the mean of 12 cuts.
[b] Calculated from the weight of the cooked meat before and after cooking.
[c] Due to its anatomical location, fat covering over the flank was considered as seam fat.

Examination of the drip composition in Table 2 shows that the rib drip, unlike the other cuts, was made up largely of lipid. This high concentration of lipid in the rib drip was probably the result of the large percentage of total fat in this cut as compared to the other cuts. Table 1 indicates that the majority of this lipid originated from the rib subcutaneous fat because of the decrease that was found in the relative percent of this depot after cooking.

TABLE 2. Physical composition of drip from cooked meat[a]

	Chuck	Rib	Round	Flank
Water added before cooking (ml)	400	0	0	150
Total drip volume[c] (ml)	978	385	530	146
% lipid[b]	18·8	75·5	32·7	42·8
% water[b]	76·9	24·5	66·0	57·2
% solids[b]	4·3	0·0	1·3	0·0

[a] Each value is the mean of 12 cuts.
[b] Expressed as a volume percent of the total.
[c] Includes water added before cooking.

Quantitative yields of lipid from muscle

The quantitative yield of lipid material from the muscle component of each cut is given in Table 3. The yield is expressed as a percentage of the total weight of the muscle. As anticipated, there is a definite increase in lipid concentration in the cooked meat. This apparent increase is probably due to loss of muscle weight by dehydration and loss of water soluble proteins during cooking.

Calculations using the lipid concentration before and after cooking and the muscle

weight before and after cooking suggest that there is essentially no loss of lipid from the muscle during cooking. These observations are similar to the results of Sartorius & Child (1938) and Lowe (1955).

TABLE 3. Amount of lipid extracted from muscle component[a,b]

	Muscle	
Cut	Raw	Cooked
Chuck	6·3 ± 0·5	10·3 ± 0·6
Rib	10·8 ± 1·0	14·8 ± 1·0
Round	5·6 ± 0·4	8·9 ± 0·6
Flank	10·4 ± 1·0	14·6 ± 1·2

[a] Expressed as a percent of the total muscle weight.

[b] Values are the mean of 12 cuts ± standard error of the mean.

Fatty acid composition of the raw and cooked lipid fractions

The fatty acid composition of the six lipid fractions of the raw and cooked chuck, rib, round and flank are shown in Tables 4, 5, 6 and 7, respectively. The eleven major fatty acids were used in the statistical analysis. C_{10} and C_{12}, and trace amounts of other unidentified fatty acids were detected but not used. $C_{14:1}$ and C_{15} were reported as a single value. The ratios of the total saturated, mono-unsaturated, polyunsaturated and unsaturated acids to saturated acids were calculated from the fatty acid means.

The fatty acid values for subcutaneous fat, seam fat and neutral intramuscular fat are similar to those previously reported (Hornstein, Crowe & Heimburg, 1961; Hornstein, Crowe & Hiner, 1967; O'Keefe *et al.*, 1968; and Terrell *et al.*, 1967). The intramuscular phospholipid values, however, were higher in saturated and lower in polyunsaturated acids than reported in previous studies (Hornstein *et al.*, 1961; O'Keefe *et al.*, 1968). This may be due to the fact that the phospholipid fraction contained trace quantities of peaks with longer retention times than $C_{20:4}$. These peaks, which made up approximately 5% of the total fatty acids in the phospholipid fraction, were probably longer chain polyunsaturated acids. These unknown peaks were not included in the calculation of fatty acid composition in Tables 4, 5, 6 and 7.

Fatty acid comparison between raw and cooked meat

The fatty acids for each raw and cooked lipid fraction were compared statistically by the paired '*t*' test. Significant differences between raw and cooked were found in only a small number of fatty acids. Since these differences followed no general pattern they were probably due to random biological variation. Therefore, we concluded that

cooking had no effect on the relative fatty acid composition of any of the beef lipids analysed.

Additional comparisons between fractions

Although differences in the percentage of saturated, mono-saturated and polyunsaturated acids between lipid fractions were not compared statistically, there were a number of apparent differences that merit mentioning. In all cuts, the seam and intramuscular neutral fractions are highest in percentage of saturated acids. In addition, the subcutaneous lipid is always highest in percentage of mono-unsaturated acids, while the intramuscular phospholipid fraction of all cuts, as expected, is lowest in saturated and mono-unsaturated acids and highest in polyunsaturated acids.

Fatty acid composition of the drip as compared to other cooked fractions

The fatty acid composition of the drip component of each cut was compared with the fatty acid composition of the other components by Duncan's multiple range test (Steel & Torrie, 1960). The purpose of this analysis was to determine indirectly which fat depot may have contributed most to the lipid portion of the drip.

In all cases the fatty acid composition of the drip was intermediate to that of the subcutaneous and seam fat. Table 8 shows the unsaturated to saturated ratios of all the lipid fractions of the cooked meat. Comparing the drip to the other lipid fractions shows that the drip was not significantly different in composition from the seam, total intramuscular or intramuscular neutral fractions. The composition of the drip was, as anticipated, significantly different from that of the intramuscular phospholipid fraction and, with the exception of the rib, was significantly different from the subcutaneous fat.

Even though the fatty acid data would indicate that the drip portion of the cooked meat originates primarily from the seam fat and that the subcutaneous lipids are less likely to be found in the drip portion, the results of the physical separation of the cuts (Table 1) indicate that the subcutaneous fat is depleted as much or more than the seam fat and would logically contribute as much lipid to the drip as the seam fat. Therefore, it is difficult to conclude that comparisons of fatty acid analyses alone, are appropriate means to identify the origin of lipids in the drip portion.

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TABLE 4. Fatty acid composition of raw and cooked *chuck* arm cuts[a]

	Fatty acid[b]													
	14	14 : 1–15[c]	16	16 : 1	17	18	18 : 1	18 : 2	18 : 3	20 : 4	% sat.[d]	% mono-enes.[d]	% poly unsat.[d]	unsat./ sat.
Subcutaneous														
Raw	3·8	2·9	27·0	6·6	1·1	11·8	44·2	1·6	1·1	0·0	44·7	52·7	2·7	1·26
Cooked	4·0	3·0	26·9	6·6	1·1	11·3	44·2	1·8*	1·1	0·0	44·3	52·8	2·9	1·26
s_D[e]	0·15	0·12	0·28	0·30	0·07	0·73	0·54	0·06	0·10	0·00				0·04
Seam														
Raw	3·8	1·8	27·7	4·1	1·1	18·2	40·8	1·8	1·0	0·0	51·4	46·1	2·8	0·96
Cooked	3·6	1·7	27·6	4·0	1·1	18·8	40·4	1·8	1·2	0·0	51·7	45·5	3·0	0·94
s_D	0·11	0·05	0·26	0·09	0·06	0·58	0·49	0·09	0·16	0·00				0·02
Intramuscular Total														
Raw	2·8	1·5	26·4	4·6	1·1	14·2	44·7	3·2	1·0	0·6	45·0	50·3	4·8	1·23
Cooked	3·1*	1·6	26·8	4·6	1·0	14·6	43·4	3·1	1·1	0·5	46·2	49·1	4·7	1·17
s_D	0·12	0·06	0·52	0·14	0·07	0·46	0·67	0·13	0·06	0·10				0·04
Intramuscular Neutral														
Raw	3·1	1·6	28·3	4·5	1·2	15·6	42·3	2·0	1·0	0·2	48·7	47·8	3·2	1·06
Cooked	3·3*	1·9	28·4	4·4	1·1	15·8	42·0	2·0	1·1	0·1	49·2	47·5	3·2	1·04
s_D	0·10	0·14	0·36	0·10	0·11	0·47	0·54	0·10	0·08	0·11				0·03
Intramuscular Phospholipid														
Raw	3·7	2·7	23·0	3·2	0·5	13·1	27·9	16·2	2·2	7·6	41·2	32·9	26·0	1·43
Cooked	2·8	2·7	23·2	3·4	0·5	13·6	29·6	14·4	2·0	7·7	41·0	34·8	24·1	1·44
s_D	0·46	0·35	1·18	0·24	0·06	0·53	2·09	1·91	0·22	1·68				0·08
Drip														
Cooked	3·5	1·8	26·6	4·7	1·2	16·7	42·6	1·8	1·1	0·0	48·6	48·5	2·9	1·05

TABLE 5. Fatty acid composition of raw and cooked 9th-10th *rib* cuts[a]

	Fatty acid[b]													
	14	14 : 1–15[c]	16	16 : 1	17	18	18 : 1	18 : 2	18 : 3	20 : 4	% sat.[d]	% mono-enes.[d]	% poly-unsat.[d]	unsat./ sat.
Subcutaneous														
Raw	3·8	2·6	27·5	5·3	1·2	12·8	43·8	1·9	1·1	0·0	46·2	50·9	3·0	1·16
Cooked	3·6	2·3	27·9	5·3	1·1	13·7	43·3	1·6	1·0	0·0	47·1	50·2	2·6	1·13
s_D[e]	0·15	0·13	0·29	0·20	0·17	0·42	0·61	0·14	0·07	0·00				0·03
Seam														
Raw	3·8	1·8	28·7	4·0	1·1	17·9	40·0	1·7	1·0	0·0	52·1	45·2	2·7	0·92
Cooked	3·8	1·8	28·2	4·5	1·1	16·5	41·3*	1·6	1·1	0·0	50·2	47·4	2·7	0·99†
s_D	0·17	0·05	0·34	0·38	0·06	0·69	0·46	0·10	0·06	0·00				0·02
Intramuscular Total														
Raw	3·3	1·5	27·9	4·1	1·0	15·8	42·0	2·4	1·5	0·4	48·5	47·3	4·3	1·06
Cooked	3·4	1·5	28·0	4·2	1·0	16·1	42·1	2·4	1·0	0·2	49·1	47·5	3·6	1·04
s_D	0·05	0·08	0·21	0·07	0·03	0·35	0·53	0·10	0·49	0·14				0·02
Intramuscular Neutral														
Raw	3·2	1·6	29·3	4·2	1·2	16·6	40·8	1·9	0·9	0·0	50·8	46·1	2·8	0·99
Cooked	3·3	1·6	29·1	4·0*	0·9	16·8	41·2	1·8	0·9	0·0	50·6	46·2	2·8	0·99
s_D	0·10	0·04	0·54	0·07	0·13	0·33	0·87	0·09	0·05	0·02				0·03
Intramuscular Phospholipid														
Raw	3·4	3·1	24·7	3·5	0·4	13·7	31·4	12·8	1·8	6·4	43·2	36·9	21·0	1·36
Cooked	3·2	3·3	24·7	3·3	0·4	12·8	29·9	13·9	2·3†	6·3	42·2	35·3	22·5	1·39
s_D	0·62	1·04	0·51	0·18	0·10	0·54	1·41	0·78	0·09	0·49				0·05
Drip														
Cooked	3·7	2·0	27·6	4·7	1·1	16·0	42·1	1·8	1·1	0·0	49·1	48·2	2·9	1·03

TABLE 6. Fatty acid composition of raw and cooked bottom *round* cuts[a]

	Fatty acid[b]													
	14	14 : 1–15[c]	16	16 : 1	17	18	18 : 1	18 : 2	18 : 3	20 : 4	% sat.[d]	% mono-enes.[d]	% poly-unsat.[d]	unsat./ sat.
Subcutaneous														
Raw	4·0	3·2	27·0	7·6	1·0	9·5	44·8	1·6	1·1	0·0	42·6	54·5	2·7	1·36
Cooked	3·8	3·3	27·6	7·4	0·9	9·8	44·4	1·9	1·0	0·0	43·2	53·9	2·9	1·33
s_D[e]	0·14	0·14	0·85	0·50	0·06	1·12	0·75	0·12	0·14	0·01				0·05
Seam														
Raw	3·9	2·0	27·6	4·5	1·0	15·9	41·9	1·8	1·2	0·0	49·1	47·8	3·0	1·03
Cooked	4·0	2·0	27·6	4·6	1·0	16·1	41·8	1·7*	1·2	0·0	49·4	47·8	2·9	1·03
s_D	0·11	0·11	0·43	0·17	0·04	0·27	0·50	0·07	0·06	0·00				0·03
Intramuscular Total														
Raw	3·2	1·7	26·9	4·9	0·9	12·9	44·4	3·2	1·1	0·6	44·5	50·5	4·9	1·25
Cooked	3·2	1·8	26·8	5·0	0·9	13·3	44·4	3·2	0·9†	0·5	44·8	50·6	4·6	1·23
s_D	0·09	0·06	0·30	0·19	0·05	0·25	0·42	0·24	0·06	0·08				0·02
Intramuscular Neutral														
Raw	3·2	1·9	30·0	5·1	1·2	13·6	42·2	1·8	0·9	0·0	48·6	48·5	2·7	1·08
Cooked	3·2	2·0	29·4	5·0	1·2	14·0	42·2	1·8	1·0	0·0	48·5	48·6	2·9	1·09
s_D	0·08	0·10	0·64	0·22	0·08	0·31	0·42	0·11	0·09	0·02				0·02
Intramuscular Phospholipid														
Raw	2·9	2·8	23·6	3·1	0·4	12·7	26·6	15·3	3·0	10·4	40·5	31·6	28·7	1·49
Cooked	1·7*	2·1	24·3	3·3	0·5	13·4	26·1	16·1	2·2	10·2	40·7	30·6	28·8	1·48
s_D	0·47	0·62	0·75	0·23	0·18	0·72	1·04	0·75	0·47	·51				0·05
Drip														
Cooked	3·7	2·2	27·6	5·3	1·1	14·5	42·6	1·8	1·2	0·0	47·6	49·3	3·0	1·09

TABLE 7. Fatty acid composition of raw and cooked rolled *flank* cuts[a]

	Fatty acid[b]													
	14	14 : 1–15[c]	16	16 : 1	17	18	18 : 1	18 : 2	18 : 3	20 : 4	% sat.[d]	% mono-enes[d]	% poly-unsat.[d]	unsat./ sat.
Seam[f]														
Raw	3·8	1·7	29·1	4·3	1·1	17·3	40·2	1·6	0·9	0·0	51·9	45·7	2·5	0·93
Cooked	3·7	1·7	29·5	4·3	1·0	16·8	40·5	1·6	0·9	0·0	51·6	46·0	2·5	0·94
s_D[e]	0·22	0·04	0·35	0·10	0·13	0·55	0·39	0·12	0·11	0·00				0·02
Intramuscular Total														
Raw	3·6	1·6	28·5	4·3	0·9	14·9	42·4	2·4	0·9	0·3	48·4	47·7	3·6	1·06
Cooked	3·7	1·6	28·3	4·2	1·0	15·1	42·8	2·5	0·8	0·2	48·6	48·0	3·5	1·06
s_D	0·10	0·05	0·18	0·16	0·06	0·24	0·62	0·06	0·08	0·07				0·02
Intramuscular Neutral														
Raw	3·5	1·8	32·1	4·3	1·1	15·3	39·4	1·5	0·8	0·0	52·6	45·0	2·3	0·92
Cooked	3·4	1·9	30·8	4·5	1·0	15·4	40·2	1·7	0·9	0·0	51·4	45·8	2·6	0·96
s_D	0·22	0·12	1·23	0·18	0·10	0·54	0·88	0·13	0·09	0·02				0·04
Intramuscular Phospholipid														
Raw	2·9	1·6	24·2	3·2	0·4	13·8	29·8	14·1	2·0	7·9	42·3	33·8	23·9	1·41
Cooked	2·9	2·3	24·2	3·0	0·4	13·4	29·2	15·0	2·1	7·4	41·6	33·6	24·5	1·43
s_D	0·55	0·87	0·95	0·22	0·09	0·83	1·26	0·92	0·13	0·85				0·06
Drip														
Cooked	3·8	1·6	28·6	4·1	1·1	17·3	40·6	1·6	0·9	0·0	51·3	45·7	2·5	0·94

[a] Each value is the mean of 12 cuts expressed as relative weight percent of total fatty acids measured.

[b] Number of carbons : number of double bonds.

[c] Approximate proportion of 2 to 1 ($C_{14:1}$ to C_{15}).

[d] Calculated from fatty acids means; differences were not analysed statistically.

[e] Standard deviation of the differences.

[f] Due to anatomical location, the fat covering the flank was considered to be seam fat rather than subcutaneous fat.

* Significant ($P < 0·05$).

† Highly significant ($P < 0·01$).

TABLE 8. Unsaturated/saturated acid ratio[a] of cooked meat[b]

	Subcutaneous	Seam	Intramuscular total	Intramuscular neutral	Intramuscular phospholipid	Drip
Chuck	1·27^{x}	0·95^{z}	1·18^{xy}	1·05^{yz}	1·45	1·06^{yz}
Rib	1·14^{x}	1·00^{xy}	1·05^{xy}	0·97^{y}	1·41	1·04^{xy}
Round	1·34^{xy}	1·03^{w}	1·24^{yz}	1·09^{zw}	1·48^{x}	1·10^{zw}
Flank	—	0·94^{x}	1·07^{x}	0·96^{x}	1·43	0·95^{x}

[a] (Percent mono-unsat. + percent polyunsat.)/percent saturated.

[b] Each value is the mean of twelve cuts. For each cut, values with the same superscript are not significantly different ($P < 0·05$).

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The bacteriology of 'scampi' (*Nephrops Norvegicus*).

II. Detailed investigation of the bacterial flora of freshly caught samples

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Summary

Forty-nine samples of freshly landed *Nephrops norvegicus*, from thirteen ports of landing, were examined bacteriologically. Total viable counts ranged from $3{\cdot}55 \times 10^3$/g—$2{\cdot}25 \times 10^6$/g at 20°C and 3×10^1/g—$2{\cdot}73 \times 10^6$/g at 37°C. Coryneform organisms were predominant in the bacterial flora with strains of the *Achromobacter–Acinetobacter* group and the *Pseudomonas, Cytophaga* and *Micrococcus* genera also present.

Introduction

The importance of the *Nephrops* fishery in the UK has already been pointed out (Walker, Cann & Shewan, 1970). As well as a good home market for 'scampi', there is now an appreciable export market, particularly to North America. As with many other foods, quality standards, particularly bacteriological, are being introduced especially where international trade is concerned. In addition, many processors are using, or would like to use, bacteriological standards as a quality control measure in their own factories.

The significance of specific bacteriological standards is debatable; the whole question of bacteriological standards for fish and fishery products was discussed recently by Shewan (1970) and Hobbs (1970). Nevertheless, it is clear that such standards are being increasingly applied and, in order to arrive at realistic and practical standards it is essential that there is a thorough understanding of the numbers and types of bacteria present on the raw material, and the effects of processing on these bacteria.

Preliminary investigations (Walker, Cann & Shewan, 1970) suggest that the initial flora and subsequent spoilage flora of *Nephrops* may well be somewhat different to that of teleost fish from the same area. It is generally accepted (Shewan & Hobbs, 1967) that, when alive and healthy, the flesh of all fish is sterile. The microbial flora develop-

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ing *post mortem* arises primarily from that present on the outer surfaces and in the intestinal tract of the live animal. Shewan & Hobbs (1967) in reviewing the available literature, also concluded that the natural flora of marine fish reflects, to a large extent, that of its environment and feed. Colwell & Liston (1962), however, expressed the view that there is a characteristic bacterial flora consistently associated with a variety of marine invertebrate animals.

Most *Nephrops* fishing grounds are far enough from shore to escape pollution from sewage effluent. It would be reasonable, therefore, to expect that the bacterial flora of freshly caught samples would reflect that of the immediate marine environment. Since *Nephrops* lives primarily on or in marine sediments, this could explain such differences in flora as might exist from that of free swimming fish. Little data are available on sediments collected on fishing grounds around the coast of the United Kingdom. However, total aerobic bacterial counts in marine sediments are generally of the order of 10^4–10^6/g (Zobell, 1938), although counts as high as 10^8/g (Kriss, 1963) and 10^9/g (Wood, 1965) have been reported. Strict anaerobes do not comprise a numerically significant proportion of sediments collected in the North Sea (Davies, 1967). Lloyd (1931) described the predominant types of bacteria in sediments from off the Clyde coast as aerobic, gram-negative asporogenous rods of the *Achromobacter* and *Chromobacterium* genera, and aerobic gram-positive spore-forming rods of the genus *Bacillus*. Bonde (1968) reported that the flora of off-shore sediments in Denmark contained a high proportion of aerobic, gram-negative asporogenous rods of the *Pseudomonas-Aeromonas-Vibrio* group along with a significant proportion of *Bacillus* spp and coliforms. These samples, however, were taken from an area subject to pollution and fresh water run off.

The bacteria usually found in sea water belong chiefly to the genera *Pseudomonas, Achromobacter, Micrococcus, Flavobacterium* and *Cytophaga* and to the coryneform group (Kriss, 1963; Scholes & Shewan, 1964; Wood, 1965).

Some data are available on the bacteria present on freshly caught Crustacea from various parts of the world. Thus Green (1949 a, b), Fieger (1950) and Fieger, Bailey & Novak (1958) published total viable counts at 20°C of 10^4–10^7/g for freshly landed Gulf shrimps and Williams, Rees and Campbell (1952) reported that, while the bacterial load carried by whole Gulf shrimp is variable, heading of the crustacean reduced the total viable count by 50–60%. The main groups of bacteria in whole Gulf shrimps are *Achromobacter, Micrococcus, Pseudomonas* and *Bacillus* (Williams, Campbell & Rees, 1952); in headed Gulf shrimps *Micrococcus, Achromobacter* and *Pseudomonas* are again common but, in this case, along with *Flavobacterium* (Campbell & Williams, 1952); while the intestinal flora comprises *Bacterium, Achromobacter* and *Micrococcus* in equal frequency (Williams & Rees, 1952).

Similar total viable counts have been reported for prawns in India (Sreenivasan, 1959) with a predominance of *Micrococcus* and *Corynebacterium* spp while Shaikhmahmud & Magar (1956) stated that the main groups are *Achromobacter* and *Micrococcus* along

with lesser numbers of *Bacillus, Bacterium, Flavobacterium, Sarcina, Pseudomonas, Serratia, Kurthia* and *Aerobacter*. In both cases, however, only a few isolates were identified.

Harris (1932) stated that the predominant types of bacteria in crab were gram-negative asporogenous rods and gram-positive streptococci, with smaller numbers of chromagenic bacteria of the genera *Pseudomonas, Flavobacterium, Micrococcus* and *Sarcina*. Alford, Tobin & McCleskey (1942) reported that, in Louisiana crab, *Micrococcus* sp predominated over smaller numbers of *Pseudomonas* and *Achromobacter* spp. Early (1967) found that, in freshly killed crabs, the white muscle contained *Achromobacter* (68%), *Micrococcus* (23%) and coryneforms (8%); the hepatopancreas had a similar flora with the addition of small numbers of *Pseudomonas* and coliforms.

The flora of fish in waters of the United Kingdom comprises mainly *Pseudomonas, Achromobacter, Micrococcus* and coryneform bacteria (Shewan & Hobbs, 1967). The taxonomy of bacteria found on fish and in the marine environment is in a very indefinite state and it is certainly not feasible to attach species names to the bacteria isolated. Shewan, Hobbs & Hodgkiss (1960) attempted to rationalize this situation for bacteria from fish and fishery products and proposed an identification scheme for the different genera. Modifications and additions to this scheme were subsequently made by Hendrie, Hodgkiss & Shewan (1964) and Hendrie & Shewan (1968) but the problem of resolving these groups of bacteria into species still remains. However, using the schemes which are available, this paper presents a more detailed investigation of the flora of freshly caught scampi from most of the commercial UK fisheries.

Materials and methods

Samples of *Nephrops norvegicus* were collected in 3–6 kg quantities depending on availability at the ports of landing. During the return journey to the laboratory they were chilled in an insulated box containing ice, sealed in polythene bags to avoid contamination with bacteria from the ice. The delay in examination of the samples from the time of collection was usually between 2–24 hr. Where a port of landing would have meant a delay greater than this, the samples were examined immediately at the port in a mobile laboratory.

Bacteriological analysis

One hundred grams of the cleaned flesh were aseptically homogenized for 2–4 min in 300 ml of chilled sterile 0·1% w/v peptone in M/15 phosphate buffer, pH 7·0. This was placed at 4°C for 30 min to allow particulate matter to settle and air bubbles to disperse. Decimal dilutions of the homogenate were then prepared using the same diluent.

Total viable counts

Total viable, aerobic counts were made on agar media using the modified Miles & Misra (1938) technique of Appleman, Bain & Shewan (1964). Because little is known

of the types of bacteria present in the flesh of scampi three media were initially employed, These were Oxoid CM 55 agar, a peptone rich medium; Oxoid plate count agar (PCA), a moderately rich medium and Oxoid CM 3 agar, a medium suitable for bacteria of non-exacting nutritional requirements. The last named medium was used as there is evidence that certain of the *Cytophaga-Flavobacterium* group of bacteria fail to grow on media of high peptone content (Mitchell, pers. comm.). Duplicate inoculated plates were incubated for 3 days at 37°C and 5 days at 20°C.

Organisms of public health significance

(a) Coliforms, Escherichia coli and faecal streptococci

These were enumerated by the use of the 'most probable number technique' and confirmed as described in the Bacteriological Examination of Water Supplies (Ministry of Health, 1956).

(b) Salmonella

Two hundred millilitres of the homogenate were added to 200 ml of double strength selenite broth and incubated for 72 hr at 37°C. After 24 and 72 hr incubation, subcultures were made on desoxycholate citrate agar and Brilliant Green agar, and these incubated for a further 48 hr at 37°C. Colonies developing on these two media and giving reactions typical of Salmonella were further subcultured, purified, and identified using the standard biochemical and serological tests for this genus.

(c) Staphylococcus aureus

A viable count for S. aureus was made by pipetting 0·1 ml quantities of dilutions of the homogenate directly on to Baird-Parker egg yolk medium (Baird-Parker, 1962) and incubating for 48 hr at 37°C. An enrichment culture was also made by inoculating 2·0 ml of the homogenate into 10% salt meat broth and incubating for 48 hr at 37°C; this was followed by subculture on Baird-Parker agar. Colonies with the typical appearance of S. aureus were further purified and identified by standard bacteriological and biochemical tests.

(d) Clostridium welchii

Cl. welchii was detected and counted using the method previously reported (Hobbs *et al.*, 1965).

(e) Clostridium botulinum

Hundred-gram samples of flesh were vacuum packed and examined for the presence of Cl. botulinum, using the method described by Cann *et al.* (1965).

Bacterial flora

Flora analyses were made by selection of colonies from the total viable counts on CM 3 agar plates incubated at 20°C. For each analysis one hundred colonies were picked off using Random Number Tables (Lindley & Miller 1962), purified and identified to the genus level using the schemes of Hendrie *et al.* (1964) and Hendrie *et al.* (1968).

Results

Samples of *Nephrops* were examined from landings at thirteen ports in Britain, twelve of which are shown in Table 1; the thirteenth was Dunbar where no flora analysis was made. Each port was visited at least once, a total of forty-nine samples being examined, of which thirty-three were uniced and sixteen iced before landing.

Bacterial counts

Comparison of thirty-seven total viable counts on the three media tested showed the CM 3 medium generally gave higher counts at both temperatures of incubation (30 cases at 20°C and 22 cases at 37°C); the differences found, however, were always less than one order of magnitude. Growth on CM 3 was slower than on the PCA and CM 55 media but pigment production of colonies was greater. Examination of the types of bacterial colonies developing on the three media showed no significant differences. Consequently, CM 3 medium was taken as the medium of choice for further work.

Total viable counts at 20°C ranged from $3{\cdot}55 \times 10^3$/g—$2{\cdot}25 \times 10^6$/g (Fig. 1). In only two instances did the count reach 10^6/g and one of these was a sample from a trawler which had been fishing principally for white fish and the *Nephrops* were known to have been stored in ice for 6 days. No significant differences in counts were noted at different times of the year, with different sizes of *Nephrops* or with port of landing. Uniced *Nephrops* carried a lower bacterial load than iced: only seven of the thirty-three former gave counts in the 10^5 range whereas nine of the sixteen latter did so.

Total viable counts at 37°C showed a similar pattern to those at 20°C (Fig. 1) but at a lower level. The counts ranged from 3×10^1/g—$2{\cdot}73 \times 10^6$/g. Again, only two counts reached the 10^6 range and the highest count apart from these was $7{\cdot}56 \times 10^4$/g. Thirty-three samples gave counts less than 10^4/g. No significant differences in counts were found from *Nephrops* of different sizes, from different ports, at different times of the year or, in this case, whether they were iced or not.

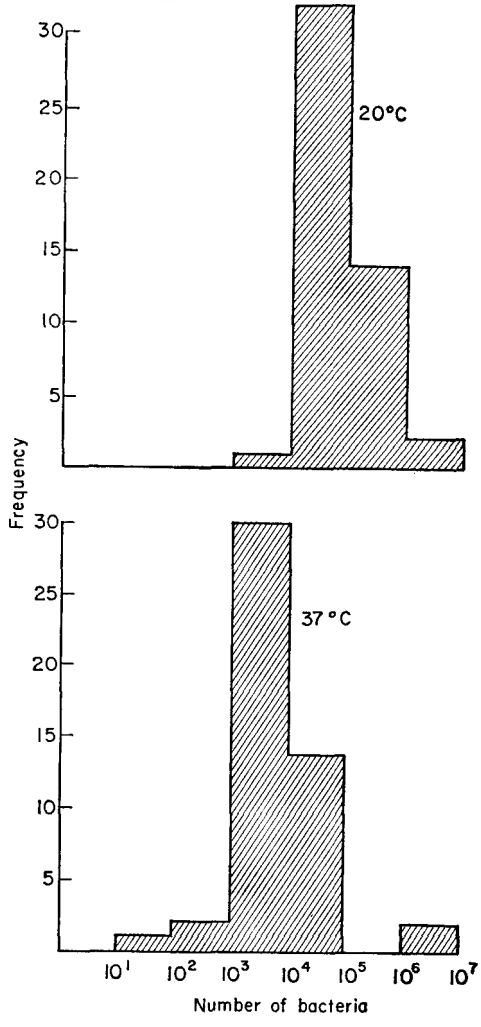
Organisms of public health significance

Coliforms, faecal coli and faecal streptococci were only detected in low numbers. Coliform organisms were present in all twelve of the samples tested, in numbers ranging from 12–750/100 g. Faecal coli were found in only six samples, in numbers ranging from 6–105/100 g. Faecal streptococci were found in seven samples in numbers ranging from

TABLE 1. Bacterial flora of *Nephrops* (%)

Port of landing	Fishing ground	Iced/ Uniced	*Pseudomonas* I	II	III	IV	*Aeromonas*	*Vibrio*	*Achromobacter*	*Acinetobacter*	*Enterobacter*	*Micrococcus*	*Staphylococcus* *aureus*	others	Coryneform	*Bacillus*	*Cytophaga-Flavobacterium*	Yeast	Unclassified	No Growth*
Lossiemouth	Westby Bank	Uniced		2					16			6	1	10	58	1	6			
Lossiemouth	Hummel Bank	Uniced							10			20	1	1	43	1	10			14
Lossiemouth	Hummel Bank	Uniced		2	7	4			13	5		22			37		7	1		2
Lossiemouth	Hummel Bank	Uniced			14	6	4		18	2		19			24		8		1	4
Buckie		Uniced	8	3	12	2	1		24	2		2		2	26		17			1
Buckie	Skate Hole	Iced	1	1	4	9		2	10			3	1	2	59		8			
Buckie	Skate Hole	Iced		3	48	13	1		24			2		1	3		5			
Peterhead	35 ml E by N off Peterhead	Iced	2	2		3			12	3		3		2	59		14			
Eyemouth	off Eyemouth	Uniced		3			1		15						60		20		1	
Eyemouth		Uniced		9	7	2	1		12	8	3	10		2	30	3	13			
North Shields		Uniced							26	10	2	13		19	23		7			
Ullapool	off Ullapool	Uniced							5	2	2	5			81		5			
Mallaig	off Mallaig	Uniced		3	2	7			16			8		4	50	2	8			
Oban	off Oban	Uniced	11	2	6				10	1		7			45		18			
Ayr		Uniced										5		93		1		1		
Ayr	Ailsacraig/ Largs Channel	Uniced			3	2			6	8		5			71		4		1	
Whitehaven	12 ml of St Bees	Uniced			1	2			21	2		14			51		6			3
Whitehaven	7 ml SE of St John's Point	Uniced	57	5	3	7	1			3		5			4		15			
Fleetwood	off Barrow	Uniced		5	2	3	1		6	1	14	4			33		16			15
Fleetwood	off Barrow	Uniced			2	1	1		44			3		1	42		6			
Fleetwood	off Barrow	Uniced	1		6	8			19	5		8			37		16			
Fleetwood	Minch	Iced	3	9	10	1			11	1					41		23	1		
Milford Haven	Minch	Iced		7	1	10			5	58		3			13		3			

* selected primary isolates non-viable on subculture



6–140/100 g. Of the food poisoning organisms tested for, only S. aureus was present in countable numbers and then in only two samples where counts of 40 and 80/g were found. The organism was detected by enrichment in a further four samples. Cl. welchii was detected only by enrichment in five samples. Salmonella and Cl. botulinum were not found.

Bacterial flora

The bacterial flora of samples from twelve ports are presented in Table 1. Coryneform organisms were the predominant group. In eight of twenty-three samples they comprised over 50% of the flora. Next in predominance were organisms of the *Achromobacter-Acinetobacter* group followed by organisms of the *Pseudomonas*, *Cytophaga* and *Micrococcus* genera. There was little variation between the iced and the uniced samples.

Discussion

It is clear from Fig. 1 that the total viable counts of bacteria on freshly landed *Nephrops* are generally from 10^3–10^5/g at 37°C and from 10^4–10^6/g at 20°C. It seems likely that much of the variation found is due to differences in procedures on board ship and in times from catching to sampling. These figures are similar to previously published counts on freshly caught crustacea. Assuming the flesh of the live *Nephrops* to be sterile, the relatively high counts probably reflect the high numbers of bacteria on their outer surfaces and the difficulties of removing adhering mud before removal of the head, claws and thorax, and exposure of the flesh to contamination. Unless the *Nephrops* can be landed live, however, beheading at sea is essential in order to prevent the detrimental autolytic changes resulting from the activities of enzymes, particularly those present in the digestive gland.

The flora analyses confirm the preliminary findings of Walker, Cann & Shewan, (1970). The predominant bacteria belong to the coryneform group with appreciable numbers of *Achromobacter* and *Acinetobacter* strains, and smaller numbers of *Pseudomonas*, *Micrococcus*, *Flavobacterium* and *Cytophaga* spp. Whilst this flora is similar to that found in marine fish generally, there is a higher proportion of coryneform organisms than found in teleost fish from the same areas (Liston, 1955; Georgala, 1958; de Silva, 1960; Shewan & Hobbs, 1967).

The total counts at 20°C on iced *Nephrops* were generally higher than on uniced samples. This is almost certainly a reflection of the fact that ice is generally not used if the *Nephrops* are landed within 24 hr of catching and that iced *Nephrops* were at least one, if not several days old before sampling.

As might be expected for a marine animal caught in unpolluted waters, organisms of public health significance were found only in low numbers. Any increase in these groups of bacteria during processing, therefore, reflects either poor hygiene or storage at too high a temperature. These groups of bacteria, for instance, do not grow at ice temperatures. The effects of processing, including ice-storage and freezing, will be discussed in Part 3 of this work.

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Some laboratory experiments on various meat preparation surfaces with regard to surface contamination and cleaning

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Summary

Laboratory experiments have been carried out to determine the bacterial plate counts on various meat preparation surfaces both before and after cleaning. The surfaces tested included wood, five proprietary cutting boards and formica. Experiments on both unused and scored surfaces showed that plate counts from wood were always greater than those from all the other boards tested indicating that the latter can be cleaned more efficiently.

Tests were made to determine the incidence of salmonellae on wooden surfaces in frequent contact with raw meat. Ten (4·3%) of 235 samples of wood scrapings were found to contain salmonellae. These results confirm that wooden surfaces can be reservoirs and distributors of salmonellae.

Introduction

Wood has been used for many centuries as a cutting surface for meat. The nature of its surface allows meat to be held firmly during preparation, and it is sufficiently hard to have a reasonable life without being so hard as to blunt the cutting edge of knives. Wood is also readily available and relatively inexpensive. However, it is notoriously difficult to clean because it absorbs blood, fat and moisture, and it also expands and contracts when washed and allowed to dry out. As a result, small particles of meat can become trapped in knife marks in the wood and may remain there indefinitely, thereby providing a reservoir for bacterial growth and cross-contamination in the processing factory, shop and kitchen. Wooden boards subjected to total immersion in hot water containing certain alkaline detergents are particularly prone to expansion and contraction and have a limited life before disintegration (Cooper & Dyett, 1967).

In recent years several new cutting surfaces have been developed, and their use in this country has become increasingly popular. The new proprietary boards or pads are usually made of (i) synthetic and/or natural rubber hardened with plastic fillers,

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(ii) high molecular weight, medium density polyethylene, or (iii) phenolic fibre laminates. They are advertised as having all the advantages of wood without its disadvantages. It is claimed that they are non-absorbent, will not crack, bend or warp, and that they are much easier to clean.

Part I of this paper describes some experiments to determine the approximate number of bacteria remaining on various meat preparation surfaces after contact with meat and after some simple cleaning procedures. All tests were carried out in the laboratory and not in the field.

Salmonella infection continues to be the most prevalent type of food poisoning in this country and it is well known that many salmonella serotypes are found regularly in raw foods of animal origin, especially meat and poultry. Because of this, it seems reasonable to assume that cross-contamination must inevitably occur in factories, shops and kitchens. It seemed worthwhile, therefore, to determine the incidence of salmonellae on wooden surfaces, especially those in frequent use as in butchers' shops. Part II of this paper describes the results of such a survey.

Part I
Experiments with cutting boards

The minced beef used in the experiments was obtained from local butchers. Samples (25 g) were incubated in 100 ml volumes of selenite F broth (Leifson, 1936), modified by replacement of lactose with mannitol and sterilization by Seitz filtration, and tetrathionate broth (medium A of Rolfe, 1946) both broths incubated at 37°C to detect the presence of salmonellae. Subcultures on bismuth sulphite agar (Oxoid) and deoxycholate-citrate lactose agar (Hynes, 1942) modified by the addition of 1% sucrose were made after 24 and 72 hr and the plates incubated for 48 hr at 37°C. The presence of salmonellae was confirmed by biochemical and serological tests.

The types of cutting boards used in this study were all unused samples, of area 35–36 sq. in., of wood (A), five proprietary boards (B–F) and formica (G). The latter was included in some of the tests for comparative reasons only and because it is widely used as a general working surface in the domestic kitchen.

The boards were washed in warm water containing an anionic detergent, thoroughly rinsed, wiped with individual paper towels and dried in an incubator at 35°C for 1 hr. They were then left uncovered on the laboratory bench for 15 min prior to the start of each experiment.

Samples of minced beef (50 g) were weighed out and placed in the centre of each board. The meat was spread over the surface of the board using a measuring cylinder, and pressed down using a sterile spatula. After 30 min the meat was removed and the excess scraped off with another spatula. The entire surface of each board was then swabbed using four calcium alginate swabs (Higgins, 1950). The first two swabs were moistened with a wetting solution containing sodium chloride (0·85%), peptone

(0·1%) and Tween-80 (0·1%) in distilled water (Mossel *et al.*, 1966) rubbed over the board and broken off into a bottle containing 9 ml of quarter-strength Ringer's solution. The other two swabs were used to remove excess moisture and meat, and were broken off into another bottle of quarter-strength Ringer's solution giving two bottles/four swabs. One ml of a 10% solution of sodium hexametaphosphate was added to each bottle, and the bottles shaken for a few minutes to dissolve the swabs. Ten-fold dilutions were made in quarter-strength Ringer's solution, and 0·5 ml volumes of appropriate dilutions spread out on the surface of duplicate blood agar plates. Plates were incubated at 30°C for 48 hr. Counts/test surface were obtained by adding the counts obtained from both pairs of swabs.

Bacterial plate counts were made in the same way after two simple cleaning procedures. In the first, the boards were immersed for 5 min in warm water(45–50°C) containing an anionic detergent: no attempt was made to scrub them. The boards were then rinsed in warm water, dried with paper towels and swabbed as described above. In the second, the boards were immersed for 5 min in warm water containing the same detergent and scrubbed for 30 sec with a plastic brush, and then rinsed and dried before swabbing.

All the above tests were made on unused, i.e. unmarked, boards. Experiments were then made with boards which had been deliberately marked in the laboratory. The surfaces of the boards were scored with a freshly sharpened butchery knife in both the horizontal and vertical directions. Each board was heavily scored for 15 min. One board (G) soon showed signs of fragmentation of the surface and was no longer usable. The soiling and recovery tests, which have been described, were repeated using the marked boards, but the cleaning procedures were different. In all six tests the boards were immersed and scrubbed in warm water containing an anionic detergent as before, but on two occasions the boards were also immersed in hypochlorite solution (200 ppm available chlorine) for 10 min prior to rinsing and drying. On these two occasions the diluent for the swabs contained 0·5% sodium thiosulphate to inactivate any carry-over of chlorine.

Results

A salmonella was detected in one of the ten samples of minced beef used; the serotype isolated was *Salmonella derby*. The significance of a single isolation from such a small number of samples is doubtful, but it did confirm that salmonellae are not infrequently found in raw meat.

Table 1 shows the plate counts obtained from swabbed surfaces of 'unmarked' cutting boards after contact with meat and after cleaning. After contact with meat, the counts from each board were fairly similar. However, there was a considerable variation in counts obtained after the two cleaning procedures. In tests 1 and 2, the

counts obtained from the wooden board were much greater than those from all the other boards. Also, the counts from one board (F) were greater than those from the other boards (C–E) probably because of the 'cloth-finish' nature of its surface. The effect of scrubbing the boards for 30 sec is clearly shown in tests 3 and 4. Low counts

TABLE 1. Bacteriological plate counts from 'unmarked' cutting boards before and after cleaning

Test	Cutting board*	Cleaning procedure	Bacterial plate counts/35–36 sq. in. at 30°C	
			After contact with meat	After cleaning
1	A	Immersion of boards in detergent solution	11×10^6	150,000
	C		3×10^6	50
	D		15×10^6	30
	E		9×10^6	90
	F		82×10^6	5,600
	G		5×10^6	150
2	A		16×10^6	60,000
	C		5×10^6	360
	D		10×10^6	260
	E		9×10^6	260
	F		30×10^6	7,000
	G		7×10^6	290
3	A	Immersion and scrubbing of boards in detergent solution	10×10^6	400
	B		2×10^6	30
	C		3×10^6	50
	D		6×10^6	50
	E		2×10^6	50
	F		7×10^6	60
	G		2×10^6	40
4	A		1×10^8	410
	B		2×10^8	80
	C		2×10^8	30
	D		3×10^8	40
	E		3×10^8	40
	F		3×10^8	90
	G		2×10^8	20

* A = wood
B–F = proprietary cutting boards
G = formica

TABLE 2. Bacteriological plate counts from 'marked' cutting boards before and after cleaning

Test	Cutting board*	Cleaning procedure	Bacterial plate counts/35–36 sq. in. at 30°C	
			After contact with meat	After cleaning
1	A	Immersion and scrubbing of boards in detergent solution	45×10^6	9,200
	B		5×10^6	240
	C		7×10^6	720
	D		7×10^6	580
	E		14×10^6	940
	F		11×10^6	200
2	A		18×10^6	6,500
	B		7×10^6	580
	C		30×10^6	680
	D		22×10^6	540
	E		11×10^6	870
	F		20×10^6	430
3	A		27×10^6	4,500
	B		4×10^6	90
	C		11×10^6	380
	D		7×10^6	480
	E		4×10^6	420
	F		7×10^6	100
4	A		11×10^6	12,500
	B		4×10^6	200
	C		2×10^6	430
	D		1×10^6	340
	E		4×10^6	350
	F		6×10^6	250
5	A	Immersion and scrubbing of boards in detergent solution, followed by immersion in hypochlorite+ solution, and rinsing	22×10^6	8,500
	B		10×10^6	20
	C		17×10^6	90
	D		27×10^6	310
	E		10×10^6	100
	F		16×10^6	330
6	A		72×10^6	6,100
	B		9×10^6	< 20
	C		22×10^6	70
	D		28×10^6	130
	E		21×10^6	< 20
	F		22×10^6	50

* A = wood
B–F = proprietary cutting boards
\+ = 200 ppm available chlorine

were obtained from all the boards, but even so the counts from wood were still four- to six-fold greater than those from all the other boards.

Table 2 shows the plate counts from swabbed surfaces of 'marked' cutting boards after contact with meat and after cleaning. In tests 1–6, the counts obtained from the wooden board were again greater than those obtained from all the other boards. In tests 5 and 6, the cleaning procedure included a 10-min immersion of the boards in hypochlorite solution (200 ppm available chlorine). The counts obtained in these tests were a little lower than in tests 1–4, with the exception of the wooden board for which high counts were still obtained. These results confirm our previous findings (Gilbert & Maurer, 1968; Gilbert, 1970) that in a cleaning procedure the use of a disinfectant is not of the first importance. Bacteria are removed from a surface more effectively by the combination of detergent activity and the physical action of scrubbing or washing: nevertheless, the use of a suitable disinfectant provides a second line of defence.

Part II
Isolation of salmonellae from wooden working surfaces

Scrapings (1–4 g) from wooden working surfaces in butchers' shops and other premises were obtained over the period March–December 1969 by arrangement with the Public Health Authorities of three London Boroughs. Samples were incubated in 15–20 ml volumes of selenite F broth (see Part I) at 37°C. Subcultures on bismuth sulphite agar (Oxoid) and deoxycholate-citrate lactose agar (Hynes, 1942) modified by the addition of 1% sucrose were made after 24 and 72 hr and the plates incubated for 48 hr at 37°C. The presence of salmonellae was confirmed by biochemical and serological tests.

TABLE 3. Salmonella serotypes and their frequency of isolation from scrapings from wooden working surfaces

Scrapings from	No. of samples examined	Positive for salmonellae No.	%	Serotype	
Butchers' shops	211	9*	4·3	*S.* 4, 12:d:—	(3)
				S. anatum	(3)
				S. dublin	(2)
				S. typhimurium	(1)
Restaurants, hotels and catering services	24	1+	4·1	*S.* 4,12:d:—	(1)
Total	235	10	4·3		

* = Wood scrapings from different butchers' shops
\+ = Wood scrapings from a Chinese restaurant

Results

Two hundred and thirty-five samples of wood scrapings were examined. Ten samples (4·3%) were found to contain salmonellae (Table 3): four samples yielded the unnamed salmonella with the antigenic structure 4,12:d:–, three samples *S. anatum*, two samples *S. dublin* and one sample *S. typhimurium*. Nine (4·3%) of the 211 samples from butchers' shops and one (4·1%) of the twenty-four samples from restaurants, hotels and catering services yielded salmonellae.

Discussion

An attempt was made to compare the various types of modern cutting board with the traditional wooden board with regard to ease and efficacy of cleaning. The results from Part I of this paper show that, after various cleaning procedures, plate counts from the modern types of cutting boards were less than those obtained from the wooden board indicating that they can be cleaned more effectively. Wood is difficult to clean, that is, it is difficult to ensure that the surface is relatively free from bacterial contamination. It should be noted that all the modern materials used were in the form of cutting boards and not chopping blocks: they are not recommended where cleavers, saws or axes are employed. Practical advice on the cleaning of wooden chopping blocks has been published elsewhere (Food Hygiene Code of Practice, No. 8, 1969).

It is uncertain whether results from laboratory tests on 36 sq. in. samples of cutting boards can be related to the much larger surface areas used in meat processing factories and shops, but they are unlikely to differ significantly. There is little doubt that the modern materials for meat preparation provide surfaces which are more easily cleaned than the traditional wooden cutting boards. The modern boards can be cleaned by immersion and scrubbing in hot water containing detergent which gives good results bacteriologically. The boards, as supplied commercially, may be detached from their supporting frames and lifted out for cleaning. Also they are reversible and when badly marked some of them can be resurfaced using sandpaper. Cooper & Dyett (1967) reported that under heavy working conditions in a meat processing factory synthetic rubber boards have lasted for at least six years, indicating that they are durable. The knife marks made in 15 min on each of the modern boards treated in the laboratory were barely visible, even though the sharp knife had been applied with considerable pressure. In contrast, the marks on the wooden board were easily seen. Using the agar-sausage technique (Ten Cate, 1963, 1965), Cooper & Dyett (1967) have also shown that the modern cutting board can be cleaned more easily than wood.

The results in Part II were not unexpected. The salmonella serotypes isolated are commonly associated with food poisoning and they have all been found in meat or poultry or both (Vernon, 1969, 1970). The importance of cross-contamination of food from other foods by means of equipment, surfaces and the hands of food handlers has

been stressed on many occasions (Hobbs, 1961; Mossel *et al.*, 1966; Gilbert, 1969; Hobbs & Gilbert, 1970; Pether & Gilbert, in preparation). The results presented confirm that wooden surfaces can be reservoirs and distributors of salmonellae. The isolation rates of salmonellae from the wood scrapings might have been greater had the samples examined been larger than 1–4 g. Because the samples were so small it was not possible to compare the use of Selenite F and tetrathionate broths as enrichment media or 37° and 43°C as enrichment growth temperatures.

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A semi-quantitative procedure for detecting Clostridium welchii in foodstuffs

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Summary

The role of *Clostridium welchii* in food poisoning is well documented. A procedure for detecting these organisms is described and this consisted of (a) making inoculations or sample dilution into cooked meat medium, (b) subculturing to sulphite agar, (c) transferring black colonies to Nagler medium, and (d) Gram-staining growth from positive reactions. All media contained polymyxin and were incubated at 44°C. Experiments showed that the method would detect a mean of approximately 0·8 *Cl. welchii* organisms on one of three occasions and approximately eight organisms on at least two of three occasions. This was more sensitive, although slower than with similar experiments using litmus milk.

Introduction

In recent years there has been a rapidly growing popularity of dried, frozen or semi-preserved 'convenience' foods. Many of these foods contain meat, meat products, raw spices or other ingredients which may have a substantial indigenous microbiological flora. In this situation correct microbiological control becomes a factor of particular importance, not least in the need to restrict numbers of food poisoning bacteria. The recognition of *Cl. welchii* as a potential food poisoning agent is comparatively recent (McClung, 1945; Hobbs *et al.*, 1953), although there have since been many documented cases of enteritis believed to be caused by these organisms. During investigations of food poisoning outbreaks, meat with a history of storage at warm temperatures, slow cooling or lengthy warming up procedures has often been shown to contain large numbers of *Cl. welchii* (Hobbs, 1965). Dehydrated soups and sauces were investigated by Nakamura & Kelly (1968) who found that ten of fifty-five samples contained *Cl. welchii*, and the authors emphasized that the method of handling such foods after rehydration was important in human health. McKillop (1959) showed that *Cl. welchii* could be isolated from the floor and window ledges of a hospital kitchen, and postulated that contamination of cooked fowls with kitchen dust was a probable

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explanation for the presence of *Cl. welchii* in the foodstuff in a particular series of outbreaks.

In earlier publications, the organism incriminated in cases of food poisoning was a heat-resistant, non-haemolytic variant of *Cl. welchii* type 'A' which produced only trace amounts of α-toxin (the 'typical food poisoning strain'). Later, β-haemolytic 'classical' strains of *Cl. welchii* type 'A' were found to cause the illness in some cases (McKillop, 1959; Hall *et al.*, 1962; Taylor & Coetzee, 1966; Sutton & Hobbs, 1968). Whilst it is true that large numbers of organisms are necessary to cause the onset of clinical symptoms, the importance of small numbers of *Cl. welchii* type 'A' in certain dried or preserved foods, such as soup-mixes, natural spices or curry preparations, has also been recognized (Mossel *et al.*, 1956; Sutton & Hobbs, 1969; Nakamura & Kelly, 1968).

Many methods have been published for detecting the ubiquitous clostridia organisms (see, for example, the book by Thatcher & Clark, 1968). Efforts to count *Cl. welchii*, a species of importance in food because of food poisoning hazards, are, however, sometimes limited to the identification of colonies from 'pour plates' or 'shake cultures'. This procedure can be very laborious if many colonies are picked and, as with any direct culture on solid media, the minimum number of bacteria detected is influenced by the dilution factor of the sample.

A method which attempts to combine selectivity with an estimate of the degree of contamination was described in a pamphlet issued by the Ministry of Health (1956). Here, a measured quantity of water sample is added to litmus milk and the mixture pasteurized, incubated and examined for the 'stormy clot' reaction, this indicating *Cl. welchii*. The disadvantages of this technique, particularly if samples other than water are used, are that some so-called 'heat sensitive' *Cl. welchii* strains might be destroyed, the medium is non-inhibitary to contaminating heat resistant organisms, not all *Cl. welchii* produce this reaction and that other clostridia (e.g. *Cl. tertium*) can give 'stormy clot' reactions.

Some workers are content to assess the hygienic quality of food by estimating the numbers of 'sulphite positive' clostridia present. This semi-selective character was described by Wilson & Blair (1924) when they found that colonies of some clostridia and certain coliforms would blacken on media containing sodium sulphite, many of the latter being inhibited if the incubation temperature was raised to 44°C, which is within the optimum temperature range for *Cl. welchii*. The sulphite blackening reaction was used by other workers when estimating the numbers of clostridia in foods using solid media (Mossel *et al.*, 1956) or broths (Gibbs & Freame, 1965) and rendered more selective by the addition of antibiotics (Mossel, 1959; Angelotti *et al.*, 1962) and incubation above 37°C (Marshall, Steenbergen & McClung, 1965).

In the food manufacturing industry, it is often useful to be able to detect *Cl. welchii*, even when the organisms are only present in relatively small numbers and are mixed

with various other species of bacteria. This type of investigation is particularly valuable in indicating bad food processing, poor raw materials and contamination from other sources. It appeared from a survey of the current literature that there was a need for a simple but effective method for detecting *Cl. welchii* in an industrial environment. The present study was, therefore, initiated to try and find a suitable combination of enrichment, selective and identification procedures for this purpose.

Experimental

Test samples

The samples used for evaluating the various procedures consisted of naturally contaminated soil, dust and foods including raw meat, meat extract, animal fat, gravy stock and natural spices.

Basic procedure

The basic procedure used for detecting *Cl. welchii* in these samples was as follows:

(i) Serial ten-fold dilutions of the samples were made in quarter-strength Ringer solution, warming or blending as necessary. A minimum of two dilutions was always used to avoid any bacteriostatic effect with high concentrations of the samples.

(ii) Aliquots of each dilution were pipetted into screwcapped bottles containing cooked meat medium (Oxoid) plus 70 units per ml of polymyxin B. sulphate ('Aerosporin', Burroughs Wellcome & Co.). The medium had first been heated at 100°C for 15 min to drive off oxygen. This step was taken not so much to assist the growth of clostridia but to restrict most contaminating aerobic organisms.

(iii) All bottles were incubated in air at 37 or 44°C for 18–24 hr.

(iv) Using a wire loop, sub-cultures were made from the broths onto third segments of sulphite agar (Wilson & Blair, 1924) plus polymyxin, as above, in petri dishes.

(v) Plates were incubated in anaerobic jars at 37 or 44°C for 18–24 hr.

(vi) If any black colonies were present, at least two from each sulphite agar segment were sub-cultured across a plate containing Nagler's medium (Oxoid Blood Agar Base No. 2, 100 ml, plus Oxoid concentrated egg yolk emulsion, 5 ml, added to the molten, cooled base before pouring. Polymyxin was also added, as before. When set, half of the agar surface was spread with gas-gangrene antitoxin (Burroughs Wellcome & Co.).

(vii) Plates were incubated as (v).

(viii) Growth exhibiting the Nagler reaction (opaque halo around colonies which is neutralized on the medium spread with antitoxin) was Gram-stained and examined for the presence of presumptive *Cl. welchii* (a stout, square-ended, Gram-positive bacillus without visible spores; *Cl. bifermentans*, another Nagler positive species, exhibits numerous spores).

(ix) The results were expressed as: *Cl. welchii* present or absent in 1 g, 0·1 g or in whatever was the weight of the original inoculum. Alternatively, if a sufficient range of sample dilutions are used, appropriate tables can be consulted to estimate the most probable number of organisms in the sample.

Effect of incubation temperature

Tests were performed in parallel, one being incubated at 37°C and the other at 44°C, to see if additional selectivity occurred at the higher temperature.

Effect of omitting sulphite agar

Whilst samples were being examined by the basic procedure, cooked meat broths were additionally sub-cultured directly onto Nagler's medium to determine whether or not the sulphite agar stage could be omitted.

Sensitivity of the basic procedure compared with litmus milk

To a solution of sterilized paprika spice was added varying numbers (as determined by 'plate' counts) of *Cl. welchii*. The strain used had originally been isolated from the untreated spice. To some mixtures were also added cultures of *Staphylococcus aureus* (NCTC 4163) *Proteus vulgaris* (NCTC 4635) and an aerobic sporing bacillus (a laboratory isolate) These infected mixtures were tested both by the basic procedure and also by adding aliquots to litmus milk, previously boiled for 15 min. All incubation was at 44°C and the litmus milks were examined each day for the 'stormy clot' reaction.

Results and discussion

Effect of incubation temperature

From the results shown in Table 1 using the complete sulphite agar procedure, it will be seen that on some occasions *Cl. welchii* was recovered at 44°C but not at 37°C. The opposite situation was not observed. These results would suggest that the higher incubation temperature might be of greater value than the lower when using this procedure.

Effect of omitting sulphite agar

Table 1 also shows that there was a slightly greater recovery of the organisms when the complete procedure was used, compared with results from cooked meat broths subcultured directly to Nagler's medium. There would, however, be a case for omitting the sulphite agar stage to give a provisional result in two days instead of three, when time saving is important.

Sensitivity of the basic procedure compared with litmus milk

Table 2 shows that the broth–sulphite–Nagler method would detect a mean of

TABLE 1. Growth of presumptive *Clostridium welchii* on Nagler medium inoculated from either cooked meat broth or sulphite agar, cultures incubated at 37 or 44°C

Sample	37°C				44°C			
	Broth		Sulphite agar		Broth		Sulphite agar	
	1 g	0·1 g	1 g	0·1 g	1 g	0·1 g	1 g	0·1 g
Raw pork	−	−	−	−	+	−	+	+
Meat extract 'A'	+	−	+	−	+	−	++	−
Chicken fat	−	−	+	−	−	+	+	+
Meat extract 'B'	+	+	±	−	−	−	+	−
Natural spice 'A'	+	−	+	−	++	−	+	−
Raw beef	+	−	+	−	+	+	++	++
Natural spice 'B'	−	−	−	−	+	++	++	++
Meat gravy	−	−	−	−	+	−	−	−
Soil	−	−	++	+	+	+	++	++
Meat extract 'C'	−	−	+	+	−	−	++	−
Natural spice 'C'	+	−	++	−	+	−	++	−
Raw chicken meat	−	−	+	−	−	−	+	−
Floor dust*	+	−	+	−	+	−	+	−

Key: ++ Presumptive *Cl. welchii*, pure growth; + Presumtive *Cl. welchii*, mixed growth; ± Positive Nagler, typical cells not seen in stained film; − Negative Nagler; * Result per 1 and 0·1 sq. in. floor.

approximately 0·8 *Cl. welchii* on one of three occasions and approximately eight of these organisms on at least two of three occasions. The 'stormy clot' reaction was not produced in litmus milk when the lower inoculum was used but developed when approximately eight *Cl. welchii* were inoculated into the medium. Single results varied during repetition of this experiment but the overall finding that the described procedure could detect very small numbers of *Cl. welchii* was confirmed. It is interesting to note that the presence of comparatively large numbers of aerobic bacteria with either procedure did not significantly alter the results. The 'stormy clot' reactions were very quick to develop and, although suffering from certain disadvantages mentioned in this paper, the litmus milk test might still be useful if used in conjunction with a more reliable procedure.

Identification procedures

Various final-stage identification tests were considered for this study but the Nagler reaction (Hayward, 1943) was chosen for its simplicity and specificity—a 'positive' result indicating either *Cl. welchii* or *Cl. bifermentans*. This reaction depends on the

TABLE 2. Growth of *Clostridium welchii* on Nagler medium or in litmus milk at 44°C, following inoculation with pure or mixed cultures of this organism

Bacteria inoculated		Culture media					
Type	Number (mean)	Sulphite/Nagler			Litmus milk		
Cl. welchii	0·8	−	−	++	−	−	−
Cl. welchii	0·8	++	−	−	−	−	−
Aerobes*	2823						
Cl. welchii	8	++	−	++	++ (1)	++ (1)	++ (1)
Cl. welchii	8	++	++	++	+	+	+
Aerobes*	2823				(1)	(1)	(1)
Cl. welchii	80	++	++	++	++ (1)	++ (1)	++ (1)
Cl. welchii	80	+	++	++	++	++	++
Aerobes*	2823				(1)	(1)	(1)

Key: Sulphite/Nagler symbols: see Table 1; Litmus milk symbols: ++ Stormy clot, pure growth *Cl. welchii*; + Stormy clot, mixed growth *Cl. welchii*; − No stormy clot. Number of days to develop reaction shown in parenthesis. * Aerobes comprise: *Staph. aureus* = 1730 cells; *Pr. vulgaris* = 106 cells; *Bacillus* = 33 cells.

production of the enzyme lecithinase C by the organisms, which causes an opaque halo to appear around the colonies when egg emulsion or plasma is present in the medium. The reaction is inhibited by specific antitoxin. Hobbs *et al.* (1953) found that all but two of many hundreds of *Cl welchii* isolates gave typical Nagler reactions, and in 1965 Hobbs reported that these organisms rarely formed spores in routine cultures, or in cooked foods, and could be recognized in stained preparations. *Cl. bifermantans* readily sporulates in culture and is thus unlikely to be confused with the former organism.

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A direct titrimetric method for the rapid estimation of sulphur dioxide in fresh pork sausage

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Summary

A method for the rapid estimation of sulphur dioxide in fresh pork sausage has been developed. A good correlation was obtained between Shipton's modification of Monier-Williams' reference method and the recommended procedure based on Potter's titration method for dehydrated cabbages. The method is applied to a sausage macerate which is twice titrated with iodine. Hydrogen peroxide is used as a specific oxidizing agent for the sulphur dioxide.

Introduction

The Preservatives in Food Regulations 1962 (S.I 1962 No. 1532) permit the presence of up to 450 ppm sulphur dioxide in fresh sausage. In practice it is usually incorporated in the mix as metabisulphite, but for the purpose of the regulations the preservative is calculated as the total sulphur dioxide (w/w). As the amount of preservative present falls rapidly, the determination in sausages has to be carried out in the factory as soon as possible after production. Conventionally the total sulphur dioxide is determined volumetrically after distillation with acid and trapping the gas produced in an oxidizing agent. For reasons of convenience and rapidity it would be useful if a method were available in which the determination is carried out on an extract or macerate without recourse to distillation.

Procedures based on that described by Monier-Williams (1927) are normally used for reference purposes. These involve reflux distillation of the sample in an inert atmosphere with acid and trapping the sulphur dioxide produced in hydrogen peroxide in which it is oxidized to sulphuric acid. The amount of acid produced is then titrated with alkali. If necessary, the result can be confirmed gravimetrically by precipitation as barium sulphate. The method has been modernized by Thrasher (1961, 1966), and Shipton (1954) in particular also paid special attention to shortening the time required for the determination. For more rapid control purposes, however, many workers employ downward distillation of the sulphur dioxide, which is titrated directly with

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iodine as it distils over into water containing starch (Preservatives Determination Committee, 1928).

The more rapid methods described involve the determination of sulphur dioxide on an extract of the food rather than a distillate. These are mostly based on a fuchsin-formaldehyde colorimetric procedure (Joslyn, 1955; Carruthers, Heaney & Oldfield, 1965) or titration with iodine (Ripper), such procedures have been mainly applied to vegetable and fruit products, especially fruit pulps and wines, and also sugar. The fact that neutral sodium sulphite does not combine with carbonyl compounds and that the hydroxysulphonic acid compounds are rapidly decomposed on treatment with alkali were used by Ripper as the basis for the determination of total sulphur dioxide in wine by direct iodine titration. This involved a cold digestion with caustic alkali and then, after acidification, rapid titration with 0·02 N iodine using starch as indicator. The addition of acetone (Bennett & Donovan, 1943; Prater *et al.*, 1944) and formaldehyde (Ponting & Johnson, 1945; Potter & Hendel, 1951) reduced errors caused by the reduction of iodine by substances other than sulphur dioxide. Prater's method involving two titrations with iodine was adapted for use with dehydrated cabbage by Potter (1954). Titration of one aliquot of the sample extract gives a measure of the total reducing substances. Instead of adding acetone (cf. Prater *et al.*, 1944) to the other aliquot, hydrogen peroxide was employed to oxidize sulphite to sulphate. It was also claimed that compared with other methods, the end-point was sharper.

The factors affecting the accuracy and reproducibility of the rapid iodometric titration for the direct determination of sulphur dioxide have been discussed by Joslyn & Braverman (1954). Apart from a tentative method described in a privately circulated BFMIRA Report (Anderton & Locke, 1956), no procedures for the rapid direct determination of sulphur dioxide in meat products were found during the authors' literature search. The purpose of this paper was to develop a method for determining the total sulphur dioxide on a water extract of fresh sausage such that it gave results in good agreement with a reference distillation procedure.

Experimental

I. *Reference method involving distillation*

The Shipton modification of the Monier-Williams method was employed (Pearson, 1970).

II. *Rapid methods applied to macerate of sample*

The various methods were applied to 10% and 20% extracts made by macerating fresh sausage samples with water and filtering (see Method II(c) below).

(a) *Rosaniline—formaldehyde colorimetric method.* The method devised for sugar (Carruthers *et al.*, 1965) was applied to the filtrate.

(b) *Direct titration with iodine.* The sample filtrate was digested in the cold for 15 min with alkali, acidified and the 'total' sulphur dioxide titrated with 0·05 N iodine.

(c) *Potter's iodine titration method.*

Reagents

0·05 N iodine
5 N hydrochloric acid
5 N sodium hydroxide
3% hydrogen peroxide
1% starch solution

Procedure

The fresh sausage sample was prepared by mincing and mixing in a pestle and mortar. 50 g comminuted sample was blended for 90 sec with 250 ml water in a mechanical blender (MSE Atomix) and filtered as rapidly as possible through a large fluted filter paper. Into each of two 250 ml conical flasks A and B were pipetted 75 ml of the 20% extract, followed by 75 ml water and 5 ml of 5 N sodium hydroxide. The solution was stirred gently using a magnetic stirrer, avoiding beating air into the solution and then allowed to stand for exactly 20 min.

To A was added 7·0 ml of 5 N hydrochloric acid and 10 ml of starch solution and, stirring gently, the liquid was titrated immediately with 0·05 N iodine to a definite blue colour (A–ml).

To B was added 7·0 ml of 5 N hydrochloric acid, 2 ml of 3% hydrogen peroxide and 10 ml of starch solution and, stirring gently, the liquid was titrated immediately with 0·05 N iodine to a definite blue colour (B–ml).

$$\text{Sulphur dioxide in sample (ppm)} = \frac{250\ (A-B) \times 1600}{75 \times \text{weight of sample taken}}$$

$$= 107\ (A-B) \text{ if 50 g is taken.}$$

Results and discussion

Preliminary findings

All results of rapid methods were compared against Shipton's reference procedure, which gave recoveries of 96–98% SO_2 when tested with sulphite solution.

The acid-bleached fuchsin—formaldehyde colorimetric method (IIa) gave much higher results than the figures given by the reference method. The reaction is interfered

with by a large number of substances and it was difficult to assess accurately the blank.

Direct titration of the extract with iodine gave very high results whereas after digestion with alkali and acidification (method IIb) fairly reasonable and comparable figures were obtained in spite of the rather poor end-point. It was apparent, however, that the blank varied considerably according to the particular sausage recipe employed. The method could only be considered satisfactory if a reliable blank were known. This would apply in routine factory control where products manufactured to the same recipe are examined continually.

The above difficulties were largely overcome by titrating the extract using Potter's method IIc and the results obtained were comparable with those given by the reference method. The method was therefore submitted to further investigation by considering factors which might influence the results with a view to getting results as close to the reference figures as possible. All the following factors were examined in relation to pork sausage extracts.

Effect of the time of maceration

A sample of fresh sausage was submitted to various maceration times ranging from ½ to 2 min. From the results in Table I, it would appear that the 'optimum' time of maceration is 1½ min. Presumably lower times gave incomplete extraction and larger periods resulted in losses due to oxidation, probably encouraged by heat and the beating of air into the liquid. A maceration time of 1½ min is therefore recommended.

TABLE 1. Effect of maceration time on the recovery of sulphur dioxide using Potter's titration

Maceration Time (min)	Volume of 0·05 N iodine used (ml)		Sulphur dioxide ppm
	Extract	Blank	
½	3·1	0·5	277
1	3·4	0·6	299
1½	3·5	0·65	304
2	3·8	1·1	288

Effect of the time of digestion with alkali

Fresh sausage samples were examined by Potter's method using cold alkali digestion times of 10 and 20 min and by Shipton's reference procedure. From the results in Table 2, it is apparent that cold digestion for 20 min gave values closer to those obtained with the reference method. In view of the alkaline pH, losses due to oxidation should be minimal and the differences are more likely to be due to the time required to release the 'bound' sulphur dioxide. Cold digestion for 20 min with alkali is therefore recommended.

TABLE 2. Effect of time of digestion of pork sausage extracts with alkali on the recovery of sulphur dioxide using Potter's titration

Sample no.	Digestion time of 10 min. Volume of 0·05 N iodine (ml) Extract	Blank	Sulphur dioxide ppm	Digestion time of 20 min Volume of 0·05 N iodine (ml) Extract	Blank	Sulphur dioxide ppm	Sulphur dioxide using reference method ppm
1	3·5	0·6	309	3·4	0·6	299	307
2	3·4	0·6	299	3·3	0·5	299	318
3	3·8	0·7	331	3·9	0·7	341	339
4	4·0	0·6	363	4·1	0·6	373	378

Effect of other factors

The use of the other binding agents such as formaldehyde and acetone produced unsatisfactory end-points in the titration. In order to save time in routine control and reduce possible losses of the preservative some samples were filtered through muslin. Although the filtration was much quicker than when paper was used, it was very difficult to decide on the position of the end-point. This was presumably due to the effect of the interfering solid matter which would pass through muslin.

Evaluation of results using the rapid method

The results obtained on twelve samples of fresh pork sausage are quoted in Table 3. From statistical analysis the means and ranges of values for the two methods are as follows:

Reference method: 341 ± 28 ppm (coeff. of variation 8·22%)
Rapid method: 336 ± 27 ppm (coeff. of variation 8·02%)

Applying the t-test, t = 2·081. There is good correlation between the two methods, and there is only about 5% probability of the results of the rapid procedure not being comparable with those using the reference technique. The recovery from sulphite solutions using the rapid method was 100%. The method gave high results, however, where the sample had badly decomposed. This corresponds to previous findings with dehydrated vegetables which had undergone lengthy storage.

Conclusions

The total sulphur dioxide of fresh pork sausage can be rapidly determined by applying Potter's titration method to the sample macerate. The results obtained are in good agreement with those from the reference method. By dispensing with the distillation

step it is possible for the analyst to cope with several samples simultaneously. Although the method does not appear to be applicable to badly deteriorated samples this is unlikely to be a serious disadvantage in practice as the factory control chemist is normally only concerned with freshly prepared choppings.

TABLE 3. Comparison of results obtained for the determination of sulphur dioxide in twelve samples of fresh sausage using the recommended rapid method and the reference procedure

Sample no.	Reference method		Rapid method		
	Volume of 0·05 N NaOH used (ml)	Sulphur dioxide ppm	Volume of 0·05 N iodine used (ml) Extract	Blank	Sulphur dioxide ppm
5	10·4	333	3·8	0·7	331
			3·8	0·6	341
6	10·8	346	3·8	0·7	331
			3·8	0·7	331
7	10·6	339	3·9	0·6	352
			3·9	0·7	341
8	10·6	339	3·8	0·7	331
			3·9	0·7	346
9	9·95	318	3·4	0·6	299
			3·3	0·5	299
10	9·6	307	3·5	0·6	309
			3·4	0·6	299
11	8·9	285	3·3	0·6	288
			3·3	0·6	288
12	12·3	394	4·2	0·6	384
			4·3	0·7	384
13	11·8	378	4·0	0·6	363
			4·1	0·6	373
14	11·0	352	4·0	0·7	352
			4·0	0·7	352
15	11·1	355	4·0	0·7	352
			4·0	0·7	352
16	10·7	342	3·7	0·6	331
			3·7	0·6	331

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The measurement of tin in canned beans by atomic absorption spectrophotometry

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Summary

The atomic absorption technique for the measurement of small amounts of tin offers advantages of speed, simplicity and freedom from interferences compared with colorimetric procedures, provided that rapid sample preparation can be achieved. Rapid methods of sample preparation for canned beans, one using concentrated hydrochloric acid and the other concentrated sulphuric acid, are described and shown to be reliable. The hydrochloric acid digestion technique coupled with atomic absorption measurements is shown to give comparable results to the catechol violet colorimetric procedure and to be both quicker and simpler to perform.

Introduction

The determination of tin in canned food is clearly important for, although there is no legal maximum for the amount that may be present, the Food Standards Committee (1953) have recommended a general limit of 250 ppm. Moreover, it is important to be able to monitor the level of tin in canned food so as to be able to assess the rate of deterioration of the container.

The almost complete lack of references relating to the application of atomic absorption spectrophotometry to the measurement of tin in food is a reflection of the difficulties involved. Rubeska & Moldan (1969) note that tin presents the greatest difficulty of all metals in the non-ferrous group. This is partly because it forms stable oxides that are difficult to dissociate, partly because sensitivity is relatively poor and partly because, until recently, tin lamps have lacked brightness. As Cameron & Hackett (1970) have indicated, the sensitivity of the atomic absorption method for elements such as copper may be increased by a factor of up to one hundred by the use of chelating agents and extraction into organic solvents, but no such increase has been reported for tin. In spite of these difficulties it was considered that the inherent advantages of the atomic absorption method—its potential accuracy, rapidity and freedom

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from interferences—made it capable of replacing and improving on traditional colorimetric procedures.

In reviewing colorimetric methods for determining tin in organic matter the Analytical Methods Committee (1967) recommended the use of the catechol violet colorimetric method for determining amounts of tin not exceeding 30 μg/sample. This method is suitable for estimating tin in canned food but suffers from the disadvantages that it is tedious and subject to many interferences. It was therefore considered that the atomic absorption method might prove superior to it, and accordingly a comparison of these methods was undertaken.

Most methods for determining tin in food are lengthy because of the need to destroy all organic matter; the catechol violet colorimetric procedure, for example, involves lengthy wet oxidation. Although wet oxidation and dry ashing have traditionally been used to destroy all organic material in atomic absorption procedures, inherently the method only requires that the tin to be determined shall be in solution and free from particles. It is therefore not essential to destroy completely all organic matter, and for suitable foods a relatively simple, rapid digestion technique may be employed. Simpson & Blay (1966) have developed such a technique, using hydrochloric acid, which may be used for foods that are easily digested. It was also considered that a modified Kjeldahl type of digestion using sulphuric acid might prove applicable to beans, and that it might offer similar advantages to the method of Simpson and Blay.

Materials and methods

Apparatus and reagents

A Perkin Elmer 290 atomic absorption spectrophotometer was used together with an Aerostyle model A air compressor and a Telsec Potentiometric Recorder. A Perkin Elmer Intensitron hollow cathode tin lamp was used in conjunction with a Boling burner and platinum–titanium nebulizer. Colorimetric measurements were made with a Unicam SP800 spectrophotometer.

A stock solution containing 500 ppm tin was made by dissolving 0·5 g high purity tin in 200 ml of hydrochloric acid (sp. gr. 1·16) and diluting to 1 litre with de-ionized water. A similar standard was prepared using sulphuric acid. Aqueous standard tin solutions in the range 2–25 ppm were made from a freshly prepared intermediate standard containing 50 ppm tin. Solutions for the catechol violet colorimetric determination were prepared in accordance with the recommendations of the Analytical Methods Committee (1967).

Sample preparation

Baked beans were prepared from haricot beans in the normal way, mixed with tomato sauce, filled into cans which were processed at 245°F for 45 min with a 20 min cooling period.

For the method of Simpson & Blay (1966) 5 or 10 g samples of homogenized baked beans were used with 25 ml hydrochloric acid solution (50 ml Analar hydrochloric acid: 22 ml of de-ionized water), the whole being brought to the boil and simmered for 5 min. The resulting solution was cooled, transferred to a 50 ml flask and made up to the mark with de-ionized water. After filtering, measurements were made directly on the filtrate. Standards were prepared so that they contained an equivalent amount of acid to the samples.

Samples of 20 or 25 g of homogenized baked beans in 500 ml Kjeldahl flasks were treated by a modified Kjeldahl procedure. After addition of 15 g potassium sulphate, 2 ml of 10% selenium dioxide solution and 40 ml concentrated sulphuric acid the flasks were heated gently until frothing ceased, then more strongly until the solution was a pale yellow colour. When just bearable to the hand 70 ml of de-ionized water was added and when cold the flasks were made up to 250 ml with de-ionized water.

Samples for colorimetric determination of tin were prepared by wet oxidation of 5 g samples of homogenized baked beans using concentrated nitric and sulphuric acids in accordance with the method of the Analytical Methods Committee (1967).

Instrumental Conditions

All measurements with the atomic absorption spectrophotometer were made under the following conditions: wavelength, 286·3 nm; spectrum band width, 0·7 nm; lamp current, 10 mA; oxidant, air; fuel, hydrogen. The use of a Telsec recorder allowed selection of suitable scale expansion.

Measurements with the SP800 were made at 552 nm.

Results and discussion

The sensitivity of tin determination by atomic absorption is poor—Gatehouse & Willis (1961) report a value of 5 ppm using an air/acetylene flame—and preliminary experiments were therefore carried out to determine optimum conditions. A comparison of air/acetylene and air/hydrogen flames showed that in both cases a fuel rich flame was required and that although hydrogen gave a slightly lower sensitivity than acetylene, this was more than compensated for by the greatly reduced background interference which permitted the use of much greater amplification of the output signal. Accordingly the hydrogen flame was used throughout at a wavelength of 286·3 nm which gave maximum absorption.

Attempts were made to increase sensitivity by complexing tin in aqueous digests using such reagents as quercetin, dithiol and ammonium pyrrolidine dithiocarbamate with subsequent extraction into methyl isobutyl ketone. As these attempts were unsuccessful all measurements were made on aqueous extracts in hydrochloric or sulphuric acid.

TABLE 1. The effect of hydrochloric and sulphuric acid on recorder peak height for a sample containing 25 μg/ml of tin.

% concentrated HCl	0	5	10	15	20	25	35	40
Peak height (cm)	6·2	5·8	5·5	5·3	5·2	5·0	4·7	4·5
% concentrated H_2SO_4	0	1	2	3	5	10	15	20
Peak height (cm)	6·2	4·8	4·0	3·4	2·9	1·9	1·3	1·1

Table 1 shows the reduction of peak height caused by these acids, particularly sulphuric acid. In order to compensate for the presence of acid in tin digests an equivalent amount of the appropriate acid was added to all standards. The sensitivity obtained for tin standards containing 2–25 μg/ml of tin made up in either hydrochloric or sulphuric acid was in the region of 2 μg/ml for 1% absorption. All calibration curves were linear.

The atomic absorption method using the hydrochloric acid digestion technique was compared with the colorimetric catechol violet method using commercial canned beans, and the results obtained are shown in Table 2.

TABLE 2. Comparison of results for the tin content of canned beans determined by colorimetric and atomic absorption techniques.

Method	Sample	Test solution	Readout	Value	Tin content of sample (ppm)	Mean (ppm)
Catechol violet	5 g in 250 ml	2 × 5 ml	Absorbance	0·35, 0·36	149, 155	152
Catechol violet	5 g in 250 ml	2 × 5 ml	Absorbance	0·32, 0·34	137. 145	141
Atomic absorption (HCl digest)	5 g in 50 ml	2 × 50 ml	Peak height (cm)	3·6, 3·5	150, 145	147

The corrosion taking place in cans of beans was monitored by measuring tin content by the atomic absorption technique using two different sampling procedures. The results of these measurements, given in Table 3, show that, as expected, the tin content of beans in plain cans rose much faster and to much higher levels than those in lacquered cans. The amount of tin in the contents of lacquered cans remained approximately constant at the level reached immediately after processing whereas that in the contents of plain cans rose rapidly for the first 4 days and then remained nearly constant. The small irregular changes of tin content with time shown in Table 3 are not significant and may be accounted for by the fact that sampling was from only two cans on any

day. Although this does not give an accurate measurement of overall corrosion because the rate of corrosion varies from can to can it is sufficient to permit a comparison of methods of sampling.

TABLE 3. A comparison of the tin content of canned beans determined by atomic absorption using two different methods of sample preparation.

Time of sampling	Tin content (ppm), HCl digest 10 g sample	Lacquered cans H_2SO_4 digest 25 g sample	Tin content, (ppm), HCl digest 10 g sample	Plain cans H_2SO_4 digest 25 g sample
Before sterilizing	25	—	25	—
After sterilizing	27	—	39	—
After 4 days	27	29	54	57
After 7 days	31	27	57	60
After 14 days	29	31	58	61
After 9 weeks	26	27	58	62
After 10 weeks	28	29	60	63

In order to compare the standard colorimetric procedure with the atomic absorption technique, comparative measurements of tin content were made on the same can of beans after 9 weeks storage. The results, based on duplicate measurements in each case, are presented in Table 4 and show that although the sulphuric acid digestion gives slightly high results (see also Table 3), the hydrochloric acid digestion gave results in close agreement with the catechol violet method.

TABLE 4. A comparison of the tin content of canned beans after 9 weeks storage as determined by colorimetric and atomic absorption techniques.

Technique	Method of digestion	Sample g	Tin content in ppm	
			Lacquered cans	Plain cans
Colorimetric	$HNO_3 + H_2SO_4$	5	24	56
Atomic absorption	HCl	10	26	58
Atomic absorption	H_2SO_4	25	27	62

Conclusions

The atomic absorption technique can be used successfully to measure the tin content of canned beans and hence to monitor the extent and progress of corrosion of the cans.

The hydrochloric acid digestion technique gives results for tin content that, in spite of relatively low sensitivity, are in good agreement (4–6%) with the catechol violet method, and it has the advantage that it is much simpler and quicker to carry out. The sulphuric acid digestion technique also gave satisfactory results, though the use of a high concentration of acid and the consequent necessity to dilute the digest before presenting it to the spectrophotometer coupled with the reduction of sensitivity brought about by the acid gave rise to small peak heights and lack of accuracy. Digestion using hydrochloric acid is therefore preferred to that using sulphuric acid for determining tin in beans, though with less easily digested canned foods the sulphuric acid method could be useful, being quicker and simpler than the colorimetric procedure.

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A single layer moisture absorption theory as a basis for the stability and availability of moisture in dehydrated foods

M. CAURIE

Summary

It has been shown that moisture absorption in dehydrated foods cannot form a layer more than a single molecule deep and the stability of the food has been shown to depend on this single layer. The use of an Aw scale as an index of available moisture in dehydrated foods has been shown to overestimate the parameter. Suggested new definitions have been expressed directly as percent moisture and as a ratio of the total moisture/bond energy or gaseous water content. It has also been shown that the laboratory method of dehydrating materials at constant temperature by lowering the ambient humidity may impair the subsequent absorption capacity of the material if the humidity is lowered below a certain critical minimum value.

Introduction

The avoidance of microbial contamination and the prevention of the growth of microbes on food are two of the major problems in food preservation. The growth of microbial contaminants on food may result in undesirable deteriorative changes in the food. However whether a given microorganism can multiply in a particular food under particular conditions usually depends on factors related to water content and its availability. The latter is believed to be proportional to the surrounding equilibrium humidity and is designated by the symbol Aw.

Early studies on the physiology of microbial growth enabled Mossel & Ingram (1955) to compile a table indicating the lowest values of Aw permitting the development of common food spoilage microorganisms. These critical water activities were further translated into 'alarm water contents' representing the highest moisture contents at which microbial spoilage would not occur. The setting up of a high 'alarm moisture content' below which no deterioration was supposed to occur obviously indicated that it was not realized that enzymes of microbial or food origin might still be reactive in the limited moisture contents of dry foods. Therefore the possible existence of a minimum value for optimum stability was not considered. A suggested

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minimum moisture content (Salwin, 1959) based on a multilayer absorption theory has lately been found to be inadequate. It is the aim of this paper to show why this is so and to demonstrate that the stability of dehydrated foods may be adequately explained in terms of a single layer absorption theory. An attempt is also made to show that the use of water activity (Aw) as a measure of available moisture is an overestimation of the parameter.

Defining a minimum moisture content for stability

In defining a minimum moisture content for the optimum stability of dehydrated foods Salwin (1959) interpreted water sorption data in terms of the BET (Brunaeur, Emmett & Teller, 1938) theory of multimolecular adsorption. The equation developed from this theory predicts the number of water molecules required to form a monolayer of firmly bound water to the surface of solid substances.

Water molecules in the monolayer of food substances are believed to be adsorbed on localized sorption sites (Cassie, 1945; Hill, 1946) which are now known to be polar functional groups of the sorbent. The mode of attachment is one molecule of water per polar functional group (Pauling, 1945; McLaren & Rowen, 1952). Recent studies (Martinez & Labuza, 1968; Karel & Nickerson, 1964; Caurie, 1970) however, have shown that the BET monolayer moisture does not offer the desired protection to dehydrated foods. Furthermore interpretation of the results of several investigators

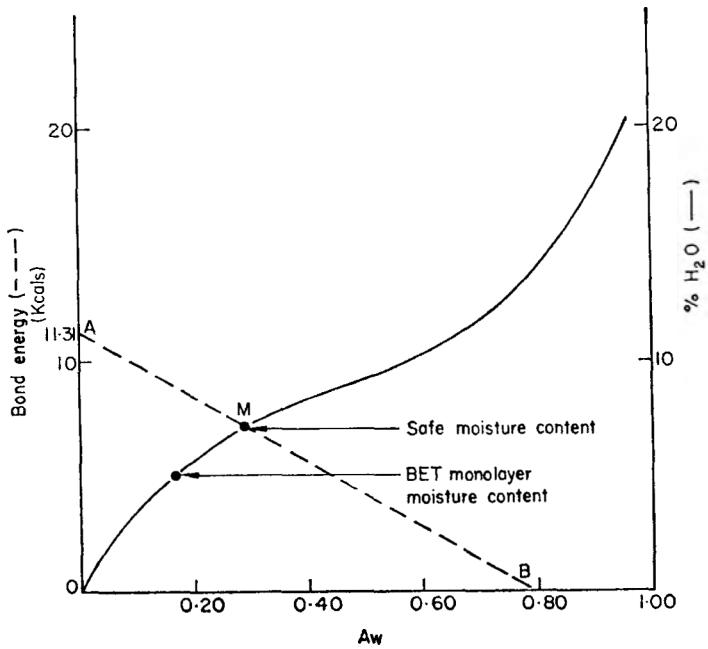

FIG. 1. Diagrammatic theoretical moisture sorption isotherm showing the relative position of Caurie's (1970) safe moisture content to the BET monolayer moisture content.

(Shaw, 1944; Benson, Ellis & Zwanzig, 1953; Benson & Seehof, 1954; Gur-Arieh, Nelson & Steinberg, 1967a; Gur-Arieh *et al.*, 1967b) reveal that sorption of polar gases including water vapour in dehydrated foods is not a surface phenomenon as is assumed by the theory from which the BET equation is derived. It is, however, one involving the penetration of the sorbate throughout the solid sorbent and is also independent of the degree of subdivision of the sorbent. This suggests that the application of the BET equation to dehydrated foods is inadequate and is more likely to underestimate the moisture content considered safe for the storage of dehydrated foods and materials.

Caurie (1970) has recently suggested an equation to predict a minimum optimum safe moisture content for the storage stability of dehydrated foods from their sorption data. This safe moisture content has been found to be constantly higher (Fig. 1) and to offer a more stable product than the BET monolayer moisture content. Thus the safe moisture contents of freeze-dried salmon (Martinez & Labuza, 1968) and orange crystals (Karel & Nickerson, 1964) calculated with this equation are considerably higher and have been shown to provide a more stable product (Caurie, 1970) than the BET monolayer moisture content.

The mechanism of stability

As a basis for stability Salwin (1959) explained that the amount of moisture held in the BET monolayer forms a protective film which adequately combines with all reactive functional groups at the adsorption sites to protect them against autoxidative reactions which are known to occur at low moisture contents. Since the BET monolayer moisture content has been shown to be lower than the moisture content considered safe for storage, it is apparent that it does not fully satisfy the definition of Salwin (1959) to cover all adsorption sites. This suggests that the type of instability noted with foods at the BET monolayer coverage of water is due to the exposure of some adsorption sites to the deterioration reactions described by Salwin (1959).

A single layer absorption theory—energy relationships and the nature of the stable moisture

Caurie (1971) has shown that as the moisture content increases with Aw the bond energy of sorbed water decreases (Fig. 1). At some critical point M (Fig. 1) on a water sorption isotherm therefore the two curves intersect. The moisture content then becomes numerically identical with the bond energy of sorbed water. This point of intersection coincides with the unavailable safe moisture content of Caurie (1970).

From the point of intersection (Fig. 1) of the two curves it will be noted that the energy of sorbed water along AB becomes for the first time numerically smaller than the total amount of moisture absorbed. The former decreases to zero at 0·784 Aw, in the case of starchy foods (Caurie, 1971), as the total moisture content increases. The

decreasing bond energy along AB (Fig. 1) down to zero is explained if it is assumed that there is an initial gaseous absorption up to a saturation point at the safe moisture content when the gaseous water molecules begin to condense at the absorption sites with increasing Aw. Since the gaseous safe moisture content is numerically equal, in K cal, to its bond energy any reduction in its value by condensation similarly reduces the bond energy by the same numerical value. Therefore the bond energy line AB (Fig. 1) above the safe moisture content estimates the amount of gaseous water molecules still left in the food at any specified temperature and Aw.

It may be observed that below the safe moisture content the number of gaseous water molecules is not identical to the bond energies but rather to the total moisture content. Indeed Brunaeur (1943) observed that at the low Aw end of sorption isotherms the number of water molecules usually corresponded to the water content of the material.

It is apparent, therefore, that the safe moisture content is the maximum number of gaseous water molecules a food material can absorb at any specified temperature and Aw. The gaseous safe moisture content later condenses at the absorption sites with increasing Aw after its attainment. Hence above the safe moisture the total equilibrium moisture content becomes a mixture of decreasing gaseous water molecules and an increasing amount of condensed water in a state of dynamic equilibrium (Fig. 2)

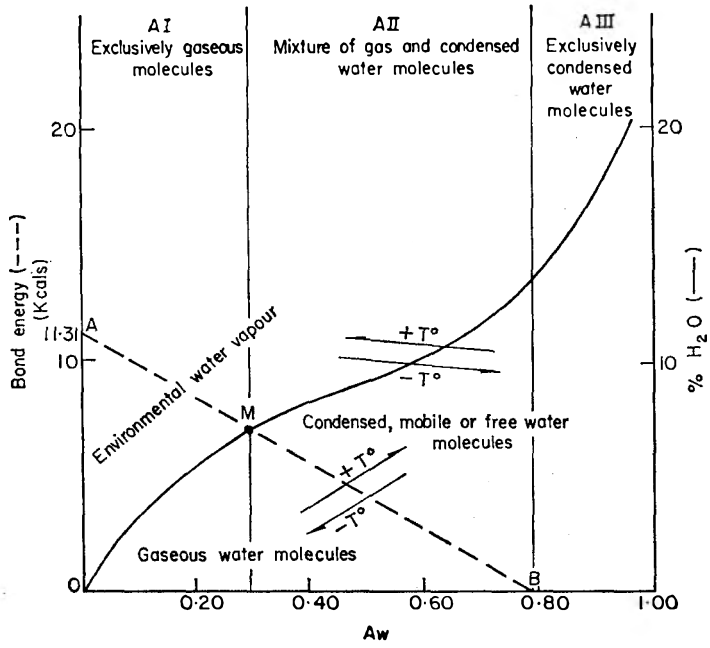

FIG. 2. Diagrammatic representation, along a theoretical sorption isotherm, of the limits of the different states of moisture and their temperature/equilibrium relationships in foods.

as the Aw increases. At 0·784 Aw in the case of starchy foods (Caurie, 1971) gaseous water molecules reduce to zero and the whole of the sorbed moisture then becomes condensed mobile free water.

Duckworth & Smith (1963) have shown that water becomes partially available and materials are able to diffuse in it shortly after the completion of the strongly bound gaseous BET monolayer. Acker (1969) and Acker & Beutler (1965) have furthermore demonstrated that enzymic reactions occur in dehydrated foods only after the completion of the BET monolayer along a path more or less parallel to the sorption isotherm. This means that the availability of condensed moisture for enzymic reactions is more or less proportional to the amount of condensed moisture held at any Aw above the BET monolayer. The results of this work essentially agree with these findings.

From this discussion it is evident that in dehydrated foods there cannot form a gaseous moisture layer more than a single molecule deep in agreement with Langmuir (1916, 1918) and Acker (1969).

Interpretation of water sorption isotherms

Caurie (1971) on the basis of water binding divided water sorption isotherms broadly into two regions, R-1 and R-2, representing areas of restricted and free water respectively. It is clear from this work (Fig. 2) that the area of restricted water (R-1) may be further divided up into two, viz. the area of exclusively gaseous absorption and the area of gaseous and condensed water absorption. Therefore on the basis of the state of water present in the food, water sorption isotherms may be divided into three clear areas (Fig. 2) viz. area I (A I) representing the area of exclusively gaseous water absorption, A II representing the area of mixed gaseous and condensed water absorption and A III representing the area of exclusively condensed water absorption.

Temperature, Aw and the protective effect of water molecules

It has been noted in this work that gaseous and condensed water molecules in a food may exist in a state of dynamic equilibrium between the Aw corresponding to the safe moisture content and 0·784 Aw for starchy foods. A second dynamic state of equilibrium exists between the environmental water vapour and condensed water molecules in the food (Fig. 2).

An increase in the ambient temperature, and therefore of the product causes a fraction of the condensed water molecules in the food to vaporize, thus raising the partial vapour pressure of the environment. The reduction in the amount of condensed moisture in this way causes a disturbance in the existing equilibrium between it and the gaseous water molecules in the food. Equilibrium is later re-established under the new conditions of reduced gaseous, condensed and, therefore, total moisture contents. At a lower ambient temperature the reverse reactions take place. These reversible equili-

brium states of moisture in the food explain the decrease in the safe moisture content of food substances with temperature.

It must be noted that a decrease in absorbed gaseous water molecules at higher temperatures does not imply a reduction in the number of absorption sites. This number remains fixed for each type of food but at higher temperatures, and in consequence higher Aw, a fraction of this number gets covered by a thin protective film of condensed water molecules. By this protective effect no absorption sites are exposed to autoxidative reactions at any Aw above the safe moisture content.

Dehydration and hysteresis

It has been stated that moisture absorption up to the safe moisture content is exclusively gaseous. Therefore, unlike conditions above the safe moisture content where gaseous water molecules at the absorption sites are in a dynamic equilibrium with condensed water molecules which may cover exposed absorption sites, no such equilibrium exists below the safe moisture layer and evaporated gaseous molecules expose the absorption sites to the deteriorative reactions described by Salwin (1959), Lea, Parr & Carpenter (1958, 1960) and Rockland (1969).

The deteriorative reactions in which the exposed absorption sites may take part will be sufficient to impair the sorption capacity of the absorption sites in proportion to the number exposed. Consequently in a subsequent absorption process at the same temperature the material will not regain its full absorption capacity. This impaired absorption capacity may partly account for the phenomenon of hysteresis exhibited by certain foods during sorption studies in the laboratory. It appears therefore that desorption below the safe moisture or some other critical value of moisture impairs the sorptive properties of some absorption sites of dehydrated foods and materials. This may be similar to what happens during freezing when water molecules removed from colloidal materials are improperly reabsorbed on thawing (Mossel & Ingram, 1955).

Measurement of available moisture in dehydrated foods

It is now firmly believed that the availability of moisture for chemical and microbiological activity is best expressed in absolute units of Aw. This idea was first suggested for liquid media by Scott (1953) who later extended it to cover dehydrated foods as well (Scott, 1957). But while the use of absolute Aw to measure available moisture may be justified in liquid media on the grounds that in homogenous liquid systems we cannot speak of variation in water binding (Acker, 1969), the situation is different in dehydrated foods where various water binding energies occur.

It has been stated in the preceding paragraphs that water content of dehydrated foods up to the safe moisture content is gaseous and does not act as a solvent. This means that the safe moisture represents a zero available moisture content. It has been

shown that gaseous water molecules begin to condense on the absorption sites (with increasing Aw) immediately after the safe moisture content has been attained (Fig. 2) thereby reducing the safe or unavailable moisture content.

From Fig. 3 it is evident that available moisture at any Aw must be equal to the difference between the total equilibrium moisture content and its corresponding gaseous fraction. The gaseous fraction of the total equilibrium moisture content may be easily estimated for starchy foods and foods containing high proportions of sugar (Caurie,

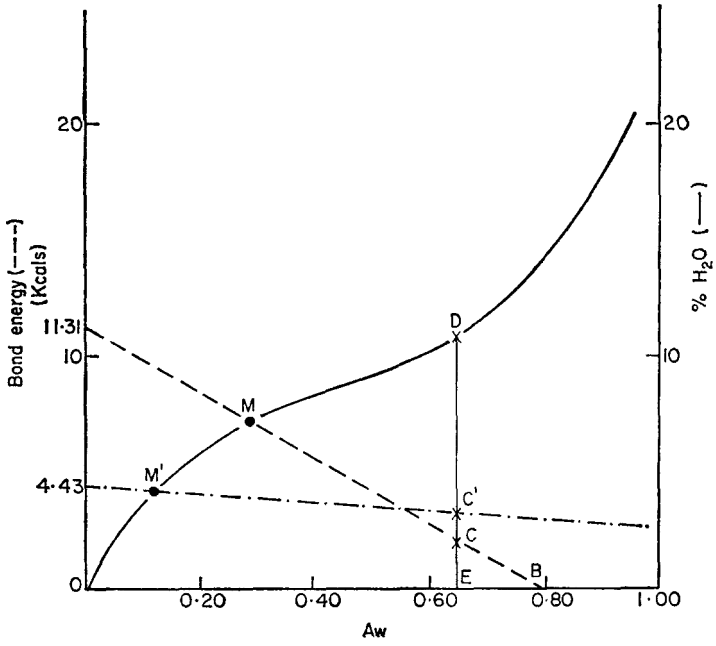

FIG. 3. Diagrammatic representation of the relative availability of moisture and the stability of starchy (– – –) and high sugar (–·–·–) foods under the same humidity conditions.

1971) by reference to Fig. 3. For example at point D (Fig. 3) on the hypothetical sorption isotherm, the corresponding gaseous fraction for starchy foods is at point C and for high sugar foods at point C′. The available moisture for the starchy food is therefore equal to DE – CE and the high sugar foods to DE – C′E when expressed as percentage moisture.

This way of measuring available moisture is in agreement with a previous statement (Caurie, 1971) that hygroscopic materials are most stable when their energy/water ratio is unity at the safe moisture content. This ratio may also be used to measure the degree of stability of a food expressed in Ew units.

The availability of moisture in foods for natural processes may be similarly defined

but using the inverse form of the ratio, that is, water/energy or gaseous water ratio. When this ratio is equal to or less than unity there is no free or mobile water. Free or mobile water is indicated when this ratio is greater than unity, the degree of availability depending then on the size of the fraction which may be expressed in We or reciprocal Ew units. Thus in starchy foods (Fig. 3) available moisture may be given by the ratio DE/CE while the corresponding stability under the same conditions is given by the inverse of this ratio, i.e. by the ratio CE/DE. The values of CE or Q have been mathematically defined for starchy and high sugar foods by Caurie (1971). At any specified temperature and humidity when the equilibrium moisture content is known the available fraction, i.e. the fraction of the moisture in excess of Q or the gaseous fraction, may be easily calculated either as percent moisture or in We or reciprocal Ew units.

Since the safe moisture content is mobile it would appear that the current use of absolute Aw units as a measure of available moisture in dehydrated foods is limited and overestimates the parameter. It is therefore suggested that available moisture is directly measured with reference to the safe moisture content on the lines outlined above.

Conclusion

The state of absorbed moisture in dehydrated foods has been shown to form the basis for stability. Also it appears that there cannot be more than one gaseous layer of absorbed moisture in dehydrated foods. Available moisture has been defined directly as percent moisture and in terms of total moisture/bond energy or gaseous water content ratio which is an inverse of the defined ratio for the degree of stability of a dehydrated food. Explanations have been offered for criteria such as the optimum moisture content and for the phenomena of hysteresis. It is hoped that the findings in this work may throw some light on some of the yet unexplained phenomena in the vast subject of water sorption.

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Book Reviews

The Use of Fungi as Food and in Food Processing (CRC Monoscience Series). WILLIAM D. GRAY. Cleveland, Ohio: The Chemical Rubber Co. London: Butterworths, 1970. Pp. v + 113, 46 figures. £5.00

'Not presume to dictate, but broiled fowl and mushrooms—capital thing!' observes one of Dickens' characters. Professor Gray would go further—a lot further. Apart from yeast and biochemists (of whom he doesn't approve), any and every aspect of fungi kindles in him a crusader's fervour. The cultivation of edible mushrooms, occidental and oriental, is dealt with in do-it-yourself detail, in 31 pages. Five pages are devoted to cheese-making. There follows a thirteen-page *catalogue raisonné* of oriental fermented foods, tantalizingly exotic in name at least: ang-khak, tempeh, sufu.

A long section (24 pages) tackles the vexed question of whether microfungi, factory-grown on waste vegetable matter spiked with ammonia, can ever usefully contribute to man's larder; here Professor Gray unfolds his vision of what can only be called the mycelilal millenium. A highly individual treatment of mycotoxins occupies a dozen pages. The book ends with a bibliography of 348 references, mercifully with full titles given, which is determinedly up-to-date: no less than 48 per cent of them were published between 1960 and 1969. Most of the articles cited are American. Indeed, it is fair to say that the book as a whole has a somewhat provincial flavour. (*I* don't know what moist angel food cake looks like, so I won't be collecting any puffballs for the table this year. (The style conveys information adequately, with less 'noise' than is usual with American texts.

About the frequent asides on man's history and pre-history, sociology, politics, food preferences, etc., it is difficult to be kind. I was especially intrigued by the suggestion that Castro 'could not have happened' in a Cuba 'well-nourished', presumably by fungal delicacies.

A mycologist's coffee-table book, then. I am a little perturbed by the unthinking way in which many scientists roll out the SCP bandwagon, and perhaps I should be more severe. But, in fact, I found *The Use of Fungi* great fun.

W. E. TREVELYAN

Progress in Industrial Microbiology. Ed. D. J. D. HOCKENHULL. London: J. and A. Churchill, Volume 9. 1971, Pp. 247. £4.

The series of volumes entitled 'Progress in Industrial Microbiology' are widely accepted as authoritative works of reference indicative of various aspects of present and future

developments in microbiology as applied to industry. Like its predecessors Volume 9 covers a wide variety of topics: biochemical fuel cells (F. D. Sisler); industrial potential of plant cell culture (Z. Puhan and S. M. Martin); rumen micro-organisms (P. N. Hobson); insecticidal activity of microbial metabolites (H. T. Huang and M. Shapiro); observations on fermentation development (D. J. D. Hockenhull); and anaerobic digestion in biological waste treatment (E. J. Kirsch and R. M. Sykes).

All the chapters emphasize the accelerating expansion of industrial microbiology. For example, a by-product of Space Research has been the renewed interest in biochemical fuel cells which Sisler defines as 'an electrochemical device in which energy derived from chemical reactions maintained by a continuous supply of chemical reactants is converted to electrical energy by means of the catalytic activity of living cells and/or their enzymes'. But whether man explores other planets or not he will continue to contaminate this one. The comprehensive review (82 pages) on anaerobic digestion of biological waste is particularly relevant, therefore, to present day problems.

The chapter by Hockenhull is a philosophical description of industrial research to illustrate how training, management and good communication can contribute towards the effective completion of a fermentation development project. Although one may not agree with all his views this is a truly rousing chapter.

The book provides a wealth of information quite in keeping with its predecessors, but it will be of limited value to food scientists and technologists. It can be recommended to lecturers and to research workers in both academic and industrial establishments.

RICHARD J. GILBERT

Chocolate, Cocoa and Confectionery: Science and Technology. By BERNARD W. MINIFIE.
London: J. & A. Churchill, 1970. Pp. viii + 624. £6.

With the small number of reference books available on chocolate and sugar confectionery this volume by Bernard W. Minifie 'based on a lifetime's practical experience in the Cadbury Group of Companies' will naturally be welcomed by those involved in the production of sweets. The field covered in about 600 pages is extremely wide and will give those who are relatively new to the subject a good introduction to the background and techniques of confectionery manufacture.

The book is presented in three parts. Part One, Cocoa and Chocolate Manufacture and Handling, covers 143 pages and includes confectionery coatings and dietetic chocolates. It is interesting to trace the points where Cadbury Ltd have devoted special effort to the solution of some of the problems involved in chocolate processing.

Part Two, Confectionery, comprises 250 pages and is largely devoted to a very comprehensive list of ingredients giving the origins, general properties, uses and, in

some instances, methods of testing the various products. Due to the number of items listed some of these are necessarily brief summaries of more comprehensive information for which references are given. The remainder of this section on Sugar Confectionery Processes and Recipes is covered in sixty-eight pages, and the treatment appears rather superficial. For example, the Microfilm cooker and Candymaker are described, but coil cookers for boiled sugar are dealt with in five lines and sweet forming and continuous centre filling processes barely mentioned.

One looks in vain for references to calculations on solubilities of mixed sugars, syrup phase compositions and ERH, although typical ERH values of confectionery products are given and the possibility of moisture transfer in assotrments is discussed. The line drawings of plant in Parts 1 and 2 are extremely useful for tracing the course of processing in unfamiliar plant, and it is only to be regretted that there are not more of them.

Part Three on General Technology devoted 128 pages to bloom, microbiological spoilage, pest control, packaging, quality control, and some general topics. In this context the comments in Chapter 25 concerning Research and Development can only be regarded as the author's own opinion and those associated with smaller companies may question the reasoning involved.

In general this is a very useful reference work, and the approach goes a long way toward filling the gap in the present range of confectionery books.

D. I. STANSELL

The Biochemistry of Fruits and Their Products—Volume I, Edited by A. C. HULME.
London: Academic Press, 1970. Pp. xviii + 620. £10.

Next in importance to the production of food is the conservation of what is produced. In order to do this effectively it is necessary to have a knowledge and understanding of the nature of foodstuffs and their behaviour in a variety of natural and artificial circumstances. The purpose of the present book is first of all to discuss the nature of fruits in general: their chemical composition, biochemistry and pre-harvest physiology. This is the content of the first volume. In the second volume the treatment will be extended to the consideration of particular fruits and it is there that their behaviours, and especially their behaviour during processing, will be treated in detail.

In this first volume, nineteen chapters deal with the constituents of fruits under such headings as sugars, lipids and volatile compounds (which include the discussion of aroma). The remaining six chapters deal with growth and preharvest factors, nutrition, hormones, ethylene and ripening, and physiological disorders. All these subjects are dealt with by recognized authorities. The treatment is admirably even and competent; it is hard to find anything to criticize either in concept or execution. But it is clearly

a work to consult rather than one to be carried around in the field, and it would be a valuable next venture if a compact, shortened version were to be produced that could be used as a text book for the student and the food technologist especially in the developing countries, where this kind of information is so much needed.

E. C. BATE-SMITH

Review of 'Soup Manufacture', Canning, Dehydration and Quick-Freezing.
By RAYMOND BINSTED and JAMES D. DEVEY.
London: Food Trade Press Ltd, 1970, Pp. 260 + xi. £4.

The third edition of this well-known book has recently been published some ten years after the second. This new volume however represents not so much an updating of earlier information as the grafting on of some one hundred pages of additional material. This includes new chapters on the Seasoning of Soups and on Technical Aspects of Raw Materials, whilst a short historical preface has also been added. In addition, all the many illustrations are new: the majority are of process and packaging machinery and represent modern practice.

The volume covers the formulation and manufacture of both canned soups and dry soup mixes, with a short section on Quick Frozen Soups, which have been little developed in this country. Contrariwise there is only passing reference to stock cubes, which have a real place in the U.K. market and are even more important on the Continent.

The chapters on canned soups cover raw materials, recipes and processing details, including a brief treatise on canning practice and machinery. There is parallel treatment of dry soup mixes, a section that has been significantly expanded in line with the development of this section of the industry in the last two decades. The caveat in regard to the recipes that they should be regarded more as pointers to development reflects a very real problem in producing a book of this type in circumstances in which commercial considerations necessitate that much of the material must come 'from the outside looking in'.

These inhibitions do not however affect such chapters as that on Legal Aspects, which has been considerably expanded and brought up to date, including a resume of the proposals for U.K. compositional standards for both canned and dry mix products. Labelling requirements are also covered.

There is brief reference to the formation of the International Association of the Broth and Soup Industry, but more recent developments in the field of international co-operation are not covered.

The new chapter on the Seasoning of Soups contributed by H. B. Heath includes a comprehensive account of the herbs that can be used for seasoning, though the further new chapter on Technical Aspects of Raw Materials in part duplicates matter carried

over from the previous edition in other chapters. The treatment of analytical techniques is uneven: with some methods, for example that on creatinine determination, being dealt with in the greatest detail. A more critical appraisal of the methods available to the analyst with particular reference to their application to soups, coupled with the citation of references to the details of the techniques, would have been more useful.

The volume, despite its shortcomings, certainly gives much useful information in compact form, and can be recommended to all who wish to extend their knowledge of this group of products.

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Metabolic Aspects of Food Safety

Edited by
Francis J.C. Roe

Can studies in laboratory animals provide assurance of the safety of food constituents and food additives for man? Or do differences between species in enzymic capability render extrapolation from the laboratory to man completely unreliable? Do differences in gut flora influence the responses of species to chemical agents? How does the evolutionary process influence the ability of different species to cope with foreign compounds? How does one measure kidney function in a rat? What is the significance of increase in liver weight unaccompanied by other changes in an animal exposed to a prospective food additive? Does this indicate adaptation or intoxication? These are some of the questions that various experts have considered in this volume. In many cases their comments are best viewed against a background knowledge of the pathology of laboratory animals, particularly rats and mice — the subject of a previous volume in this series (Cotchin & Roe 1967).

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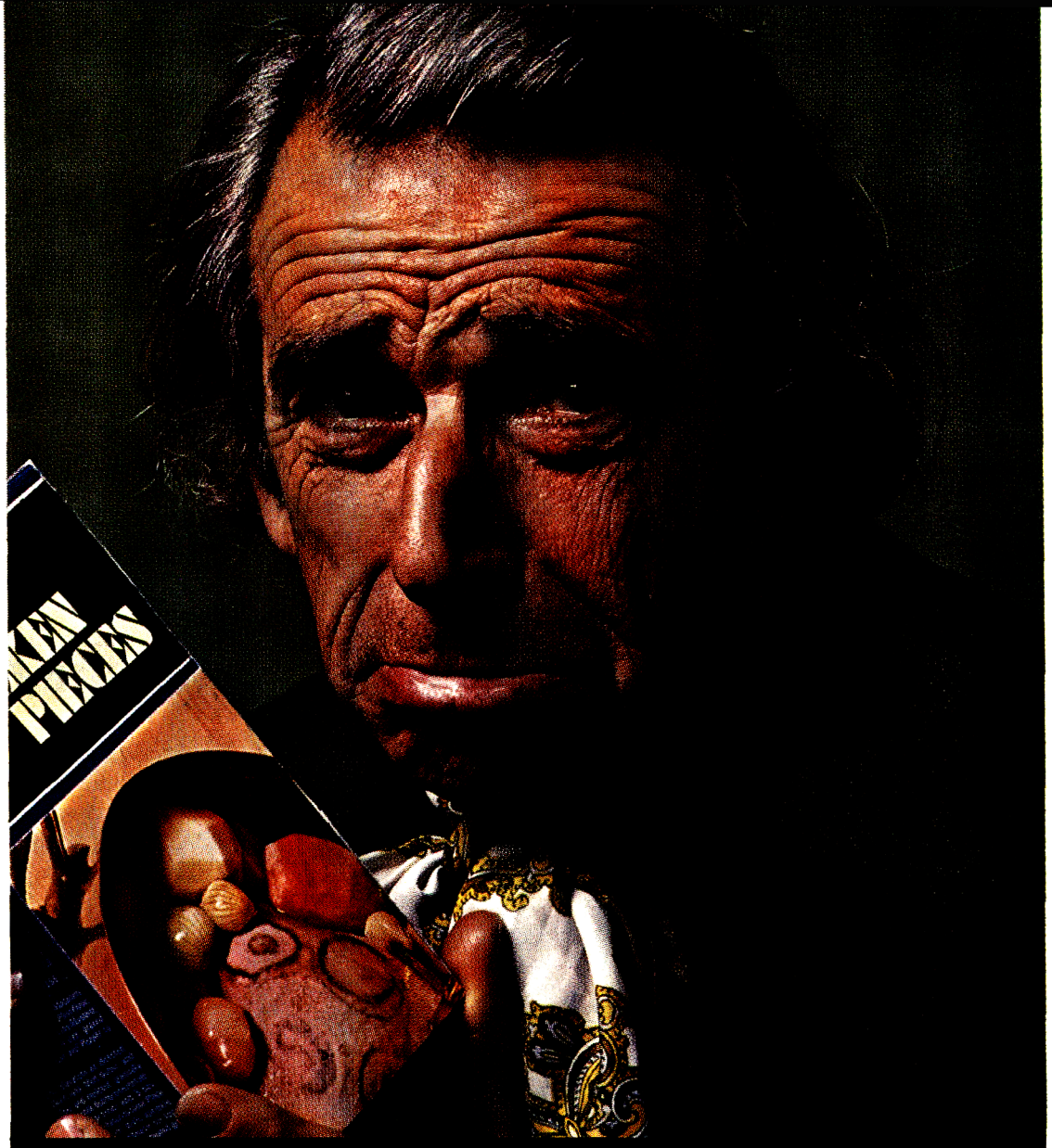

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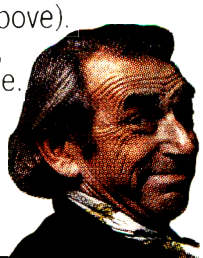

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gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	µg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr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

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