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Prediction of the effect of temperature on water sorption isotherms of food material

H. A. IGLESIAS AND J. CHIRIFE

Summary

An empirically modified two-parameter multilayer adsorption equation is shown to take into account the effect of temperature on the water sorption isotherms of some food materials. Characteristic parameters of the sorption equation for each food tested are presented.

The equation should be of value in predicting the shelf life behaviour of food products at different storage temperatures.

Introduction

A fundamental characteristic of food materials which influences almost every aspect of the dehydration process and the storage stability of the dried product, is its water sorption isotherm. For this reason, equations for fitting water sorption isotherms are of special interest in the prediction of drying phenomena (King, 1968), in predicting nutrient retention during dehydration (Labuza, 1972), or in predicting the shelf life of a dried product in a packaging material (Karel, Mizrahi & Labuza, 1971; Labuza, Mizrahi & Karel, 1972). Several mathematical equations have been reported in the literature for describing water sorption isotherms of food materials, and Labuza (1968); Agrawal, Clary & Nelson (1969), more recently Nellist & Hughes (1973) reviewed the applicability of most of them. Each of the models reported, empirical, semi-empirical or theoretical, have had some success in reproducing equilibrium moisture content data. However, only a few of them have been able to describe adequately the temperature dependency of the isotherm. One of the earliest models relating temperature, water activity and moisture content is that of Henderson (1952),

$$1-A_w = \exp\,(-g.T.\,X^n) \qquad (1)$$

where A_w = water activity = p/p_o, g, n are constants, X = moisture content, dry basis, and T = temperature.

However, it was later observed that the term T in eqn (1) does not eliminate the temperature dependency of constants g and n (Pichler, 1957; Day & Nelson, 1965;

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Agrawal *et al.*, 1969; Fito & Sanz, 1974). Day & Nelson (1965) omitted the term T in Henderson's equation and related constants g and n to an empirical power function of temperature. The proposed four parameter equation is

$$1 - A_w = \exp\ (-j_1\ T^{h_1}.X^{j_2 T^{h_2}}) \tag{2}$$

where j_1 and j_2 are constants and h_1 and h_2 are constants.

Chung & Pfost (1967) proposed a model of the form,

$$\ln A_w = \frac{-A}{RT} \exp\ (-B\ X) \tag{3}$$

where A and B are constants, but as noted by Agrawal *et al.* (1969) and Nellist & Hughes (1973) the use of the RT term does not eliminate the temperature dependency of constants A and B.

Chen & Clayton (1971) proposed a four parameter equation,

$$A_w = \exp\ (-k_1\ T^{m_1} \exp\ (-k_2\ T^{m_2}\ X)) \tag{4}$$

where k_1, k_2 are parameters and m_1, m_2 are parameters, which when applied to some cereals showed a better fit than that of Day & Nelson's (1965).

The objective of this study was to develop a simple equation for relating equilibrium moisture content, water activity and temperature. For this purpose, a two-parameter multilayer adsorption equation developed by Halsey (1948) was empirically modified to take into account the effect of temperature.

Development of modified equation

Recently, we have shown (Iglesias, Chirife & Lombardi, 1975) that a multilayer adsorption equation originally developed by Halsey (1948), could be used to describe the water sorption behaviour of a very wide variety of foods and food components. Halsey's equation is

$$p/p_o = \exp\left(-\frac{a}{RT}\theta^{-r}\right) \tag{5}$$

where a and r are parameters, $\theta = X/X_m$, X = equilibrium coverage, gram sorbed/100 g solid, and X_m = monolayer value, same units as X.

For purposes of curve fitting, eqn (5) may be put in the form,

$$\ln \ln p_o/p = -r \ln \theta + \ln \frac{a}{RT}. \tag{6}$$

It was previously observed that eqn (6) did not permit the mathematical prediction of the temperature dependency of the isotherm, when applied to various food materials. Consequently, the term RT was not taken into account and the following equation

was proposed (Iglesias *et al.*, 1975):

$$\ln \ln p_o/p = -r \ln \theta + \ln a' \tag{7}$$

where $a' = a/RT$, with both parameters, a' and r depending on product characteristics and temperature. A plot of ln ln p_o/p versus ln θ should be a straight line from which the parameters r and a' may be calculated. If X_m is not desired to be used, eqn (7) will also fit the experimental data yielding a different value for parameter a''.

Eqn (7) would be more useful in some cases if it had a built-in temperature factor. This is done in the following way.

For the purposes of generality, Eqn (7) is written as,

$$\ln \ln p_o/p = -r \ln X + \ln a'' \tag{8}$$

where X replaced θ. In this way, eqn (7) becomes independent of the monolayer value, which it has been shown (Iglesias & Chirife, 1975) to be temperature dependent. The first attempt to use eqn (8) for predicting the effect of temperature could have been to suppose both parameters a'' and r, as an empirical function of temperature, as it was done by Day & Nelson (1965) with Henderson's equation, and Chen & Clayton (1971) with Chen's equation. A simplified approach was tried here. In this, parameter r is held as a constant for each material, allowing a'' to become temperature dependent. On applying eqn (8) to a number of literature data of sorption isotherms at several temperatures, the parameter r is now held constant for each product. The value of r was chosen at an intermediate temperature when only three isotherms were available, or at 25 °C when more than three were investigated. Parameter a' was calculated using a linear regression program in an IBM 360/50. 128 K computer. The results are shown in Table 1 characterized by letter A. Analysis of the results, shown in Table 1, indicates that parameter a' may be related to temperature by an empirical function of the form,

$$a'' = \exp (bT + c) \tag{9}$$

where T = temperature (°C) and b and c are constants.

Eqn (9) may be written,

$$\ln a'' = bT + c \tag{10}$$

and constants b and c were calculated by a least squares analysis. Once the values of b and c were obtained, the values of a' were recalculated through eqn (9) and the results are shown in Table 1 indicated by letter B. We have now a resulting expression relating equilibrium moisture content, water activity and temperature,

$$p/p_o = \exp (-e^{bT+c}X^{-r}). \tag{11}$$

A statistical analysis of all values obtained, which is not included here, indicated the goodness of fit for the proposed eqn (11). Calculated and experimental water sorption isotherms for various food materials at several temperatures are plotted in Figs 1 and 2 and show the degree of applicability of the proposed equation.

TABLE 1. Parameters a'' and r for the proposed equation (11)

Product	Specifications	Temperature (°C)	Range of A_w		r	a''	Reference
Chicken, raw	Ads. -freeze dried	5	0·10–0·70	A	1·072	11·03	Wolf, Spiess & Jung (1973)
				B	1·072	11·15	
		45	0·10–0·70	A	1·072	7·461	
				B	1·072	7·181	
		60	0·10–0·70	A	1·072	5·914	
				B	1·072	6·089	
Corn	Desorption	4·5	0·20–0·80	A	2·522	496·8	Chen & Clayton (1971)
				B	2·522	504·7	
		15·5	0·20–0·80	A	2·522	395·3	
				B	2·522	394·5	
		30	0·20–0·80	A	2·522	290·5	
				B	2·522	285·2	
		38	0·20–0·80	A	2·522	235·7	
				B	2·522	238·4	
		50	0·20–0·80	A	2·522	191·3	
				B	2·522	182·3	
		60	0·20–0·80	A	2·522	139·8	
				B	2·522	145·7	
Fish protein concentrate	Adsorption	25	0·20–0·80	A	2·132	72·04	Rasekh, Stillings & Dubrow (1971)
				B	2·132	72·11	
Fish protein concentrate	Adsorption	35	0·20–0·80	A	2·132	62·05	
				B	2·132	61·90	
		42	0·20–0·80	A	2·132	55·56	
				B	2·132	55·63	
Laurel	Adsorption	25	0·05–0·70	A	1·318	10·97	Wolf *et al.* (1973)
				B	1·318	11·18	
		45	0·05–0·70	A	1·318	7·50	
				B	1·318	7·17	
		60	0·05–0·70	A	1·318	5·01	
				B	1·318	5·14	
Nutmeg	Adsorption	5	0·10–0·70	A	1·892	44·03	Wolf *et al.* (1973)
				B	1·892	46·53	
		25	0·10–0·70	A	1·892	32·37	
				B	1·892	29·61	
		60	0·10–0·70	A	1·892	12·97	
				B	1·892	13·42	
Paranut	Adsorption	5	0·10–0·70	A	1·581	6·01	Wolf *et al.* (1973)
				B	1·581	6·33	
		25	0·10–0·70	A	1·581	4·61	
				B	1·581	4·25	
		60	0·10–0·70	A	1·581	2·05	
				B	1·581	2·12	

TABLE 1 *continued*

Product	Specifications	Temperature (°C)	Range of A_w		r	a''	Reference
Thyme	Adsorption	5	0·10–0·70	A	1·496	21·43	Wolf *et al.* (1973)
				B	1·496	22·12	
		25	0·10–0·70	A	1·496	17·89	
				B	1·496	17·16	
		45	0·10–0·70	A	1·496	13·66	
				B	1·496	13·31	
		60	0·10–0·70	A	1·496	10·60	
				B	1·496	11·00	
Wheat flour	Adsorption	20·2	0·12–0·89	A	2·259	173·7	Bushuk & Winkler (1957)
				B	2·259	174·1	
		30·1	0·13–0·90	A	2·259	152·9	
				B	2·259	156·8	
		40·8	0·13–0·90	A	2·259	141·1	
				B	2·259	140·0	
		50·2	0·15–0·90	A	2·259	126·1	
				B	2·259	126·8	

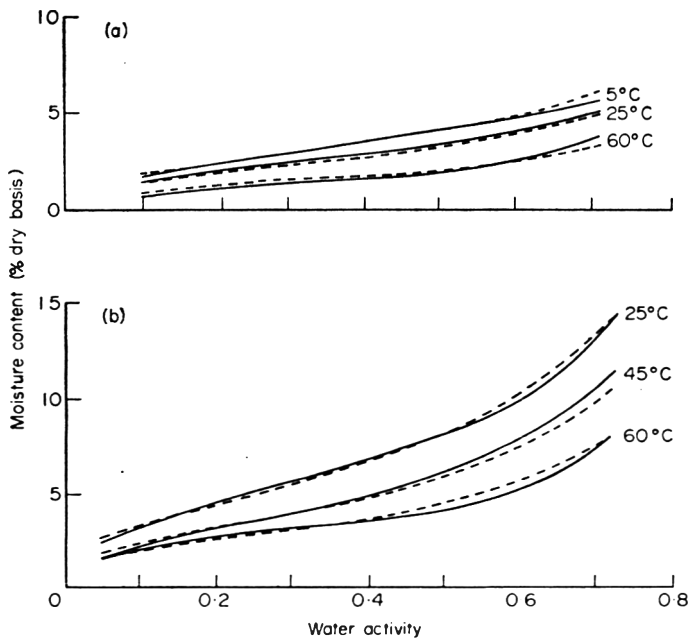

FIG. 1. Comparison of experimental and calculated data: (a) Paranut (Wolf *et al.*, 1973); (b) Laurel (Wolf *et al.*, 1973). ——, Experimental; – – –, calculated.

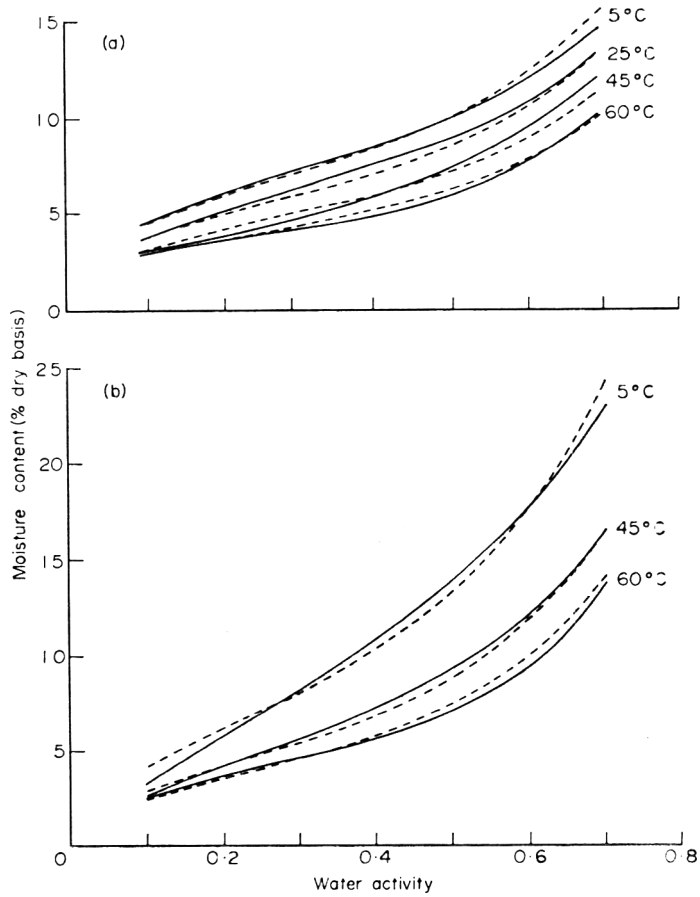

FIG. 2. Comparison of experimental and calculated data: (a) Thyme (Wolf *et al.*, 1973); (b) raw chicken (Wolf *et al.*, 1973). ——, Experimental; – – –, calculated.

Conclusions

It was was found that the following equation

$$p/p_0 = \exp\,(-e^{bT+cX-r})$$

may be used to predict reasonably well the effect of temperature on water sorption isotherms of some food materials and the corresponding values of the constants b and c are shown in Table 2. Obviously, the proposed equation may not be as accurate, and with a wide range of application to both products and water activity, as it could be if both parameters, a″ and r were allowed to be a function of temperature. However, the merits of eqn (11) should be judged considering that it is simpler than the usual

TABLE 2. b and c constants of equation (11)

Product	Specification	b	c
Chicken	Adsorption	−0·0110	2·466
Corn	Desorption	−0·0224	6·324
Fish protein concentrate	Adsorption	−0·0152	4·659
Laurel	Adsorption	−0·0222	2·969
Nutmeg	Adsorption	−0·0226	3·953
Paranut	Adsorption	−0·0199	1·945
Thyme	Adsorption	−0·0127	3·160
Wheat flour	Adsorption	−0·0106	5·373

four-parameter equations reported in the literature (Day & Nelson 1965; Chen & Clayton, 1971). This simplicity comes from the smaller number of parameters which need to be determined in order to characterize the effect of temperature on the isotherms. This is valuable for some engineering calculations where simplicity is required. No conclusion should be drawn about the physicochemical mechanism of water sorption from the observed fitness of eqn (11), because it was empirically modified.

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Comparison of methods of freshness assessment of wet fish

III. Laboratory assessments of commercial fish

J. R. BURT, D. M. GIBSON, A. C. JASON AND H. R. SANDERS

Summary

Samples of cod were obtained during four commercial fishing trips and stored in boxes and in bulk storage. After landing, freshness assessments were made by a sensory panel, two instrumental methods—Torry Fish Freshness Meter and Intelectron Fish Tester V—and two chemical methods—the determination of hypoxanthine and trimethylamine indices.

The results from the chemical methods were in close agreement with those previously obtained on experimental fish. The instrumental methods were strongly affected by the type of storage.

Introduction

In parts I and II (Burt *et al.*, 1975, 1976) results of sensory, instrumental and chemical methods of freshness assessment were reported on fish of known history, caught and stored under closely defined and observed conditions. The present investigations were designed to determine the effect of normal commercial catching and storage conditions on each of the methods. In contrast to the experimental runs reported in Papers 1 and 2, no control was exercised over the catching procedure; all fish were caught and gutted in normal commercial operations. Some fish were boxed under the same conditions that had been used in the experimental runs, other fish were tagged and stored in bulk or shelved. It has been common practice for fish caught during the earlier part of a distant-water voyage to be placed in bulk storage several layers high, while the later catch was stored on shelves in single layers. No distinction is made in the subsequent analysis between these types of storage; both are referred to as bulk storage.

During the experimental runs, fish were caught in as few hauls as possible in order to attain the greatest possible uniformity. They were then stored for different periods of time until tested. To investigate the effect of normal commercial conditions, which include storage on board ship for different lengths of time, the fish were laid down at intervals during the voyage, and after landing were tested within as short a time as possible. For each run testing was completed within two days.

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Materials and methods

Seasons and grounds

Four runs were carried out during 1969 using three trawlers fishing different grounds and landing at different ports (Table 1). Runs C and D were successive trips by one vessel.

Storage

Two boxes of cod of each of two size groups were laid down from one haul on each sampling day. Sampling days were chosen such that six ages-in-ice spread over the whole catching period were obtained. Boxing and icing conditions were the same as for the experimental runs. Except during run B, when only boxed fish was carried, bundles of fish from the same hauls that had been used for the boxed fish were placed in bulk storage. On each sampling day, one bundle of each size group was prepared by tying twelve fish together and attaching an identifying label. The bundles were stored with the remainder of the same haul.

TABLE 1. Origin and disposition of catches

Run	Date of catch	Ground	Port of landing	Place of assessment	Days-in-ice
A	June	Iceland	Grimsby	Hull	6–14
B	September	Shetland	Aberdeen	Aberdeen	2–10
C	September–October	Barents Sea	Hull	Hull	7–17
D	November	Barents Sea	Hull	Aberdeen	8–18

The labelled bundles had to be identified and recovered during normal unloading procedure on the markets. This proved difficult in Hull; in run C, bundles from only four of the six sampling days were recovered, most of those from run D were lost.

Assessment

All freshness assessments were carried out under laboratory conditions in Hull or Aberdeen (Table 1). Fish were transported by lorry to the place of assessment. Testing was carried out on the day of landing and the subsequent day, except in run D, when tests were delayed by one day by the time spent in transit.

The ranges of ages-in-ice of the fish when tested (Table 1) were constrained by the lengths of the voyages from the fishing grounds.

The methods used were the same that had been used on experimental fish: assessment by a sensory panel (Shewan *et al.*, 1953), measurement by Torry Fish Freshness Meter (TFM) (Jason & Richards, 1975), by Intelectron Fish Tester V (IFT) (Hennings, 1963, 1965) with temperature compensation (Gibson & Shewan, 1971) and the deter-

mination of hypoxanthine concentration (Jones *et al.*, 1964; Burt, Murray & Stroud, 1968; Burt, Stroud & Jones, 1969) and trimethylamine concentration (Murray & Burt, 1964; Murray & Gibson, 1972). Hypoxanthine concentration expressed as weight in milligrams per 100 g of fish was converted to hypoxanthine index (HXI), where $HXI = \log_{10}$ (concentration $+5$); trimethylamine concentration as weight in milligrams of nitrogen per 100 g of fish to trimethylamine index (TMI), where $TMI = \log_{10}$ (concentration $+1$).

Preparation and testing were the same as for the experimental fish, except that because of time limitations the sensory panel assessed raw odour (RO) and cooked flavour (CF) only. In contrast to the procedure for experimental fish, fish of different ages-in-ice were assessed at a single session of the panel. Run A was assessed by a panel whose membership was completely different.

TABLE 2. Standard deviations of boxed fish

	RO	CF	TFM	IFT	HXI	TMI
Within-box-layer	0·35	0·29	7·2	9·3	0·065	0·122
Between-boxes	See text		1·6	2·4	0·029	0·043

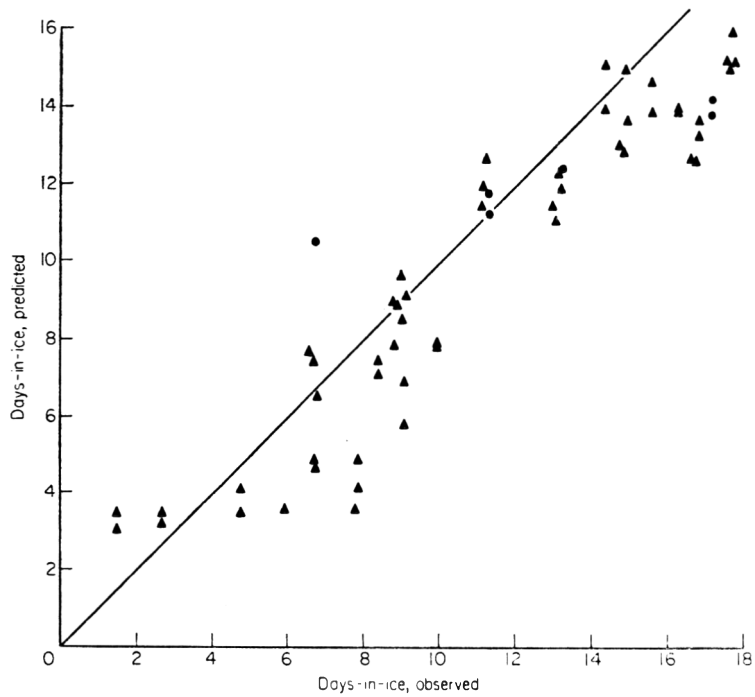

FIG. 1. Ages-in-ice predicted from raw odour scores and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

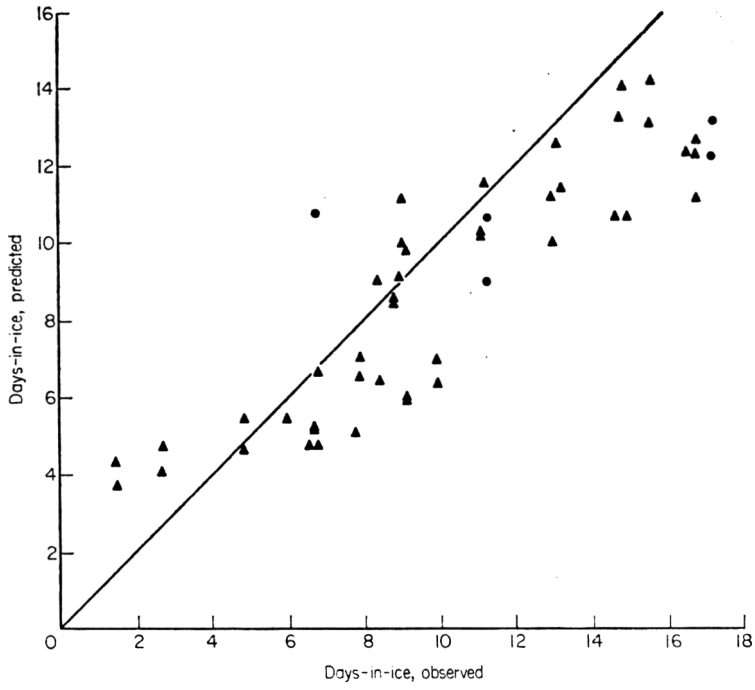

FIG. 2. Ages-in-ice predicted from cooked flavour scores and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

Each fish was tested by TFM and IFT, six fish from each batch (box or bundle) by HXI, TMI and RO, and three fish by CF. The boxes from run B contained few, but very large, fish. When only three fish were in a box they were tested by all methods, otherwise four fish were tested by the chemical and sensory methods.

TMI results for fish stored for less than seven days, when the rate of trimethylamine production is low, were not included in the analysis of results.

Results and discussion

Boxed fish

Within-box-layer standard deviations were calculated for all tests (Table 2). All tests, except TMI, showed an increase over the values for experimental fish. The largest increase (more than 40%) was for RO; this may at least partly be due to the different taste panel procedures mentioned earlier. TMI showed a decrease.

Between-box standard deviations were significant for all non-sensory tests, including HXI which had no significant deviation for experimental fish. The sensory tests had significant deviations only in run A, 0·26 for RO, 0·50 for CF. Pooled values have therefore not been calculated for these tests.

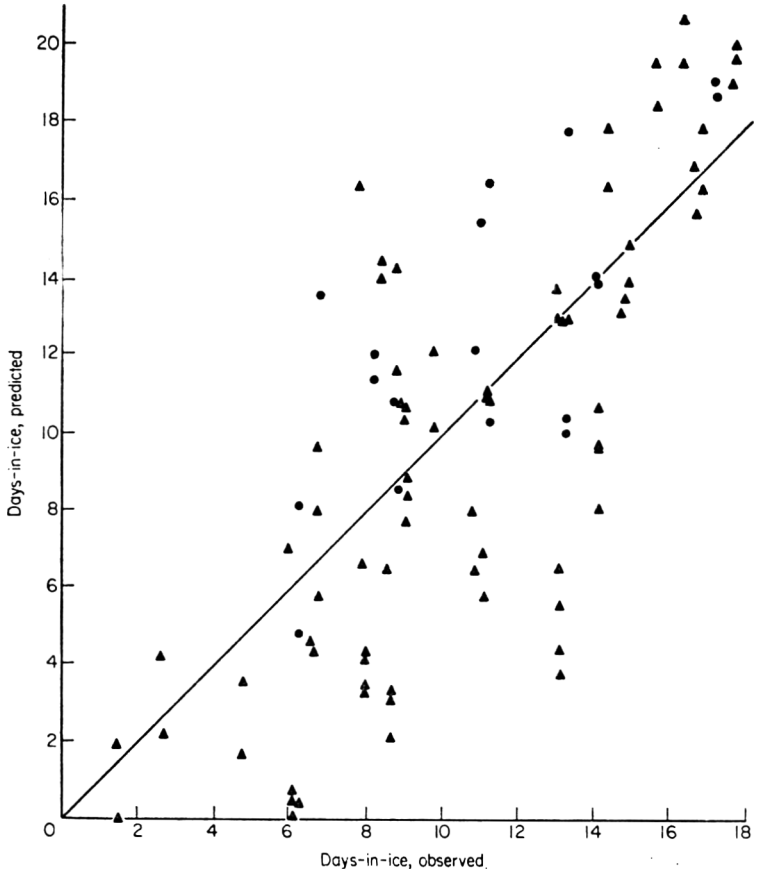

FIG. 3. Ages-in-ice predicted from Torry Fish Freshness Meter readings and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

Data for a full analysis of variance were available only for run A. The results were in general agreement with those on experimental fish. Size effect was significant only for the chemical tests but not for the instruments, layer effect for the instruments but not for the chemical tests. For RO, layer but not size effect was significant, in contrast to the results for experimental fish.

Predictions of age-in-ice were made for each box from the box means using the regression parameters obtained on experimental fish. For each test, the predicted ages-in-ice have been plotted against the observed ages-in-ice (Figs 1–6). Run A, with its different sensory panel, has been omitted from the plots of sensory tests.

For a comparison of the separate runs, mean deviations of observed from predicted ages-in-ice have been calculated (Table 3). Differences between runs are least for the sensory and the chemical tests, highest for TFM. Chemical tests are best at predicting

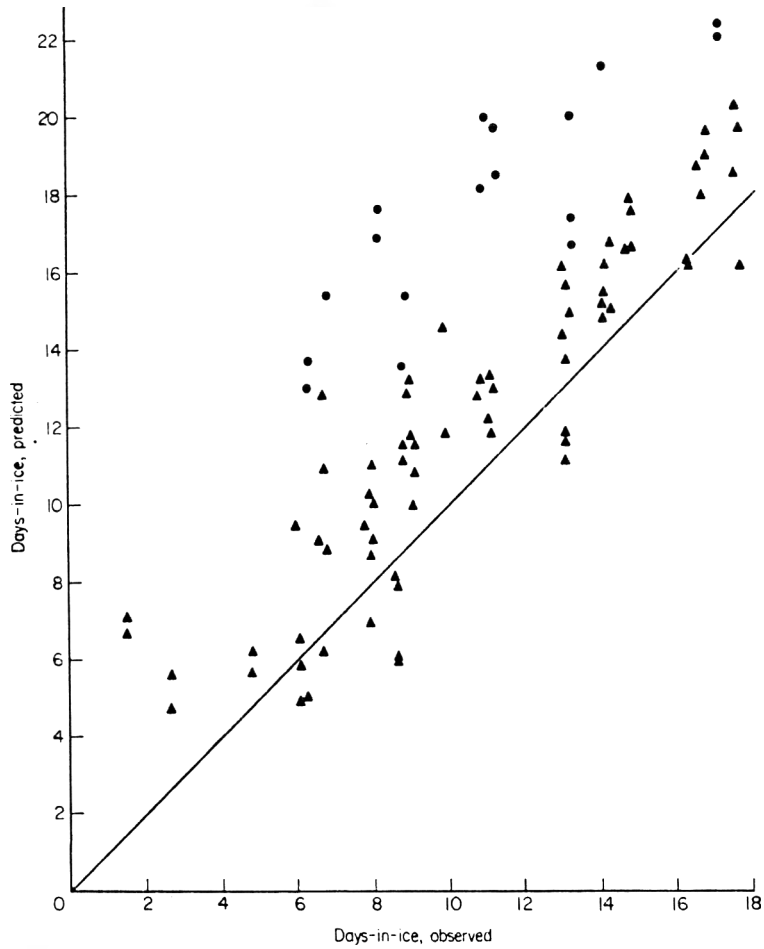

FIG. 4. Ages-in-ice predicted from Intelectron Fish Tester V readings and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

ages-in-ice from the relationships obtained on experimental fish. Sensory tests show larger deviations and in particular under-estimate the ages-in-ice of fish stored longer than about fourteen days and over-estimate the age of very fresh fish. IFT tends to over-estimate ages-in-ice at all ages: for the same age-in-ice lower readings are obtained on commercial fish. This effect may be explained by greater physical damage caused to the commercial fish by rougher treatment. It has been observed that abrasion of the skin lowers the readings of IFT and TFM. In run A, for unexplained reasons, TFM gives higher readings than expected.

The difference in behaviour of the different tests precludes the direct use of the calibration relations between the tests which were obtained on experimental fish.

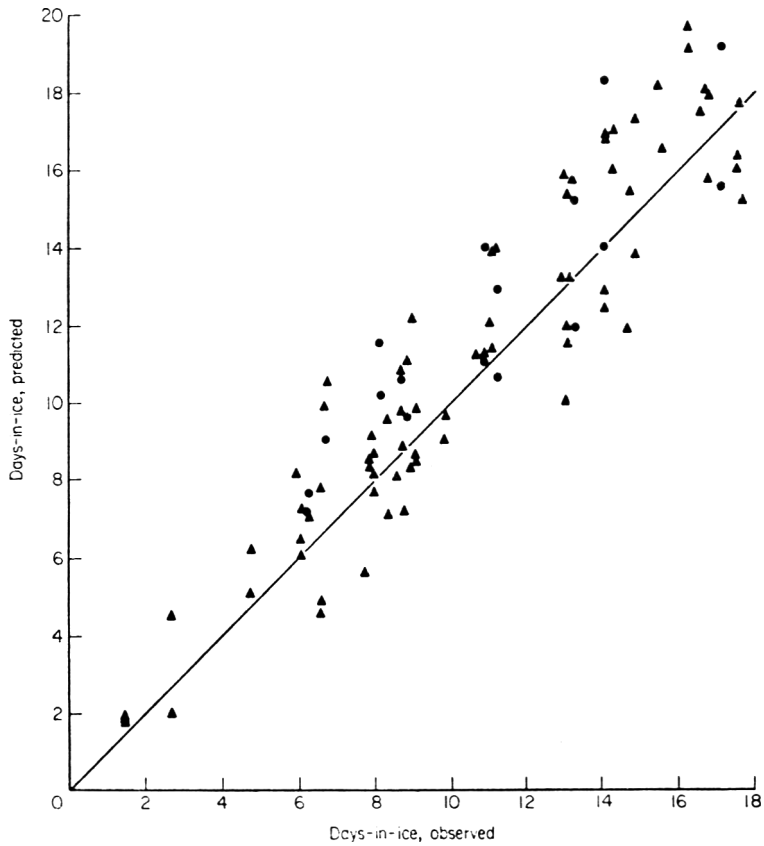

FIG. 5. Ages-in-ice predicted from hypoxanthine index and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

Regression analyses with age-in-ice as the independent variable were carried out for the separate runs and the regression coefficients, which express the change in reading or index for one day increase in storage time, were calculated. For the non-sensory tests the regression coefficients were smaller for commercial than for experimental fish. Values pooled over all runs are shown in Table 4. The decrease from the values for experimental fish is least for TMI (2%) and most for TFM (16%). No figures are given for the sensory tests: the use of differently constituted sensory panels and the behaviour of the tests at the extreme ends of the age-range preclude the calculation of a single regression coefficient. The sensitivity of a test, defined as the standard deviation expressed in units of equivalent days-in-ice, increases with increasing standard deviation and decreasing regression coefficient. For all tests except TMI sensitivities are thus larger than for the experimental fish and larger sample numbers are consequently needed. Table 4 also lists the sample numbers required to estimate batch means to within the equivalent of ± 1 day-in-ice with 95% confidence.

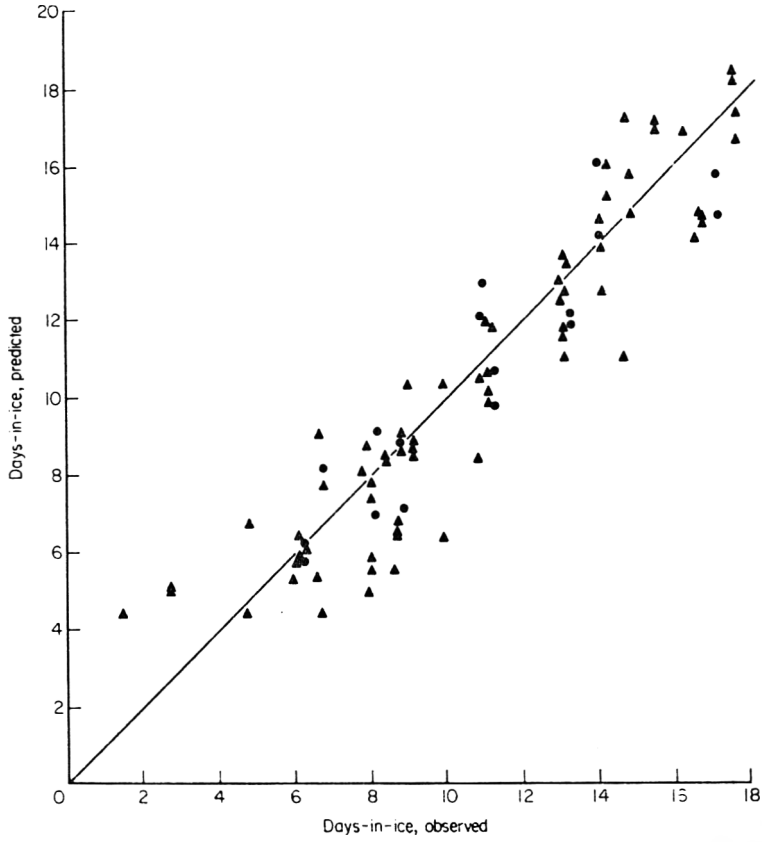

FIG. 6. Ages-in-ice predicted from trimethylamine index and observed ages-in-ice. ▲, Box; ●, bulk; ———, line of perfect agreement.

Bulked fish

Within-batch (bundle) standard deviations did not differ significantly from the within-box-layer standard deviations, except for TFM with a pooled value of 8·9. This increase is greater than is accounted for by the layer-effect.

There were significant differences between the means of corresponding batches in boxes and bulk for all tests in the two runs for which sufficient bulk samples were recovered with the exception of TMI, run C. The bulked fish showed greater spoilage than the boxed fish, except for HXI, run C. The means of the differences have been converted to equivalent days-in-ice (Table 5) by the use of the regression coefficients on experimental fish. The differences were greatest for the instruments (4 to 6 days). The sensory tests showed differences of 1 to 3 days; the advantage of boxing over bulking had previously been estimated at 1 to 1½ days increase in overall storage life under commercial conditions (Anon, 1964).

TABLE 3. Mean deviation (observed–predicted), days-in-ice

Run	RO	CF	TFM	IFT	HXI	TMI
Boxed						
A	(3)	(5)	5	0	0	1
B	1	1	0	−3	0	*
C	1	2	0	−3	−1	0
D	2	1	−4	−1	0	0
Bulked						
A	(0)	(4)	−1	−7	−2	0
C	1	2	−3	−7	0	1

Figures in parentheses were obtained by a differently constituted sensory panel.
* Run B, which contained mostly fresh fish, was excluded from TMI calculations.

TABLE 4. Regression coefficients, sensitivities and sample numbers

		TFM	IFT	HXI	TMI
Regression coefficient	Boxed	−2·5	−3·9	0·039	0·110
	Bulked	−1·9	−3·1		
Sensitivity	Boxed	3·0	2·5	1·8	1·2
	Bulked	4·8	2·4		
Sample number	Boxed	34	24	13	5
	Bulked	87	42		

TABLE 5. Mean difference between boxed and bulked fish, equivalent days-in-ice

Run	RO	CF	TFM	IFT	HXI	TMI
A	3	1	6	6	1	1
C	1	1	4	5	−1	0

Figures 1 to 6 also show the predicted ages-in-ice of bulked fish plotted against the observed ages; Table 3 lists the mean deviations. Any discrepancy between Tables 3 and 5 is caused by the fact that Table 3 is calculated for all boxes and Table 5 only for those boxes for which a corresponding bulked batch was recovered.

The regression coefficients of bulked fish were lower than those of boxed fish for the instrumental and sensory methods, thus increasing the sensitivities and sample numbers still further (Table 4).

The chemical tests are again least affected by the difference in treatment and the relationships obtained on experimental fish can be applied to both boxed and bulked commercial fish.

Conclusions

The relationships between the chemical indices (hypoxanthine and trimethylamine) and storage time obtained on cod caught and stored under controlled experimental conditions are closely followed in fish caught commercially, stored in boxes or in bulk storage. Differences between commercial runs are small and the same relationships can be used under all these conditions.

Instrumental readings (Torry Fish Freshness Meter and Intelectron Fish Tester V) are generally lower—indicating apparent greater spoilage—on commercially caught fish, and lower on fish stored in bulk than on boxed fish. The rate of fall in readings with storage time is less for commercial than for experimental fish, and less for bulked than for boxed fish, thus increasing the sample numbers required for the same precision. The differences between commercial runs are considerable, particularly for the Torry Fish Freshness Meter, so that a given reading may correspond to widely different ages-in-ice in different runs.

Physical damage to the skin and the underlying tissues is the probable reason for the differences in instrumental readings obtained on commercially caught fish. Such damage is not part of spoilage as generally understood, namely autolytic and bacterial decay of the flesh. Instrumental readings on fish which have suffered little or no damage, such as experimental fish, show close relationships with results from chemical and sensory methods. The different behaviour of the instrumental methods does not, however, invalidate their use as methods of freshness assessment, provided that the instruments are calibrated appropriately to the kind of fish to be tested.

The sensory tests are less discriminating on commercial fish than on experimental fish, but no firm conclusions can be drawn on the respective contributions to this effect from the state of the fish and the different conditions of operation of the sensory panel in the experimental and the commercial situations.

The interrelations between the tests are altered as a consequence of the difference of behaviour in their relationships with age-in-ice. These interrelations are not reproduced here because they add little to the conclusions drawn.

Acknowledgment

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The effects of fibre, starch damage and surfactants on the baking quality of wheat/cassava composite flours

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Summary

Sieving of cassava flour yielded a fraction suitable for use in wheat/cassava bread. The degree of starch damage of the cassava flour had no effect on baking quality. Cassava flour did not exhibit diastatic activity, nor did its presence affect the diastatic activity of wheat flour. However, the addition of 0·25% of malt to the composite flour caused an increase in loaf volume. Partial replacement of wheat flour by cassava flour, therefore, is limited mainly by the consequent deficiency of diastatic enzymes. Calcium stearyl lactylate, at 2/7% of flour weight caused a significant increase in loaf volume, but the addition of glyceryl monostearate had no effect on loaf volume.

Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the genus Manihot of the natural order Euphorbiaceae. It is widely grown for its edible tubers which serve as a staple food in many tropical countries. In recent years, several workers have carried out investigations aimed at incorporating cassava products into bread (Pringle, Williams & Hulse, 1969; Kim & De Ruiter, 1968; Dendy, Clark & James, 1970). With cassava starch this does not present a serious problem, but bread qualities deteriorate with the addition of about 10% of cassava flour. Cassava flour differs from cassava starch in that it contains other constituents besides starch. It is the purpose of this work to study some of the factors affecting the use of cassava flour in bread.

Materials and methods

Source and preparation of cassava products

Some samples of cassava tubers were supplied by the Federal Department of Agriculture, Moor Plantation, Ibadan. Other samples were purchased from Starch

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Products, Slough. The dried chips were comminuted and milled in a C. & N. Laboratory Mill.

Determination of diastatic activity

Diastatic activity was determined by the A.A.C.C. method (1962a). Damaged starch was assessed by the A.A.C.C. method (1962b).

Baking procedure

Water absorption was determined by a Simon research water absorption meter. The bread-making formulae and the baking procedure are as shown in Table 1. Two methods, Activated Dough Development (ADD) and the Chorleywood Bread Process (CBP) were employed.

TABLE 1. Breadmaking formula and processing procedures

(a) Chorleywood Bread Process (CBP)

Flour	1134 g	(100%)
Yeast (compressed)	26 g	2·2%
Salt	19·9 g	1·8%
Fat	8·6 g	0·7%
Water (approx.)*	680 cc	60%
Ascorbic acid (as 5·75 cc of 1% soln)	50 ppm	0·005%
Bromate (as 2·87 cc of 1% soln)	25 ppm	0·0025%
Soya flour†	4·05 g	0·33%

(b) Activated Dough Development process (ADD) (formula as above, but with the addition of 35 ppm of L-cysteine hydrochloride)

Dough processing procedures

	CBP	ADD
Mixing	5WH/lb dough	1WH/lb dough
Rest	—	15 min
Dough temperature	86–88°F	86–88°F
Dividing	By hand	By hand
Intermediate proof	10 min	10 min
Final proof	*c.* 50 min‡	*c.* 48 min‡
Oven temperature	440°F	440°F
Baking time	40 min§	40 min§

* Cold water is used for CBP. The volume is dependent on the water absorption of the flour.

† Enzyme active, i.e. retaining lipoxygenase activity.

‡ Proving was done to a height of 4 in in the tin. Water was placed in a tray to provide humidity in the proving chamber.

§ No steam injection was employed.

Bread assessment

After baking, the bread was allowed to cool at room temperature and assessed the following day. The loaves were weighed, loaf volumes calculated and, in some cases, the loaves scored for crust and crumb characteristics.

Sieving of flour into fractions

Cassava flour was milled in a C. & N. Laboratory Mill and sieved in a laboratory plansifter. Sieves numbers 24GG, 32GG, 5XX, 10XX and 12XX* were placed one above the other on the plansifter, in order of decreasing pore size, with the coarsest on top. About 100 g of flour was placed on the uppermost (coarsest) of the chosen nest of sieves, and a rubber plansifter ball was placed on each sieve. The plansifter was operated for 5 min. Three fractions were collected: (1) material passing 5XX but retained on 10XX; (2) material passing 10XX but retained on 12XX; and (3) material passing 12XX. About 65% of the total milled whole tuber was in fraction (3).

Milling of cassava flour to different levels of damaged starch

Preliminary experiments showed that cassava tubers milled in a C. & N. Laboratory Mill had suffered starch damage to the extent of about 3%. Milling in the Brabender Quadrumat Junior, on the other hand, caused a high proportion (about 12%) of the starch granules in cassava flour to be damaged.

Results and discussion

Diastatic activity of cassava products

As can be seen from Fig. 1, cassava flour does not exhibit diastatic activity, nor does its presence affect the diastatic activity of wheat flour.

Effect of sieving on the characteristics of bread baked from composite flour

The results presented in Table 2 show that in the ADD Process, the finest fraction (passes 12XX) gave the highest loaf volume, and the coarsest fraction gave the lowest (1390 cc versus 1170 cc). By comparison, the unfractionated cassava flour gave a loaf volume of 1252 cc. The coarsest fractions also gave low loaf scores. The loaf score of composite flours containing 30% 12XX fraction was only slightly inferior to that of wheat/cassava starch (70 : 30) composite. Similar results were obtained in the CBP

* Sieve no.	Meshes per mm	Aperture (mm)	Bolting surface (%)
24GG	9	0·850	60
32GG	12·25	0·611	55
5XX	26	0·250	44
10XX	43	0·129	31
12XX	49·5	0·112	29

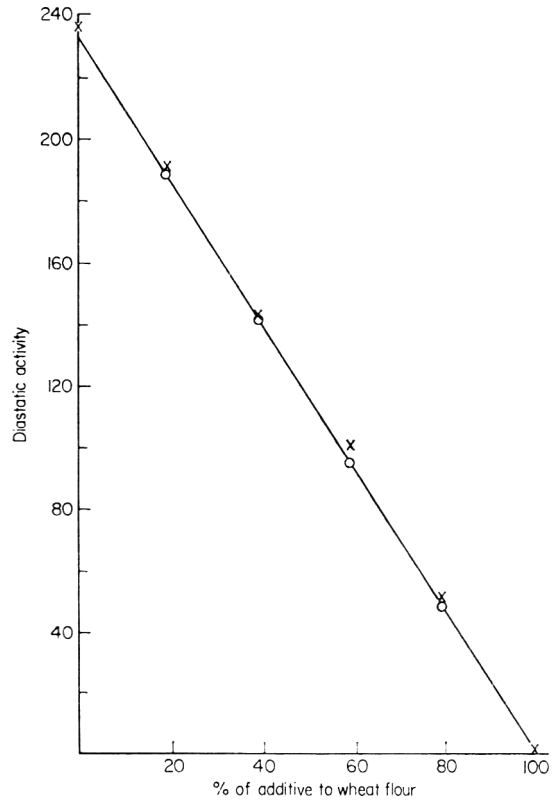

FIG. 1. Diastatic activity of composite flours. ×, Wheat/cassava flour; ○——○, theoretical data for wheat flour with an inert additive.

TABLE 2. Effect of sieving on the characteristics of bread baked from composite flours

	Loaf vol. (cc)	Loaf sp. vol.	% of control	Loaf score*
(a) CBP process				
Manitoba wheat (control)	1515	4·03	100	100
Wheat+30% cassava starch	1435	3·97	98·5	84
Wheat+30% CF (passed 12XX)	1368	3·68	91·3	77
Wheat+30% CF (5XX to 12XX)	Not baked (failed to prove)			
(b) ADD process				
Manitoba wheat (control)	1438	3·65	100	100
Wheat+30% cassava starch	1415	3·58	98·1	87
Wheat+30% cassava flour (CF)	1252	3·21	87·9	64
Manitoba wheat (control)	1495	3·95	100	100
Wheat+30% CF (5XX to 10XX)	1170	2·94	74·4	22
Wheat+30% CF (10XX to 12XX)	1223	3·09	78·2	34
Wheat+30% CF (passed 12XX)	1390	3·59	90·9	76

Process. Loaf scores showed the same trends as loaf volumes but differences were more pronounced.

Microscopic examination of the fractions

Examination of the fractions under the microscope showed that the fraction passing 5XX but retained on 10XX consisted of starch granules and granules embedded in fibrous material. The fraction passing 10XX but retained on 12XX was similar but with a much lower proportion of fibrous material. The finest material (passed 12XX) was virtually free from fibrous material. The coarsest fraction, which had the greatest number of fibres, gave the poorest quality loaf, both in volume and in texture. This suggests that the presence of fibres is largely responsible for the inferior baking quality of cassava flour, compared with that of cassava starch, when they are separately incorporated into wheat flour.

The first fraction (passed 12XX) was virtually free from fibres. Other substances, mainly sugars, proteins, amino acids and lipids, are of course present in this fraction. The possibility that cassava proteins may interfere with wheat gluten cannot be ruled out completely, but this effect will be small compared with that of the fibres.

Effect of starch damage on the volumes of loaves made from wheat/cassava composite flours

The results of baking tests carried out by the ADD process are presented in Table 3. These indicate that the degree of starch damage of the cassava product does not affect the loaf volume of the bread made from composite flours within the range of damaged

TABLE 3. Effect of starch damage on the volumes of loaves made from wheat/cassava composite flours (ADD process)

	Loaf vol. (cc)	Loaf sp. vol.	% of control
(a) CF milled in the Brabender Quadrumat Junior to 13% starch damage			
Manitoba wheat	1575	4·45	100
Wheat + cassava starch (70 : 30)	1337	3·65	81·6
Wheat + CF (70 : 30)	1250	3·56	80·0
(b) CF milled in a C. & N. Mill to 3% starch damage			
Manitoba wheat	1575	4·12	100
Wheat + cassava starch (70 : 30)	1320	3·30	80·1
Wheat + CF (70 : 30)	1260	3·24	78·6

* In assessing 'Loaf score' points were assigned on the following basis: loaf volume, 10; loaf shape, 10; crust colour and bloom, 10; pile, 5; crust colour and sheen, 10; crumb grain, 10; crumb softness, 10; crumb stability, 10; taste and eating quality, 15; keeping quality, 10.

starch contents studied, since differences in loaf specific volumes of 1·4 to 1·5% relative to the respective controls are considered to be within experimental error.

The loaf volumes of breads made from composite flours are less than those from the control wheat. The differences can be attributed mainly to differences in gluten content. The greater the level of cassava product in the composite mixture, the less the level of gluten. In the case of 30% addition, the gluten content drops from 14% in the control to 9·8% in the wheat/cassava composite mixture.

The inferior baking performance of the wheat/cassava composite flours may be explained in part by the role of wheat gluten in bread making. Wheat proteins form a network of films, presumably of lipo-protein complexes, in which starch granules are embedded. These films constitute the walls of gas vesicles which maintain their integrity in the face of carbon dioxide and moisture vapour diffusion. The presence of fibres will interfere with the formation of these films and form weak points in the continuous matrices of the gas vesicles.

A relation between loaf volume and wheat flour protein content has been reported by several investigators (Finney, 1943; Finney *et al.*, 1950; Fiffeld, 1950; Pence & Elder, 1953). It is clear that, for any dough in which wheat gluten is reduced, the loaf volume will also be reduced. Lack of an adequate amount of gluten will also lead to coarse crumb structure.

Effect of surfactants

The effects of various commercial emulsifiers (obtained from Food Industries Ltd) on the performance of composite flours were studied in both ADD and CBP processes. These emulsifiers are already well known to increase the volumes of wheat flour bread significantly, but the present study sought to determine whether this effect could be extended to composite flours. The results are presented in Table 4.

Calcium stearyl lactylate (Admul Lactyl) as a replacement for 2/7 of the fat in the formula brought about a slight increase in loaf volume in the CBP process. Higher levels of CSL did not cause any further increase in loaf volume, nor was any effect obtained by similar substitutions of fat by CSL in the ADD process.

Glyceryl monostearate (Hymono SF/33) as partial replacement for the fat in the formula had no effect on the volumes of breads made from composite flours, either in the ADD or in the CBP process.

Effect of malt and emulsifiers

The addition of malt resulted in a considerable increase in loaf volume, greater than 100 cc compared with the control. An even greater increase could be obtained by the simultaneous part substitution of formula fat with CSL. A similar effect was obtained with a glycerol ester of fatty acids and diacetyl tartaric acid (Admul Data 175) in place of CSL, but in this case the texture of the bread was more open.

The addition of malt led to an increase in loaf volume presumably by furnishing maltose for the enhanced generation of carbon dioxide. As shown in Fig. 1 the cassava products used in this study lacked diastatic enzymes. Cassava used at the 30% level

TABLE 4. Effect of added surfactants made from composite flours

	Shortening/Emulsifier	Loaf vol. (cc)	Loaf sp. vol.	% of control
(a) Effect of calcium stearyl lactylate (CSL) on loaf volumes				
CBP process				
Wheat (control)	8·6 g fat	1388	3·68	100
Wheat/CF (70 : 30)	8·6 g fat	1254	3·24	88·0
Wheat/CF (70 : 30)	7·5 g fat + 1·1 g CSL	1313	3·49	94·8
Wheat/CF (70 : 30)	6·4 g fat + 2·2 g CSL	1318	3·44	93·5
Wheat/CF (70 : 30)	4·3 g fat + 4·3 g CSL	1333	3·44	93·5
ADD process				
Wheat (control)	8·6 g fat	1379	3·69	100
Wheat/CF (70 : 30)	8·6 g fat	1160	3·00	81·3
Wheat/CF (70 : 30)	7·5 g fat + 1·1 g CSL	1175	3·06	82·1
Wheat/CF (70 : 30)	6·4 g fat + 2·2 g CSL	1125	2·94	79·7
(b) Effect of glyceryl monostearate (GMS) on loaf volumes				
CBP process				
Wheat (control)	8·6 g fat	1388	3·68	100
Wheat/CF (70 : 30)	8·6 g fat	1254	3·24	88·0
Wheat/CF (70 : 30)	7·5 g fat + 1·1 g GMS	1259	3·24	88·0
Wheat/CF (70 : 30)	6·4 g fat + 2·2 g GMS	1250	3·22	87·5
Wheat/CF (70 : 30)	4·3 g fat + 4·3 g GMS	1266	3·26	88·6
ADD process				
Wheat (control)	8·6 g fat	1390	3·70	100
Wheat/CF (70 : 30)	8·6 g fat	1150	2·94	79·4
Wheat/CF (70 : 30)	6·4 g fat + 2·2 g GMS	1125	3·02	81·6
Wheat/CF (70 : 30)	4·3 g fat + 4·3 g GMS	1125	2·94	79·4
(c) Effect of CSL or DATA, with or without added malt, on loaf volumes				
ADD process				
Wheat (control)	8·6 g fat	1385	3·68	100
Wheat/CF (70 : 30), no malt	8·6 g fat	1150	2·94	79·9
Wheat/CF (70 : 30), 2·7 g malt	8·6 g fat	1250	3·26	88·6
Wheat/CF (70 : 30), 2·7 g malt	6·4 g fat + 2·2 g CSL	1292	3·36	91·3
Wheat/CF (70 : 30), 2·7 g malt	3·2 g fat + 3·2 g DATA	1340	3·42	92·8

in the dough would therefore bring about a 30% reduction in diastatic enzymes, which could be at least partly compensated for by the addition of malt.

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The histochemical demonstration of soya products in foodstuffs

F. O. FLINT AND Y. A. LEWIN

Summary

A histochemical staining method for showing the carbohydrate constituents of plant tissue was applied to dried soya bean, textured soya proteins and commercial meat and cereal products containing soya. A description including photomicrographs of the cellular material characteristic of soya bean and its products follows. This shows the incorporation of cellular elements in textured soya products and in foodstuffs to which material of soya origin had been added.

Introduction

The use of soya proteins in the food industry is becoming more widespread. They are now employed in many dehydrated, canned and frozen 'convenience foods' both as an inexpensive extender for meat and as a functional ingredient (Rakosky, 1970). Techniques developed over the last fifteen years are used to give the soya bean protein a fibrous structure and 'meaty' texture. The many industrial patents indicate the wide interest in this field (Gutcho, 1973).

Soya materials are available in three main forms as set out in Table 1 (Wolf & Cowan, 1971).

Soya grits and soya flour especially are used extensively by the baking industry and also form key ingredients in cereal, dietary and infant foods (Lockmiller, 1972). Con-

TABLE 1. Composition of soya products

Material	Protein (N × 6·25) (%)	Carbohydrate (%)
Soya flours and grits	40–55	35 (approx.)
Soya concentrates	65–70	15–20
Soya isolates	90–95	—

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centrates are also used in baked goods but more widely in the meat industry to reduce shrinkage on cooking as well as to increase the protein content.

There are two main methods of manufacturing textured products from soya protein (Wolf & Cowan, 1971). The first involves extruding an alkaline dispersion of soya protein isolate through spinnerets into an acid coagulating bath. The resulting fibres are stretched to give them a chewy texture. After the addition of colouring and flavouring materials, the fibres are cooked and products closely resembling meat can be obtained. By varying the orientation of the fibres, meat, seafoods, nuts and vegetables can be simulated (Robinson, 1972).

In the second process a mixture of soya flour, water and flavourings is subjected to heat and pressure then extruded into the atmosphere. If this is done under reduced pressure, an expanded product is obtained. This product can be made much more cheaply than the spun protein because the less expensive soya flours and grits are used. For a low cost meat substitute in such products as soups, comminuted meat products and 'convenience foods' expanded soya products are normally used in preference to the more expensive spun soya proteins. The addition of soya protein to meat products increases the protein nitrogen value without contributing to the meat content. Although it is a legal requirement for food manufacturers to declare this addition it is also possible for a manufacturer to take advantage of the economy of soya protein without declaration.

In the publicity which soya products have received there has been some emphasis on cheapness and a consequent tendency to associate soya protein with low quality meat products. In fact foods containing soya protein could well have a higher nutritional value than similarly priced products which contain a high proportion of skin or other connective tissue. A method of demonstrating the presence (or absence) of soya material is therefore desirable.

Spun soya products are readily identified by their regular fibrous appearance (Coomeraswamy & Flint, 1973). Soya flours, grits and concentrates can be identified by the cellular material they contain. Where the cellular material is in an amorphous state the full periodic acid Schiff technique (McManus, 1946) as described in standard histochemical textbooks (Chayen, Bitensky & Butcher, 1973) should be followed carefully to avoid false positive results. This staining of carbohydrate material in cell debris present in T.V.P. is well illustrated in Linke (1969). Where the technique is used to demonstrate morphological detail a shortening of the standard histochemical method by the omission of sulphite rinses may be justified. The method of Coomaraswamy & Flint (1973) is such an abbreviated technique and in the present work it was further shortened by the omission of the fixation stage for all the soya containing products.

Materials and methods

To provide authentic material of soya origin dried soya beans and samples of three different commercial soya products were used. These included 'Imitation bacon bits'

and 'Hamburger granules' made solely from soya flour and a textured protein product containing wheat gluten and egg albumen in addition to soya flour. The products chosen to show soya material associated with meat included two dehydrated convenience meals described as 'Beef Italienne' and 'Beef curry' and a frozen minced beef product. The cake chosen to show the use of soya flour in baked goods was a sucrose free fruit cake marketed for diabetics.

All the samples except the soya bean were rehydrated and without fixation fresh frozen sections (10 μm thick) were cut at $-18°C$ in a cryostat. This method was not successful for the rehydrated soya bean which proved too friable for sectioning. It was necessary to fix the rehydrated soya bean in formol saline (4% w/v aqueous formaldehyde containing 0·9% sodium chloride) and to embed in 25% gelatine. The method used for this is described in standard textbooks of histological technique, e.g. Culling (1963).

Staining technique

The method used to show the presence of soya elements depends on the histochemical demonstration of the carbohydrate fraction using a modified version of the periodic acid Schiff (PAS) technique (McManus, 1946). The soya bean contains virtually no starch but its cell walls are rich in polysaccharide material and the histochemical demonstration of this provides a ready means of positively identifying cellular material derived from the different parts of the bean. The staining schedule was that of Coomaraswamy & Flint (1973). In all cases protein was counterstained with the acid dyestuff light green (C.I. No. 42095).

Results

Figure 1 shows a cross-section of the soya bean seed coat and a portion of the cotyledon. It has been included for comparison with the photographs of soya bean sections. Plate 1(a) and (b) show part of a section of gelatine embedded soya bean (stained PAS/Light green). The outer epidermal layer of palisade cells can be seen. The underlying layer of hourglass cells is shown more clearly in Plate 1(b). These cells have a tendency to separate from each other and from the adjoining layers. The indistinct region beneath the hourglass cells consists of several layers of compressed parenchymous cells. The next single layer of cells is the aleurone layer. This is intact and clearly visible in Plate 1(a) and (b). The cell walls are thick and heavily PAS positive. The aleurone layer and the underlying layer of thin-walled compressed cells make up the endodermis (characteristic of the soya bean). The cells described so far are all part of the hull or seed coat. The surfaces of the cotyledons are covered with an epidermis made up of small cubical cells filled with aleurone. This layer can be seen in Plate 1(a) and (b) but is broken in several places. The bulk of the cotyledon is made up of thin-walled elongated cells containing protein and oil.

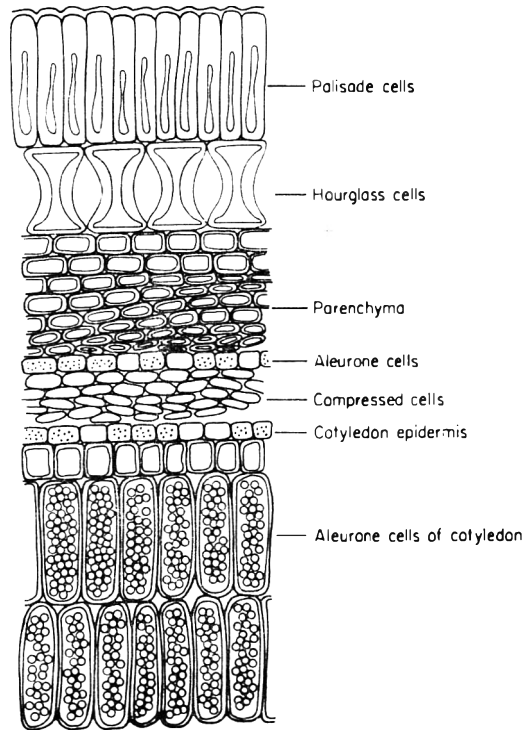

FIG. 1. Portion of soya bean, cross-section (from Markley, 1950).

Imitation bacon bits (*Plate* 1(*c*))

This product is described as containing soya in the form of soya flour. All the sections examined contained large fragments of cellular material. Plate 1(c) demonstrates the expanded nature of the product and includes a particle of the hull from a soya bean. The outer layer of palisade cells and a few associated hourglass cells can be seen and below this the layers of cells which form the seed coat.

Hamburger granules (*Plate* 1(*d*))

This product also prepared from soya flour is much more compact than the textured bacon bits. Much of the PAS positive material is amorphous and the particles of obvious cellular origin tend to be smaller than those found in the bacon bits product. All the sections showed small groups of palisade cells. One such group can be seen in the centre of Plate 1(d).

Textured product containing soya protein (*Plates* 2(*a*) *and* (*b*))

In addition to soya protein this sample was known to contain wheat protein and egg albumen. The texture is intermediate between the rather dense 'Hamburger granules'

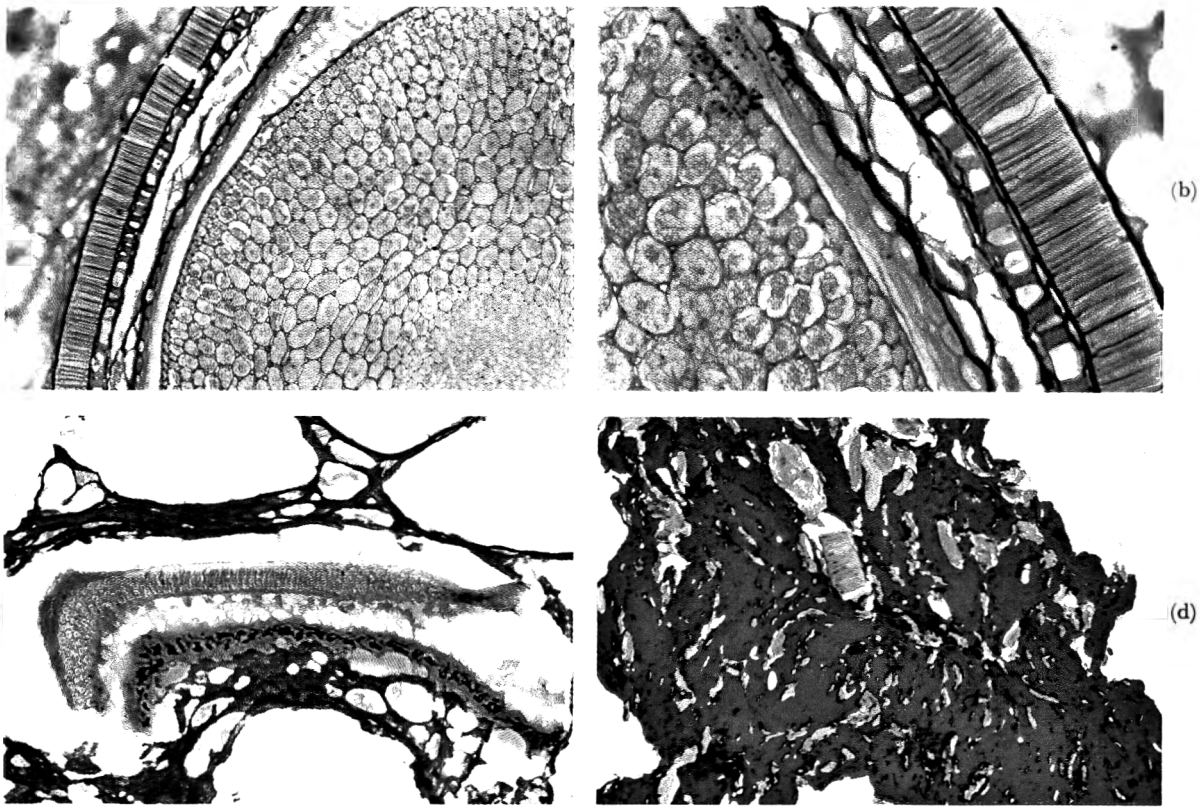

PLATE 1. (a) and (b) Section of gelatine embedded soya bean (stained PAS/light green) ((a): ×100, (b): ×235); (c) Imitation bacon bits (×100); (d) Hamburger granules (×100).

(*Facing p.* 140)

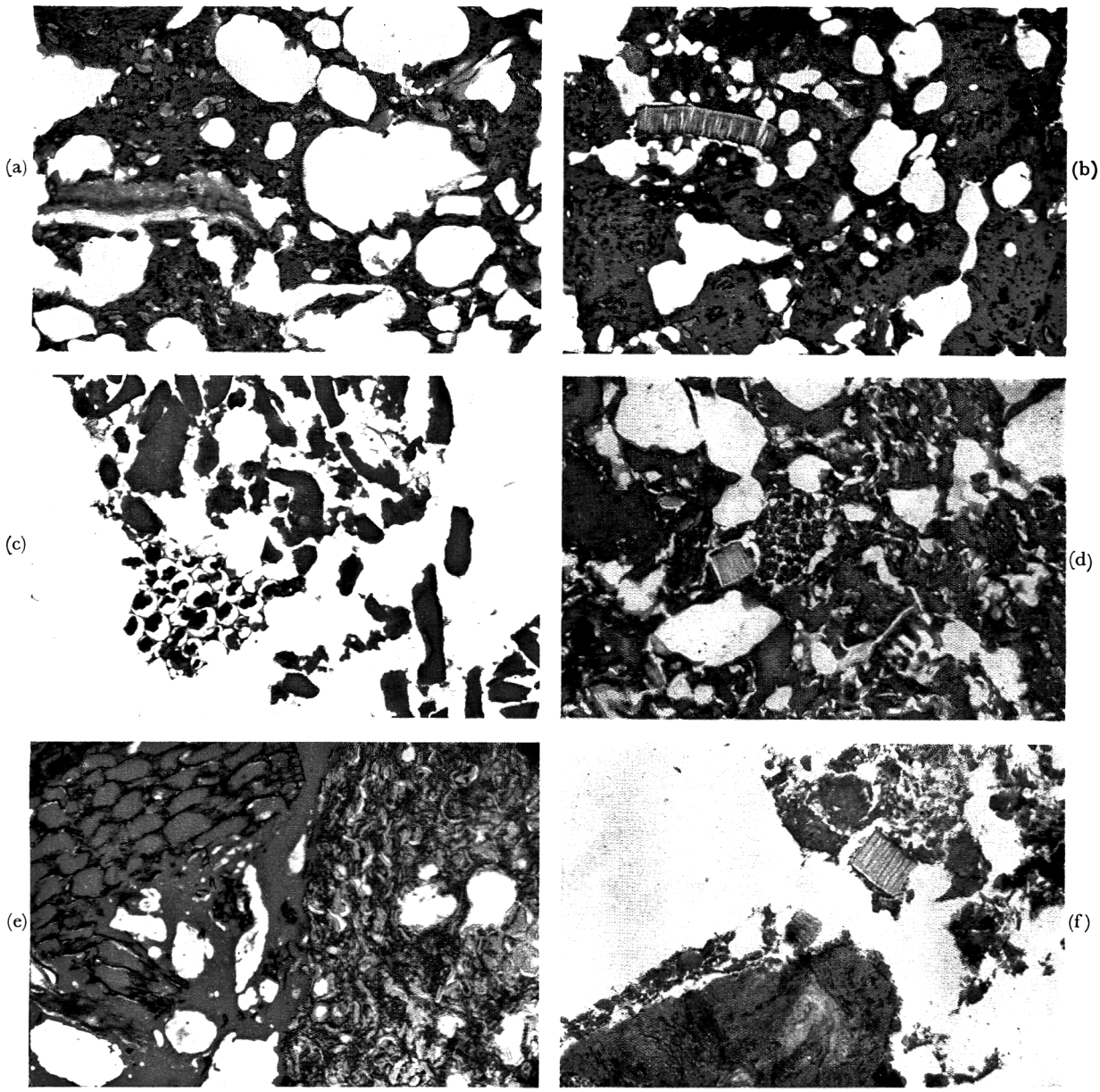

PLATE 2. (a) and (b) Textured protein product containing soya (× 100); (c) Convenience meal 'Beef Italienne' (× 100); (d) Convenience meal 'Beef curry' (× 100); (e) Frozen comminuted meat product (× 100); (*f*) Diet cake containing soya flour (× 100).

and the expanded 'Bacon bits'. Isolated cellular particles and much amorphous carbohydrate material were seen. Plate 2(a) shows some of the compressed cells of the endosperm and Plate 2(b) shows a small group of alisade cells.

Convenience meal 'Beef Italienne' (Plate 2(c))

Evidence of soya material was found in one sample of this product but none was found in a second sample purchased at a later date. This may have been due to experimentation with ingredients by the manufacturer or insufficient sampling for microscopy. Plate 2(c) shows some of the larger protein containing cells of the cotyledon present in the first sample.

Convenience meal 'Beef curry' (Plate 2(d))

The sample of 'Beef curry' sectioned contained a large amount of PAS positive material, a high proportion of which was in a cellular form. At least three distinct cell types can be seen in Plate 2(d): centrally aleurone cells of the cotyledon and palisade cells are present and to the lower right of these cotyledon epidermal cells.

Frozen minced beef product 'Steaklet' (Plate 2(e))

This product contained isolated groups of soya cells including palisade cells and cotyledon cells and also rusk, the starch constituent of which is PAS positive. Plate 2(e) shows a group of the cotyledon cells on the left and a large fragment of rusk showing the gelatinized but still recognizable starch granules on the right-hand side.

Diet cake containing soya flour (Plate 2(f))

The sugar free cake contained much PAS positive material, most of it being partially gelatinized wheat starch, but here and there small groups of soya cells. Palisade cells and aleurone cells were most abundant. Plate 2(f) shows two groups of palisade cells and clusters of PAS positive cooked starch granules.

Discussion

The carbohydrate materials of the soya bean may be demonstrated in foodstuffs, which contain soya flour, by the periodic acid Schiff staining technique. The palisade cells are the most common form detected in foodstuffs. In products consisting mainly of textured soya flour, large fragments of cellular material are frequently found, e.g. 'Bacon bits'. In foodstuffs in which soya flour is not the major ingredient smaller groups of soya cells are observed, e.g. the diet cake and the range of meat products examined.

As well as palisade cells, hourglass cells and cells of the aleurone layer and cotyledon were seen in some of the samples. The palisade cells and cotyledon cells were the most distinct and easy to recognize. The characteristic hourglass cells were only observed in the Bacon bits samples.

The PAS method is a histochemical technique for demonstrating carbohydrates and when used for histochemical purposes precautions are necessary to prevent reoxidized fuchsin giving false positive results. The method used here bases the identification of soya material on its morphology and so a simplified version of McManus method is appropriate. The method is only applicable to soya flours, grits and extrudates or to products which contain them. Spun protein does not contain cellular material and cannot be detected in this way.

There is a greater need to demonstrate the presence of soya flour and grits in foods because these are cheap source of protein and more likely to be added to low cost comminuted products as a substitute or extender for meat. The higher cost of soya protein isolates will itself inhibit the misuse of these products. With the present world food shortage and the high cost of meat in developed countries the use of proteins from soya beans must increase. It would be convenient to have a method of detecting soya protein *per se*. This can be approached by studying the chemical make-up and structure of soya protein molecules and the development of a histochemical staining technique specific for this unique protein. Current work has this aim.

Acknowledgments

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J. Fd Technol. (1976) **11**, 143–159.

Methylamino acids as indices in meat products

I. The development and validity of an analytical procedure

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Summary

A method is suggested for determining the 3-methylhistidine and N^{ε}-methyllysine content in meat and meat products using an ion exchange chromatography technique. 3-methylhistidine titres showed less variation within a sample than titres of N^{ε}-methyllysine. Concentrations of both 3-methylhistidine and N^{ε}-methyllysine in beef, lamb, pork and whale hydrolysates are given. In beef and lamb, the 3-methylhistidine concentrations have a mean value 6·0 ($\pm$ 0·7) mg/g N. The concentrations in pork varied between 5 and 56 mg/g N, an increase in concentration being related to an increase in age, whilst those of whalemeat were consistently high (>300 mg/g^{N}). A dipeptide of 3-methylhistidine and β-alanine, as found in whalemeat extracts, appears to be present in pork and can readily be removed from both pork and whale by a prior washing procedure. The levels of the residual, protein-bound 3-methylhistidine are then similar to those found in beef and lamb. Interspecies variation in N^{ε}-methyllysine is less marked. 3-methylhistidine and N^{ε}-methyllysine were absent from the non-meat proteins analysed.

The similar interspecies value for the 3-methylhistidine bound to actin and myosin is tentatively suggested as an index of meat content in meat products.

Introduction

During the last decade the use of non-meat proteins in meat products has become well established. Although these substitutes may not detract from the nutritive quality, texture or flavour of the product, they could lead to the replacement of expensive animal protein by less expensive protein of vegetable or microbial origin. To inform the consumer as to the nature of the product purchased, the analyst requires specific techniques to ascertain the exact proportions of non-meat and meat protein present.

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Although various methods have been proposed for the detection and determination of non-meat proteins, including serological, chemical, enzymic and electrophoretic procedures (Smith, 1974; Pearson, 1975) their application is limited with processed products due to the effect on the constituent proteins of the severe treatments (e.g. heat) which the product receives. These methods are orientated towards the identification of specific non-meat proteins, and when more than one type of non-meat protein is present, numerous methods for specifically identifying each non-meat protein would be necessary in order to assess meat contents. If, however, meat content could be directly calculated from an assessment of the myofibrillar protein present, the analysis of meat products could be considerably simplified and would be feasible even with those which had been severely processed.

Hibbert & Lawrie (1972) suggested the use of 3-methylhistidine as a possible indicator of meat protein content since it was thought to be a characteristic constituent of the myofibrillar proteins, it was heat stable and was thought to be present at similar levels in the common meat species. 3-methylhistidine was estimated by ion exchange chromatography, and when the method was applied to mixtures of beef and textured soya protein which had been subjected to commercial sterilizing procedures in cans, a satisfactory correlation was found between the 3-methylhistidine content and the percentage of meat in the mixture.

Methylamino acids, however, are not limited solely to muscle proteins. Thus N^{ϵ}-methyllysine has been found in histones and cytochrome c (Park & Kim, 1967; Delange, Glazer & Smith, 1969), and 3-methylhistidine is believed to be present in certain plant material where contractile proteins similar to actin and myosin have been identified. Pollard & Korn (1972) report the presence of both 3-methylhistidine and N^{ϵ}-methyllysine in the presumed actin protein of *Acanthamoeba castellanii* although neither was found to be present in the myosin-like protein. Actin- and myosin-like molecules are now believed to be present in most types of cells within the body, and are probably important for cell mobility and cell division (Adelstein, 1974). However, the concentration of such contractile-like proteins is of an exceedingly low order in comparison with the concentration of myofibrillar proteins in meat, and can be neglected.

3-methylhistidine is thought to be an integral part of the myofibrillar proteins, being found in both actin (Johnson, Harris & Perry, 1967; Asatoor & Armstrong, 1967) and myosin (Johnson & Perry, 1970). The latter suggest that the 3-methylhistidine content of myosin varies somewhat, being rather higher in white skeletal muscle than red skeletal and smooth muscle. It is, however, known to be absent from cardiac and foetal myosins (Kuehl & Adelstein, 1970). The 3-methylhistidine content of actin is thought to be more consistent. Although free 3-methylhistidine is not believed to occur in the sarcoplasm, it is known that 3-methylhistidine may be found combined with β-alanine, as the dipeptide balenine (or ophidine) in whalemeat extract (Carisano, 1964; Cocks, Dennis & Nelson, 1964), and in certain reptiles (Crush, 1970).

N^{ϵ}-methyllysines are present only in the sub-fragment 1 portion of the myosin

molecule, being absent from the actin molecule (Kuehl & Adelstein, 1969; Hardy *et al.*, 1970). The latter suggest that only mono-N^{ϵ}-methyllysine and tri-N^{ϵ}-methyllysine are present, and that di-N^{ϵ}-methyllysine is absent.

The aim of the present study was to investigate further the potential of 3-methylhistidine and N^{ϵ}-methyllysine as valid indices of meat protein content. By slight modification of the ion exchange chromatographic method of Hibbert & Lawrie (1972), it was hoped to ascertain the levels at which 3-methylhistidine and N^{ϵ}-methyllysine occur in mammalian muscle, and whether they represent a sufficiently constant constituent of the meat to permit their use as accurate indices of lean meat protein, specifically differentiating it from proteins from other sources.

Materials

Standard solutions were made up using authentic L-3-methylhistidine and N^{ϵ}-methyl-L-Lysine hydrochloride (Sigma Chemicals). N^{ϵ}-methyllysine was assumed to be mono-N^{ϵ}-methyl-L-Lysine (MW 196·7).

All meat samples used were purchased fresh from either the local butcher or slaughterhouse. Whalemeat samples were donated privately.

Methods

Ion exchange chromatography

Chromatographic separation of the methylamino acids was effected using a 27·5 × 1 cm column of Technicon Chromobeads Type A resin (Hibbert & Lawrie, 1972). Temperature of the column was maintained at 30 °C and elution was achieved using a pH 5·28 sodium citrate (0·35 M Na^{+}) buffer containing B.R.I.J. 35 (Polyoxyethylene lauryl ether) detergent (5 ml 50% per 2-l buffer). Flow rate was maintained at 30 ml/hr, a single chromatographic separation taking 8 hr to complete, using a micrometering pump Series II, containing two buffer delivery units in series, thus allowing two resin columns to be run simultaneously. (These columns will be referred to as column I and column II.)

The samples (2 ml) were pipetted directly on top of the column: these were then adsorbed on to the resin surface, before elution with the buffer. After the passage of 125 ml of eluant, fractions of 1·5 ml were collected by an L.K.B. machine. Colour was developed in the individual fractions using ninhydrin (Moore & Stein, 1954), and the absorption measured at 570 μm.

The columns were maintained by regeneration (0·2 N NaOH) and equilibration with buffer after each chromatographic separation.

Preliminary studies, using the authentic amino acids, were carried out to investigate the effect of column length and pH on the separation of the methylamino acids during

ior. exchange chromatography. A column length of 27 cm was found most suitable, since when using greater lengths, elution took too long, and using smaller lengths, resolution of N^{ϵ}-methyllysine from lysine was not satisfactory. Of the pH values tried, pH 5·28 was most satisfactory since, at pH 5·79, elution of 3-methylhistidine occurred along with ammonia. pH 5·28 allows separation of both N^{ϵ}-methyllysine and 3-methylhistidine: 1-methyllysine elutes prior to histidine. Using these conditions of separation N^{ϵ}-methyllysine eluted after 132 ml, and 3-methylhistidine after 204 ml.

A standard curve was produced for each methylamino acid separated on each column, from known concentrations of the authentic methylamino acids. For 3-methylhistidine all titres were calculated from peak height × width at half-height, and corrected using an external standard (0·1 μmol histidine). For N^{ϵ}methyllysine, however,

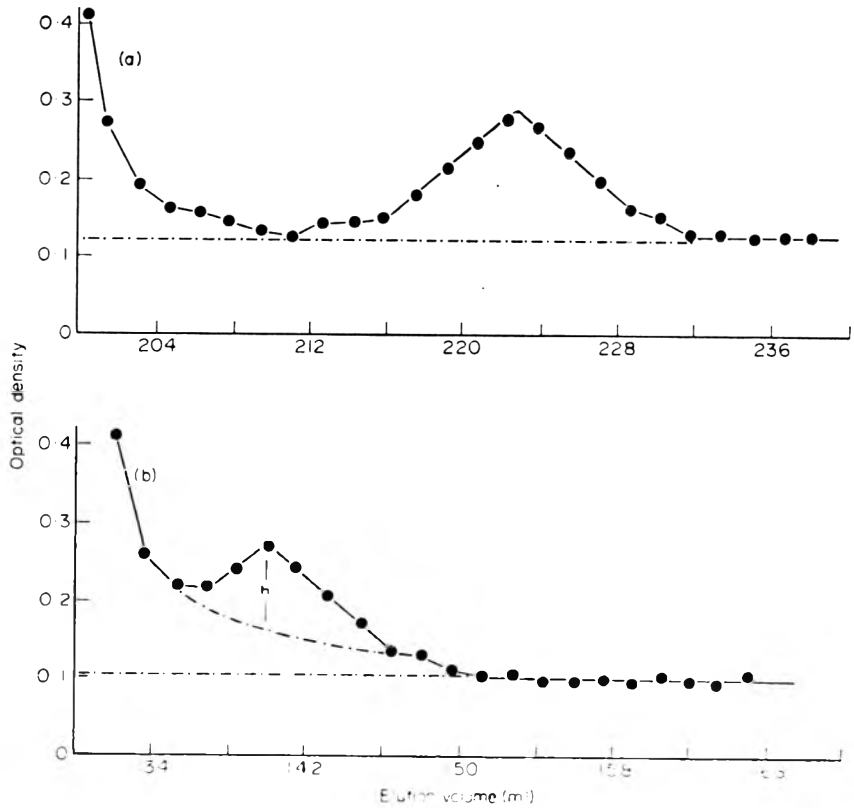

FIG. 1. The chromatographic separation of (a) 3-methylhistidine and (b) N^{ϵ}-methyllysine from a meat hydrolysate using a 27·5 × 1 cm column of Technicon Chromobeads Type A resin, and eluting with a pH 5·28, 0·35 M Na^{+} sodium citrate buffer at a flow rate of 30 ml/hr. Temperature was maintained at 30°C. Analysis was carried out using Column II.

titres were calculated from peak height (h) alone, since overlapping of the N^{ϵ}-methyllysine peak by lysine sometimes occurred (see Fig. 1). The straight line equations for each calibration curve are shown below.

3-methylhistidine	column I	$y = 21{\cdot}0288x - 0{\cdot}8$
	column II	$y = 16{\cdot}528x + 0{\cdot}4$
N^{ϵ}-methyllysine	column I	$y = 38{\cdot}0962x - 1{\cdot}6$
	column II	$y = 17{\cdot}0223x + 1{\cdot}3$

Sample preparation

Most meat samples were obtained from animals within 3 hr post mortem. Excess visible fat was removed from the tissues, and the latter minced. Fat was removed from the minced sample by extraction with hexane, after which the sample was divided for hydrolysis, nitrogen determination and collagen determination. The sample was hydrolysed by placing 1·5 g in 200 ml of 6 N HCl and refluxing for 72 hr. The hydrolysate was filtered twice through Whatman No. 2 filter paper, and evaporated to dryness twice using a rotary evaporator; the hydrolysate was made up to a final volume of 10 ml with distilled water. A 2-ml aliquot of the hydrolysate (~ 70 mg protein) was used for each determination. Results are expressed in milligrams of methylamino acid per grams of nitrogen, where the nitrogen content had previously been corrected for collagen content. This is necessary when large amounts of connective tissue are present in the sample, which would otherwise give a misleading value for total nitrogen.

Nitrogen was determined by macrodigestion and microdistillation (Pearson, 1970). Collagen was quantitatively estimated by the method of Woessner (1961).

Since possible losses could occur during hydrolysis and subsequent volume reduction, known concentrations of both 3-methylhistidine and N^{ϵ}-methyllysine were hydrolysed for 72 hr by refluxing with 200 ml of 6 N HCl, after which the hydrolysate was filtered and reduced to a final volume of 10 ml. A 2-ml sample was applied to the column and analysis for 3-methylhistidine and N^{ϵ}-methyllysine undertaken.

Analysis was carried out on the *longissimus dorsi* muscle from four lambs of the same age, in order to investigate the reproducibility of determinations. Samples were prepared for ion exchange chromatography as previously described, and determinations were made on both column I and column II for comparison.

The effect of interfering compounds on 3-methylhistidine and N^{ϵ}-methyllysine determination

(*a*) *Carbohydrate.* 5%, 10% and 15% sucrose solutions were mixed with an equal volume of minced, defatted beef steak. Hydrolysates were then analysed for 3-methylhistidine and N^{ϵ}-methyllysine.

(*b*) *Salt.* Salt is used extensively in meat products. At high ionic concentration it causes solubilization of the myofibrillar proteins. Since this could lead to loss of 3-methylhistidine and N^{ϵ}-methyllysine during determinations it appeared desirable to

assess the extent to which the procedure might be invalidated. An 18% salt solution was chosen for study, for although only 2–3% salt may be found in sausage meat, concentrations of this order may be used during the curing of pork. Minced, defatted beef (*longissimus dorsi*) muscle was subjected to four treatments, from which hydrolysates were made and analysed for 3-methylhistidine and N^{ϵ}-methyllysine. The four treatments were as follows: (a) minced sample suspended in an 18% salt solution; (b) minced sample suspended in an 18% salt solution and then dialysed; (c) minced sample suspended in distilled water; and (d) minced sample dialysed against distilled water. Excess liquid was removed prior to hydrolysis by vacuum filtration.

The identification and determination of 3-methylhistidine and N^{ϵ}-methyllysine in mammalian muscle

Meat samples were prepared as previously described. Certain meat samples were washed prior to hydrolysis, being extracted by either distilled water or a sarcoplasmic buffer (0·039 M sodium borate buffer, containing 0·025 M potassium chloride, pH 7·1). The minced, defatted meat was placed in 3 vol. (3 × weight of sample in millilitres) of extractant, homogenized for 2 min, and centrifuged at 600 *g* for 15 min to remove excess solution. The treatment was repeated as required. Portions of the residue were then allocated for hydrolysis, nitrogen determination and collagen determination as previously indicated. A 2 ml aliquot of the hydrolysate (10 ml) was analysed for methylamino acid content.

Identification of soluble 3-methylhistidine by paper chromatography

Ten grams of minced sample were stirred in 30 ml of hot water (90 °C) for 30 min. The solution was filtered through Whatman No. 1 filter paper to remove any solid material. Fat and soluble proteins were removed from the filtrate by treatment with hexane and 10% trichloro-acetic acid respectively. The filtrate was again filtered and reduced in volume using a rotary evaporator to a final volume of 5 ml; 4 ml of the latter was employed for quantitative determination (fraction A), and the remaining 1 ml for paper chromatography. The latter fraction was made up to 100 ml and dialysed against distilled water to remove salts. This solution was then evaporated to a final volume of 2 ml (fraction B).

Identification of the dipeptides (and their amino acid constituents) present in the extracts, was carried out using two-dimensional ascending chromatography in a modified Whatman Chromatography tank, a support being placed in the bottom of the tank on which the solvent trough is rested. The solvents used were butanol-acetic acid-water (12 : 3 : 5), followed by an 80% phenol solution buffered at pH 5·28.

From the previously dialysed fraction (B) an (0·1 ml) aliquot was pipetted directly on to the right-hand corner of the 27-cm-square Whatman 3 MM paper, and dried prior to placing the paper in the solvent. Each solvent travelled 21 cm before the paper was removed. All experiments were carried out at room temperature.

The dipeptide spots were located by spraying the papers with a 0·2% solution of ninhydrin in acetone, containing 2% pyridine, and placing in an oven at 110°C. The papers had been previously dried for 48 hr to remove all traces of phenol. Unknown spots were identified by reference to standards, on the basis of spot colour and Rf values.

Once identified, those spots believed to contain methylhistidine were cut out and eluted with 2·5 ml of distilled water, 2·5 ml of 6 N HCl was added and the extract placed in an oven at 110°C for 3 hr. The hydrolysate was evaporated to dryness and applied to the paper. The same solvents were used for development as before. Unknown spots were located and identified by reference to ion exchange chromatography standard amino acids.

One aliquot (2 ml) of the hot water extract fraction (A) was applied directly to the resin column, the other (2 N) being hydrolysed in a sealed tube at 110°C for 8 hr by the addition of 5 ml of 6 N HCl. The hydrolysate was evaporated to 5-ml and a 2 ml sample applied to the resin column.

A hydrolysate was also prepared from minced meat samples after a hot water extract had been made. Aliquots were allocated for hydrolysis, nitrogen determination and collagen determination as previously described. The hydrolysate was rotary evaporated to final volume 10 ml, and a 2-ml sample applied to the resin column.

The assessment of methylamino acids in non-meat proteins

Non-meat proteins were hydrolysed as for meat proteins when in the form of fibres. Powdered proteins, however, were hydrolysed by the addition of 5 ml of 6 N HCl to 0·5 g sample in a sealed tube and then hydrolysed for 72 hr at 110°C. The hydrolysate was filtered, and then reduced in volume, and made up to a final volume (5 ml) with distilled water. A 2-ml aliquot was used for ion exchange chromatography.

Results

From a chromatographic separation of a mixture of authentic amino acids it was found that 3-methylhistidine and N^{ϵ}-methyllysine emerge as distinct peaks from their parent amino acids. When actual meat samples were applied to the column (Fig. 1), 3-methylhistidine still emerged as a separate peak, although the N^{ϵ}-methyllysine peak appeared immediately after lysine, sometimes as a small peak on the trailing edge of the lysine peak. It was found in one lamb (*longissimus dorsi*) sample (Table 5), certain pork samples (Table 6), and certain whale samples (Table 7) that N^{ϵ}methyllysine appeared as two separate peaks, rather than one, as was usually observed. This was assumed to be due to the separation of both N^{ϵ}-mono-methyllysine and N^{ϵ}-tri-methyllysine. When only one peak is present it was thought to be due to either both types of N^{ϵ}-methyllysine eluting together or (when lower readings occurred) to a single species in the latter case. (N^{ϵ}-tri-methyllysine is the most likely since it elutes after N^{ϵ}-mono-methyllysine.)

From the results in Table 1 it can be seen that in both columns less than ±3% losses occur during hydrolysis of a sample. The results in Table 2 show mean concentrations for 3-methylhistidine and N^{ϵ}-methyllysine from four lamb (*longissimus*

TABLE 1. Losses of 3-methylhistidine and N^{ϵ}-methyllysine which occur during hydrolysis

Column I				Column II			
3-methylhistidine		N^{ϵ}-methyllysine		3-methylhistidine		N^{ϵ}-methyllysine	
μmol before hydrolysis	μmol after hydrolysis	μmol before hydrolysis	μmol after hydrolysis	μmol before hydrolysis	μmol after hydrolysis	μmol before hydrolysis	μmol after hydrolysis
0·50	0·49	0·30	0·29	0·50	0·50	0·30	0·31
% recovery							
97·3		97·6		100		103·3	

TABLE 2. The variation of 3-methylhistidine and N^{ϵ}-methyllysine determinations within one sample using two resin columns, and between four similar samples of lamb (*longissimus dorsi*), nine months old

Sample % N (corrected for collagen)	Column	3-methylhistidine		N^{ϵ}-methyllysine		Ratio 3-MeHis.: N^{ϵ}-MeLys.
		Elution vol. ml	mg/g N	Elution vol. ml	mg/g N	
A	II	214	7·06	136	2·87	2·45
3·044%	I	206	6·08	135	2·46	2·47
	II	216	6·29	136	2·83	2·22
	Mean	—	6·48	—	2·72	2·38
		St. D = (±0·50)		St. D = (±0·23)		
B	I	216	7·27	—	—	
3·061%	II	224	6·46	138	2·54	2·54
	I	212	6·35	138	2·46	2·58
	II	214	6·85	138	1·67	4·10
	Mean	—	6·73	—	2·22	3·07
		St D = (±0·35)		St. D = (±0·39)		
C	II	222	6·79	142	1·81	3·75
3·247%	I	212	6·76	140	2·03	3·33
	II	216	6·13	138	2·03	3·02
	I	206	6·74	138	2·55	2·64
	Mean	—	6·60	—	2·11	3·18
		St D = (±0·33)		St. D = (±0·27)		
D	I	212	6·48	138	2·46	2·63
3·039%	II	218	6·49	138	2·21	2·94
	I	208	6·54	135	2·36	2·77
	II	218	6·36	140	1·53	4·16
	Mean	—	6·47	—	2·14	3·13
		St. D = (±0·06)		St D = (±0·36)		

dorsi) samples of the same age, after four (2 ml) samples of the same hydrolysate (10 ml) have been analysed, two samples being analysed using column I and two using column II. Comparison of the 3-methylhistidine titres for each sample, show that differences between results, using different columns, appeared minimal. However, variations of 10% occurred between titres using the same column. Only in lamb (*longissimus dorsi*) D is the standard deviation of the mean titre less than $\pm 0{\cdot}1$. Comparison of mean concentrations between the four lamb (*longissimus dorsi*) samples gives a standard deviation of $\pm 0{\cdot}1$, for a mean of 6·57 mg 3-methylhistidine/g N. Comparison of N^{ϵ}-methyllysine titres, both between and within samples, show more variation. Samples A and C give mean titres where standard deviation is $\pm 0{\cdot}25$, whilst for samples B and D the standard deviation of the mean is $\pm$ 0·36 in both cases. The mean titres are similar in samples B, C and D, (2·2 mg/g N), however, that of A is much higher (2·7 mg/g N), thus the standard deviation between mean concentrations is higher ($\pm 0{\cdot}3$) than for those of 3-methylhistidine ($\pm 0{\cdot}1$). The ratio of 3-methylhistidine to N^{ϵ}-methyllysine varies between 2·2 to 4·1 within a sample, and 2·4 to 3·1 for mean ratios between samples.

The effect of interfering compounds on 3-methylhistidine and N^{ϵ}-methyllysine determination

(*a*) *Carbohydrate.* From the results shown in Table 3, it can be seen that the presence of soluble carbohydrate (sucrose) has little effect on 3-methylhistidine determinations

TABLE 3. The effect of carbohydrate (sucrose) concentration on the 3-methylhistidine and N^{ϵ}-methyllysine determination in beef

Treatment	3-methylhistidine mg/g N	N^{ϵ}-methyllysine mg/g N	Ratio 3-MeHis. : N^{ϵ}-MeLys.
No sucrose added	6·53	3·53	1·85
5% sucrose added	6·44	2·92	2·20
10% sucrose added	6·75	3·10	2·18
15% sucrose added	6·16	2·88	2·14

up to an addition of 10% sucrose. When 15% sucrose is present however, the 3-methylhistidine content is reduced, although this reduction is within the variation encountered between samples (Table 2). Similarly, the N^{ϵ}-methyllysine content is reduced as the concentration of sucrose added increases. The losses of the latter are of a greater order

than those of 3-methylhistidine, as indicated by the increase in the ratio of 3-methylhistidine to N^{ϵ}-methyllysine.

(*b*) *Salt.* The results in Table 4 show that considerable losses of 3-methylhistidine and N^{ϵ}-methyllysine occur in the presence of 18% salt, when the treatment does not include

TABLE 4. The effect of salt concentration and dialysis on 3-methylhistidine and N^{ϵ}-methyllysine determination in beef (*l. dorsi*)

Treatment	3-methylhistidine mg/g N	N^{ϵ}-methyllysine mg/g N	Ratio 3-MeHis. : N^{ϵ}-MeLys.
No salt added, dialysed	6·49	3·20	2·03
18% salt added, dialysed	6·13	3·69	1·66
No salt added, not dialysed	6·87	3·23	2·13
18% salt added, not dialysed	3·24	1·76	1·84

preliminary dialysis—almost 50% of both amino acids being removed. When the sample is dialysed after treatment with 18% salt, although slight loss of 3-methylhistidine occurs, an increased N^{ϵ}-methyllysine titre is noticed. Thus dialysing appears to counteract the confounding effect of salt.

The identification and determination of 3-methylhistidine and N^{ϵ}-methyllysine in mammalian muscle

The concentrations of both 3-methylhistidine and N^{ϵ}-methyllysine found in beef and lamb are given in Table 5. 3-methylhistidine contents are similar for both beef and lamb. Concentrations for beef show a mean value of 5·9 mg/g N (S.D. $\pm$ 0·7), whilst those of lamb show a mean value of 6·1 mg/g N (S.D. $\pm$ 0·7). It is noted, however, that certain samples for both beef and lamb do have 3-methylhistidine concentrations < 5 mg/g N. Determinations for N^{ϵ}-methyllysine in both beef and lamb are more variable, concentrations ranging from 2·1 to 3·8 mg/g N, although most results are within the range 2·2 to 2·7 mg/g N. The determination of N^{ϵ}-methyllysine is complicated by the presence of two types, N^{ϵ}-mono and N^{ϵ}-tri-methyllysine within mammalian

TABLE 5. 3-methylhistidine and N^{ϵ}-methyllysine in beef and lamb samples

Sample	3-methylhistidine mg/g N	N^{ϵ}-methyllysine mg/g N	Ratio 3-MeHis.: N^{ϵ}-MeLys.
Lamb (chump chop)	5·40	2·22	2·43
Lamb (*l. dorsi*) I	4·44	2·37	1·87
Lamb (*l. dorsi*) II	6·09	2·10, 2·62	2·90, 2·32
Lamb (*l. dorsi*) A	6·52	2·72	2·40
Lamb (*l. dorsi*) B	6·76	2·22	3·05
Lamb (*l. dorsi*) C	6·61	2·11	3·13
Lamb (*l. dorsi*) D	6·47	2·14	3·02
Lamb (leg) red	6·41	3·81	1·68
Lamb (leg) white	5·84	2·22	2·63
Beef (leg) A	5·87	2·64	2·22
Beef (leg) B	6·02	1·47, 2·62	4·10, 2·98
Beef (steak)	6·48	3·53	1·84
Beef (*l. dorsi*) A	4·79	2·22	2·15
Beef (*l. dorsi*) B	6·87	1·96, 2·54	3·50, 2·70
Beef (shin)	5·42	2·38	2·28

muscle, and as such the ratio of 3-methylhistidine to N^{ϵ}-methyllysine shows a wide variation.

Determinations for pork samples are given in Table 6, and unlike beef and lamb, the concentration of 3-methylhistidine varied considerably, ranging from 6 to 56 mg/ g N. Since it was found that the 3-methylhistidine content of pork (*longissimus dorsi*) could be reduced from 19 mg/g N to 5 mg/g N by prior washing of the meat sample, the presence of a water soluble form of 3-methylhistidine was suggested. N^{ϵ}-methyllysine however, showed no comparative variation, determinations being similar to those obtained for beef and lamb.

TABLE 6. 3-methylhistidine and N$^{\epsilon}$-methyllysine in porcine muscles

Sample	3-methylhistidine mg/g N	N$^{\epsilon}$-methyllysine mg/g N	Ratio 3-MeHis.: N$^{\epsilon}$-MeLys.
Pork (Chump)	6·86	2·19	3·13
Pork (Spare rib)	5·70	2·92, 3·79	1·95, 1·50
Pork (*l. dorsi*) IV (twelve weeks)	11·82	2·13, 2·79	5·55, 4·24
Pork (*l. dorsi*) I (six months)	16·61	2·58	6·44
Pork (*l. dorsi*) II (six months)	19·95	2·52	7·92
Pork (*l. dorsi*) II washed × 3 water	5·21	2·26, 2·65	2·31, 1·47
Pork (*l. dorsi*) III (three years)	56·20	2·27	24·76

In Table 7 data for whalemeat are given. As expected, these titres are very high in respect of 3-methylhistidine (367 mg/g N) due to the presence of 3-methylhistidine in the dipeptide balenine. The effect of prior washing of the whalemeat, using different washing procedures, is also shown, from which it is clear the 3-methylhistidine may be reduced (dependent on the washing procedure given) to a value of 6 mg/g N. In order to

TABLE 7. The effect of different washing treatments given to whole whalemeat prior to hydrolysis on 3-methylhistidine and N$^{\epsilon}$methyllysine titres

Sample	3-methylhistidine mg/g N	N$^{\epsilon}$-methyllysine mg/g N	Ratio 3-MeHis.: N$^{\epsilon}$-MeLys.
Whalemeat I (Norwegian) Whole	368·36	No results obtained	—
Whalemeat I Washed × 3 water	9·65	—	—
Whalemeat I Washed × 3 Sarcoplasmic Buffer	6·07	3·15, 5·20	1·93, 1·17
Whalemeat II (Norwegian) Whole	366·37	2·06, 3·09	178, 119
Whalemeat II Washed × 1 water	13·27	2·36	5·62
Whalemeat II Washed × 3 water	8·30	1·88, 3·05	4·41, 2·72

obtain values representative of bound 3-methylhistidine, the washing procedure had to be repeated thrice, using sarcoplasmic removal buffer rather than water. Table 6 shows that a thrice washing with water is sufficient to remove all soluble 3-methylhistidine from pork. Clearly, if present, soluble 3-methylhistidine can successfully be removed.

As with pork, N^{ϵ}-methyllysine titres show no comparable variation, concentrations being similar to those obtained for beef and lamb, even after a given washing procedure. Since the presence of soluble 3-methylhistidine had been indicated in pork as well as whale, more detailed examination of the soluble fraction of certain samples was carried out, using both ion exchange and paper chromatography.

The results given in Table 8 confirm the presence of 3-methylhistidine in the hot water extract (fraction A) of both pork and whale samples, although not in lamb. The

TABLE 8. The occurrence and identification of soluble 3-methylhistidine in meat using ion exchange chromatography

Sample	3-methylhistidine			
	Hot water extract (Fraction A) μmol/g	Hot water extract (Fraction A) (Hydrol.) μmol/g	Whole Hydrolysate mg/g N	Whole Hydrolysate after HWe mg/g N
Lamb B (*l. dorsi*)	None detected	None detected	6·76	No sample available
Pork (*l. dorsi*) III	None detected	1·66*	56·20	17·15
Pork (*l. dorsi*) IV	None detected	0·41	11·82	9·34
Whalemeat (Norwegian) I	None detected	0·34*	368·36	No sample available
Whalemeat (Norwegian) II	None detected	26·49	366·37	76·89

* Hot water extract (HWe) was dialysed prior to hydrolysis.

3-methylhistidine is only detectable in the hydrolysed hot water extract using ion exchange chromatography. None was detected in the hot water extract prior to hydrolysis, thus indicating that the 3-methylhistidine is present as a peptide (proteins having been removed by preliminary treatment of the extract with trichloroacetic acid).

Further analysis was carried out to determine the form of the peptide in which soluble 3-methylhistidine was thought to be present. The results of the examination of hot water extracts (fraction B) using paper chromatography are given in Table 9.

Table 9b shows that three dipeptide spots were obtained for each sample of pork, whilst two were found in lamb and in each sample of whale. Spot 1 is common to all species, and by comparison of standard Rf values and spot colour (Table 9a), appears to be carnosine (β alanyl-histidine). Identification of spots 2 and 3 was less definite;

TABLE 9. (a) The Rf values and colour of known dipeptides and their constituent amino acids using paper chromatography

Standard	Rf value	Colour
Carnosine (β-alanyl-L-histidine)	0·41	Green
Anserine (β-alanyl-L-1-methylhistidine)	0·75	Green
Histidine	0·18	Purple
β-alanine	0·40	Blue-purple
1-methylhistidine	0·54	Green
3-methylhistidine	0·75	Grey-purple

(b) The identification of histidine dipeptides present in meat using paper chromatography

Sample	Spot 1 Rf colour	Spot 2 Rf colour	Spot 3 Rf colour	Suggested dipeptides present
Lamb (*l. dorsi*) B	0·43 Green	0·80 Green	—	Spot 1 Carnosine Spot 2 Anserine or 3-methylhistidine dipeptide
Pork (*l. dorsi*) III	0·45 Brown	0·72 Brown	0·82 Brown	Spot 1 Carnosine Spot 2 Anserine Spot 3 3-MeHis. dipeptide
Pork (*l. dorsi*) IV	0·32 Green	0·78 Green	0·85 Green	Spot 1 Carnosine Spot 2 Anserine Spot 3 3-MeHis. dipeptide
Whalemeat I	0·41 Green	0·82 Green	—	Spot 1 Carnosine Spot 2 Anserine or 3-methylhistidine dipeptide
Whalemeat II	0·36 Green	0·79 Green	—	Spot 1 Carnosine Spot 2 Anserine or 3-methylhistidine dipeptide

(c) The constitution of 3-methylhistidine dipeptides present in meat using paper chromatography

Sample	Amino acid spots obtained by hydrolysis of spots (2 and 3) separated in 9(b)		
	Spot (a) Rf colour	Spot (b) Rf colour	Suspected amino acids present
Lamb (*l. dorsi*)	0·33	0·51	(a) β-alanine
B	Purple	Green	(b) 1-methylhistidine
Pork (*l. dorsi*)	0·41	0·79	(a) β-alanine
III	Purple	Purple	(b) 3-methylhistidine
Pork (*l. dorsi*)	0·41	0·60	(a) β-alanine
IV	Purple	Blue-purple	(b) 3-methylhistidine or 1-methylhistidine
Whalemeat	0·42	0·70	(a) β-alanine
I	Purple	Grey-purple	(b) 3-methylhistidine
Whalemeat	0·42	0·68	(a) β-alanine
II	Purple	Grey-purple	(b) 3-methylhistidine

but, by comparison with standards, it would seem likely that spot 2 was anserine (β alanyl-1-methylhistidine) in the lamb sample, and balenine (β alanyl-3-methylhistidine) in the whale sample, and that spot 3, as found in the pork samples, was the possible 3-methylhistidine dipeptide.

When spots 2 and 3 were hydrolysed, identification of their constituent amino acids was possible (Table 9c). 3-methylhistidine and β-alanine were found to be present in both samples from pork and whale, whilst in lamb, only β-alanine and 1-methylhistidine were found. Since no anserine was identified in whale, no 1-methylhistidine would be expected to be present; however, anserine was thought to be present in pork samples (spot 2) and thus when both spots were hydrolysed some 1-methylhistidine should be identified. When Rf values of spot (2 and 3)(b) are examined for pork samples, it is evident that in pork sample (*longissimus dorsi*) IV, the Rf value is mid-way between those expected for 3-methylhistidine and 1-methylhistidine, indicating that both may be present, but are not distinguished due to incomplete separation. However, in pork sample (*longissimus dorsi*) III the Rf value of spot (2 and 3)(b) is very similar to that of the standard 3-methylhistidine, suggesting that only the latter is present.

Certain non-meat proteins were analysed for the presence of 3-methylhistidine and N^{ϵ}-methyllysine. Neither amino acid could be detected in any of the samples. These were spun soya protein fibres, whole egg powder, granogen soya milk, soya fluff powder, casein powder, and gelatin.

Discussion

The mean titre of 3-methylhistidine (~6 mg/g N) for beef and lamb accords with that obtained by Hibbert & Lawrie (1972). Moreover, the amino acid was present at more or less constant levels within the different muscles sampled (Table 5). It therefore shows more promise as an index of meat than does N^{ϵ}-methyllysine, the concentrations of which range more widely. Analysis of various non-meat proteins—egg, milk, soya—revealed that neither 3-methylhistidine nor N^{ϵ}-methyllysine were present. To consider a titre of ~6 mg 3-methylhistidine/g N as signifying that the protein of a presumed meat product is entirely derived from lean meat requires, additionally, that the 3-methylhistidine is present only as an integral part of the polypeptide chains of the principal myofibrillar proteins, actin and myosin.

It was therefore disturbing to find that the concentration of 3-methylhistidine in pork was not only much more variable than in lamb and beef but attained very high values which were impossible to reconcile with their being associated with the myofibrillar proteins. It seemed that the amino acid must be present in some other form. It was well known of course, that whale muscle contains soluble 3-methylhistidine as the dipeptide, balanine (β-alanyl-3-methylhistidine: Carisano, 1964; Cocks *et al.*, 1964), and such was found in this species (Tables 7, 8 and 9), but only the dipeptides carsonine and anserine had hitherto been reported in pork.

Closer examination of the phenomenon by ion exchange and paper chromatography, showed that pork contains a third dipeptide (Tables 9b and c) which, whilst absent from the muscles of younger pigs, appears gradually to replace anserine as the age of the animal increases. Whether this has a dietary or physiological explanation remains to be elucidated. Fortunately, it could be demonstrated that, where soluble 3-methylhistidine occurred, it could be removed by washing meat samples before hydrolysis (Tables 6 and 7), and residual values were then similar to those of lamb and beef.

The need for a preliminary treatment of meat products before determination of methylamino acids is further exemplified in Tables 3 and 4. Whereas the presence of soluble carbohydrate (sucrose) caused no significant loss of methylamino acids, high concentrations of salt (as may be found in certain meat products) may lead to low titres of methylamino acids. This reflects solubilization of myofibrillar proteins during initial washing, and it may be avoided by dialysing samples against distilled water.

The variation of 3-methylhistidine titres within a given sample, although not large, is clearly a matter of concern. It seems likely to be due, however, to the modes of applying hydrolysate to the column and of calculating 3-methylhistidine concentrations from the graphical plots obtained after column separation. These calculations require accurate interpretation of peak areas, assessment of the true base line and the use of an effective standard. Since an external standard only indicates variation in the ninhydrin reaction and cannot account for within-column differences, an internal standard would appear preferable. Although S-β-(4 pyridylethyl)-L-cysteine has been suggested for such purposes (Cavins & Friedman, 1970) its elution position is much later than that of

3-methylhistidine and its use would thus greatly prolong running time. Greater accuracy would thus appear to depend upon further standardization of the experimental technique generally.

The basic premises involved in suggesting the use of 3-methylhistidine as an unequivocal index of the protein of lean meat, namely that it is not found in non-meat proteins and that it is present at a characteristic level in the former, have been borne out, in essentials, by the present investigation. It is clear, however, that the procedure must be applied with some circumspection and that it must be further developed before it can be routinely employed in analysing unknown samples which have been processed too severely for electrophoretic identification of the proteins. Moreover, many more proteins need to be examined. Such studies will form the basis of subsequent publications.

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Microbial profile of dehydrated cured mutton mince from raw material to the finished product

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Summary

Total plate count, thermophiles, coliforms, staphylococci, streptococci and moulds of dressed carcasses brought from the local slaughterhouse and at different stages in the preparation of pre-cooked dehydrated mutton mince were studied. The sources of contamination and necessary precautions to be adopted are discussed.

Introduction

Meat and meat products provide highly favourable media for the growth and multiplication of bacteria. Although the flesh of healthy, live animals is almost free from bacteria (Haines, 1937), during slaughter the defensive barrier of the skin and mucous membranes is lost and the meat gets contaminated with various microorganisms from external sources (Mallman & Churchill, 1946; Jensen & Hess, 1941; Ayres, 1955). Environmental plant sanitation thus becomes extremely important and this has been emphasized by a number of workers (Weinzirl & Newton, 1914; Reith, 1926; Haines, 1937; Jensen & Hess, 1941; Kirsch *et al.*, 1952; Drewniak *et al.*, 1954; Ayres, 1955; Thornton, 1968). Although a lot of developments have taken place in the meat industry in the economically advanced and industrialized countries of the world, the production of safe, clean and wholesome meat still meets with great difficulties in India (Krishnaswamy & Lahiry, 1964). Being a tropical country with temperatures during the major part of the year ideally suitable for the growth and multiplication of bacteria, the bacterial contamination in non-refrigerated meat is bound to be high. The raw meat sold in retail markets in India is mostly non-refrigerated, but information on the incidence and types of microflora present in non-refrigerated market meat is rather meagre (Krishnaswamy & Lahiry, 1964; Bachhil & Ahluwalia, 1973).

Over 55% of the total quantity of meat in India is from sheep and goat. Apart from fresh mutton a number of varieties of processed meat, mostly in the canned and dehydrated form, has been marketed. These are mainly intended for the use of the Defence Services. During the last few years there has been a rapid increase in the range

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and quantity of these products. Of late, most of the meat is cured in order to retain the attractive colour of fresh meat and to have a longer shelf life in addition to reducing the total microbial load curing is known to modify the initial microbial flora.

Dehydrated meats are ideal for military rations because of their light weight and their storage stability at room temperature. If the product is pre-cooked it can be prepared for eating with a minimum of time and effort and the bacterial load will be reduced still further. If the product is precured also, the attractive colour of fresh meat is retained. Such a pre-cooked, dehydrated cured mutton in minced form has been developed in this laboratory for use of the Defence Services either as such or as components of convenience mixes in the various types of dehydrated mixes such as cutlet mix, curry mix, sausage mix, etc (Kadkol *et al.*, 1972). As the reconstitution is carried out with very little heat treatment, the microorganisms that are present in the dehydrated mutton mince will be of great practical significance from the health point of view when the product is to be consumed. It is important, therefore, to understand at which point in the preparation of this product the meat is most liable for bacterial contamination. At various stages, from the dressed carcass to the mutton mince, bacterial counts were made, and, furthermore, examinations of the hands of the workers and of the environment were carried out, in order to pinpoint the stages where extra precautions should be taken.

Materials and methods

Six batches of pre-cooked dehydrated, cured mutton mince were made for the studies on a laboratory semi-pilot plant scale. Each batch was made with five or six mutton carcasses in the weight range of 6–10 kg each, purchased from the local market which were deboned, cut, cured and made into pre-cooked dehydrated mutton mince as per the methods of Das *et al.* (1973) further modified by V. K. Mathur *et al.* (personal communication). The method in brief is as follows. The carcasses were washed under running tap water and deboned. After deboning the meat was washed and minced in a Hobart mincer using a disc of 10 mm diameter holes. As the mincing was not allowed to continue for more than 15–20 min at a stretch, the temperature of the mince varied only from 29–31 °C. After each batch, the mincer was washed thoroughly and steamed for 15 min so that contamination from one batch is not carried over to another batch. Further at the end of each day the mincer was sterilized. The minced meat was then mixed with sodium nitrite at a concentration of 200 ppm and potassium citrate at 2500 ppm and allowed to cure at room temperature for 1 hr; 1% sodium chloride and 1% sodium bicarbonate were added on the minced meat weight basis and mixed thoroughly and allowed to cure for half an hour at room temperature. The meat was then cooked in an open pan until it softened and all the gravy was absorbed, the time taken being approximately 1 hr; it was minced again using the same mincer but with a

different disc of diameter 6 mm and dried in a cabinet drier at 65–70°C for 2½ hr with a tray loading of 1·6 kg/m².

Samples were drawn at different steps in the process of its preparation namely, (1) dressed carcass; (2) dressed carcass after washing; (3) deboned and washed meat made into mince; (4) cured meat; (5) cooked meat; (6) cooked and minced meat; (7) dehydrated, cured, minced meat; (8) packed in cans under nitrogen. In addition, microbiological counts for the environment was also carried out initially, in the middle of the day and towards the close of the day.

In the case of the carcass, samples were drawn from eight areas namely, hind quarter, loin, shoulder region, abdominal region, middle quarter, scrag, fore quarter and axillar. Sampling was done by scooping the muscle with a sterile stainless steel scooper of 5 cm diameter to a depth of 1 cm, cutting off the muscle with a sterile knife, mixing all these eight scoops in a Waring blender and from the well mixed material triplicate samples each weighing 10 g were mixed thoroughly in ¼ Ringer's solution. Serial dilutions were made in the same diluent and the viable counts are expressed as numbers of colonies per gram of the sample. The average count of all the carcasses for each batch was calculated and is presented in Table 1, column 4. In the case of deboned minced meat prior to curing, meat from all the carcasses was mixed thoroughly and three representative samples each weighing 10 g were taken for microbial analysis and the average of these three analysis are given in Table 1, column 5. Sampling for making counts of cured meat, cooked meat and cooked and minced meat were also carried out in a similar way. Analysis was carried out for total plate count, thermophiles, coliforms, staphylococci, streptococci and moulds as per standard methods (American Public Health Association, 1966). Total plate counts were done on dextrose tryptone yeast extract agar and incubated at 37 °C for 48 hr. Thermophiles were enumerated on the same medium, but incubated at 55 °C for 48 hr. Coliforms were analysed on violet red bile agar at 37 °C for 24 hr, staphylococci on medium 110 at 32 °C for three days, further characterized by a coagulase test, pigmentation and mannitol fermentation and streptococci on maltose azide tetrazolium agar (Harrigan & MeCance, 1966). Moulds were enumerated on potato dextrose agar acidified to pH 4·5 at the time of pouring by adding 1% tartaric acid and incubating at 32 °C for four days. *Clostridium perfringens* was estimated on sulphite polymyxin sulphadiazine agar using the procedure of Thatcher & Clark (1968). Cultures were isolated and identified according to the methods of Breed, Murray & Smith (1964). Atmospheric contamination was determined by exposing 95 × 17 mm Petri dishes poured with dextrose tryptone agar for 10 min at three different times of each day (Stringer, Bilskie & Naumann, 1969). The microbial counts were expressed as number of colonies per plate and their significance worked out.

The pH values were determined by mixing thoroughly 10 g of meat in 90 ml of distilled water and measuring the pH of the supernatant liquid by a glass electrode. Chemical analysis of the final product were carried out as per A.O.A.C. methods (1970) and are given in Table 4.

Results and discussion

A perusal of Table 1 shows that the initial bacterial load of meat as it is sold in the local market varies between $1{\cdot}5 \times 10^5$ to $1{\cdot}6 \times 10^6$ and except in one case falls within the suggested standards (Frazier, 1967) and are comparable to the bacterial loads reported by Burzynska *et al.* (1971) and Krishnaswamy & Lahiry (1964). Bachhil & Ahluwalia (1973), however, obtained much lower values for various tissues. This may be due to the fact that these authors had collected the samples immediately after skinning and evisceration of the carcass, thus eliminating the further atmospheric, handling and transport contamination to which it is normally subjected to before it reaches the consumer. Maintaining high hygienic standards, Rawal *et al.* (1973) could obtain an extremely low average total plate count of 1785 for dressed carcasses in a Defence slaughter house. As expected, washing reduced the total bacterial load to approximately half. The minced meat had a total count of $7{\cdot}7 \times 10^5$–7×10^6. Weinzirl & Newton (1914) and Leferve (1917) found total counts of 10^7 in minced meat whereas Tiwari & Maxy (1971) obtained $3{\cdot}5 \times 10^7$. Rao (1970) surveying samples from market and butcheries from the Defence Organization, found a lower number from the Defence sources. This may be due to stricter inspection in the latter case. Rawal *et al.* (1973) also got a very low figure in a military slaughter house. The increase of bacterial load in minced meat as compared to the washed carcasses could be due to various factors such as surface contamination from the mincing machine and bacterial multiplication during the time interval. Fragmentation of the tissues with the liberation of juices and the agitation caused during mincing could also add to quicker multiplication. As these washing and mincing operations are done at room temperature there will be rapid multiplication of the bacteria. As observed by Ayres (1960) the level of initial contamination combined with the holding temperature and time will profoundly influence its final bacterial quality.

The pH of the meat showed wide variation ($6{\cdot}44 \pm 0{\cdot}41$, $n = 30$) and did not show any correlation with its total plate count. Coefficient of correlation was $-0{\cdot}0178$ and was not significant. Perhaps, as observed by Bate-Smith (1948), a high pH value may exist in muscles for physiological reasons quite unconnected with bacteriological contamination.

Curing brought down the bacterial population further. This is due to the combined effect of sodium chloride and sodium nitrite (Jensen, 1954). The prolonged cooking reduced the microbial load to an insignificant level as is indicated by the low count in the cooked meat. The increased load in the cooked and minced samples and also in the dehydrated product must have come as after processing contamination such as handling, atmosphere, mincing machine surfaces, etc. Moulds were present only in the case of cans packed under nitrogen in the order of 55, 5, 10, 10, 5 and 25 colonies per gram respectively in the various batches. As the nitrogen packing is done in a separate room the mould contamination must have been introduced from there. The

TABLE 1. Microbial load at different stages of preparation of dehydrated, cured mutton mince (log of viable count/g)

Types of organisms	Batch no.	Dressed carcass	Dressed carcass after washing	De-boned and minced meat	Cured meat	Cooked meat	Cooked and minced meat	Dehy-drated and minced meat	Packed
(1) Total plate count	I	5·68	5·45	6·85	More than 4	3·72	3·84	1·65	1·85
	II	6·04	5·85	6·23	4·86	Less than 1	1·18	2·34	2·5
	III	5·18	5·9	5·89	5·34	Less than 1	1·78	1·7	2·02
	IV	6·22	4·9	6·18	5·58	2·15	2·11	—	1·54
	V	5·74	5·48	6·23	4·93	1·85	1·65	—	1·78
	VI	5·89	5·6	6·4	5·26	Less than 1	1·48	1·54	1·93
(2) Thermophiles	I	—	—	—	—	—	—	Less than 1	1·48
	II	—	—	—	—	—	—	2·06	2·32
	III	1·79	—	Less than 1	Less than 1	Less than 1	1·18	1·3	1·34
	IV	1·66	—	1·18	Less than 1	Less than 1	Less than 1	—	1·18
	V	1·53	1·3	1·18	Less than 1	Less than 1	Less than 1	—	1·18
	VI	1·88	1·6	1·65	1·93	Less than 1	Less than 1	1·18	1·3
(3) Staphylococci	I	5·04	4·7	4·52	—	Less than 1	Less than 1	Less than 1	1·74
	II	5·15	4·9	5·01	4·32	Less than 1	Less than 1	1·48	1·9
	III	4·32	4·18	4·86	4·15	Less than 1	1·6	1·3	1·6
	IV	5·04	—	4·04	3·97	1·65	1·6	—	1·18
	V	4·68	4·4	4·76	3·43	1·81	Less than 1	—	1·4
	VI	4·34	4·11	4·52	4·00	Less than 1	Less than 1	Less than 1	1·18
(4) Coliforms	I	3·92	3·7	4·76	3·79	Less than 1	Less than 1	Less than 1	Less than 1
	II	4·32	4·18	4·4	2·54	Less than 1	Less than 1	Less than 1	Less than 1
	III	3·08	2·78	2·92	2·86	Less than 1	Less than 1	Less than 1	Less than 1

TABLE 1 *continued*

Types of organisms	Batch no.	Dressed carcass	Dressed carcass after washing	De-boned and minced meat	Cured meat	Cooked meat	Cooked and minced meat	Dehy-drated and minced meat	Packed
	IV	4·50	—	4·58	3·82	Less than 1	Less than 1	—	Less than 1
	V	4·75	4·48	4·63	3·01	Less than 1	Less than 1	—	Less than 1
	VI	5·11	4·9	—	4·28	Less than 1	Less than 1	—	Less than 1
(5) Streptococci	I	3·92	3·65	3·9	3·85	1·54	1·65	Less than 1	Less than 1
	II	4·38	—	3·4	2·65	Less than 1	Less than 1	Less than 1	Less than 1
	III	3·59	3·08	3·26	2·6	Less than 1	Less than 1	Less than 1	Less than 1
	IV	4·15	—	3·36	3·3	Less than 1	Less than 1	—	Less than 1
	V	4·04	3·78	3·86	1·3	Less than 1	Less than 1	—	Less than 1
	VI	3·84	3·42	3·53	3·2	Less than 1	Less than 1	Less than 1	Less than 1
(6) *Clostridium perfringens*	I	2·08	1·9	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
	II	2·12	1·9	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
	III	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
	IV	Less than 1	—	Absent in 10 g	Less than 1	Absent in 10 g	Absent in 10 g	—	Absent in 10 g
	V	1·96	1·74	2·54	Absent in 10 g	Absent in 10 g	Absent in 10 g	—	Absent in 10 g
	VI	2·9	Absent in 10 g	2·75	2·5	Absent in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g

—, Not done.

putrefactive anaerobe, *Clostridium perfringens* was present in four cases of raw mutton. Thermophilic counts were low to negligible in all the cases. However, staphylococci, coliforms and streptococci are present in all the samples and their number is fairly high in the majority of them.

TABLE 2. Analysis of variance table for aerobic plate count of atmosphere in relation to time

Sources of variation	df	m.s.
Batches	4	13700·88
Time	2	11727·43*
Batches × time	8	3482·18
Error	15	132·34

* Significant at 0·1% level.

TABLE 3. Mean aerobic plate count of atmosphere in relation to time (no. of colonies/plate)

A	10.00 hours	50·4	±3·64 (15 df)
B	14.00 hours	107·0	
C	17.00 hours	45·3	

A–B, 56·6*; A–C, 5·1 NS; B–C, 61·7*.
* Significant at 0·1% level.

Studies on the atmospheric contamination reveal (Tables 2 and 3) that the bacterial load of atmosphere increased as the work progressed, reached a maximum towards 14.00 hours and again decreased by evening. Statistical treatment indicated that the 14.00 hours values were significantly higher ($P = 0{\cdot}001$) than 10.00 hours and 17.00 hours values. As the aerial contamination is dependent on materials present in the working room, the contamination from this will be minimum towards evening as the meat reached the final stages of cooking and dehydration. Swabs taken from workers' hands during different periods of the day also showed bacterial contamination, the organisms resembling those from meat.

No significant correlation could be found between log total plate count and log coliforms of dressed carcass (Table 1) ($r = 0{\cdot}7424$, $n = 6$). Although on a cursory glance staphylococci appear to be in large numbers wherever total plate count was high, when the coefficient of correlation was calculated, this also was not significant ($r = 0{\cdot}6282$, $n = 6$). However, a significant correlation could be found between log total plate count and log streptococci ($r = 0{\cdot}8274$, $p < 0{\cdot}05$, $n = 6$) indicating thereby that streptococci could be taken as an index of bacteriological quality of meat.

Pseudomonas, *Micrococcus* and *Bacillus* were the common genera present in the carcases. The first two only dominated up to the cooking stage. But in cooked, dehydrated, minced mutton *Bacillus* and *Micrococcus* dominated and *Pseudomonas* was almost absent. As the cooked meat is almost sterile, the slight increase in the bacterial load of the cooked, minced, dehydrated product obviously must have come from the hands of the workers, the surfaces of equipments and also to some extent from the atmosphere. The general character of the bacterial flora in these may be more dependant on the bacterial

TABLE 4. Percentage of proximate composition and nitrite content of dehydrated cured mutton mince

Batch no.	Moisture	Protein N×6·25	Fat	Salt	$NaNO_2$ in ppm
1	5·6	83·3	18·0	2·3	27·0
2	5·6	75·0	11·2	1·9	31·7
3	6·8	79·0	10·7	2·1	35·0
4	4·2	79·8	11·9	2·1	31·8
5	2·6	79·2	12·4	2·0	35·8
6	7·5	79·3	11·8	1·8	26·9

flora of the raw meat as all the cross contamination must have come from this. The almost total absence of *Pseudomonas* in the dehydrated meat confirms the inhibitory effect of the curing salts on this organism reported earlier (Kitchell & Ingram, 1966; Patterson, 1963).

Though the total plate count in the raw meat is comparable to some of the suggested standards the heavy contamination with staphylococci, coliforms and streptococci indicate the necessity to observe more hygienic precautions while handling and marketing the meat. As the ambient temperature is ideal for the multiplication of bacteria normally present in meat, the mincing and curing operations should be carried out in a low temperature room. Since cross contamination can take place from the hands of workers and from equipment, proper precautions should be taken to clean these immediately after each operation. The results of the study have indicated that even with heavily contaminated raw meat it is possible to obtain pre-cooked dehydrated cured mutton mince of very low counts if proper hygienic precautions are taken during all the stages of operations involved in the preparation.

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Biochemical changes in vacuum packaged beef occurring during storage at 0–2°C

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Summary

Lactic acid, total carbohydrate and alcohol-precipitable carbohydrate concentrations in vacuum packaged beef stored at 0–2° for nought to nine weeks were generally low, although the fluctuation in concentrations from week to week could be broadly correlated with changes in the proportions of certain groups of microorganisms in the microflora. The changes in the concentrations of these constituents were not considered useful criteria of the overall bacteriological condition of vacuum packaged beef. Certain ninhydrin-positive compounds in beef increased considerably during storage in vacuum packages, probably mainly peptides and amines. An acidic odour noticeable on opening vacuum packages of beef after storage for three to four weeks or longer was attributed in part to the presence of short chain volatile fatty acids, mainly acetic acid.

Introduction

Spoilage of meat by microbiological or autolytic action is generally accompanied by biochemical alterations in the constituents of meat and these have been used to assess indirectly the extent of spoilage. Generally, estimations of biochemical changes measure the degree of spoilage which has already taken place and no reliable method has yet been devised which will indicate incipient spoilage or which will give an estimate of the probable shelf-life of the meat (Turner, 1960; Ingram & Dainty, 1971). The degree of microbiological contamination is a fairly reliable indicator of the state of freshness of the meat but bacterial counts are time-consuming and are therefore inconvenient for industrial quality control procedures (Pearson, 1968).

Physico-chemical tests used to determine the degree of spoilage of fresh meat include pH measurement (Turner, 1960; van Logtestijn, 1965; Bentler, 1972) and connected with this, the titratable alkalinity (Shelef & Jay, 1970). These methods, together with extract release volume (ERV) measurement (Jay, 1964) have been applied to vacuum

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packaged meat (Sutherland, Patterson & Murray, 1975) but were found to be unsuitable for assessing both the microbiological and keeping quality of meat matured in this way.

Dye reduction tests have been used to measure the degree of spoilage of fresh meat (Rodgers & McCleskey, 1957; Saffle *et al.*, 1961). Investigations of the degradation of nitrogenous substances in meat include studies of changes in total volatile nitrogen (Pearson, 1968), ninhydrin-positive material (Turner, 1960; Jay & Kontou, 1964) and amino acid content (Locker, 1960; Gardner & Stewart, 1966a, b; Jay & Kontou, 1967), levels of nucleotides (Jay & Kontou, 1967), and total non-protein nitrogen (Ockerman *et al.*, 1969).

Investigation of changes in other chemical constituents of fresh meat such as glucose, glycogen and lactic acid have been made by Gardner (1965) while Lea, Parr & Jackson (1969) examined variations in carbohydrates, haem pigment, creatine, lactic acid and release of H_2S in spoiling poultry meat.

In the present communication investigations of the changes in some of the constituents of vacuum packaged meat were made in order to correlate these changes with the corresponding microbial numbers and species.

Methods

Preparation and storage of the meat

Striploins (*M. longissimus dorsi*) from a Friesian heifer of 9½ cwt liveweight were 'boned-out' in the abattoir, and after microbiological sampling, each was cut across the grain of the meat into six equally sized blocks, slicing from the lean side through to the fat side. Each of the twelve blocks was individually vacuum packaged in a film having the following properties: water vapour transmission rate 5·8 g/m²/24 hr/90% RH/40°, and O_2 transmission rate 30 cm³/m²/24 hr/litre atmosphere/0% RH/22°. The CO transmission rate was stated by the manufacturer to be *c.* five times the O_2 transmission rate under the same conditions. The blocks of meat were stored in the laboratory, fat side down, at a temperature of 0–2° and a relative humidity of 98–100% obtained by placing a tray of water in the incubator. Each week up to nine weeks of storage one block of meat was withdrawn at random and after checking that the package had not leaked, the packaging material was removed aseptically and the meat placed on a sterile stainless steel tray for sampling.

Microbiological sampling

Sterile stainless steel tubes, 10 cm² in cross-sectional area were pressed firmly on to the lean surface of the block of meat, and 20 ml of sterile 0·5% (w/v) peptone water (Oxoid L37) poured into the tube. The enclosed surface of the meat was scraped vigorously with a sterile long handled metal spoon for 2 min and the mixture withdrawn by means of a sterile pipette into a sterile glass container (method of Williams 1967).

Ten-fold dilutions of the sample liquid were prepared by the method of Murray (1956) and five drops each of 0·02 ml, from suitable dilutions were spread on the surface of previously prepared plates of selective and non-selective agars. Presumptive *Microbacterium thermosphactum* were enumerated on the medium of Gardner (1966) after incubation in air for 3 d at 25°, while lactobacilli and related catalase negative organisms were enumerated on the medium of de Man, Rogosa & Sharpe (1960) after incubation for 5 d at 30° in reduced oxygen tension conditions, achieved by burning a candle to extinction in the closed incubation jar.

Titratable acidity/alkalinity of the meat (*T.A.*)

An aqueous extract of the meat was prepared by homogenizing meat (10 g) with distilled water (100 ml) for 2 min at full speed on an MSE 'Atomix' blender (MSE Ltd, Crawley).

The homogenate was filtered through a Whatman No. 1 filter paper and a 20-ml aliquot used for the determination. The quantity of 0·02 N HCl required to reduce the pH of the homogenate to 5·0 was recorded, using a pH meter to determine the end-point. The determination was repeated three times with further aliquots and the mean of the four determinations calculated. This method was described by Shelef & Jay (1970).

Preparation and storage of meat extracts

Aqueous extracts. The method of Jay (1964) was employed to estimate the extract release volume (ERV) of the meat. Lean meat (25 g) was homogenized with distilled water (100 ml) for 2 min at full speed on an 'Atomix' blender. The homogenate was filtered through a folded Whatman No. 1 filter paper and the volume of liquid released in 15 min was recorded. After filtration aliquots (4 ml) of the filtrates were freeze-dried in glass ampoules, sealed and retained at −20° until required. Protein was precipitated from the rehydrated extract using trichloracetic acid (TCA), adding 0·25 ml TCA (50 g/100 ml) to 4 ml extract. The supernatant (aqueous protein-free meat extract) was used as soon as possible after centrifugation.

Alcoholic extracts. These were prepared according to the method of Gardner (1965) based on a technique described by Awapara (1948). Meat which had been retained at −20° until needed, was homogenized with industrial methylated spirit in the ratio 10 g meat to 6·0 ml methylated spirit resulting in a final alcohol concentration of 80 ml/100 ml aqueous phase (the moisture content of the meat was calculated to be 75 g/100 g). The precipitated proteins were removed by centrifugation, the volume of the alcoholic supernatant measured and shaken with three volumes of chloroform to remove the fat. The upper aqueous layer, containing amino acids from the meat, was removed with a Pasteur pipette and used as soon as possible.

Meat exudate. Exudate or 'drip' within the vacuum package was removed by pipette immediately on opening the package. Protein was precipitated by adding 0·25 ml TCA

(50 g/100 ml) to 1 ml of exudate and centrifugation was carried out in screw-capped bottles to minimize loss of volatile components. The protein-free supernatant was used immediately for gas chromatographic analysis of volatile components.

Lactic acid determination. The aqueous extracts (1 ml) were treated by the van Slyke (1917) procedure, involving the addition of $CuSO_4.5H_2O$ (20 g/100 ml) solution (1 ml) and solid $Ca(OH)_2$ (*c.* 1 g) to remove substances which might interfere with the lactic acid determination. Lactic acid was estimated using the method of Barker & Summerson (1941). The optical densities (O.D.) of the treated extracts were read at 565 nm using an SP 600 spectrophotometer (Pye Unicam Ltd, Cambridge) against a reacted blank of distilled water.

Total carbohydrate. Anthrone reagent, containing anthrone (0·5 g/100 ml), thiourea (1·0 g/100 ml) and concentrated H_2SO_4 (72 ml/100 ml) was prepared (Carrol, Longley & Roe, 1956). Aqueous protein-free meat extract (0·1 ml) diluted with distilled water (0·4 ml), was added to 5 ml anthrone reagent and the tubes heated in a boiling water bath for 15 min (Roe, 1955). After cooling, the O.D. of each sample was read at 620 nm. A standard graph was prepared using 25–300 μg/ml glucose in saturated benzoic acid solution.

Alcohol-precipitable carbohydrate (*APC*). Aqueous protein-free meat extract (0·2 ml) was added to aqueous ethanol (1 ml; Carrol *et al.*, 1956) stored overnight at 4°, the deposit redissolved in 1 ml distilled water and treated with anthrone reagent. The standard graph for total carbohydrate was used to estimate the APC but the values were first multiplied by 0·9 to convert the glucose value to glycogen (Carrol *et al.*, 1956).

Determination of changes in ninhydrin-positive material (*NPM*). Alcoholic meat extract (25 μl) were applied to corners of two sheets of chromatography paper (10 × 10 in Whatman No. 1). The papers were developed by the ascending technique, in the first dimension using *n*-butanol, pyridine, water (equal volumes) solvent and in the second dimension using phenol solution (200 ml) ammonia (1 ml) solvent (Smith, 1960). The papers were treated after drying with ninhydrin in acetone (0·2 g/100 ml) followed by heating at 100° for 5 min. From each paper, circles of 1 cm diameter were excised from each of the main regions of NPM, A-H as shown in Fig. 3. A circle of 1 cm diameter only of area H was cut out since at weeks 1–5 this area contained all of the ninhydrin positive material at this position. The cut areas were eluted individually in 5 ml methanol for 24 hr at 4°. The O.D. of each eluate was read at 509 nm against a control prepared from a blank piece of paper cut from the same chromatogram as the NPM. The mean of the duplicate O.D. readings for each pair of chromatograms was calculated. This procedure was carried out for vacuum packaged meat stored for nought to nine weeks.

GLC qualitative analysis of volatile components of meat exudate. Deproteinized exudate from six samples of meat which had been stored for eight weeks in vacuum packages and a control sample of fresh meat which had been vacuum packaged and stored overnight at 2° (to obtain some exudate) were examined by GLC for volatile fatty

acids. The following volatile acids (*c.* 0·001–0·002 M) were chromatographed both individually and as a mixture: acetic, propionic, *iso*-butyric, *n*-butyric, *iso*-valeric, *n*-valeric and caproic. Formic acid was not detected by the flame ionization detector. Some lower alcohols, i.e. methanol, ethanol, *n*-propanol, *n*-butanol and amyl alcohol and also diacetyl, were also injected for analysis.

Operating specifications of gas chromatograph

Chromatograph: Phillips PV 4000
Column dimensions: 3 m × 2 mm i.d. stainless steel coil
Solid support: 100–120 mesh acid-washed Chromosorb W
Stationary phase: 10% FFAP (Free Fatty Acid Phase; Phase-Sep Ltd, Deeside Industrial Estate, Queensferry, Flintshire)
Temperature of column: 150°
Carrier gas: Nitrogen, flow rate 30 ml/min
Detector: Flame ionization
Attenuation: 5×10^2
Recorder: Leeds & Northrup Speedomax W, −0·1, +1·0 mV FSD
Sample size: 5 μl
Analysis time: 6 min (to *n*-caproic acid, C_6)

Results and Discussion

Lactic acid levels varied from week to week, with no marked increase or decrease (Fig. 1). While there was no net accumulation of the compound, despite increasing

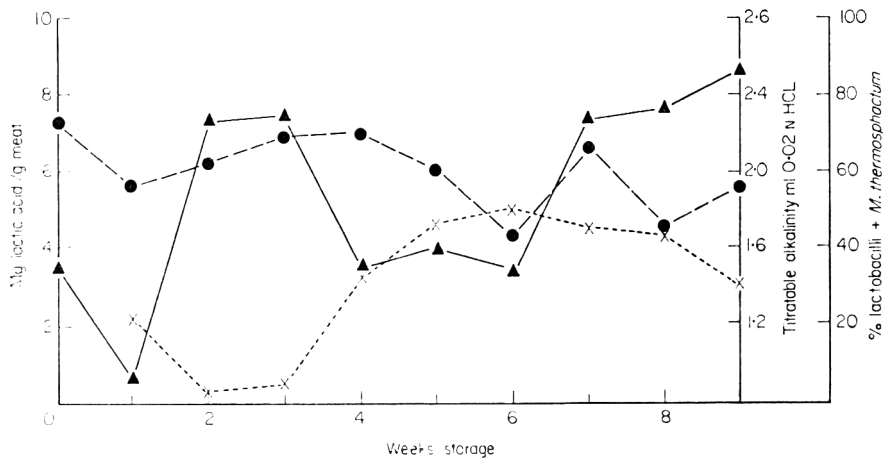


FIG. 1. Lactic acid levels, titratable alkalinity and $\log_{10}$ numbers of Gram positive, acid-forming bacteria on meat matured in vacuum packages. ▲, % lactobacilli + *M. thermosphactum*; ×, titratable alkalinity; ●, lactic acid.

numbers of lactic acid-producing organisms on the meat (Sutherland, Patterson & Murray, 1975), the pattern of increase and decrease of lactic acid concentration followed broadly that of the percentage of lactobacilli + *M. thermosphactum* present in the microflora (Fig. 1). This correlation of lactic acid concentration with the proportion of lactic acid bacteria in the microflora rather than actual numbers may be due to the further metabolism of lactic acid by the remainder of the microflora. Lactic acid determination is therefore not a useful method for evaluating the overall bacteriological condition of vacuum packaged beef; this was also concluded by Gardner (1965) for spoiling minced meat and Lea *et al.* (1969) for stored chicken meat. The titratable alkalinity (TA) appeared to be inversely related to the lactic acid, although other acids produced by microorganisms could also affect the TA. Metabolism of amino acids leading to production of ammonia, however, would counteract any change in pH or TA.

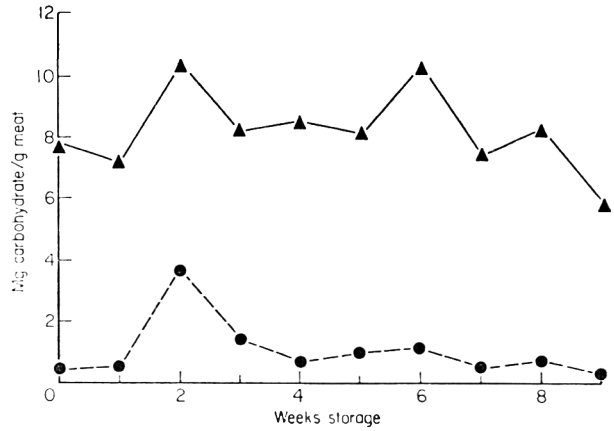


FIG. 2. Levels of total and alcohol-precipitable carbohydrate in meat matured in vacuum packages. ▲, total carbohydrate; ●, alcohol-precipitable carbohydrate.

The total carbohydrate value of vacuum packaged meat throughout the storage period was similar to that of fresh meat (*c.* 10 mg/g; Lawrie, 1966). Changes in the total carbohydrate content were also small and variable, with no particular trend of increase or decrease (Fig. 2). The APC comprised *c.* 12% of the total carbohydrate level at each week and the pattern of change was similar. While the variation in carbohydrate levels did not correspond to the increase in total bacterial numbers, the increase in total carbohydrate at week 2, which appeared to be accounted for by APC, coincided with a high proportion (66%) of Gram-positive, catalase-negative bacteria (i.e. excluding *M. thermosphactum*) on the meat, identified as lactic cocci, some of which are noted for polysaccharide production, e.g. *Leuconostoc*, *Pediococcus* spp. Polysaccharide (slime) may have been synthesized by these species effecting a conversion of carbohydrate to APC with little loss through fermentation. At week 6, the total carbohydrate

again attained a high value, but there was no correspondingly high level of APC, and the lactic cocci constituted a lower proportion of the microflora isolated. The flora at week 6 comprised 50% Gram-negative, non-fermentative rods, a higher percentage of such organisms than usual. In the absence of oxygen, or the very low levels present in vacuum packaged meat, they would presumably be incapable of converting carbohydrate to acidic products and hence the carbohydrate would remain at a high level. As with lactic acid, variation in carbohydrate level cannot be used to measure the overall bacteriological condition of vacuum packaged meat.

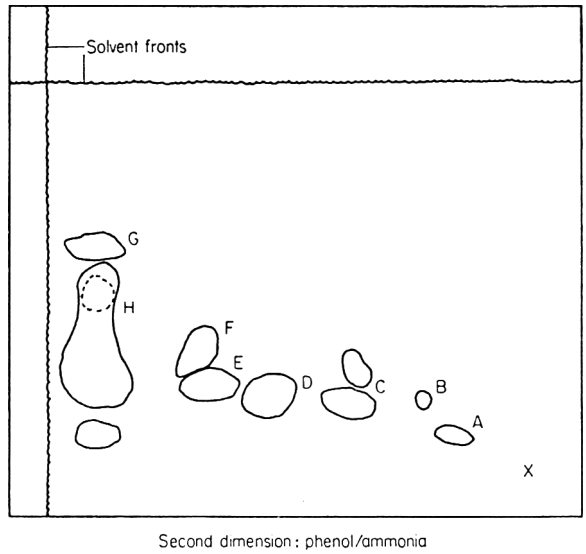
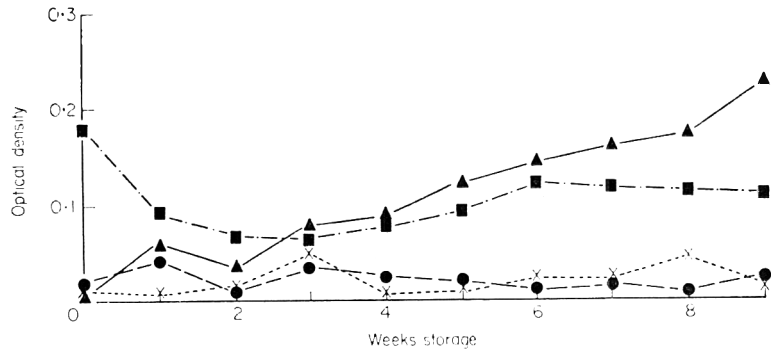
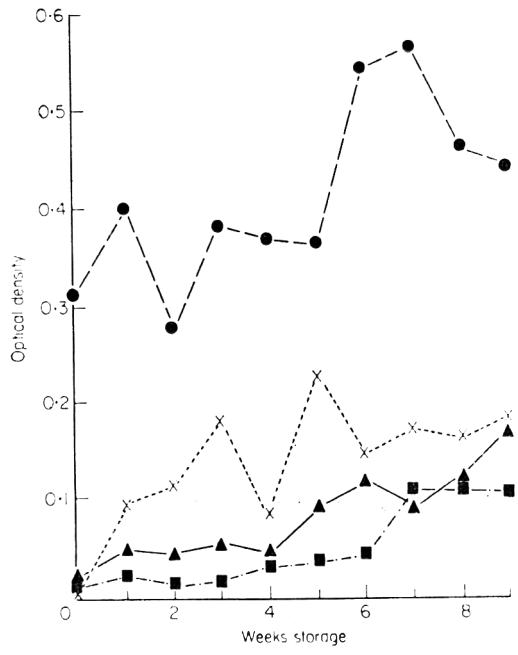


FIG. 3. Regions of ninhydrin positive material from meat matured in vacuum packages after nine weeks.

The changes in O.D. of the eluted regions of NPM A-H, illustrated by the chromatogram of the 9-week extract in Fig. 3, are given in Figs 4a and b. Initially, NPM was concentrated in regions A, D, E and G and although nearly all regions had become apparent at week 1, most showed a decrease in O.D. at week 2. At this stage, as mentioned above, the flora consisted largely of lactic cocci. Such nutritionally-demanding organisms may have depleted the available amino acids and peptides. Thereafter, there was a sustained increase in NPM to week 9, probably resulting from autolysis and microbial degradation of protein. Region H although only a small area in weeks 1–6, increased considerably in area thereafter and was probably an agglomeration of amino acids, peptides and α-amines (all of which are positive with ninhydrin) which failed to separate in the solvents used, so only a single representative area, present in all chromatograms was excised. Region F corresponds to a site to which many amines migrate in



(a)



(b)

FIG. 4. (a) Changes in optical density of eluates from 1 cm diameter circles in regions A-D of ninhydrin positive material of meat during nine weeks of storage in vacuum packages. ●, A; ×, B; ▲, C; ■, D. (b) Changes in optical density of eluates from 1 cm diameter circles in regions E-H of ninhydrin positive material of meat during nine weeks of storage in vacuum packages. ●, E; ×, F; ▲, G; ■, H.

the solvent systems used (Smith, 1960) and the results suggest that there was a small but steady increase in the level of amines in the vacuum packaged meat during storage at 0–2°.

The increase in NPM may be caused by the release of peptides and α-amines as a

consequence of the degradation of meat protein, although Sutherland *et al.* (1975) considered microbial proteolytic activity was unlikely in the low oxygen tension in evacuated packages, in view of the results obtained when representative isolates of the microflora were examined for their proteolytic activity.

The sour/acid odour observed on opening vacuum packaged beef was considered to be caused by volatile compounds arising from microbial metabolism. GLC analysis of deproteinized exudate from stored meat showed that a number of lower carboxylic acids were present, including acetic acid in greatest quantity, with smaller amounts of propionic acid and *iso*-butyric acid and virtually negligible quantities of *n*-butyric and *iso*-valeric acids and no caproic acid was detected. The peak heights corresponded approximately to the heights given by 0·001–0·002 M solutions of the acids in water. However, considerable amounts could have been lost on opening the package and during deproteinization owing to the volatility of such acids. Nevertheless, lower carboxylic acids occurred in the free form in vacuum packaged meat, and contributed to the odour although they may not have been solely responsible for it. Two unidentified peaks emerged before acetic acid: these were also present in fresh meat exudate which contained in addition a small amount of acetic acid and traces of the other volatile acids. The source of the acids seems unlikely to have been the carbohydrate since this remained low and relatively constant. However, deamination of amino acids can lead to the formation of carboxylic acids, e.g. acetic from glycine, propionic from alanine, *iso*-butyric from valine, although other more complex pathways may also be involved. Metabolic routes are highly complex and inter-related, the metabolic end-products of one group of organisms providing a suitable substrate for another group and it is therefore possible that a number of the Gram-positive, catalase-negative (or weakly catalase-positive) isolates, collectively termed 'lactobacilli', may be species of *Butyribacterium* and *Propionibacterium*, organisms more generally associated with cheese. These organisms could be responsible for the conversion of lactic acid to volatile carboxylic acids.

In conclusion it may be said that the lactic acid and carbohydrate contents of vacuum packaged beef changed little during storage for nine weeks and hence did not reflect the increase in microbial numbers. There was, however, an accumulation of NPM and also of volatile fatty acids which contribute to the acidic odour observed when vacuum packages are opened. A possible reason for the small changes in the levels of the constituents of meat recorded in these studies when compared with the number of bacteria present on the meat after storage *in vacuo*, is that while the surface of the meat was sampled for bacteriological examination, whole steaks, 2–3 cm thick, were homogenized to produce aqueous and alcoholic extracts. Thus changes in the constituents of meat brought about by microbial action at the surface, may have been masked by dilution. The results obtained, however, are more representative of the commercial situation in contrast to other work (e.g. Jay, 1964; Gardner, 1965) in which the spoilage of minced meat was studied, where a very large surface area of

meat was available for bacterial growth, and there were comparatively short diffusion paths for microbial nutrients, waste products and extracellular enzymes.

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Formation of nitrosylmyoglobin in bacon involving lactate dehydrogenase

K. S. CHEAH

Summary

When cured bacon was exposed to the air the metmyoglobin (MetMb) induced was reconverted to nitrosylmyoglobin (NOMb) by repacking the bacon *in vacuo* and storing the packs for 1–2 weeks at 5°C. The experimental evidence suggested that NOMb formation involved MetMb reduction by NADH formed from NAD^+ and lactate by lactate dehydrogenase (EC 1.1.1.27). All the components of this system were found to be present in bacon. The observation was supported by experiments showing *in vitro* NOMb formation using lactate dehydrogenase, NAD^+, MetMb and nitrite at pH values normally found in bacon.

Introduction

There are three hypotheses for the formation of nitrosylmyoglobin (NOMb) in cured meat products such as bacon. In all of these, metmyoglobin (MetMb) is considered to be the pigment that has to be reduced prior to NOMb formation. The reduction of MetMb has been postulated to be carried out either chemically by SH-groups, non-enzymatically by NADH in the presence of coenzymes or enzymatically by mitochondrial enzymes (see Möhler, 1973). The last hypothesis postulates the transfer of the nitrosyl group of nitrosylferricytochrome *c* by NADH-cytochrome *c* reductase to MetMb to form nitrosylmetmyoglobin (NOMetMb) which is subsequently reduced to NOMb by mitochondrial enzymes in the presence of NADH and nitrite (Walters, Casselden & Taylor, 1967).

The present investigation shows that in cured bacon lactate dehydrogenase can participate in NOMb formation by reducing NAD^+ to NADH which in turn reduces MetMb.

Experimental

Reagents

Sodium salts of ADP, ATP, malate, pyruvate and succinate and myoglobin were obtained from Sigma Chemical Corp.; lactate dehydrogenase (rabbit muscle) and NAD^+ from Boehringer. All other reagents were of analytical grade.

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Methods

The bacon was either purchased from commercial sources or processed (experimental bacon) at the Meat Research Institute by the Wiltshire method (Taylor, Dant & French, 1973) at 4°C using a brine solution containing 2000 ppm sodium nitrite and 25% NaCl. After maturation for seven days at 4°C, the bacon was sliced, vacuum packed in polyvinylchloride film and stored at 4°C before use. The formation of the brown colour of MetMb was induced by unpacking and exposing the cured bacon to air in the light at 5°C for one to two days. Reconversion of the MetMb to the original pink colour of NOMb was induced by repacking the bacon *in vacuo* and storing the packs at 5°C for one to two weeks.

Homogenates were prepared by mincing the bacon and then homogenizing the mince with a Thomas teflon-pestle glass homogenizer in the appropriate reaction medium. Oxygen uptake was measured either with the Clark oxygen electrode [Yellow Spring Biological Monitor (Model 53)] or with the Warburg apparatus. Lactate dehydrogenase (EC. 1.1.1.27) activity was assayed by the formation of NADH at 340 nm using the Aminco-Chance dual-wavelength/split-beam spectrophotometer. NAD^+ estimation was carried out as described by Klingenberg (1963). Other experimental details are described in the legends to the tables and figures.

MetMb was freshly prepared by oxidizing the pigment with ferricyanide and then removing the excess oxidizing agent by chromatography using a column of Sephadex G-25 previously equilibrated with 50 mM phosphate buffer (pH = 6·80).

Reflectance spectra were measured relative to barium sulphate and the dominant wavelength λ_d (a measure of hue) was calculated from the C.I.E. tristimulus values (Wyszecki & Stiles, 1967).

The microbiological examination was carried out as described by Taylor & Shaw (1975) using Plate Count Agar (PCA, Oxoid) plus 4% NaCl. The bacterial colonies were counted after incubation at 25°C for five days.

Results and discussion

Table 1 represents a typical experiment where NOMb was converted to MetMb in bacon by exposing slices wrapped in polyvinylchloride film to air for two days at 5°C in the light. The combined effect of oxygen and light caused the colour to change from pink to brown (λ_d decreased from 592 to 586 nm) and the reflectance spectra clearly showed that the brown colour was due to the formation of MetMb (strong absorption at 630 nm). When the faded bacon was repacked *in vacuo*, i.e. reducing conditions and stored at 5°C, the surface colour became pink (λ_d increased from 586 to 589 nm). The absorption band at 630 nm was no longer distinct indicating that MetMb was being reconverted to NOMb. The commercial bacon sample also showed the same phenomenon.

TABLE 1. Conversion of MetMb to NOMb in bacon

Longissimus dorsi (bacon)	pH	NO_2 (ppm)	Colour (bacon)	Oxygen uptake (natoms O/min/g bacon)	
				Malate + pyruvate	Succinate
7 days (5°C) vacuum packed	5·7	490	Pink (NOMb)	0	0
7 days (5°C) vacuum packed, exposed 2 days (5°C) to air	5·7	449	Brown (MetMb)	0	0
7 days (5°C) vacuum packed, exposed 2 days (5°C) to air, re-vacuum packed and stored 7 days (5°C)	5·8	338	Pink (NOMb)	0	0

The oxygen uptake was measured with the oxygen electrode at 25°C in a total volume of 3·0 ml containing 0·6 g bacon homogenate as compared with 1·0 g bacon homogenate with the Warburg apparatus. The reaction medium contained 50 mM phosphate buffer at pH 5·8. NOMb and MetMb were identified spectroscopically by Dr D. B. MacDougall. The viable count (Log_{10} per gram) of salt-tolerant bacteria ranged from 2·44 to 2·75.

TABLE 2. Non-enzymatic oxidation of NADH and NADPH by MetMb

Additions	Oxygen uptake (natoms O/min)
Succinate (45 μmol)	0
Malate (76 μmol) + pyruvate (90 μmol)	0
NAD^+ (17 μmol)	0
NADH (17 μmol)	302
NADPH (15 μmol)	285

Oxygen consumption was measured polarographically at 25°C in a total volume of 2·50 ml. The reaction medium contained 48 μmol MetMb in 50 mM sodium hydrogen maleate—50 mM NaOH buffer (pH = 5·60).

The commercial and the experimental cured bacon used in our experiments for the conversion of MetMb to NOMb did not consume oxygen (as judged by polarographic measurements over a period of 10 min with the oxygen electrode) even in the presence of either succinate or malate *plus* pyruvate.

However, when the oxygen uptake experiments were repeated over a period of 6 hr with the Warburg apparatus, a minute amount of oxygen was consumed by the bacon homogenate. At 37°C, the rate of oxygen uptake was about 3 μl of oxygen per gram of bacon per hour. This low activity could be due to a non-enzymatic reaction between MetMb and NADH or NADPH as demonstrated in Table 2. Both NADH and NADPH

could react with MetMb resulting in oxygen consumption. Neither succinate nor malate *plus* pyruvate could react with MetMb under our experimental conditions.

The conversion of the induced MetMb to NOMb in cured bacon (Table 1) could only occur after MetMb reduction, indicating that a reducing system was still active in the bacon. Our experiments showed that bacon contained a functional lactate dehydrogenase capable of reducing exogenous NAD^+ to NADH (about 260 nmol NADH formed per gram of bacon), which in turn could be completely re-oxidized stoichiometrically by exogenous MetMb. Table 3 illustrates a typical experiment carried out at pH 5·80. In addition to lactate dehydrogenase, bacon also contained NAD^+ (range from 34 to 364 nmol per gram bacon) and lactate (range from 69 to

TABLE 3. Formation of NADH in bacon homogenate and its re-oxidation by MetMb

Additions	NADH (nmol) re-oxidized
MetMb (7·8 nmol)	7·2
MetMb (70 nmol)	68·0

NADH formation was monitored at 340 nm using the Aminco-Chance spectrophotometer at 22°C. The sample cuvette contained 2·80 ml (0·23 g) bacon homogenate in 50 mM phosphate buffer (pH = 5·8) to which 0·20 ml NAD^+ (27 mM) was added. The reference cuvette contained the same volume of bacon homogenate and 0·20 ml phosphate buffer.

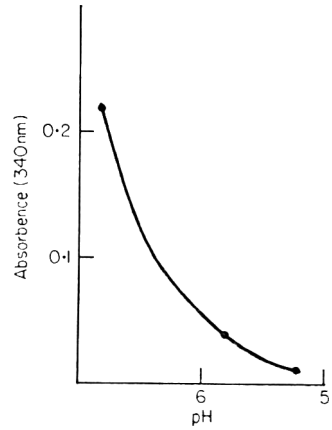


FIG. 1. Effect of pH on lactate dehydrogenase activity. Lactate dehydrogenase activity was assayed by following the formation of NADH at 340 nm using the Aminco-Chance Spectrophotometer. The sample cuvette contained 0·5 ml lactate (0·41 M), 0·2 ml NAD^+ (27 mM) and 0·02 ml lactate dehydrogenase (2 mg/ml) in 50 mM phosphate buffer (total volume = 2·72 ml). The reference cuvette contained all the constituents except lactate.

99 μmol per gram bacon). Thus, bacon contained all the necessary constituents for the generation of NADH for MetMb reduction.

Experiments carried out in model systems without bacon showed that lactate dehydrogenase could be involved in MetMb reduction. The experimental data showed that lactatede hydrogenase could function at pH values observed in bacon, and also that NOMb could be formed from MetMb in the presence of NAD^+, nitrite and lactate dehydrogenase. Figure 1 illustrates the effect of pH on lactate dehydrogenase activity measured by the formation of NADH at 340 nm. The data showed that the enzyme was still operating even as low as pH 5·3 in keeping with our previous observation that this enzyme could function at the pH values of bacon.

Figure 2 shows the formation of NOMb in model experiments in the absence of bacon at pH 5·80. The result was typical of each of the experiments conducted at three different pH values (5·50, 5·80 and 6·50). The data show that NOMb (574 nm) was only formed (– – –) if all the necessary components (lactate dehydrogenase, NAD^+, MetMb and nitrite) were present. NOMb was detectable 15 min after nitrite addition but not all the MetMb (635 nm) was converted to NOMb even up to four and a half days after nitrite addition. In the absence of nitrite (–·–·–), the MetMb was reduced and converted to MbO_2 (582 nm), as oxygen was available. The control experiment (——) which contained only MetMb showed a typical MetMb spectrum.

Our experimental data suggest that the lactate dehydrogenase in bacon, which contained all the necessary constituents for NADH formation, was the most likely

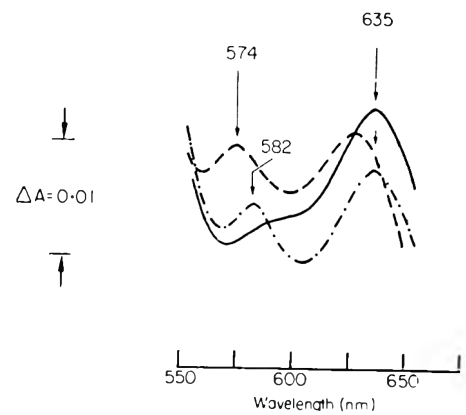
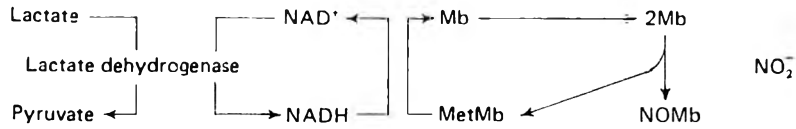


FIG. 2. Formation of NOMb from MetMb involving lactate dehydrogenase. The spectra were recorded with the Aminco-Chance spectrophotometer at 22°C. The test (– – –) contained 0·5 ml lactate (0·4 M), 0·4 ml NAD^+ (27 mM), 0·1 ml lactate dehydrogenase (2 mg/ml), 26 nmol MetMb and 6 mg nitrite in 50 mM phosphate buffer (total volume = 3·10 ml). The reaction mixture in a Thunberg cuvette was bubbled with oxygen-free N_2 for 30 min before nitrite addition. The first control (–·–·–) contained all the additions except nitrite, and the second control (——) contained only MetMb and buffer. The spectra illustrated were recorded 47 hr after nitrite addition.

system responsible for MetMb reduction prior to NOMb formation. The following scheme illustrates the pathway for NOMb formation from MetMb.



The formation of NOMb from Mb was described by Koizumi & Brown (1971) and was similar to that observed with Hb by Brooks (1937). The operation of lactate dehydrogenase for generating NADH for MetMb reduction prior to NOMb formation does not necessarily exclude other possible systems that might also be capable of reducing MetMb in cured bacon.

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The proteins of intermediate moisture meat stored at tropical temperature

III. Differences between muscles

Z. A. OBANU, D. A. LEDWARD AND R. A. LAWRIE

Summary

Intermediate moisture (i.m.) meats prepared from selected muscles of two ewes, one sow, five hens and two goats all underwent protein breakdown and complexing reactions during storage at 38 °C. The relative rates of these reactions varied from sample to sample. These reactions included colour changes culminating in the formation of 'Maillard' type brown pigments. This discolouration may well limit the storage life of these meats. It was apparent within three to nine weeks at 38 °C.

At 28 °C all the reactions occurred far more slowly.

One bull muscle which was also examined proved atypical in that although proteolysis occurred there was no evidence of any complexing and, up to nine weeks' storage, no evidence of any non-enzymic browning.

Introduction

It has previously been shown that during the processing and storage, at 38 °C, of cook-soak-equilibrated intermediate moisture (i.m.) beef, marked changes in the nature of the proteins occur (Obanu, Ledward & Lawrie, 1975a, b; Obanu & Ledward, 1975). Solubility and electrophoretic studies indicated that there was both crosslinking and breakdown of the proteins during storage (Obanu *et al.*, 1975a) and that these changes affected the eating quality of the i.m. beef (Obanu *et al.*, 1975b); Obanu & Ledward, 1975).

It seems probable that the ultimate quality of the stored i.m. meats will depend on the relative rates of the crosslinking and degradative reactions and different samples may well respond differently to processing and storage. The present study was undertaken to check on the generality of the results obtained on i.m. steer *longissimus dorsi* muscles stored at 38 °C (Obanu *et al.*, 1975a, b; Obanu & Ledward, 1975) by studying the behaviour of muscles selected from goats, ewes, sow, hen and bull when processed and stored at 38 °C. As 38 °C is a maximal tropical temperature some muscles were also

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stored at 28 °C (a median tropical temperature) to give some insight into the temperature dependence of the reactions.

Materials and methods

The muscles used in the present study were:

(i) *Longissimus dorsi* from a six-month goat (female): Goat A;
(ii) *Longissimus dorsi* from a three-month goat (female): Goat B;
(iii) *Longissimus dorsi* from a four and a half-year ewe: Ewe A;
(iv) *Longissimus dorsi* from a three and a half-year ewe: Ewe B;
(v) *Longissimus dorsi* from a two and a half-year sow;
(iv) Combined breast muscles from five hens, each about six months old;
(vii) Combined leg muscles of each of the hind legs from Goat A.

These muscles were chosen as they typify the range of meats which may be preferred in a developing tropical country (Obanu, 1975). Some tests were also performed on i.m. samples prepared from the *longissimus dorsi* muscle of an eleven-month old bull. Only a limited number of tests were possible on this muscle as it was primarily prepared for some nutritional studies.

Preparation and storage of the samples

The intermediate moisture meat samples (1 cm^3) were prepared and processed to yield samples of a_w 0·85 (Obanu *et al.*, 1975a). All samples were stored in air-impermeable cryovac PVDC bags at 38 °C; in addition some of the hen and sow samples were also stored at 28 °C. Uncooked samples of the muscles were blast frozen at −20 °C and stored at −0 °C for use as controls.

Samples were taken at intervals of three weeks, frozen in liquid nitrogen and pulverized with dry ice. Some 1 cm^3 meat was retained for texture, moisture, colour and thiobarbituric acid (TBA) determinations.

Analytical methods

The variables determined at each sampling were moisture content, pH, solubility in 0·1 M KCl, solubility in 3% SDS plus 1% β-mercaptoethanol, hydroxyproline release (by measurement of the solubility in Ringers solution at 77 °C), texture and TBA value. The techniques used have already been described (Obanu *et al.*, 1975a, b). Electrophoretograms of the SDS/β-mercaptoethanol soluble proteins of selected samples were prepared (Obanu *et al.*, 1975a).

Reflectance spectra and solubility of the haematin complexes in pyridine (40% v/v) were determined as before (Obanu & Ledward, 1975). Non-enzymic browning is obviously an important factor affecting the storage life of these meats (Obanu &

Ledward, 1975). However, due to the insolubility of the brown pigments formed in the products, and the contribution of the haematin pigments to the overall browness, it was not thought possible to objectively assess the degree of non-enzymic browning, but subjective assessment of colour changes was attempted (Obanu & Ledward, 1975).

Results

Moisture content and pH

The moisture content of all the samples after processing was a little greater than that of the steer samples studied previously (Obanu *et al.*, 1975a). It ranged from 45·8% in the *longissimus dorsi* of one of the ewes to 50·8% in the *longissimus dorsi* muscle of one of the goats (Table 1).

As previously found for steer *longissimus dorsi* (Obanu *et al.*, 1975a) all the samples lost some moisture on storage at 38 °C (Table 1).

In all these samples a decrease in pH was observed over twelve weeks of storage at 38 °C. The decreases were from 5·7 to 5·4 and 6·0 to 5·6 in the goat *longissimus dorsi* muscles, 5·8 to5·6 and 5·8 to 5·4 in the goat leg muscles, 5·7 to 5·3 and 5·7 to 5·4 in the

TABLE 1. Moisture contents (% w/w) of i.m. meats stored at 38 °C

Storage time (weeks)	Goat A *l. dorsi*	Goat B *l. dorsi*	Goat A leg	Ewe A *l. dorsi*	Ewe B *l. dorsi*	Hen breast	Sow *l. dorsi*	Sow* *l. dorsi*
0	46·3	50·8	49·5	45·8	47·6	49·7	48·8	48·8
3	44·6	49·7	46·4	45·0	45·4	49·4	50·6	48·7
6	42·6	45·3	44·2	41·3	—	39·2	39·7	43·4
9	40·5	40·8	47·1	39·4	43·4	39·0	43·4	44·7
12	33·9	39·5	43·8	38·7	41·4	43·9	42·8	48·3

* These samples were stored at 28 °C.

TABLE 2. Effect of storage at 38 °C on the solubility of i.m. meats in 0·1 M KCl expressed as (soluble nitrogen × 100/total nitrogen)

Storage time (weeks)	Goat A *l. dorsi*	Goat B *l. dorsi*	Goat A leg	Ewe A *l. dorsi*	Ewe B *l. dorsi*	Hen breast	Sow *l. dorsi*
0	6·6	5·4	5·7	6·8	6·2	5·8	7·4
3	7·0	6·4	7·9	10·1	7·3	9·5	6·1
6	7·5	7·2	8·7	6·6	8·7	9·4	7·4
9	10·7	12·2	10·5	8·4	8·9	10·1	9·0
12	11·0	11·7	11·1	7·8	8·3	10·7	9·2
Fresh meat cooked in water	10·8	9·7	9·9	10·5	10·3	12·5	12·0

ewe muscles, 5·8 to 5·3 in the hen muscles and 5·6 to 5·2 in the sow muscle. No decrease was observed in the steer sample previously examined (Obanu *et al.*, 1975a).

Solubility in 0·1 M *KCl*

No protein nitrogen was solubilized in any of the samples as shown by the absence of a precipitate on the addition of 10 ml of 20% trichloroacetic acid (TCA). The non-protein nitrogen (NPN) extracted by 0·1 M KCl usually decreased on processing and then increased during subsequent storage at 38 °C (Table 2). This behaviour was also found in steer (Obanu *et al.*, 1975a). At 28 °C the increases in KCl soluble nitrogen were much less marked, there being no apparent increase in the sow *longissimus dorsi* while the hen samples only increased from 5·8 to 7·9% over twelve weeks.

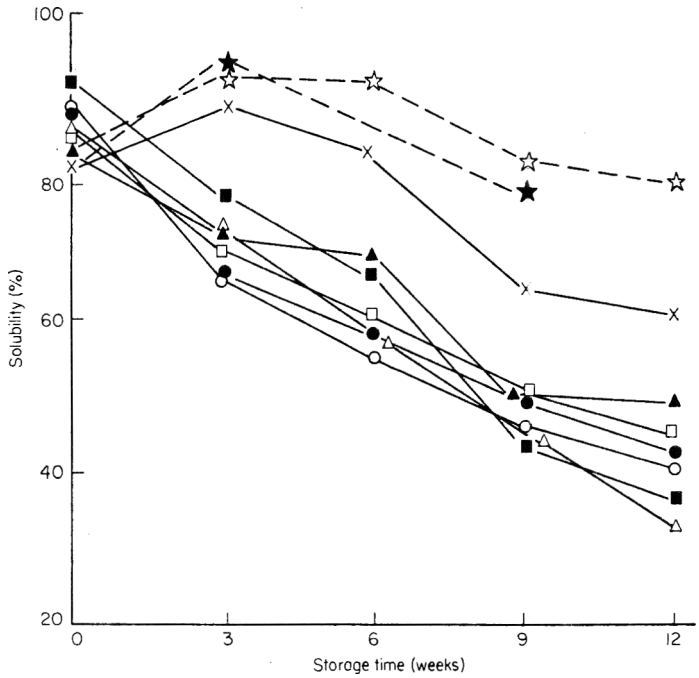

FIG. 1. Solubility of nitrogen in 3% SDS + 1% β-mercaptoethanol during storage at 28 and 38 °C. Solubility is defined as (soluble nitrogen × 100/total nitrogen). ○, goat A *longissimus dorsi* at 38 °C; ●, goat B *longissimus dorsi* at 38 °C; □, goat A leg at 38 °C; ■, ewe A *longissimus dorsi* at 38 °C; △, ewe B *longissimus dorsi* at 38 °C; ▲, sow *longissimus dorsi* at 38 °C; ×, hen breast at 38 °C; ☆, sow *longissimus dorsi* at 28 °C; ★, hen breast at 28 °C.

Solubility in SDS/β-mercaptoethanol

Results on the solubility of the i.m. meats in 3% SDS/1% β-mercaptoethanol are summarized in Fig. 1. It is apparent that, at 38 °C, all the i.m. samples show decreased

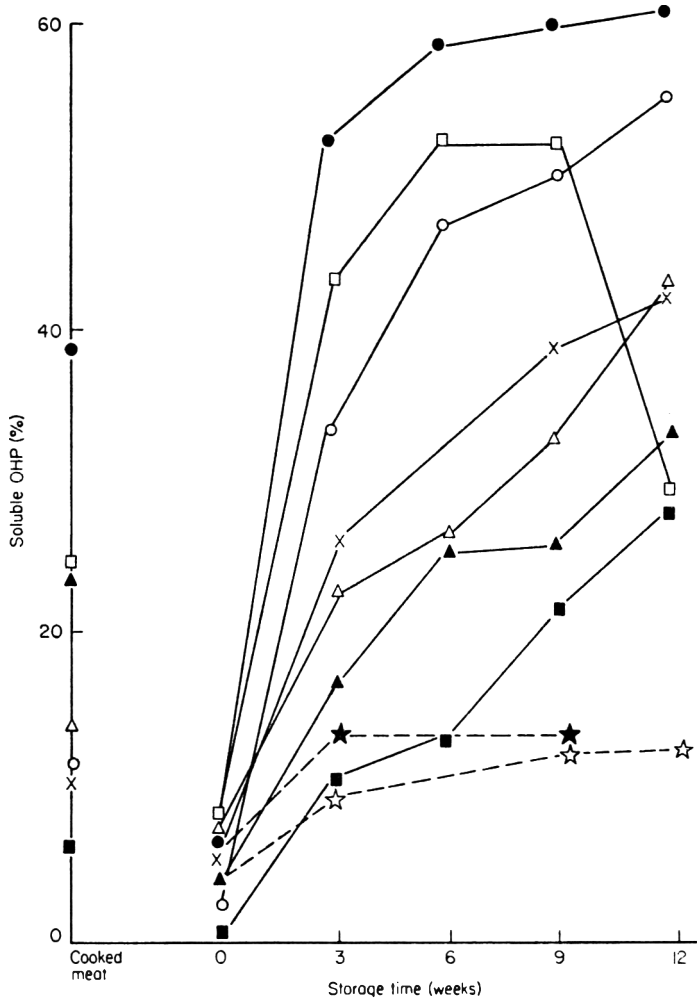

FIG. 2. Solubility of OHP in different i.m. samples during storage at 28 and 38 °C. Solubility is defined as (soluble OHP × 100/total OHP). ○, goat A *longissimus dorsi* at 38 °C; ●, goat B *longissimus dorsi* at 38 °C; □, goat leg A at 38 °C; ■, ewe A *longissimus dorsi* at 38 °C; △, ewe B *longissimus dorsi* at 38 °C; ▲, sow *longissimus dorsi* at 38 °C; ×, hen breast at 38 °C; ☆, sow *longissimus dorsi* at 28 °C; ★, hen breast at 28 °C. The values for the cooked meat were determined on meat freshly cooked in water at the same temperature as that used to prepare the i.m. samples, i.e. 70 °C.

solubility on prolonged storage. This was also found for the steer samples studied previously (Obanu *et al.*, 1975a). At 28 °C the changes were far less marked and both the sow and hen samples showed slight increases from nought to six weeks followed by a steady decrease in solubility (Fig. 1). This behaviour is similar to that observed in steer *longissimus dorsi* at 38 °C (Obanu *et al.*, 1975a).

Collagen breakdown

As shown in Fig. 2 there is a marked decrease in soluble hydroxyproline (OHP) following processing but on storage the amount solubilized increases, at different rates, in all the samples. These results are in general agreement with those reported for the steer *longissimus dorsi* i.m. samples (Obanu *et al.*, 1975a).

Electrophoresis

Electrophoretograms of selected samples all showed the patterns expected from the solubility data (i.e. samples showed electrophoretograms consistent with increased insolubility) as previously observed (Obanu *et al.*, 1975a).

Texture measurements

Shear force measurements on the samples are summarized in Fig. 3. It is seen that, as with the steer (Obanu *et al.*, 1975b), there is a rapid tenderization of the samples during the first three weeks of storage. The three-week values of the samples vary from about 40% (goat leg muscle) to 75% (goat *longissimus dorsi*) of the initial shear force areas, cf. the steer samples previously studied (Obanu *et al.*, 1975b).

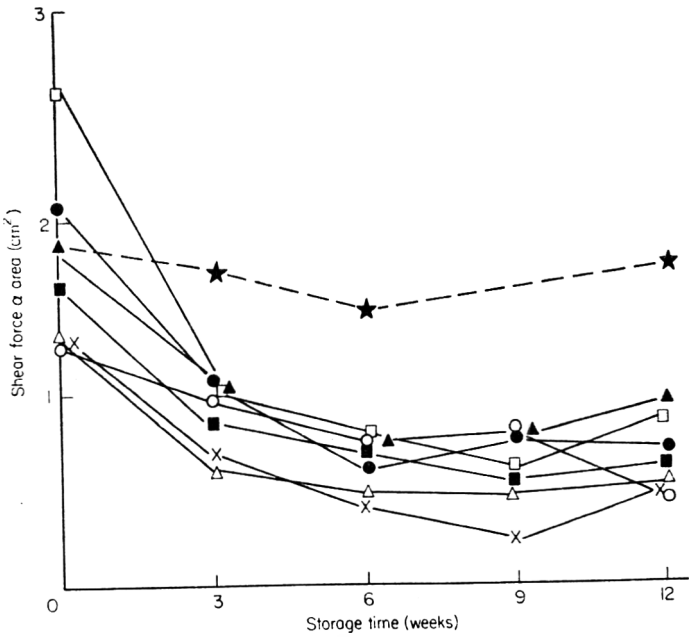

FIG. 3. Effect of storage at 38 °C on the tenderness (measured as a shear force area (cm^2) of i.m. meats. ○, goat A *longissismus dorsi*; ●, goat B *longissimus dorsi*; □, goat A leg; ■, ewe A *longissimus dorsi*; △, ewe B *longissimus dorsi*; ▲, sow *longissimus dorsi*; ×, hen breast; ★, sow *longissimus dorsi* at 28 °C.

At 28 °C the changes were far less marked; the three-week values of the sow and hen samples being 91 and 86% of the initial shear force values.

TBA value

As can be seen from Table 3 the variation in TBA value of the different samples during storage is complex. Some yield an initial decrease followed by an increase (goat legs and ewe B *longissimus dorsi* at 38 °C) others an initial decrease over three weeks and then little change (goat *longissimus dorsi* and sow *longissimus dorsi* at 38 °C while ewe A *longissimus dorsi* and hen breast at 38 °C and all the samples at 28 °C display increases in TBA value during the first three to six weeks of storage.

TABLE 3. TBA* values of i.m. meat samples during storage at 38 °C and 28 °C

Storage time (weeks)	Storage at 38 °C							Storage at 28 °C	
	Goat A *l. dorsi*	Goat B *l. dorsi*	Goat A leg	Ewe A *l. dorsi*	Ewe B *l. dorsi*	Sow *l. dorsi*	Hen breast	Sow *l. dorsi*	Hen breast
0	13·1	12·5	3·2	1·4	3·1	9·0	1·4	9·0	1·4
3	3·9	3·0	0·7	5·2	1·2	4·3	3·4	9·8	8·4
6	3·7	3·9	4·0	5·6	5·1	3·6	3·8	7·4	—
9	4·2	3·0	5·0	3·9	4·2	3·6	3·6	5·9	6·2
12	2·8	3·0	4·2	3·0	3·9	3·1	3·3	4·8	—

* TBA value is defined as milligrams malonaldehyde/1000 g of meat.

Colour measurements

After processing the samples exhibited reflectance spectra typical of cooked meat (Obanu & Ledward, 1975) with absorption peaks (reflectance minima) at about 415, 540 and 640 nm. In all samples, the spectra changed during three weeks of storage to that typical of free haematin with a single, broad absorption peak at 380–400 nm. These results agree with those observed on the steer *longissimus dorsi* samples (Obanu & Ledward, 1975).

As found with the steer samples (Obanu & Ledward, 1975) only limited solubility of the haematin of the goats and ewes was achieved in 40% pyridine and the amount extracted decreased during storage (Fig. 4). The pigment concentration in the sow and hen was too low to allow meaningful results to be achieved.

Subjective evaluation by three observers of the colour of samples stored at 38 °C for different times indicated that browning occurred in the hen and sow muscles after three weeks, and in the goats and ewes after six to nine weeks. This compares with a time of about twelve weeks in the steer samples (Obanu & Ledward, 1975). These differences in time may not be real as the natural brown colour of the ewe, goat and

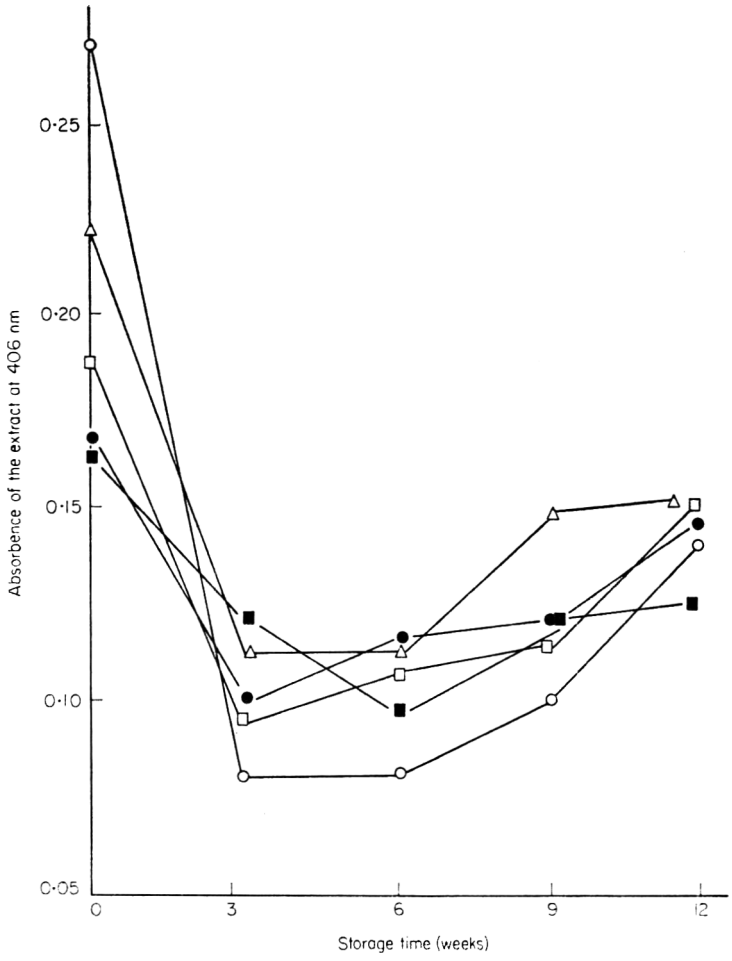

FIG. 4. The extractability by pyridine (40% v/v) of the haematin complexes in i.m. meats during storage at 38 °C. ○, goat A *longissimus dorsi*; ●, goat B *longissimus dorsi*; □, goat A leg; ■, ewe A *longissimus dorsi*; △, ewe B *longissimus dorsi*.

steer samples may mask the first sign of non-enzymic browning. On tasting, the bitterness normally associated with non-enzymic browning of the samples was not detected but this may be because the glycerol disguised the flavour.

Browning was apparent in the sow and hen samples stored at 28 °C after six weeks' storage.

The bull *longissimus dorsi* gave several results which differed markedly from those of the steer muscles previously examined (Obanu *et al.*, 1975a, b; Obanu & Ledward, 1975) and differed also from those of the goats, ewes, sow and hens in this study. Unlike these muscles, the bull *longissimus dorsi* showed an increase in 0·1 M KCl-soluble

nitrogen (all NPN) on processing (from 3·8% in ordinary cooking to 6·3% in cook-soak equilibration), and a further very rapid increase to 13·5% within three weeks of storage at 38 °C. Volodkevich texture measurements showed that very marked tenderization occurred, the three-week shear force area being only 15% of the initial value. That marked protein breakdown was occurring was also indicated by the rapid release of soluble OHP from these samples. The percentages of soluble OHP obtained were 11·6, 39·9, 63·4 and 64·5 at nought, three, six and nine weeks respectively. The bull muscle showed no appreciable decrease in solubility in SDS/β-mercaptoethanol (96·3 to 90·2%) during storage at 38 °C for nine weeks and the electrophoretograms showed no apparent differences throughout storage. Also, unlike all other samples studied, the spectra of the bull *longissimus dorsi* i.m. samples did not change during nine weeks of storage at 38 °C and the extractability, by pyridine, of the haematin from the bull i.m. meat was high and did not decrease appreciably during storage; the absorbances of the extracts at 406 nm were 0·542, 0·558, 0·542 and 0·485 at nought, three, six and nine weeks of storage respectively. Subjective assessment of the samples indicated that no marked colour change occurred during nine weeks' storage.

Discussion

The processing and storage changes found in all but the bull i.m. samples were similar to those observed with meat obtained from a steer (Obanu *et al.*, 1975a, b; Obanu & Ledward, 1975) and are indicative of both proteolysis (e.g. increased NPN and release of OHP on storage) and crosslinking (insolubility in SDS/β-mercaptoethanol and 40% v/v Pyridine) occurring. The results obtained with the bull are unusual as, although there is evidence of proteolysis occurring, there is no evidence for any of the complexing reactions taking place. Work is at present in progress to confirm, or not, whether this atypical behaviour is common to all bull meat.

Consideration of the results for the muscles other than bull indicate that the rate of the breakdown and complexing reactions varies from muscle to muscle.

Consideration of the rate of NPN and OHP release (Table 2, Fig. 2) suggest that the relative rates of general protein breakdown and collagen breakdown are similar, the rates decreasing in the order Goat B, Goat A, Hen, Ewe B, Sow, Ewe A which comparing the goat and ewe results, suggests that the younger the animal the more rapid the breakdown. It should be noted though that the amount of soluble OHP in the goat leg muscles actually decreases after six to nine weeks (Fig. 2). The previous study on steer *longissimus dorsi* samples suggests that in this muscle the proteolysis is very rapid (Obanu *et al.*, 1975a, b).

One would expect the solubility in SDS/β-mercaptoethanol to reflect the result of both the crosslinking and breakdown reactions and thus if the crosslinking rates are similar, the solubility might be expected to remain highest in the order steer, goat and hen. This relationship is found for the steer and hen (Fig. 1; Obanu *et al.*, 1975a) but

all the goat samples rapidly became insoluble. Thus goat muscle appears to be more susceptible to the crosslinking reactions than the others. Also it would be expected that the texture of the samples would reflect this balance between breakdown and crosslinking but the inherent variations in shear force values and possible complex effects of the two reactions on texture make meaningful comparisons difficult.

The nature of the degradative and polymerization reactions is not yet known, but the changes in the TBA values during storage suggests that carbonyls may play a role. It has recently been shown that malonaldehyde (which is a product of lipid oxidation and reacts with TBA) could react with collagen to yield stable crosslinks (Svadlenka, Davidkova & Rosmus, 1975). If these TBA reactive carbonyls are such essential reactants it is not surprising that the concentration of 'free' carbonyls varies from sample to sample and behaves differently from sample to sample with storage (Table 3).

From this study it would appear that, at 38 °C, the storage life of i.m. meat varies from three to twelve weeks depending on the samples, non-enzymic browning, due to some of the chemical changes described in this paper, being the factor which defines the shelf life. If this colourization is not objected to then the storage life may be longer. The effect of these changes on nutritional quality is at present being studied.

This work has also shown that at 28 °C (a medium tropical temperature) these chemical changes occur much more slowly so that in practice the storage life may be expected to be in excess of six weeks for even the most susceptible samples, i.e. the white muscles of pork and hen.

Acknowledgments

Financial support from the Association of Commonwealth Universities is gratefully acknowledged.

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(*Received* 8 *September* 1975)

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Book Reviews

Principles of Meat Science. By J. C. FORREST, E. D. ABERLE, H. B. HEDRIK, M. D. JUDGE and R. A. MERKEL.
San Francisco: W. H. Freeman, 1975. Pp. 417. $17.50.

In the Preface the authors indicate that the text is intended to serve as an introductory course on Meat Science for students and to supplement another volume by the same publishers entitled *The Science of Meat and Meat Products*. They also state that it is oriented towards the meat industry in the United States; and, indeed, after reading the book, one feels that *The Principles of Meat Science and Meat Technology (with particular reference to U.S.A.)* would have been a more appropriate title.

The Science of Meat and Meat Products was last published in 1971; and the present text, apart from its main intention, incorporates more recent views on many topics within this fast-changing field of knowledge. Being jointly written by its five authors, instead of being a compilation of the views of two dozen contributors on various aspects of the subject, it also provides a more coherent and, in general, more logical account of the scientific aspects of meat.

The subject is presented in three parts. The first, 'Introduction', outlines the background to meat as a food, and the goals in animal production, in two chapters. 'Muscle and associated tissues' is the topic of Part II. In three chapters, the authors give a most useful account of muscle structure and formation at a level suitable for the intended readership. It is excellently illustrated with diagrams and microphotographs. The only criticism is that there is rather more histological and physiological detail, both on muscle itself and on the associated tissues, than seems strictly necessary.

The remaining twelve chapters of the book constitute Part III, 'Meat science'. These take the reader from a consideration of the conversion of muscle to meat, factors affecting post mortem change and the properties of fresh meat, through the principles of meat preservation and on to cooking and nutritive value, meat grading and the identification of meat cuts.

All these features are clearly described; but it would perhaps have been more logical had the subject of Chapter 9, 'Principles of meat processing' (largely concerned with extraneous and derivative operations such as sausage formulation and spices), followed those in Chapters 10 and 11, which deal respectively with 'Microbiology, deterioration and contamination' and 'Methods of storing and preserving meat'.

The last four chapters (on meat inspection, grading, byproducts and the identification of wholesale and retail cuts) in so far as they refer particularly to the USA meat industry, are not directly relevant to UK conditions. Moreover, their inclusion (together with much of Chapter 9) under the heading 'Meat science' can be questioned. The topics are more properly the concern of those studying meat technology and butchery.

Probably because the book is designed to be introductory, the bibliography is limited.

Most of the facts presented in the text, therefore, are not related to the original work from which they were derived. On the one hand, of course, this enhances the readability of the text. On the other hand, there are clearly advantages in being able to follow up readily any topics of particular interest, especially where views, contrary to the generally accepted ones, are expressed. Fortunately the text is well founded and is virtually free from controversial matter. The statement on p. 92, however, to the effect that 'all muscle fibers appear to be red at birth, but shortly thereafter some of them differentiate into white and intermediate fiber types' may be seriously questioned.

The only typographical error detected appears on p. 74, in Table 3.3, where the total percentage of unsaturated fatty acids in the subcutaneous fat of sheep is represented as 62·3% instead of 42·3%.

In contemplating an expenditure of the order of $18 the student commencing meat science might well feel that the second edition of *The Science of Meat and Meat Products*, notwithstanding its more advanced but somewhat less logical and less recent approach, was marginally preferable. Nevertheless, the authors have produced an excellent, readily understood, modern text, which should be in all libraries concerned with the science and technology of foods. It can be strongly recommended.

Individual books should be examined before purchase, as it has been noted that the reproduction of the print is not perfect in all copies.

RALSTON LAWRIE

Fish and Shellfish Processing: Food Technology Review No. 22. By M .T. GILLIES. New Jersey: Noyes Data Corporation, 1975. Pp. x + 3. US $36.00.

This book is a further addition to the Noyes Data Corporation series of surveys of US Patents from 1960 to 1974. It serves a useful purpose in providing extracts from patents in the field of fish and shellfish processing thus making it simple for the research worker or designer to search the patent field quickly.

The author has set out to review each patent and record the relevant technical details without including the legal jargon and this has certainly been achieved. However, it is a pity that in such a useful work the review is merely presented and no critical assessment is made of the subject matter of the patent. It is therefore necessary to have some expert knowledge before maximum value can be obtained from this volume.

The subject matter is logically divided into seven chapters enabling the patents to be grouped together and indexes of the authors, companies and patent numbers are provided. Some criticism of any grouping can always be made but in this work sufficient sub-headings are provided to enable the reader to locate a subject readily.

The introduction to some of the sections may be misleading in the overall world context and it must be remembered that not all of the inventions which are the subject of US Patents originate in the United States and conversely US Patents are also of

interest throughout the world. For example, the introduction to the section on sardines might mislead the non-expert into believing that the process referred to is the only process for canning sardines.

The patent by Jason and Sanders on electrical resistance thawing of blocks of frozen fish is included in the chapter on 'Tuna and tuna-like species' although the two examples of thawing given in the patent are of $1\frac{5}{8}$-inch thick blocks of herring and white fish fillets. Although it has not been possible to read the whole text other items may be similarly misplaced.

This volume will therefore be a useful reference for the expert who will be able to discern from the review whether he requires to obtain the full specification.

M. R. HEWITT

Food Process Engineering. By H. A. LENIGER and W. A. BEVERLOO. Dordrecht: Reidel, 1975. Pp. x + 552. Cloth U.S. $39, Paper U.S. $19.

As the authors inform us in the Preface, this book has resulted from many years of teaching engineering aspects of food technology at the Agricultural University of Wageningen, The Netherlands.

The book is divided into four chapters. Chapter 1 is a short introduction outlining the character of food processing and food process engineering. The differences between food processing and chemical processing are stressed; certain concepts such as 'processes' and 'processing', are explained; and certain chemical engineering terms used in later chapters are introduced.

In Chapter 2, the elements of fluid dynamics, fluid/particle mechanics, heat transfer and mass transfer are developed along traditional chemical engineering lines. Though the authors do consider that this chapter might serve as a refresher course and have omitted the derivations of many of the equations, those who are less mathematically inclined might find some of the content formidable.

Chapter 3, 'Mechanical operations', applies the fluid and particle mechanics introduced earlier to those chemical engineering unit operations in which heat and/or mass transfer are not primarily involved. Handling of materials, mixing of materials, size reduction of materials, size enlargement of materials, separation of solids from liquids, separation of solid/gas mixtures, separation of solids, separation of liquid/gas mixtures and separation of liquids are among the topics covered.

Chapter 4, 'Physical operations', is concerned with those operations in which heat transfer and/or mass transfer considerations are all important. The practical aspects of heat exchange are first discussed in some detail, followed by a study of the physical methods of food preservation including heat preservation, low temperature preservation and dehydration. The second part of this chapter is concerned with physical separations including concentration, extraction, distillation and crystallization and concludes with a brief account of some miscellaneous separation methods.

Though containing much material of real interest to practising food process engineers, this book is primarily a textbook. It comes, therefore, as something of a surprise to find no references to either general textbooks or to the increasing volume of literature in this field. With the publication of *Food Process Engineering*, another book joins the relatively small number of works attempting to deal with food process engineering as a subject in its own right. For this reason alone this book will be very welcome.

J. R. BUTTERS

Seiyû Roku: On Oil Manufacturing. By OKURA NAGATSUNE (translation from the nineteenth century Japanese text by Eiko Ariga).
New Brunswick: Olearius Editions, 1974. Pp. 80. £3.40.

Okura Nagatsune was born in 1768 on the Japanese island of Kyushu, and came from farming stock. During a life of nearly ninety years he published a number of books in the field of agricultural technology, whose clear aim was to enable the farmer to broaden his sources of income and to obtain some added value from processing his harvested crops. He formed a one man agricultural extension service and, since several of his books were reprinted, he is thought to have exerted a significant influence.

This book is however not merely a practical guide couched in simple language. It contains twentysix illustrations by one of the foremost wood block artists of the day, Matsukawa Hanzan.

The present translation, and its publication are at the initiative of Professor Carter Litchfield, who is well known, both for his original contributions to the chemistry and biochemistry of oils and fats, and as a bibliophile with an extensive collection of printed works on oils and fats technology. Dr Litchfield found a first edition of Okura's book in the Burndy Library in Norwalk, Connecticut and formed the ambition to have it translated and to share its delights with us.

The subject dealt with by Okura is the extraction by pressing of rapeseed and cottonseed oil and a simple alkali refining treatment. Three variations of processing, prevailing in different districts of Japan, are described and fully illustrated. The processing consisted of drying of the seeds by the sun and by parching over a fire, pounding to a powder and sieving, steaming and pressing. The press cake was recycled twice through the pounding, parching and pressing procedures to obtain maximum yield of oil.

Attention is paid to the tools required and their quality, and cost calculations for each process are presented. Finally there are lists of prices to be expected for the oil after allowing for its transport to the market.

The text and illustrations of the original were printed from hand carved wooden blocks. The present book contains faithful reproductions of these blocks, with an English translation of the text, expertly done by Eiko Ariga. In addition there is a

biographical note on Okura by Professor Richard C. Rudolph and a comparison with western oil technology of the same period by Dr Litchfield.

The book has been printed, bound and presented in a near reproduction of the traditional Japanese methods.

This unusual book cannot be judged by the yardsticks usually employed in these pages. It is a most desirable possession with a very wide appeal to the book lover, as an example of the (to us) unfamiliar printing and binding conventions of another civilization. To anyone interested in the graphic arts, the quality of the drawings is most striking. Using very simple lines the artist shows in each human figure an energetic devotion to the task in hand.

To anyone interested in the history of food technology the drawings and the text enable comparisons to be made in two ways, with European methods of the same time, and with modern processes. Although the equipment and methods shown are primitive and laborious, there is careful weighing and measuring at each step, and one's heart warms to see the quality control man seated on a platform above the dirt of the factory floor, peering into a row of porcelain dishes to estimate the oil colour after refining, and with a tasting spoon poised in his hand.

In short, the *Seiyū Roku* is an ideal gift, and if it has come too late for this Christmas, we all have birthdays, and if they are too far off, then there are many occasions for an 'unbirthday' present.

We have good reason to be grateful to Dr Litchfield for making accessible to us at so reasonable a price a fascinating work of the time during which Japan had completely cut itself off from the west, and look forward to further imprints of Olearius Editions.

K. G. BERGER

Fruit and Vegetable Juice Processing: Food Technology Review No. 21. By J. K. PAUL.
New Jersey: Noyes Data Corporation, 1975. Pp. x + 277. US$36.

This review of 165 US patents deals with all aspects of fruit and vegetable juice processing. The eight sections include manufacturing techniques, concentration processes including freeze concentration, dehydration, freeze drying, stabilization processes using chemical additives, flavours from juices, juice enhancers and miscellaneous processes.

The section on manufacturing techniques includes apple, citrus, grape and tomato juices together with two patents on vegetable juices. The most interesting section deals with concentration processes and includes osmotic processes as well as freeze concentration. Dehydration techniques reported cover drum, foam-mat spray and vacuum drying and a range of freeze drying processes, viz. fluidized bed, spray and foam drying.

The next sections give details of antioxidant compositions, stabilization using chemicals such as benzohydroxamic acid and polyphosphates, clarification methods, cloud stabilization, deaeration and sterilization. This is followed by essence recovery opera-

tions by continuous condensation, distillation or extraction techniques, and a section on juice flavour enhancers. Company names, inventors' names and patent number indices are also given. The last index is particularly interesting as it enables one to compare the contents with those of other Noyes Food Technology Reviews. The result of this is that fourteen of the patents have been reviewed previously in Review No. 1, ten in No. 2 and no fewer than seventy-eight in Fruit Juice review No. 15, 1970. Thus nearly half the patents contained in this book have already been described in previous volumes.

The remainder of the patents give a useful picture of the developments up to September 1974. Those who are concerned with juice processing or handling will find this volume interesting. Librarians who have already purchased previous volumes will have a problem in deciding whether or not to purchase this volume.

S. D. HOLDSWORTH

Practical Meat Inspection, 2nd edn. By ANDREW WILSON.
Oxford: Blackwell Scientific Publications, 1975. Pp. viii + 236. £4.75.

This second edition is much the same as the first one apart from a few added paragraphs and the chapters dealing with poultry and rabbits having been rearranged. The chapter on physiology is extremely brief and very elementary. The author explains in the preface that this is as much as a meat inspector need know of the subject. It seems rather strange that this is considerably less than is required in the Institute of Meat, Meat Trades Final Certificate, which is the main qualifying requirement for entry to the R.S.H. Certificate in Meat Inspection Course. One would have assumed that a Meat Inspector required to have a greater knowledge, particularly of physiology, than a meat trader.

It is a pity that the author did not explain post mortem anaerobic glycolysis when dealing with rigor mortis to give the student a better understanding of the post mortem changes. To suggest that pancreatice juices contain only three enzymes and that intestinal digestive juices contain only four enzymes could be misleading to students. The author, in common with many other writers, refers to the exposed surfaces of the adductor and semi membranosus muscles of a hind-quarter as the gracilis muscle. It would have been useful to have included a description of the carcass characteristics of young bulls and young boars, in chapter four, since such carcasses are now becoming more frequently encountered in the Meat Industry and this trend is likely to increase. The explanations relating to 'dark cutting beef' would seem to be out of line with the more recent publications on this subject. The post mortem fall of pH in carcass is always quoted as much less than 48 hr (usually 10–14 hr) and the authors explanation of decomposition at the end of chapter five does not seem to conform to the explanations given in other publications.

The chapters on specific disease and the disease caused by animal parasites are concise and informative. The student can obtain essential information without being subjected to an excessive amount of detail. This applies equally to the chapters on

Poultry and Rabbits. The statement that 'benign tumours are frequently multiple, several arising in different parts of the body' would seem to conflict with other authorities such as *Veterinary Pathology* by Smith & Jones.

The book *Practical Meat Inspection* is, in my view, a useful introductory textbook on the subject but a great deal more and deeper knowledge is required by a Meat Inspector than the mere scratching of the surface of the subject as this book does.

F. J. MALLION

Books Received

Spirits, Aperitifs and Liqueurs: Their Production. By S. M. TRITTON. London: Faber & Faber, 1975. Pp. 82. £2.35.

A brief introduction to the production of spirits, aperitifs and liqueurs with detailed recipes.

Microbial Aspects of the Deterioration of Materials. By D. W. LOVELOCK and R. J. GILBERT (Eds). London: Academic Press, 1975. Pp. xiii + 211. £8.50.

A collection of papers, read at a meeting of the Society for Applied Bacteriology, on the microbiological changes produced in a wide variety of non-food materials.

World Food Problem: A Selective Bibliography of Reviews. By M. Rechcigl Jr. Cleveland: C.R.C. Press, 1975. Pp. xiv + 211. £25.90.

A comprehensive bibliography of mainly English language review articles on all important facets of the world food problem, covering such questions as the state of nutrition, present and future food resources, food quality, food hazards and environmental aspects.

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

Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted current practice. Conversion factors to SI units are shown where appropriate.

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
Calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 *T*°C + 32

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

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