Volume 14 Part 3 September 1976

JOURNAL

OF THE

ASSOCIATION OF PUBLIC ANALYSTS

Survey of Pesticide Residues in Lettuce 1 July, 1974–30 June, 1975

Report prepared by R. S. Hatfull, County Laboratory, Stafford, ST16 2LG, on behalf of the Association of Public Analysts

This Report gives the results obtained during the twelve month period, 1 July, 1974–30 June, 1975. It was planned by the Association of Public Analysts, following the publication of the Joint Survey of Pesticide Residues in Foodstuffs, 1 January, 1972–31 December, 1972 (third year), which indicated a need for a further investigation of the incidence of residues of pesticides in lettuce, with particular reference to thiocarbamates and PCNB (Quintozene).

This further Survey has been shown to have been fully justified by these results, which have confirmed the opinion expressed in the earlier Survey of a need for control of these compounds.

Introduction

As distinct from the earlier Surveys[1,2,3] which were organised jointly by the Association of Public Analysts and the Local Authority Associations, this Survey was organised solely by the Association of Public Analysts.

Both the Second[2] and the Third[3] Reports made reference to the finding of substantial amounts of pesticide residues in lettuce, in particular of thiocarbamates and PCNB (Quintozene) and following a discussion of these findings at the Annual Conference of the Association of Public Analysts in the Spring of 1974 it was decided to carry out a further 12 months Survey of lettuce, commencing on 1 July, 1974.

Apart from the desire to start this Survey as soon as practical, the period of the Survey was chosen so as to cover first, the main crop of outdoor grown lettuces and subsequently those intensively grown under glass for the autumn, winter and spring markets. In accordance with this, the results are separately reported for each of the four three-month periods July—September, October–December, January–March and April–June.

Arrangements were made, by the individual members of the Association, with the Enforcement Officers of local authorities for samples of lettuce to be included in the normal sampling programmes of the participating authorities and the ready co-operation of the Enforcement Officers and their respective authorities is gratefully acknowledged.

Organisation

The Survey was organised on lines very similar to those of the previous Surveys[1,2,3] with the addition of a further Zone, Zone 8, to cover Northern Ireland. It had been hoped to include Scotland in the Survey, but technical difficulties and the uncertainty consequent upon local government reorganisation in Scotland prevented this from being achieved.

Full details of the areas covered by the Zones were given in the Report of the First Survey[1] but, for convenience, are summarised as follows, account being taken of changes brought about by local government reorganisation.

Zone	Participating Authorities
1	London Boroughs of Southwark, Sutton, Merton, Richmond, Kingston-upon-Thames, Croydon, Harrow, Lewisham
2	Counties of Surrey, Kent, Hampshire
3	Counties of Avon, Somerset, City of Cardiff
4	Counties of Durham, Cleveland, Tyne & Wear, Humberside, Northumberland
5	Counties of Cheshire, Lancashire, Metropolitan Counties of Liverpool and Manchester. Counties of Clwyd, Gwynedd
6	Counties of Staffordshire, Salop, Hereford and Worcester, Derbyshire, Leicestershire. West Midlands Metropolitan County
7	Counties of Norfolk, Suffolk, Essex, Cambridgeshire, Borough of Southend-on-Sea
8	Counties of Antrim, Armagh, Bambridge, Belfast, Castlereagh, Coleraine, Limavady, Newton Abbey, Omagh

Sampling

Samples were taken by local authority Inspectors as informal samples under the Food and Drugs Act, 1955.

Public Analysts were asked to report, if possible, the source of each sample variety and whether grown in the open or under glass.

Pesticides Sought

The Survey was primarily aimed at obtaining information about the incidence of residues of thiocarbamates but some work was also carried out on PCNB (Quintozene) residues and some Public Analysts additionally sought a wider range of pesticides.

Reporting Limits

The reporting limits applied were those of the Third Survey[3], but given not as parts per thousand million (parts per 10^9) but as numerically equal nanograms/gram (ng/g).

Thiocarbamates	500	nanograms/gram
PCNB	20	,,
Organo-chlorine compounds	10	,,
DDT and allied compounds	20	,,

Methods of Analysis

Before analysis, the root, if present, was removed and any obviously inedible leaves discarded. The lettuces were not washed unless there was contamination by soil and then the washing was kept to a minimum.

Thiocarbamates were determined by the procedure given in the Report of the Third Survey[3]. In this method the thiocarbamate is converted to carbon disulphide by acid hydrolysis, removed by distillation and absorbed in a diethanolamine reagent containing copper and the resultant yellow colour is related to standards prepared from carbon disulphide. For the purposes of this Report the results as CS_2 have been converted to ethylene *bis*dithiocarbamic acid on the basis that one molecule of ethylene *bis*dithiocarbamic acid yields two molecules of carbon disulphide.

PCNB (Quintozene) was determined by the general procedure of Hamence, Hall and Caverley[4], but using a column of 2 per cent. XE-60 to ensure effective separation from BHC.

Results

All amounts are given as nanograms/gram (ng/g) related to the lettuce in its natural fresh state.

An entry of "Nil" means that the pesticide sought was either not detected or was present in an amount less than the reporting level.

An entry of a dash (-) indicates that that particular pesticide was not sought.

The origin of samples in Tables I–IV is indicated by:

* U.K.;
** imported;
*** origin unknown.

It should be noted that because of the variability of recovery rates (e.g. that for different compounds of the thiocarbamate group was found to vary from less than 50 per cent. to approximately 100 per cent.) none of the experimental results for thiocarbamates have been corrected for recovery, and all such results therefore represent minimum amounts. Thiocarbamates that were identified included "Zineb", "Maneb" and "Thiram".

It is emphasised that Tables I–IV give details of *positive* findings only and that these Tables must not, therefore, be used to make *negative* deductions. For example if, for a particular Zone at a particular time, six out of ten samples were found to contain pesticides then the results for those six samples are given. The remaining four samples will have contained none (or less than the reporting limit) of those pesticides *that were sought*, but since all pesticides were not sought by all Public Analysts it must not be taken that the four samples were necessarily free from pesticides.

TABLE I

PESTICIDES IN LETTUCE

First Period: 1 July, 1974–30 September, 1974

Total samples examined:		120
Samples with residues of:	thiocarbamates	10
	PCNB (Quintozene)	1
	thiocarbamates and PCNB	0
	other pesticides	3

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)			Origin of samples
			Thiocarbamates	PCNB	Others	
1	5	0	nil	nil	nil	***
2	12	2	nil	nil	Aldrin 150 BHC 26	***
3	19	0	nil	22	nil	
4	4	0	nil	nil	nil	
5	40	1	1900	nil	nil	*
6	29	8	4200	nil	nil	*
			560	nil	nil	***
			560	nil	nil	***
			540	nil	nil	***
			840	nil	nil	*
			1100	nil	nil	***
			700	nil	nil	*
			700	—	—	***
7	4	0	nil	nil	nil	
8	7	3	nil	nil	BHC 20	***
			nil	nil	BHC 10	***
			530	nil	nil	***

TABLE II

PESTICIDES IN LETTUCE

Second Period: 1 October, 1974–31 December, 1974

Total samples examined:		140
Samples with residues of:	thiocarbamates	58
	PCNB (Quintozene)	10
	thiocarbamates and PCNB	1
	other pesticides	2

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)			Origin of samples
			Thiocarbamates	PCNB	Others	
1	4	2	44,600	33	nil	***
			nil	69	DDE 40	***
2	20	7	nil	82	DDT 860 TDE 63	*
			nil	nil	BHC 12	*
			nil	21	BHC 11	*
			nil	22	nil	*
			nil	97	nil	*

TABLE II *continued*

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)			Origin of samples
			Thiocarbamates	PCNB	Others	
			nil	56	nil	**
			nil	71	nil	**
3	16	5	nil	49	nil	***
			nil	20	nil	***
			880	nil	nil	***
			8400	nil	nil	***
			nil	nil	BHC 160	***
					DDE 300	***
					DDT 2300	***
4	40	32	17,500	nil	nil	***
			27,000	—	—	***
			47,600	—	—	***
			100,000	—	—	***
			840	—	—	***
			61,400	—	—	***
			62,800	—	—	***
			209,000	—	—	***
			1400	—	—	***
			6690	—	—	***
			840	—	—	***
			700	—	—	***
			58,600	—	—	***
			46,000	—	—	***
			32,000	—	—	***
			78,100	—	—	***
			25,100	—	—	***
			66,900	—	—	***
			7000	—	—	***
			1260	—	—	***
			4880	—	—	***
			97,600	—	—	***
			37,700	—	—	***
			223,000	—	—	***
			2800	—	—	***
			23,700	—	—	***
			255,000	—	—	***
			7950	—	—	***
			60,700	—	—	***
			19,500	—	—	***
			237,000	—	—	***
			100,000	—	—	***
5	22	12	2650	nil	BHC 10	***
			1790	nil	nil	*
			2800	nil	nil	***
			9800	nil	nil	***
			63,000	nil	nil	***
			54,600	nil	nil	***
			39,200	nil	nil	***
			12,300	nil	nil	***
			20,300	nil	nil	***
			164,000	nil	nil	***
			8540	nil	nil	***
			36,400	nil	nil	***
6	22	7	3500	nil	nil	*
			56,000	nil	DDT 1100	***
			28,000	nil	—	***
			28,000	nil	—	***

TABLE II *continued*

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)			Origin of samples
			Thiocarbamates	PCNB	Others	
			3300	nil	—	***
			23,000	nil	—	***
			28,000	nil	—	***
7	9	3	nil	880	—	***
			570	nil	—	***
			1400	nil	nil	*
8	7	2	11,100	nil	nil	***
			1320	nil	nil	***

TABLE III

PESTICIDES IN LETTUCE

Third Period: 1 January, 1975–31 March, 1975

Total samples examined:		148
Samples with residues of:	thiocarbamates	82
	PCNB (Quintozene)	21
	thiocarbamates and PCNB	10
	other pesticides	6

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)				Origin of samples
			Thiocarbamates	PCNB	Others		
1	12	11	2660	nil	nil		*
			16,100	40	nil		**
			nil	50	nil		**
			nil	25	nil		***
			29,400	21	nil		*
			nil	33	nil		**
			nil	66	nil		**
			50,400	nil	nil		**
			nil	26	nil		**
			nil	32	nil		*
			nil	30	BHC	12	**
2	20	10	1200	nil	nil		**
			158,000	nil	nil		*
			39,000	—	—		*
			1500	—	—		*
			nil	98	nil		*
			8400	nil	BHC	60	*
			nil	nil	BHC	100	*
			nil	73	nil		*
			nil	nil	BHC	130	*
			nil	48	nil		*
3	18	9	nil	36	nil		***
			nil	140	nil		***
			nil	100	nil		***
			nil	20	nil		***
			nil	20	DDE	22	***

TABLE III *continued*

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)				Origin of samples
			Thiocarbamates	PCNB	Others		
			nil	nil	DDE	30	***
					BHC	30	
			nil	nil	DDE	50	***
			nil	nil	DDE	50	***
			nil	nil	DDE	68	***
4	47	41	620	nil	nil		***
			780	nil	nil		***
			670	nil	nil		***
			57,400	—	—		***
			15,400	—	—		***
			123,000	—	—		***
			36,400	—	—		***
			7980	—	—		***
			25,200	—	—		***
			4480	—	—		***
			63,000	—	—		***
			9100	—	—		***
			19,600	—	—		***
			32,200	—	—		***
			4060	—	—		***
			10,100	—	—		***
			2940	—	—		***
			410,000	—	—		***
			5600	—	—		***
			7840	—	—		***
			7000	—	—		***
			1680	—	—		***
			4620	—	—		***
			123,000	—	—		***
			3640	—	—		***
			4480	—	—		***
			9800	—	—		***
			28,000	—	—		***
			3920	—	—		***
			198,000	—	—		***
			153,000	—	—		***
			57,400	—	—		***
			53,200	—	—		***
			16,800	—	—		***
			35,000	—	—		***
			11,200	—	—		***
			30,800	—	—		***
			13,200	—	—		***
			4200	—	—		***
			47,600	—	—		***
			13,600	—	—		***
5	11	10	33,600	—	—		***
			23,800	—	—		***
			4900	—	—		***
			111,000	—	—		***
			30,800	nil	DDT	75	***
			8680	nil	nil		***
			5320	—	—		***
			12,700	nil	nil		*
			590	nil	nil		**
			1150	nil	nil		***

TABLE III *continued*

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)			Origin of samples
			Thiocarbamates	PCNB	Others	
6	20	13	22,400	nil	nil	**
			61,900	nil	nil	*
			22,400	220	nil	*
			44,800	100	nil	*
			nil	670	nil	***
			133,000	nil	nil	***
			2660	1200	nil	***
			3780	100	DDT 60	*
			3920	100	nil	*
			120,000	nil	nil	***
			35,000	nil	nil	*
			28,700	nil	nil	**
			2800	—	—	***
7	16	14	1400	nil	nil	**
			nil	110	—	***
			nil	30	—	***
			500	nil	—	***
			nil	840	—	***
			6550	nil	—	***
			29,400	—	—	***
			12,600	1300	—	***
			nil	260	—	***
			nil	260	—	***
			5180	220	—	***
			950	nil	—	***
			1120	nil	—	***
			5180	1820	—	***
8	4	1	590	nil	nil	***

TABLE IV
PESTICIDES IN LETTUCE

Fourth Period: 1 April, 1975–30 June, 1975

Total samples examined:		103
Samples with residues of:	thiocarbamates	16
	PCNB (Quintozene)	2
	thiocarbamate and PCNB	2
	other pesticides	2

Zone	Samples examined	Samples containing pesticides	Pesticides found (*ng/g*)				Origin of samples
			Thiocarbamates	PCNB	Others		
1	7	1	504,000	nil	nil		**
2	12	6	nil	nil	BHC	40	*
			nil	nil	BHC	80	*
			nil	170	TCNB	35	*
			nil	190	TCNB	38	*
			3080	nil	nil		*
			4590	nil	nil		*
3	27	1	10,900	nil	nil		***
4	28	5	22,400	—	—		***
			1400	—	—		***
			25,200	—	—		***
			86,800	—	—		***
			670	nil	nil		*
5	10	2	4760	nil	nil		***
			11,500	290	BHC	470	***
					DDE	120	
					DDT	350	
6	14	4	1960	nil	nil		*
			740	nil	nil		*
			780	nil	nil		*
			28,900	100	nil		*
7	1	0	nil	nil	nil		
8	4	1	590	nil	nil		***

TABLE V

SUMMARY OF TABLES I–IV

Period	Samples examined	Thiocarbamates	PCNB	Thiocarbamates and PCNB	Pesticides other than thiocarbamates and PCNB*	Pesticides absent	Total samples containing pesticides
July–September 1974	120	10 (8·3%)	1 (0·8%)	0 (—)	3 (2·5%)	106 (88·4%)	14 (11·6%)
October–December 1974	140	57 (40·8%)	10 (7·1%)	1 (0·7%)	2 (1·4%)	70 (50·0%)	70 (50·0%)
January–March 1975	148	72 (48·6%)	21 (14·2%)	10 (6·8%)	6 (4·0%)	39 (26·4%)	109 (73·6%)
April–June 1975	103	14 (13·6%)	2 (1·9%)	2 (1·9%)	2 (1·9%)	83 (80·7%)	20 (19·3%)
Totals	511	153 (29·9%)	34 (6·7%)	13 (2·5%)	13 (2·5%)	298 (58·4%)	213 (41·6%)

* Samples containing thiocarbamates and/or PCNB *and* other pesticides are excluded from this heading but included under the other appropriate headings.

Additional Information

It was originally planned also to classify the results according to:

(a) type or variety of lettuce;
(b) whether U.K. grown or imported;
(c) whether grown in the open or under glass.

In the event, however, such additional information was seldom readily available and this aspect of the Survey is thus of limited value.

TYPE OR VARIETY OF LETTUCE

Only nine samples were positively identified as of the "Cos" type. A very few were identified as "Density" and "Webbs Wonderful" but the vast majority were described only as "Cabbage" or "Flat".

From this limited information there were no indications that the type or variety had any relationship to pesticide usage.

U.K. OR IMPORTED

	U.K.	Imported	Origin unknown	Totals
No pesticides	84	9	205	298
Thiocarbamates	22	7	124	153
PCNB	10	7	17	34
Thiocarbamates and PCNB	6	1	6	13
Pesticides other than thiocarbamates and/or PCNB*	5	0	8	13
Totals	127	24	360	511

* Samples containing thiocarbamates and/or PCNB are included in the other categories.

WHETHER GROWN IN THE OPEN OR UNDER GLASS

Grown in the Open

21 lettuces were known to have been grown in the open—19 in the U.K. and 2 of unknown origin. Of these only one, of U.K. origin, contained pesticides (780 ng/g of thiocarbamates, 4·8 per cent. of such samples).

Grown under Glass

13 lettuces were known to have been grown under glass—6 in the U.K. and 7 imported.

Of these, 6 (5 U.K. and 1 imported) contained pesticide, 46 per cent. of such samples:

Origin	Pesticide (*ng/g*)	
U.K.	Thiocarbamate	29,400
	PCNB	21
U.K.	PCNB	82
	DDT	860
	TDE	63
Imported	Thiocarbamates	12,000
U.K.	,,	158,000
U.K.	,,	54,600
U.K.	,,	2100

Conclusions

This survey shows that during the 12-month period July 1974–June 1975, 41·6 per cent. of lettuces contained significant amounts of pesticide residues. There was a marked seasonal variation in that such residues were much more evident during the six months from October to March, than at other times of the year.

The results indicate that such residues mainly arise from the use of pesticides on lettuces grown under glass and it seems fairly certain that crops grown during the six months October–March would be under glass.

Although the Survey was aimed at obtaining information firstly upon the use of thiocarbamates and secondly upon the use of PCNB, certain limited information was also obtained upon the use of other pesticides. The use of such other pesticides in lettuces was found to be more common than might have been supposed and up to five different pesticides were observed in individual samples. There was even one recorded instance of the presence of Aldrin, the general use of which was discontinued some years ago.

The amounts of pesticides found, and in particular of thiocarbamates, could only be considered as excessive. Amounts of thiocarbamates in excess of 10,000 ng/g were common and the highest amount found, (504,000 ng/g (0·05 per cent.)) could have resulted only from gross misuse of this pesticide.

References

1. "Joint Survey of Pesticide Residues in Foodstuffs sold in England and Wales (First Year) 1 August, 1966–31 July, 1967." The Association of Public Analysts, London, 1969.
2. "Joint Survey of Pesticide Residues in Foodstuffs sold in England and Wales (Second Year) 1 August, 1967–31 July, 1968." The Association of Public Analysts, London, 1971.
3. "Joint Survey of Pesticide Residues in Foodstuffs sold in England and Wales (Third Year) 1 January, 1972–31 December, 1972." The Association of Public Analysts, London, 1975.
4. Hamence, J. H., Hall, P. S., and Caverley, D., *Analyst*, 1965, **90**, 649.

J. Assoc. Publ. Analysts, 1976, **14**, 87–95

Microbiological Standards for Food: Report of a Meeting

At a meeting arranged jointly by the Association of Public Analysts and the Analytical Division (N.W. Region) of the Chemical Society and held at Heskin Hall, Wigan, on 3 October, 1975, with Dr L. S. Bark of Salford University in the Chair, papers were read by Mr R. B. Sparnon (Findus Foods Ltd.) and Dr B. Jarvis (B.F.M.I.R.A.). Both speakers warned the audience that their papers were not in a form suitable for publication, mainly because they made use of as yet unpublished material. This report gives summaries of their papers.

Microbiological Standards for Frozen Foods

R. B. Sparnon

Technical Manager, Findus Ltd, Grimsby, South Humberside

The first pre-requisite towards defining microbiological quality is accurate identification of the micro-organisms considered relevant. Secondly, the qualitative identification of these organisms in the food under examination must be followed by quantification; the difficulties met with in this stage are discussed in this lecture. Thirdly, having defined product quality in terms of either shelf life (spoilage organisms) or public health risk (pathogenic organisms), the objective is to relate the quantitative measurements of the organisms counted to the corresponding level of either kind of risk. There is no evidence that this approach is being taken to the problem; rather, criteria are being based on data from routine analyses by industrial microbiologists which represent actual commercial practice. These criteria therefore are not strictly related to what one could determine as an acceptable product quality in terms of shelf life or public health risk.

As regards accurate identification, microbiology has its roots in the study of disease, and the amount of data available about a particular organism tends to reflect its importance as a cause of disease, so that (e.g.) staphylococci, salmonellae and coliform organisms are among those most studied in the food industry. The principle of identification of an organism is the selection of characteristics that are peculiar to that organism and of tests that will define those characteristics. When defined sufficiently, the characteristics are held to have identified the organism. The chosen characteristics are mainly biochemical in nature, so one can only fingerprint, so to speak, the organisms sought. It is therefore very important that the reproducibility of the test procedures shall be of a high order.

In a recent collaborative investigation[1], different strains of pseudomonads were sent to several laboratories, with details of 17 different tests for distinguishing pseudomonads. The results obtained in the various laboratories

were compared. The variation between laboratories in two important tests (urea-hydrolysis and oxidase activity) was found to exceed the variation between strains of pseudomonads. This was not unexpected, but it emphasises the need for rigid standardisation of microbiological techniques.

Most diseases associated with *E. coli* occur in children and are of the gastro-enteritis type. Fourteen strains have been identified as associated with such conditions, but there is also the general coliform group that is used as an indicator of the possible presence of pathogens. *Coli*-indicator tests nearly always figure in bacteriological standards, both national and international, but many different tests are used for identifying and classifying coliforms, and some time ago the frozen food industry began to wonder whether all the bacteria reported as coliforms really were coliforms. An investigation was therefore made[2] in which 11 commonly used test procedures were checked to see what proportion of the organisms they "identified" were in fact true positives. The tests were applied to frozen fish, mousse and beans, and it was found that the confirmation of coliform organisms was 100 per cent. only with 5 of the tests, and even then only with beans. The results as a whole showed a variation from 100 down to 18 per cent. The details have not yet been made available outside the industry.

It is unusual for a laboratory to select a particular technique for a particular food, so the efficiency of identification might vary from 100 to 18 per cent. in a very unfortunate laboratory, and the data that have been accumulated over the years must contain inaccuracies of this magnitude. The science of the identification of micro-organisms has thus not yet progressed to the point where consistently accurate methods for calculating numbers of specific organisms can be applied. There is an urgent need for investigations directed towards the selection of the best techniques for each organism and each type of food.

Now let us consider quantification. When microbiology was medically based, it was mainly concerned with identification; but when industrial uses for micro-organisms developed (e.g. in brewing, cheese-making and effluent disposal) the need for a different outlook became clear. Then, when food processing brought microbiological problems in its wake, yet another need emerged—so to control the microbial count in food that it was always wholesome, as regards both freedom from spoilage and an acceptably low health risk.

Unfortunately, the problem of enumerating bacteria is very confused—there is none of the precision of chemical estimation about it. Using as an example the familiar "Most Probable Number" concept in the coliform field, when one says that the technique used has indicated a M.P.N. of 20 organisms per g, one is really saying that one can have 95 per cent. confidence that the true count lies between 7 and 89! This is bad enough, but the conclusion emerging from another study being conducted into counting techniques is that the between-laboratories variation exceeds the between-techniques variation.

The objective of all this is establishment of the relationship between microbial counts and product quality. Where do we draw the line among our data and say that this is where spoilage is threatened or a public health risk arises? It is valid to try to use the data available to draw a limit line; but the British

Government's opinion is that quantitative microbiology is not sufficiently advanced for microbiological standards to become part of the law.

Because of the growth of international trade in food, one finds that pressures for standardisation are developing, and microbiology has not been immune from such pressures. Proposed standards keep emerging from different parts of the world and most of them are based on the concept of "Good Commercial Practice". This means that all the available data are examined and, by application of some mathematical formula, one then finds what is called "The Norm", e.g., a line drawn two standard deviations away from the mean, and the resulting number is proposed as a standard.

Quality control in factories is often based on using this kind of procedure as a means of assessing the day-to-day acceptability of a product and of recognising when the variation in the microflora of the product becomes unacceptable. The techniques used and their relationship to the product are constant, so numerical comparisons are meaningful. There is no relationship, however, between such a standard and the overall assessment of product quality. Research that will relate numbers of organisms with spoilage risk or health risk still needs to be done with each of the many kinds of food to which standards might be applied.

There is currently awaited a second edition of a manual[3] prepared by the Fruit and Vegetable Preservation Research Association. The committee that took the decision to produce this manual undertook to examine all the major products of the frozen food industry, and their published data can probably be taken as a good indication of the norms of the industry and how the norms for different food were related to each other at that time. Since all the testing was done in one laboratory using well-defined techniques, these may be the first set of figures that can be used to compare the state of the industry with any standards that may be proposed.

A body now actively investigating the subject of standards, the Thatcher Committee, bases its proposals[4] on a system that employs two factors—big M and little m, each representing a bacteriological count. The principle is to obtain a minimum fixed percentage of counts below little m and a maximum percentage between little m and big M. No count is allowed to exceed big M. These standards usually relate to a Standard Plate Count (SPC) at a stated temperature and time of incubation, but in addition, limits (usually nil only in the case of salmonellae) may be given for specific organisms. Thus, for boneless frozen meat, little m is 5×10^5 and big M is 10^7 SPC per g and salmonellae should be absent. When this standard was first put forward, boneless frozen meat produced in the U.K. would not have reached it in 18 per cent. of the batches examined, while samples from non-European countries almost all complied with the SPC standard but tended to fail as regards the absence of salmonellae. The standards of this kind so far proposed have all been based on "good commercial practice", so that, as previously pointed out, they are not related to spoilage or health risks.

Similar standards are suggested for frozen fish and fish products. Here "little m" and "big M" are 10^6 and 10^7 per g respectively, and staphylococcus is required to be absent. The annual market for such products in Britain is

150,000 tons, and there are no documented cases at all of food poisoning involving breaded pre-cooked fish products, fish fingers, fish cakes, cooked prawns, kippers, and all the other such products included. All products analysed were found to comply with the proposed standards.

The International Committee on Microbiological Standards for Food has proposed standards for blanched frozen vegetable that are not generally attained by the industry. These standards, and the actual attainment, are given in Table I.

TABLE I

ATTAINMENT OF PROPOSED MICROBIOLOGICAL STANDARD

(*standard plate counts per gram of sample*)

Food	Per cent. of samples complying with standard (a), (b) and (c)		
	Below 10^4 Min. 40 per cent. (a)	10^4 to 10^6 Max. 60 per cent. (b)	Above 10^6 Nil (c)
Peas	21	79	nil
Sliced green beans	29	91	nil
Broad beans	24	39	37
Sprouts	62	35	nil

Note that with fish products the upper limit is 10^7 organisms, whereas with the above vegetables it is 10^6 per g. Thus, the limits inserted into the standards probably reflect the results obtained in practice rather than the risk involved.

On the other hand, consider frozen prepared meat products like pies, sliced beef, etc., the market amounting in all to about 100,000 tons per year and considered by the trade to be the one carrying the greatest risk. None of these products in the survey gave SPC's as high as the proposed standard (not more than 40 per cent. between 10^5 and 10^6 per g). Coliform organisms (supposed to be absent from all samples) were occasionally found in steak and kidney pies and in chicken pies, however. Again, in Washington some years ago, the Codex Alimentarius Hygiene Committee proposed a standard for pre-cooked and partially cooked frozen foods based on maximum total counts of 100,000, coliforms 20 and staphylococci 100 per g. (These figures were subsequently withdrawn for reconsideration.) Had these standards applied to the foods examined in the survey, the discarded foods would have amounted to 16,000 tons, worth about £12 million. It is hard to believe that such a quantity of food jeopardised anyone's health; the solitary case of food poisoning attributed to any of the products mentioned above involved sliced beans. It is not possible therefore to conclude that there is any relationship between the proposed standards and the public health risk.

Until further research provides a more accurate means of assessing microbiological quality, bacterial counts can be used on a routine basis in industry to indicate consistency of bacteriological performance. Significant departures

from the norm deserve investigation. To draw specific conclusions relating to specific bacteriological counts demands further development of the science of microbiology.

References

1. "A study in test reproducibility between laboratories: Report of a Pseudomonas Working Party." Edited by P. H. A. Sneath and Vera G. Collins. *J. Antonie v. Leeuwenhoek*, 1974, **40**, 481.
2. Research by the Fruit and Vegetable Preservation Research Association, Chipping Campden (unpublished).
3. "A Manual of Methods for the Bacteriological Examination of Frozen Foods," Fruit and Vegetable Preservation Research Association, Chipping Campden, 1975.
4. Thatcher, F. S., and Clark, D. S., "Micro-organisms in Food," Univ. of Toronto Press, Canada, Vol. I, 1968 and Vol. 2, 1974.

Analytical Microbiological Control of Non-Frozen Foods

B. JARVIS

Leatherhead Food R.A., Randalls Road, Leatherhead, Surrey, KT22 7RY

Non-frozen foods comprise various groups characterised by the process applied and the subsequent stability of the product, i.e. perishable; semi-preserved; preserved; and "commercially sterile" foods. The microbiological control of these commodity groups is similar in principle but different problems exist for individual groups and for specific products within a group.

The aim of the food manufacturer is to produce "acceptable" foods with a satisfactory shelf life, free from any actual or potential hazard to the health of the consumer. Food-associated hazards range from the presence of natural and environmental toxicants (e.g., mercury, pesticides, etc.) to the presence of food-poisoning organisms (e.g., salmonellae) or toxic microbial metabolites (e.g., aflatoxin). All foods are initially contaminated to a greater or less extent with micro-organisms which could cause spoilage or food poisoning if extensive growth were to occur in the food. It is the role of the factory quality assurance department, backed by routine chemical and microbiological analysis, to ensure that the degree of microbial or chemical contamination is acceptable for a particular commodity and that the product, as manufactured, conforms in all other respects with the requirements of the various food laws and regulations. However, once the food has been distributed to wholesale or retail outlets the manufacturer rarely has any control over the conditions of storage of the food—hence the present-day interest of consumer and manufacturer alike in the introduction of *meaningful* open-date coding systems, etc.

The growth of micro-organisms in any substrate is governed by the chemical and physical parameters of the substrate. Factors such as temperature of storage, water activity (a_w), pH value of the product, the amount and type of chemical preservative (if any) and the conditions of processing and packaging, all play a role in controlling microbial growth. By varying such parameters the shelf lives of perishable foods can be extended and their safety enhanced.

Examples are the reduction of water activity by dehydration or by the use of salt, sugar or humectant; use of refrigeration for transportation and storage of "perishable" foods; reduction of pH value by microbial fermentation (e.g., cheese) or by addition of acid; pasteurization of milk, fruit juices and alcoholic beverages to destroy vegetative forms of spoilage and pathogenic organisms; thermal sterilization of foods to destroy both vegetative and spore forms of micro-organisms, thereby ensuring a shelf-stable commodity. The effects of the different preservation methods depend on the nature of the food commodity and on the levels and types of contaminating organisms present in the food. No acceptable method of food preservation will cover up the use of grossly unsatisfactory raw materials or unhygienic processes; however, the presence of certain organisms in foods may not always be disadvantageous and it is essential to define carefully the acceptable and unacceptable levels of contamination by particular micro-organisms for a particular commodity.

TABLE I

MICROBIOLOGICAL HAZARDS ASSOCIATED WITH FOODS

Type of hazard	Degree of hazard	Representative groups of organisms
No direct health hazard	(Utility)	Spoilage organisms (e.g., as indicated by SPC*)
Health hazard	Low, indirect	Indicator organisms (e.g., coliforms)
	Moderate, direct, limited spread	*B. cereus*, *Cl. perfringens*, *Staph. aureus*
	Moderate, direct, widespread	*Salm. typhimurium* and other salmonellae *Shigella* spp., *Vibrio parahaemolyticus*, enteropathogenic *E. coli*
	Severe hazard	*Cl. botulinum*, *Salm. typhi*, *Salm. paratyphi*, *Vibrio comma*, Infectious hepatitis virus

* SPC = Standard Plate Count.

The I.C.M.S.F.[1] has classified health risks from food-associated micro-organisms into various categories (Table I). Moderate hazard (limited spread) organisms are typified by *B. cereus*, which is new as a hazard in this country and arises because of the manner of handling cooked rice in many Chinese restaurants. It is a common contaminant of rice and can survive cooking to multiply to potentially hazardous numbers if the cooked rice is left to cool slowly and is not stored at refrigeration temperatures[2]. *Cl. perfringens* (*welchii*) is the prime cause of food poisoning associated with canteens and other communal kitchens. *Staph. aureus* is associated with food handlers; it occurs commonly on the skin and in the mucosal membranes of many people and can produce a thermo-stable enterotoxin when it grows in foods.

Moderate hazard (widespread) organisms are predominantly species of

Salmonella, the most common cause of food poisoning in the U.K. The organisms occur in many animal feedstuffs and are commonly present on raw meats and meat products[2,3]. Other organisms may also present a hazard and enteropathogenic strains of *E. coli* are of increasing potential in this area.

Because of the control traditionally employed by the food manufacturing industry, severe health hazards of microbiological origin are rare in the U.K. but can occur from time to time. There has been no case of human botulism in this country since 1956 and none from U.K.-manufactured foods since the 1920's but several outbreaks have occurred in the U.S.A. and in Europe so that vigilance must never be relaxed. Changes in the legal tolerances for nitrite and nitrate as preservatives in cured meats could replace a potential hazard from nitrosamines by a very real hazard from botulism[4]. Other organisms in this hazard group (e.g., *Salm. typhi* and *Salm. paratyphi*) are of importance because of their rapid spread (e.g. the Aberdeen typhoid outbreak). Shigella dysentery and cholera may be associated with international travellers and immigrants. Infective hepatitis A is the newest potential severe food-borne hazard[5] but at present no tests for the causal agent exist.

The degree of hazard may be modified by processing and/or by post-process storage and handling conditions. In selecting tests for microbiological quality assurance and control it is essential to recognize the potential degree of hazard associated with contamination of particular types of food commodity. Sampling plans must take into consideration both the feasibility of testing and the types of organism likely to be encountered in the food and should ideally be as simple as possible.

Much effort is presently being expended on the establishment of standard methods and microbiological "standards" for foods both nationally and internationally. Organisations such as B.S.I., I.S.O., Codex Alimentarius and I.C.M.S.F. are all involved in producing microbiological standards and standard methods for foods. Furthermore, we are presently facing the introduction of microbiological standards in various draft E.E.C. Directives for foods. In the U.K., legislative requirements have been aimed at protecting public health by ensuring the adequacy of processes, using simple biochemical tests (e.g. the phosphatase test for pasteurised milk) rather than by applying quantitative microbiological standards, which are costly to implement and which relate more to assessment of product "quality", unless specific tests are made for pathogens. "Quality" standards are not new—reputable manufacturers and retailers have used "house standards" or "purchasing specifications" for many years.

It is essential to realize that "quantitative" microbiology is not an exact science. Problems of variation in the distribution of organisms make truly representative sampling difficult; enumeration methods vary in their reproducibility so that the most accurate assessment cannot be considered to be statistically meaningful to better than half a log unit, i.e. a colony count of 1×10^6 organisms per g indicates a contamination level between 3×10^5 and 3×10^6. Consequently a "Pass or Fail" (2-class) standard is meaningless for quantitative analyses and a 3-class standard is more acceptable. Such a standard would require that of n samples per batch tested for a particular

group of organisms, none may exceed a maximum (M) level (e.g., 10^5 organisms per g), but that a maximum number c samples may exceed a lower level (m) (e.g., 10^3 organisms per g). In setting the levels for M, m, n, and c cognisance must be taken of the degree of hazard associated with a particular product, with the achievable levels of contamination under good manufacturing practice (G.M.P.) and with the number of samples which it is physically and economically possible to test in the factory laboratory and/or in a regulatory control laboratory. In some cases the degree of hazard might be such that $m = M$ and that $c = 0$, so that a two-class plan would effectively be used. An example of this situation might be the desirable objective of exclusion of salmonellae from certain ready-prepared foods which will not be cooked before consumption. In such a situation one is testing for *presence or absence* of the organism in a *given weight of sample*, not for specific numbers. However, it is meaningless to require "total absence" of pathogens; the quantity of food from which the organisms must be absent *must* be stated and an appropriate sampling plan must be used.

Commercial "house standards" have been published for particular purposes which provide useful guidelines to industrial attitudes. For example, Goldenberg and Elliott[6] have published house standards for meat pies and other products: after storage for 24 hours at room temperature, plate counts of less than 200 per g are classed as "satisfactory", between 200 and 10,000 "acceptable but investigate" and if 50 per cent. of pies have counts greater than 10,000 per g the category is "stop production". For sausages similarly stored, up to 500,000 organisms per g is considered acceptable but no sample should exceed 2,000,000. Salmonellae have to be absent from 25 g samples and coagulase-positive *Staph. aureus* are not to exceed 1000 per g. The I.C.M.S.F.[1] has issued "guidelines" for many food commodities based on the "little m" and "big M" principle with rigorous sampling schedules. Several draft E.E.C. directives also contain microbiological standards, many of which are meaningless; at least one contains the requirement "Mycotoxins—none permitted at any level"!

If standards with legislative backing are to be introduced it is essential that certain criteria are met. The standards should (1) be related primarily to the degree of hazard associated with contamination of a specific commodity or group of commodities; (2) be based on standardized methods and culture media; (3) be set realistically so that they are achievable under G.M.P.; (4) be enforceable by establishment of appropriate control laboratories staffed by qualified and experienced microbiologists (not chemists); and (5) be seen to be of value in improving or maintaining the quality of foods offered for sale to the consumer.

References

1. International Commission for Microbiological Standards for Foods (ICMSF). "Micro-organisms in Foods. Sampling for Microbiological Analysis: Principles and Specific Applications". University of Toronto Press, Canada, 1974.
2. Hobbs, B. C., "The Microbiological Safety of Food. Food poisoning in England and Wales". Ed. B. C. Hobbs and J. H. B. Christian. Academic Press, London, 1974, pp. 129–142.

3. Harvey, R. W. S., "The Microbiological Safety of Food; Salmonella-contaminated animal feed in relation to infection in animals and man." Ed. B. C. Hobbs and J. H. B. Christian. Academic Press, London, 1974, pp. 9–17.
4. Jarvis, B., and Walters, C. L., "Nitrite in trouble," *Nature*, 1972, **240**, 171.
5. Chaudhuri, A. K. R., Cassie, G., and Silver, M., "Outbreak of Type-A Hepatitis in Greater Glasgow." *Lancet*, 1975, (ii) (7927), 223–225.
6. Goldenberg, N., and Elliott, D. W., "The Microbiological Safety of Food; The value of agreed non-legal specifications". Ed. B. C. Hobbs and J. H. B. Christian. Academic Press, London, 1974, pp. 359–368.

J. Assoc. Publ. Analysts, 1976, **14**, 97–103

An Approach to Air Pollution Monitoring with Limited Resources

G. J. DICKES

Scientific Adviser's and Public Analyst's Laboratory, Avon County Council, Canynge Hall, Whiteladies Road, Bristol BS8 2PR

Despite the complexity of many problems involving sampling and analysis of ambient air for the presence of pollutants, a simple approach can sometimes yield much useful data with which an analyst can give an opinion. Five examples are given where this philosophy has been applied.

Public Analysts are being called upon, with increasing frequency, to help and advise District Council Environmental Health Departments on matters concerning the monitoring of air pollutants. This is primarily a result of the passing of the Control of Pollution Act, 1974[1], under which certain Regulations will be made, establishing the District Councils' responsibility for cleanliness of air. District Environmental Health Officers often pose apparently complex problems in areas where there is little previously recorded work. Local authorities are at present required to exercise economy. Non-technical officials within some authorities do not always appreciate the value of information which can be obtained from simple tests[2]. This paper outlines some simple approaches to problems which have occurred when the Laboratory and Environmental Health Inspectors have co-operated using limited resources.

A procedure has been adopted in The Avon Scientific Adviser's Laboratory for tackling air pollution problems and it is applied to each new problem. It consists of five stages.

1. Appraisal.
2. Consideration of the type of sampling which is desirable (or practical).
3. Assessment of the analytical facilities available.
4. Action.
5. Results and conclusions.

APPRAISAL

(a) What is the nature of the hazard?
(b) Is it urgent?
(c) Is the pollutant, or its source, known?
(d) Is the problem there now, or is it forecast?
(e) Who is the complainant?
(f) Does it require a personal visit?

THE TYPE OF SAMPLING

Analytical techniques in environmental analysis are reasonably well documented, but guidance about efficient and meaningful collection of samples is not. A decision about sampling the pollutant is often a most critical step.

It is convenient to categorise pollutants into gas, solid and liquid, and to consider the available sampling methods.

(a) *Gases*

Monitoring, i.e. sampling and analysis in one unit, can be achieved by means of manually operated gas-sampling kits which are commercially available. These give a direct read-out of the qualitative or semi-quantitative concentrations of most of the gaseous pollutants. This is useful for urgent spot checking. For long term monitoring, continuous gas monitors for specific gases such as carbon monoxide, sulphur dioxide, nitrous fumes and hydrocarbons are available commercially, but the capital outlay with some of these instruments can be considerable.

The most versatile method of continuous gas sampling is by drawing the contaminated sample by means of a diaphragm pump through a trapping liquid for inorganic gases, or through an adsorbing solid for organic gases,

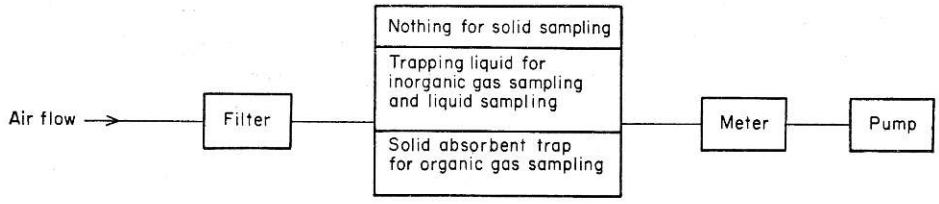

Fig. 1. A simple modular air sampling arrangement. Note: any combination of solid, liquid or gas sampling can be used, provided the pump has the capacity to achieve a satisfactory flow rate.

the volume being recorded on a meter. Figure 1 is a diagrammatic representation of a simple modular air sampling kit which includes gas, solid, and liquid sampling, or any combination of them.

(b) *Solids*

A cheap way of sampling particulate matter in air is by deposition. Many receptacles have been used for this, e.g. the Standard Deposit Gauge, the dry deposit gauge, petri dish and vegetation deposition. The main disadvantage of these collecting devices is that they do not give pollutant concentration and therefore a standard cannot be applied. Furthermore, they do not give the total of the airborne matter. On the other hand, these methods can be used as indicators of the extent of solid pollution.

A more satisfactory way of sampling airborne particulate matter is through a suitable filter medium, drawing air by diaphragm pump and metering the gas flow (see Figure 1).

(c) *Liquids*

Liquids, such as acid mists, can be sampled by using filtration or liquid

trapping methods as indicated in Figure 1. The liquid sample will either be on the filter or in the trapping liquid or in both.

ASSESSMENT OF ANALYTICAL FACILITIES

The choice of analytical technique is governed by what is available in the laboratory, and will obviously influence the quality and quantity of the sample to be obtained. There are many occasions when it would be advantageous to use such techniques as mass spectrometry, neutron activation, X-ray fluorescence spectrometry and electron microscopy, but since these are not commonly available, it is necessary to plan the sampling to fit the techniques that are available.

(a) *Gases*

The inorganic gases, e.g. sulphur dioxide, sulphur trioxide, nitrous fumes and ammonia, contained in trapping liquids can be analysed spectrophotometrically, by titrimetry or by selective ion electrodes.

The organic gases, e.g. hydrocarbons, esters, alcohols, aldehydes and ketones, trapped on adsorbents have to be desorbed either by heating or by solvent elution or extraction. Preferred techniques for both qualitative and quantitative analysis include gas chromatography, thin layer chromatography, high-pressure liquid chromatography and infra-red spectrometry.

(b) *Solids*

Particulate matter may be first examined microscopically. This is desirable in problems arising from pollution by the combustion of coal and oil and in problems concerning quarrying. Microscopic examination of fibrous deposits is normally essential, e.g. asbestos, the analysis of which usually involves X-ray diffraction.

Metal particulate matter is most frequently analysed by atomic absorption spectrophotometry. Salts such as sodium chloride and ammonium sulphate can be conveniently analysed by classical wet chemical methods.

(c) *Liquids*

As for gases and solids, classical chemical or common instrumental methods are used, depending on the nature of the liquid pollutants.

ACTION

Having selected the types of sampling and analysis, the magnitude and timescale of the exercise must be decided upon, in co-operation with the Environmental Health Officer, answers to the following questions being required.

(a) How many sampling points are necessary?
(b) How long is it necessary to sample either continuously or intermittently?
(c) How accurate and sensitive do the measurements need to be?

RESULTS AND CONCLUSIONS

Results are obtained in concentration units where possible, i.e. p.p.m. or weight per unit volume (e.g. mg per cubic metre). Where possible, meteoro-

logical data such as wind direction, wind speed, rainfall and cloud cover are taken into account.

There are no legal standards in the U.K. for the concentration of pollutants in ambient air. The analyst, with the community physician, must provide local standards. These can be based on his experience, a comparison of pollutant concentration with that in a control sample, or standards from other countries.

The Threshold Limit Values of the American Conference of Governmental Industrial Hygienists[3] form a rough basis for standards and cover most known air pollutants. These values strictly only apply to factory air breathed by a man working a forty hour week in a normal working life. Since a man works approximately one quarter of the week, a quarter of the Threshold Limit Values might be taken as a rough standard. If a factor of ten is included in order to protect the physically weakest people, i.e. the very young, the very old and the abnormally susceptible, it would be better to use one fortieth of the Threshold Limit Values. This approach to arriving at a standard is probably invalid with short-term monitoring (hours or days), as it is based on lifetime periods. It might be of significance in monitoring for periods of several months. While this is an incomplete summary of a complex subject, yet for the immediate purpose of this work, such a standard could give an idea of the order of magnitude of maximum permissible concentrations of pollutants in air.

Examples Where the Philosophy Has Been Applied

FORMALDEHYDE FROM A UREA-FORMALDEHYDE FOAM FILLING OF CAVITY WALLS

Appraisal. Several council house tenants complained of a pungent odour and acute irritation to eyes and throat not long after the walls of their houses had been filled with urea-formaldehyde foam. Monitoring of the pollutant was required urgently, which necessitated a personal visit. The odour appeared to be that of formaldehyde.

Sampling. Because of the urgency of the problem, it was decided to use a manually operated gas sampling kit using a formaldehyde indicator tube.

Analytical facilities. Not applicable in this case.

Action. The air in several rooms of the worst-affected houses was monitored, both close to the walls, which frequently showed damp patches, and in the middle areas.

Results and conclusion. The monitoring confirmed the presence of formaldehyde in most of the rooms and that it was coming from the walls, but the concentrations were less than one fortieth of the Threshold Limit Value. Nevertheless, the presence of the pollutant in the houses was having a distressing effect on the inhabitants and measures were taken by the District Environmental Health Department in conjunction with the contractors to alleviate the problem.

INCINERATION OF WASTE SLUDGE FROM ELECTROPLATING TANKS

Appraisal. The problem concerned the possible contamination of the atmosphere with metal or metal oxide fume as a result of incineration of sludge. The possible pollutants were cadmium, chromium, nickel and zinc. A visit was made to plan the monitoring sites.

Sampling. The ideal arrangement would have been continuous air sampling at a rate of approximately 10–15 litres per minute at sites surrounding the emission source. Only one apparatus capable of doing this was available, and it was placed on the NE side of the emission source, in the hope that there would be a prevailing SW wind. At the same site, and at three other sites, viz., NW, SE and SW of the emission source, moss was hung in bags as qualitative deposition collectors.

Analytical facilities. Atomic absorption spectrophotometry was available for the determination of cadmium, chromium, nickel and zinc in both filters and moss.

Action. The air was monitored during a period of one week before the incineration began, then for the incineration period, when the wind direction was right, and for one week afterwards.

Results and conclusions. None of the four metals were found in the filters or in the qualitative moss samplers in any quantity greater for the incineration period than for the periods before or afterwards.

VEHICLE EXHAUST EMISSION NEAR A SCHOOL

Appraisal. The Parent-Teacher Association of a school complained that the vehicle exhaust fumes of heavy traffic nearby, particularly when the children were arriving and leaving, were a health nuisance. The problem had to be dealt with reasonably quickly, but was not urgent. The possible pollutants were lead, carbon monoxide, nitrous fumes and polynuclear hydrocarbons. A personal visit was necessary to assess the best sites for monitoring.

Sampling. It was decided to monitor the air continuously inside and around the school for lead and carbon monoxide in the first instance. Tests for nitrous fume and polynuclear hydrocarbons would have been added if the concentrations of lead and carbon monoxide had been found to be high. It was desirable and practical to monitor for one month using continuous air samplers for particulate lead (siting two monitors outside the school and one inside a classroom). Another sampler was used on a control site about one mile away.

It would have been desirable to monitor carbon monoxide at all the sites but this was not possible. As a compromise, one carbon monoxide monitor was borrowed for three weeks and sited outside the school.

Analytical facilities. Atomic absorption spectrophotometry was available for the determination of lead. No facility was required for the carbon monoxide sampling since the borrowed non-dispersive infra red instrument gave a direct read-out.

Action. The air was continuously monitored for lead during one month, changing the filters twice daily. The air was continuously monitored for carbon monoxide during the latter three weeks of the period.

Results and conclusions. The lead concentrations in the air surrounding the school were no higher than those at the control site and were less than a fortieth of the Threshold Limit Value. The lead concentration in the classroom was very low. The carbon monoxide concentrations were also normal and less than a fortieth of the Threshold Limit Value.

PARTICULATE MATTER AND PHENOLIC DEPOSITION NEAR A FACTORY

Appraisal. The occupants of houses near a factory which manufactures products of tar distillation complained that the factory was depositing smuts over the area surrounding the works. The problem was intermittent and several personal visits revealed that the houses and gardens most affected were those which were down-wind of the works. The vegetation appeared to have been attacked by some chemical agent.

Sampling. The type of sampling considered to be most desirable was continuous air sampling at 10–15 litres a minute using a sodium hydroxide trap for the collection of the phenolic substances. In addition, the collection of the black particulate deposit was desirable and was conveniently made using petri dishes.

However, a continuous air sampling apparatus was not available and so a large amount of broad-leaved vegetation was used as a deposition collector.

Analytical facilities. A microscope was available to study the black particulate and gas chromatography to detect phenols and phenolic acids.

Action. Petri dishes were strategically positioned around the works and were replaced after the collection of a reasonable amount of solid matter. This collection was made four times. The collected matter was examined microscopically and then extracted for analysis by gas chromatography. Broad-leaved plants and nettles which appeared to have been attacked were gathered on three separate occasions and analysed by gas chromatography. Weather conditions were noted during the sampling period.

Results and conclusions. There was no evidence of phenols, cresols, naphthols or any organic acids in the particulate deposit in the petri dishes or on the vegetation. Oily droplets were noted in all the petri dish deposits and there was a greater concentration of droplets close to the factory and a generally greater concentration in a down-wind direction.

It was concluded that there was no significant phenolic emission but that there was definite evidence of oily smuts coming from the factory.

CHECK ON POSSIBLE FLUORIDE EMISSION NEAR AN INDUSTRIAL COMPLEX

Appraisal. An investigation was required on behalf of the District Environmental Health Department at the request of the District Veterinary Consultant. There were at least two possible fluoride emitters in the industrial complex, viz. a brickworks and also a chemical works known to be using hydrofluoric

acid in considerable quantity. The veterinary interest was in the possibility of deposited fluoride on grazing land nearby and its possible adverse effects on the health of cattle and horses. Personal visits were made as part of the planning of the investigation.

Sampling. The most satisfactory way of sampling was considered to be continuous air sampling at 10–15 litres per minute. The samplers would be positioned in such a way that, in conjunction with weather data, any possible emission source would be pinpointed. The particulate fluoride would be trapped on a filter and gaseous fluoride would be contained in an alkaline trap.

This desirable sampling method was not practical owing to lack of apparatus, but there was a small network of Standard Deposit Gauges in the area which could be used as collectors. In addition, sampling of grass from the nearby grassland was carried out.

Analytical facilities. Microdistillation and a fluoride selective ion electrode apparatus were available.

Action. The insoluble matters and the solutions of five Standard Deposit Gauges and two control Gauges were analysed for fluoride over a period of six months and the weather data was noted in that time. The grassland in the area, and in a control area were also sampled and analysed.

Results and conclusions. Results showed that the soluble and insoluble fluoride contents of the rain water near the industrial complex were significantly higher than those of the controls. There was also strong evidence to show that the sole source of the fluoride was the chemical works. The grassland in the industrial area showed only slightly elevated levels of fluoride when compared with the control samples.

References

1. "Control of Pollution Act", H.M.S.O., London, 1974.
2. "Annual Report of the Lancashire County Analyst", 1974, County Hall, Preston, p. 238.
3. "Threshold Limit Values for 1973", Department of Employment, H.M. Factory Inspectorate, London, 1973.

J. Assoc. Publ. Analysts, 1976, **14**, 105–110

Studies on the Effect of Heat Processing on Various Spoilage Values of Meat and Fish

Part I. Fat Spoilage Values of Beef

D. Pearson, R. J. Bonam and D. Rasanayagam

National College of Food Technology (University of Reading), Weybridge, Surrey

Samples of beef dripping were heated in air and processed in cans (in the absence and presence of water) at temperatures up to 116°C. There were minimal increases in the free fatty acids, Kreis number, peroxide value and the TBA number. The results obtained on the fat extracted from minced beef cooked in water showed a similar pattern. When minced beef was canned at 116°C each of the four values decreased, but they tended to increase on storage. In view of its greater consistency it is suggested that a maximum FFA figure (1·5 per cent. as oleic acid calculated on the extracted fat) might be of some value for assessing the acceptability (freshness) of canned beef.

It has previously been shown that when beef is heated there is a considerable rise in the total volatile nitrogen (TVN), rising in canned products to as high as 280 mg of N per 100 g of protein[1]. Such findings indicated that, although the TVN figure could be used to assess the acceptability of raw meat[2,3], it was inapplicable to heat-processed products. As fat rancidity values have also been recommended as possible spoilage criteria[4], it was decided to investigate to what extent they altered when meat was heated. Most of the previous work in this field has involved the heating of "pure" vegetable oils rather than animal fats present in meat as such. Also such workers have tended to concentrate attention on those rancidity values related to oxidative deterioration rather than the FFA, which may in general be more reliable for indicating acceptability of fresh meat[3,4].

Several factors have been shown to encourage acceleration of oxidation of lipids, e.g. heat or the presence of water, salt or traces of metals, haematin compounds, photochemical pigments and oxidative enzymes. Antioxidants and synergists on the other hand tend to retard rancidity development. Artman[5] has published a review covering the possible mechanisms which occur in fats due to the application of heat. When first heated, fat gains in weight due to oxygen absorption and the peroxide value increases. Water, if present, encourages fat hydrolysis with liberation of FFA. The glycerides with shorter chain and unsaturated acids appear to be the most susceptible to hydrolysis. The presence of steam in a deep fryer, by partially removing some FFA, has sometimes reduced the rate of oxidation of the fat[6,7]. On the other hand

the addition of water to a hot fat increases the proportion of polymeric material and the FFA[7]. Water also affects the formation of carbonyls in heated fats[8].

It is probable that the factors effecting changes in the fat in intact muscle are more complex and there may be interaction between various components in the flesh. There is evidence that for meat, the phospholipids oxidise to a greater extent than the neutral triglyceride fat[9]. In this work it was decided to follow changes on heating commercially prepared beef dripping, with and without the addition of water, before examining the more complex picture of meat also containing lean tissue.

It would be useful to compare the results obtained with the tentative "just spoiling" and acceptability limits published previously for raw beef[4] (Table I). This would be appropriate in view of the chromatographic work of Anderson *et al.*[10], who found that there were no significant differences between the lipids of raw and cooked meat. Further Lea[11] has pointed out that food processing is normally carried out at temperatures lower than those required for thermal polymerisation and there is little opportunity for serious deteriorative changes in the lipid[12]. Most other cooking processes however involve some exposure to atmospheric oxygen, and autoxidation and oxidation-induced polymerisation are possible. Lea[11] added however that the parallel development of off-flavour may well not be noticed by individuals with less sophisticated palates.

TABLE I
CRITICAL LIMITS FOR FAT SPOILAGE VALUES IN RAW BEEF[4]

	Value at "just spoiling" stage
FFA (as per cent. oleic acid)	1·8
Kreis number (see text)	4·7
Peroxide value (mequiv/kg)	4·5
TBA (Method II) (mg malonaldehyde/kg)	1·8

Experimental Procedures

HEATING AND PROCESSING

Heating of beef dripping up to 130°C

In each experiment 4 × 60 g samples of factory prepared beef dripping were heated on a hotplate in separate glass beakers to 25, 50, 75, 96 and 130°C respectively. Rancidity values were determined on each as well as on untreated samples after 15, 30, 45 and 60 minutes at these temperatures.

Heating of beef dripping in cans at 116°C

To investigate the effect of water, 40 g of beef dripping and 160 ml water were added to 8 oz cans, which were then processed at 116°C in a rotating retort for 0, 15, 30, 45, 60, 75 and 90 minutes. The ratio of water to fat was chosen to correspond approximately to that in canned meats. After processing, the water was drained off before dissolving the fat in chloroform for the analysis. Rancidity values were also determined on corresponding samples of beef dripping processed similarly, but without the addition of water.

Cooking of minced beef

Cold water was added to the minced beef using a ratio of water to meat of 3:2. A series of such mixtures was heated in beakers over a hotplate, with constant stirring, for periods of 5, 10, 15 and 20 minutes.

Preparation of canned minced beef

Four ounces of meat were added to 8 oz cans and filled with hot water to give a headspace of $\frac{1}{4}$ in to $\frac{3}{8}$ in. The clinched cans were exhausted for 55 minutes at 96°C, sealed and processed at 116°C for 85 minutes before cooling in water.

SAMPLING

Samples from cooked minced beef were mixed with the cooking water in a mechanical macerator. Canned meats were comminuted in a mincer and then mixed with a pestle in a mortar.

ANALYTICAL PROCEDURES

Water was determined by drying at 100°C to constant weight.

Fat was estimated on the dried residue obtained in the water determination by extraction with light petroleum (b.p. 40–60°C) in a Bolton extractor.

Fat spoilage values were mostly estimated by macerating the sample with chloroform,[13] filtering and using the dried filtrate for the determination of FFA[13], peroxide value (Sully method)[14], Kreis test[13] and thiobarbituric acid value (Method I)[14] as previously described. Particularly with the samples including lean meat (minced beef, commercial canned samples, etc.), the distillation procedure of Tarladgis *et al.*[15] was used for the TBA value (Method II), as Method I would exclude lipids not extractable with chloroform.

Results and Discussion

EFFECT OF HEATING COMMERCIAL DRIPPING UP TO 130°C

From the large number of determinations made after heating samples of factory-prepared dripping at 5 different temperatures up to 96°C for 15, 30, 45 and 60 minutes, the alterations found in the rancidity values were not

TABLE II
RANGES OF FAT SPOILAGE VALUES OF SAMPLES OF BEEF DRIPPING HEATED IN BEAKERS AT TEMPERATURES UP TO 130°C

Beef dripping sample	Free fatty acids, as per cent. oleic acid	Kreis number (see text)	Peroxide value, *milliequivalents/kg*
Range for untreated samples	0·70–1·9	1·8–4·6	2·0–3·8
Maximum found for samples heated for up to one hour at various temperatures up to 96°C	2·3	5·0	4·1
Maximum found for samples heated for up to one hour at 130°C	2·3	6·0	12·2

significantly different from one another. In Table II only maxima are recorded for all the samples heated at 25, 50, 75 and 96°C. As the changes seemed rather greater as the temperature was raised above 96°C however, the maximum values for determinations after heating the fat to 130°C are given separately. The increase in values from "fresh" was not as great as it would appear from the maximum and minimum figures recorded in Table II. For example, the greatest changes in the values found for individual samples were as follows: FFA 1·1–2·2 (1 hr at 96°C), Kreis number 1·8–5·0 (1 hr at 96°C) and peroxide value 2·3–4·1 (1 hr at 50°C). Taken in conjunction with previous correlations with organoleptic findings[4] it would appear that the free fatty acids show the most significant and consistent increases when the fat is heated in the presence of oxygen.

EFFECT OF HEATING COMMERCIAL DRIPPING IN CANS AT 116°C

The temperature of 116°C was chosen as canned meats are commonly processed at this temperature. Batches were processed in the retort together and individual cans were removed after 15, 30, 45, 60, 75 and 90 minutes. There was little difference in the results between the samples processed in the presence of water and those processed alone. Taken over the 90-minute heating periods, there was little alteration in the FFA and the rise in the Kreis number and peroxide value was not sufficient to be of organoleptic importance[4]. Of slightly greater significance was the increase in the TBA number (Method I), which tended in each of the "runs" to rise to an almost constant maximum (16·8–18·5) before decreasing slightly to about 13 at the end of the 90 minutes processing. Similar studies applied to fat rendered at butcher's shops showed comparatively high values after purchase and only very small alterations after processing. This would indicate that beef fat, which is relatively saturated, reaches maximum values fairly readily.

The samples of heated dripping with FFA figures above 1·5 per cent. (Table II) also had relatively high values prior to treatment.

EFFECT OF COOKING MINCED BEEF IN WATER

Nine samples of minced beef were examined for fat spoilage values after purchase and after cooking in water for 5, 10, 15 and 20 minutes. The results recorded in Table III show the range of figures found for the freshly purchased

TABLE III
RANGES OF FAT SPOILAGE VALUES OF 9 SAMPLES OF MINCED BEEF COOKED IN WATER FOR PERIODS OF UP TO 20 MINUTES

Minced beef sample	Water, *per cent.*	Fat, *per cent.*	Free fatty acids, as per cent. oleic acid	Kreis number (see text)	Peroxide value, *milli-equivalents/kg*	TBA number (Method II), *mg malonalde hyde/kg*
Freshly purchased	55·5–67·9	9·8–20·2	0·2–1·6	1·1–6·6	1·1–12·1	1·0–3·8
Maxima for corresponding samples cooked in water for periods of up to 20 minutes	—	—	1·7	8·1	15·6	4·6

meat and the maxima for the corresponding samples cooked in water. The FFA, Kreis number, peroxide value and TBA number all showed increases due to heating in water for up to 20 minutes. The maximum figures are approximately of the same order as those found for "pure" dripping heated in air (Table II). The main difference is that the minced beef samples showed a smaller increase in FFA, but slightly higher maximum peroxide values and Kreis numbers.

EFFECT OF CANNING MINCED BEEF AT 116°C

Table IV shows the results obtained with minced beef when raw, after canning and after storing the canned products for one month at room temperature (approx. 20°C). Contrary to other results found, each of the values actually decreased during canning, although there were increases apparent after storage. It is difficult to explain the low values for FFA, as loss by decomposition or volatilisation is unlikely during processing in sealed cans. It is possible however that volatile components could have escaped from the clinched cans during exhausting for 55 minutes at 96°C. On the other hand as the processing temperature (116°C) is not higher than the boiling points of the fatty acids, volatilisation seems unlikely. From the analytical point of view the FFA may be more difficult to extract due to binding during processing with the denatured protein. Another possibility is that the acids may be partially neutralised by the ammonia formed[1]. It should be noted however that after one month's storage the various values increased above the figures found after canning. One aspect which is obvious from the results is that the FFA, unlike the TVN[1], does not increase markedly during the heat processing.

The FFA figures for both the laboratory (Table IV) and commercially (Table V) canned meats did not exceed the maximum of 1·5 per cent. (as

TABLE IV
EFFECT OF CANNING AND STORAGE OF 5 SAMPLES OF MINCED BEEF AT 116°C

Batch number	Condition of sample	Fat, *per cent.*	Free fatty acids, as per cent. oleic acid	Kreis number (see text)	Peroxide value, *milli-equivalents/kg*	TBA number (Method II), *mg malonaldehyde/kg*
1	Raw	2·7	1·0	4·0	1·5	3·2
	Canned	3·6	0·8	1·1	1·2	0·8
	Canned and stored	3·8	1·5	1·2	3·9	2·4
2	Raw	19·6	0·7	3·3	2·8	2·7
	Canned	12·0	0·4	1·7	1·6	1·0
	Canned and stored	12·4	1·0	7·6	3·0	2·3
3	Raw	5·8	1·4	4·1	6·2	1·7
	Canned	6·4	0·9	2·5	4·8	0·4
	Canned and stored	6·9	1·5	3·8	7·2	1·2
4	Raw	17·8	1·2	7·4	7·9	5·3
	Canned	12·8	1·0	2·8	3·0	1·4
	Canned and stored	10·0	1·4	3·2	11·5	2·2
5	Raw	12·4	0·8	2·4	2·0	1·4
	Canned	11·2	0·4	1·4	1·3	0·9
	Canned and stored	10·8	0·9	2·2	2·4	1·2

Storage: each canned sample was stored for one month at room temperature (approx. 20°C).

oleic acid when calculated on the extracted fat) recommended for raw beef (Table I). In view of the inconsistencies sometimes found with raw beef[4] it is

TABLE V
FAT SPOILAGE VALUES OF COMMERCIALLY CANNED MEATS

Sample	Water, *per cent.*	Fat, *per cent.*	Free fatty acids, as per cent. oleic acid	Kreis number (see text)	Peroxide value, *milli-equivalents/kg*	TBA number (Method II), *mg malonaldehyde/kg*
Stewed steak "A"	67·1	10·5	1·4	4·6	2·5	1·6
Beef steak	65·2	14·4	0·5	2·1	1·5	1·2
Minced beef	70·8	10·7	1·2	3·0	1·0	1·7
Stewed steak "B"	58·2	16·2	0·7	2·5	1·2	0·7
Roast beef in gravy	65·0	6·6	1·3	4·2	2·8	2·2
Stewed steak with gravy	69·1	11·4	0·6	2·7	1·8	1·5

hardly surprising that occasional canned samples gave relatively high figures for the fat spoilage values related to oxidative changes. As haem catalysis of oxidation is partly a result of breakdown of peroxides, what is measured analytically, at least with the peroxide value, is a balance between formation and decomposition.

All the canned beef samples mentioned in this paper were classed as being organoleptically acceptable.

Conclusions

Samples of beef fat and minced beef heated in air showed slight but measurable increases in fat spoilage values, i.e. FFA, Kreis number, peroxide value and the TBA number. As with raw meat[4] the changes in the FFA were more consistent than with other values.

When beef was canned at 116°C each of the four values decreased prior to rising again during storage. All samples canned in the laboratory and commercially gave figures which did not exceed the maximum of 1·5 per cent. FFA (as oleic acid on the extracted fat) recommended previously for the acceptability of raw beef. In view of the greater inconsistency found with the three other values and the unsuitability of the TVN[1], the FFA figure seems to have possibilities for giving some idea as to the condition of canned beef.

References

1. Pearson, D., and Lee, K. L., *J. Assoc. Publ. Analysts*, 1973, **11**, 28.
2. Pearson, D., *J. Sci. Food Agric.*, 1968, **19**, 366.
3. Pearson, D., *ibid.*, 1968, **19**, 556.
4. Pearson, D., *ibid.*, 1968, **19**, 553.
5. Artman, N. R., "Advances in Lipid Research", Paoletti, R., and Kritchevsky, D. (Editors), Academic Press, New York, 1969, **7**, 245.
6. Lea, C. H., "The oxidative deterioration of food lipids", In "Lipids and Their Oxidation", Schultz, H. W. (Editor), Avi, Conn., 1962, p. 3.
7. Perkins, E. G., and van Akkeren, L. A., *J. Amer. Oil Chem. Soc.*, 1965, **42**, 782.
8. Dornseifer, T. P., Kim, S. C., Keith, E. S., and Powers, J. J., *ibid.*, 1965, **42**, 1073.
9. Love, J. D., and Pearson, A. M., *ibid.*, 1971, **48**, 547.
10. Anderson, D. B., Breidenstein, B. B., Kauffman, R. G., Cassens, R. G., and Bray, R. W., *J. Food Technol.*, 1971, **6**, 141.
11. Lea, C. H., *Chemy Ind.*, 1965, p. 244.
12. Roubal, W. T., *J. Amer. Oil Chem. Soc.*, 1963, **40**, 215.
13. Pearson, D., *J. Assoc. Publ. Analysts*, 1965, **3**, 76.
14. Pearson, D., *ibid.*, 1974, **12**, 73.
15. Tarladgis, B. G., Watts, B. M., Younathan, M. T., and Dugan, L., *J. Amer. Oil Chem. Soc.*, 1960, **37**, 44.

J. Assoc. Publ. Analysts, 1976, **14**, 111–112

A Note on the Freezing Point of Goats' Milk

G. V. JAMES
Western Counties Laboratories, 8 Worrall Road, Bristol

Goats' milk is now being offered for sale, packed in cartons containing one pint, over a widely scattered area of South Wales and the West Country, and the contents of individual cartons may be the product of a single goat or bulked from herds.

In 1972, two samples were received giving very differing results of analysis which raised doubts as to the authenticity of the samples (Table II, samples a and b). A search in the literature produced the information shown in Table I.

TABLE I
PUBLISHED ANALYTICAL DATA FOR GOATS' MILK

Reference No.	Fat, *per cent.*	Total solids, *per cent.*	Solids not fat, *per cent.*	Ash, *per cent.*	Freezing point, °C
(1)	4·6	14·0	9·4	0·8	—
(2)	3·2 –5·5	12·54–14·35	8·5 –10·03	0·65–0·87	—
(3)	2·95–7·25	11·96–18·40	8·39–11·17	0·75–1·04	−0·595– −0·550
(4)	4·5 –5·3	13·6 –14·6	9·1 – 9·2	0·96–0·99	−0·578– −0·559
(5)	4·7 –6·65	13·02–15·88	8·28– 9·23	0·74–0·80	−0·570– −0·552
(6)	3·1 –4·75	11·39–13·64	8·29– 8·69	—	−0·575– −0·571

A wide variation in composition is evidently possible and according to Davis[2] the composition will vary between breeds of goats and between morning and evening milking, the evening milk being richer in fat. A poorer quality

TABLE II
RESULTS OF ANALYSES OF RETAIL SAMPLES OF GOATS' MILK

Sample	Fat (Gerber), *per cent.* *w/w*	Total solids, *per cent.* *w/w*	Solids not fat, *per cent.* *w/w*	Ash, *per cent.* *w/w*	Chloride as Cl, *per cent.* *w/w*	Freezing point, °C
a	3·5	11·59	8·09	0·66	—	—
b	3·1	15·00	11·90	0·55	—	—
c	3·4	11·67	8·27	0·63	—	—
d	3·7	12·21	8·51	0·63	—	−0·565
e	3·2	11·05	7·85	0·61	—	−0·562
f	3·2	11·39	8·19	0·75	—	−0·550
g	3·4	11·68	8·28	0·795	0·114	—
h	3·5	10·82	7·32	0·94	0·141	—
i	4·3	12·63	8·33	0·775	0·112	—
j	3·4	11·69	8·29	0·63	0·095	—
k	3·2	11·39	8·19	0·75	0·111	−0·550
l	3·7	12·21	8·51	0·63	0·093	−0·565
m	3·2	11·05	7·85	0·61	0·087	−0·562
n	3·0	10·49	7·49	0·87	0·127	−0·572
o	3·5	13·08	9·38	1·02	0·151	−0·578

of milk results as the animal ages. He further points out that legal limits in force in Gibraltar require at least 3·5 per cent. fat and 8·0 per cent. of solids not fat.

Further samples from retail outlets were examined, the results of analyses being given in Table II (samples c–e). Sample c was a formal sample. The low ash and solids not fat figures suggested some irregularity in view of the figures obtained by other authors. Therefore an "Appeal to Goat" sample was arranged (sample f) from which it was seen that the sample c was obviously genuine.

Davis[2] states that chlorides are higher in goats' milk than in cows' milk and this may explain the apparent difference in results obtained with a low ash figure and a high freezing point depression. Further samples were obtained in order that this could be investigated. The results are given in Table II (samples g–o) and include the figure for chloride.

The chloride figures are higher than in cows' milk and average 14·7 per cent. of the ash compared with 14·3 per cent. of the ash of cows' milk[2]. In my opinion the chloride figure is hardly sufficient to cause the change of freezing point but other ions also may be responsible.

During the collection of these samples a request was received for a Coliform Count and Methylene Blue test to be made on one of these samples, which gave the following results:

Methylene Blue	Satisfactory—Grade I
Count on agar at 37°C	1 colony per ml
Count on agar at 22°C	No colonies per ml
E. coli	Present in 10 ml Absent in 1 ml

In this sample, at least, the bacteriological quality was high.

Much more work needs to be done but this short note may be of assistance to others who may be interested.

My thanks are due to Inspector M. Richards of the Trading Standard Department of Gwent County Council for obtaining some of the samples and to Mr Myland who allowed an extensive experiment to be made on his herd.

References

1. Nicholls, J. R., "Aids to the Analysis of Food and Drugs", 6th Edn., Baillière, Tindall & Cox, London, 1942, p. 1.
2. Davis, J. G., and MacDonald, F. J., "Dairy Chemistry", 5th Edn., Griffin, London, 1953, pp. 97–101.
3. 1938 Report of Director of Food and Drugs Department of Public Health, Commonwealth of Massachusetts, *Analyst*, 1940, **65**, 226.
4. Bagnall, H. H., *Analyst*, 1943, **67**, 148.
5. Whittle, E. G., *ibid.*, 1943, **67**, 247.
6. Hutchinson, A. J., *private communication*, 1973.

J. Assoc. Publ. Analysts, 1976, **14**, 113–117

Food Microscopy

(An Annotated Bibliography)

PART IID. MAJOR INGREDIENTS: FATS AND OILS

G. W. WHITE AND MISS A. J. SHENTON

Lyons Central Laboratories, 149 Hammersmith Road, London W14 0QU

Fats and oils occur in natural foods and also constitute one of the major ingredients in many manufactured foods, including baking products, confectionery, dairy products, margarine and many other different types of "spread".

The main sources of edible fats and oils are vegetable fats (~49 per cent.), animal fats (~46 per cent.) and marine oils (~5 per cent.). Vegetable fats and oils are obtained from the appropriate seeds and fruits either by mechanical expression or solvent extraction. The crude oil so obtained is then neutralised, bleached and deodorised. Animal fats, such as beef fat, mutton fat and lard are obtained by rendering the fatty tissues of the animals. In addition to these basic methods of manufacture, many different processing methods are used to alter the basic properties of fats. These processing methods may alter the *chemical composition* as in (a) blending, (b) hydrogenation, (c) interesterification, (d) glycerolysis and (e) fractionation or may profoundly affect the *physical form* or crystal structure of the fat as in (f) solidification, (g) tempering and (h) ageing or storage.

Microscopical studies have been carried out on bulk fats, on fats in emulsions or as powders, and on fats in more complex products, where it is necessary to show their presence by the use of histochemical reagents, normal stains or fluorescent stains. The microscopical properties which can be studied in bulk fats include crystal habit, size and extent of aggregation, inclusions, the effects of reagents, thermal properties, polymorphism and the effects of composition and processing on their microstructure. Some early workers have used crystal habit to detect the adulteration of lard with beef fat, but the method is not altogether reliable. Studies have been carried out on crystal size in fats; the production of very fine crystals is favoured by a high rate of nuclei formation coupled with a low linear crystallisation velocity. The extent of aggregation and network formation in fats is a vital factor controlling their rheological properties, and is an aspect arousing increasing interest. Apart from crystals, other "inclusions" in fats that have been studied microscopically are air bubbles (plasticised fats) and water droplets (butter and margarine). Some attempts have been made, in chemical microscopy, to use the saponifica-

tion reaction and also the formation of fatty acid crystals as a diagnostic tool for fat identification, but these methods are very limited.

Thermal properties of fats that can be studied with a microscope hot-stage include melting points, refractive index of the melt, supercooling and crystallisation behaviour. Many fatty acids and glycerides show the phenomenon of multiple melting points on heating, due to polymorphism—the occurrence of more than one crystalline form. Considerable work has been done in this field with the polarising microscope and hot-stage, used in conjunction with other methods such as cooling and heating curves, dilatometry, X-ray diffraction, infrared spectroscopy and nuclear magnetic resonance. Generally, the triglycerides show 4 polymorphic forms (α, β', β'' and β), but in some cases (e.g. cocoa butter) up to 6 forms have been found. Another thermal parameter, which has some advantages over the melting point, is the critical mixing temperature (CMT) or critical solution temperature (CST); the sample and a test liquid are introduced into a capillary which is heated on a micro hot-stage, and the CMT is the temperature at which the meniscus between the two liquids disappears. The method is said to be diagnostic.

The crystalline characteristics of fats can be modified either by altering their molecular composition or by variation of their physical processing, and microscopical studies in both areas have been reported. Where processing is concerned, many studies have been made, over the last ten years, of the process for the production of quick-tempered shortenings, using scraped-surface heat exchangers. The relationship between rheological properties and microstructure is of considerable practical importance.

Fats and oils are also met with in the form of oil-in-water emulsions, as in cream, ice cream, salad dressings, milk, etc., and in the form of water-in-oil emulsions as in butter and margarine. The microscope is extensively used in measuring the size of the globules in such emulsions, and in the study of their stability to heating and cooling, freezing and thawing. Supercooling in the oil phase of oil-in-water emulsions is also of practical importance and has been studied microscopically.

Free-flowing powdered fats have been made by spray-setting and by spray-drying of oil-in-water emulsions, and the microscope can be used to study their particle size and size distribution, particle shape and air inclusions.

The following references constitute a selection covering these many aspects.

1. Weygand, C., and Gruntzig, W., "The Polymorphous Systems of Natural Monoacid Triglycerides", *Z. Anorg. Chem.*, 1932, **206**, 304.
2. Butcher, C. H., "The Microscopy of Food Products, 3. Lard", *Food*, 1934, **3**, 176.
3. Butcher, C. H., "Industrial Microscopy, VI. Oils, Fats, Waxes, etc.", *Ind. Chemist*, 1934, **10**, 495.
4. Greene, L. W., "Chemical Microscopy of Fats and Waxes, I. (A Review)", *Oil and Soap*, 1934, **11**, 31.
5. Mehlenbacher, V. C., "Fat and Oil Microscopy", *Oil and Soap*, 1936, **13**, 277; *C.A.*, 1937 **31**, 281.
6. Greene, L. W., "Chemical Microscopy of Fats and Waxes. III. Castor Oil", *Amer. J. Pharm.*, 1937, **109**, 67.
7. Greene, L. W., "Chemical Microscopy of Essential Oils", *Drug Cosmetic Ind.*, 1938, **43**, 156; *C.A.*, 1938, **32**, 8072.
8. Greene, L. W., "Chemical Microscopy of Fats and Waxes, IV. Cotton Seed Oil", *Cotton and Cotton Oil Press*, 1938, **39**, (14), 3.
9. Greene, L. W., "Chemical Microscopy of Fats and Waxes, V. Chaulmoogra Oil", *Amer. J. Pharm.*, 1938, **110**, 492.

10. Greene, L. W., "Chemical Microscopy of Essential Oils, II. Bitter Almond Oil", *Amer. J. Pharm.*, 1939, **111**, 10; *C.A.*, 1939, **33**, 3068.
11. Greene, L. W., "Chemical Microscopy of Essential Oils, III. Perfumery", *Essent. Oil Record*, 1939, **30**, 309; *C.A.*, 1940, **34**, 855.
12. Sutton, R. W., Barraclough, A., Mallinder, R., and Hitchen, O., "The Examination of Lard", *Analyst*, 1940, **65**, 623 [see also comments on this paper by Williams, K. A., *Analyst*, 1940, **65**, 633].
13. Brown, J. B., "Low Temperature Crystallisation of the Fatty Acids and Glycerides", *Chem. Rev.*, 1941, **29**, 333.
14. Quimby, O. T., "Application of Microscopy to Polymorphism of Tristearin-Type Fats", *Anal. Chem.*, 1948, **20**, 686.
15. Lutton, E. S., "Review of the Polymorphism of Saturated Even Glycerides", *J. Amer. Oil Chem. Soc.*, 1950, **27**, 276.
16. Quimby, O. T., "Microscopic Appearance of Polymorphic Forms of One-acid Triglycerides" *J. Amer. Chem. Soc.*, 1950, **72**, 5064.
17. Vaeck, S. V., "Research on the Polymorphism of Some Natural Fats. I. Thermic and Microscopic Study of Cocoa Butter", *Int. Choc. Rev.*, 1951, **6** (4), 100.
18. Mohr, W., and Mohr, E., "The Crystal Forms of the Solid Constituents of the Butterfat in Lard and Butter", *Molkerei u. Käserai-Zeitung*, 1954, **5**, (46), 1508; *C.A.*, 1955, **49**, 3432.
19. Hoerr, C. W., "Microscopy of Fats and Oils", *J. Amer. Oil Chem. Soc.*, 1955, **32**, 659.
20. Hoerr, C. W., and Waugh, D. F., "Some Physical Characteristics of Rearranged Lard", *J. Amer. Oil Chem. Soc.*, 1955, **32**, 37.
21. Hutton, J. F., Matthews, J. B., and Scarlett, N. A., "The Structure of Lubricating Greases, under the Light Microscope in Relation to Their Performance", *J. Inst. Petroleum*, 1955, **41**, 163; *C.A.*, 1955, **49**, 9915.
22. McCrone, W. C., "Fusion Methods in Chemical Microscopy", Interscience Publishers, York, 1957.
23. Griebel, C., "The Microscopical Examination of Fat-Producing Animal and Plant Raw Materials and Their By-Products", Analyse der Fette und Fetteprodukte, Kaufman, H. P., Vol. 2, Springer-Verlag, Berlin, 1958.
24. Gupta, L., "Ringed Appearance of Lipid Spheres under the Phase Contrast Microscope", *Nature*, 1958, **182**, 537.
25. Hanssen, E., "Variations in the Microstructure of Fat through Various Methods of Cooling", *Fette Seifen Anstrich.*, 1958, **60**, (8), 658.
26. De Man, J. M., and Wood, F. W., "Polarised Light Microscopy of Butter Fat Crystallisation", Inter. Dairy Congress, Proc. 15th Congr. London, 1959, **2**, 1010; *C.A.*, 1960, **54**, 4951.
27. Schulman, J. H., Stoekenius, W., and Prince, L. M., "Mechanism of Formation and Structure of Micro Emulsions by Electron Microscopy", *J. Phys. Chem.*, 1959, **63**, (10), 1677.
28. Tverdokhleb, G. V., "Specific Heat of Ripe Cream in Relation to Polymorphous Transformation of Milkfat", *Mol. Prom.*, 1959, **20**, (6), 24.
29. De Man, J. M., "The Crystallisation Behaviour of Milkfat", *J. Dairy Sci.*, 1960, **43**, 839.
30. Hoerr, C. W., "Morphology of Fats, Oils and Shortenings", *J. Amer. Oil Chem. Soc.*, 1960, **37**, 539.
31. Moreno, J. M. M., Herrerra, C. G., Delgado, E. M., Valle, C. J. Del, and Hidalgo, L. D., "Some Characteristics of Membranes Protecting Oil Emulsions in Protein Solutions", *J. Amer. Oil Chem. Soc.*, 1960, **37**, 582; *C.A.*, 1961, **55**, 2146.
32. Pearse, A. G. E., "Histochemistry, Theoretical and Applied", 2nd Ed., J. & A. Churchill Ltd, London, 1960: Fat-Soluble Colorant Methods for Lipids, p. 302.
33. Singleton, W. S., Benerito, R. R., and White, J. L., "Factors Affecting Oil Particle Size in the Freezing and Thawing of Fat Emulsions", *J. Amer. Oil Chem. Soc.*, 1960, **37**, 88.
34. Stoeckenius, W., Schulman, J. H., and Prince, L. M., "The Structure of Myelin Figures and Microemulsions as Observed with the Electron Microscope", *Kolloid Z.*, 1960, **169**, 170.
35. Moreno, J. M. M., Catalina, F., and Herrerra, C. G., "Electron Microscopic Studies of Anion-Active Membranes in Oil-in-Water Emulsions", *Fette, Seifen, Anstrich.*, 1961, **63**, 915.
36. Okada, M., "Crystal Growth in Butterfat Observed by the Electron Microscope", *J. Appl. Phys.* (Japan), 1961, **30**, (11), 824; *D.S.A.*, 1964, **26**, 331.
37. Belousov, A. P., and Vergelesov, V. M., "Polymorphic Transformations in Milkfat", *Mol. Prom.*, 1962, **23**, (3), 4.
38. Belousov, A. P., and Vergelesov, V. M., "On Certain Peculiarities of Crystallisation of Milkfat in Cooling", Inter. Dairy Congress Proc. 16th Congr., Copenhagen, 1962, **B**, 49.
39. Belousov, A. P., and Vergelesov, V. M., "Polymorphism in Butterfat", Inter. Dairy Congress Proc. 16th Congr. Copenhagen, 1962, **B**, 122.
40. Chapman, D., "The Polymorphism of the Glycerides", *Chem. Rev.*, 1962, **62**, 433.
41. McKnight, L. M., and Wood, F. W., "The Influence of Butterfat Precrystallisation on the Hardness of Gold'n Flow Type Butter", Inter. Dairy Congress, Proc. 16th Congr. Copenhagen, 1962, **B**, 33.

42. Stoeckenius, W., "Some Electron Microscopical Observations on Liquid Crystalline Phases in Lipid-Water Systems", *J. Cell Biol.*, 1962, **12**, 221.
43. Wallis, T. E., "Textbook of Pharmacognosy", J. & A. Churchill Ltd, London, 1962: Notes on Lard, p. 512.
44. De Man, J. M., "Polymorphism in Milkfat", Review Article No. 111, *D.S.A.*, 1963, **25**, 219.
45. Moran, D. P. J., "Phase Behaviour of Some Palmito-Oleo Triglyceride Systems", *J. Appl. Chem. (London)*, 1963, **13**, 91; *C.A.*, 1963, **58**, 12777.
46. Vergelesov, V. M., and Belousov, A. P., "Polymorphism of the Triglycerides of Some Higher Fatty Acids and Natural Mixtures of Triglycerides", *J. Phys. Chem. (U.S.S.R.)*, 1963, **37**, 1082.
47. Wilton, I., and Wode, G., "Quick and Simple Methods for Studying Crystallisation Behaviour of Fats", *J. Amer. Oil Chem. Soc.*, 1963, **40**, (12), 707.
48. Bangham, A. D., and Horne, R. W., "Negative Staining of Phospholipids and Their Structural Modification by Surface Active Agents as Observed in the Electron Microscope", *J. Mol. Biol.*, 1964, **8**, 660.
49. De Man, J. M., "Physical Properties of Milkfat", *J. Dairy Sci.*, 1964, **47**, (11), 1194.
50. Ferrans, V. J., "The Fluorescence Microscopy of Lipids", *Dissert. Abstr.*, 1964, **24**, 3049; *C.A.*, 1964, **60**, 16187.
51. Fischer, R., "The Critical Mixing Temperature in the Analysis of Fats (Micro-Method)", Proc. 1st World Fat Congress, Hamburg, 1964, II, 111.
52. Keinert, J., "Cocoa Butter Characteristics in Correlation with the Precrystallisation of Fatty Melts", *Int. Choc. Rev.*, 1964, **19**, (10), 446; *BFMIRA Abstr.*, 1965, **18**, 68.
53. Okada, M., "Transformation of a Tristearin Crystal From the α to the β Form by the Melt Grown Method", *J. Electronmicroscopy (Tokyo)*, 1964, **13**, 180.
54. Okada, M., "Morphological Observation of the Crystal of Triglycerides by Electron Microscope", *J. Electronmicroscopy (Tokyo)*, 1964, **13**, 87.
55. Rubio, M., Catalina, F., Moreno, J. M. M., Herrera, C. G., and Valle, C. J. Del, "Interfacial Problems in Olive Oil Extractions. IV. Electron Microscopy of Olives", *Anal. Real. Soc., Espan. Fis. Quim. Ser.* B., 1964, **60**, 825.
56. Fukada, E., and Masuzawa, M., "Crystallisation Behaviour and Mechanical Properties of Fats", *J. Soc. Mat. Sci.*, 1965, **14**, (139), 40.
57. Hoerr, C. W., and Ziemba, J. V., "Fat Crystallography Points Way to Quality", *Food Engng*, 1965, **37**, (5), 90.
58. Lovegren, N. V., and Feuge, R. O., "Solidification of Cocoa Butter", *J. Amer. Oil Chem. Soc.*, 1965, **42**, 308.
59. Lutton, E. S., "Phase Behaviour of Aqueous Systems of Monoglycerides", *J. Amer. Oil Chem. Soc.*, 1965, **42**, 1068.
60. Schmid, H. H. O., Mangold, H. K., and Lundberg, W. O., "Characterisation and Identification of Lipids by Their Critical Solution Temperatures", *J. Amer. Oil Chem. Soc.*, 1965, **42**, 372.
61. Barrall, E. M., and Guffy, J. C., "The Polymorphism of Tristearin", Preprints (General Papers), Divn Petroleum Chem. Inc., *Amer. Chem. Soc.*, 1966, **11**, (3), 261.
62. Moreno, J. M. M., Herrera, C. G., Janer, C., Pereda, J., and Flores, V., "Effect of Surfactive Agents on Olive Oil Extraction", *Afinidad*, 1966, **23**, (246), 527; *C.A.*, 1967, **66**, 10644.
63. Reigel, G. W., and McMichael, C. E., "The Production of Quick Tempered Shortenings", *J. Amer. Oil Chem. Soc.*, 1966, **43**, 687.
64. Williams, K. A., "Oils, Fats and Fatty Foods: Their Practical Examination", 4th Edn, J. & A. Churchill, London, 1966: Microscopic Examination for Beef, Lard and Hydrogenated Fats, p. 120.
65. Casley-Smith, J. R., "Some Observations on the Electron Microscopy of Lipids", *J. Roy. Micr. Soc.*, 1967, **87**, 463.
66. Chapman, D., Williams, R. M., and Ladbrooke, B. D., "Physical Studies of Phospholipids. VI. Thermotropic and Lyotropic Mesomorphism of Some 1,2-Diacyl-Phosphatidylcholines (Lecithins)", *Chem. Phys. Lipids*, 1967, **1**, 445.
67. Heintze, W., "Optical Polarisation Phenomena of Microscopic Globules", *Milchwissenschaft*, 1967, **22**, (2), 69; *C.A.*, 1967, **67**, 2211b.
68. Imamura, M., Niiya, I., Takagi, K., Okada, M., and Matsumoto, T., "Deterioration of Oils and Fats of Hardened Coconut Series. I. Chemical Change and Crystal Growth of Hardened Coconut Oil at Different Temperature Storage. II. Correlation Between Crystal Growth and Hydrolysis", *J. Japan Oil Chem. Soc.*, 1967, **16**, 506 and 551 respectively.
69. Walstra, P., "On the Crystallisation Habit in Fat Globules", *Neth. Milk Dairy J.*, 1967, **21**, 166; *D.S.A.*, 1968, **30**, 346.
70. Cornish, R. M., "Studies of Glyceryl Monostearate", *J. Soc. Cosmetic Chemists*, 1968, **19**, 109.
71. Drachenfels, H. J. Von, "Microscopic Observation of the Boundary Interface of Lecithin-Water", *Fette, Seifen, Anstrich.*, 1968, **70**, 486.
72. Meara, M. L., "Research Work on Oils and Fats", *Food World*, 1968, **3**, (5), 16.

73. Pajor, Z., Pandula, E., Peres, T., and Stark, J., "Electron-Microscopic Investigation of the Structure of Ointments. II. Examination of W/O and O/W Emulsion Ointments", *Fette, Seifen, Anstrich.*, 1968, **70**, 182.
74. Anon., "Lipid Helices", *Nature*, 1969, **221**, 1198.
75. Buchheim, W., "Quantitative Study of the Reaction Between Osmium Tetroxide and Lecithin and Similar Substances with Regard to the Interpretation of Electron Microscope Pictures of Myelin Structures", *Kieler Milchw. Forsch. Ber.*, 1969, **21**, (4), 491; *D.S.A.*, 1970, **32**, 255 [1762].
76. Fluck, D. J., Henson, A. F., and Chapman, D., "The Structure of Dilute Lecithin-Water Systems Revealed by Freeze-Etching and Electron Microscopy", *J. Ultrastructure Research*, 1969, **29**, 416.
77. Glauert, A. M., and Lucy, J. A., "Electron Microscopy of Lipids. Effects of pH and Fixatives on the Appearance of a Macromolecular Assembly of Lipid Micelles in Negatively Stained Preparations", *J. Microscopy*, 1969, **89**, 1; *C.A.*, 1969, **71**, 78074n.
78. Berger, K. G., "Fats as Structural Components of Foods", *Food Manufacture*, 1970, **45**, (5), 60.
79. Buchheim, W., "The Molecular Order of Bi-Refringent Fat Globules", *Milchwissenschaft*, 1970, **25**, 223.
80. Deamer, D. W., Leonard, R., Tardieu, A., and Branton, D., "Lamellar and Hexagonal Lipid Phases Visualised by Freeze-Etching", *Biochimica Biophysica Acta*, 1970, **219**, 47.
81. Flack, E. A., and Krog, N., "The Functions and Applications of Some Emulsifying Agents Commonly Used in Europe", *Food Trade Review*, 1970, **40**, (8), 27.
82. Jewell, G. G., and Meara, M. L., "A New Rapid Method for the Electron Microscopic Examination of Fats", *J. Amer. Oil Chem. Soc.*, 1970, **47**, 535.
83. Okada, M., "Crystal Growth and Physical Properties of Oil and Fat by the Use of Electron Microscopy", *J. Japan Oil Chem. Soc.*, 1970, **19**, 600; *Food Sci. Tech. Abstr.*, 1971, **3**, 12N, 526.
84. Tverdokhleb, G. V., and Vergelesov, V. M., "Factors Controlling Crystallisation of Milkfat", Proc. 18th Inter. Dairy Congress, 1970, **1E**, 211; *Food Sci. Tech. Abstr.*, 1970, **2**, 12P, 1671.
85. Vaughan, J. G., "The Structure and Utilisation of Oil Seeds", Chapman and Hall, London, 1970.
86. Zugibe, F. T., "Diagnostic Histochemistry", The C. V. Mosby Company, Saint Louis, 1970: Lipid Staining, p. 51; Electron Microscopy of Lipids, p. 58.
87. Beresteyn, E. C. H., and Walstra, P., "Effect of Globule Size and Properties upon the Crystallisation of Milkfat", *Officiel Org. K. ned. Zuivelbond*, 1971, **63**, (47), 1112; *D.S.A.*, 1972, **34**, [2287].
88. Castro, R. de, and Vazquez, R., "Microscopical Examination under Polarised Light of Hog Backfat and its Mixtures with Hydrogenated Fat", *Grasas y Aceites*, 1971, **22**, 463; *Food Sci. Tech. Abstr.*, 1972, **4**, 7N 320.
89. Friberg, S., "Liquid Crystalline Phases in Emulsions", *J. Colloid Interface Sci.*, 1971, **37**, (2), 291.
90. Wilton, I., and Friberg, S., "Influence of Temperature-Induced Phase Transitions on Fat Emulsions", *J. Amer. Oil Chem. Soc.*, 1971, **48**, 771.
91. Eins, S., "Electron Microscopy of Mesomorphic Structures of Aqueous Lipid Phases, III. The Phosphatidylserine-Water System", *Chem. Phys. Lipids*, 1972, **8**, 26.
92. Lutton, E. S., "Lipid Structures", *J. Amer. Oil Chem. Soc.*, 1972, **49**, (1), 1.
93. Shcherbakov, V. G., and Silantev, L. V., "Electron Microscopic Study of the Distribution of Oil in Sunflower Seed Cells During Grinding", *Maslozhirovaya Promyshlenost*, 1972, **38**, (1), 5; *Food Sci. Tech. Abstr.*, 1973, **5**, (2), 2N 99.
94. Chayen, J., Bitensky, L., Butcher, R. G., "Practical Histochemistry", John Wiley & Sons, London, 1973: Reactions for Lipids, p. 80.
95. Lundberg, B., "The Visualisation of a Lamellar and a Cubic Mesophase in the Monoolein-Water System by Freeze-Etching", *Chem. Phys. Lipids*, 1973, **11**, 219.
96. Berger, K. G., and Pollitt, R. J. M., "Oils and Fats. The Physical Structure of Shortenings", Proc. Symp. of IFST, 7–8 March, 1974, p. 21.
97. Culling, C. F. A., "Handbook of Histopathological and Histochemical Techniques", 3rd Edn, Butterworth & Co., London, 1974; Lipids (Fats, Lipoids, Lipins), Ch. 16, p. 351; Fluorescent Methods for Lipids, Ch. 32, p. 613.
98. Jacks, T. J., Yatsu, L. Y., and Hensarling, T. P., "Extraction of Lipids from Cottonseed Tissue. V. Ultrastructural Effects of Extraction with Hexane-Acetic Acid", *J. Amer. Oil Chem. Soc.*, 1974, **51**, 169.
99. Ostanina, A. V., and Aleshin, S. N., "Study of Fat Globule Membranes using Optical and Electron Microscopes", *Mol. Prom.*, 1974, (**2**), 23; *Food Sci. Tech. Abstr.*, 1974, **6**, 8P 1075.
100. Pollitt, R. J. M., "Rheology of Shortenings", 5th European Symposium on Food Rheology in Food Processing and Food Quality, Dechema Monographien, Nr 1505–1536, Band 1974, **77**, 363, Verlag Chemie GMBH, Weinheim/Bergstrasse.