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Objects of the Journal. The Journal covers a wide field ranging from pure research in the various sciences associated with food to practical experiments designed to improve technical processes. While the main object is to provide a forum for papers describing the results of original research, review articles are also included. Selected papers read at ordinary meetings of the Institute are published either in full or in summary, as a separate section of the Journal. The Editor welcomes papers from food scientists and technologists. These must be of a high standard of original research or comprehensive reviews of specialized sections of food science or technology.

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Food research in the United Kingdom

C. L. CUTTING

I. The food industry

Production

More is spent in Britain on food than on any other commodity group, yet in 1961–62 when the last survey was made only about £5M was spent on food research; £634M, including £213M by private industry, was spent on research and development as a whole.* These were 2·2 and 0·7% of the gross national product respectively (Advisory Council on Scientific Policy, 1962). Expenditure on research and development in relation to output was lower in 'food, drink and tobacco' than in any other group of manufacturing industries with the exception of 'wood, paper and printing' (Department of Scientific and Industrial Research, 1960).

About half the total of £5557M spent on food in 1964 was estimated to have been processed in some way (£2750M to £2850M) (Central Statistical Office, 1965a). With increasing affluence the importance of food in the national expenditure has declined in relation to other items from 31% in 1957 to 25% in 1964; although expenditure on food is expected to rise by £500M (at 1964 prices) by 1970, it will then only account for 23% of the total (The National Plan, 1965). Weekly expenditure on food was 33s 0d per head in 1964 (Central Statistical Office, 1965b), or 100s 4d per household (average 3·06 persons), *plus* 12s 1d per household (totalling £551M) for meals bought away from home (Ministry of Labour, 1965).

It is instructive to review the relative importance of the different classes of food commodity. Table 1 lists the values of principal types of food consumed at home in 1963, assuming an estimated population of 53,673,000. It is necessary to differentiate between: (a) the preparation and distribution of fresh (i.e. unprocessed) foods, and (b) food manufacturing, processing, preservation or compounding.

In 1964, 621,000 people were employed in the food processing industry. Between now and 1970 this number is expected to fall only 0·1% annually. The anticipated increase in output of 1·6% per annum per head is less than half the national average of 3·9% for all manufacturing industry.

The food manufacturing industry partly overlaps the drink industry and consists of broadly distinguishable sections of which Table 2 gives the gross and net output, or

* The corresponding figures for 1965, recently published, are £756·6M and £327·8M (Report on Science Policy, Cmnd 3007, H.M.S.O.).

Author's address: British Food Manufacturing Industries' Research Association, Leatherhead, Surrey.

TABLE 1. Household expenditure on principal foods, 1963

	£ million		£ million
Milk—liquid	424	Sugar	122
Milk—processed and cream	47	Preserves, jam, marmalade and other sweet spreads	46
Cheese—natural and processed	96	Sugar confectionery	116
Meat—carcase, including poultry and offals	761	Cocoa and chocolate	186
Meat—processed, canned, etc.	516	Flour	35
Fish—fresh	77	Bread	286
Fish—processed	17	Buns, cakes and flour confectionery	149
Fish—quick frozen	18	Biscuits (excluding chocolate biscuits)	89
Fish—canned, cooked and other products	84	Chocolate biscuits	27
Eggs	214	Breakfast cereals	41
Fats—butter	187	Other cereal products	41
Fats—margarine, lard and other fats and oils	92	Puddings	17
Fruit—fresh and quick frozen	238	Dried pulses	7
Fruit—canned	75	Table jellies	8
Fruit—juices	13	Soups, canned and dehydrated	36
Fruit—dried, including nuts	25	Meat and vegetable extracts	15
Vegetables—potatoes	138	Tea and coffee	158
Vegetables—potatoes—pre-packed	19	Coffee powders and essences	41
Vegetables—potatoes—crisps and chips	67	Brand food drinks	11
Vegetables—fresh green, peas, beans, roots, etc.	147	Soft drinks	99
Vegetables—quick frozen	26	Ice-cream	34
Vegetables—canned and other products (excluding beans)	46	Invalid and infant foods	8
Vegetables—canned beans	31	Other miscellaneous food items	16
Pickles	23	Approximate total	4969

Note. A factor of 11·629 has been used to convert pence per week to £ million a year total. Source: Ministry of Agriculture, Fisheries and Food, 1965.

'added value' (from Board of Trade Census of Production in 1963, the results of which are just becoming available). Total net output is over £850M. Independent firms in most sections have declined appreciably in number since the last census (1958). In the chocolate and sugar confectionery industry there were 300 manufacturers in 1964 as against 600 in 1958; total production remained about the same but labour fell about 15% (Johnson, 1965). In 1963, the ten largest companies made 86% of the chocolate. The turnover of the dozen or so largest food processors is now over £1000M; of the five largest milling and baking groups £700M, and of the two largest dairy groups £300M (*The Times* Review of Industry and Technology, 1965). About twenty large groups therefore account for about one-third of all food sales, and make a net profit before interest and tax of around £150M.

TABLE 2. Size of various sections of the food manufacturing industry (1965)

	Goods produced and work done (£ million)	Net output (£ million)	No. of firms (1958)	Total No. of employees (1964) (thousands)	No. of scientists and technologists (1964)†
1. Grain milling	332	89	543	35	320
2. Bread and flour confectionery	296	153	2037	125	10
3. Biscuits	129	58	108	44	60
4. Bacon-curing, meat and fish products	243	80	979	69	160
5. Milk products (including butter and cheese)	221	43	411	26	230
6. Sugar	228	38	23	15	130
7. Cocoa, chocolate and sugar confectionery	260	112	664	101*	–
8. Fruit and vegetable products	235	91	378	58	130
9. Animal and poultry foods	285	72	761	19	160
10. Margarine	54	7	27		
11. Starch and miscellaneous food industries	127	50	307	36	430
12. Brewing and malting	612*	157*	311	79*	–
13. Spirit distilling and compounding	234*	43*	69	46*	–
14. Soft drinks, British wines and cider	97	53	782	55	90
15. Vegetable and mineral oil and fats	159*	20*	124	12*	–
16. Gelatine, adhesives, etc.	17*	6*	77	5*	–
Total of 1–11 and 14	2477	856	–	483	1720

* 1958.

† Excluding technical assistants; source, Ministry of Labour.

– Not available.

Imports and exports

Imports of food are essential to Britain and these in 1964 were about £1500M or 27% of all U.K. imports. More than half these imports were unprocessed foods, such as meat, fresh fruit and unrefined sugar. Much could not be produced in Britain or was raw material for further manufacture. The manufactured food imports have an essential bearing on the pattern of food research in Britain and are therefore listed in Table 3 (Trade and Navigation Accounts, 1965).

TABLE 3. Values of U.K. imports of manufactured foods (1964)

	£ million
Cereals, milled	14
Biscuits, cakes and other cereal preparations	4
Bacon	112
Canned beef and veal	23
Canned bacon, hams and other pig products	36
Canned poultry and other prepared and preserved meat	11
Canned salmon	29
Canned crab, pilchard, sardines and all other fish and fish preparations	11
Preserved milk, cream, etc.	14
Butter	157
Cheese	38
Sugar, unrefined	131
Sugar, refined and all other sugars	5
Fruit, dried	19
Fruit, canned and other preserved	51
Fruit, juices	7
Vegetables, preserved and preparations	38
Miscellaneous foods and food preparations	39
Total	739

TABLE 4. Values of U.K. exports of manufactured foods (1964)

	£ million
Cereals, milled, breakfast and malt	5
Biscuits and cakes	8
Meat products	4
Fish products	5
Milk products	12
Solid sugar, molasses, etc.	34
Sugar confectionery	13
Cocoa and chocolate products	9
Fruit and vegetables, dried, etc.	5
Jam	2
Soups	1
Sauces	1
Margarine	2
Coffee products	2
Miscellaneous	6
Total	109

Table 4 gives manufactured food exports for 1964. These are considerably smaller than imports, but are still appreciable. Half the figure of over £100M was accounted for by sugar and confectionery (Cocoa, Chocolate and Confectionery Alliance, 1965).

II. Types of food research organization

Three interests are interwoven in the history of British food research, namely Government, industry and universities, probably in that order of importance (Society of Chemical Industry, 1965).

Organized food research in the U.K. perhaps started in 1912 at the National Institute for Research in Dairying under the University College of Reading but with support from the Development Fund. Nevertheless the Laboratory of the Government Chemist, which, amongst other things, is concerned with legislative aspects of foods, began in 1842 but was established as an independent organization in 1911. When the Department of Scientific and Industrial Research (D.S.I.R.) was set up in 1917, to remedy deficiencies in British industrial science, the urgent need to reduce food losses through spoilage was recognized in the formation of the Food Investigation Board.

The Government also gave financial encouragement to British manufacturers to establish industrial co-operative Research Associations; the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades was one of the first to be set up in 1919. A fruit and vegetable canning research laboratory was instituted at Chipping Campden in association with the University of Bristol in the same year. This became a D.S.I.R.-aided Research Association in 1952 and is now the Fruit and Vegetable Preservation Research Association (F.V.P.R.A.).

The Low Temperature Research Station (L.T.R.S.) was set up by D.S.I.R. in 1922 in conjunction with the University of Cambridge. Its initial interests were principally in relation to the transport and storage of imported Commonwealth produce in refrigerated shipping space. The Research Association of British Flour Millers (R.A.B.F.M.) was set up in 1924 and the botulism outbreak of 1922 led to the opening of the British Food Manufacturers' Research Association (B.F.M.R.A.) in 1925. The Ditton Laboratory (D.L.) was opened in Kent in 1928 and the Torry Research Station (T.R.S.) in Aberdeen in 1929 to investigate problems of marketing fruit and fish respectively. Both began as off-shoots of L.T.R.S., under the Food Investigation Board. Sub-stations of Ditton and L.T.R.S. were set up in the wholesale markets of Covent Garden and Smithfield before 1939, and the Humber Laboratory was started as an out-station of T.R.S. in 1950. The Pest Infestation Laboratory (P.I.L.) was established in 1940 to study grain storage in wartime. Just before the Second World War, the Government also set up the Scientific Adviser's Division of the Ministry of Food, now under the Ministry of Agriculture, Fisheries and Food (M.A.F.F.). In 1959 L.T.R.S., D.L. and P.I.L. were transferred to the Agricultural Research Council (A.R.C.) which already

largely financed the N.I.R.D. and the Hannah Dairy Research Institute (H.D.R.I.) in Scotland.

The Chocolate, Confectionery and Jam Research Association and B.F.M.R.A. amalgamated in 1946 to found B.F.M.I.R.A. The British Baking Industries Research Association (B.B.I.R.A.) was also established in 1946 and the British Gelatine and Glue Research Association (now G.G.R.A.) 2 years later.

The Tropical Products Institute (T.P.I.) which was formerly under the aegis of the Imperial Institute and was partly concerned with foods, joined D.S.I.R. in 1949. The Government Chemist's Laboratory, previously directly under the Treasury, also joined D.S.I.R. about this time. The British Industrial Biological Research Association (B.I.B.R.A.) was set up in 1960 to pursue the toxicological problems of foodstuffs.

D.S.I.R., A.R.C. and the Medical Research Council (M.R.C.) were responsible to the Lord President of the Council who subsequently also became Minister for Science.

It was announced in 1962 that meat research formerly carried out at L.T.R.S. is to be transferred to a new Meat Research Institute (M.R.I.) near Bristol and close to the University Veterinary School. The livestock and livestock products industries agreed to share half of the cost through a statutory levy. The rest of L.T.R.S. and the Ditton Laboratory are to be amalgamated in a new Food Research Institute (F.R.I.) at Norwich, close to the University of East Anglia.

The Trend Committee, in its Report on an Enquiry into the Organization of Civil Science in 1963, stated that, as regards research into the processing of food (other than fish), both home produced and imported, 'we believe that the existing arrangements are not entirely satisfactory and that they should be examined in more detail' (Cmnd 1963). Such an examination has not yet been completed.

After the 1964 General Election, the A.R.C. was placed under the Department of Education and Science whilst T.R.S. and all the R.A.s became the responsibility of the new Ministry of Technology. The T.P.I. joined the new Ministry of Overseas Development.

Some teaching institutions are also engaged in food research. After the war the London County Council's Smithfield Institute became the nucleus of a National College of Food Technology (N.C.F.T.) which is now to become a department of the University of Reading. The Royal Technical College, Glasgow (now the University of Strathclyde) set up a food science department in 1950 to be followed by the Universities of Leeds, Reading and Nottingham. The Borough Polytechnic, later a College of Advanced Technology, and a number of other educational institutions also carry out food research.

III. Food research in industry

When the older food research organizations were founded it was relatively rare for food companies to engage in research, apart from quality control and testing. The

situation has now completely changed, to some extent as a result of the influence of state-aided food research.

Estimates of industrial expenditure on food research and development have been made by: (a) the Department of Scientific and Industrial Research (1960) in 1958, (b) the National Institute of Economic and Social Research for the Federation of British Industries (1961) in 1959–60, and (c) the Advisory Council on Scientific Policy (1962) in 1961–62, of which the last is most exhaustive.

These estimates are rather unsatisfactory because (a) and (c) combine food with drink and tobacco and even (c) is 4 years old. More up-to-date figures may, however, soon be available. The food industry employs 75% of the total labour force in the food, drink and tobacco industries, and this factor has therefore been applied to the published figures in Table 5. Nevertheless it is realized from the results of (b) that this is not a very satisfactory procedure.

TABLE 5. Recent estimates of food research and development expenditure in the U.K.

Source	Current expenditure (£M)				Capital expenditure (£M)			Total (£M)	Qualified man-power on research and development	Average expenditure per qualified employee (£)
	Salaries and wages	Materials and equipment	Other	Total	Land and buildings	Plant	Total			
(a) D.S.I.R. (1958)*	1·5	0·3	0·8	2·6	—	—	0·9	3·5	530	6642
(b) F.B.I. (1959–60)	—	—	—	2·5	—	—	—	—	539	4557†
(c) A.C.S.P. (1961–62)*	2·9	0·7	0·8	4·4	0·2	0·7	0·9	5·3	735	7200

* Assuming expenditure on 'Food' represents 75% of that on 'Food, drink and tobacco' throughout.
† Based on current expenditure only.

The Federation of British Industries' (1961) survey perhaps suggests that expansion of industrial research since 1945 has been due more to increase in the size of existing industrial research laboratories, than to an increase in the number of food manufacturing firms engaged in research.

Allowing for increase in the costs of research since 1961–62, and some increase in scale, it may be guessed that the food industry is currently spending £7M to £8M on its own research and development. Of this perhaps one quarter is 'research' and three-quarters 'development', assuming that food manufacture presents the same pattern as the average for manufacturing industry as a whole; of the 'research' about one-eighth would be 'basic' (i.e. about £0·5M) and the rest 'applied'. These figures are generally supported by the rather scanty returns (nine firms, all save one employing more than 2000) to the Federation of British Industries (1961) survey on this question, which claimed 12% of a total expenditure of £822,000 on 'basic research', 19% on 'minor improvements', 20% on 'major improvements', 34% on 'new products', and 12% on 'technical services'. The remainder was unspecified.

The most remarkable single development in industrial food research in post-war Britain has been the enormous expansion in the scale of research by Unilever Ltd. Over 700 people were reported to be working in 1963 at their central food research laboratory at Colworth House, near Bedford (Wilkinson & Taylor, 1963). Three-quarters of these people were working on human and the rest mostly on animal foods (Cole & Tempel, 1964). The total now spent by Unilever on research, including central fats and oil research, is probably not less than £2½M a year; this is about one-third of the total estimated for the entire U.K. food industry.

There is reason to believe that well over half of all other food manufacturing firms, including very large ones, apparently do no research at all. More than half of all laboratory activity is not in fact research but control of quality and raw material. Most of the rest have only one or two people engaged in research. Less than one firm in ten employ more than ten persons on research and very few, if any, more than one hundred. The Federation of British Industries (1961) suggest that a research and development department which employs fewer than three qualified scientists and engineers, that is, a research unit of about ten altogether, 'cannot really undertake original research and development work, but must confine itself to the assimilation of research undertaken elsewhere and to "trouble-shooting" within the firm'. On this basis 90% of all food firms are incapable of real research. Perhaps 50% of all scientific personnel reported as engaged on research in the food industry are employed in units of under ten persons. This serves to accentuate the role of the Research Associations on whom the majority of even medium-sized firms (with 300–2000 employees) must rely for technical and scientific information.

Some 'sponsored research' organizations are also operating increasingly in the food field (Woodward, 1966). These probably account now for a part of the food industry's 'external expenditure' on research, including R.A.s, which in 1958 (Department of Scientific and Industrial Research, 1960) and in 1959–60 (Federation of British Industries, 1961) was less than £300,000. The Brewing Industry Research Foundation, although concerned with a 'drink' rather than a 'food', has an annual income of £160,000 entirely from industry (Society of Chemical Industry, 1965).

Although a significant number of individual private consultants are employed by manufacturers, they do little research.

Comparisons with other countries are difficult and data scanty. The National Institute of Economic and Social Research (1962), however, has compared our food research expenditure for 1959 with that in the U.S.A., where 85 $M (or £30M at 2·8 $ = £1) were being spent internally, including Government expenditure. On the basis of a more realistic 'research exchange rate' of 6·3 $ = £1 the U.S.A. was still spending 4·9 times as much as the U.K. and employing 3·5 times as many qualified scientists and engineers on food research. The growth index (1935 = 100) for the food industry in the U.S.A. was 210 in 1958, compared with 170 in the U.K. Research expenditure in

relation to net output was 1·7 times as high in the U.S.A. and 1·9 times as many scientists were employed in relation to total employment in the food industry. Thirty-five 'large' U.S. food firms employing more than 5000 spent an average of 1·8 $M in performing research whereas in the U.K. twenty-one firms employing more than 2000 spent an average of 0·6 $M, i.e. a ratio of 3·0 : 1. As the latter is calculated at the 'research exchange rate' of 6·3 $ = £1, it represents just under £100,000 per firm and, of course, was largely accounted for by a single firm.

IV. Food Research Associations

The six food R.A.s were set up at different times. Their particular fields are shown in Table 6, which also gives details of the Government-controlled Research Institutes (G.R.I.s). R.A.s are primarily financed voluntarily by member firms on an agreed subscription basis related in some way to the size of the concern. The Government, formerly through D.S.I.R. but now through the Ministry of Technology, supplements industrial income for co-operative research by a grant which may be as high as 30s for £1 (i.e. 150%) for a new organization, such as B.I.B.R.A.; with well-established R.A.s the figure may be 50% (e.g. B.F.M.I.RA.) or less (R.A.B.F.M.).

The R.A.s are autonomous and controlled by councils elected by members and on which the Government is represented. It is official policy to stiffen progressively the terms of grant in order to induce industry to subscribe increasing sums (Department of Scientific and Industrial Research, 1962). Nevertheless the total Government contribution to the food R.A.s has also been increasing steadily during the past few years.

The programme of a Research Association is determined by the governing council on the advice of the Director of Research whose proposals must interpret the needs expressed by members. The Ministry of Technology, however, continuing the policy of D.S.I.R., makes its support conditional on there being a 'well-balanced distribution

TABLE 6. U.K. State-aided food research laboratories

Name	Location	Date of foundation	Main foods or topics concerned†
1. Research Association of British Flour-Millers (R.A.B.F.M.)	St. Albans	1923	Cereals and flour; nutrition
2. British Baking Industries Research Association (B.I.B.R.A.)	Chorleywood	1946	Bread, cakes and biscuits
3. British Food Manufacturing Industries Research Association (B.F.M.I.R.A.)	Leatherhead	1919, 1925 } 1946	Meat products (bacon, hams, sausages, pies, etc.); canned and similar fish products; pickles and sauces; jams and preserves; edible oils and fats; miscellaneous products; cocoa, chocolate and sugar confectionery

TABLE 6 (*continued*)

Name	Location	Date of foundation	Main foods or topics concerned†
4. British Industrial Biological Research Association (B.I.B.R.A.)	Carshalton	1960	Toxicity testing of additives used in food manufacture (and cosmetics)
5. Fruit and Vegetable Preservation Research Association (F.V.P.R.A.)	Chipping Campden	1919	Canned and quick-frozen fruits and vegetables
6. Gelatine and Glue Research Association (G.G.R.A.)	Birmingham	1948	Gelatine (and glue)
Government Research Institutes			
Agricultural Research Council (Department of Education and Science)			
7. Low Temperature Research Station (L.T.R.S.)	Cambridge	1922	Meat (including bacon and hams), eggs, poultry, fats, fruit and vegetables
8. Ditton Laboratory To be succeeded by:	Nr. Maidstone	1928	Fresh fruit, vegetables, cut flowers
7(a). Food Research Institute (F.R.I.)	Norwich	1968	All above foods except meat
8(a). Meat Research Institute (M.R.I.)	Bristol	1967–68	Carcase meat (including bacon and hams)
9. Pest Infestation Laboratory (P.I.L.)	Slough	1940	Insect pests of stored foods
10. *National Institute for Research in Dairying (N.I.R.D.)	Shinfield	1912	In part: milk products
11. *Hannah Dairy Research Institute (H.D.R.I.)	Ayr	1931	In part: milk products
Ministry of Technology			
12. Torry Research Station (T.R.S.)	Aberdeen (and Hull)	1929	Unprocessed fish and some fish products, including fish meal
13. Government Chemist	London, etc.	1911	In part: food analysis
Ministry of Agriculture, Fisheries and Food			
14. Food Standards, Science and Safety Division	London	1939	Fresh meat, etc. (now transferred to M.R.I.) and food defence
Ministry of Overseas Development			
15. Tropical Products Institute (T.P.I.)	London	1949	In part: concerned with tropical foods

* Not Government-owned, but receive 95% of income from the Government.

† See 'Additional References'.

of effort. . . . The Department holds the view . . . that the Association should keep prominently in mind the importance of conducting an adequate volume of long-range research'.

Table 7 compares staff and expenditure of R.A.s and G.R.I.s in 1963–64, in relation to output of the relevant sections of the industry given in Table 2, or otherwise estimated. Table 8 similarly compares expenditure on equipment and the total area of various laboratories.

Subscribing members of food R.A.s number from less than 100 for G.G.R.A. and R.A.B.F.M. to over 1000 for B.B.I.R.A., which includes many small bakers. Some large diversified companies belong to a number of R.A.s, perhaps several food R.A.s, as well as P.A.T.R.A. for printing and packaging advice and B.S.I.R.A. for scientific instruments.

Members, in return for their subscriptions, receive special research reports, current literature abstracts and the benefits of comprehensive information, library loan and practical advisory and analytical services for some of which they pay extra. Some 15,000 enquiries a year are answered and over 10,000 samples reported on (Society of Chemical Industry, 1965).

Non-food manufacturers can associate with the work to varying extents. Although practice varies, in general neither results nor advisory services are available to non-subscribers.

V. Government food research

Government-financed laboratories are listed in the lower part of Table 6, with further details in Tables 7 and 8.

The two Dairy Research Institutes, although independent foundations with industrial subscribers, as in a Research Association, nevertheless receive 95% of their income from the Agricultural Research Council. The new Meat Research Institute is unique in receiving half its income by statutory levy.

Fixing research priorities so as best to serve the food industry presents special difficulties for a Government Institute without organizational relationship with industry. Some G.R.I.s take advice in various ways in considering the research to be undertaken, but in general the research worker decides. Publication is chiefly in the scientific and technical literature, although some institutes also operate an advisory service, notably Torry for the fish industry.

Some further Government-controlled research involving food, such as the Infestation Control Division of the Ministry of Agriculture, Fisheries and Food, is not listed in Table 6. In addition, other Government Research Institutes, such as the National Engineering and Warren Spring Laboratories, direct their research programmes towards the apparent needs of British industry. The food industry, as one of the largest and less advanced technically, has naturally attracted attention in considering, for

TABLE 7. Size of state-aided food research establishments (1964)

	Staff		Income from industry (£000)	Expenditure (£000)		Approximate 1963 output of industry served (£million)		Expenditure as % net output
	Total	Graduate		Total[1]	Per graduate	Goods produced and work done	Net output (=added value)	
Research Associations								
1. R.A.B.F.M.	62	21	66	100	5·0	332	89	0·11
2. B.B.I.R.A.	70	23	49	111	4·8	425	212	0·06
3. B.F.M.I.R.A.	104	51	75	173	3·4	1750	500	0·035
4. B.I.B.R.A.	61	17	53[3]	94[2]	5·5	..... Spread		
5. F.V.P.R.A.	39	13	27	49	3·8	120	50	0·1
6. G.G.R.A.	22	8	24	34	4·2	20	8	0·4
Total 1–6	358	133	294	561	4·2	2650	860	0·07
Government Research Institutes								
7. L.T.R.S.	154	55	N.A.	229 (314)[4]	4·2	..... Spread		
8. D.L.	48	25	N.A.	111 (120)[4]	4·4	600[6]	–	–
8A. M.R.I.				48[4,5]	–	550	–	–
9. P.I.L.	105	40	N.A.	188 (338)[4]	4·7	..... Spread		
10. N.I.R.D.	86	29	N.A.	186	6·3	221	43	0·5
11. H.D.R.I.	21	8	N.A.	34	4·3			
12. T.R.S.	116	38	N.A.	279	7·3	110	110	0·25
13. M.A.F.F.	28	13	N.A.	68	5·2	..... Spread		
14. T.P.I.	61	17	N.A.	95	5·6	..... Spread		
Total 7–14	619	205	–	1238	5·5	–	–	–
Total 1–14	977	338	–	1799	5·0	–	–	–

[1] Figures for Government Institutes exclude share of headquarters staff and costs.
[2] Not yet fully operative. 1964 income £126,000 and bank overdraft £87,000.
[3] Including about £25,000 from food and confectionery manufacturers.
[4] 1964–65 figures, including capital.
[5] Not yet fully operative. Total income £300,000.
[6] Estimated from Annual Abstract of Statistics (Central Statistical Office, 1965c).
N.A. Not available.

TABLE 8. Facilities of state-aided food research laboratories (1964)

Research laboratory	Equipment expenditure 1963–64 (£000)	Total area (ft^2)
Research Associations		
R.A.B.F.M.	2·6	22,200
B.B.I.R.A.	2·8	20,000
B.F.M.I.R.A. (1965)	5·7	19,000
B.I.B.R.A.	7·3*	23,000
F.V.P.R.A.	2·4	15,850
G.G.R.A.	0·6	6,000
Government Research Institutes		
L.T.R.S.	13	26,400
D.L.	14†	12,270
F.R.I.	–	(99,000)‡
M.R.I.	–	(73,000)‡
P.I.L.	4	29,200
T.R.S.	14	100,000

* Depreciation only; £75,000 allocated for equipping new laboratories in 1964 and 1965.

† Special circumstances, for transfer to F.R.I.

‡ Projected in plans.

example, future basic research on pump design and mixing. Work carried out for individual firms must be paid for but in fact in 1958 the Select Committee on National Expenditure was very critical of the relatively minute financial support obtained in this way for the research carried out in D.S.I.R. stations, including Torry Research Station.

VI. Food research by statutory food bodies

The six statutory food Boards and Authorities, financed compulsorily by their respective industries, all sponsor research and development aimed at improving the position of the British producer. Most also carry out some on their own account. This is sometimes encouraged by £1 for £1 Treasury support. Much of this research is biological in emphasis, but some is technological and is carried out through R.A.s, G.R.I.s, universities or sometimes industry. Table 9 gives an estimate of this sum, already mostly accounted for in Table 7.

TABLE 9. Expenditure on research by statutory food bodies (1964–65)

Body	Total (£000)	Processing (£000) (Estimated)	Comments
British Egg Marketing Board	188	–	Including quality control projects
Herring Industry Board	7	7	5% of income—work at B.F.M.I.R.A.
Milk Marketing Board	81	10	B.F.M.I.R.A., N.I.R.D.
Pig Industry Development Authority	127	20	B.F.M.I.R.A., M.R.I., Leeds University
Potato Marketing Board	118	20	L.T.R.S., B.F.M.I.R.A., Cambridge University, National Institute of Agricultural Botany
White Fish Authority	295	20	T.R.S., B.F.M.I.R.A.
Total research and development	816	*c.* 100	

A Cereals Authority is now being constituted and a Meat and Livestock Commission is intended to replace and extend the functions of P.I.D.A., including responsibility for collecting the Meat Research Levy for the A.R.C.'s M.R.I. This Commission will also have extensive powers to sponsor other research and it is suggested that statutory food bodies could play a much greater part in satisfying the research and development requirements of the industries whose long-term interests they are empowered to represent.

VII. Food research by universities

Five universities with food science departments carry out food research as part of the main purpose of graduate and post-graduate instruction and training, and at least eight other universities and colleges are also working on food. A number of university staff engage in part-time consultancy with food manufacturers. This involves a certain amount of research. A few Government food Research Institutes are sited in close contact with universities.

Total university expenditure on food research has probably been underestimated at £150,000 (Society of Chemical Industry, 1965). Although these activities are a useful contribution, however, they cannot be as consistent and continuous, or comparable in scale or direction, with those of the established food research organizations.

VIII. Discussion

(a) *How best to satisfy the needs of industry*

The object of all scientific and technological research on food is, or should be,

not only to increase knowledge but also to provide information that the food industry can use to improve products, produce new ones and reduce costs. The contributions of industry, R.A.s, G.R.I.s and universities to this economic objective are adequately summarized elsewhere (Society of Chemical Industry, 1965) and in the annual reports and publications of the various organizations (see 'Additional References'). As in most other fields of human endeavour, however, most of the leading personnel would consider that performance in their sector would be more effective with a little more financial support. But the 'pay-off' of all research takes time to substantiate. It is always difficult convincingly to justify in detail the economic value of increased investment in research. Or equally to defend the present position.

Although there are obvious dangers in over-rigid planning with lines of demarcation, some improvement in the present situation is possible. A major reassessment is required of the functions of the different types of body and how they can best fulfil modern needs.

Food manufacturers could with advantage themselves carry out more research, and at least build up more units of the size required even to understand, interpret and utilize the results of research carried out elsewhere.

Much of the work in many industrial laboratories is necessarily short-range in scope and limited to overcoming immediate production problems without probing their basic causes. Where a firm is doing some longer-range research, it can assimilate better the results of state-aided research. Those who maintain their own research laboratories and can tackle their own short-term and *ad hoc* problems therefore tend to make good use of the R.A.s. Although R.A.s help smaller firms by means of *ad hoc* work, 'trouble-shooting' and advice, and some demands for this type of activity are unsatisfied, nevertheless R.A.s, like G.R.I.s, cannot be confined to short-term investigations alone. Although these are what industry mostly requires in order to solve production problems, they can only be tackled effectively by workers with practical experience of the scientific problems and not merely relying on books and papers.

Most R.A.s are anxious to increase the proportion of their long-range work and only if this can be done will they retain the interest and financial support of the larger firms able to indulge in substantial research efforts of their own. The Government, as the major financial contributor, should be able to exercise its influence to a greater extent to redress the balance without infringing the autonomy of R.A.s.

It is always difficult to decide where to draw the line between research aimed at overcoming current difficulties and longer-range (so-called 'basic') research to provide a better background understanding, and also to decide how much effort should be put into original work and how much into issuing advice based on published, but insufficiently known, facts.

The thesis that the best scientific discoveries are made by scientists finding their own problems appeals little to those who have to demonstrate to industry the

immediate usefulness of their work. It is often too costly to continue an investigation for industry once it has yielded sufficient results, or shows no promise of doing so, after spending the amount budgeted. Major achievements in the food industry have been due more to practical men than to basic research workers. However, the latter would often make much greater economic impact if more practically orientated. Research for the food industry would, in fact, gain more immediately from greater technological resources than from intensified fundamental research. It could then repeat the success of the Torry kiln, freezing fish at sea and the Chorleywood process. Knowledge of the industry and its problems is as important as a good knowledge of science. G.R.I.s must not be cut off from the industry and if the distinction between R.A.s and G.R.I.s is to remain it must be on the basis of commodity rather than depth of study or application.

It is in this type of 'application research and development' where Britain generally is backward. Engineering workshops and pilot-plant required are, however, expensive compared with the classical type of laboratory work, now rapidly becoming out-of-date.

Although it might be argued that the food industry, with help from the food machinery industry, can solve its own problems, the vision and persistence of outside organizations were necessary in the three instances quoted above. There are many other problems to be solved. The food machinery industry has no co-operative research organization of its own, although such a service could be highly advantageous.

An R.A. has the considerable advantage of close relationship and contact with its industry, although closer contact with the industrial problems of manufacturers in operating factories would be advantageous. Manufacturers are, of course, understandably secretive about some procedures and would not therefore welcome too inquisitive an R.A. The scope of R.A.s is, hence, inclined to be limited to investigation of specific problems, rather than extended to original study of entire processes.

It is clear from the shortage of good staff and their cost, and the rising complexity of equipment, that research expenditure cannot continue to rise at the present rate and it must soon be realized that the extent of overlapping that must occur in industrial research is too expensive. Industrial research and publicly supported laboratories must integrate their activities and exchange results, thus permitting a more concentrated attack on the essential basic problems.

Planning must still allow for initiative and individualism. There is already national and international co-operation in basic research between various public laboratories, but more co-ordination is required between these and industrial research laboratories. A significant contribution was made by B.F.M.I.R.A. in 1965 in that its members, through the medium of the R.A., are now sharing the results of their own investigations in the field of process control.

Industry would not wish the autonomy of their R.A.s to be prejudiced by increased Government financial assistance but Government support could be restricted, for example, to long-range items, or provision of special facilities or buildings, which determine scientific health and performance. Industry could retain the primary responsibility for research, development and advice concerned with utilization. This official help could perhaps be by extension of the so-called 'special assistance' and 'earmarked grants', without, however, some of their restrictive conditions.

On broad issues affecting consumers (who finally have to pay for everything), R.A.s no less than G.R.I.s must take a detached and scientific view, although their greater technical knowledge often makes them more sympathetic to the manufacturers than are the 'authorities'. R.A.s must also guide their members, even when they have to give them unpalatable advice.

Government food legislation, with the laudable aim of consumer protection, both in food safety and standards, is sometimes proposed in the absence of some of the relevant facts. R.A.s often have to provide facts based on research before it can be seen whether proposed legislation is reasonable or practicable. B.I.B.R.A. is engaged on the safety of food colours and other additives, and B.F.M.I.R.A. is currently concerned with proposed standards for products including sausages and meat pies. The preparation of a Code of Practice for canned fruit and vegetables has been part of recent work done by F.V.P.R.A.

(b) *Co-ordination and collaboration*

In addition to collaboration of R.A.s with industry, much interchange occurs between individual research workers in other organizations. This assists the work of the various independent and autonomous bodies, complements their efforts and reduces unjustifiable overlap. B.F.M.I.R.A., which covers the broadest front of all, has various degrees of contact, consultation and co-operation with fifteen out of the sixteen other laboratories listed in Table 6.

Food R.A.s, being primarily financed voluntarily, can only operate effectively if they perform a unique service that industry cannot obtain elsewhere more cheaply or for nothing. Their viability therefore depends on proper relationships with other food research organizations. Some overlap is perhaps unavoidable but this should be kept to a minimum in commodities where some work is done at public expense and some by R.A.s.

R.A.s must assume the primary responsibility for bridging the gap between the food industry and research on basic properties and composition of the raw materials carried out at G.R.I.s and university food science departments. The A.R.C. has proposed that joint R.A. liaison officers might be established at the Council's two new food institutes but it is unlikely that R.A.s will be able to afford this luxury.

The Directors of the six food R.A.s meet together to discuss matters of mutual interest and so now do the six Chairmen. Joint meetings of Chairmen and Directors take place when required.

The Consultative Committee of Directors of Food Research Organizations established by D.S.I.R. to provide a common forum for Directors of Government-aided food research laboratories, has continued to meet since A.R.C. assumed responsibility for most of the Government food research. This committee does not provide for discussion of policy.

Each organization, whatever its structure, nevertheless has its own job to do, however conceived and controlled, and is therefore flexible only within certain limits.

(c) *Financial considerations*

In 1964 a total of more than £2M, including capital, was spent on centralized food research by Government and industry. This is at least of the same order as the comparable research (as differentiated from development) being carried out by industry on its own (see section III above).

The total national expenditure on food research and development, both public and private, is therefore something like £10M. Expenditure by the A.R.C. on purely agricultural research is also now about £10M a year, with industry doing considerably less. The National Agricultural Advisory Service to farmers also employs several hunred scientists and costs several million extra. Government marine biological research costs over £1M a year of tax payers' money.

The six food R.A.s, which spent just over £0·5M, therefore accounted for about one-quarter of the total amount spent on state-aided food research. This fraction has been diminishing and will continue to do so largely because of increased capital expenditure.

Regular annual subscriptions to their R.A.s by the food manufacturers themselves were about £225,000. This is only about 10% of the total cost of state-aided food research, and less than the annual cost of any of the major G.R.I.s.

A Government grant of £1 for £1 would greatly assist the food R.A.s but the official policy is 'progressively stiffening grant terms to established associations' (Department of Scientific and Industrial Research, 1962), apparently to encourage manufacturers to subscribe more. The result is that although industrial income is slowly increasing, the cost of maintaining even existing levels of research is rising faster. R.A.s are therefore not fully effective and industrialists are consequently unwilling to increase the R.A. subscriptions (Federation of British Industries, 1963). The Federation, now Confederation, of British Industries (1965), appears to consider that if a Research Association is justifying its existence to its industry, Government help should gradually be withdrawn. Some co-operative research organizations, for example the Brewing Industry Research Foundation, already thrive without a Government subsidy. It has also been asked

(Anon., 1965) whether it is a proper function of a Research Association to attempt to sustain small and inefficient producers.

Although many food R.A. members admit that productivity or exports have been aided, research is not so obviously essential to them as, for example, advertising, and they are therefore reluctant to pay more for it. About £3M is spent annually on advertising breakfast cereals, £3M on canned soups, and over £4M on the various pet-foods (*The Financial Times*, 1963, 1964, 1965).

A further factor militating against increased voluntary industrial support is the Meat Industry (Scientific Research Levy) Order, 1963, for financing the Meat Research Institute, whereby levies of £75,000 a year are paid by the meat products industry which has for 40 years supported B.F.M.I.R.A., currently with £15,000 a year. Although industry has little or no say in Government food research, it would not want to pay an R.A. to do work that Government laboratories might be doing anyhow. Furthermore there is an Advisory Committee for the Meat Research Institute on which leading representatives of the industry and others serve in a personal capacity.

The expansion, rebuilding and re-equipping of the G.R.I.s at Aberdeen (T.R.S.), Bristol (M.R.I.), Norwich (F.R.I.) and Slough (P.I.L.) alone must be costing around £4M. No food R.A. has reserves sufficient to finance building expansion; raising funds from members for building is as difficult as it is for anything else, so that B.I.B.R.A. (and P.A.T.R.A.) have had to borrow heavily.

There is considerable disparity in the public and co-operative research expenditure on various commodities (Table 7). The greatest proportionate expenditure supported by an industry is about 0·4% on gelatine and glue research in which food uses are only a minority interest.

Research on milk products (0·5%) and fish (0·25%) both financed by Government are followed by fruit and vegetable canning and quick freezing (0·1%) and flour milling (0·1%), both at R.A.s. Research on baking is about 0·06% of the net output of the industry, whilst expenditure by B.F.M.I.R.A. on work on meat products, chocolate and sugar confectionery, jam and pickles, edible oils and fats and miscellaneous food products, amounts to about 0·03–0·04%. The biological testing at B.I.B.R.A. cannot be attached to particular commodities, and draws much of its support from outside the field of food manufacture.

R.A.s are faced with acute financial problems, which are preventing them from helping the British food industry to the full. Table 7 shows that in 1964 the expenditure per graduate of the fourteen comparable food research organizations (Society of Chemical Industry, 1965) ranges from £3400 to £7300, with an average of £5000. The average for G.R.I.s is £5500 compared with £4200 for the R.A.s Furthermore the figures for G.R.I.s do not include headquarters costs.

All these figures however, are low if compared with the average expenditure in 1961–62 in the food industry of £7200 per qualified research and development scientist and

engineer (Advisory Council on Scientific Policy, 1962). Even this figure was below that for manufacturing industry as a whole, which, excluding the aircraft industry, spent £8500 per head.

This is a reflection of relative salary scales, career prospects, ratio of technical assistants to graduates, equipment, laboratory space and facilities generally, which determine the all-crucial vitality of a research organization (Mitchell, 1965). R.A.s are finding it difficult to compete with conditions in Government food science.

Costly scientific equipment is essential today for a laboratory which is not to 'go out of business academically and industrially' (Melville, 1964). Food research, as in other fields, requires access to a radio-isotope laboratory, electron microscope, mass spectrograph, nuclear magnetic resonance and electron spin resonance and automatic analysis generally. Yet expenditure on scientific equipment in the R.A.s has been only 2–3% of the budget; Government food research organizations of comparable size spend three times as much (see Table 8). Published figures show that for the food industry the figure is well over 10% (Advisory Council for Scientific Policy, 1962). In accommodation and facilities the R.A.s are also working under considerable difficulties.

The optimum size of the co-operative research effort in a particular section of an industry cannot be decided in abstract—it depends on how much more or less effective an organization would prove to be with more or less resources at its disposal. It could always be argued that the present situation represents the resultant rough evaluation by industry of the financial support that is justified.

It might equally be asked how the size of the Government's food research effort is decided. The Rt Hon. Aubrey Jones suggested that there 'is scope for greater Government action [in research] than there now is' provided that one can 'define more precisely what it is that Government in the civil field should undertake'. At the same time Sir Harold Roxbee Cox, now Lord Kings Norton, Chairman of the Metal Box Co. which has strong links with the food industry, speaking as Chairman of D.S.I.R.'s Research Council, referred to the need for rationalization of where Government responsibilities lie in research generally, and concluded that 'there is work going on in Government Stations which might be more appropriate to R.A.s—work in R.A.s which might be more appropriate to Government Stations'. There seems food for thought here, not least in the field of food research.

Industry believes that amalgamations of R.A.s would result in more economic working (Federation of British Industries, 1963; Confederation of British Industries, 1965). Table 7 shows that the smaller food R.A.s serving self-contained sections of the industry receive proportionately greater support, and it may be feared by their industrial supporters that minority interests might become submerged in a larger organization. Furthermore, common facilities and administration would probably require constituent groups to be close together, and involve formidable difficulties and costs in rebuilding. The amalgamation in 1946 which led to B.F.M.I.R.A. was of two organizations already

sharing the same Director and building. A recent Working Party considered that although the amalgamation of R.A.B.F.M. and B.B.I.R.A., both concerned in the use of flour, was practicable, it would neither result in appreciable economies in operation nor in itself solve the problem of rising running costs of $7\frac{1}{2}\%$ a year required merely to maintain the present level of activity of a research organization.

Amalgamations, possible new members including foreign members or more sponsored work (as advocated by D.S.I.R.), are not therefore likely to solve the financial problems of the R.A.s. Increased subscriptions nearly always lead to resignations of members, and a reduction rather than an increase of effort is more liable to result.

The latest report of the Confederation of British Industries (1965) on R.A.s nevertheless infers that 'rationalization and amalgamation' should be considered not only for R.A.s but also for Government laboratories which could adopt elements from the structure of R.A.s with their close relations with industry. Amalgamation on this basis presents greater attractions, but it would require a policy for food research which hitherto is not apparent.

Perhaps the division of function between Government Research Institutes, financed almost entirely from taxes, and the R.A.s also requires reconsideration. The R.A.s were founded and financed by the manufacturing sectors of industry. Research and advisory services for farmers have always been provided free, presumably in order to get them to accept them at all. Similarly, distribution problems of unprocessed foods and dairy products have also been dealt with by G.R.I.s.

This was perhaps justified because food manufacturers were aware of the need for scientific understanding of their processes and were therefore prepared to raise funds for research with the bait of a Government grant at a time when production and marketing interests were too traditional to have any such appreciation. It was therefore justifiable for the Government to intervene and initiate research in a backward industry. In 1966 this distinction may no longer be justifiable.

The prime example is Torry Research Station, founded in 1929 to study the technical problems of a backward industry. The fish handling and processing industry, however, which is now highly concentrated, could well pay for its own quota of research either directly or through its statutory bodies which already levy on it. The same consideration applies to the dairy industry.

In this inequitable situation sections paying little or nothing for their research are getting much more done for them than sections that have for long voluntarily subscribed to co-operative research at R.A.s. Less than £100,000 is spent by B.F.M.I.R.A. on research on chocolate and sugar confectionery (sales of £300M annually), sausages (£90M) and meat pies (£40M). Torry spends nearly £300,000 a year on fish worth only about £50M at landing and even after all processing and distribution probably does not exceed

£150M. In the first instance the relevant sections of industry pay about £60,000 and Government £30,000, whilst the Government pay practically the entire £300,000 for fish, the industry making only a token contribution through the statutory bodies.

Most R.A.s have benefited from the A.R.C. special research grants and the Ministry of Technology's 'ear-marked' grants for special new projects. These are, however, no substitute for the regular income required to maintain the normal programme and make forward planning possible. Directors of Research of food R.A.s, who are struggling continually with financial difficulties, would welcome any improved financial arrangement which would make some planning possible.

Any section of industry can apply under the 1947 Act for permission to finance research by statutory levy on all manufacturers in that section. The Ministry of Technology, and previously D.S.I.R., encourages R.A.s to do this in order to place the finance of research on a more stable basis. The Government can also initiate such a levy. The costs of co-operative research would then be shared by all manufacturers who could, of course, demand and expect the services they require. Most R.A.s at present necessarily issue much of their work to members only, in confidential reports. With better finances, R.A.s could publish results for all to use when this was in the public interest. All food research would then be on the same basis as part of the national scientific information service.

Even when members of an R.A. are agreed on a levy however the M.A.F.F. will only sanction it with the Board of Trade if they are satisfied that there is sufficient support from the industry as a whole. A recent request by B.B.I.R.A. for a statutory levy, therefore, although it was supported by trade federations was rejected.

The Meat Research Levy, of course, makes it very difficult to raise another research levy from the same firms. A meat pie manufacturer who already pays the levy on the meat, and may in future have to pay a levy on the flour used in the pastry, is unlikely to support a further levy for B.F.M.I.R.A., which is doing research on meat pies. One comprehensive levy to cover all food research rather than a number of separate sectional levies might be preferable.

The mechanics of levying on an industry so diverse as the food industry presents complications. In the case of meat it is relatively easy to deduct a small sum from the subsidy paid to the farmer on every beast. Sacks of flour and even numbers of cans present little difficulty. A common basis for chocolate or pickles introduces problems, although the ingenuity of the revenue departments could surely solve them.

The Industrial Training Act provides a precedent for a levy on an industry for a common purpose, although the principle of a 'draw-back' to offset a firm's own effort would not seem as appropriate in the case of research. 'A considerable extension of the compulsory levy for research associations' has been advocated by P.E.P. (1963). Whatever is done financially for R.A.s must not lessen contacts, with obligations to and confidence of their industry.

(d) *Government responsibility for food research*

The Government-aided food R.A.s opted to stay with D.S.I.R. in 1959 when the A.R.C. took over general responsibility for much food research. D.S.I.R. is now absorbed into the Ministry of Technology and the A.R.C., once, like D.S.I.R., responsible to the Minister for Science, is now part of the Department of Education and Science.

In consequence an integrated policy for Government food science, of which the R.A.s are a part, is difficult. The Food Research Advisory Committee, which includes leading food industrialists, advises the Ministry of Agriculture, Fisheries and Food on research matters (other than fish) and recommendations are then transmitted to the Agricultural Research Council.

IX. Conclusions

(1) British food science and technology has much to its credit, but further efforts and expenditure are called for both from food industry and Government if the maximum contribution is to be made to the solution of the country's economic problems, by providing the nation with the cheapest and best food and making the best use of home resources.

(2) Although the food technological research conducted at Government expense is rightly expanding, there is need likewise for planned growth in the level of activity of food R.A.s.

(3) Detailed examination, recommended by the Trend Committee, of the 'not entirely satisfactory' arrangements for food processing research (other than fish) should pay special attention to the proper financing of food R.A.s so that their scientific and technological effectiveness is raised to a maximum. The statutory food Boards and Authorities should ensure by appropriate financial arrangement that the industries they administer are provided with the requisite food science and technology research.

(4) Measures of rationalization should be proposed that will integrate food research without lessening the value of the R.A.s to the industries at present supporting them.

(5) Industries should be induced to value and make use of the food research work which Government has hitherto carried out substantially without charge.

(6) Government food Research Institutes would benefit from the stimulus of greater organizational contact with the food industry.

(7) Policy must be implemented so as to instil into food research organizations the 'sense of urgency' called for by the Federation of British Industry (1963) and yet provide them with the resources and independence to enable them to tackle problems not recognized by industry.

(8) The combination of the best features of all systems seems to be: (i) Applied research programmes and advisory (or 'extension') activities to be geared to the contributions from industry and submitted to industrial representatives through Advisory Councils and technical committees to establish priorities and practical orientation and to ensure publicity for application of results; (ii) Government contributions sufficient to encourage more basic, long-term or speculative investigations than industry can sponsor; (iii) in both cases, funds necessary to meet accepted requirements of programmes to be guaranteed within normal budgetary framework; (iv) industrial contributions to be provided by statutory bodies or trade associations, so as to represent all interests likely to benefit, and to be supplemented by £1 for £1 from the Government; and (v) R.A. type machinery for two-way consultation between industry and research workers to be extended so as to give recognition and fulfilment of short-, medium- and long-term needs of the food industry.

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The spoilage flora of eviscerated chickens stored at different temperatures

ELLA M. BARNES AND MARGARET J. THORNLEY

Summary. The methods used for isolating and characterizing the Gram-negative spoilage bacteria of chickens are described and discussed.

When a comparison was made of the spoilage flora of chickens stored at 1, 10 and 15°C it was confirmed that pigmented and non-pigmented strains of *Pseudomonas* predominated at 1°C together with the chromogenic organism hitherto described as *Pseudomonas putrefaciens*. On the other hand, the main spoilage organisms at 15°C were found to be strains of *Acinetobacter* and *Enterobacteriaceae*, the latter being difficult to relate to known species.

Introduction

When eviscerated chickens are stored at chill temperatures, it has been shown that the predominant spoilage organisms are strains of pigmented and non-pigmented *Pseudomonas* (Ayres, Ogilvy & Stewart, 1950; Barnes & Shrimpton, 1958; Ayres, 1960; Nagel *et al.*, 1960). Also present, but generally in much smaller numbers, are Gram-negative non-motile cocco-bacilli which have been classified in a variety of ways. Thornley, Ingram & Barnes (1960) described them as *Achromobacter*, Ayres *et al.* (1950) stated that they resembled *Alcaligenes viscosus*, whilst Nagel *et al.* (1960) assigned them to the *Achromobacter/Alcaligenes* group without attempting to place them specifically in either genus. More recently, Thornley (1966) has shown that these strains are distinct in many properties from the motile, peritrichous species *Achromobacter* or *Alcaligenes*, and considers that they should be classified as *Acinetobacter* (Brisou & Prévot, 1954). As a result of these and other recent investigations concerning the Gram-negative spoilage flora of chilled poultry, meat and fish (Shewan, Hobbs & Hodgkiss, 1960) improved methods have been developed for isolating and differentiating these organisms. These methods are discussed below and have been used to compare the spoilage flora of chickens stored at 10 and 15°C with those stored at 1°C, as little is known of the spoilage flora of chickens held without refrigeration.

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Materials and methods

(a) *Origin of samples*

All the chickens were taken from a large factory eviscerating, chilling and freezing about 20,000 birds per day. They were taken consecutively from the processing line after they had passed through a mechanical chiller and were then loosely packed in polythene bags which were opened during storage for odour assessment. In all, thirty chickens were used, of which twenty-one were examined bacteriologically.

(b) *Isolation and identification of the spoilage flora*

Bacteriological examination of the chickens. The sampling techniques used for eviscerated chickens and for equipment in the processing plant generally have been described by Barnes & Shrimpton (1958). Dilutions of the sample are prepared with a solution containing 0·1% peptone and 0·5% sodium chloride (Straka & Stokes, 1957). Portions of 0·025 ml of each dilution are then spread over one-quarter of a Petri dish containing Difco heart infusion agar which has previously been dried for $1\frac{1}{4}$ hr at 50°C. For a total viable count, plates are incubated for 3–4 days at 20°C, or for counting only psychrophiles 14 days at 1°C is used.

It is important to use the dilution medium of Straka & Stokes (1957), since some of the organisms decrease in numbers in Ringers solution. For psychrophilic organisms it is necessary to use a surface counting method rather than pour plates, since some of these are destroyed at temperatures below that of melted agar.

Other media have been compared with heart infusion agar for counting the spoilage organisms at 1 and 20°C and of these, Oxoid tryptone soya agar and blood agar gave an equal count. Hartley's digest agar has been found to give variable results but was improved by the addition of 0·3% yeast extract. When Paton's agar (Paton, 1959) was tested, pigmented and non-pigmented strains of *Pseudomonas* grew, but not *Acinetobacter*. Using the medium of King, Ward & Raney (1954) for the detection of maximum fluorescin production by pseudomonads all the organisms grew, but only a proportion of colonies subsequently identified as pigmented pseudomonads showed fluorescence on the plates initially.

Isolation and maintenance of cultures. Inocula from colonies from the highest dilution showing growth on the count plates are transferred to Difco heart infusion broth; those from 20°C counts are incubated at 20°C for 2 days, while those from 1°C counts are incubated at 1°C until turbid. The culture is then streaked on heart infusion agar to obtain single colonies and the plates incubated at 20°C for 2–3 days. Typical colonies are picked into heart infusion broth and incubated overnight at 20°C. This broth culture is then used to make the stock culture, and for a number of tests described below.

Considerable difficulty has been encountered in keeping some of these organisms alive on agar slopes stored at 1 or 5°C. This is particularly the case with motile peritrichous strains (possibly *Achromobacter* or *Alcaligenes*) and even some of the pseudomonads have died after a few months storage on heart infusion agar. A medium more favourable to survival has been the culture collection medium of Vera G. Collins (personal communication), containing in 1 litre distilled water: Laboratory Lemco 3 g, Proteose-peptone (Difco) 5 g, Bacto-tryptone (Difco) 1 g, Agar (Difco) 15 g; pH 7·0–7·2.

Preliminary identification tests.

1. The 24-hr broth culture is examined in a wet film (no coverslip) using a low power phase contrast objective, for general appearance and motility. The film is then dried and Gram stained.

2. A loopful of the broth culture is streaked across a plate of King, Ward & Raney's agar and two antibiotic tablets, containing 10 μg oxytetracycline and 2·5 i.u. penicillin, are placed on each streak, 4 cm apart. Six strains can be tested per plate. After overnight incubation at 20°C, sensitivity to the antibiotics is recorded, and the plates are examined under ultraviolet light for fluorescence. Pigment production is also noted, and part of the culture is removed for the oxidase test of Kovacs (1956). A few *Acinetobacter* strains grow poorly on this medium, and in these cases the antibiotic test is repeated using heart infusion agar.

3. The broth culture is inoculated (by stabbing) into two 1 oz bottles of Hugh & Leifson's medium containing glucose (Hugh & Leifson, 1953) and also into the arginine medium of Thornley (1960). One bottle of glucose medium and the arginine medium are sealed with vaseline. Changes in pH in these media are noted during incubation at 20°C for 14 days. Oxidative or fermentative reactions are determined from the behaviour in the glucose media, and the producton of alkaline conditions in the arginine medium is noted.

With the above combination of tests, it is possible to differentiate the strains of *Pseudomonas* and *Acinetobacter* from other Gram-negative organisms, in particular the *Enterobacteriaceae* and *Aeromonas* (see Table 1). Flavobacteria are usually separated at the outset from these genera by the yellow colour of their colonies on the heart infusion agar plates. A further group of spoilage organisms producing brownish colonies were found to be identical with *Pseudomonas putrefaciens* as described by Long & Hammer (1941). This organism has lateral as well as polar flagella so should not be included in the genus *Pseudomonas* (Thornley & Barnes, 1964). Its reactions in the screening tests are shown in Table 1.

Additional plating tests are valuable for these and other groups and some can be carried out using replica plating.

TABLE 1. Differentiation of *Pseudomonas*, *Acinetobacter* and other Gram-negative bacteria

Test	*Pseudomonas*		*Pseudomonas putrefaciens*	*Acinetobacter*	*Enterobacteriaceae*	*Aeromonas*
	Group 1*	Group 2*				
Motility	+		+	−	+ or −	+
Flagella	Polar		Polar and lateral	None	Peritrichous	Polar
Fluorescence (King *et al.*, 1954)	+	−	−	−	−	−
Penicillin (Shewan, Hodgkiss & Liston, 1954)	Resistant		Resistant	Sensitive (10% resistant)	Resistant	Resistant
Oxidase (Kovacs, 1954)	+		+	+ or −	−	+
Mode of attack on glucose (Hugh & Leifson, 1953)	Oxidative		Oxidative or inert	Oxidative or inert	Fermentative	Fermentative
Arginine (Thornley, 1960)	+		−	−	+ or −	+

* Groups of Shewan *et al.* (1960). 1, Pigmented; 2, Non-pigmented.

Replica plating tests. These are based on the technique of Lederberg & Lederberg (1952), by which an inoculum from the colonies on one agar plate (the master plate) is transferred by means of a sterile velvet pad to identical positions on a series of replica plates which are then incubated to allow growth. This method can be applied to any plate test, and for these organisms is used in the following way.

The master plate of heart infusion agar is inoculated at twelve points in a regular pattern with different cultures by dipping a straight wire into a 24-hr broth culture and stabbing into the plate. This is incubated overnight at 20°C and then replicated on to plates of the following media, with the incubation temperature and time shown.

(1) Oxoid MacConkey 3 (37°C for 24 hr). Red colonies indicate lactose-positive *Enterobacteriaceae*.

(2) Nutrient agar + 30% skim milk (20°C for 4 days). This gives good pigment production with *Flavobacteria* (Shewan *et al.*, 1960) and shows clear zones for casein-digesting organisms.

(3) Heart infusion agar (1°C for 14 days). Growth indicates psychrophilic strains.

(4) Heart infusion agar (20°C for 4 days, incubated anaerobically in a McIntosh and Fildes jar). This eliminates the obligate aerobes.

The medium of King *et al.* (1954) for maximum fluorescin production has also been used by replica plating in some experiments, and the antibiotic tests can also be done in this way.

Results

To determine changes in the flora during storage six chickens were examined initially and the rest stored at either 1, 10 or 15°C until off odours were detected. At this stage six more chickens were examined from both the 10 and 15°C storage. Only three chickens from 1°C storage were examined as detailed analyses had been made previously on chickens stored at this temperature by Barnes & Shrimpton (1958). The total viable counts (Table 2) were found to be about 10^5 bacteria/cm^2 for the initial samples, and between 10^7 and 10^8/cm^2 after spoilage.

TABLE 2. Changes in the kinds of spoilage organisms on chickens stored at 1, 10 and 15°C

Treatment	Total count at 20°C (bacteria/cm^2)	% Distribution of groups													
		Total No. of strains	Micrococci	Gram-positive rods (catalase positive)	Gram-positive rods (catalase negative)	Streptococci	Flavobacteria	*Pseudomonas putrefaciens*	*Entero-bacteriaceae* L+ ‡	*Entero-bacteriaceae* L− ‡	*Aeromonas*	*Pseudomonas* (pigmented)	*Pseudomonas* (non-pigmented)	*Acinetobacter*	Unidentified
Initial	$9{\cdot}5 \times 10^4$* (six birds)	58	50	14	—	—	14	—	8	0	—	2	—	7	5
After storage at 1°C (10–11 days)†	$6{\cdot}0 \times 10^7$* (three birds)	40	—	—	—	—	—	19	—	3	—	51	20	7	—
After storage at 10°C (3–4 days)†	$5{\cdot}7 \times 10^7$* (six birds)	80	4	—	4	6	—	4	3	12	4	21	12	26	4
After storage at 15°C	$6{\cdot}2 \times 10^7$*	69	—	4	2	8	—	4	10	17	6	9	2	34	4

* Geometric mean.

† Approximate time for the development of 'off odour'.

‡ L+, Lactose fermented; L−, Lactose not fermented.

The percentage of the different types of bacteria occurring initially and after each treatment was determined from the study of 247 strains. A very heterogeneous collection of organisms was present initially with micrococci forming half of the total population and catalase positive Gram-positive rods (probably corynebacteria) and flavobacteria forming the second largest groups. The pseudomonads and acinetobacters together comprised less than 10% of the population.

After storage at 1°C, 71% of the population consisted of pigmented and non-pigmented strains of *Pseudomonas*. The next major group (19%) were chromogenic Gram-negative bacteria identical with *Pseudomonas putrefaciens* (see above).

After storage at 10°C, the pseudomonads were still the most numerous group although only present as 33% of the total, with *Acinetobacter* as the second largest group (26%) and *Pseudomonas putrefaciens* present as a small proportion. The *Enterobacteriaceae* formed 15% of the flora.

The 15°C storage flora was again different, as the largest group present was *Acinetobacter* (34%) followed by strains of *Enterobacteriaceae* (27%) with *Pseudomonas* strains of less importance (11%).

The strains of *Enterobacteriaceae* isolated after spoilage at all three temperatures were nearly all able to grow at 1°C. They were mainly lactose negative, and of the lactose positives, only a few strains could be identified as *Escherichia*. All the others were difficult to place in any group, although a number of strains showed most resemblance to a lactose-negative *Aerobacter*. Similar strains were described by Eddy & Kitchell (1959).

Discussion

In these experiments the chickens stored at 1°C spoiled with pigmented and non-pigmented strains of *Pseudomonas*, thus confirming earlier results (Ayres *et al.*, 1950; Barnes & Shrimpton, 1958). *Pseudomonas* strains formed a much smaller proportion of the spoilage flora at 10°C whilst at 15°C they were only 11%. Although at 15°C the bacteria were still predominantly Gram-negative, the majority of these were either *Acinetobacter* or *Enterobacteriaceae*. This change with the temperature of storage is at variance with the results of Ayres *et al.* (1950).

It follows from these differences that it is inadvisable to relate the results of 1°C storage experiments to those of 15°C storage. For example, some of the oxygen impermeable films used for packaging poultry which have been shown to extend the storage life at 1°C by retarding the growth of pseudomonads (unpublished data) may not be so effective when a high proportion of the spoilage flora is facultatively anaerobic, as was found at 15°C.

Little is known of these atypical strains of *Enterobacteriaceae* as food spoilage organisms but they have previously been isolated as the predominant organism on slimy pork stored at 15°C (Eddy & Kitchell, 1959; Kitchell & Ingram, 1959).

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Brown discoloration in pre-rigor cut fish fillets

T. R. KELLY AND W. T. LITTLE

Summary. The surface browning in pre-rigor processed cod fillets has been shown to be caused by the formation of a denatured form of methaemoglobin produced from fish blood contaminating the surface. Browning is less likely to occur in fillets cut in rigor or later because a pre-rigor cut surface exudes a coagulum in which blood is trapped and from which it cannot be easily removed by washing. The observation that Ocean Perch is more liable to surface discoloration has been explained in terms of differences in blood distribution in the muscle tissue. Cod haemoglobin differs markedly from mammalian haemoglobin in that oxyhaemoglobin changes to deoxyhaemoglobin as the pH is reduced below 8. Mammalian haemoglobin is stable at all pH values between 10 and 4.

It is suggested that surface browning in fish fillets can be prevented by using correct bleeding procedures before filleting, or by filleting in rigor or later.

Introduction

The development of the modern processing trawler has brought in its wake quality problems not previously encountered with traditional iced fish. One of these is the surface discoloration frequently found in sea-frozen fillets. In the last few years we have seen many examples of pre-rigor processed fillets which showed marked brown surface discoloration after thawing from the frozen state or after cooking from the frozen state. Some species are more liable to this than others, for example Ocean Perch (*Sebastes marinus*) fillets are much more frequently discoloured than cod (*Gadus morhua*) fillets.

The discoloration of fish during processing or storage has been reported. Tarr (1950) explained the brown discoloration which occurred on heating white fleshed fish to 220°C in terms of the Maillard reaction. Such a reaction is common in vegetables that contain considerable quantities of carbohydrate. Venolia, Tappel & Stansby (1957) doubted whether a Maillard reaction would cause extensive discoloration in fish muscle, which contains only very small quantities of carbohydrate. These workers traced the

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development of 'rust' browning on the surface of frozen fish to an oxidative deterioration of the flesh and oil of the fish.

Brown & Tappel (1958) related the brown discoloration in fresh and frozen fish to the conversion of oxymyoglobin to brown metmyoglobin. Oxymyoglobin occurs in the brown muscle of fish, which in cod is on the skin side of the fillet. This would not explain discoloration on the cut surface.

In meat chemistry it is well established that both haemoglobin and myoglobin may lose an electron, becoming oxidized to the corresponding brown 'met' pigments. This transformation occurs through a two-step process beginning with the deoxygenation of oxymyoglobin to yield myoglobin. It should be emphasized that this change is readily reversible. The myoglobin formed, with the haem iron in the ferrous state, is very readily oxidized to the ferric compound metmyoglobin, which is responsible for the undesirable 'brown' colours.

Jones (1965) has stressed the importance of correct bleeding procedures in preventing discoloration. Jones states that blood expressed from a pre-rigor cut fillet is trapped in a 'coagulum' and is very resistant to washing. He also observed that surface blood is very much less apparent in fillets cut from fish in rigor or later.

Our experience is much in agreement with these observations. We have found that surface blood in a fillet, or the contamination of a fillet surface with a blood solution, always produces a brown discoloration. With pre-rigor cut fillets, blood exuded into the surface is difficult to wash off, whereas in rigor-cut fillets it can be washed off quite easily.

This paper describes how this brown discoloration on fish surfaces is produced and puts forward an explanation for the differences between pre-rigor and in-rigor cut fillets. Differences between species in suceptibility to browning are also discussed.

Experimental material

Cod (*Gadus morhua*) samples were processed and frozen at sea under normal commercial conditions or prepared from aquarium cod. The aquarium cod were held for at least 6 weeks from capture and fed on squid.

Ocean Perch (*Sebastes marinus*) used for the iron analyses were normal commercial sea-frozen fillets. The other work on Ocean Perch was done on iced fish 7–10 days after catching.

Fish haemoglobins were prepared by separating the erythrocytes from freshly-drawn citrated blood, washing them with saline solution and then haemolysing with cold distilled water. The cell debris was removed by centrifuging at 0°C to yield a clear solution of unpurified fish haemoglobin. These stock solutions were used for oxygen equilibrium and kinetic studies without further purification.

Experimental methods

Oxygen equilibrium measurement

Portions of the stock solutions were diluted to 50 ml with phosphate buffers (K_2HPO_4 and KH_2PO_4) to give a range of solutions between pH 6 and 8. The final concentration was 0·05 M with respect to the phosphate ion.

The absorption spectra of these solutions in the wavelength range 450–650 mμ were measured on a recording spectrometer at room temperature (18°C) within 1 min of preparing the solution.

The fraction of total haemoglobin in the oxyhaemoglobin form was calculated from extinction measurements at 573 mμ using the expression:

$$\% \ HbO_2 = \frac{E_{obs} - E_{Hb}}{E_{HbO_2} - E_{Hb}} \times 100$$

where E_{obs} is the observed extinction at 573 mμ,

E_{HbO_2} is the extinction at 573 mμ of solutions with pH 8,

E_{Hb} is the extinction at 573 mμ after complete reduction produced by the addition of a few grains of sodium dithionite.

Autoxidation kinetics

Haemoglobin solutions of equal concentration in 0·05 M phosphate buffers were stored in tubes at +5°C. From time to time samples were withdrawn and diluted in a concentrated solution of dipotassium mono hydrogen phosphate. This raised the pH to about 8, thus converting any deoxhyaemoglobin to oxyhaemoglobin and effectively stopping the reaction. The absorption spectrum was read immediately and the fraction of pigment remaining in the ferrous form was then estimated from the expression:

$$X_{Fe^{2+}} = \frac{E_{obs} - E_{Hb^+}}{E_{HbO_2} - E_{Hb^+}}$$

where $X_{Fe^{2+}}$ is the fraction of unoxidized pigment,

E_{obs} is the extinction of the solution at 573 mμ,

E_{Hb^+} is the extinction of the solution after complete oxidation by the addition of a minute quantity of potassium ferricyanide,

E_{HbO_2} is the extinction of the solution at zero time.

Plots of log $X_{Fe^{2+}}$ against time were drawn and the first order rate constants for the reaction were calculated from the slopes of the lines.

Kinetic measurements were repeated at 15°C.

Determination of iron content

Iron in fish muscle was determined by the method described by Birno & Hansen (1962).

Qualitative test for haemoglobin

A qualitative test for the presence of haem pigments on fish fillets was devised by adapting the method of Kohn & O'Kelley (1955). Haem pigments on the surface of the fillet produced an immediate intense blue when the *o*-tolidine–hydrogen peroxide mixture was sprayed on.

Results and discussion

Presence of blood pigments on the cut surface

The complicity of haem in the surface browning of fillets was investigated by measuring the iron content on the surface and in the interior of discoloured Ocean Perch fillets.

TABLE 1. Iron content of Ocean Perch fillets

	Cut surface Fe (ppm)	Interior Fe (ppm)
Discoloured fillet	6·2	1·7
Normal fillet	2·5	1·8

The large difference in the iron content between the surface and interior of a brown discoloured fillet is good evidence for implicating haemoglobin as a cause of browning. However, the possibility that the iron was not present as haem iron must be considered.

The *o*-tolidine–hydrogen peroxide reagent is a specific stain for haem pigment and when this was sprayed on to discoloured fillets an immediate intense blue surface stain was produced. Normal fillets gave a weak blue stain which took some minutes to develop.

These observations, together with the iron results, prove the presence of blood pigments on the surface of discoloured fillets.

When cod fillets, free of blood, were dipped into various dilutions of whole cod blood, discoloration was obtained with solutions as dilute as 1 part of blood in 500 parts of water. Intensification of the discoloration was related to an increase in blood concentration of the solution.

We next investigated the reasons why fish haemoglobin, which in its natural state is red and soluble, should so readily produce an insoluble brown discoloration.

Sensitivity of fish haemoglobin to pH

Cod haemoglobin differs from mammalian haemoglobin in an important aspect. Whereas solutions of the latter remain bright red at all pH values between 10 and 4,

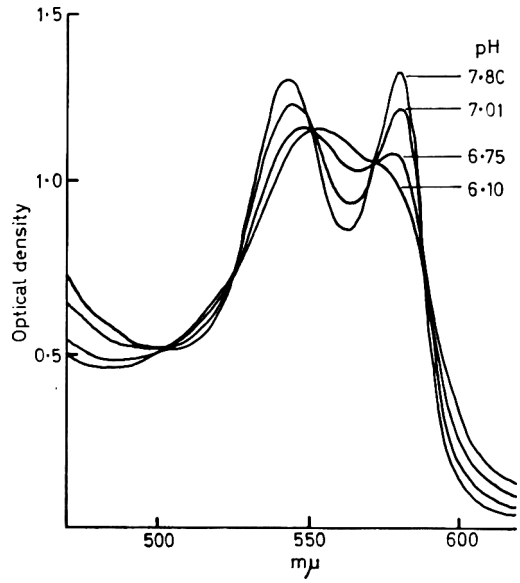

FIG. 1. Adsorption spectra of cod haemoglobin in the pH range 7·8–6·1.

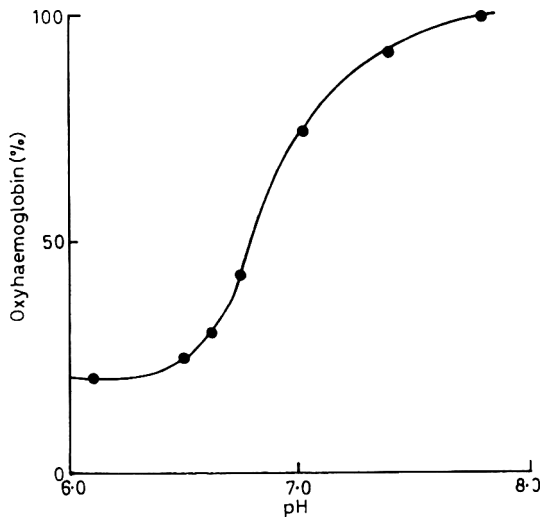

FIG. 2. Influence of pH on the oxyhaemoglobin content of cod haemoglobin.

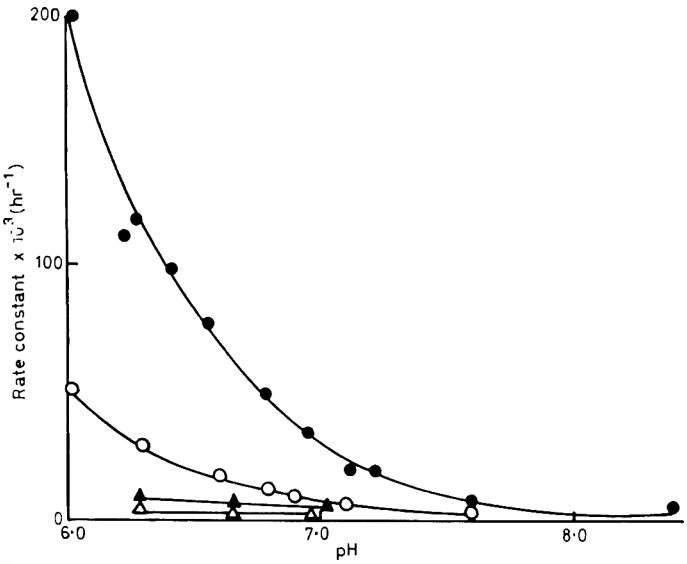

FIG. 3. Effect of pH on the rate of autoxidation of cod haemoglobin. ○, 0·05 M PO_4 at 5°C; ●, 0·05 M PO_4 at 15°C; △, mammalian at 15°C; ▲, mammalian at 25°C.

solutions of the former change from red to purple as pH is lowered from 8 to 6. Spectrophotometric examination showed that cod oxyhaemoglobin changes to deoxyhaemoglobin as the pH is reduced below 8 (Fig. 1). The way in which pH controls the composition of the blood is shown in Fig. 2. In the pH range 6·8–6·4 the oxyhaemoglobin content falls off rapidly to a constant value of 20%. As a consequence we would expect a much enhanced rate of autoxidation of fish haemoglobins in the normal range of post-mortem muscle pH.

Autoxidation of cod haemoglobin

This has been followed at +5 and +15°C in the pH range 8–6. The results are shown in Fig. 3. As predicted by the work on pH sensitivity, the rate of autoxidation is very dependent upon pH, the rate increasing as the pH decreases. A 10°C rise in temperature increased the rate of reaction by a factor of 4.

For comparative purposes, the rate constants for the oxidation of mammalian haemoglobin at 15 and 25°C are included in Fig. 3. At 15°C the rates of oxidation for cod haemoglobin are between 30 and 50 times as great as those of mammalian haemoglobin in the pH range 7–6·4, which is the normal post-mortem pH.

The pH sensitivity of cat fish, dab, plaice, whiting and haddock haemoglobins have also been investigated. The composition of solutions of these haemoglobins are shown in Fig. 4; they have a fairly wide range of pH sensitivity. The curves fall into three

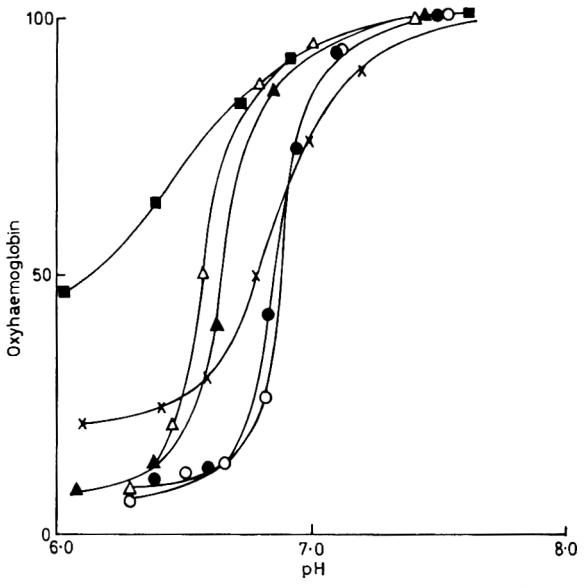

FIG. 4. pH sensitivity of fish haemoglobin. ×, Cod; △, plaice; ▲, dab; ○, whiting; ●, haddock; ■, catfish.

groups. The haemoglobins of the round fish, haddock, whiting and cod, are most sensitive to pH. Those of flat fish deoxygenate at lower pH than those of round fish. Cat fish haemoglobin is the least pH sensitive.

The production of methaemoglobin is followed by the appearance of a brown precipitate which is insoluble in water or salt solution. A dried sample of this substance had the following composition:

$$N = 14{\cdot}8\%, \; Fe = 0{\cdot}33\%$$

The iron content of haemoglobin based on a molecular weight of 68,000 is 0·329%. The precipitate therefore seems to be simply a denatured form of methaemoglobin in which the porphyrin ring remains attached to the globin.

Having determined the mechanism of blood discoloration, we then set out to explain the species variations in susceptibility to browning as well as the reasons for the more severe discoloration of pre-rigor cut fillets than rigor or later cut fillets.

Post-mortem bleeding of fish

Measurements were carried out at sea to compare the volume of blood which flowed from freshly killed cod and Ocean Perch. The average results of these measurements are given in Table 2.

Cod is much more easily bled than Ocean Perch and therefore at the time of filleting

TABLE 2. Volume of blood obtained from freshly killed cod and Ocean Perch

	ml blood / kg body wt	Total blood volume (Published) (ml/kg)	
Cod	9·0	19·0	Ronald *et al.* (1964)
Ocean Perch	3·5	—	

there will be more blood in Ocean Perch tissue than in cod tissue. This is substantiated by the observations made during the filleting of freshly caught Ocean Perch. At the time of cutting the fillets were free from blood. However, shortly after filleting blood oozed from the cut surface and contaminated it. Cod fillets did not bleed to this extent.

The distribution of blood within cod and Ocean Perch can be demonstrated by injecting a solution of Indian ink into the back vessels of post-rigor fish. Sections cut from such injected fish are shown in Plate 1(a) and (b).

The difference between the two is immediately obvious. In cod there is a system of extremely fine vessels while in Ocean Perch there is a much more clearly defined branched system of relatively large blood vessels. Ocean Perch would therefore be expected to have a greater proportion of its blood within the muscle tissue than cod, and consequently at the time of filleting more blood will be available for contaminating the fillet.

Differences between pre-rigor and post-rigor cut fillet surfaces

A freshly killed cod was bled by washing the main blood vessel with water. A solution of Indian ink was then injected into the blood system and the fish filleted while still in the pre-rigor state. One of the resulting fillets is shown in Plate 2(a). Staining at the cut surface was very pronounced. Plate 2(b) shows this on a magnified section of the cut surface. Although Indian ink, like haemoglobin, is miscible with water, the staining on the surface was not readily removed by washing. This demonstrates that pre-rigor fillets can be readily stained at the cut surface by any pigment discoloration.

Microscopic examination of sections of pre- and post-rigor cut fillet surfaces shows a considerable difference. In the pre-rigor tissue the cell contents of the cut surface cells have exuded out and appear to have fused into a continuous network from which individual cells cannot easily be isolated (Plate 2c). The post-rigor cut surface cells do not show this phenomenon. The cells are flaccid and can be easily separated right up to the cut surface (Plate 2d).

Discussion

The brown surface discoloration of fish fillets is due to the formation of a denatured form of methaemoglobin. The methaemoglobin is produced by the autoxidation of

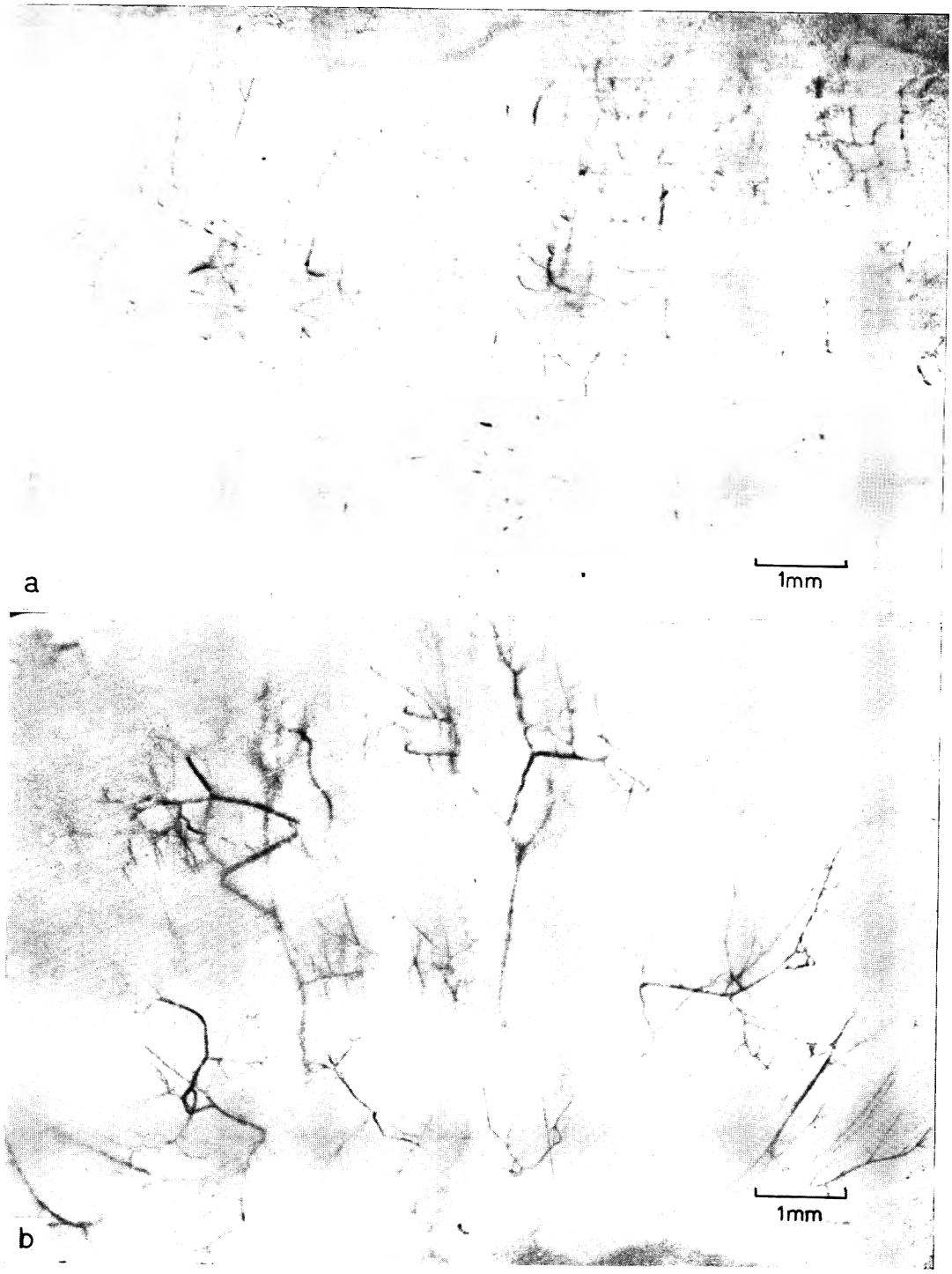

PLATE 1

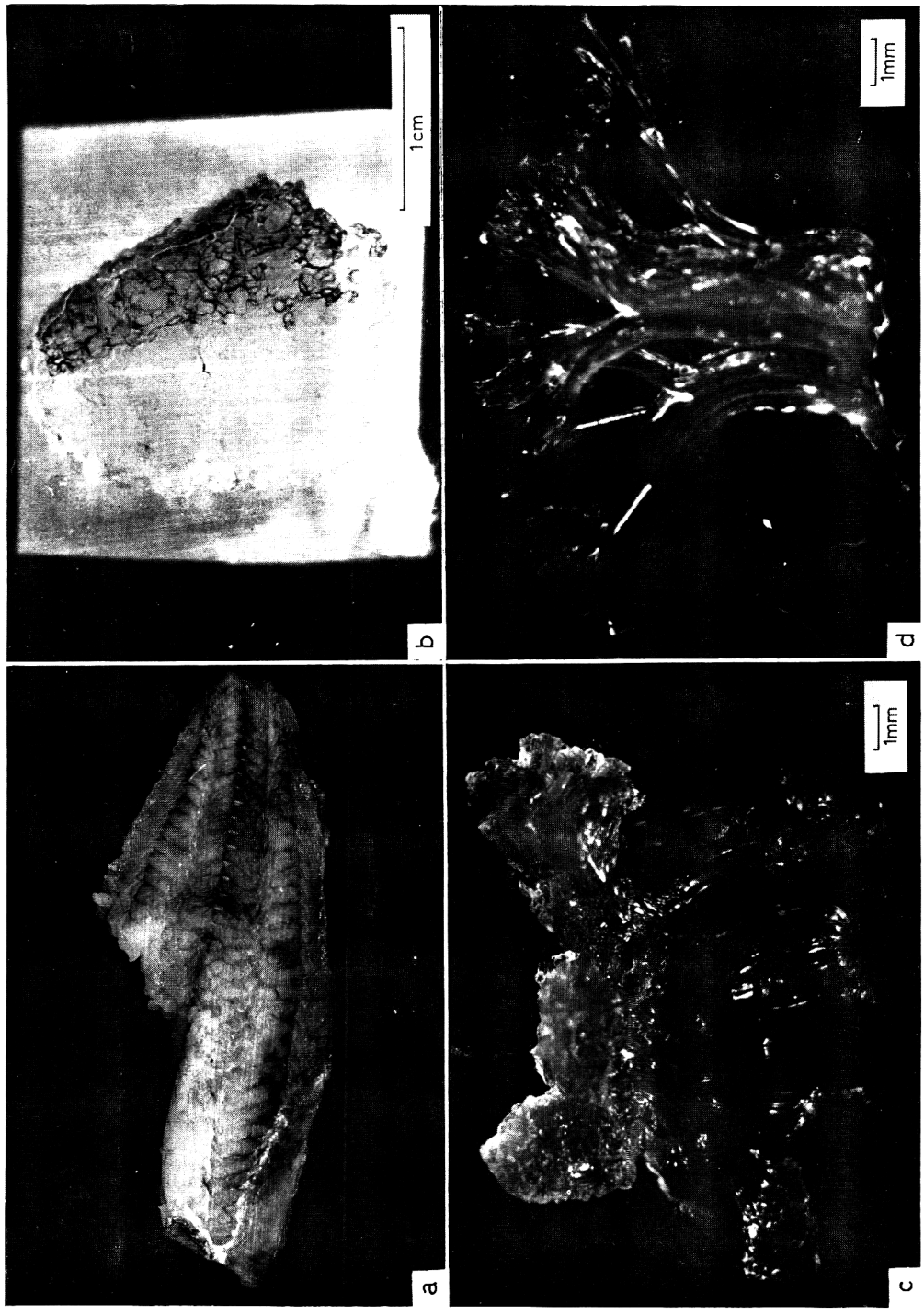

PLATE 2

oxyhaemoglobin. In fish the rate of autoxidation depends on the pH. Therefore fish muscle which falls to a low ultimate post-mortem pH will be more liable to discoloration than fish muscle with a high post-mortem pH. This sensitivity of cod haemoglobin to pH is very interesting. It means that in period of stress, when lactic acid is being secreted into the blood stream causing its pH to drop, the cod haemoglobin will become more and more deoxygenated and less capable of transporting oxygen. The end result of this could be anoxia. The catfish is much better equipped in this respect and this probably explains its greater tenacity and ability to survive the stresses of capture.

It would be relatively simple to combat discoloration if we only needed to remove surface blood. However a comparison between pre-rigor and post-rigor cut surfaces has shown that in a pre-rigor cut surface cell contents are exuded and form a coagulum in which blood may be trapped. This trapped blood is 'protected' from removal by washing and will cause discoloration. Post-rigor cut surfaces do not produce this coagulum and hence any blood on the surface may be washed off before it has had the opportunity to become oxidized.

Examination of the blood distribution in cod and Ocean Perch shows that the latter has more blood in its muscle tissue than cod. Also because Ocean Perch is spiny it is seldom gutted before filleting. These facts would explain the high evidence of browning in pre-rigor cut fillets of this species.

Surface browning in fillets can therefore be prevented by using correct bleeding procedures before filleting, or by filleting in rigor or later.

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A new method for counting bold and blind seeds in raspberry jam and for computing blindness rate constants

J. C. DAKIN AND J. TAMPION

Summary. A new technique and cell for counting bold and blind seeds in raspberry jam is described. Using this method it was found that the rate at which seeds become blind in a jam follows a logarithmic course. This is analogous to the thermal death rate for bacteria and consequently the standard mathematical concepts for dealing with this phenomenon may equally be applied to blindness development. An expression D_s, equivalent to the decimal reduction time for bacteria, is used to compare the rates of blindness development in experimental jams. This represents the time in weeks for 90% of the seeds to become blind. The new methods are illustrated by results obtained from experimental jams.

Introduction

One of the faults experienced during the storage life of raspberry jam is the slow displacement of air from the seeds and their infusion with syrup. As a result the seeds lose their opacity and tend to merge with the surrounding jam. This fault, which is termed 'blindness' by the trade, gives the jam a dull, old appearance and the illusion of a low fruit content.

Investigations into the cause and elimination of undesirable characteristics in food products are considerably facilitated if a means of accurately measuring the extent of development of the fault can be found. In past work by Colquhoun (1960) on the problem of blindness of seeds in raspberry jam two methods of assessing the extent of blindness have been described. One involves a direct observation and estimation of the seeds in a flat-sided jar and the other the counting of blind and bold seeds through a superimposed window laid against the jar side. Both these methods suffer from the defect that they depend on the judgement and experience of the observer. Furthermore, the blind seeds are not readily visible in the jam and consequently bold seeds are more likely to be counted than blind seeds—a tendency accentuated with depth—and results will normally be underestimates of the number of blind seeds present.

The new method of measuring the numbers of blind and bold seeds described in this

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report minimizes the personal judgement factor and overcomes the difficulties associated with depth.

Having obtained reliable counts on a jam over a period of time the problem exists of how to treat the figures and to express them in a manner which truly reflects the rate at which the seeds are becoming blind in the jam. This presents some difficulties and the new method reported here represents the culmination of a number of attempts to overcome these. In order to illustrate its use in practice a brief investigation into the blindness development in jams prepared from fresh and sulphited fruit is reported. The results obtained are in themselves interesting in that they provide information on the degree of uniformity of the seed population in the jam.

Part I. A new method of counting bold and blind seeds in raspberry jam

The apparatus is essentially similar to an enlarged version of a haemocytometer cell and is mainly constructed from $\frac{1}{8}$ in. sheet Perspex. The cell, consisting of a base plate and cover, upon which is etched a grid to facilitate counting, is illustrated with dimensions in Fig. 1. The jam under test is first well stirred, to ensure that the seeds are evenly distributed, and a sample of approximately 15 g is then placed in the enclosed area on the base plate. The cover is placed on top of the jam and pressed evenly downwards until it meets the cavity walls on the base plate. This causes the jam to fill

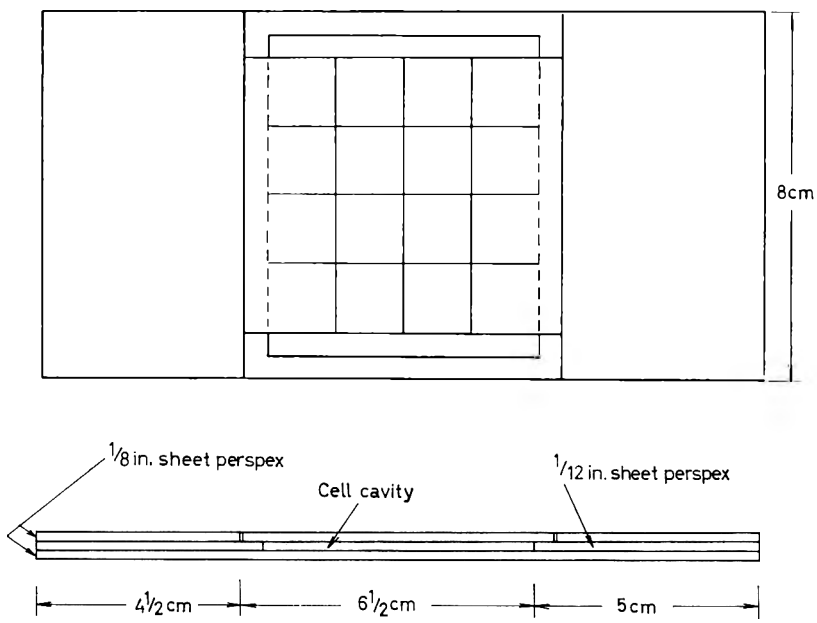

FIG. 1. Raspberry seed counting cell.

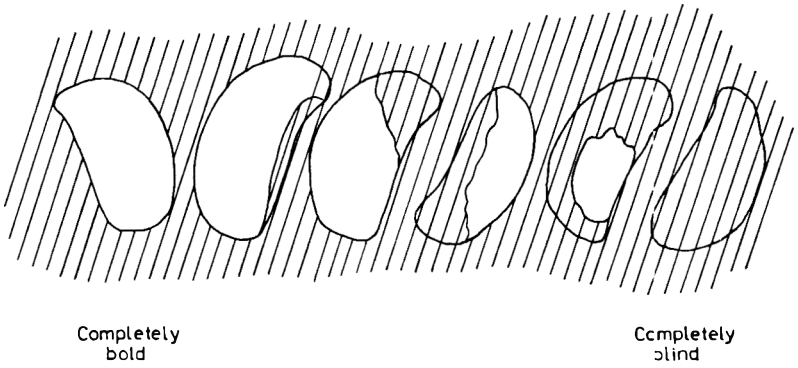

FIG. 2. Diagrammatic representation of raspberry seeds in various stages of infiltration.

the cell cavity completely, surplus exuding at either end. The cell is then placed on two supports which hold it 3 in. above a sheet of white paper. This is illuminated and with the aid of a hand tally counter of the push-button type the total number of seeds is counted, i.e. using transmitted light. This count is then repeated. The number of bold seeds is next determined using light reflected from the seeds. This is achieved by placing a black background under the counting chamber. This count is also duplicated. Two further samples of the same test jam are then similarly assessed. From the sums

TABLE 1. Sample blindness counts of the seeds in raspberry jam

Cultivar	Sample plate No.	Jar 1			Jar 2		
		Total seeds counted	Bold seeds counted	Calculated % bold seeds	Total seeds counted	Bold seeds counted	Calculated % bold seeds
Malling Exploit	1	80	32	40·0	104	34	32·7
		86	27	31·4	103	32	31·1
	2	84	24	28·6	71	15	21·1
		82	26	31·7	71	15	21·1
	3	104	31	29·8	89	26	29·2
		105	29	27·6	87	23	26·2
Norfolk Giant	1	71	68	95·8	76	69	90·8
		71	66	93·0	76	68	89·5
	2	80	74	92·5	74	72	97·3
		79	77	97·5	73	70	95·9
	3	72	67	93·1	69	66	95·7
		71	65	91·5	72	64	88·9

of these six pairs of figures the percentage of bold seeds is calculated. Because the seeds differ with respect to the amount of infiltration it is necessary to set a limit above which the seeds are not counted as bold. This has been fixed at a level of 50% infiltration. Various examples of infiltrated seeds are illustrated in Fig. 2. The distinction between blind and bold seeds is relatively clear in practice and good agreement between counts can be obtained from the same slide by different observers.

Sample counts from two jams one predominantly bold (cv. Norfolk Giant) and the other mostly blind (cv. Malling Exploit) are given in Table 1. In neither case were differences between plates or jars of the same jam significant at the 1% level ($P = 0{\cdot}01$). The number of samples taken and the method itself would therefore appear to be adequate for estimating the extent of blindness development.

Part II. The rate of development of blind seeds in raspberry jam stored at constant temperature

The development of blindness in the seed population in raspberry jam is not a sudden occurrence but takes place over a period of weeks, months or even years. The manner in which biological populations change with time may provide information relating to their composition and degree of homogeneity. The following study of the rate of blindness development was therefore carried out to investigate this aspect.

Materials and methods

Jams were prepared from fresh fruit of the cultivar Malling Jewel and two samples of sulphited pulp (cv. Malling Exploit) one of which had 0·6% tannic acid incorporated at its preparation in order to observe the influence of a protein precipitant on the rate of blindness development. The following recipe was employed: 750 g fruit; 1600 g sugar; 150 ml pectin solution (3·5% pectin dissolved in 19% sugar solution); 240 ml water; boiled to an end weight of 2500 g in approximately 10 min. The jams were then filled into ½-lb jars, capped, steam sterilized and stored at 30°C. At intervals sample jars were removed and the total and bold seeds counted by the technique described in Part I.

Results

When the results were complete it was recognized that the rate at which seeds changed from bold to blind probably followed an exponential course. The results were therefore plotted on semi-logarithmic graph paper and are shown in Fig. 3. It will be clearly seen that, within the limits of experimental error, the plots are linear in character, thereby confirming that an exponential course of development prevailed, i.e. a constant percentage of the remaining bold seeds became blind for each unit of time.

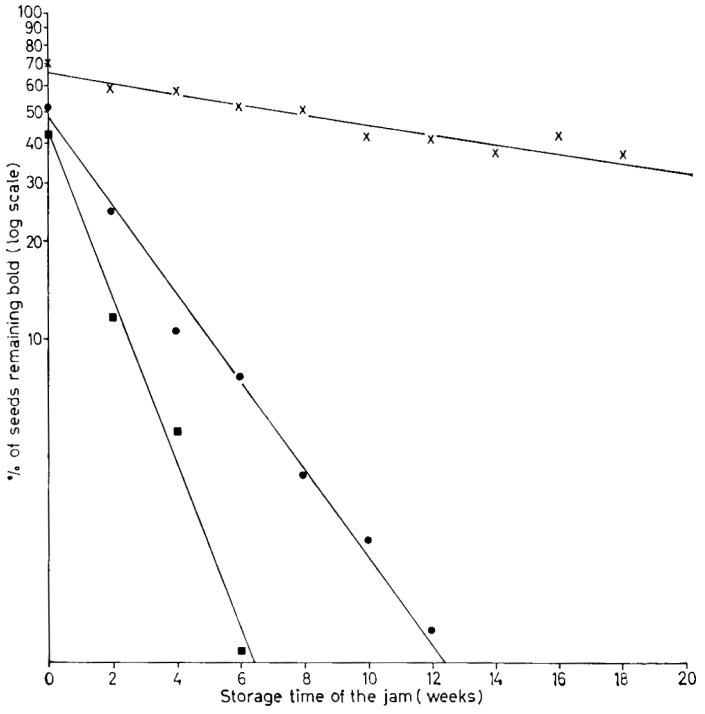

FIG. 3. Semi-logarithmic plots of the development of blind seeds in raspberry jam. ×, Malling Jewel fresh fruit; ■, Malling Exploit sulphited (control); ●, Malling Exploit sulphited (treated). Storage temperature 30°C.

Discussion

The fact that the rate at which raspberry seeds become blind follows an exponential course suggests that, with regard to the tendency to become blind, the seed population is homogeneous. This, in turn, supports the view that the principal factor controlling susceptibility to blindness is inherent rather than environmental, because, had the latter been the case, the influence of environmental factors on the seed population would be unlikely to be sufficiently even to prevent a divergence from the exponential character of blindness development. It thus appears that the breeding of raspberry varieties resistant to blindness would be the most satisfactory method of control in practice.

Although it seems likely that the principal factor controlling blindness is of hereditary origin, nevertheless the rate of blindness development can be modified by changes in manufacturing procedure. Having established a satisfactory method of following the course of development it was therefore necessary to find a mathematical technique for handling and expressing the results in order to facilitate their comparison in future experiments.

Considering the mathematical interpretation of the graphical results it follows that:

$$\log n_1 - \log n_2 = t/D_s \qquad (1)$$

where n_1 and n_2 are the initial and final number of bold seeds respectively, t represents the storage time in weeks and D_s is a constant.

The alternative form of equation (1) gives:

$$D_s = t/\log\,(n_1/n_2)$$

from which it is clear that D_s represents the time required for one logarithmic cycle, i.e. a 90% loss of bold seeds. By analogy with bacteriological techniques D_s may conveniently be termed the decimal reduction time.

As a practical example the results for Malling Exploit sulphited (control) jam from Fig. 3 gives:

$$D_s = \frac{6{\cdot}4}{\log\,(43/1)} = \frac{6{\cdot}4}{1{\cdot}633} = 3{\cdot}92 \text{ weeks}$$

By substituting in this same equation the calculated D_s value it is possible to obtain the time required for any given reduction in the quantity of bold seeds by simply inserting appropriate values for n_1 and n_2.

In practice, of course, the decimal reduction time D_s can normally be read directly from the graph as indicated above. Thus for the three jams the following results are obtained:

Type of jam	Decimal reduction time (D_s) in weeks
Malling Exploit sulphited (control)	3·9
Malling Exploit sulphited (treated)	7·4
Malling Jewel fresh fruit	56·9

Although the use of the equations enables the calculation of decimal reduction time (D_s) from a minimum of two successive readings taken on the jam over a reasonable period of time, in practice the reliability of individual readings is insufficient to make this procedure acceptable. It is therefore preferable to calculate decimal reduction times by taking a series of readings over a period of time, plotting the results and reading the values directly from the graph.

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Bending, shrinkage and texture of cod frozen at sea

R. M. LOVE

Summary. Newly-caught cod were fixed in bent positions with various degrees of curvature during the onset and resolution of rigor mortis. It was found that even in those bent in a complete circle, head and tail touching, there was no significant denaturation of the proteins, neither was the texture found to be tough on tasting. The importance of the results in relation to freezing fish at sea is discussed.

Introduction

The modern practice of freezing fish on the ships that caught them, very soon after death, gives rise to problems not previously encountered. Insufficient bleeding leads to a product with a darker flesh that may be unattractive to the buyer, and phenomena associated with rigor mortis can adversely affect the raw and cooked texture.

Rigor mortis is an irreversible contraction of muscle tissue that occurs soon after death. If the fish is still intact, opposing muscles pull against each other and the animal becomes rigid, but the bony structures to which they are attached prevent any actual change in length. After a further period (about 30 hr in cod stowed in crushed ice) the muscle relaxes again ('resolution of rigor mortis') and if the fish is now filleted it yields an acceptable product.

If, however, it was filleted before the onset of rigor mortis, the subsequent contraction of the loose muscle results in a shrinkage which, in a way at present imperfectly understood, causes the texture of the cooked product to become tough and 'rubbery'. In a similar way, if thin strips of muscle from newly-killed fish are quickly frozen before rigor mortis sets in, and then rapidly thawed, they undergo extensive shrinkage ('thaw rigor'). The texture again becomes tough, much fluid is exuded, and a diminution in the extractability of the protein in 5% sodium chloride, long used as a criterion of deterioration (Dyer, French & Snow, 1950) becomes very pronounced (Love, 1962).

All undesirable changes – toughness, fluid exudation and diminution in salt-extractability – are therefore associated with a change in length.

It was noticed that the fillets from a cod that had passed through rigor mortis while lying in a curved shape were of unequal length, the one from the 'inside' of the curve

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being permanently shortened and exhibiting on the cut surface the corrugations characteristic of shrunk muscle.

In cases where fish are to be frozen at sea as fillets, it is clearly desirable to allow the fish to pass through rigor mortis before filleting, so as to avoid shrinkage. The intention of the present work was to find out whether fish which had been lying bent during this period suffered deterioration in the proteins of the 'inside' fillet or not, i.e. whether it was necessary for the fish to be laid out straight after catching.

Materials and methods

The experiment was carried out in the laboratory of the research ship '*Sir William Hardy*' with cod (*Gadus morhua* L.) caught on 6 June 1965 off the East Coast of Iceland. The fish from one haul, all about 65 cm long, were divided into four groups of ten fish. The fish in the first group were laid out as straight as possible and covered with crushed ice. String was threaded through holes in the nose and tail of each fish in the other three groups, so that they could be held with any desired degree of curvature. Those of the second group were tied so that the middle of each fish made an angle greater than 90°. The third group were curved similarly, but at an angle less than 90°, while those in the fourth group were given the greatest possible curvature, head and tail overlapping.

All groups were left, covered in melting ice, so that rigor mortis set in and then resolved. After 4 days, five fish from each group were filleted. Samples were taken from the region of greatest curvature of the inside and outside fillets, and dissected free from connective tissue (Ironside & Love, 1958). The samples from the five outside fillets of a group were pooled, chopped with scissors and mixed, and two 1 g aliquots taken for estimation; the inside fillets were sampled similarly. The homogenization, separation and measurement of soluble and insoluble protein nitrogen have been described previously (Ironside & Love, 1958).

Taste panel assessment was not carried out at sea.

The remaining five fish from each group were frozen at −40°C, and taken back to Torry Research Station in the frozen state. After 6 weeks at −29°C they were thawed in still air at about 15°C, filleted and tested as described above. Inside and outside fillets from the various groups were also compared, using the Triangle Technique, by a group of tasters (members of the laboratory staff not specially 'trained') after steam-cooking for 30 min without added seasoning.

Results and discussion

The results of protein extractability determinations are shown in Fig. 1. It is quite clear that no matter what curvature was imposed on the fish during the rigor period, there is no significant difference between the extractabilities of the proteins of the

elongated and the foreshortened fillets. Freezing and cold storage followed by slow thawing caused an overall reduction in extractability, but still no difference between the two halves of a fish.

Taste panel assessments for texture showed no significant differences between the two halves. This is not surprising, since it is now well established that taste panel

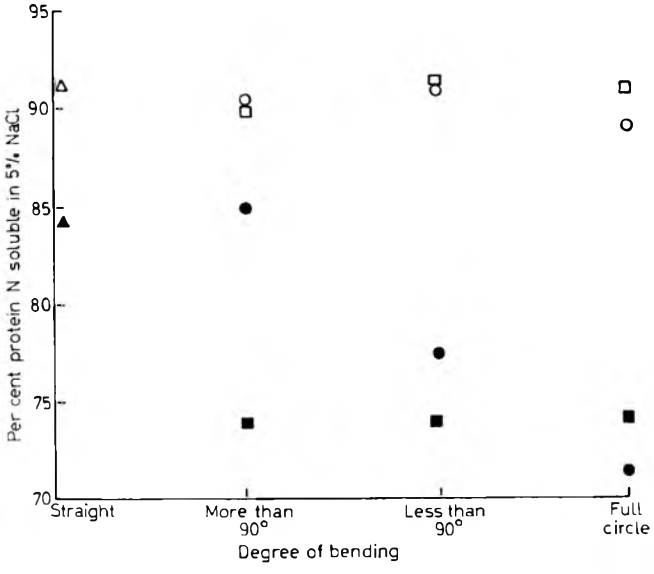

FIG. 1. Protein extractability of cod muscle after going through rigor mortis with various degrees of stretching or shrinking. Triangles, fillet kept straight, neither stretched nor shrunk; squares, 'inside' i.e. shrunk fillet; circles, 'outside' i.e. stretched fillet. Open symbols show the situation in unfrozen fish; solid symbols refer to the same conditions after freezing, storage and thawing.

rating follows protein extractability quite closely (Dyer & Morton, 1956). The only difference found was in the appearance, since the 'outside' fillets sometimes showed more gaping between the myotomes.

It is clear from these results that pre-rigor cod muscle still attached to the skeleton cannot shrink enough, even if the fish is bent in a complete circle, to affect the cooked texture adversely. This is in complete contrast to fillets taken from the bone before rigor mortis, which becomes rubbery.

In the context of freezing at sea, we may conclude that cod can be frozen as whole fish at any time after catching without adversely affecting the texture. If there is a delay before they can be frozen, they can be left, chilled in ice, without any special

care being taken as to their orientation. They may be frozen as fillets provided that they first passed through rigor mortis as whole fish. Filleting pre-rigor fish for freezing is risky, because if the fillets should enter rigor before they are frozen they will usually shrink, exude fluid and become tough, especially if they are warm or much handled (Dyer & Fraser, 1961). Besides, there is now a considerable body of opinion that fish frozen intact will keep better than those frozen as fillets (Piskarev, Bornovalova & Luk'yanitsa, 1961; Nakamura & Tokunaga, 1961; King, 1962). While this may be due to drying out, which would affect fillets more quickly than whole fish, these authors claim it is because the mechanical handling initiates deterioration in the protein.

Acknowledgments

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The use of tasters for investigating cold storage deterioration in frozen fish

R. M. LOVE

Summary. Frozen cod cold-stored at −14°C were tasted for texture changes by an untrained panel, using a simple comparison technique that did not require the use of memory. It was found that the fish had to be kept for about 3½ weeks at −14°C before the tasters could clearly detect toughening, and a nomogram, constructed from objective data, is given to show the equivalent times at other temperatures.

Introduction

The texture of cold-stored fish muscle steadily deteriorates, at a rate governed by the temperature (Reay, 1939), seeming tougher, 'drier' and more fibrous in the mouth when eaten. The whole subject of texture appreciation in the mouth is an extremely complex one, involving both mechanical and auditory stimuli and being influenced by psychological factors. It has been reviewed in its various aspects in a symposium (*Texture in Foods*, 1960) and some further information is given by Matz (1962) On the other hand, measurements of physico-chemical phenomena which develop alongside the texture changes, such as the decrease of protein extractability in 5% sodium chloride (Dyer, French & Snow, 1950) or the increase in the resilience of the muscle cells (Love & Mackay, 1962) have been developed into tests for cold-storage deterioration which are reasonably reproducible and are usually more convenient to carry out than tasting assessment. However, the fundamental question remains: What is the smallest amount of deterioration, demonstrable by objective methods, which can be detected by anyone eating the fish? This question is all the more important since a number of treatments, e.g. double freezing (Love, 1962) or freezing to very low temperatures (Love & Elerian, 1963), have been shown by the objective techniques to damage fish protein, but it is not known how important such effects would be to someone intending to eat the product.

The present work is an attempt to answer this question.

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Material

Cod (*Gadus morhua* L.) about 20 in. long were caught by trawl-net within a radius of about 30 miles from Aberdeen during the months of September and October 1959.

This species is very suitable for studies on cold-storage deterioration, since the proteins deteriorate in the frozen state more quickly than in any other common European food-fish apart from the whiting, *Gadus merlangus* L. (Love & Olley, 1964).

Methods

After the removal of the guts, and within a day of being caught, the cod were frozen in an air-blast freezer at −30°C. They were sprayed with water after freezing so as to form a protective film of ice, and stored in wooden boxes at −14°C for various periods. They were thawed by leaving at room temperature (about 18°C) overnight, and then filleted.

The anterior halves of each fillet were used for tasting, being steamed in enclosed glass casseroles for 30 min without added seasoning. Samples were presented to the tasters in pairs which differed as to length of storage time. The taster was merely required to state which of the two seemed the tougher. Eight comparisons, four in the morning, four in the afternoon, were made under code by each taster for each pair of conditions. There were from seven to ten tasters: these were not specially 'trained' personnel, but were any laboratory staff who happened to be available. Thus between each pair of conditions, for example 3 weeks as against 5 weeks at −14°C, there were from fifty-six to eighty comparisons made. The number of times that the sample known by the experimenter to have been stored for the longer period was marked 'tougher' was then expressed as a percentage of the number of comparisons. On this basis, '50%' represented inability to distinguish between a pair.

The first approach to the problem had been by way of a scoring system. The tasters were given a cooked sample of cod stored for more than 30 weeks at −14°C, which was to be scored '1', and also with a sample of fish that had been frozen and immediately thawed without storage, to be scored '10'. The coded sample of cod, which had been cold-stored for a certain period, was presented with these two standards to be scored with some intermediate value. The inclusion of standards in each trial probably reduced errors arising from imperfect recollection of textures. Fish stored at weekly intervals from 0 to 20 weeks at −14°C were tasted in this way, and the scores averaged. It had been hoped to assess the sensitivity of the panel by a statistical analysis of the curve, but the results showed so much scatter than they were deemed useless for this purpose. While assessments of fish flavours can be made by a panel with sufficient agreement to be useful (Ehrenberg & Shewan, 1953), the assessment of texture changes is obviously more difficult. The approach was accordingly modified to the much simpler system outlined above, where no attempt was made to assign a numerical score to the fish.

Results

Fig. 1 shows the results. Several points, each the outcome of at least fifty-six comparisons, are shown for each difference value on the abscissa. This is because, for example, '5 weeks difference at −14°C' could be the difference between fish stored for 0 and 5 weeks, or 1 and 6 weeks, 2 and 7 weeks, etc. Comparisons were therefore made between fish of constant difference in storage time, but with different degrees of deterioration, to see whether the panel showed any marked decline in sensitivity as

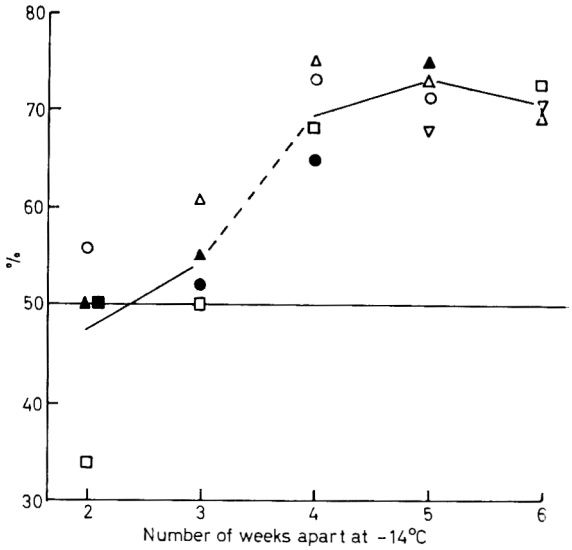

FIG. 1. Percentage of taste comparisons in which the fish of a pair stored the longer at −14°C was judged to be the tougher after cooking. 50% represents inability to distinguish between the pair. Each point is calculated from the results of fifty-six to eighty comparisons. The symbols represent different storage times of the sample in a pair stored for the *shorter* time, thus: ▽, 0 weeks at −14°C; ○, 1 week; △, 2 weeks; □, 3 weeks; ●, 4 weeks; ▲, 5 weeks; ■, 7 weeks. For example, the open square on the graph opposite a value of 4 on the abscissa shows the comparison between cod fillets stored for 3 weeks and 7 weeks at −14°C. The curve joins points which are the overall averages for each value on the abscissa, having regard to the total number of individual comparisons made.

deterioration proceeded. Obviously if they had been asked to distinguish between fish stored for 30 and for 35 weeks at −14°C they would not have been able to do so, since the deteriorative changes would have gone to completion in both samples (Love & Ironside, 1958). However, in the range studied, the panel seemed to distinguish the same differences equally well under differing degrees of deterioration – for example the difference between fish stored between 5 and 10 weeks at −14°C was distinguished as clearly as between 0 and 5 weeks.

Fig. 1 shows that the panel could not distinguish between fish with differences in

storage time of 2 or of 3 weeks, although after 3 weeks there were signs that differentiation was beginning.

After 4 weeks at $-14°C$ a clear distinction was made. Curiously, it did not improve as the difference in storage time increased beyond 4 weeks, and a value of 100% was never reached. At best, the distinction could only be made about three times out of four. By contrast, the 'cell fragility' test can distinguish between batches of cod only 2 weeks apart at the same temperature, nine times out of ten (Love & Mackay, 1962).

Discussion

From these figures, the point at which tasters start to be able to detect a definite toughening of texture lies somewhere between 3 and 4 weeks at $-14°C$ in the case of cod. Now the deterioration of frozen cod muscle has been studied at a number of temperatures by objective methods with considerable accuracy, and the relationship between the temperatures has been worked out (Love, 1962). If we regard the minimum storage period for detection at $-14°C$ to be $3\frac{1}{2}$ weeks, then with this information we can construct a nomogram showing how long cod can be stored at any temperature before toughening is detectable in the steam-cooked product by untrained personnel.

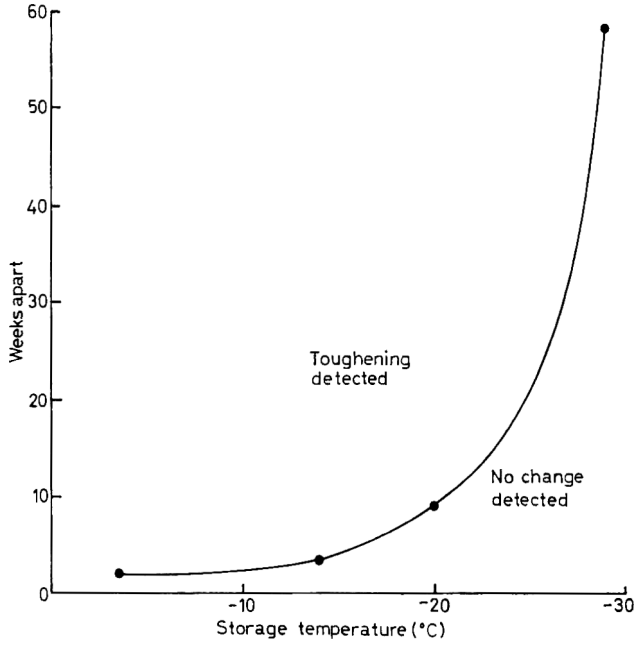

FIG. 2. Nomogram showing the number of weeks apart a pair of cod fillets must be stored at any temperature before a difference in texture can be detected between them by an untrained taste panel, about three times out of four.

This is shown in Fig. 2. It should be emphasized that these are 'steady' temperatures – those in commercial cold-stores tend to fluctuate due to opening the doors, etc., so that the time corresponding with the nominal temperature of the cold store would be shortened. Also, the fish must be protected against desiccation ('freezer-burn') which rapidly causes the texture to toughen even after a very short time in the cold-store.

Finally, the scheme applies to cod only. To establish the corresponding values for other species of fish would require separate tasting tests, since extrapolation of the results obtained by objective methods might be misleading.

Moorjani, Montgomery & Coote (1960) found that if the salt-extractability of the muscle of two batches of *Nemadactylus macropterus* which had been cold-stored differed significantly at the 5% level, or better, then tasters could also distinguish between them. If the difference was only significant at the 10% level, they could not be distinguished by tasting. White *et al.* (1964) found that a small trained panel could distinguish toughness differences in light turkey meat which differed in Warner–Bratzler shear force by 4 lb in a 9–22 lb range. Similarly, from the present work, we can say that in the 0–10 weeks range at $-14°C$, an untrained panel can distinguish cod which differ in cell fragility values of more than 0·3–0·15, or in protein solubilities greater than 20–10%, depending on the extent of deterioration. It is not possible to give a single value in either case, because both quantities decline as an asymptotic curve, not a straight line. However, the figures show that the deterioration occurring after, for example, freezing to very low temperatures followed by storage would be noticed by ordinary people eating the fish after steam-cooking.

It must be borne in mind that the method of preparation of the fish can profoundly influence these findings – if the fish are fried in batter, or made into fish pie or fish cakes, the perception of cold storage deterioration is greatly reduced.

Acknowledgment

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The effect of sodium chloride, potassium nitrate and sodium nitrite on the recovery of heated bacterial spores*

T. A. ROBERTS AND M. INGRAM

Summary. The ability of aerobic and anaerobic spores, after various degrees of heating, to produce macrocolonies in media containing different concentrations of sodium chloride, potassium nitrate and sodium nitrite has been examined. The effect of different heat treatments, roughly from F_0 = 0·0015 to F_0 = 1·5, on the inhibitory levels of these curing ingredients, showed that the more severe treatments were capable of rendering surviving spores considerably more sensitive to subsequent inhibition by concentrations of the order found in cured meats. To produce this effect, less heating was needed with two *Bacillus* strains than with three of *Clostridium*. Sodium chloride and potassium nitrate had quantitatively similar effects. The inhibitory effect of sodium nitrite increased roughly ten-fold from pH 7 to pH 6.

Introduction

To maintain a product of good appearance and texture, the heat treatment given to canned cured meats is minimized, and is frequently so low as to have, in itself, only a small lethal effect on bacterial spores. Although viable bacterial spores, both aerobic and anaerobic, may in consequence often be isolated from such products, as is well known, their excellent record as regards unrefrigerated shelf stability and public health indicates that the spores present are incapable of normal development while they remain in the product. However, numerous papers describing the stabilizing effects of curing ingredients have, in general, thrown little light on the exact conditions and mechanism which prevent the outgrowth of such bacterial spores; although it is generally agreed that: (i) with the heat treatments commonly used, the spores will develop if the meat is without curing salts, and (ii) unprocessed spores introduced from outside are likely to grow in the cured meat; from which it has long been plain that control depends on some combined effect of heating and the salts.

Earlier workers had investigated the effect of the presence of salts on the direct effect of heating, but this influence of salts must be small. Jensen & Hess (1941) suggested that bacterial spores were more sensitive to heat in the presence of curing ingredients. More comprehensive, later, studies led to the conclusion that this was incorrect (Yesair & Cameron, 1942; Stumbo, Gross & Vinton, 1945a, b; Silliker,

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Greenberg & Schack, 1958). Similarly, unpublished results from this laboratory have shown conclusively that up to 6% (w/v) sodium chloride alone is without effect on the heat resistance of spores of the strain of *Cl. sporogenes* used below.

In the present experiments, accordingly, spores were heated to various degrees in the absence of salts, and then transferred to nutrient media containing the relevant salt in different concentrations to see how far the effect of the salts might lie specifically in hindering the subsequent development of a previously heated spore.

Materials and methods

Organisms and preparation of spore suspensions

The organisms used were *Clostridium sporogenes* PA 3679 (NCTC 8594); a strain of *Clostridium* resembling *Cl. oedematiens*, isolated from a blown can of luncheon meat (*Clostridium* I); an unidentified species of *Clostridium* isolated from bacon (*Clostridium* II); *Bacillus subtilis* and a strain of *Bacillus* (referred to as *Bacillus* F) isolated from a blown can of lightly processed bacon and known to be unusually resistant to sodium chloride and nitrite (Eddy & Ingram, 1956).

Spore suspensions of the anaerobes were prepared, using the method of the National Canners' Association (Reed, Bohrer & Cameron, 1951), in a medium comprising pork extract, peptone, tryptone, dextrose, and K_2HPO_4. After incubation for 1 week at 37°C, followed by 2 weeks at 30°C, spores were harvested by centrifugation, washed three times with distilled water, then suspended in distilled water and heated at 80°C for 30 min to kill vegetative cells. All the heated spore suspensions were stored at 1°C.

Spore suspensions of the aerobes were obtained by washing the surface growth from Hartley's digest agar contained in Roux bottles, after incubation at 37°C for 7 days. Spores were harvested in distilled water, shaken vigorously to break up clumps, then washed in distilled water three times, heated at 80°C for 30 min, and stored at 1°C, as for the anaerobes.

Heat treatment of the spores

Prior to plating in the different media, the approximate heat resistance of each spore suspension was determined, in order to arrive at a suitable level of inactivation for the purpose of the experiment.

Heat resistance was determined by total immersion of small volumes of spore suspension (0·01–0·1 ml) contained in ampoules in a thermostatically controlled water bath; or, for temperatures exceeding 100°C, in either an oil-bath or a small pressure vessel which had a rapid come-up time and could be rapidly cooled by spraying with water. Large volumes, up to 50 ml, used during the main experiments, were heated at temperatures up to 100°C by total immersion in a water-bath, and at temperatures exceeding 100°C in the small pressure vessel. The design of these experiments was not critically dependent on exact times of heating.

To permit rough comparison of the various heat treatments, they have been approximately calculated as arbitrary F_0 values, on the common assumed basis of $z = 18°F$.

Viable counts

With the anaerobic spores, decimal dilutions were made in quarter-strength Ringer's solution + 0·1% sodium thioglycollate. Replicate 1-ml samples were placed in test tubes containing 10 ml Reinforced Clostridial Agar (RCA) (Oxo Ltd, London) containing varying amounts of sodium chloride, potassium nitrate or sodium nitrite. A black glass rod was added to facilitate subsequent counting of colonies (Ingram & Barnes, 1956) and, after solidification, the contents were sealed with a deep layer of agar containing the appropriate concentration of the salt. Incubation at 37°C was continued up to 14 days.

With aerobic spores, duplicate 1-ml samples of decimal dilutions in quarter-strength Ringer's solution were pour plated in Hartley's digest agar containing varying concentrations of the curing salts. Plates were overpoured with a thin layer of aqueous agar plus the appropriate salt concentration to prevent colonies spreading. Incubation at 37°C was continued for at least 48 hr, and up to 14 days where growth was slowed by the higher concentrations of the curing salts.

In both counting methods, sodium chloride and potassium nitrate were added to the agar before the final autoclaving. Sodium nitrite was added in solution just before the medium was used (at 50°C).

Results

The effect of incubation on nitrite concentration was checked by examining plates containing initial concentrations of 0, 100, 200, 400, 800, 1200 and 1600 ppm (i.e. mg $NaNO_2$/l—this basis is used throughout). Freezing at −20°C broke the gel and the released liquid was filtered off after thawing. Nitrite was determined colorimetrically (Eddy, 1958) in duplicate plates at pH 5·6, 6·6 and 7·6. A control series, frozen and thawed immediately after pouring, was compared with plates incubated for 48 hr at 37°C before freezing. No fall in nitrite concentration occurred in this 48 hr; except at pH 5·6, when a fall of 20% occurred.

pH (checked by capillators) was little affected by incubation.

False low counts were occasionally obtained upon plating a ×10 dilution of the heated suspension. This was overcome by placing an inoculum of 1 ml in 100 ml of counting medium instead of the usual 10 ml. Washing the heated spore suspension in quarter-strength Ringer's solution failed to remove the inhibitory factors.

The effect of sodium chloride on recovery of heated anaerobic spores

Table 1 gives the recoveries of viable spores of *Cl. sporogenes* PA 3679 in RCA containing up to 10% (w/v) added sodium chloride, after heating at 80°C for 20 min

TABLE 1. The effect of sodium chloride on recovery of heated spores of *Cl. sporogenes* P.A. 3679

% NaCl added to RCA medium	No. ($\log_{10}$) of spores recovered/ml in medium indicated after heating at:			
	80°C for 20 min	100°C for 20 min	115°C for 5 min	115°C for 6 min
0	7·18	5·48	4·67	2·91
1·0	7·11	5·36	3·87	2·83
1·5	—	—	3·71	1·00
2·0	7·20	5·23	3·58	0·70
2·5	—	—	3·58	—
3·0	—	—	3·08	—
3·5	—	—	2·48	—
4·0	6·72	4·48	0·70	—
5·0	6·48	5·04	—	—
6·0	6·28	5·08	—	—
6·5	4·48	3·15	—	—
6·75	3·10	2·36	—	—
7·0	2·48	< 1·00	—	—
10·0	< 1·00	—	—	—

(F_0 = 0·0015), 100°C for 20 min (F_0 = 0·17), 115°C for 5 min (F_0 = 1·3), or 115°C for 6 min (F_0 = 1·6).

These recoveries are compared on a percentage basis in Fig. 1. In this and subsequent Figures spores given a particular heat treatment are regarded together, the number, developing as macroscopically visible colonies on medium with particular salt concentrations, being represented as percentages of the number in absence of salt.

TABLE 2. The effect of potassium nitrate on recovery of heated spores of *Cl. sporogenes* P.A. 3679

% KNO_3 added to RCA medium	No. ($\log_{10}$) of spores recovered/ml in medium indicated after heating at:				
	80°C for 20 min	100°C for 20 min	108°C for 15 min	115°C for 5 min	115°C for 6 min
0	6·86	5·75	5·30	4·61	2·91
0·5	6·92	5·81	—	—	—
1·0	6·99	5·77	—	4·28	3·46
1·8	—	—	5·54	—	—
2·0	6·90	5·75	—	4·64	3·15
3·0	—	—	5·43	4·00	2·23
3·5	6·69	5·43	—	—	—
4·0	—	—	5·04	3·40	1·18
5·0	5·74	4·34	2·00	< 1·00	< 1·00
6·0	—	—	< 1·00	—	—
6·5	< 2·00	< 2·00	—	—	—

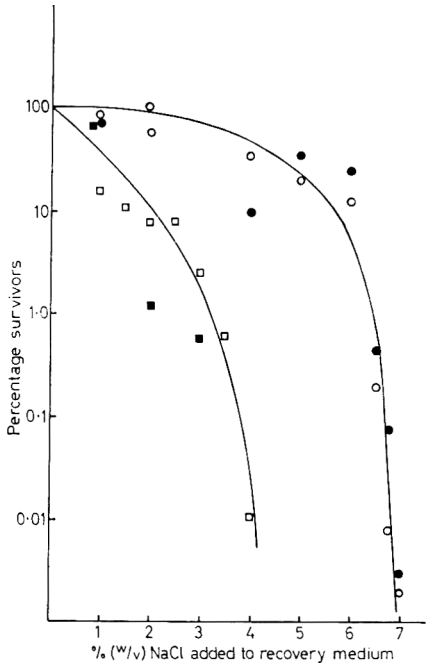

FIG. 1

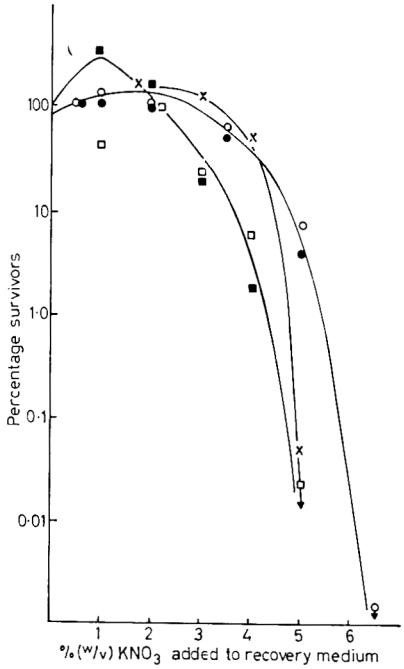

FIG. 2

FIG. 1. The effect of sodium chloride on recovery of heated spores of *Cl. sporogenes* P.A. 3679. ○, Heated at 80°C for 20 min; ●, 100°C for 20 min; □, 115° C for 5 min; ■, 115°C for 6 min.

FIG. 2. The effect of potassium nitrate on recovery of heated spores of *Cl. sporogenes* P.A. 3679. ○, Heated at 80°C for 20 min; ●, 100°C for 20 min; ×, 108°C for 15 min; □, 115°C for 5 min; ■, 115°C for 6 min.

Spores of PA 3679 which had been subjected to 80°C for 20 min (to kill only vegetative cells, i.e. nominal 100% survival), or to 100°C for 20 min (equivalent to 2·0% survival), possessed a similar tolerance to sodium chloride in the recovery medium: 4% (w/v) depressing the recovery to 10–35% and 6½% (w/v) to 0·2–0·5%, of that in RCA. However, heating at 115°C for 5 min (0·31% survival) or 6 min (0·0055% survival) substantially increased the susceptibility of the surviving spores to subsequent inhibition in the plating medium. After these treatments, recovery in medium containing 3% (w/v) sodium chloride was reduced to about 2·5% of that in absence of salt; whereas with spores subjected to the milder heat treatments (80°C/20 min; 100°C/20 min) 3% (w/v) sodium chloride barely depressed the count significantly.

The effect of potassium nitrate on recovery of heated anaerobic spores

Table 2 gives recoveries of viable spores of *Cl. sporogenes* PA 3679 in RCA containing

up to 6·5 % (w/v) added potassium nitrate, after heating at 80°C for 20 min ($F_0 = 0{\cdot}0015$), 100°C for 20 min ($F_0 = 0{\cdot}17$), 108°C for 15 min ($F_0 = 0{\cdot}8$), 115°C for 5 min ($F_0 = 1{\cdot}3$), or 115°C for 6 min ($F_0 = 1{\cdot}6$). These recoveries are compared on a percentage basis in Fig. 2.

Spores heated at 80°C for 20 min (i.e. 100% survival) or at 100°C for 20 min (7·7% survival) possessed a similar tolerance to potassium nitrate in the recovery medium, 4% (w/v) being slightly inhibitory and higher concentrations markedly so. After heating at 108°C for 15 min (2·7% survival) there was no change in the tolerance of surviving spores to concentrations of potassium nitrate up to 4% (w/v) but survivors were more sensitive to higher concentrations. In the presence of 5% (w/v) potassium nitrate, recovery was only 0·05% of that in RCA without nitrate, compared with 4–7·5% after the two lighter heat treatments.

More drastic heating at 115°C for 5 min (0·56% survival) and 115°C for 6 min (0·011% survival) slightly increased the sensitivity to potassium nitrate in the plating medium but the overall sensitization was considerably less than that caused by sodium chloride.

There was repeated evidence of a small stimulation of recovery of PA 3679 by concentrations of potassium nitrate up to 2% (w/v) after heating at 80°C for 20 min and 100°C for 20 min, a more marked effect of 2 and 3% (w/v) after heating at 108°C for 15 min, and a very marked stimulation by 1% (w/v) after heating at 115°C for 6 min (Fig. 2).

There was no such stimulation with *Clostridia* I and II, results for which are presented in Tables 7 and 8. In other respects, their behaviour was similar to that of *Cl. sporogenes* PA 3679.

The effect of sodium nitrite on recovery of heated anaerobic spores

Table 3 summarizes the recoveries of spores of PA 3679 in RCA containing up to 1200 ppm of sodium nitrite at pH values between 5·6 and 7·5; data compared on a percentage basis in Fig. 3. Whether heated at 80°C for 20 min ($F_0 = 0{\cdot}0015$) or 100°C for 20 min ($F_0 = 0{\cdot}17$), the increase in sensitivity with decreasing pH is very marked. After 80°C for 20 min, recovery at pH 7·5 is depressed to about 1% of that in RCA by 300–400 ppm sodium nitrite. Reducing the pH to 7·0 results in a similar depression of recovery by 125–200 ppm, and at pH 6·5 a slightly lower concentration is equally effective. After heating at 115°C for 5 min ($F_0 = 1{\cdot}3$) growth of the surviving spores (0·9%) was inhibited rather more readily at pH 6·8 and 6·2, about 50 ppm being sufficient to reduce recovery to 1% of that in RCA.

Similar results were obtained with *Clostridium* I after heating at 80°C for 20 min, or 108°C for 20 min ($F_0 = 1{\cdot}0$), recovery at pH 6·8 equivalent to 1% of that in nitrite-free RCA being obtained using 350–400 ppm sodium nitrite in the former instance, and 75 ppm in the latter.

TABLE 3. The effect of sodium nitrite on recovery of heated spores of *Cl. sporogenes*

pH	ppm $NaNO_2$ added to RCA medium	No. (log_{10}) of spores recovered/ml in medium indicated after heating at:		
		80°C for 20 min	100°C for 20 min	115°C for 5 min
7·5	0	6·65	5·95	—
	200	6·38	4·64	—
	400	5·56	4·08	—
	800	3·40	2·78	—
	1200	<2·00	<2·00	—
7·0	0	6·72	5·78	—
	100	5·85	4·04	—
	200	4·79	3·91	—
	400	<2·00	<2·00	—
6·8	0	—	—	4·61
	10	—	—	3·60
	20	—	—	3·40
	30	—	—	3·30
	40	—	—	2·30
	50	—	—	1·78
	60	—	—	1·30
	70	—	—	<1·00
6·5	0	6·75	5·52	—
	50	4·42	4·94	—
	100	<2·00	<2·00	—
6·2	0	—	—	4·61
	10	—	—	3·57
	20	—	—	2·95
	30	—	—	2·46
6·0	0	6·42	4·23	—
	50	<2·00	<2·00	—
	100	<2·00	<2·00	—
5·6	0	—	—	2·48
	10	—	—	<1·00
	20	—	—	<1·00

The effect of sodium chloride on the recovery of heated aerobic spores

Table 4 summarizes the effects of including up to 16% sodium chloride in the recovery medium for *B. subtilis*, heated at 80°C for 20 min (F_0 = 0·0015) or at 95°C for 45 min (F_0 = 0·12); and for *Bacillus* F after 80°C for 20 min or 90°C for 30 min (F_0 = 0·025). Comparison on a percentage basis is made in Fig. 4. The *Bacilli* were considerably more tolerant than the *Clostridia* studied to the inclusion of sodium chloride in

TABLE 4(a). The effect of sodium chloride on recovery of heated spores of *Bacillus subtilis*

% NaCl added to medium	No. (log_{10}) of spores recovered/ml in medium indicated after heating at:	
	80°C for 30 min	95°C for 45 min
0	8·83	6·34
1	8·96	5·46
2	8·96	5·63
4	8·78	5·20
8	8·11	4·49
12	4·30	< 1·00
16	< 1·00	< 1·00

TABLE 4(b). The effect of sodium chloride on recovery of heated spores of *Bacillus* F

% NaCl added to medium	No. (log_{10}) of spores recovered/ml in medium indicated after heating at:	
	80°C for 30 min	90°C for 30 min
0	8·95	6·56
2·5	8·89	5·76
5·0	8·79	4·46
10·0	7·91	3·11
15·0	7·17	2·20

TABLE. 5. The effect of sodium nitrite and pH on the recovery of heated spores of *Bacillus subtilis*

ppm $NaNO_2$ added to medium	No. (log_{10}) of spores recovered/ml in medium indicated after heating at:							
	80°C for 30 min				95°C for 45 min			
	pH 7·6	pH 6·6	pH 6·2	pH 5·6	pH 7·6	pH 6·6	pH 6·2	pH 5·6
0	8·87	9·00	8·89	8·83	6·30	6·55	6·04	6·23
100	8·85	8·64	—	8·76	6·25	6·11	—	5·65
200	8·87	8·55	8·91	5·00	5·55	6·08	5·74	< 1·00
400	8·49	8·59	8·74	< 1·00	5·47	6·42	5·54	—
600	—	—	3·71	—	—	—	1·83	—
800	8·46	8·43	< 1·00	—	5·17	5·55	< 1·00	—
1600	8·74	8·43	—	—	5·47	4·88	—	—

TABLE 6. The effect of sodium nitrite and pH on recovery of heated spores of *Bacillus* F

ppm $NaNO_2$ added to medium	No. (log_{10}) of spores recovered/ml in medium indicated after heating at:											
	80°C for 30 min				90°C for 30 min				100°C for 35 min			
	pH 7·6	pH 6·6	pH 6·2	pH 5·6	pH 7·6	pH 6·6	pH 6·2	pH 5·6	pH 7·6	pH 6·6	pH 6·2	pH 5·6
0	8·81	8·59	8·89	9·92	6·52	5·92	5·76	5·23	3·11	2·90	2·74	2·11
100	8·83	8·30	—	8·76	6·17	6·00	—	4·74	—	—	—	1·00
200	8·76	8·58	8·80	—	5·97	6·17	5·73	—	—	—	2·14	—
400	8·70	8·63	8·71	—	6·04	5·89	5·81	—	3·28	3·06	< 1·00	—
600	—	—	2·56	—	—	—	—	—	—	—	—	—
800	8·53	8·63	1·54	—	5·85	6·14	—	—	3·23	3·11	—	—
1200	—	—	—	—	—	5·50	—	—	3·23	2·78	—	—
1600	8·53	8·76	—	—	6·07	—	—	—	3·14	2·40	—	—

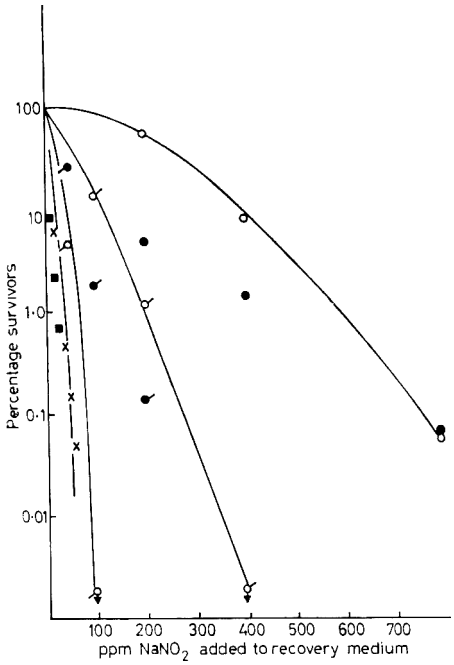

FIG. 3

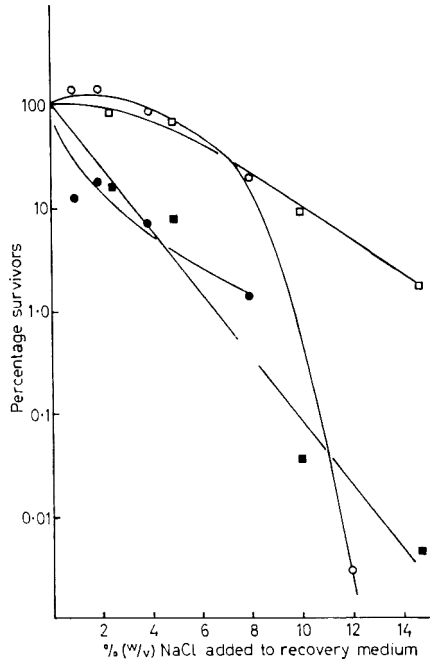

FIG. 4

FIG. 3. The effect of sodium nitrite on recovery of heated spores of *Cl. sporogenes* P.A. 3679. ○, Heated at 80°C for 20 min; ●, 100°C for 20 min: no tag, pH 7·5; right-hand tag, pH 7·0; left-hand tag, pH 6·5; ×, 115°C for 5 min (pH 6·8); ■, 115°C for 5 min (pH 6·2).

FIG. 4. The effect of sodium chloride on recovery of heated spores of *B. subtilis* and *Bacillus* F. *B. subtilis*: ○, Heated at 80°C for 30 min; ●, 95°C for 45 min. *Bacillus* F: □, Heated at 80°C for 30 min; ■, 90°C for 30 min.

the recovery medium; but after heating, a reduction in this tolerance was observed, similar to that observed with the *Clostridia*. The inclusion of up to 4% sodium chloride in the recovery medium was without effect on the recovery of *B. subtilis* spores subjected to a treatment equivalent to $F_0 = 0{\cdot}0015$; but after heating to $F_0 = 0{\cdot}12$, recovery in a medium containing 1, 2 or 4% sodium chloride was reduced to about 10% of that in RCA.

A similar reduction in salt tolerance was evident with *Bacillus* F after heating at only $F_0 = 0{\cdot}025$ (Fig. 4).

The effect of sodium nitrite on recovery of heated aerobic spores

Tables 5 and 6 summarize data on the recovery of spores of *Bacillus subtilis* and *Bacillus* F, in the presence of concentrations of sodium nitrite up to 1600 ppm at pH values within the range 5·6–7·6, after different heat treatments.

At pH 7·6, after heating at 80°C for 30 min (F_0 = 0·0024), spores of *B. subtilis* were able to produce colonies in the presence of nitrite concentrations as great as 1600 ppm (Fig. 5), and this also occurred at pH 6·6. Reduction of the pH to 6·2 had a remarkable effect on the ability of spores surviving the heat treatment to produce colonies. Recovery was similar at concentrations up to 400 ppm, but 600 ppm permitted less than 0·001% of the surviving spores to produce colonies. At pH 5·6 the inhibitory effect of sodium nitrite was even more marked, and in the presence of 200 ppm sodium nitrite, recovery was only 0·016% of that in the absence of nitrite. Heating at 95°C for 45 min (F_0 = 0·12) did not greatly affect this recovery pattern (Fig. 6) except that recovery was slightly lower at pH 7·6 and 6·6 than after the lighter heat treatment.

Similar trends were observed with *Bacillus* F after heating at 80°C for 30 min (F_0 = 0·0024), 90°C for 30 min (F_0 = 0·025), or 100°C for 35 min (F_0 = 0·27). After F_0 = 0·0024 (Fig. 7) concentrations of sodium nitrite up to 1600 ppm did not greatly depress

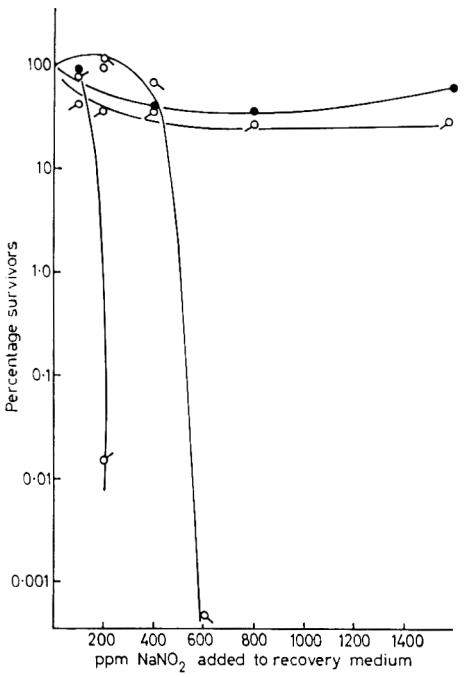

FIG. 5

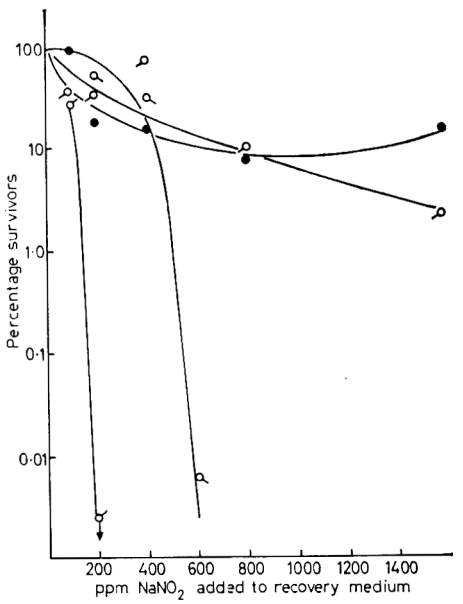

FIG. 6

FIG. 5. The effect of sodium nitrite on recovery of spores of *B. subtilis* heated 80°C for 30 min. ●, pH 7·6; ○: tagged bottom left, pH 6·6; tagged bottom right, pH 6·2; tagged top right, pH 5·6.

FIG. 6. The effect of sodium nitrite on recovery of spores of *B. subtilis* heated at 95°C for 45 min. ●, pH 7·6; ○: tagged bottom left, pH 6·6; tagged bottom right, pH 6·2; tagged top right, pH 5·6.

TABLE 7. The effect of sodium chloride, potassium nitrate and sodium nitrate on recovery of heated spores of *Clostridium* I [No. ($\log_{10}$) of spores recovered/ml in medium indicated after heating for 20 min at 80°C (A) and 108°C (B)]

% NaCl added to RCA medium	A	B	% KNO_3	A	B	ppm $NaNO_2$ (pH 6·8)	A	B
0	7·62	3·25	0	7·62	2·57	0	7·62	3·25
0·25	—	3·23	1	7·46	2·54	10	—	2·30
0·50	—	2·98	2	—	2·15	20	—	2·17
0·75	—	2·77	3	7·07	1·54	30	—	1·70
1·00	7·36	2·47	4	—	< 1·00	40	—	1·70
1·50	—	1·81	5	6·77		50	7·59	< 1·00
2·0	—	1·00	7	2·30		100	7·11	
3·0	7·11	—	8	1·40		150	6·44	
5·0	6·79	—	9	1·47		200	5·34	
7·0	5·97	—	10	2·44(?)		250	3·34	
8·0	< 1·00	—				300	3·38	
						350	3·17	
						400	2·66	
						450	2·38	
						500	2·23	
						600	2·14	

TABLE 8. The effect of sodium chloride, potassium nitrate and sodium nitrite on recovery of heated spores of *Clostridium* II

% NaCl added to RCA medium	Spores*	% KNO_3 added to RCA medium	Spores*	pH	ppm $NaNO_2$ added to RCA medium	Spores*
0	3·61	0	4·36	6·8	0	4·11
					50	3·47
1	3·14	1	4·30		100	2·30
					150	1·00
3	3·42	3	4·30			
				6·0	0	3·00
5	2·59	5	2·30		50	< 1·00
6	2·20	6	< 1·00	5·8	0	2·70
					50	< 1·00

* No. ($\log_{10}$) of spores recovered/ml in medium indicated after heating at 115°C for 5 min.

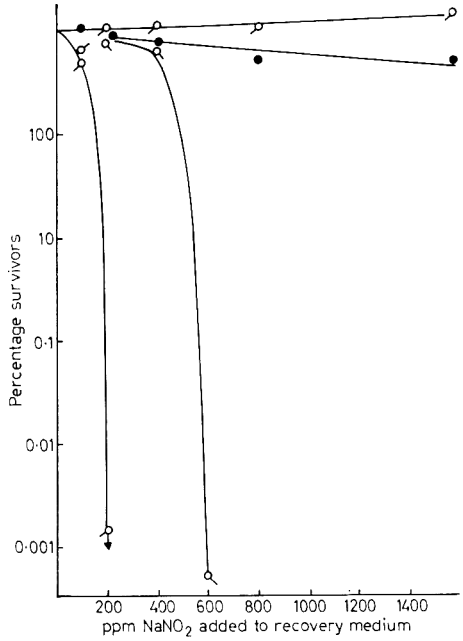

FIG. 7

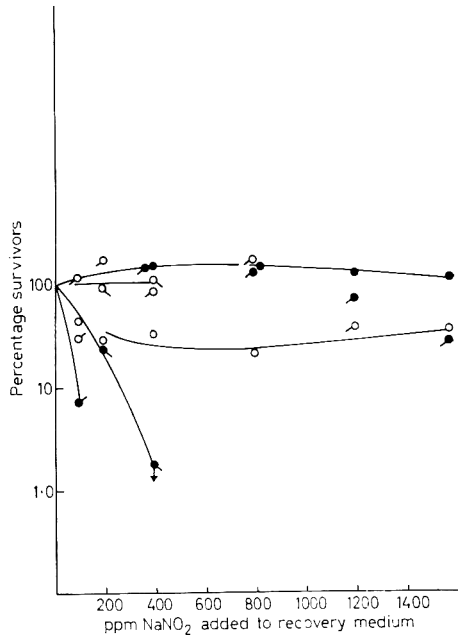

FIG. 8

FIG. 7. The effect of sodium nitrite on recovery of spores of *Bacillus* F heated at 80°C for 30 min. ●, pH 7·6; ○: tagged bottom left, pH 6·6; tagged bottom right, pH 6·2; tagged top right, pH 5·6.

FIG. 8. The effect of sodium nitrite on recovery of heated spores of *Bacillus* F. ○, Heated at 90°C for 30 min; ●, 100°C for 35 min; no tag, pH 7·6; tagged bottom left, pH 6·6; tagged bottom right, pH 6·2; tagged top right, pH 5·6.

the recovery of surviving spores at pH 7·6 and 6·6 However, a reduction in pH to 6·2 resulted in virtually all the surviving spores becoming sensitive to concentrations of sodium nitrite over 400 ppm, yet remaining unaffected by concentrations up to 400 ppm. A further drop in pH to 5·6 resulted in no growth in concentrations exceeding 100 ppm. After more severe heating, $F_0 = 0{\cdot}27$ (Fig. 8), *Bacillus* F still gave good recovery at pH 7·6 and 6·6 even in the presence of 1600 ppm sodium nitrite. The effects of sodium nitrite at pH 6·2 and 5·6 were similar to those on *Bacillus subtilis*.

Discussion

The processing of canned cured meats, in relation to bacterial spores, has been reviewed by Riemann (1963). Both aerobic and anaerobic spores are present in cured meats (e.g. Brown, Vinton & Gross, 1960); and although numbers of the latter may be as low as 10^{-2} to 10^{-3} per g (Riemann, 1963; Steinkraus & Ayres, 1964), it appears from the relative ease with which *Bacilli* and *Clostridia* may be isolated from some products

(Ingram, 1952; Ingram & Hobbs, 1954), that the incidence of contamination is at times relatively high. Spores of *Bacilli* are reported to be inactivated by approximately $F_0 = 0{\cdot}1$–$0{\cdot}6$ and those of *Clostridia* by $F_0 = 0{\cdot}6$–$1{\cdot}0$ (Brown *et al.*, 1960). The lighter heat treatments used for cured meats are therefore unlikely to have much effect on the load of *Clostridium* spores, and significant numbers of viable spores might be expected to survive in the finished product as is actually observed.

It seems clear, from the observations in this paper, that an important factor in the stability of such moderately heated cured meats arises from the ability of curing salts to interfere with some stage in the germination and development of surviving heated spores, at concentration levels which would be ineffective with unheated spores. It is reasonable to suppose that the phenomenon is a general one, since it has been observed with distinctly different strains of *Bacillus*, and several of *Clostridium*. It would seem worth while to verify that it occurs, in particular, with *Cl. botulinum* and *Cl. perfringens*.

It is equally clear that the details may differ in different cases; for example, the effective concentrations obviously depend on the organism in question, while we have seen mild activation phenomena with one strain of *Clostridium* only. A broad comparison of Figs. 1 and 4 suggests that *Bacilli* are rendered more sensitive to sodium chloride by lower heat treatments than are *Clostridia*. Whereas $F_0 = 0{\cdot}12$ (*B. subtilis*) and $F_0 = 0{\cdot}025$ (*Bacillus* F) caused a considerable reduction in salt tolerance (Fig. 4), $F_0 = 0{\cdot}17$ did not produce a detectable change in the salt tolerance of *Cl. sporogenes* (Fig. 1). Thus, although initially *Bacilli* are often present in greater numbers than *Clostridia*, their spores are generally more sensitive to heat and their tolerance to salt is considerably reduced by comparatively mild heating. It is interesting that this heat sensitization was most pronounced with the organism (*Bacillus* F) otherwise most resistant to sodium chloride. Obviously, organisms with both a high inherent sensitivity and, in addition, high degree of sensitization by heat, are not likely to be recovered from heated cured products.

The above observations could explain the fact, well attested in practice, that a process which controls small numbers of spores is likely to fail with larger numbers. Any particular combination of heating and curing ingredients will, apparently, permit only a certain minute proportion of the spores to develop. If the initial numbers are so small that this minute proportion of them is a negligible number, the process will be satisfactory; but as the initial numbers rise, so will the importance of this fixed proportion, until the absolute number of failures becomes appreciable and the process is deemed to fail.

The findings of Shipp (1962) that mildly heated spores of *Cl. sporogenes* germinated in samples containing 3% sodium chloride, or 2·7% sodium chloride + 0·5% sodium nitrate, but failed to grow in a residual concentration of 167 ppm sodium nitrite at pH 5·7 can be explained entirely by reference to the present study.

In the case of sodium chloride Gould (1964) has shown, with lightly heated spores

of several species of *Bacillus*, that salt concentrations of about 15% are needed to prevent germination, while 4–7% (depending on the species) suffice to prevent out-growth. Similarly with spores of *Clostridium*, circumstantial evidence suggests that the inhibition occurs at the outgrowth of the germinated spore. Mundt, Mayhew & Stewart (1954) observed germination of 90% of the spores of *Cl. sporogenes* in the presence of 8% sodium chloride, and at pH values down to 5·3; but this germination was not followed by vegetative growth. Riemann (1957) found that spores which have lost their heat resistance remain resistant to sodium chloride until outgrowth occurs. Vegetative cells are much more sensitive to sodium chloride than are spores (Mundt & Kitchen, 1951). All this suggests that the effectiveness of sodium chloride, in combination with heat as with radiation (Roberts, Ditchett & Ingram, 1965), lies in preventing out-growth of sensitized spores after they have germinated, because the concentrations used in the present study appear too low to have inhibited germination, though this point awaits direct confirmation.

From the broad similarity between the effects of sodium chloride and potassium nitrate, it seems possible that their influence is in some way osmotic, and perhaps depends fundamentally on the activity of water in the system. This point also deserves more careful verification. In any case, the concentrations of potassium nitrate in cured meats are much below (*ca.* one-tenth) those of sodium chloride, hence it appears that its separate effect must be negligible in practice. Roughly, its effect might be regarded as that of an equal concentration of sodium chloride; as has previously been indicated by others, though on rather different evidence (Bullman & Ayres, 1952).

The effect of sodium nitrite has certainly a different basis; not only are the concentrations involved much smaller, but they are very much dependent on pH. Where we were fortunate to obtain a suitable range of observations, for example in Fig. 3, they appeared consistent with the view that the effective agent is undissociated nitrous acid. For example, Fig. 3 indicates that for spores heated at 80°C for 20 min, the respective concentrations of nitrite needed to reduce the percentage survival to 1%, at pH 7·5, 7·0 and 6·5 respectively, were about 575, 200 and 50 ppm; which is not far from the concentration ratio $10 : \sqrt{10} : 1$ which would be expected on that basis. Similar, though less well defined, relations were indicated with other organisms, for example in Fig. 8 for a *Bacillus*. Gould (1964) has reported a similar pH-dependent effect of nitrite (0·03% $NaNO_2$ at pH 6) in inhibiting outgrowth, and at slightly higher concentrations germination, of *Bacillus* spores. It appears fortunate that the pH of canned meats is normally not far from 6, a degree of acidity sufficient to make nitrite highly effective, but not sufficient to make it decompose rapidly; quite small differences in pH, arising for example from additions of phosphate, might apparently have an important effect on the bacteriological stability of cured meats.

Combinations of these curing salts might conceivably have effects greater than the sum of those indicated here for the individual substances. It also seems that the effect

of salt itself might well bear some relation to pH, though Gould (1964) did not observe this, and that it might be influenced by incubation temperature (Schmidt & Segner, 1964). These matters too deserve more extensive investigation. The present experiments have altogether neglected the effect of temperature, having been conducted for convenience entirely at 37°C, near the optimum for the bacteria tested. It may be that storage temperature is not, at least in the U.K., a factor obviously contributing towards the good commercial and public health record of most canned cured products, because refrigerated storage is never enforced, and seldom even recommended. But it seems probable that the sensitization here reported might be affected, at least in magnitude, by the temperature prevailing while the damaged spore is struggling against the inhibitory salts; because such phenomena are known with both heated (Wheaton & Pratt, 1961) or irradiated (Wheaton, Pratt & Jackson, 1959; Freeman & Bridges, 1959) bacteria on ordinary media.

Nevertheless, though various supporting investigations remain to be made, the phenomena described here seem, for the first time within our knowledge, to offer a quantitatively satisfying explanation of the surprising effectiveness of modest heat treatments in preventing the development of bacterial spores in cured meat products.

Acknowledgments

This work was largely carried out in 1958–60 by Miss J. M. H. Stapp, working under the supervision of the late Dr B. P. Eddy, the project having been suggested by M.I. The data have recently been completed, analysed and prepared for publication by T.A.R.

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PROCEEDINGS
OF THE
INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

Report of a Symposium on 'Food Additives as Food Modifiers' held at the National College of Food Technology, Weybridge, on Tuesday, 28 September 1965

Food Additives and Contaminants

A. KEKWICK

Recent great advances in food technology have demanded a new approach to the problem of food additives. Legislation tended to be prohibitive but this is now being

displaced by the prescription of permitted lists of additives which will eventually embrace all classes of additives and contaminants. Such permitted lists have two advantages: they offer protection to the consumer that his health will be unimpaired, and protection to the manufacturer from malicious gossip or litigation.

Two factors are usually taken into account in considering any addition of an additive to a permitted list: the need for the additive and its safety in respect of human health. These are not separate requirements but are essentially interdependent. The need for any particular additive may vary greatly. In its best form it extends the shelf life of an article to be used as food; an important consideration in a world increasingly short of food. It may carry through from an essential part of processing or it may increase consumer acceptance. There is a clear difference in the importance of each need.

A further difficulty is encountered in considering the need for an additive. Some additives arise as by-products of another industry, e.g. malic and fumaric acids from the plastics industry. These may or may not be important, but the trade will not use them until they are placed on a permitted list and authority will not place them on it as no need for them has been established. Such a situation may become restrictive unless appreciated on all sides.

It is important to realize that safety (freedom from hazard) cannot be proved scientifically since it is essentially a negative. The expensive and comprehensive series of toxicity tests recently suggested by the Ministry of Agriculture, Fisheries and Food (*Memorandum on Procedure for Submissions on Food Additives and on Methods of Toxicity Testing*, Her Majesty's Stationery Office, 1965) seeks rather to define what form the hazard from any food additive might take. It embraces the necessity for a specification, acute toxicity data to define the target organs, long-term testing at levels well above any likely intake, tests for carcinogenicity and the provision of data relevant to the effects of the additive or similar substance in man. Only when the hazard is defined can it be set against the importance of the need for the additive. Many difficulties arise from failure of the industry to appreciate this point about 'safety'. Too often one hears the argument that the substance occurs naturally in human metabolic cycles and therefore must be 'safe'. This is only true if all the components of the cycle are also added; cyanide, for example, occurs during certain human metabolic cycles but would scarcely find favour among any of us as a food additive.

Even after the expensive toxicity tests have all been carried out by competent toxicologists, definition of the hazard to man may still be very difficult. For example, difficulties may be encountered over specification. Commercial samples of diethylene glycol monoethyl ether may contain up to 30% of ethylene glycol which is in itself highly toxic to man. There is little doubt that it could be prepared in a form which contains less than 5% but it must be emphasized that no specification for such a preparation exists at present. Until it does, it would be irresponsible to add it to a permitted list.

Another general difficulty arises in relation to the quality of toxicological data. For example, a publication appeared recently purporting to show that triethyl citrate produced convulsions in cats. On the face of it, it seems highly improbable that this effect could be attributed to the substance itself, since other citrate salts have been widely studied in animals and used in man. Until this matter is cleared up it surely would be wrong to expect authority to add this substance to a permitted list, at least without a warning that this finding must be cleared up. The alleged production by BHT of congenital deformities in rats in 1950 must have taken many thousands of pounds and, what is more important, many hundreds of hours of skilled labour to refute.

Another difficulty arises when animal toxicity data have to be extrapolated to man. Many conditions to which laboratory animals are immune, such as certain allergies, are common in man. The mouse eats one-tenth of its body weight daily, man about one-hundredth; the laboratory animal is almost immune to damage to its central nervous system even with heavy metals while it is man's most vulnerable organ. This only lists a few of the important differences in order to show that they exist and is certainly not an attempt to denigrate screening of food additives in the laboratory, particularly when this is carried out by knowledgeable toxicologists who are fully aware of these shortcomings.

Again, pressure is sometimes exercised to put an additive on the permitted list because it has been included on that of another country. While in general this may be acceptable, it should be realized that in countries with a narrow economy which are often primary food exporters, the type of pressure which may be exerted to obtain inclusion on a list may be political or quasipolitical and certainly may not be in the best interests of the consumer here. It is obviously desirable in this field that agreements about permitted lists should be international, but it is much more doubtful whether it would be prudent at the present time to accept international reciprocity.

Another difficulty is that posed by the question of multiple additives. If permitted lists in themselves are restricted to one additive for each particular technological task (as many would wish), this must in time have the effect of restricting further research into improving these important substances. On the other hand, if more than one additive to perform a single task is permitted, the position immediately arises that a manufacturer can use them in combination. If he does so, they should, theoretically, be tested toxicologically in combination since, though the effect on the food may be advantageous, the effect on the consumer may be the reverse. If more than one additive performing the same function is on a permitted list and they are to be used in combination, the industry should hold itself responsible at least for seeking the advice of a toxicologist and in a few cases further testing will be found to be necessary.

While the food industry seems to me to be vividly aware of its great opportunities to help feed a hungry world, it is at times completely blind and emotionally resistant to the idea that it can do harm to the consumer. I have puzzled over this and I would

like tentatively, and in this audience humbly, to put forward a reason which may explain it. It may be that the highly skilled technologists in the industry are mainly trained chemically, i.e. to deal with the food itself, and not biologically. If there is any substance in this suggestion it could be easily remedied even within this building. For example, in recent months the industry submitted the wish to have a large number of coal tar derivative colouring matters included on the permitted list. These, with some others added for other reasons, totalled forty-two in number. Toxicological data were non-existent in the case of ten of them, and completely useless in the case of a further eleven. This surely indicates a failure to realize that these colouring matters may endanger health. More recently some 1100 flavourings were suggested as necessary to be included on a permitted list. Among the suggestions was coumarin. This substance, whose actions are well known in both animals and man, can promote bleeding. It could be argued that several tons of food would have to be taken to produce such an effect, but it was not realized that several thousand people are being given this or similar substances to produce the same effect following a coronary thrombosis. These patients walk a tight-rope between normality and serious haemorrhage. They are carefully advised to avoid aspirin or any aspirin-containing medicines and to avoid foods which naturally contain a large amount of coumarin. Surely it cannot be doubted that it would be irresponsible to suggest putting coumarin on a permitted list of flavourings.

This is a rapidly growing and important industry; important to the world at large; important to the consumer; important to the nutritionist; and important to each of you. Perhaps the difficulties encountered in this field at present have been overstressed but this has been done in the belief that they can only be overcome when they have been defined and discussed both within the industry and outside.

Discussion

Professor A. G. Ward (Leeds) asked whether arrangements for testing food additives in this country are on a sufficient scale. Of the three main laboratories engaged in this work in the U.K., one belonged to a commercial firm, the second carried out sponsored work and the third was a co-operative Research Association. The only other substantial organization worked solely on irradiation. Where work was carried out for a particular firm it would not be published in the normal way; this could involve costly procedures and duplication to make results public. *Professor Kekwick* thought facilities in this country inadequate. More work of the standard of that done at BIBRA was needed. There should also be more freedom of publication within the industry.

Dr M. Stein (Nottingham) questioned the permitted use of additives such as sulphur dioxide and wondered whether the disadvantages of chemicals were not slightly

exaggerated. *Professor Kekwick* replied that sulphur dioxide, if submitted today, should get clearance since he believed it did not persist in foods and one was concerned only in what persisted into the final product. *Mr R. Butler* (Ashford) said that sulphur dioxide residues occurred in sugar confectionery and the Japanese would accept no more than 30 ppm. Food additive legislation did not seem to consider level of usage; for example, if coumarin is prohibited then strawberries, which contain it, should be prohibited. The difficulty of forbidding the use of 20 ppm sulphur dioxide in biscuits when much larger quantities are permitted in sausages, soft drinks and other foods, was mentioned by *Professor J. Hawthorn* (Strathclyde), while *Dr J. J. Wren* (Lyons Laboratories) thought that people were too conscious of cost. Cysteine might be preferable to sulphur dioxide in biscuits as being more functional and less objectionable toxicologically, but at present it was too costly. *Professor Kekwick* agreed, adding that rather inconstant and indeterminate additives sometimes came from the trade, often which varied in composition from batch to batch. It was almost impossible to advise on toxicity of such additives.

Professor Kekwick, replying to *Mr J. W. Selby* (Leatherhead), said that toxicity information from the American FDA is available to authorities in the U.K. provided the work was not done under contact to a particular firm. In reply to *Mr N. Wookey* (Rank Organization), he said that most laboratories used rodents as test animals, although a non-rodent species is preferred for 90-day and acute toxicity tests. The metabolism of the laboratory rodent has been very well studied but does not differ very greatly from that of man.

Food Additives as Food Modifiers

G. A. H. Elton

Introduction

Food additives have been defined by Dr J. R. Nicholls in an address to the Royal Society of Health in 1958 as substances which are not traditionally used as foodstuffs but which might be added for specialist purposes. The term 'food additives' is preferred to 'chemical additives' since in the widest sense all substances are chemicals of one sort or another.

Classification

Additives are used in food for a variety of purposes which may be broadly classified into five main groups:

(a) Substances which are added to extend the useful life of the food and to delay the onset of deterioration.

(b) Materials which alter the organoleptic characteristics of the food, improve its taste, colour, texture, etc.

(c) Materials which facilitate processing of the food, permitting more efficient manufacturing methods to be used.

(d) Materials which are added for nutritional purposes.

(e) Materials which occur in the food, due to accidental contamination.

The subject of the use of food additives is one on which there have been almost interminable discussions over the last many years, and one which arouses a great deal of emotional discussion, particularly in the popular press.

Food additives will now be considered under the five headings to try to establish whether or not there is a good case for the use of these materials.

(a) *Materials used to extend the useful life of the product.* This heading covers such materials as anti-mould and anti-bacterial agents used to retard the growth of micro-organisms, antioxidants which inhibit oxidative rancidity, anti-staling agents, etc. For example, it is now permitted to add small amounts of propionic acid or its salts to bread in order to inhibit the growth of mould spores on it. The growth of the bacteria responsible for the development of rope may also be retarded by the addition of small quantities of acetic acid to bread. Similarly, in the case of cake, certain preservatives are permitted by law for the inhibition of mould growth. A recent development which we have been studying in our laboratories is the use of carbon dioxide atmospheres for prolonging the mould-free shelf-life of cake. It is perhaps arguable whether this comes under the normal definition of an additive, but it seems unlikely that the use of carbon dioxide for food preservation will be open to objection.

The fats used in the manufacture of baked products normally exhibit sufficient stability to oxidation for oxidative rancidity not to be a problem, but in certain lines, e.g. biscuits intended for export markets, the use of antioxidants is sometimes desirable in order to ensure that rancidity is not a determining factor on storage life.

In addition to deterioration due to the growth of micro-organisms, the shelf life of bread is limited by its ability to stale and here anti-staling agents such as glyceryl monostearate perform a useful function, either by reducing the actual rate of staling or by extending the time required to reach an unacceptable level of staleness.

Under this heading can be included some materials which might legitimately be considered under heading (e), accidental contamination. This refers to materials such

as insecticides, fumigants, etc., which are used to disinfect raw materials such as wheat, and which have found their way through into the final product. It is not intended that such additives be present in the final food, though in effect they often amount to *deliberate* additions since it is known that carry-over occurs, and in the case of the systemic insecticides and fungicides it is inevitable that it should do so. It is perhaps of interest to refer here to some unforeseen hazards involved in the use of insecticides or fungicides, which have recently received a great deal of attention in the U.S.A. and elsewhere following the publication of Rachel Carson's book *Silent Spring*. Nevertheless, given the assurance that testing methods are adequate, it would be almost universally accepted, particularly in under-developed countries where food is in short supply, that the use of any reasonably safe additive which will increase the supply of food available to the population is justified. It has been estimated that of all food grown in the world some 20% is lost due to the depredations of insects, rodents and other pests, so that any methods of reducing this spoilage, such as better husbandry and hygiene precautions, the use of selected insecticides, etc., is to be welcomed. In the more developed countries where food is generally not in short supply the need for the use of food additives to increase the useful life of the food arises from the tendency for the processing of foods in such countries to be concentrated in centralized production units which make for more efficient production. As a result of this, food is often distributed over a wide area and, despite all efforts, may sometimes not reach the consumer as quickly as would be the case in a community with a stronger agricultural bias. In such cases, the controlled and careful use of food additives of this type can make available to the public a much wider range of palatable products than would otherwise be the case.

(b) *Materials which alter the organoleptic characteristics of the food.* These comprise those substances which perhaps most obviously modify food characteristics. Their use is largely confined to the developed countries, and they include such materials as colourings, flavourings, emulsifiers and stabilizers, humectants, etc. Most of these are already subject to control by government legislation, and an official report on the use of flavouring agents has just appeared.

There are sound physiological reasons why food should be made as attractive and appetizing as possible and colourings and flavourings are widely used for this purpose. Enhanced palatability may also be brought about by modifications in texture or consistency, and such changes may also assist the production of convenience foods for use in the home. A large number of foodstuffs are colloidal emulsions or foams, e.g. creams, sponges, etc., so that emulsifiers and stabilizers, natural or synthetic, play an important part in their preparation.

The use of colourings and flavourings may also be desirable to standardize the appearance and flavour of products made from raw materials of varying characteristics, and

occasionally to retain an appearance and flavour to which the consumer has become accustomed when it is necessary to use a raw material of different origin for political or economic reasons. In extreme cases the additive may enable a texture to be obtained which cannot be achieved by other means as with the use of chlorine-treated flour in high-ratio cake.

Such alterations in organoleptic characteristics as have been referred to above are fully justifiable and would be accepted by the majority of reasonable people as of benefit to the consumer. However, the use of additives for these purposes should never be such that the consumer is misled into thinking the product is of higher quality, or of greater nutritive value, than it actually is. Nor should such additives ever be used to conceal faulty processing techniques.

(c) *Materials which facilitate processing of food.* The manufacture of prepared and semi-prepared foods today frequently involves the use of large-scale production techniques with a high degree of mechanization. This is inevitable in modern society where such techniques are becoming increasingly important in keeping down the cost of foodstuffs. Food additives used as processing aids are of value in making feasible the use of such large-scale production methods. Their use for these purposes may range from fairly obvious applications, such as the use of an additive to facilitate the free flow of a powdered material or a stabilizer to ensure that a filling cream remains stable in consistency over long periods, to more sophisticated and sometimes more controversial applications. For example, the additive may be used to overcome ingredient variations and hence ensure that a product of even composition and quality is produced. The baking industry provides a number of examples of such applications. It is desirable in breadmaking that doughs of approximately uniform rheological consistency are produced at the mixing stage. This is not always easy to arrange because of variations in flour quality, but alterations, which can be predicted from laboratory tests, of the amounts of the so-called oxidizing improvers, enable these variations in consistency to be overcome with consequently increased uniformity in the bread produced. Similarly, in the conventional breadmaking process the fermentation characteristics of the flour have a considerable effect on the resultant bread. Such characteristics may vary widely and for this reason it is often necessary to supplement the natural enzymes in the flour by making small additions of α-amylase, sometimes in the form of malt flour, sometimes in the form of an enzyme concentrate. Some advantage is gained, particularly under American conditions, by the addition to bread doughs of small quantities of proteolytic enzymes. Such enzymes achieve their effect on rheological properties by modifying the wheat protein as do oxidizing improvers, though by a different mechanism.

The rheological properties of biscuit doughs are a major determinant of the characteristics of the finished biscuit. Here it is desirable to produce a dough with minimal

elastic recovery, but this again is influenced by the characteristics of the flour used. Such absence of elasticity enables distortion of the finished biscuits to be minimized, and can be achieved by the treatment of the flour with small amounts of sulphur dioxide. The sulphur dioxide does not remain as such in the finished biscuits. These desirable visco-elastic properties of biscuit doughs may also be achieved by the use of proteolytic enzymes, but it is necessary to exercise careful control of their action.

(d) *Materials which are added for nutritional purposes.* An excellent example of the addition of food additives for nutritional purposes is given by the statutory requirement in this country for the addition of token nutrients to flour. All flour, with the exception of wholemeal flour, is required by law to contain minimum amounts of vitamin B_1, nicotinic acid, iron and calcium. This is achieved by the miller by making suitable additions before the flour is sold. It may be that the majority of the population on a good mixed diet do not benefit from these additions but, in the opinion of the government advisers, there are certain elements of the population, particularly among the older age groups, who would suffer from malnutrition if the addition of these substances were discontinued.

A further interesting example is the possible addition of amino acids to foodstuffs containing vegetable protein with a view to upgrading the nutritional value of that protein to the equivalent of first-class animal proteins, e.g. it has been demonstrated recently that the addition of lysine to bread produces a great improvement in the protein dietary value of this foodstuff. It is relevant here to consider the use of additives, the sole purpose of which is to reduce the nutritive value of a food. In this category are included cellulose derivatives which are used as an aid to slimming by reducing the calorific value of certain foods. Similarly, the use of the artificial sweeteners, saccharin and cyclamates, achieves the same purpose. In a similar category falls the use of special additives for dietetic purposes. For example, the addition of sorbitol to jam for diabetics.

Another recent and highly controversial example of an additive being used for nutritional purposes is the addition to some public water supplies of trace amounts of fluoride.

The addition of substances to food for nutritional purposes is a matter for careful consideration by experts. In this country, the government is showing an increasing tendency to protect the consumer from nutritional claims based on false premises, or unjustifiably exaggerated.

(e) *Accidental contaminants.* This heading can include a very wide range of substances. One example would be slight contamination from oil used to lubricate food processing machinery. For this reason, parts of machinery used in the baking industry which are likely to come into contact with food are lubricated with purified mineral oil and there are regulations governing the maximum level of mineral oil which can

occur in bread. Other accidental contaminants can arise in a variety of ways. Recently there was a case of the occurrence of 1 ppm of silicone from barrier hand cream in sponge cakes. The presence of this material, which is well known as a good anti-foaming agent, was disastrous, producing complete collapse of the sponges.

There is one aspect of the problem of accidental contaminants which has recently been attracting considerable attention. The packaging of foodstuffs is being carried out on an ever-increasing scale, and generally this development is to be welcomed because of its contribution to improved hygiene and elimination of a number of potential hazards which have existed hitherto. However, a very wide range of packaging materials is now employed and new ones are likely to emerge quite regularly, and the possibility must be examined of contamination of the foodstuffs from these materials by such processes as leaching of specific constituents employed in their manufacture. For example, plasticizers used in preparing plastic films may be volatile and their chemical constitution must therefore be carefully considered. Wrapping papers containing traces of copper and iron may catalyse the development of oxidative rancidity if used to wrap fat or fat-containing materials. These subjects and related ones are being studied by various groups including a Committee of the Institute of Packaging on which various Research Associations and other bodies are represented.

All responsible food processing firms take the utmost care to ensure that accidental contamination of foodstuffs is eliminated altogether or reduced to an absolute minimum. The legislating authorities are rightly insistent on the maintenance of a high standard of hygiene and careful manufacturing practice, and the occurrence of accidental contamination of food is nowadays very rare.

Research on food additives

A great deal of research on the pharmacology, toxicology and general safety aspects of food additives is in progress in various laboratories throughout the world. One might particularly mention the U.S. Food and Drug Administration and the British Industrial Biological Research Association. Semi-empirical development work on the production of new food additives for specific purposes is also going on in many industrial and official laboratories. However, it seems that there is scope for more research on the mechanism of the action of additives of various types. Research of this kind could lead in some cases to the development of more efficient additives or to the more economical utilization of existing additives or possibly, in certain cases, to the elimination of additives and their replacement by other methods of modification of the food. In some cases where the mechanism of action of additives has been studied, it has been found that they exert their influence through a most complicated chain of events. For example, small amounts (75 ppm) of ascorbic acid (vitamin C) are used in some bread-making processes to increase loaf volume. The mechanism of the action of this substance is roughly as follows: the ascorbic acid is converted by atmospheric oxygen (with

the assistance of the enzyme ascorbic acid oxidase) into dehydroascorbic acid. Dehydroascorbic acid can (with the assistance of the enzyme dehydroascorbic acid reductase) oxidize sulphydryl groups which are present in the protein of the flour. The reduction in sulphydryl group content which is brought about reduces the breakdown of the three-dimensional protein network present in the dough, which would normally occur as a result of sulphydryl–disulphide exchange.

There appears to be an ample quantity both of ascorbic acid oxidase and of dehydroascorbic acid reductase naturally present in flour to maintain this oxidation–reduction cycle. In this way, the reducing agent ascorbic acid acts as an 'oxidizing' improver with properties very similar to those of substances such as potassium bromate, potassium iodate and ammonium persulphate. However, from the pharmacological point of view, ascorbic acid is regarded as being more acceptable by the Food Standards Committee and as a result of the development of new processes its use in breadmaking is spreading rapidly in this country.

Other examples of cases where we could benefit from fundamental research on the mechanism of the action of additives include the fields of anti-mould agents, anti-staling agents, emulsifiers, enzymes, etc. It is pleasing to note that fundamental research on colours and flavours is to form part of the programme of the new Food Research Institute at Norwich.

Legislative aspects

It is appropriate at this point to refer to one important legislative aspect of the use of food additives. These substances are now so widely used throughout the world at one stage or another in food production and processing that they are inevitably present in foods exported from one country to another. Unfortunately, the legislation controlling the use of additives in particular countries shows large variations which may lead to grave difficulties in the exporting of a given foodstuff.

Since the war, there has been an increasing consciousness of the need to unify regulations. The Common Market countries have decided to co-ordinate their food laws and have made considerable progress in this direction, but a much more ambitious attempt to unify food legislation is that initiated jointly by the Food and Agriculture Organization of the United Nations and the World Health Organization. These bodies have created a Codex Alimentarius Commission which aims at comprehensiveness on a world basis. It is to be hoped that this body will be successful in removing at least some of the problems at present arising from variations in the laws governing the use of additives in different countries. No one will dispute the necessity for thorough examination of additives before passing them for use in foods but, subject to this overriding safeguard, food additives have a useful and indeed essential part to play in the food industries of the world.

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Discussion

Dr J. B. M. Coppock (Spillers Ltd) said that since the whole question of food additives is fraught with potential toxicological dangers, great care is taken. Nevertheless, there can be risks even in some of the most innocent substances. Dr Elton discussed flour and bread improvers and could have touched on the Chorleywood Bread Process, which uses ascorbic acid (vitamin C) as the improver. One can take less objection to vitamin C than to most substances, nevertheless it here is acting as an oxidizing agent, not as vitamin C, and one may ask whether it should be tested toxicologically like other flour treatments. Other natural substances, such as amino acids, may be used for fortifying animal foods and there is danger that too much may lead to imbalance with resulting physiological effect on tissue growth. He asked Professor Kekwick's views on the use of lysine and other amino acids as food additives. He mentioned also the use of substances which delay or prevent disease, e.g. coccidiostats, and direct growth stimulators such as arsanilic acid and nitrohydroxyphenylarsonic acid. These latter have been found to help in overcoming a soil selenium problem in the U.S. and indeed were growth stimulants. Provided such drugs are withdrawn at the right time, all may be well but there may be risks in delay. There are perhaps further risks depending on whether poultry metabolize organic arsenic completely or whether some remains in the faeces, which may then be used for manuring, e.g. mushroom beds. The soil organisms might perhaps decompose organic arsenic compounds and produce inorganic arsenic hazards.

He asked Professor Kekwick's opinion on whether more official control of the use of these substances was required. Chemists at university learnt little toxicology; he believed potential industrial chemists should be given some training to make them realize the dangers.

Professor A. Kekwick replied that most of the submissions over a number of years had been drawn up by chemists with no biological training. Biological training should be part of the course for chemists going into food technology. Protein and amino acid imbalances and the diseases caused by them are well known. Antibiotics, which are the chief growth stimulants here, are carefully controlled by regulations.

Mr J. W. Selby (Leatherhead) mentioned the use of ascorbic acid as a colour preservative in cooked cured meats.

Dr Elton agreed on the need for research into the mechanism of the action of additives. More efficient additives might be developed. Ascorbic acid, a good example of this need, operates as a bread improver in a complex manner. Ascorbic acid oxidase naturally in flour, with atmospheric oxygen, converts ascorbic acid to dehydroascorbic acid. This efficient oxidizing agent reacts with sulphydryl groups in protein producing cross-linking which prevents disulphide–sulphydryl interchange. It does this through another enzyme naturally in flour, dehydroascorbic acid reductase. Thus ascorbic acid, naturally a reducing agent, has an oxidizing action in flour.

Dr M. Stein (Nottingham) mentioned that sulphur dioxide might be combined in food but released during digestion, but *Dr Elton* replied that in biscuit flours sulphur dioxide was normally at the low level of 20 ppm and could not be detected in the finished product. The Food Standards Committee in the Bread and Flour Report, 1960, stated that they found no evidence suggesting health hazards at 20 ppm in biscuit flours.

Dr W. E. Elstow (Weston Research Laboratories) asked what constituted an additive as distinct from a food. *Dr Elton* defined food additives as substances not traditionally used in food. *Dr A. E. Bender* (London) said one function of the food scientist was to spread information to the public, fellow scientists and particularly pure chemists whose products might be used in food processing.

Dr Elton, in reply to *Dr J. Tampion* (Epsom), said that variations in climate, soil, etc., made it difficult to overcome completely biological variation, but that research was continuing on improvement of wheat quality, its disease resistance, yield, suitability for bread making, etc.

Phosphates as Food Modifiers

C. W. Tod

The phosphates used as food additives are mainly inorganic salts, particularly sodium and calcium salts, but potassium, ammonium and iron phosphates are all included to some extent in foodstuffs. There are also some organic phosphates in use; there are two types of starch phosphates and there is the food emulsifier permitted in the U.K. which is a glycerol ester of fatty acid and phosphoric acid.

In the inorganic compounds we are dealing with a series of phosphates, the simplest

being the orthophosphates, which are the salts of orthophosphoric acid, a tribasic acid. It is possible to produce three orthophosphates for each metal and in fact with some metals there are also basic salts. Of the sodium orthophosphates, only the mono- and disodium phosphates are usually considered in food, the trisodium phosphate being rather too alkaline, although it has been suggested at times for the processing of acidic cheeses.

The next in the series is pyrophosphoric acid, in which there are two phosphorus atoms per molecule. This has four replaceable hydrogen ions in each molecule and they can be replaced separately although the common salts are those in which two and four hydrogen ions are neutralized.

Continuing up the series there is tripolyphosphoric acid or triphosphoric acid; although products can be made in which partial neutralization is achieved the only common tripolyphosphate is the pentasodium salt. This is produced in considerable quantities for incorporation in domestic synthetic detergents but there are also some food uses for it.

The next in the series, with four phosphorus atoms per molecule, cannot be prepared as a crystalline sodium salt but its presence in solution can be demonstrated. With more complex salts than the tripolyphosphate the products are usually glasses. These are prepared by melting monosodium orthophosphate, with more or less disodium phosphate, and as one would expect, they can have a range of molecular weights. It is possible commercially to obtain the sodium glasses with an average molecular weight from about 400 up to about 2500 but the product most commonly sold on the British market has an average molecular weight of about 1200.

An even more complex phosphate used in the food industry is the potassium Kurrol salt, which can have a molecular weight running into millions. This is a very complex phosphate with some cross-linking of the chains when it is produced by melting phosphates containing less potassium than is required for KH_2PO_4. It is insoluble in water but can be made to dissolve in solutions of sodium or ammonium salts to give viscous solutions.

Phosphates include a whole range of products all based on repeating P–O–P–O chains. Although the glasses are usually called metaphosphates they are essentially not true metaphosphates and are really polyphosphates since they must have either an excess of Na_2O or H_2O to end the chains. The only true metaphosphates commonly available are the tri- and tetrametaphosphates, which are cyclic products and were at one time thought to have little commercial use; the trimetaphosphate has, however, been used in the production of one of the starch phosphates, in which it is a cross-linking agent.

Although there are a number of other phosphorus compounds available as sodium salts, in general they have no interest for the food manufacturer, an example is hypophosphite, which has been used as a pharmaceutical for many years.

What are the properties of the phosphates which make them of interest to industry in general and the food industry in particular? The one responsible for the biggest industrial use is undoubtedly sequestering power, by which condensed phosphates are able to take metal ions into soluble complexes. The first real appreciation of the value of this property arose in America, where one of the glassy phosphates was applied as a means of softening water by the formation of complexes of calcium and magnesium which resisted precipitation by soap.

Other metal ions of interest which are sequestered are iron, copper, and zinc. The stability constants of the complexes which these metals form are lower than those with EDTA but the sequestering power is sufficiently great to make it of importance to some purposes.

A property which the phosphates also possess is that of being useful buffers. This applies essentially to the lower phosphates as the phosphate glasses have no appreciable buffering capacity, although they acquire it by conversion to ortho- and pyrophosphates when they are hydrolysed.

Another property of interest is emulsifying and dispersing power, which can be demonstrated on some oils. Phosphates are, however, by no means what one would describe as emulsifying agents for the production of stable emulsions.

The complex phosphates will also precipitate proteins as everyone who has tested for so-called metaphosphate with egg albumen solution will remember.

With regard to the safety-in-use in foodstuffs of these phosphates, it is of interest to consider what the F.A.O./W.H.O. Expert Committee has to say on the phosphates. They virtually treat all phosphates alike on the assumption that in the body in a medium of low pH and the presence of enzymes they are all degraded to the orthophosphate. This is certainly a very reasonable assumption to make and is in accord with the available evidence. F.A.O./W.H.O. have two levels of intake, one 'unconditional' up to 30 mg/kg body weight/day and the other 'conditional', 30–70 mg/kg. As far as can be seen this would allow quite a reasonable margin between what is used in this country and the maximum suggested intake.

After reviewing the properties which the phosphates possess, it is perhaps rather surprising to think that the biggest use of phosphates in food depends essentially on the availability of solid acidic compounds. This is in the aeration of baked goods where two phosphates are commonly applied over the whole world. These are acid calcium phosphate and acid sodium pyrophosphate. These two phosphates provide quite a difference in rate of reaction with bicarbonate, the acid calcium phosphate being particularly fast and the acid sodium pyrophosphate giving one of the slowest rates of reaction of any practical product. The importance of having a slow rate of reaction for some purposes is in the first place so that too much gas will not be lost as the baked goods are being formed and also that there should be some left to provide what bakers call 'late lift'—in other words there must be some

carbon dioxide available for expansion in the oven when the dough is nearly set.

One interesting feature of the slow reactivity of the pyrophosphate is that it is only demonstrated if one determines the rate of reaction of the pyrophosphate with bicarbonate in milk or when other calcium salts are present. The reaction with bicarbonate in water alone is rapid.

As a simple division between the applications of these two phosphates one might say that the acid calcium phosphate is usually applied in self-raising flour for domestic use and the pyrophosphate in the commercial bakery. In addition to this, however, it is customary to have pyrophosphate in domestic baking powder where the acid calcium phosphate, if used alone, does not provide sufficient stability in the tin. Here it is also sometimes desirable to add a faster product along with the acid sodium pyrophosphate, and for this purpose it is customary to add a small amount of acid calcium phosphate or perhaps tartaric acid.

Of the uses which depend on the sequestering property of the complex phosphates, one of the simplest applications is that in which they prevent the appearance of magnesium ammonium phosphate crystals (struvite) in canned shell fish. Here one appears to be dealing simply with the sequestering of magnesium, although some doubt could be cast on this by an unsuccessful trial we had with pyrophosphate, known to be at least as good a sequestering agent for magnesium as the more complex phosphate. However, this failure might be due to low solubility of pyrophosphate in the broth.

An interesting use is in the prevention of the blackening of potatoes. This discoloration occurs after cooking and it seems to be agreed that iron is a component of the dark colour which is produced. The suggestion is that the iron in the potato is present in the ferrous form, which is more easily sequestered by complex phosphates than is the ferric. Apparently during cooking the *o*-diphenols with iron form colourless compounds, which on exposure to air change to the coloured ferric compounds. At all events, it has been shown that by dipping the potatoes before cooking in a 2% acid sodium pyrophosphate solution there is a reduction of blackening; the minimum effective level is said to be 1·5%.

One interesting example of sequestering power is that in which phosphates have been used to help to reduce rancidity in fats. There have been a number of papers from American workers, particularly Betty M. Watts, in which complex phosphates have been suggested as synergists of the usual antioxidants and presumably they are acting as sequestering agents for trace elements such as copper.

Another application which might very well depend on sequestering is in the processing of various meats. From the literature, there is sufficient evidence to believe that the sequestering of calcium, magnesium or possibly zinc could perhaps be playing some part. This has been hinted at in some German papers on this subject but contradicted in others.

The phosphates generally used in the treatment of meat are the pyrophosphate, tripolyphosphate and glassy phosphates, although this is also one of the products for which the complex potassium Kurrol salt is used.

In ordinary meat there are two applications, one in the treatment of hams, where phosphates are added to the pickle; a limitation here is that very complex phosphates such as the potassium Kurrol salt are not soluble in brine and equally so is the pyrophosphate, so that one is restricted to substances like tripolyphosphate and the glasses. Here the main claim is that phosphates reduce cooking losses but there are a number of subsidiary claims.

The other major use is in sausage manufacture. This was almost certainly first done in Germany where they were considering the type of sausage in which the meat itself provides the binding power. It was found that the addition of the phosphate to old meat restored the binding power normally present in fresh meat. How it does this and whether the phosphate itself is a binding agent have been the subject of a number of papers, mostly in the German literature. However, Dr Bendall has also published a paper on this subject in which he came to the conclusion that the action of pyrophosphate was due to its ability to split the link between the two components of actomyosin.

Emulsifying power has also been suggested as a reason for using phosphates in sausages and perhaps there is more justification for considering this property in the 'British sausage', in which a cereal binder is used. One of the claims for phosphates in this type of sausage is that there is a reduced loss of fat in cooking and there are also claims for improved taste. The reduced loss of fat may be due to increased extraction of water-soluble, heat-coagulable protein which could improve emulsification (or dispersion).

In addition to ordinary meat, however, fish is quite often treated with phosphates prior to freezing. The intention here is to reduce thawing drip and this claim seems to be very well substantiated, particularly by work in the U.S.A. The 1964 report of the Torry Research Station also mentions that treatment of wet fish before packing prevents the separation of water even when the fish has not been frozen and thawed. This is almost certainly an application of the same phenomenon. Incidentally Torry suggest in the same report that the reduction of thawing drip is due to swelling of the muscle cells on the outside which prevents the loss of the water from the fillet.

Another meat which can be treated is chicken and there has been some application in the U.S.A. to frozen cooked chicken, this being the only form in which phosphate treatment is allowed in that country. It is true that when fresh chicken is frozen and thawed the amount of drip is less than when the phosphate is not present in the soaking liquor but this appeared to us from our tests to be due to the reduction by the phosphate of the water uptake. One interesting claim here, however, is the greater stability

against oxidative deterioration and we have certainly demonstrated this by the TBA test.

The recommended concentrations of solutions are as follows: for fish a 10% or 12% solution and for chicken about a 6% solution. For hams 5% phosphate in a 10–15% brine and for sausages 0·5% on the weight of the meat is suggested.

Another group of applications is those in the dairy industry. One is in the production of evaporated milk where in order to arrest coagulation it is common practice to add disodium phosphate. The amount is quite small, around 0·025% of the anhydrous salt. For this application orthophosphate is favoured.

On the other hand, there are references in American literature to the use of polyphosphates in high-temperature short-time milk concentrates. Here it is said that polyphosphates are particularly effective in retarding age-thickening of these concentrates, and one phosphate said to be particularly suitable is a short-chain glass.

For many years now phosphates have been added in the processing of cheese, in which cheese is melted and a solution of a phosphate mixed in before the cheese is deposited in the moulds. There is a good deal of empirical information on processed cheese manufacture and there is certainly a variety of emulsifying agents in common use, citrates and tartrates as well as several phosphates. Orthophosphates are fairly commonly used but so also are the pyrophosphates and the phosphate glasses.

It is fairly general practice to vary the alkalinity of the emulsifying solution according to the acidity of the original cheese but there is also a specific difference with condensed phosphates which are thought to cause cross-linking of the protein molecules and give a stiffer cheese.

Betty M. Watts and two other workers also describe the effect of sequestering agents in improving the whipping qualities of non-fat milk solids. Glassy phosphates and tripolyphosphate are said to be superior to the other agents tried; they also improve the whipping properties of dried whole eggs and soya bean flour.

An interesting use in connection with milk products is in instant puddings. Here milk is made to gel in the presence of tetrasodium pyrophosphate and pregelatinized starch. This makes a cold-setting pudding available in a very short time. It is general practice to add, in addition to the tetrasodium pyrophosphate, an accelerator, usually an orthophosphate or a calcium salt. There seems to be some specific action of the tetrasodium pyrophosphate which has been surmised to be an interaction with the casein. The type of tetrasodium pyrophosphate also seems to be critical.

The milk used for this instant pudding is of importance and we know that if one removes all the calcium by softening then the gelling does not occur. Equally if we use a condensed phosphate with high sequestering power for calcium salts, such as a phosphate glass or tripolyphosphate, the gelling effect does not take place.

Discussion

Mr Selby (BFMIRA) said that although they had done considerable work on phosphates in sausages they had been unable to throw light on the mechanism involved. Significantly less fat was lost on cooking but the sausages developed a firm texture found unattractive by some tasters. If used to prevent struvite in canned fish, the polyphosphate would be largely hydrolysed during retorting and the sequestering power would have to persist for many months, since struvite formation occurs slowly. Dr Taylor (BFMIRA), who was not present, regretted the complicated patent situation.

Mr Tod replied that the basic patents on meat processing with phosphate will end in 1967. The glassy phosphates would probably be largely hydrolysed during autoclaving but short-chain phosphates which sequester magnesium as effectively might still be left.

Dr H. Fore (Weybridge) thought the user of food chemicals, not the manufacturer, should be ultimately responsible for safety-in-use. Perhaps chemical manufacturer and food manufacturer could jointly finance the testing of a new additive.

Mr Tod, answering *Professor Hawthorn* (Strathclyde), said that addition of phosphate to food was increasing only *pro rata* with the population. A rapid post-war rise due to greater use of baking powder when eggs were scarce had not been sustained. The average U.K. figure was 150 mg P/person/day; an adult eating considerable amounts of sausages, scones, instant puddings and drinking certain soft drinks might digest 1·3 g P/day as added phosphate. He agreed with *Miss M. Dixon* (Arthur D. Little Ltd) that tetrasodium pyrophosphate probably gave a taste to instant puddings.

Mr H. J. Palmer (Beckenham) thought phosphate emulsifiers functioned in meat as in cheese processing. In other words, addition of phosphates to meat, is only of use if the meat is heat-treated. The Germans long ago during manufacture of processed cheese tried adding metaphosphate; this slowly hydrolysed and after a few months the cheese became sour, acid and granular. *Mr Tod* replied that in hams phosphates reduced cooking losses but the effect was noticeable on raw fish. Phosphate glasses were still sometimes used in processing of cheese; alkaline phosphates could be used to prevent excessive lowering of pH.

Mr T. McLachlan (London) asked how a small quantity of polyphosphate sequestered a much larger quantity of magnesium ammonium phosphate. Also, how phosphates emulsified fat in meat if phosphates increased the amount of coagulable protein.

Mr Tod answered that according to Gorton Pew Fisheries Co.'s patent (USP 2,555,236) the quantity of glassy phosphate for canned shellfish was fifty times the magnesium content of the fish; this is above the usually accepted figures for sequestration of magnesium. Phosphates increase the amount of soluble protein and presumably therefore improve emulsion stability; this effect may persist after the cooking and coagulation of the protein. The effect in reduced jelly formation with hams and fat loss in sausages is well established.

Enzymes as Food Modifiers

W. W. Bryce

The nature of enzymes

Enzymes are biological catalysts, elaborated by all living cells, and they exert the necessary control of the sequence of chemical reactions which serve to produce and maintain living matter. Once elaborated by a cell, an enzyme can act independently of the cell if a suitable environment is provided.

Sources of industrial enzymes

It is one of the problems of the commercial enzyme producer to choose suitable cellular material with a high content of the desired enzyme and a minimum content of other enzymes which might interfere in a given application. It is essential that such starting material should be non-toxic, or non-pathogenic, and should not give rise to toxic products in the expected application.

The principal industrial enzymes and their sources are given below:

(a) Plants (malt diastase, papain, bromelain, ficin and urease).

(b) Animal organs (pancreatic enzymes, pepsin, catalase and rennin).

(c) Micro-organisms (amylases, proteinases, pectinase, glucoseoxidase and cellulase).

Micro-organisms are the preferred source of enzymes since they are less subject to production and supply problems which may occur with plant and animal sources.

It may be anticipated, however, that as knowledge increases use will be made of tissue culture techniques to grow animal and plant cells, as a source of enzymes, in much the same way as micro-organisms are cultivated today.

In utilizing micro-organisms for enzyme production the basic skill of the producer

consists in the selection and screening of organisms, and their maintenance. Further essentials are the selection of environmental conditions for growth and enzyme production, such as medium composition, air or oxygen supply, temperature and pH control, etc.; and the maintenance of aseptic conditions. It should be emphasized that optimum conditions for growth are not necessarily those for maximum enzyme production and sometimes a compromise has to be effected.

Enzyme recovery and purification

It is first necessary to obtain a clear aqueous solution of the enzyme. In the case of animal or plant material it may have been first dried at low temperature or frozen to ensure stability of the enzyme content during transport to the enzyme manufacturer. Subsequently the material may require mincing, or de-fatting with solvents, prior to extraction with water or suitable buffer solutions, and filtering. The exocellular enzymes produced by the culture of micro-organisms are released into the fermentation medium, and may be recovered in solution by filtration in the presence of a filter aid.

In some instances, however, the enzymes are endo-cellular. In such cases the organisms are recovered by filtration, and the filtrate is discarded. The cells are then slurried in water or salt solutions and are allowed to autolyse, or are destroyed by mechanical means. The cell debris is then removed by filtration or centrifugation to yield a clear extract.

Subsequently the enzyme extract is concentrated at low temperature under vacuum. The concentrate may then be spray dried or dried on an inert carrier to give a solid concentrate. The choice of drying technique depends on the characteristics of the enzyme system, and on whether a completely soluble product is desirable.

If a liquid product is desired it may be obtained by dilution of the concentrate to a standard enzyme activity. More purified products may be obtained by precipitating the enzyme from the liquid concentrate by means of solvents such as isopropyl alcohol or acetone. In such cases the process must be carried out in the minimum of time, at low temperatures, since prolonged contact with solvents frequently leads to high losses due to enzyme inactivation.

The precipitation is usually carried out in the presence of carrier materials such as lactose or starch, in order to obtain a precipitate with the desired properties. It is recovered by filtration or centrifugation, and the recovered cake is dried at low temperature. Throughout all processing, temperature and pH must be carefully controlled to maintain maximum enzyme stability, and the addition of reducing agents or other stabilizing agents may be necessary.

Subsequently the dried material is ground, sifted, and utilized in the formulation of enzyme products for particular applications. This formulation usually involves blending with inert materials, such as salt, starch or sugars, to adjust the enzyme activity to the level desired for application.

A more detailed description of the production and use of microbial enzymes for food processing is contained in a recent paper (Beckhorn, 1965).

Some properties of enzymes

In conducting research in enzyme chemistry, and in making kinetic studies with pure substrates, it is necessary to use enzymes of a far greater purity than those used in industry. Many enzymes have been purified to the point of forming crystalline materials which possess maximum activity, and which appear homogeneous when a variety of tests are applied to them.

In all instances these purified enzymes have been shown to be proteins, which may or may not be associated with a non-protein prosthetic group. The non-protein prosthetic groups may be divided into two classes: (a) specific coenzymes, usually organic molecules of somewhat complicated structure, which play a part in the enzyme reaction itself, often as carriers or transferers of some chemical grouping, and (b) cofactors of a very simple nature such as inorganic ions which in various ways bring the enzyme protein itself into a catalytically active state.

The primary structure of the enzyme protein is formed by the linkage of amino acids through peptide bonds to give a long polypeptide chain. The secondary structure is formed by the polypeptide chain assuming a spiral or helical structure throughout the whole, or greater part of its length.

The tertiary structure is formed by the folding of the helix into a globular, ellipsoid or similar form. This structure is stabilized partly by hydrogen bonding between the folds of the helix, and by Van der Waals forces between non-polar groups in amino-acid side chains. Salt linkages and disulphide bonds are also involved in maintaining the specific tertiary structure. The tertiary structure of a protein is the most labile, but for many enzymes small changes in this structure are reversible under mild conditions. Thus changes in pH of the aqueous environment over a narrow range may bring about such a reversible modification of the tertiary structure, due to changes in the induced charges on the amino-acid side chains. Larger pH changes are liable to bring about irreversible changes in both secondary and tertiary structures, i.e. denaturation of the enzyme.

Excessive heat also brings about denaturation of enzymes. However, within limits, the effect of raising the temperature of an enzyme reaction is to increase the reaction rate, as is the case in other chemical processes. As a rough generalization, the rate of increase is $1\frac{1}{2}$–2 times for each 10°C rise in temperature.

The optimum temperature for an enzyme reaction is reached when the effect of faster reaction rates at the higher temperature is balanced by the rate of enzyme denaturation. Due to the effect of continued enzyme denaturation at higher temperatures, the longer the time of the reaction, the lower is the optimum temperature.

It will be apparent that pH, temperature, and time of reaction are dependent variables, and that therefore optimum conditions will vary with the desired application. A certain degree of trial and error is therefore necessary in arriving at the most convenient and economical use of commercial enzymes.

It is frequently observed that the presence of the substrate serves to protect the enzyme partially from adverse conditions of heat or pH. Thus, provided only a moderate degree of modification of the substrate is required, and the duration of treatment is short, adequate enzyme action will often take place under conditions in which a simple enzyme solution would be destroyed.

Enzyme specificity

There are now nearly 1000 well-defined enzymes. However, despite this large number, they are known to catalyse only about six reaction types. The multiplicity of enzymes is due to their specificity for parts of the substrate other than the groups acted upon.

It is believed that the substrate is temporarily combined with the enzyme, and that as the substrate is approached by the enzyme, it induces a change in the coiling of the enzyme protein, i.e. the tertiary structure. In the case of the true substrate, the catalytic centre of the enzyme is located in the right place relative to the sensitive bond in the substrate which is to be broken, and hence the reaction can proceed (Koshland, 1960).

If an attractive group is missing from the substrate, a portion of the enzyme molecule is not held in the correct orientation, and no reaction takes place.

The amino acids involved in the active centre of the enzyme are not necessarily arranged in sequence in the enzyme molecule. Due to the coiling of the tertiary structure, they may be brought into close proximity spatially, although they may be separated on the polypeptide chain.

The spatial orientation of the component amino acids of the active centre is, of course, influenced by the flexibility of the enzyme protein and of its substrate. The degree of flexibility will influence the degree of specificity, i.e. whether the enzyme is able to attack only one compound giving complete specificity, or a group of similar compounds, that is group specificity.

It is the specificity of action of enzymes, coupled with the ability to stop their action by heating or pH changes, which commends their use to the food manufacturer. Time is limited so we must now turn to some practical aspects of the application of enzymes in the food industry.

Some suggestions as to when enzymes may be of benefit to the food technologist

(a) When a food processing plant is being automated, or a change is being made from a batch process to a continuous process.

(b) When as a result of (a) a stricter time schedule has to be adhered to, and greater uniformity in material, and processing is desired.

(c) When work study on plant processes suggests the need for improving texture, solubility, clarity, filterability, ease of pumping of processed materials. Enzyme induced alteration in bulk density, or removal of variations in thickness of finished goods, etc., may contribute to the elimination of packaging problems.

(d) When the possibility exists that the mild hydrolysis of one component of a mixture will simplify and improve utilization of others, improving taste, colour or texture in the end product, or altering viscosity.

Some common faults in applying enzymes

The following analysis of the more common sources of difficulty may be helpful.

(a) Inadequate attention to storage. Commercial enzymes should be stored under cool and dry conditions.

(b) Inadequate housekeeping, e.g. failure to reseal containers, or the accumulation of several partially used containers with considerable headspace sometimes leads to loss of enzyme activity due to aerial oxidation, etc.

(c) Inadequate attention to duration of treatment, temperature and pH control. Seasonal temperature changes in processing plant are sometimes neglected, particularly if the process is being operated at or near room temperature.

When scaling up processes, due attention should be paid to heat content of new vessels, and the longer heating and cooling times which may be required.

(d) Inadequate mixing or stirring to promote a homogeneous mix may lead to incomplete and variable enzyme activity.

(e) The use of open-ended steam pipes for heating may lead to local overheating and inactivation of enzymes, and dilution with condensate may be a variable quantity.

(f) The use of copper service pipes, in an otherwise inert plant; the use of thermometers with unsuitable metal casing, may lead to enzyme inactivation by metal poisoning. A particular example is the case where process water may have been standing in contact with such materials over the week-end.

Some enzyme applications

The food technologist concerned largely with starch based raw materials is well served by the enzyme producer in that there is a wide range of enzymes available capable of producing changes in viscosity of starch solutions, then through intermediate stages of dextrin formation, to complete breakdown with the formation of maltose and glucose. Enzymes available include fungal and bacterial amylases, malt α- and β-amylases, pancreatic amylase, fungal amyloglucosidase, etc., and these may be utilized to obtain the desired results over a wide range of pH and temperature conditions.

Such enzyme materials find use in the milling, baking and confectionery industries, in brewing, in glucose and glucose syrup production, and in the removal of starch haze from such materials as pectin, also in the preparation of predigested foods for infants and invalids, and digestive aids.

The food processor who is concerned with the modification of proteins has a number of aids in the form of proteolytic enzymes such as papain, bromelain, ficin, pancreatin, rennin, pepsin, fungal and bacterial proteases. However, knowledge concerning protein composition and structure is less advanced than that relative to starch and starch products. The number of types of enzyme susceptible bonds is greater in the protein molecule than those in starch components, and knowledge concerning the bond breaking abilities of the various proteases is less certain. For this reason those successes in the application of proteolytic enzymes to food processing that have occurred have largely been achieved by a process of trial and error. It is often found that a mixture of proteolytic enzyme shows to greater advantage than does enzyme material from one particular source. Among the applications may be noted the chillproofing of beer, cheese production, meat tenderization, and the modification of hard wheat flours.

Among other enzyme applications in the food industry may be mentioned: (a) the application of a mixture of pectolytic enzymes to the clarification, filtration, and concentration of fruit juices; (b) the use of glucose oxidase in the removal of glucose from whole eggs and egg albumen prior to drying. The same enzyme may also be applied to the removal of oxygen from the headspace in bottled and canned goods; and (c) the use of invertase in the production of soft centre chocolates and fondants.

Discussion

Mr H. J. Bunker (Twickenham) said it is well known that cell-free enzymes from yeast can produce alcohol from sugar. Experiments are being made to develop this to the large-scale brewing stage. He then commented on the removal of protein material, which is essential for producing 'brilliant' beers, since otherwise potential 'haze' or 'cloudy' complexes may form. Proteolytic enzymes are therefore useful but must not be overdone. If all nitrogenous constituents are broken down to amino acids, the beer will not appeal to the consumer because retention, and perhaps texture, of the 'head' depends on some of the nitrogenous materials.

Mr F. Shaw (Norwich) mentioned a case where a breakdown product, although proved non-toxic by biological tests did not comply with the legal regulations because it was not on the permitted list of substances. He asked whether such breakdown products, formed by normal accepted food modification processes, should be regarded as additives.

Mr Bryce replied that, as he had already said, the enzyme should be derived from a non-toxic source, and the products should also be non-toxic. To extend control to exclude the formation of certain non-toxic products is a debatable matter. Possibly the argument would hinge on whether the enzyme responsible was innate in the processed material or whether it was added. If added, then possibly the use of a purer and more specific enzyme might obviate the difficulty.

Dr A. E. Bender (London) asked whether in the production of enzymes problems from mutations were met. In using the mould *Neurospora crassa* as a possible source of *l*-amino acid oxidase, he had encountered a strain which produced the *d*-enzyme; it had mutated. Both these enzymes were later found to be produced at different stages of the growth of the mould.

Mr Bryce replied that bacteria, unlike moulds, gave little trouble in enzyme production. Fermentation times were generally too short for spontaneous mutations to make any serious difference to plant yields, although stored inocula may present problems. The storage medium on which the organism is grown and maintained has a considerable bearing on mutation and loss of ability to produce enzymes. The problem is tackled by programmed selection of high yielding strains, and storage of master cultures under mineral oil, on sterilized soil, or lyophilized.

Dr J. J. Wren (Lyons Laboratories) asked Mr Bryce first whether he could name any beneficial application of lipases or lipoxidases, that is, purified preparations in food products, and secondly whether lipase activity in preparations or proteases, amylases, etc., presented problems in their application.

Mr Bryce answered that the action of lipases is generally to be avoided and production and application has not been widely studied. He could think of only two possible applications, neither operated in this country; flavour promotion in processed cheese, and the rapid maturing of chocolate milk crumb.

The major use of lipoxidase is in bleaching carotene pigments in flour. It is normally added as a natural component of soya flour, a rich source; a purer more active material would probably be uneconomic. Lipase in microbial enzymes could be avoided by careful selection of organisms, growth medium, and processing.

Mr S. H. Cakebread (Lyons Laboratories) asked first whether any commercial use has been made of synthetic enzyme action, secondly on what foodstuffs, other than *Brassicas*, flavour regeneration had been investigated.

Mr Bryce replied that on the synthetic side he could only give the manufacture of dextrans, although he had heard of experiments with maize, peas, pineapples, tomatoes

and carrots. He believed the difficulty of using flavour regeneration enzymes might be to stop the action in time.

Professor A. G. Ward (Leeds) asked about the complexity of the plant proteolytic enzyme systems. He had noticed that sequence studies were being done on a material called papain which presumably was a component of papain as commercially produced. He asked what degree of complexity there is in this type of enzyme system. In this case it would presumably be a major component of the commercial papain isolated as a pure enzyme for sequence work.

Mr Bryce replied that commercial papain certainly contains a mixture of three enzymes—papain, chrymopapian and lysozyme, which can be separated (Cayle, Saletan & Lopez-Ramos, 1964). Bromelain from pineapple shows four optima at pH 4·5, 5·5, 7·0 and 8·5, regardless of the type of substrate used. This implies four proteinases at least.

Mr T. McLachlan (London) asked how one could speak of the specificity of enzyme action when ten or twelve enzymes can be used to hydrolyse starch and even more to break up proteins. He also asked about the action of enzymes in pre-tenderizing meat in animal slaughter.

Mr Bryce said that the specificity of enzymes resides in their mode of action and in the nature of the specific linkages in the substrate, which they are capable of breaking, as well as the substrate they attack. Both enzyme and substrate must be purified to demonstrate specificity of action. Confusion arises because commercial enzymes are sometimes mixtures and natural food materials are mixtures of different substrates.

There are two possible methods of applying proteolytic enzymes to the tenderizing of meat: (a) by injection just prior to slaughter, and (b) in rehydrating meat which has been freeze dried previously. Neither appears to be used commercially in Britain. Enzyme powder or solution may also be applied to the cut meat but adequate penetration is a problem.

Physiological Effects of Food Additives as Food Modifiers

MAGNUS A. PYKE

The possibly harmful physiological effects of food additives have been intensively studied during recent years, and elaborate and exhaustive tests developed and applied.

There is little doubt that the evidence for the freedom of synthetic chemical additives from the likelihood of possessing any measurable toxic influence on the people consuming them is very much stronger than is the evidence for the safety of a wide variety of natural, untreated foods, ranging from nutmeg to plaice. It would, therefore, be fruitless to rehearse once again the facts showing for one foodstuff after another that the additives used as modifiers, as distinct from such substances as synthetic vitamins which are added for a specific nutritional purpose, have no significant physiological effect at all. Their possible physiological effect on the diet as a whole is, however, a much more interesting and important topic.

The factor which makes the present period of history different from any others that have gone before is the speed with which technological change affects society. During the short historical period of a single generation, changes of major significance have occurred in food technology. The situation, therefore, has now been reached for the first time when large numbers of individuals may subsist for the whole of their lives on a diet made up of items, each one of which has been processed and packaged by a food technologist. Even though we can be assured that no measurable trace of toxic material has been introduced it is, nevertheless, worth considering whether by using technologically modified food products, the pattern of nutrients has been changed sufficiently to affect the physiological value of the diet as a whole.

The diet as a whole

Nowadays, should there be a week of heavy snowfall in the north-east of Scotland, which is an event which occurs every 4 or 5 years, military helicopters are called out to mount an air-lift to supply the inhabitants of the area with the food to which they are accustomed. Twenty years ago or less, the people would have made provision for such an emergency by laying down stores of flour, oatmeal, salted herrings and butter. Today, they, like the rest of the community, subsist on such technologically sophisticated commodities as cornflakes, bread from plant bakeries, spread easily with high-quality margarine, preserves, frozen or dehydrated vegetables, pre-packed meat or poultry. It is already commonplace to be served with frozen or dehydrated peas at a country restaurant in July. And the economics of catering and food manufacture and distribution alike make it not unreasonable for the Kent caterer to serve in June quick frozen strawberries from his wholesaler rather than fresh strawberries from his garden.

I do not wish to imply that each separate manufactured food that I have mentioned is not of the highest quality and that every food additive incorporated in it as a food modifier has not successfully undergone the most stringent tests for safety-in-use. The new problem, however, to which I suggest our attention should be drawn, is that there is now an existing possibility that all or the major part of the entire diet eaten may have been so produced.

We have passed far beyond the state of knowledge when McCarrison (1927) described the results of feeding young rats for 6 months on either a Sikh diet, on which they throve and bred very well, or on a diet of poor ignorant Westernized people, on which they did very badly. But even with our present knowledge of nutritional requirements, it would demand considerable fortitude and a strong conviction in the completeness of modern science to undertake to subsist for a period of years solely on even such a carefully designed food combination as United States Army K-rations.

Lacunae in nutritional knowledge

When we discuss the physiological implications of the use of food additives as food modifiers and omit the possibility of direct toxicological effects which are being investigated with such diligence and thoroughness, we are, in fact, considering their influence on nutrition. And recent events in the history of the science of nutrition would make it naïve to imagine that we already possess sufficient knowledge to enable us to foresee every eventuality for the future. Food technologists were undoubtedly taken aback when in 1946 Sir Edward Mellanby presented evidence implicating nitrogen trichloride, an additive which for at least 25 years had been widely used as a flour improver to modify the structure of bread, in the development of nervous symptoms in dogs. It is now history that this 'improver' reacts with the amino acid, methionine, to produce a toxic substance, methionine sulphoximine, and history does not repeat itself. Nevertheless, it is also true to say that nutritionists, as distinct from toxicologists, were themselves surprised, after a generation of study of vitamins and the deficiency diseases which arise when adequate trace-amounts of them are not present in diets, when Cecily Williams (1933) recorded that some protein or amino acid deficiency was a causative factor in the appearance of one of the most widespread and serious nutritional diseases, namely, kwashiorkor. Malnutrition of this degree of obviousness is not to be expected in people eating diets in which products of the food-manufacturing industries form a major part, nevertheless we must bear in mind that the 'modifying' effect of improvers such as nitrogen trichloride, chlorine dioxide, even ascorbic acid, is a chemical change in the flour protein.

If the nutritionists were embarrassed to discover that a food additive used to modify protein structure might produce an effect of considerable physiological significance and that after 50 years study of deficiency diseases, a condition of widespread distribution could be occurring of which inadequate protein of appropriate chemical composition was the cause, they were perhaps even further nonplussed when Ancel Keys (1952), among others, presented evidence to suggest that certain fats, particularly those containing more of the saturated fatty acids, might be concerned in coronary heart disease. Previously it had been considered that the physiological function of fats in the diet was three-fold: to serve as a vehicle for fat-soluble vitamins, to provide a con-

venient source of calories, and to add to the palatability of food. Bread and butter is an agreeable comestible; bread and water means hardship. The increase in fat consumption in parallel with the increase in wealth was considered nutritionally good.

In the last 10 years all this has changed. The exact function of fat is unknown in spite of intense research into the physiological role of unsaturated fatty acids, into vitamin E and other antioxidants which protect them, into the potentially toxic effect of monomers and dimers formed from unsaturated fatty acids by high temperatures applied during frying and roasting (Ferestone *et al.*, 1961) and into the effect of different fats on the level of cholesterol in the blood and its possible relationship with ischaemic heart disease. Whatever the exact facts may turn out to be, there is a substantial weight of statistical evidence to show that the incidence of such heart disease in a population is related to the amount of saturated fat the people eat. Jolliffe & Archer (1959) showed that the data collected up to then demonstrated that the death rate from degenerative heart disease was related statistically to the proportion of saturated fat in the diet and was also related to the proportion of animal protein and to the number of telephones per 100 persons. That is to say, besides the direct evidence about unsaturated fatty acid, vitamin E and cholesterol, coronary heart disease is also linked with the economic status of the people eating the diet.

The whole matter, however, is something which food technologists should be aware of. The use of food additives such as GMS and emulsifiers in general which modify the consistency of foodstuffs and affect the amount of fat in a commodity and its state of dispersion may clearly have a bearing on the physiological effect of the fatty component of the diet as a whole.

The modification of the consistency of margarine is an extreme example of the use of food technology and of a variety of additives—lecithin, soaps, GMS, monostearin sulphoacetate, polymerized oxidized oil—apart from the adjustment of the hardness of the fats and oils used by control of hydrogenation. Already, margarine manufacturers have given serious consideration to the possible physiological significance of the level of saturated and unsaturated fatty acids in their product. Meanwhile, until the facts are finally settled, they must continue to do so.

Sugar

The presence or absence of sugar is clearly an appropriate topic to raise in any general discussion of the use of additives to modify the consistency of foods. The proportion of sugar present in soft drinks, for example, has a direct effect on their viscosity. Consequently, a sweetening agent used in place of part of the sugar must be recognized as a food modifier. Similarly, the conversion of sucrose to invert sugar may change a solid fondant into a liquid. Recent discoveries relating to the metabolism of carbohydrate

have once more brought surprises for the nutritionists in an area of knowledge where they had tended to assume that no more remained to be discovered.

For example, Winitz (1965) and his colleagues recently published a report of experiments in which a number of volunteers were fed—if that is the correct term to use—for 19 weeks solely on clear solutions containing every nutrient they were known to require. This regimen was particularly designed for the convenience of men travelling in a semi-prone position in a space capsule for whom the minimum of insoluble and indigestible dietary residue would be, in every sense of the word, for their convenience. The food technologist, however, may consider this ration merely one example of the totally manufactured diet to which I have already made reference.

The classically trained nutritionist has been brought up to believe that the familiar carbohydrates, the monosaccharides, glucose and fructose, the disaccharides, sucrose and maltose, the dextrins and the starches possess equal nutritional value. Provided the necessary amounts of B-vitamins are available, it is generally assumed that, when they form part of a normal diet, each is of the same physiological value contributing approximately 4 cal/g, when allowance is made for any loss or gain of hydroxyl groupings. During the course of their trials with liquid diets, however, Winitz and his colleagues found that the total serum cholesterol levels of all their experimental subjects fell from an average of 226 mg to a value of 151 mg/100 ml. The sole source of carbohydrate in the diet was glucose. When, however, during a 2-week period the glucose was changed to sucrose, a sharp rise in serum cholesterol followed in all men in the experiment. This unexpected phenomenon provides striking confirmation of the hypothesis first put forward by Yudkin (1957) that the consumption of excessive amounts of sugar and not of fat is correlated with an increased incidence of ischaemic heart disease.

Here then is another occasion when the food technologist, who might, for purely technical reasons, wish to modify the flow-characteristics of a product by changing its sugar content, finds himself introducing a factor of physiological significance for the consumer. It is worth noting that the liquid diet administered by Winitz and alleged to be complete in every nutritional respect was only tested over a 19-week experimental period. This represents, say, 0·5% of a lifetime of only fit young male adults. The information obtained, striking though it was, was not of the same order of certainty as that collected about the possible harmful physiological effect of a particular additive, which will have been administered to animals of both sexes and of more than one test species for several generations.

Obesity

It is, of course, of the highest importance to ensure that substances employed as food additives exert no adverse physiological effect if they are consumed for prolonged

periods or even for an entire lifetime. The work done by national and international bodies on food colours, preservatives, flavours, emulsifiers, antioxidants and food modifiers alike to ensure physiological harmlessness is, therefore, well justified. But the search for symptoms of chronic or acute signs due to the presence of specific compounds in the diet must be assessed as part of the study of other physiological effects which are more obvious and frequent. In a recent article, Gubner (1957), of the Bureau of Medical Research Equitable Life Assurance Society of U.S., reviewed evidence which showed that the diet consumed by the United States community caused a high incidence of over-weight and he also presented hard statistical data implicating this over-weight with coronary heart disease, angina and diabetes.

Food additives used as food modifiers can clearly play a part in this situation. Liquid paraffin is no longer used as a component of foods on the grounds that it *could* form a solvent for vitamin A from other dietary ingredients, which would therefore be lost by the body—although avitaminasis-A due to paraffin drinking has never been demonstrated in man—and also because prolonged ingestion may lead to invasion of the liver by droplets of hydrocarbon. On the other hand, methyl cellulose, which is accepted as a food ingredient, makes, like paraffin, no nutritional contribution. Foods, therefore, which contain as 'modifiers' a significant proportion of 'nutritional placebo' like methyl cellulose may serve to make a desirable physiological contribution by helping to reduce the incidence of obesity.

Similar physiological benefit may also be obtained from additives which increase the proportion of the two best obesity-inhibiting agents in manufactured foods, namely air and water. It may well be, therefore, that flour improvers, which enable the baker to produce a well-set-up and well-aerated loaf, or GMS and other additives which permit a large volume of air to be beaten into confectionery, may prove themselves to be of physiological benefit by reducing the calorific value of a unit volume of food. Similarly, methyl cellulose and other additives which facilitate the manufacture of highly aerated products will correspondingly reduce the ingestion of fat, sugar and calories alike.

Additives such as acetylated monoglyceride, which form a coating on foods and prevent the loss of moisture (Benton, 1965), will similarly, by reducing the calorific value per mouthful, play a part at least in preventing over-weight with its consequent harmful physiological effects.

Conclusion

The British Industrial Biological Research Association is now working to ensure that additives used in food do not introduce any toxicological hazard. To do this, the scientists undertake prolonged and detailed studies. Their reward is to obtain negative results. But while this work is in progress things do not stand still. The food habits of society change, partly due to the efforts of food technologists and partly due to the

economic advance and social change occurring in the community. Scientific understanding of nutrition advances as well. The thesis proposed in this paper is that the main physiological effects caused by food additives used as food modifiers are more likely to be those arising from the composition and adequacy of the diet as a whole than any due to the specific chemical composition of a particular compound. This being so, I should like to see facilities and finance provided for Dr Golberg and his colleagues to extend their researches so that they could carry out long-term investigations into the health, vigour and long life of their animal species—of which man is the most important—as well as into the non-appearance of harm.

Discussion

Dr A. E. Bender (London) said that, within the context of this symposium, the last paper posed the main problem facing the nutritionist, a double problem of over-nutrition in one part of the world and undernutrition in the other part. Food technology, as Dr Pyke has shown, now offers a wide choice of very attractive foods nutritionally. The wide choice available, however, causes over-eating with the consequences of obesity. Since improved technology, plus improved methods of food preparation by domestic scientists, is a cause of over-eating perhaps food should be made less attractive. There are two ways of looking at present gaps in knowledge of nutrition. Nutritional discoveries in the past 70 years have shown how to prevent deficiency diseases, and health in the western world has greatly improved. Nevertheless, common diseases such as diabetes and heart disease, or minor complaints such as constipation and fatigue are increasing, and it may be asked, although no answer is forthcoming, whether the wrong kinds of food are being eaten—malnutrition rather than undernutrition.

Diet that produces a rapid growth rate in both animals and man can be compiled but it is not known whether different quantities or ratios of nutrients, or addition or subtraction of essential fatty acids, sugar or other nutrients would benefit health. Peoples in different parts of the world are apparently equally healthy on quite different diets. Little is also known about individual variation. Some individuals can tolerate doses causing ill-effects in others. This might be a factor in the problem of food additives. For example, a fifth of the subjects examined in a recent report could drink coffee immediately before going to bed with no effects at all, but another fifth were kept awake for several hours on the same dose. A very very small fraction, about seventy cases a year out of 800,000 babies, are hypersensitive to vitamin D. A dose as small as 1000 i.u.—25 μg—gives rise to the symptoms of hypercalcaemia.

In reply to *Professor Ward*, *Dr Bender* said that he was probably right in assuming that one tended to eat to calorie requirements, to eat 200 calories equivalent of bread rather than two rounds of bread. Puffing up a loaf might fool the consumer for a time

but he might soon realize that he was hungry and take two and a half rounds instead of two. Total calorie intake is the important factor, and food additives that merely puff up food in some way probably do not have a real effect, except perhaps on a subject obese mainly through sitting and eating 'chocolate creams'. Such a person is probably eating merely to satisfy some mouthfeel, oral fixation or emotional disturbance. Carboxymethyl cellulose decreases intake by filling the stomach with something inert before the meal. This appears to work in the short run, but Professor Charlotte Young says that the failure rate is enormous. She rarely manages to slim more than a quarter of the people who offer themselves and most return a year later and are back to their original weight. These devices for slimming, whether appetite depressent drugs, stomach-filling materials or even metabolic stimulators like thyroid, seems to work only for a limited time. People then revert to normality which means eating too much and putting on weight. The most important thing, it is generally agreed, is to modify eating habits for life but, of course, people will not do this, and so these devices may therefore be of some assistance on a slimming regime.

Mr T. McLachlan (London) recalled Dr Pyke's remarks that the ingestion of liquid paraffin may withdraw vitamin A from the body. After operations on the colon, massive doses of liquid paraffin are generally given, and although in the form of so-called agar emulsions, the quantity of agar is negligible and plays no part in the intestines. Substances in the diet used to reduce calorie intake must be attractive. Wood cellulose to increase the bulk in the diet of diabetics, proved to be useless. Dr Pyke spoke of the advantages of glucose over sucrose; presumably, public analysts had been wrong in fighting, for nutritional reasons, the use of liquid glucose in jam and excessive quantities of air in ice-cream.

Dr M. Stein (Nottingham) was puzzled by the discovery that the intake of additional sugar upset Claude Bernard's theory of maintenance of internal milieu. This presumably demands the calling into action of enzymes and pathways to cope with any upsetting of equilibrium. Does this not mean that any fructose would disappear in a 24 hr day?

Dr Bender replied that the quantities used in these experiments have been really excessive. Dr McDonald at Guys Hospital fed a pound of sugar or a pound of starch a day and showed changes in the blood fats. In animal experiments usually 60% of the diet is sugar, starch, or some other ingredient. On the other side are attempted explanations of increasing heart disease; since sugar consumption has risen parallel with increasing incidence the suggestion is that the sugar intake might be in some way involved. In attempting to explain why sugar behaves differently from starch, one may suggest that the difference must be fructose. As Dr Stein said, the body changes its

metabolic pathway in order to deal with the fructose, and possibly this change of the metabolic pathway might have other effects, like a higher rate of cholesterol or fat synthesis. It is suggested that when there is a high concentration of fructose perhaps alternative metabolic pathways become more important.

In reply to a further question by *Dr Stein*, *Dr Bender* said that too much of anything can upset equilibrium in some way. It seems to be that a little of everything seems to be 'safe' but the moment 'too much' of anything is taken then changes can be seen. One must admit that what consitutes 'too much' is not known.

Mr Boxley asked about the nutritional results of removing or adding salts to foods. Thus salts are removed in the production of pure sugar, but nitrites and nitrates are added to bacon. Sodium chloride is very largely added to food, about 1% on the average, yet some people have to live on a salt-free diet.

Dr Bender replied that about 15 times as much salt is ingested as required. The requirement is about 1 g/day and the average intake is about 15 g—which possibly means that some individuals may eat 5 g and others 45 g. The excess apparently is always excreted but one cannot be certain that such a large excess is harmless. There are reports, although they should perhaps not be given too much weight, that rats fed salt in their young days develop hypertension later, and those kept almost free from salt do not have this later disability. It may be quite wrong to extrapolate to man and to suggest that hypertension is connected with our salt intake.

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Closing remarks by Mr T. McLachlan

One of the first things needed, and which so far no country seems to have, is a good definition of the word 'additive'. It is useless to speak about the addition of chemicals to food, since all food is made up of a mixture of chemicals. Similarly one cannot rely solely on food grown on compost fertilized soil. Chemicals are and must be used so

long as they are safe and do not upset the balance of the body or of the soil. In some continental countries the addition of colour to canned peas is prohibited, but few British people will eat canned peas unless they are coloured. During the last war there was a slight shortage of fat in the diet, which the Danes tried to compensate by the introduction of pork in natural juice. This contained from 20% to 30% of loose fat. At first it was popular, but people soon rebelled and it has now been replaced by luncheon meat, in which the fat is emulsified by the use of emulsifiers and stabilizers – all additives.

It is commonly said that substances used for many years may be regarded as safe, but this is doubtful. Cigarette smoking was considered safe until recently. Coumarin was used as a flavouring for many years and is now condemned. The Food Additives Committee have suggested that oil of bitter almonds must not be used, and one wonders whether bitter almonds themselves should therefore be prohibited as a food. The addition of hydrogen to fat is the use of an additive. If used incorrectly it may be dangerous but, if properly controlled by the hydrogenaton of a portion of the fat, the resulting product is more attractive and perhaps more digestible. One wonders whether the indiscriminate use of phosphates in foods is safe. When crematoria were opened the excess of acid phosphates in the soil caused trouble with the lawns and flower beds and had to be neutralized with lime. Is it not possible that excessive additions of phosphates to the diet may be equally harmful in the long run? Professor Ward asked whether many more animal laboratories are required for the examination of food additives. These are absolutely essential, but must be large and properly equipped in every way.

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Papers to be published in future issues

On odour classification. By R. Harper.

Early British patents for food preservation methods. By C. G. Tucker.

A survey of pH, and ultimate pH values of British progeny-test pigs. By J. R. Bendall, A. Cuthbertson and D. P. Gatherum.

The incidence of watery muscle in commercial British pigs. By A. McM. Taylor.

Relation between oxygen tension, biosynthesis of ethylene, respiration and ripening changes in banana fruit. By L. W. Mapson and J. E. Robinson.

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Some applications of differential thermal analysis to oils and fats. By K. G. Berger and E. E. Akehurst.

The heat resistance of anaerobic spores in aqueous suspension. By T. A. Roberts, R. J. Gilbert and M. Ingram.

The evolution of the food technologist. By J. R. Blanchfield.

J. Fd Technol. (1966) **1**, 166.

Errata

KELLY, K., JONES, N.R., LOVE, R.M. & OLLEY, J. (1966) Texture and pH in fish muscle related to 'cell fragility' measurements. *J. Fd Technol.* **1**, 9.

Page 12, Fig. 2: The scale on the vertical axis of graph (b) should read from 0 to 1·2, as in graph (a), and not from 1·0 to 2·2 as printed.

NORRISH, R.S. (1966) An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups. *J. Fd Technol.* **1**, 25.

Page 28, Fig. 2: In the first line of the legend the word 'sorbitol' should read 'sucrose'.

Page 31, Table 6: A minus sign should be inserted in the headings of the third and fourth columns of the table.

GRIFFITHS, M.H.E. (1966) Systematic identification of food dyes using paper chromatographic techniques. *J. Fd Technol.* **1**, 63.

Page 65, Table 3: The R_F value of Patent Blue in Solvent 10 should read 0·4 and not 0·04 as printed.

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TENTATIVE PROGRAMME, 1966–67

Date	Place and time	Subject	Speakers
29 September	J. Sainsbury Ltd, Basingstoke	Ladies' Evening (Visit)	
5 October	College of Technology Blackpool	Symposium: 'Developments in Production, Processing and Packaging of Meat'	N. of England Branch Meeting
28 October	Grimsby College of Technology, Grimsby, Yorks. (9.30 a.m.)	Symposium: 'Transport of Frozen Food'	
3 November	Borough Polytechnic, Borough Road, London, S.E.1 (Tea 6.30 for 7.00 p.m.)	'Cost reduction in the Food Industry'	L. Simmens, M.SC., F.R.I.C.
28 November	The University of Strathclyde, Glasgow	Film and Talk 'Spices'	H. Heath and T.S.E. Powell (Bush, Boake, Allen Ltd)
29 November		A.G.M. North of England Branch	
6 December	Borough Polytechnic, Borough Road, London, S.E.1 (Tea 6.15 for 6.45 p.m.)	Annual General Meeting	W. Rowan Hare (President, F.M.F.)
January	The University, Reading		
February	The University, Leeds (N. of England Branch Meeting)	Film and Talk 'Spices'	H. Heath and T.S.E. Powell (Bush, Boake, Allen Ltd)
2 March	(Joint Meeting: Institute of Packaging)	'Vacuum and Gas Packaging of Foodstuffs'	
March	Carlow, Ireland (Joint Meeting: Carlow Scientific Council)		
5–6 April	Church House, London, S.W.1.	Symposium: 'Quality Control in the Food Industry'	
April	Gas Showrooms, Manchester (N. of England Branch Meeting)	Wine/Cheese Tasting Meeting	Dr Buckle (Coates Ltd) Mr Peacock
18 May	(Joint Meeting: Royal Society of Health)	'Man and his Food'	Sir William Slater

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

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