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Early history of the Institute of Food Science and Technology

J. D. MOUNFIELD
President of the Institute

It was during the early part of 1961 that a group of former students of the National College of Food Technology at Weybridge in Surrey asked the Principal to discuss with them the possibility of forming an organization to establish and safeguard the professional status of the new food technologists being trained at the College. The Principal gave encouragement to the idea and decided to call an open meeting of all interested persons so that opinions for and against could be freely expressed.

This meeting was held at the National College on 15 February 1962 and the proposal that an Institute of Food Technology should be established was first formally made by J. E. Morrison, a postgraduate student of the College, on behalf of himself and his colleagues. The possibility of forming a similar organization had been discussed in the early 1930s but no steps had been taken to inaugurate one when the outbreak of the 1939 war halted further progress.

During the 30 or so years between these two attempts the food industry had been served technically, and served well, by chemists, biochemists, microbiologists, physicists and others who entered the industry, acquired a knowledge of food and gradually took on the role of food technologists. Very few, if any, had been given systematic training for their work and their transformation into food technologists was, in consequence, a slow process. Each one tended to develop along relatively specialized lines and because of this the true function of the food technologist was never well defined. To some he was the backroom chemist who from time to time pronounced on the quality of the firm's products; to others he was the general factotum who spent most of his time sorting out the irritating day-to-day problems of the factory. Relatively few regarded him as a key person who could and should be responsible for the planning, development and control of large-scale food processing operations, for the design of new products and plant, and for the improvement of existing products and processes.

It was perhaps not surprising therefore that, on the introduction of systematic courses of education in food technology and food science, the students who qualified should look for something more than a paper qualification, that they should look as

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well for professional recognition in order to establish a rightful and clearly defined place for themselves in the food industry. The function of the food scientist in classifying, using and extending knowledge about food fitted into a reasonably familiar pattern but that of the food technologist had still to be understood and accepted by many both inside and outside the industry.

This situation was the background to the first open meeting in February of 1962 when about 200 interested persons attended and for about 3½ hr discussed the advantages and disadvantages of forming a professional organization for themselves.

Dr J. D. Mounfield, who was voted into the chair, said that after a formal motion had been proposed and seconded he would first ask those who were in favour of a new organization to make known their reasons and afterwards would invite opposing speakers for their views. The proposer of the motion 'that an Institute of Food Technologists be formed in the United Kingdom' said there were two main reasons why he thought this should be done. The first was that the occupation of food technology was not yet recognized in this country and the formation of a professional body was needed to establish and to raise the status and the standard of the technologist. The second reason was that existing organizations were not able to meet this need.

Immediate support for the proposal was forthcoming from a number of speakers, both young and old, for the reasons given by the proposer of the motion, but there were others who opposed it on the grounds that a new organization was unnecessary, that existing organizations adequately met the needs of both food scientists and food technologists, and that a new body would merely harm existing ones. Arguments on both sides were put forward earnestly and vigorously but with good humour. Several speakers drew attention to the benefits to food scientists and technologists in the United States which had flowed from the establishment of their own Institute of Food Technologists. Other speakers pointed out that the American Institute was not a qualifying body and, for that reason, did not form a valid comparison. One or two industrial representatives felt there was clearly a need for a new and specific person — a food technologist — and that he required special training and separate recognition as a professional worker. They thought the proposed new organization would give him this recognition.

At the end of the meeting a resolution was passed by a large majority that 'an *ad hoc* committee be formed to consider the establishment of an organization representative of food scientists and technologists in this country and to report to a further meeting in London not later than 31 December 1962'.

The committee of eleven elected for the purpose consisted of:

Dr J. D. Mounfield (Principal, National College of Food Technology, Weybridge) — Chairman.
Dr E. C. Bate-Smith (Director, Low Temperature Research Station, Cambridge).

Mr J. R. Blanchfield (Group Chief Chemist, Co-operative Wholesale Society Preserves Group).
Mr J. Bodmer (Superintendent, H. J. Heinz Company Ltd).
Dr S. Cotson (Lecturer, Department of Chemistry and Food Technology, Borough Polytechnic).
Professor E. L. Crossley (Department of Dairying, University of Reading).
Dr C. L. Cutting (Director, British Food Manufacturing Industries Research Association, Leatherhead).
Mr J. E. Morrison and Mr J. A. Rowe (former students of the National College).
Dr. W. G. Wearmouth
Mr A. H. Woollen (Editor, *Food Manufacture*).

The Committee subsequently held three meetings and prepared a Report which was first circulated to all those who attended the first open meeting in February 1962, and was later presented to a second open meeting held on 6 February 1963. Fog delayed its presentation on the agreed date in December 1962.

In its Report the *ad hoc* Committee made the following observations and recommendations:

Need for a new organization

(1) After having considered and discussed the views expressed at the first open meeting, opinions communicated to the Committee, and views expressed privately and in the press, the Committee consider that no existing body adequately meets the needs and interests of food scientists and technologists in the United Kingdom, and that a new organization should be formed.

(2) The Committee consider that this organization should have the following characteristics:

(a) That it should be an independent professional institution rather than a learned society.

(b) That it should establish qualifications and conditions for membership and that membership should be open to all food technologists and scientists with the requisite qualifications.

(c) That it should have the power to become an examining body conducting its own examinations, should its Council consider this desirable.

(3) The Committee recommend:

(a) That if there is a majority in favour at the meeting on 5 December 1962 at which its report will be presented, then that meeting should constitute itself the Inaugural Meeting of the new organization.

(b) That those present at that meeting should be immediately eligible to become Inaugural Members of the new organization.

(c) That an Inaugural Council of fifteen members be elected at that meeting, from whom the Council may subsequently elect a Chairman, Secretary and Treasurer.

Aims and form of the new organization

(1) The committee consider that the aims of the new organization should be:

(a) To promote and further the knowledge, development and application of science and technology to every aspect of food for the well-being of society.

(b) To be the professional body of food scientists and technologists in the United Kingdom.

(c) To collaborate with other bodies concerned in the various disciplines represented in food science, and with other organizations concerned in the various aspects of food technology.

(d) To promote the dissemination of knowledge and ideas amongst food scientists and technologists.

(e) To further the education and training of food scientists and technologists.

(2) The committee consider that the Council of the new organization should initiate appropriate activities to further the above aims. In the committee's view, these activities should include the publication of a Journal.

(3) The committee do not consider themselves called upon to make detailed recommendations regarding the Constitution of the new organization, although they make the following general recommendations (in addition to those listed in the previous paragraph).

(a) That the new organization should comprise at least two grades of membership, one of which should be student membership, the qualifications for these to be decided by the Inaugural Council.

(b) That Inaugural Membership, open to all food scientists and technologists, should cease on the adoption of the Constitution.

(c) That the Inaugural Council should be charged with the task of preparing a draft Constitution and of framing by-laws in accordance with the general principles adopted at the Inaugural Meeting.

(4) The committee recommend that Inaugural Members be required to contribute the sum of one pound (£1).

(5) The committee recommend that the name of the new organization be 'The Institute of Food Science and Technology'.

At the second open meeting on 6 February 1963, which was attended by about 300 people, the above Report was fully discussed and accepted with only minor amendments. The following motion was carried unanimously by those present:

'That in the opinion of this meeting no existing body adequately meets the needs and interests of food scientists and technologists in the United Kingdom and that a new organization should be formed'.

The meeting decided that, as recommended, the new organization should be an independent professional institute and not a learned society and that it should establish qualifications and conditions for membership and that membership should be open to all food scientists and technologists with the requisite qualifications. It decided that the organization should have the power to become an examining body, conducting its own examinations if necessary.

The meeting declared itself to be the inaugural meeting of the Institute of Food Science and Technology and it agreed that any *bona fide* food technologist resident in the United Kingdom could be accepted as an inaugural member on payment of the sum of £1. It was further decided that inaugural membership should be temporary and should cease at the first Annual General Meeting.

At this point, the Chairman said he thought the next step should be the appointment of an Inaugural Council charged with the task of drafting a Constitution for the new Institute. He suggested that it should consist of fifteen members. These suggestions were

accepted and the meeting elected the following to serve as members of the Inaugural Council, with power to co-opt as they considered necessary:

Dr J. D. Mounfield, Dr E. C. Bate-Smith, Mr A. H. Woollen, Professor E. L. Crossley, Dr E. H. Steiner, Dr D. B. Smith, Dr S. M. Herschdoerfer, Mr A. E. Billington, Mr I. M. V. Adams, Mr J. R. Blanchfield, Mr E. Druce, Mr E. M. Learmonth, Mr G. S. T. Bamford, Mr H. R. Binsted, Mr D. H. Fry.

It was further decided that the quorum for Council meetings should be eight and that the Council should elect its own chairman, secretary and treasurer.

Before the meeting ended, the chairman invited discussion on the aims and policy of the new Institute so that the new Council could be given guidance in the drafting of a Constitution. Helpful suggestions were made on many aspects of the work of the Institute. These included opinions about the qualifications and experience regarded as necessary for membership, about the desirability of publishing a Journal and about the holding of professional examinations. It was agreed that membership should be restricted to individuals and should not be open to organizations and that a high standard of competence should be required for the various grades of membership. Lastly, it was agreed that the next Council should be elected by postal ballot. The Inaugural Council was urged to avoid delay in drafting and presenting a Constitution to the inaugural members.

At the first meeting of the Inaugural Council, Dr J. D. Mounfield was elected Chairman, Professor J. Hawthorn Vice-Chairman, Professor E. L. Crossley Honorary Treasurer and Mr A. H. Woollen Honorary Secretary. Five meetings of the Council were held between 4 March and 11 December 1963 and the work of drafting a Constitution proceeded. From the outset an attempt was made, with the aid of legal advice, to draft the Constitution in such a way that it would be acceptable to the Board of Trade and would thus enable the Institute to limit its financial liabilities after the manner adopted by many businesses and professional organizations. This involved the drafting of a Memorandum and Articles of Association together with appropriate by-laws. It soon became clear to the Council, however, that to follow the legal advice being given to it would lead to unacceptable delay and the clauses of the various documents were therefore re-drafted by Council and amalgamated to form a single document. This change in procedure led to delay in presenting the draft Constitution to the main body of members and it was not until 19 March 1964 that a general meeting of Inaugural Members could be called together. A long and stimulating meeting was held on that day, the time being devoted mainly to a detailed consideration of the proposed Constitution. A number of amendments which had been submitted previously in writing, were discussed. Some were accepted and some rejected but in the end the meeting adopted a Constitution which was substantially the one put forward by the Inaugural Council. The final document was a formidable one consisting of seventy-eight Clauses which it was hoped would cover most eventualities.

Finally, the Inaugural Members endorsed the action of the Inaugural Council in appointing a Qualifications Committee (later called Membership Committee) to make a start on the many applications for membership which had already been received. It was agreed that the first constitutional Council should be elected from among those accepted Fellows and Associates whose applications had been received by 1 May 1964.

Perhaps the most important clauses in the Constitution which the Inaugural Meeting endorsed were those defining grades of membership as these were the ones most likely to affect the status of the Institute and its future effectiveness as a professional body. The decisions taken in the establishment of these Clauses illustrate better than anything else the responsible attitude adopted by the members towards the standing of the Institute they were creating. The relevant Clauses are as follows:

6. *Grades of Membership.* The Members of the Institute shall be divided into the following grades: Students, Licentiates, Associates, Fellows, and Honorary Fellows. Only Associates, Fellows and Honorary Fellows who were Associates or Fellows at the time of their election as Honorary Fellows, who shall be known as Voting Members, shall have the right to vote on any matter relating to the affairs of the Institute.

7. *A Student Member* shall be a person not under 18 years of age who at the time of application is a student under such conditions as shall satisfy the Council. Each student who does not apply for and attain a higher grade of membership within 5 years of the date of his admission as a Student Member shall cease to be a Student Member on the expiration of such period, provided nevertheless that the Council may, on receipt of a written application from the Student, allow him to remain a Student Member for a further period not exceeding 5 years.

8. *A Licentiate* shall be a person not under 21 years of age who:

(a) *either* has an appropriate university degree or academic or professional qualification approved by the Council as being equivalent thereto;

(b) *or* academic or professional qualifications accepted by the council as evidence of adequate educational attainment;

(c) *or* has passed such examination as the Institute may hold for the purpose;

(d) *or* satisfies the Council that by virtue of his experience in the field of food science and technology, by the responsibility of his employment and by any additional evidence acceptable to Council that he is a proper person to be admitted.

9. *An Associate* shall be a person not under 24 years of age who:

(a) *either* has an appropriate university degree with first or second class honours or equivalent academic or professional qualification or has passed such examination as the Institute may hold for the purpose *together with* at least 3 years appropriate experience in food science or food technology;

(b) *or* has an appropriate degree or academic or professional qualification accepted by the Council as evidence of adequate educational attainment *together with* at least 4 years appropriate experience in food science or food technology;

(c) *or* has in the opinion of the Council an established reputation as a food scientist or food technologist acquired by his experience and attainments.

10. *A Fellow* shall be a person normally not under 33 years of age who possesses the necessary qualifications for Associate Membership *and* who has not less than 10 years appropriate experience in food

science or food technology *and* has produced evidence to the satisfaction of the Council either that he has made a substantial contribution to the science or technology of food or that he has reached a position of seniority and authority in the profession acceptable to the Council.

11. *Honorary Fellows.* The Council shall have power to elect Honorary Fellows. Honorary Fellows shall be such persons and shall enjoy such privileges as may from time to time be determined by the Council.

These Clauses were framed to demand a high but not impossibly high academic attainment and a significant though not unduly long period in a post providing relevant experience and responsibility, the aim being to ensure such a degree of competence on the part of each member as to command the respect of the industry.

Throughout the summer of 1964 the Membership Committee was fully occupied in its task of dealing with the 393 applications for membership received up to 31 May. By the autumn the Inaugural Council had approved the election of ninety-seven Fellows, 119 Associates and 120 Licentiates and had approved the arrangements for the election by postal ballot of the first Council. This was the situation when the first Annual General Meeting of the Institute was held at the Borough Polytechnic in London on 7 December 1964. At that meeting, which was attended by about 100 members, the Chairman announced the result of the ballot and declared the following elected:

President: Dr J. D. Mounfield.
Vice-President: Professor J. Hawthorn.
Honorary Treasurer: Professor E. L. Crossley.
Honorary Secretary: A. H. Woollen.
Members of Council: I. M. V. Adams, T. E. Bashford, A. E. Billington, J. R. Blanchfield, S. H. Cakebread, Dr J. G. Davis, E. Druce, H. B. Heath, Dr S. M. Herschdoerfer, Dr M. Ingram, T. McLachlan, Dr D. B. Smith, Dr E. H. Steiner, M. Spencer and Professor A. G. Ward. (At a subsequent meeting of the Council Dr E. C. Bate-Smith was co-opted to the Council.)

The President then reviewed the events leading to the Annual Meeting, after which a long and useful discussion took place on the future activities of the Institute. From among the many suggestions put forward the one receiving most support was that a new Journal should be published as soon as possible. The Council promised to try to implement the proposal.

At the first meeting of the elected Council the President proposed that separate Committees should be set up to decide and control policy and to study and develop the various functions of the Institute. It was agreed that the following should be formed:

1. A General Purposes Committee to control policy and finance (Chairman, The President; Secretary, A. H. Woollen).

2. A Membership Committee to consider and recommend candidates for election to the Institute (Chairman, The President; Secretary, A. E. Billington). This Committee was a continuation of the Qualifications Committee appointed in March 1964.

3. A Programme Committee to recommend and arrange programmes for Ordinary Meetings of the Institute (Chairman, T. McLachlan; Secretary, E. Druce).

4. A Publications Committee to plan the publication of a new Journal and to issue Newsletters or Proceedings of the Institute (Chairman, Dr J. G. Davis; Secretary, S. H. Cakebread).

5. An Education Committee to survey existing courses of education in food science and technology in Colleges and Universities and to advise on careers and examinations (Chairman, Professor J. Hawthorn; Secretary, H. B. Heath).

It has been through its various Committees that the Institute has organized its normal activities and provided for its future development. The Council's first actions were to ask the Programme Committee to plan and arrange a series of meetings, symposia and social events covering 2 years and to ask the Publications Committee to plan the publication of a new Journal. It is in no small measure due to the energy and enthusiasm of Mr T. McLachlan and Dr J. G. Davis, Chairmen of the respective Committees, that these two objectives have been achieved in so short a time.

The first Ordinary Meeting of the Institute was held at the Borough Polytechnic on 18 March 1965 when films were shown, and the first provincial meeting, arranged by Mr J. R. Blanchfield, was held in Manchester on 29 April 1965. From the latter meeting sprang the first Branch of the Institute, the Northern England Branch, which came into formal existence on 20 July 1965.

It can now perhaps fairly be said that the Institute has established its potential as a major force in the field of food science and technology and there is every reason to believe that it will continue to adopt a mature attitude towards its responsibilities and will exercise a beneficial influence on the development of the food industry in the new era into which we are entering.

J. Fd Technol. (1966) **1**, 9–15.

Texture and pH in fish muscle related to 'cell fragility' measurements

K. KELLY,* N. R. JONES,† R. M. LOVE† AND J. OLLEY†

Summary. The cell fragility method relates the protein denaturation of cold-stored fish to the optical density of a muscle homogenate. A high $E_{\frac{1}{2}\,cm}$ value has been claimed to indicate a good quality frozen fish while a low one would indicate considerable cold-storage deterioration. Three independent groups of workers have now shown that the initial optical density of a muscle homogenate may vary considerably. It appears to be correlated with the pH of the muscle, which in turn is related to the texture. It has been found that a fish with a high pH has a soft texture and gives a low cell fragility reading, while a high reading is given by fish with low pH, which have a firmer texture.

At low temperatures denaturation of the muscle is slight during cold-storage, so that this pH effect may well predominate in determining the optical density of the homogenate. Thus, while the cell fragility technique is not invalidated as a research tool, it can give misleading information when used on commercial samples of unknown pH.

Introduction

Three groups of workers have independently and for different reasons been studying the texture of cod. K.K. was interested in the cold-storage behaviour of commercially caught cod, N.R.J. was studying practical methods of fillet freezing on fish of varying physiological conditions, and R.M.L. and J.O. were basically concerned with the problem of deciding whether the cell fragility method or the soluble protein method of determining protein denaturation was most closely related to textural changes and free fatty acid production. The initial experiments of the three groups have led them to the same conclusions, and the results have been summarized together in this paper.

Methods

pH measurement. K.K. measured the pH of a 2 : 1 water fish homogenate at 20°C. N.R.J. the pH of a 5 : 1 homogenate containing 5×10^{-3} M-iodoacetate at 15°C,

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† Torrey Research Station, Aberdeen.

R.M.L. and J.O. the pH of a 2 : 1 homogenate containing $2{\cdot}5 \times 10^{-3}$ M-iodoacetate at 20°C. All measurements were done with a glass electrode. The small samples for pH measurements were taken adjacent to those used for cooking owing to the variation in post-rigor pH down the length of a fish.

Cell fragility was determined by the method of Love & Mackay (1962).

Texture. The fish were steamed for 30 min in a casserole. K.K. used a trained panel of ten individuals and an arbitrary scale of 0–4 from soft to tough with the 'normal' as the middle of the range. N.R.J. used an arbitrary scale of 1–5 of increasing toughness: (2 = 'normal') with a panel of five laboratory workers untrained in textural evaluation, but who might be expected to react as average consumers. R.M.L. and J.O. used the tentative firmness score sheet for frozen cod of Baines & Shewan (1965) with a scale from 0 to 6 and 2 as the normal value, comparable to fresh unfrozen cod. The panel contained four trained members.

Microscopy

A sample of the homogenate from the cell fragility measurement was transferred to a slide and placed under a cover slip immediately; delay caused coagulation of the fibrils. Photographs were taken under phase contrast with the Zeiss ultraphot.

Experimental material

The fish used in the experiments of K.K. were landed commercially at Aberdeen and had just passed through rigor. The fish were filleted and sorted according to pH before air-blast freezing at −29°C and storing in polythene pouches. The fish caught in December 1963 were stored at −29°C for 1 year. To confirm the trend of results a further batch caught in December 1964 was stored for 1 month at −29°C. The fish in the experiments carried out by N.R.J. were caught in May 1963 and held in an aquarium and either fed or starved for 8 weeks. Sub-groups were frozen before, during and after rigor as fillets. The fillets were held at −20°C for 2 weeks prior to examination. The fish used by R.M.L. and J.O. were caught between February and May 1965 by the Station's research trawler. The February samples were examined unfrozen and the May samples were divided into paired fillets. pH, taste panel and cell fragility measurements were carried out on one fillet, the other fillet being frozen in an air-blast freezer, thawed and examined the following day. Twelve fish were selected for the May experiments to give a size range from 40 to 112 cm. The first experiment in February involved fish of approximately 60–80 cm. The second experiment involved fish in the 40–50 cm range. There was no selection of fish with regard to pH and a natural scatter is represented. It has subsequently been found that while small fish

almost invariably have a higher pH, the pH of large fish can cover the whole range.
All frozen fish were thawed overnight at 4°C.

Results

The effect of pH on the organoleptic texture ratings of fish muscle is shown in Fig. 1(a) and (b). The toughness of the product was inversely related to pH. This effect was independent of the size of the fish as the results in Fig. 1(b) were obtained from the fish ranging from 40 to 112 cm in length. The cell fragility values of the fish were inversely related to pH also, both in commercial samples stored either for 1 month or 1 year at −29°C and in samples where the pH was artificially changed by starvation

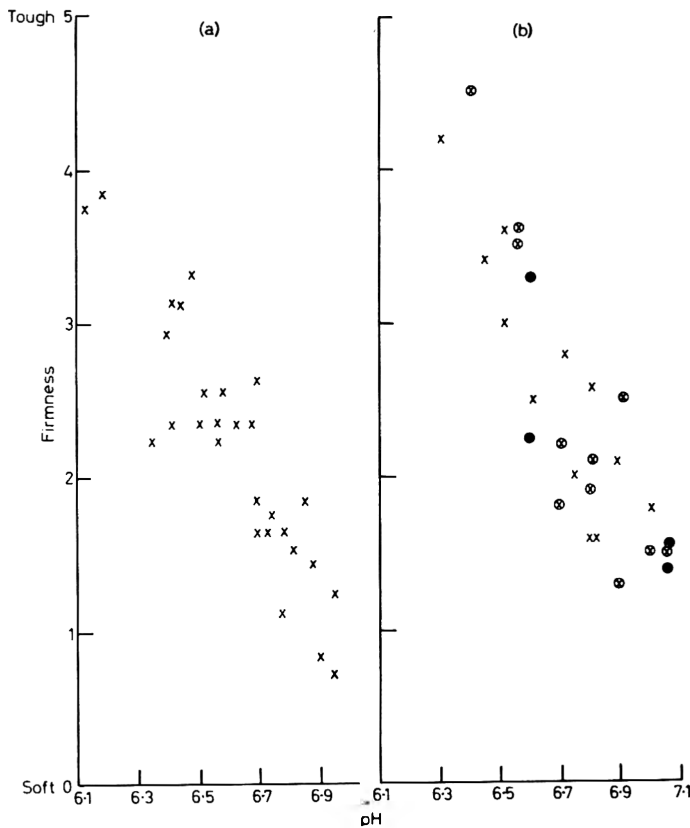

Fig. 1. Taste panel ratings of fish correlated with pH. (a) Unilever fish. Caught December to January 1965. Frozen, stored 3 days at −29°C. (b) Experiments of R.M.L. and J.O. ×, Fresh post-rigor fillets caught in May; ⊗, frozen paired fillets thawed next day; ●, larger fresh post-rigor cod caught in February.

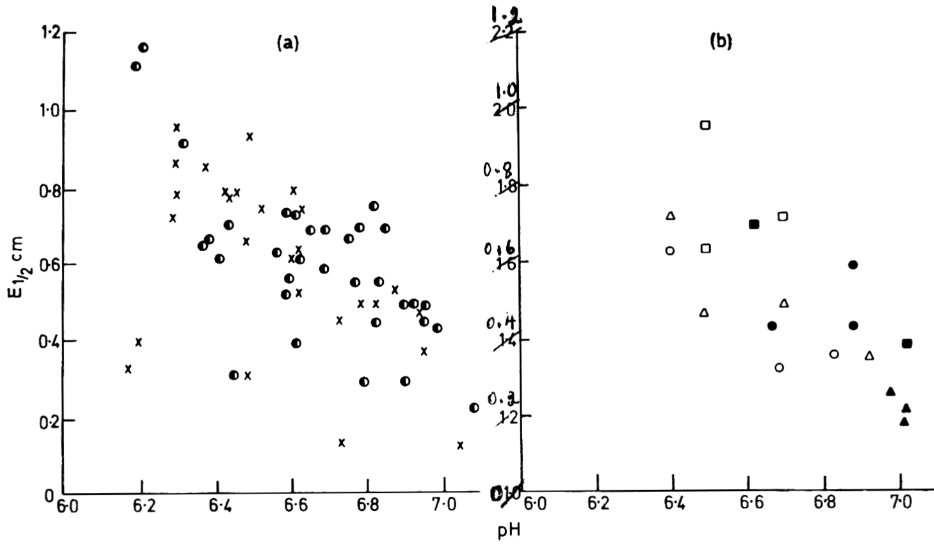

Fig. 2. Correlation of cell fragility with pH. (a) Experiments of K.K. Post-rigor cod frozen and stored for 1 month (◐) and for 1 year (×). (b) Experiments of N.R.J. Open symbols, fed fish; closed symbols, starved fish. Symbols represent fish frozen pre-rigor ○ ●, post-rigor □ ■, and in rigor △ ▲. Fish stored 2 weeks at −20°C after freezing.

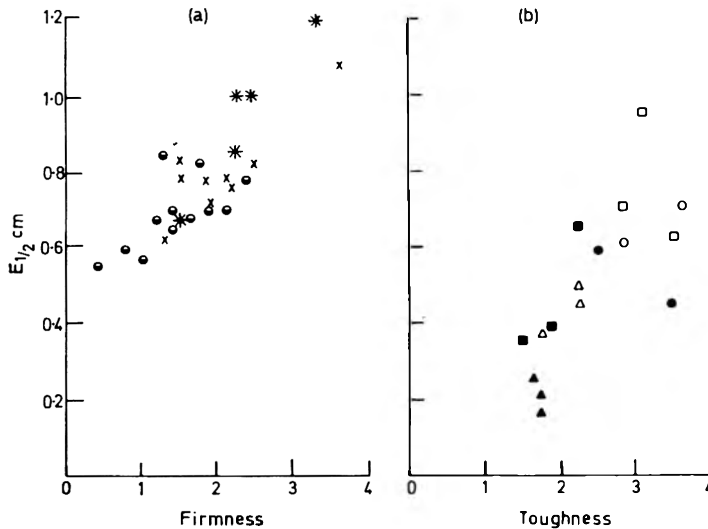

Fig. 3. Correlation of cell fragility with texture. (a) Experiments of R.M.L. and J.O. Fresh post-rigor cod, larger fish caught in February (*), smaller fish caught in February (◒) and fish caught in May (×). (b) Experiments of N.R.J. Symbols as for Fig. 2(b). Note N.R.J.'s scale starts at 1 and not 0.

PLATE 1. Phase contrast microscopy of the formaldehyde homogenates from the cell fragility tests. ×550. Experiments of R.M.L. and J.O. Large cod caught in February unfrozen. (a) Cell fragility $E_{\frac{1}{2}\,cm}$ = 1·2, pH 6·6. (b) Cell fragility $E_{\frac{1}{2}\,cm}$ = 0·65, pH 7·1.

(*Facing p.* 12)

(Fig. 2(a) and (b)). Toughness was related directly to cell fragility (Fig. 3(a) and (b)). K.K. obtains low cell fragility results for pre-rigor fillets independent of pH, and the conditions of N.R.J. for obtaining a high cell fragility after freezing a low pH fish pre-rigor may have been critical.

Plate 1 shows the phase contrast pictures of fish of high and medium pH. The differences are subtle but accounted for a variation of 0·6 $E_{\frac{1}{4}\,cm}$. The higher pH fish show a tendency to form formaldehyde homogenates with shorter wider fibrils which were more difficult to focus on, thus giving a more diffuse impression of the individual sarcomeres. The background fluid was also less clear. Love & Mackay (1962) described the *misty* appearance of the suspension fluid after cell fragility determinations on very starved fish and a different appearance of the particulate suspension. They noted low $E_{\frac{1}{4}\,cm}$ readings but attributed them solely to protein loss and did not relate them to a continuous regression of results with pH. The $E_{\frac{1}{4}\,cm}$ values for a given pH were higher than in Fig. 2 where the fish had been frozen. Love (1962) noted a substantial drop in cell fragility values on freezing of cod.

Discussion

The effect of pH on the water binding capacity and hence the tenderness of meat has been known for several years (Deatherage, 1963; Hamm, 1960), but the significance of pH in fish has tended to be overlooked. This is due to the fact that the pH range of post-rigor fish is one order of magnitude higher than in meat, so that, hitherto, fish has been thought to be out of the range of low pH effects. However, Little (1965) expressed the opinion that the texture of fish was directly related to pH and Murray, Jones & Burt (1964) found that the accumulation of lactic acid in the muscle of frozen shrinking pre-rigor fillets was positively correlated with toughness. These observations have been amply confirmed by the present work.

The results from the three groups of workers are not strictly comparable because each group has used an independent texture panel and K.K. a different cell fragility blender from the Torry group (see Fig. 4). However, the trends within the three groups are strikingly consistent. Cutting (1953) quotes the initial range of pH of Gadoids to be about 6·3–7·0. Equating the firmness score of Baines & Shewan (1965) to this pH range would give ratings of from 1 to 4·5, i.e. from soft and mushy to tough and chewy. These workers equate a rating of 2 with the impression of firmness given by an average range of post-rigor fresh fish. The present paper indicates that fresh values may vary widely about this average. The full extent of such variation in terms of fishing ground, species, etc., remains to be established.

Cold storage of frozen fish leads to toughening which appears to be related to a side-to-side aggregation of the myofibrils (Love *et al.*, 1965). This phenomenon has been measured objectively by the cell fragility method (Love & Mackay, 1962). Figs. 1–3

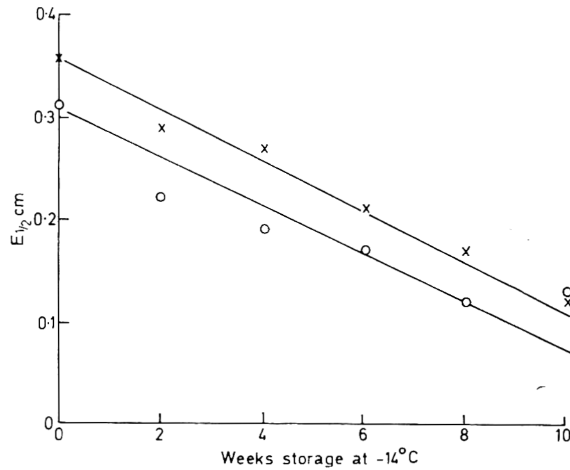

FIG. 4. Decrease in cell fragility of a high pH fish at higher storage temperatures. ×, Torry Research Station equipment; ○, Unilever equipment.

show that with fish of variable initial pH a high reading of $E_{\frac{1}{2}\,cm}$ from 1 to 1·2 indicates a tougher product independently of whether the fish was fresh or frozen, while a low reading 0·1–0·5 indicates a soft and mushy product. That is, toughness and cell fragility values are still shown to be closely related, but in this case it is in a sense contrary to that occurring in the frozen state. This has been discussed in a recent paper (Love *et al.*, 1965). However, it is apparent that when fish are stored at −29°C denaturation by aggregation of fibrils is slow and therefore the influence of pH may be the dominant one (Fig. 2). At higher temperatures, as for example −14°C, aggregation is more rapid and a fall in cell fragility values here indicates an increase in the denaturation of the muscle.* Fig. 4 illustrates cold storage changes in high pH (i.e. soft) fish. Although on the basis of earlier work an $E_{\frac{1}{2}\,cm}$ of 0·3 would be taken to indicate material that had suffered from prolonged cold storage, it represents in the present instance a freshly frozen soft product. Whether the low figure is the result of the influence of pH (myofibril fragility) or cold-storage denaturation [myofibrillar aggregation (Love *et al.*, 1965)] is of course immediately apparent on inspecting the homogenate. After cold storage this fish denatured and the cell fragility decreased. Further work is required to determine whether fish of high and low pH denature at the same rate in the cold store and whether the toughening of a sloppy product from high pH fish would make it more acceptable.

* It has recently been shown (Robertson & Love, 1965) that frozen cod in consumer packs taken from retail cabinets at point of sale show very low values in the cell fragility test, as compared with the wholesale material. The cause of this was the high storage temperature employed, so that the low values were the result of cold-storage aggregation and pH played little, if any, part.

It is thus apparent from the present studies that there can be serious pitfalls in the determination of textural quality in frozen fish of unknown pH by the cell fragility method. It may well be, however, that a practicable compensation for pH can be worked out, with consequent improvement in the performance.

Acknowledgments

Dr T. R. Kelly organized the texture ratings in Fig. 1(a) and Dr J. J. Connell and his team the firmness ratings in Figs. 1(b) and 3(a).

The work described by N.R.J., R.M.L., and J.O. in this paper was carried out as part of the programme of the Ministry of Technology.

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Nature and orientation of ice in frozen fish

A. AITKEN

Summary. The ice component of frozen fish muscle has been examined by X-ray diffraction. Only the common hexagonal form was observed, but line intensities indicated strong orientation of the crystallites. The *c*-axis is shown to be perpendicular to the direction of freezing; the muscle fibre direction does not appear to affect the direction of orientation to a noticeable extent.

Introduction

It has long been recognized that some of the water in biological materials is tightly bound to proteins or carbohydrates and is not readily frozen or removed by drying, though Love & Elerian (1963) have recently shown that some of this water can be irreversibly unbound by freezing to low temperatures. The less strongly bound, readily frozen, water has been studied mainly with regard to its location in the tissue. The present study deals with the nature of this ice in frozen fish muscle.

Moline *et al.* (1961) have suggested that discrepancies between observed and computed specific heats of meat and fish may be a consequence of the formation of vitreous ice instead of the common hexagonal modification. Dowell, Moline & Rinfret (1962) have shown that, in gelatin gels, the three polymorphic forms of ice stable at atmospheric pressure, hexagonal, cubic and vitreous, can all be formed depending on the gelatin concentration and the rate of freezing. The conditions of formation of these structural modifications in pure water have been examined by Dowell & Rinfret (1960) and their structures reviewed (Lonsdale, 1958; Blackman & Listgarten, 1958; Brill, 1962). Luyet, Tanner & Rapatz (1962) have argued that, in rapidly frozen gelatin gels, the formation of cubic ice need not be postulated; all X-ray diffraction patterns could be attributed to mixtures of vitreous ice with hexagonal ice of specific habit.

The present work was initiated to determine whether the formation of a less common form of ice might explain some unusual dielectric properties of frozen fish (A. C. Jason, unpublished results).

Methods

An X-ray diffractometer (Philips Type PW 1051) was used with Cu Kα radiation to obtain powder patterns of specimens of frozen fish. A cooled specimen holder, designed

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by Smith (1961), was modified to enable a 20 × 10 mm specimen, 2 mm thick, to lie in the correct sample plane. With the exception of the first sample examined, all specimens were cut from fresh, unfrozen, post-rigor cod (*Gadus callarias* L.), the direction of the muscle fibres being, as closely as possible, aligned with one of the axes of the sample (Fig. 1). It was very difficult to obtain exact alignment and the specimens, after freezing and X-ray examination, were inspected by teasing the fibres under a hand lens to determine the actual fibre orientation. The first sample used was cut, in a cold chamber, from a large frozen cod fillet which had been frozen under commercial conditions and stored at −29°C for 3 months.

Freezing of the unfrozen samples was arranged so as to be unidirectional, i.e. the directions of maximum temperature gradient at all points in the sample were parallel. Three freezing directions were used together with three fibre directions (Fig. 1). Only eight of the nine possible combinations were examined. Freezing from the

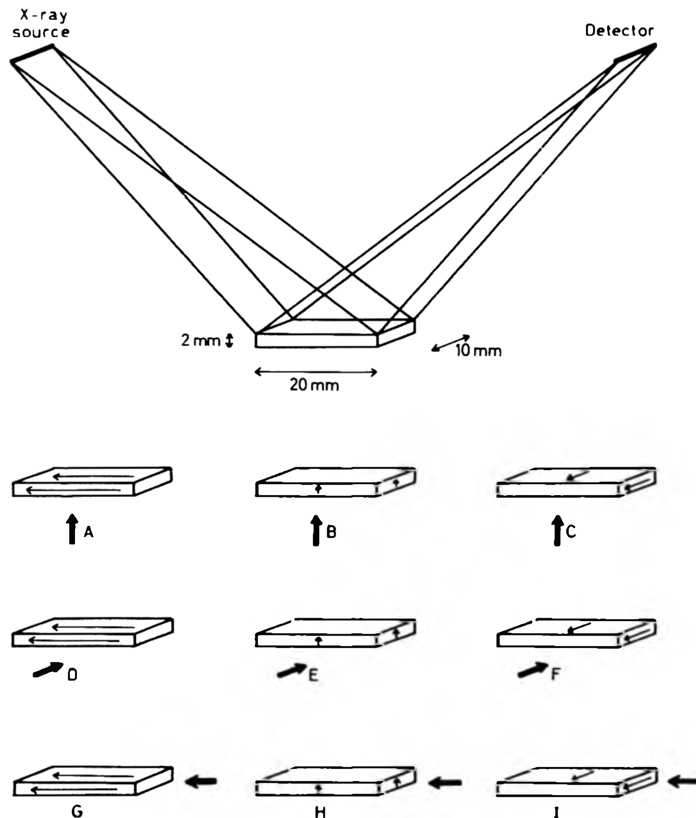

Fig. 1. Top: Orientation of sample dimensions with respect to X-ray beam. A–I: Combinations of fibre directions and freezing directions; arrows on blocks indicate fibre direction, heavy arrows indicate freezing direction (i.e. normal to cold surface); I was not examined.

20×10 mm face was achieved by laying the specimen, on this face, on the bottom of an aluminium container which was then dipped into solid CO_2/acetone. The specimen was covered during freezing by a frost cover (Smith, 1961), to prevent condensation on the surface. The freezing time, indicated by a visible increase in opacity of the upper surface, was about 5 sec. The face of the specimen exposed to the X-ray beam was normally the one distant from the freezing surface. In order to freeze from the 20×2 mm or the 10×2 mm face, the sample was supported vertically in an aluminium container between two blocks of insulating material with the appropriate face in contact with the bottom of the container, which was then dipped into solid CO_2/acetone. Immediately after freezing, the sample was covered by a frost cover. The samples were stored in solid CO_2 until required for X-ray examination. The sample holder was cooled to below $-60°C$ before inserting the sample and held at this temperature.

Owing to the difficulty of cutting wet fish accurately to size, the sample thickness sometimes varied. This caused a slight deviation of the positions of the X-ray reflections by up to $0{\cdot}7° \ 2\theta$ but this did not lead to any difficulty in identifying reflections.

Results

The first specimen examined, cut from commercially frozen fish, gave an X-ray pattern which was clearly that of the common hexagonal modification, which is thermodynamically stable at this temperature, though the relative intensities of the reflections differed somewhat from those of pure hexagonal ice (Dowell & Rinfret, 1960). It was suspected that this was due to some degree of preferred crystallographic orientation of the ice crystals. Such orientation could be caused either by the high degree of orientation of the muscle fibres and their components (Bourne, 1960), or by the possible directional nature of freezing, or both. The combinations of fibre direction and freezing direction illustrated above were therefore examined.

Two characteristic diffraction patterns were obtained—Type I and Type II—which were clearly related to the hexagonal pattern of Dowell & Rinfret (1960). Fig. 2 is a diagrammatic representation of peak heights, while in Fig. 3 a direct trace of a Type I pattern is given to show the uniform background. Type I patterns were given by specimens of classes A, B and C: Type II by classes D, E, F, G and H. The patterns of Fig. 2 have been chosen to show the highest degrees of orientation observed in the series of experiments. There was some variation from sample to sample in the degree of orientation but the differentiation into the two types was always clear. It is clear that the direction of freezing has the predominant influence on the pattern obtained. For a given freezing direction, the slight differences in X-ray pattern between samples of the same fibre direction (i.e. replicate samples) are comparable to those between samples of different fibre directions. Thus the fibre direction has no substantial effect on the crystallite orientation.

The uniform background of the traces (Fig. 3) suggests that no significant amount of vitreous ice is formed. The patterns of Type II have, as their strongest line, that of cubic ice (Dowell & Rinfret, 1960) but the relative intensities of the other lines do not suggest the presence of substantial amounts of this modification. Since cubic ice is formed only at high freezing rates it is unlikely that specimens giving Type II, which necessarily have a lower mean freezing rate than those giving Type I, could have any cubic ice.

A gelatin gel (20%) specimen was also examined; it was frozen from the 20 × 10 mm face and the orientation proved to be slight. On the other hand, distilled water frozen from the same face showed very marked orientation of Type I.

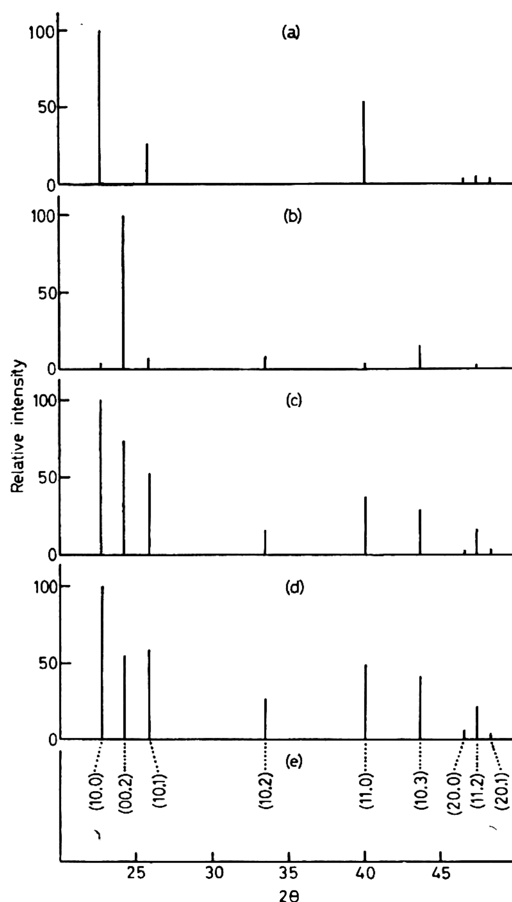

FIG. 2. X-ray powder diffraction patterns (diagrammatic): (a) Type I (present investigation); (b) Type II (present investigation); (c) hexagonal ice (Dowell & Rinfret, 1960); (d) hexagonal ice (calculated, see 'Discussion'); (e) indices of spacings.

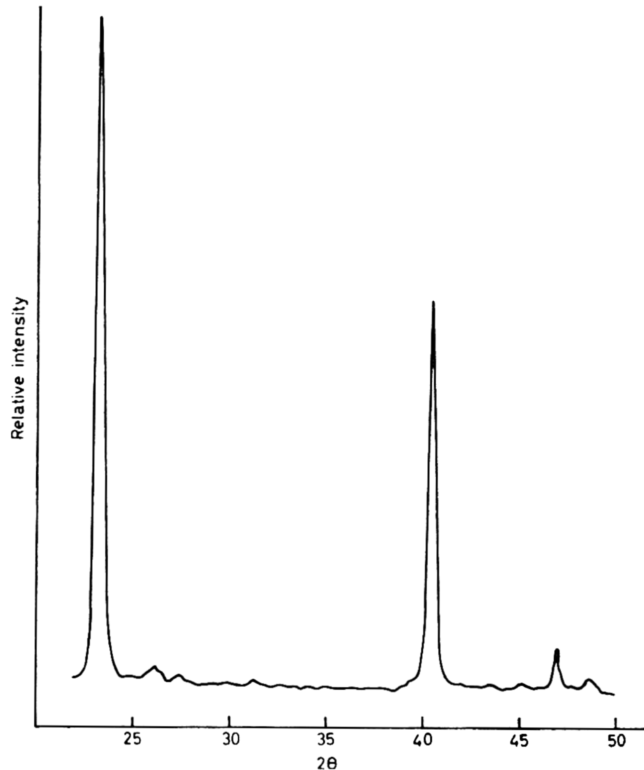

FIG. 3. Trace of Type I diffraction pattern to show uniform background.

Discussion

All patterns examined have been that of hexagonal ice, the normal modification. As the freezing rates employed in this work have been very much higher than normal commercial rates it seems very improbable that any polymorphic form other than the hexagonal one could arise under normal conditions. It thus seems unlikely in this case that the crystal structure of the ice phase can be responsible for the specific heat or dielectric constant anomalies mentioned in the Introduction.

The discovery of marked orientation may have significance in a wider field. The two types of oriented pattern were compared in Fig. 2 with Dowell & Rinfret's data, taken by implication to be free from preferred orientation. The latter pattern agrees substantially with a pattern, published by the same group (Dowell *et al.*, 1962, Fig. 3), of hexagonal ice formed by recrystallization of vitreous ice and probably unoriented. There is a remarkable paucity of published powder patterns of ice. The ASTM Index (1963) pattern is that of Dennison (1921) and is highly oriented. To check the absence

of orientation in Dowell & Rinfret's data, the powder pattern of ice has been calculated by standard methods (International Tables for Crystallography, 1962). Only oxygen atoms were included and atomic co-ordinates from Lonsdale (1958) were used. Temperature corrections were applied; the root mean square amplitude of vibration of the atoms was also taken from Lonsdale (1958). Absorption corrections were not applied. The calculated pattern is given in Fig. 2 and agreement with Dowell & Rinfret (1960) is satisfactory in view of the uncertainty arising from lack of absorption correction and from neglect of the hydrogen atoms.

The very marked orientation observed in the present work both with fish and distilled water emphasizes the importance of having a completely random pattern available for comparison when drawing conclusions from relative intensities. Many of the patterns of Luyet *et al.* (1962) are readily explained as being due to hexagonal ice of a moderate degree of orientation.

The nature of the observed orientation can be seen by considering the strongest line in each of the pattern types. In Type I it is the (10.0)—very occasionally (11.0)—while in Type II the strongest line is (00.2). In Type I the (00.2) spacing is normally very weak, while in Type II the (10.0) is present at moderate intensity. These facts are consistent with alignment of the crystallites with their *c*-axes predominantly at right angles to the direction of freezing. Thus in specimens of classes A, B and C, which give Type I patterns, the great majority of the crystals have their *c*-axes parallel to the plane of the sample. In classes D, E, F, G and H, giving Type II patterns, the *c*-axes of a substantial number of crystallites will be perpendicular to the specimen plane giving rise to a strong (00.2) reflection. Fig. 4 shows crystals in the orientations required to give the strongest lines of Type I and Type II.

While the diffractometer is not ideally suited to the study of preferred orientation, and it is not possible to draw diagrams showing the complete spatial distribution of the crystal axes, some further information can be obtained from the lines of the X-ray

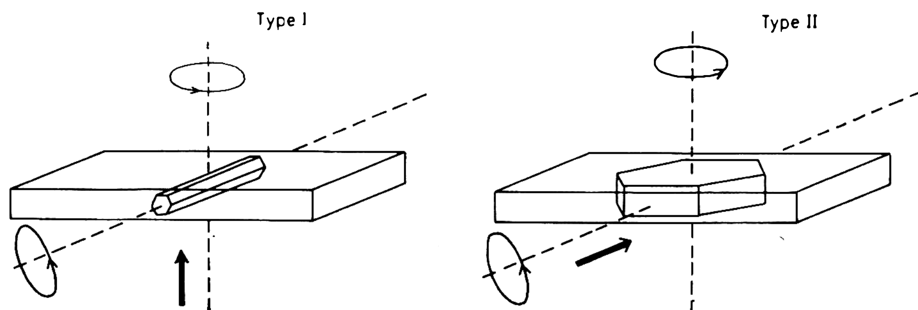

Fig. 4. Muscle blocks (orientation with respect to X-ray beam as in Fig. 1) showing crystallites in orientations required to give the strongest lines of Type I and II patterns: heavy arrows show freezing directions, dashed lines indicate axes of apparently random orientation.

pattern other than the strongest. For example in Type I patterns the mean ratio of the intensities of the (10.0) and (11.0) lines is 1·82 ± 0·75 (S.D.); this ratio is not significantly different from Dowell & Rinfret's value of 2·56. There is therefore random orientation of the crystallites around their *c*-axes. Again, orientation of the *c*-axis within the plane normal to the direction of freezing can be considered. Both in classes A, B and C and in D, E and F there are no reproducible differences in the relative strengths of the (00.2) line and the (10.0) and (10.1) lines according to the fibre direction. Thus the orientation of the *c*-axis appears to be random in the plane perpendicular to the freezing direction. The axes of apparently random orientation are indicated in Fig. 4.

Considerable interest in ice crystal orientation has recently arisen in connection with the properties of sea ice (Kingery, 1963) and it is generally agreed that, although at the surface of the ice the *c*-axes tend to be vertical, at a depth of about 5 cm the predominant *c*-direction is horizontal (perpendicular to the temperature gradient) in agreement with the present results. Pounder (1963) has clearly shown how this can be explained on the assumption of more rapid growth at right angles to the *c*-axis than along it. Megaw, indeed, showed in 1934 that in pure water the fastest growth was normal to the (11.0) plane. On a free water surface growth at right angles to the *c*-axis gives rise to hexagonal plates which float with the *c*-axis vertical and thus control the surface orientation. Only at greater depths does the effect of growth rate begin to control the orientation. In fish, on the other hand, there is no possibility of 'gravitational' orientation as on a free water surface and the orientation actually observed is set up much more quickly. The specimens of classes A, B and C were generally examined on the face remote from the original freezing surface. Two specimens were studied also in the inverse position. The X-ray patterns from both faces were almost identical, the face nearest the freezing surface showing only slightly less orientation. Thus the state of preferred orientation is established at the freezing surface.

The possible crystallographic orientation of the ice phase in frozen biological materials should in future be taken into account when any anisotropic properties are being examined. These will include refractive index, thermal conductivity, electrical conductivity, dielectric constant, elastic properties and many others. Crystallographic orientation does not necessarily bear any relationship to external shape of crystals. It is possible then that ice crystals formed under different conditions but externally identical may be of different orientation and have different properties.

Acknowledgments

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J. Fd Technol. (1966) **1**, 25–39.

An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups

R. S. NORRISH

Summary. An apparatus is described for determining the equilibrium relative humidity (e.r.h.) of confectionery syrups and the results obtained are compared with those of other workers. The identity of e.r.h. with the thermodynamic activity is pointed out and an equation for the activity coefficient as a function of concentration is derived. The agreement between e.r.h. values calculated by means of this equation and experimental results is demonstrated and some of the advantages of the use of equations of this type are discussed, including the ability to draw significant conclusions about the nature of the solvent–solute interaction.

Introduction

In the confectionery industry the equilibrium relative humidity (e.r.h.) of the products (i.e. the ratio of the partial pressure of water in equilibrium with the product to the saturated vapour pressure of water at the same temperature) is of some importance, since it governs the direction and ultimate extent of moisture transfer to or from the ambient atmosphere and therefore affects the shelf life. Since the e.r.h. can be modified by the addition of various 'humectants', a project was initiated to determine the effect on e.r.h. of the addition of these substances to sucrose solutions. The humectants studied were dextrose, fructose, invert sugar, glucose syrups, sorbitol and glycerol.

The equilibrium relative humidities of binary aqueous solutions of sucrose and the humectants mentioned have already been reported in the literature: for sucrose (Whittier & Gould, 1930; Thieme, 1934; Scatchard, Hamer & Wood, 1938; Pouncey & Summers, 1939; Grover, 1939, 1947; Robinson & Stokes, 1949; Heiss, 1955); for dextrose, fructose and invert sugar (Cleland & Fetzer, 1944; Grover, 1947; Heiss, 1955; Taylor & Rowlinson, 1955; Jongen, 1956a); for glucose syrups (Cleland & Fetzer, 1944); for sorbitol (Griffin, 1945; Anon., 1955; Jongen, 1956b; Jenkins, 1960); and for glycerol (Scatchard *et al.*, 1938; Cleland & Fetzer, 1944). Some data are also reported in International Critical Tables (1926). While the agreement between different

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investigators is on the whole fairly satisfactory, Whittier & Gould (1930) and Pouncy & Summers (1939) report considerably lower values for sucrose than the other workers, and the results for sorbitol and glycerol both show a considerable scatter, particularly at the higher concentrations. Furthermore there are very few published data on multicomponent mixtures. It was decided, therefore, to redetermine the values for all the binaries plus as many multicomponent systems as were necessary to confirm the validity of the correlating equation to be described.

Results

The experimental method adopted depended on the change of resistance of a ceramic pellet (doped and fired titanium dioxide, originally developed by S.I.R.A.) with change of relative humidity and the apparatus used is shown in Fig. 1. The pellet,

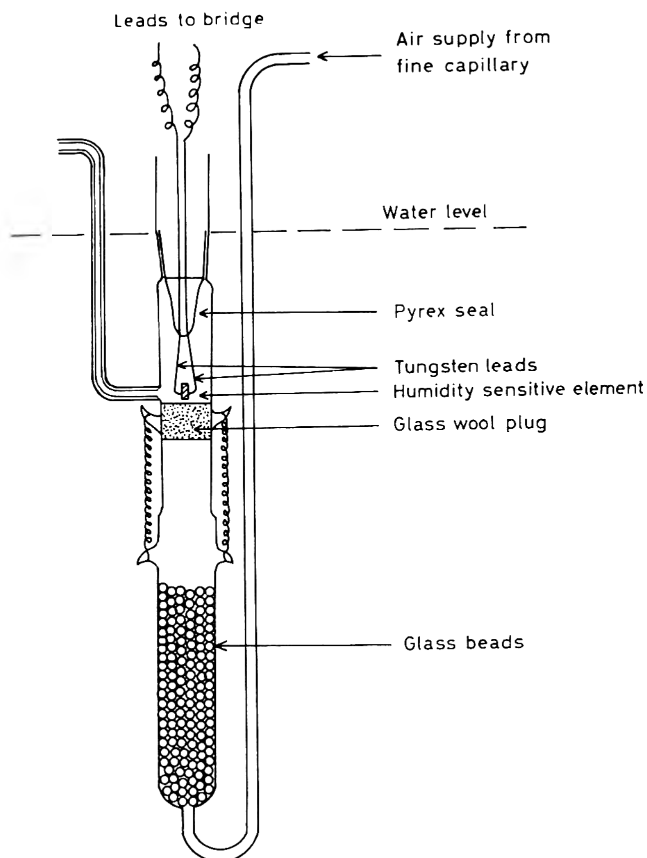

FIG. 1. Diagram of apparatus.

approximately 7 mm diameter and 1 mm thick and provided with conductive coatings on each face to which fine wires were attached, was connected by means of these wires to the two tungsten wires sealed through a B.19 cone. The cone, with pellet attached, was mounted in a B.19 socket on top of the measuring cell, a tube 7 cm long provided with a capillary outlet in line with the pellet and carrying a B.19 cone at its lower end. The lower half of the cell was filled with glass wool and was mounted on top of a glass column 10 cm long and 2·5 cm diameter filled to a height of 7 cm with 2·5 mm diameter glass beads and provided with an inlet at the lower end. The joint between the cell and column was secured with springs.

The whole apparatus was immersed in a water bath controlled at 25°C to better than 0·05°C. Air was supplied to the inlet at the bottom of the apparatus via a fine capillary connected to a 1 litre Buchner flask, the pressure in which was maintained manually by the operation of a rubber aspirator bulb.

The sample (7·5 ml) was poured into the column and allowed to trickle over the glass beads. After placing the measuring cell in position the apparatus was lowered into the bath and left for not less than 30 min to come to temperature equilibrium. The Buchner flask was then pressurized so that a very slow stream of air passed through the sample, over the wetted glass beads and into the measuring cell, whence it was vented to atmosphere via the capillary outlet.

The pellet formed one arm of a Wheatstone bridge energized from an 8 V, 50 cycle supply, the out-of-balance current being detected by a vibration galvanometer. The bridge was balanced every 5 or 10 min until no further change of resistance occurred. The time taken to come to equilibrium depended on the sample and on the initial state of the pellet, but was typically about half an hour.

The apparatus was calibrated using saturated salt solutions, the equilibrium relative humidities of which have been determined by Wexler & Hasegawa (1954) who state that the uncertainty in their values varies from 1·2% at high values to 0·2% at low values of relative humidity. Precautions were taken to avoid the effects of hysteresis and long-term calibration shifts by making all measurements from the same direction and by calibration checks before and after each series of measurements. It was confirmed that no error resulted from failure to reach true equilibrium since no difference could be detected between an experiment in which the air supply to the apparatus was saturated with water and one in which it was first dried over concentrated sulphuric acid. The effect of evaporation of water from the sample during an experiment was also negligible since it can be calculated that even if the air supply was perfectly dry the solids content of the sample could not have changed by more than 0·6% per hour in the worst case. The chief limitation on the accuracy of the results was the insensitivity of the bridge at low humidities. Between 95 and 90% the sensitivity was of the order of $\pm$0·1% relative humidity but fell to not much better than $\pm$2% e.r.h. between 55 and 50% e.r.h. For this reason no measurements were attempted below 50%. It is

concluded from the above considerations that the absolute overall accuracy of the measurements is about 1–2% e.r.h. over the range 50–100% e.r.h.

The results obtained, together with those of other investigators are shown in Figs. 2–7 and Tables 1–5. It will be seen that the agreement between different workers is generally good.

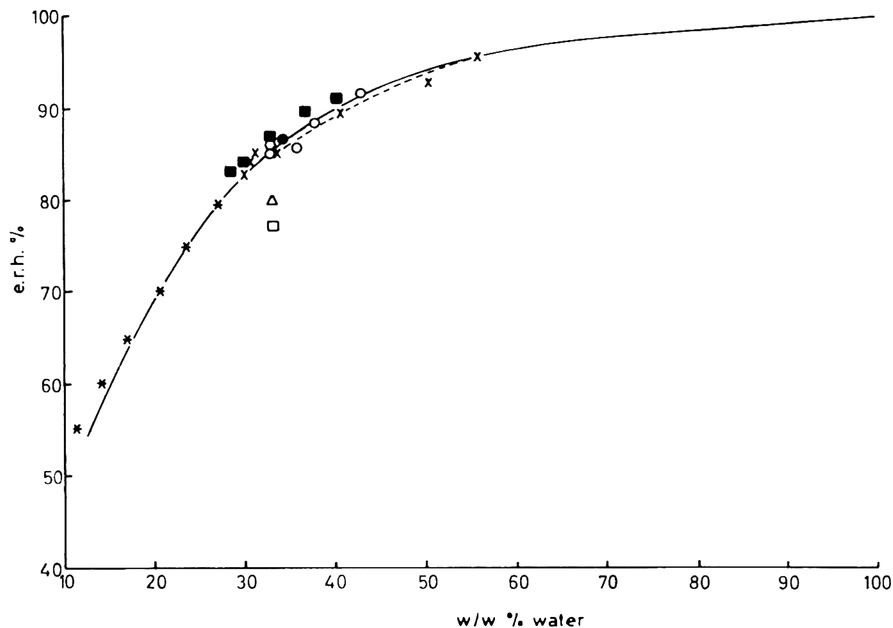

Fig. 2. Equilibrium relative humidity (e.r.h.) of aqueous sorbitol solutions. Line calculated from equation (1) with $K_2 = -2{\cdot}60$. Symbols used are: ×, Heiss (1955); ■, Grover (1947); ✱, Thieme (1934); △, Pouncey & Summers (1939); □, Whittier & Gould (1947); ●, International Critical Tables (1926, pp. 293, 365); ○, this research; ----, Robinson & Stokes (1949) and Scatchard, Hamer & Wood (1938).

Discussion

A number of correlating equations have been proposed in the literature. Those of Grover (1940) and Money & Born (1951) are probably the best known in the confectionery industry; that of Dunning (1951), valid only for pure sucrose solutions, is less well known. However, these equations are in many ways unsatisfactory. Firstly they are all entirely empirical so that no significance can be attached to the values of the adjustable constants which appear in them and no confidence can be placed in their use outside the range over which they have been fitted to the experimental results. Secondly none of them can be adapted to give linear plots, which are not only a con-

venience, but make for more accurate interpolation and particularly extrapolation. Furthermore, Grover's equation, which for sucrose solutions can be written:

$$\text{e.r.h. }\% = 104 - 10\frac{m_s}{m_w} + 0{\cdot}45\left(\frac{m_s}{m_w}\right)^2$$

where m_s and m_w are the weights of sucrose and water respectively, cannot possibly be valid at the extremes of concentration, since it extrapolates to 104% e.r.h. at 100% water and infinity at 0% water. Neither Grover's equation nor that of Money & Born is thermodynamically consistent in the sense that the Gibbs–Duhem equation can be

TABLE 1. Results for water–sucrose–sorbitol

w/w(%)			e.r.h.(%)	
Water	Sucrose	Sorbitol	Experimental	Calculated
37·6	51·7	10·7	88·0	87·3
41·2	44·2	14·6	88·5	89·0
35·0	46·7	18·3	85·0	85·0
45·7	29·0	25·3	88·6	89·5
49·1	24·9	26·0	91·5	91·0
30·0	63·0	7·0	81·6	81·9
30·0	56·0	14·0	81·0	81·0
30·0	49·0	21·0	80·2	80·5
30·0	42·0	28·0	79·9	79·9
30·0	35·0	35·0	79·6	79·3
30·0	21·0	49·0	79·3	77·7
30·0	14·0	56·0	79·0	77·2
30·0	7·0	63·0	78·3	76·5

TABLE 2. Results for water–sucrose–dextrose

w/w(%)			e.r.h.(%)	
Water	Sucrose	Dextrose	Experimental	Calculated
35·4	61·0	3·57	85·8	86·8
35·0	55·8	9·20	85·8	86·0
34·2	52·2	13·6	85·6	85·0
36·0	47·7	16·3	85·8	85·9
36·7	43·6	19·7	86·3	86·1

satisfied without postulating absurd functional relationships between the activity coefficients of the solutes and concentration. It was considered that a thermodynamically based equation free of the above objections, particularly if it could be generalized to include multicomponent systems, would be valuable not only for the practical reason that it would reduce the amount of experimental work required to define a given system, but also because of the possibility of gaining an insight into the mode of interaction of the components, and would be the first step towards predicting the behaviour of systems for which no experimental data are available.

TABLE 3. Results for water–sucrose–glycerol

w/w(%)			e.r.h.(%)	
Water	Sucrose	Glycerol	Experimental	Calculated
15·7	32·5	51·8	52·5	44·5
18·8	38·7	42·5	53·0	53·4
19·8	40·7	39·5	57·5	55·6
20·1	41·4	38·5	57·3	56·8
20·4	42·2	37·4	58·5	57·6
22·5	46·3	31·2	61·8	62·9
24·2	49·8	26·0	65·7	66·8
26·4	54·4	19·2	71·5	72·2

TABLE 4. Results for sucrose solutions containing 16·2 w/w(%) confectioners glucose solids

w/w(%) water	D.E. value	e.r.h.(%)	
		Experimental	Calculated
27·6	64·7	80·7	80·5
	54·8	82·2	81·5
	45·4	81·6	81·9
	37·1	82·9	82·1
32·5	64·7	84·3	85·0
	54·8	86·1	86·0
	45·4	87·1	86·5
	37·1	87·0	86·6

The defining equation for equilibrium relative humidity is: e.r.h. $= p_1/P_1$ where p_1 is the partial pressure of water and P_1 the saturated vapour pressure at the same temperature. Assuming ideal behaviour in the vapour phase (a very good approximation for the range of temperatures and pressures of interest), $p_1 = P_1 x \gamma_1$, where x_1 is the mole fraction of water in the solution and γ_1 is its activity coefficient. Equilibrium relative humidity is therefore numerically equal to $x_1 \gamma_1$, the thermodynamic activity

TABLE 5. Results for some complex mixtures

Substance		w/w(%)		
Water	30·2	31·8	31·8	35·9
Sucrose	48·0	45·5	45·5	45·5
64·7 D.E. glucose solids	9·9	—	—	6·1
Sorbitol	4·0	13·6	—	9·1
Glycerol	4·0	—	9·1	—
Dextrose	3·9	9·1	13·6	—
Invert sugar	—	—	—	3·4
e.r.h. (%) Experimental	82·5	82·5	80·6	85·9
e.r.h. (%) Calculated	80·8	82·1	80·0	86·2

TABLE 6. Values of K_2 and free energy of mixing for various sugars

Substance	Molecular weight*	K_2	$\Delta \bar{F}^E_\infty$ (cal/mole)
Sucrose	342	- 2·60	- 3·55
Glucose D.E. 32·8	505	- 2·48	- 3·38
42·0	460	- 2·31	- 3·15
55·0	396	- 2·25	- 3·06
64·0	353	- 1·96	- 2·66
83·4	260	- 1·64	- 2·23
90·7	225	- 1·30	- 1·77
Sorbitol	182	- 0·85	- 1·16
Invert sugar, dextrose, fructose	180	- 0·70	- 0·95
Glycerol	92	- 0·38	- 0·52

* Apparent molecular weight for the glucose syrups.

of the water in solution. The way in which the e.r.h. varies with concentration is therefore known once the dependence of the activity coefficient on concentration is specified. By definition:

$$RT \ln \gamma_1 = \Delta \bar{F}_1{}^E \tag{1}$$

where $\Delta \bar{F}_1{}^E$, is the partial molal excess free energy of mixing which in turn is given by:

$$\Delta \bar{F}_1{}^E = \frac{\partial(N \Delta F^E)}{\partial N_1} \tag{2}$$

where N_1 is the number of moles of water in N moles of solution and ΔF^E is the excess free energy of mixing per mole of mixture. The problem thus reduces to finding a relation between ΔF^E and concentration. For an ideal solution the excess free energy

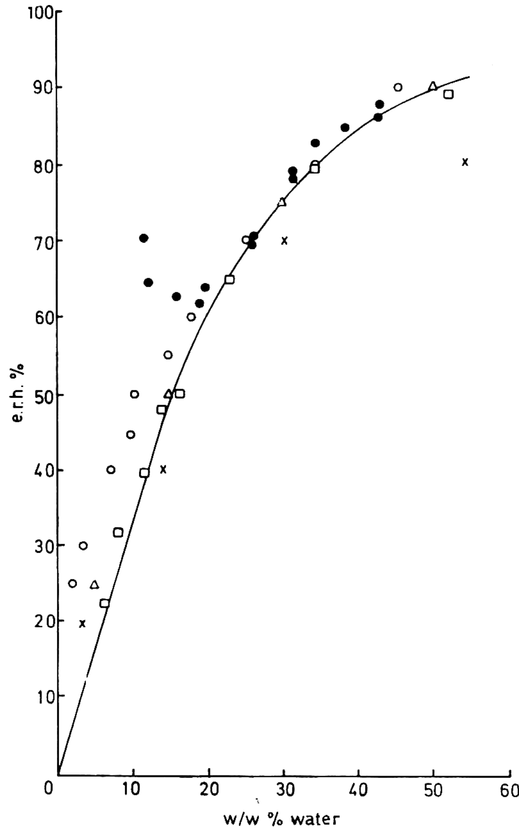

Fig. 3. Equilibrium relative humidity (e.r.h.) of aqueous sorbitol solutions. Line calculated from equation (5) with $K_2 = -0{\cdot}85$. Symbols used are: ○, Jongen (1956b); ×, Anon. (1955); △, Jenkins (unpublished); □, Griffin (1945); ●, this research.

is zero for all concentrations. If the solution is not ideal the simplest possible assumption one can make for a binary mixture is:

$$\Delta F^E = C_{12}\, x_1\, x_2 \qquad (3)$$

where C_{12} is a constant and x_1, x_2 are the mole fractions of the two components. This implies that the free energy change is a result of binary interactions only and is symmetrical in the sense that the components are interchangeable. From equations (1), (2) and (3)

$$RT \ln \gamma_1 = C_{12}\, x_2^2 \qquad (4)$$

or, more conveniently,

$$\log \gamma_1 = \log \frac{\text{e.r.h.}}{x_1} = K_2 x_2^2 \qquad (5)$$

where $K_2 = 0{\cdot}434\, C_{12}/RT$.

A plot of log γ_1 versus x_2^2 should thus be a straight line of slope K_2 through the origin. For all the binary solutions investigated this was found to be the case. The solid lines in Figs. 2–6 have been calculated using the values of K_2 obtained in this way (Table 6) and it will be seen that these curves represent the experimental data quite adequately.

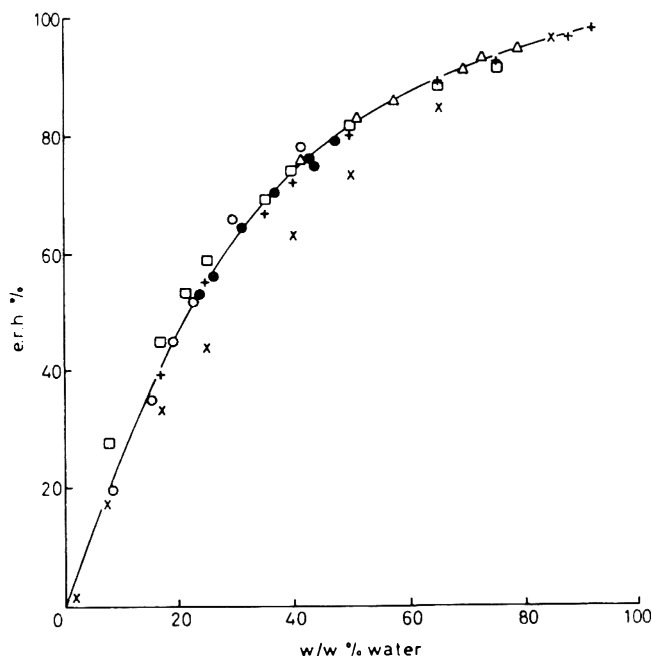

Fig. 4. Equilibrium relative humidity (e.r.h.) of aqueous glycerol solutions. Line calculated from equation (5) with $K_2 = -0{\cdot}38$. Symbols used are: ×, +, International Critical Tables (1926, pp. 291, 293); ○, Cleland & Fetzer (1944); □, Grover (1940); △, Scatchard *et al.* (1938); ●, this research.

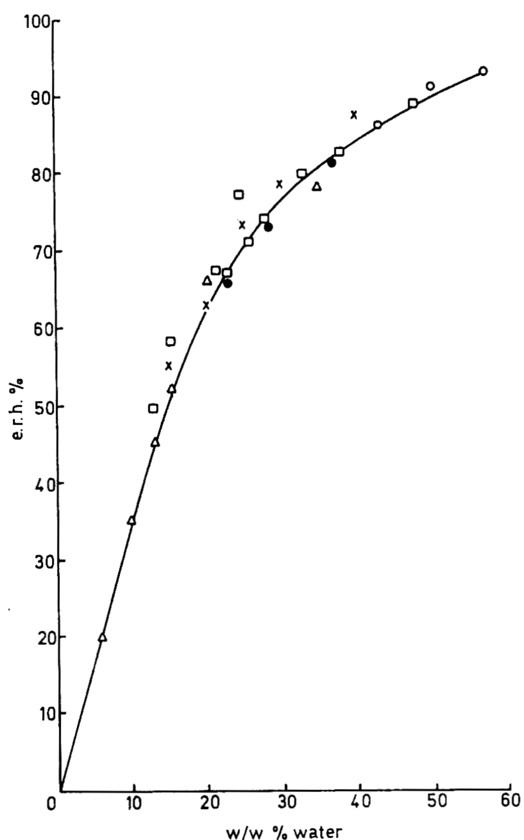

FIG. 5. Equilibrium relative humidity (e.r.h.) of aqueous invert sugar solutions. Line calculated from equation (5) with $K_2 = -0{\cdot}70$. Symbols used are: ○, Heiss (1955), dextrose; ●, Heiss (1955), fructose; △, Cleland & Fetzer (1944), invert; ×, Jongen (1956a), invert; □, Grover (1947), invert.

Generalizing equation (3) to include multicomponent solutions we have

$$\Delta F^E = C_{12}\,x_1\,x_2 + C_{13}\,x_1\,x_3 + C_{23}\,x_2\,x_3 + \ldots \tag{6}$$

giving

$$RT \ln \gamma_1 = C_{12}\,x_2{}^2 + C_{13}\,x_3{}^2 \ldots + (C_{12} + C_{13} - C_{23})\,x_2\,x_3 + \ldots \tag{7}$$

If now the further assumption is made that

$$C_{12} = (\beta_1{}^{\frac{1}{2}} - \beta_2{}^{\frac{1}{2}})^2 \text{ etc.} \tag{8}$$

where β_1 and β_2 are some unspecified properties of the pure components, equation (7) reduces to

$$RT \ln \gamma_1 = (C_{12}{}^{\frac{1}{2}}\,x_2 + C_{13}{}^{\frac{1}{2}}\,x_3 + \ldots)^2 \tag{9}$$

or

$$\log \gamma_1 = (K_2{}^{\frac{1}{2}}\,x_2 + K_3{}^{\frac{1}{2}}\,x_3 + \ldots)^2 \tag{10}$$

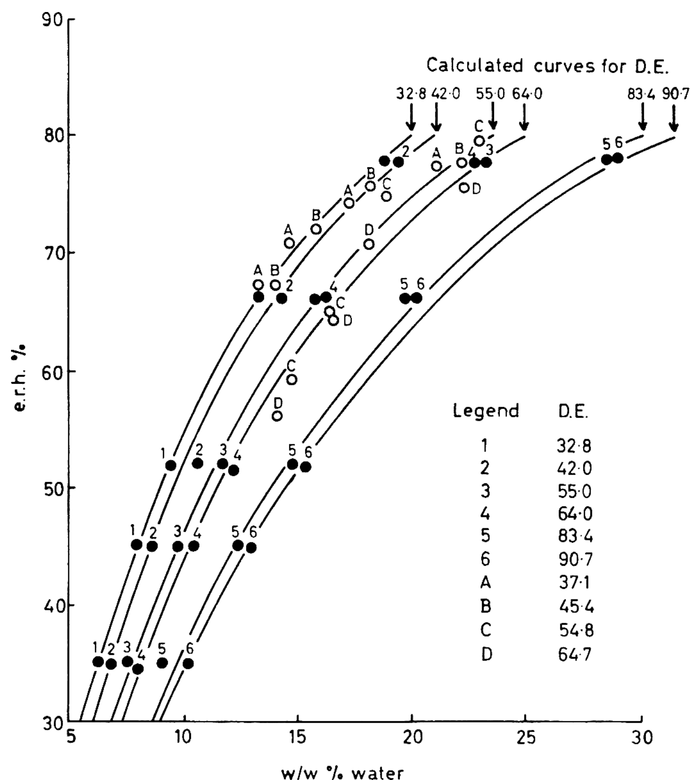

FIG. 6. Equilibrium relative humidity (e.r.g.) of aqueous confectioners glucose solutions. Symbols used are: ●, Cleland & Fetzer (1944); ○, this research.

where the Ks are identical with those for the separate binary solutions. If this equation is valid therefore, no experimental data need be determined on multicomponent mixtures once the binary values are known. In fact values of e.r.h. calculated by equation (10) are in good agreement with the experimental results, as may be seen by comparing the calculated and experimental figures in Tables 1–5.

It should be noted that the correlation of e.r.h. values for glucose syrups is complicated by the fact that these are actually muticomponent mixtures, which should strictly be represented by equation (10) rather than by equation (5). The exact composition of these syrups is, however, not usually known, nor are data on the component binaries available, so that a 'working' molecular weight must be used in conjunction with equation (5), this molecular weight being that for which K_2 varies least with change of concentration. These 'working' molecular weights were found to be given by

$$\text{Molecular weight} = 660 - 4{\cdot}8\ (\text{D.E.})$$

where D.E. is the dextrose equivalent of the syrup. Molecular weights given by this equation are in good agreement with those calculated by Grosso (1963) over the middle range of D.E. values but higher at low D.E. values and vice versa.

Table 6 lists the values of moleculer weight used, the values of K_2 and the values of $\Delta \bar{F}^E_\infty$, the partial molal excess free energy of mixing at infinite dilution.

Apart from the practical value of the correlating equations in interpolation, extrapolation, detection of faulty data and in reducing the amount of experimental data required to characterize a given system, both the sign and the magnitude of the derived values of $\Delta \bar{F}^E_\infty$ and the symmetric relationship between ΔF^E and concentration, yield significant information about the mode of interaction between water and the substances studied. The correlating equation proposed for binary systems is formally identical with the 'regular solution' equation as developed for example by Hildebrand (1929) and Hildebrand & Scott (1964), but this latter (derived on the assumption of an ideal entropy of mixing) yields only positive values of the excess free energy. The factors leading to positive free energies of mixing must be operative in the solutions considered here, particularly the contribution from the excess entropy of mixing due to the great disparity in size between the molecules of water and solvent, so that the large negative values actually found indicate a very strong association between solute and solvent,

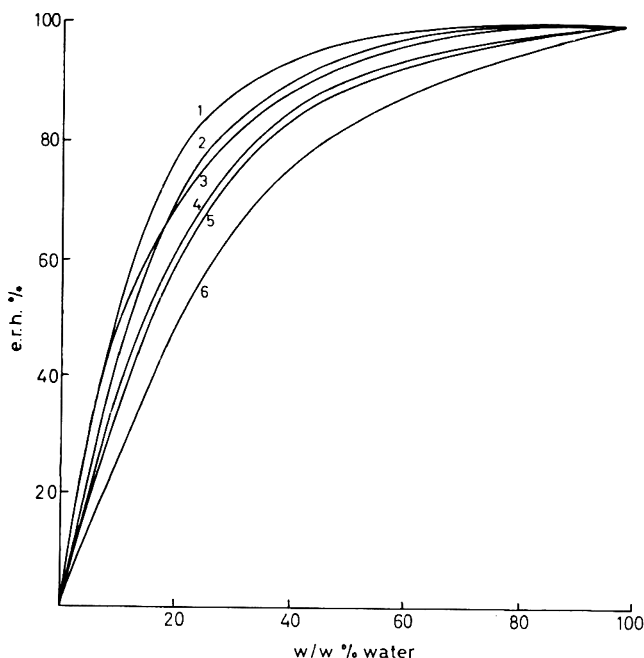

Fig. 7. Comparison of humectants. 1, 42 D.E. glucose; 2, 64 D.E. glucose; 3, sucrose; 4, invert sugar, dextrose and fructose; 5, sorbitol; 6, glycerol.

presumably due to hydrogen bonding. Dunning, Evans & Taylor (1951) find the partial molal heat of dilution of sucrose and water at infinite dilution to be $-3{\cdot}3$ kcal/mole; the free energy change from Table 6 is $-3{\cdot}6$ kcal/mole so that the $T\Delta S$ contribution to the free energy change is only $+0{\cdot}3$, much smaller than would be expected if the degree of order in the mixtures were not at least as great as in the pure substances themselves. Nevertheless, it would seem to be mistaken to postulate the existence of definite hydrates, since there are no discontinuities in the vapour pressure of the solutions.

The overwhelming effect of hydrogen bonding in determining the properties of these solutions presumably explains their relatively simple behaviour, since the mixtures can be regarded to a first approximation as consisting simply of hydroxyl groups. Consequently, one might expect to find a correlation between ΔF^E (and hence K_2) and the number of such groups in the molecule. This correlation is shown in Fig. 8, from which it is seen that, with the exception of sucrose, the relationship is approximately linear. The anomalous behaviour of sucrose is doubtless connected with, among other things, the presence of two intra-molecular hydrogen bonds in the sucrose molecule. Such intra-molecular bonds would not contribute to the sucrose–sucrose interaction energy but could possibly be broken in aqueous solution to form sucrose–water bonds.

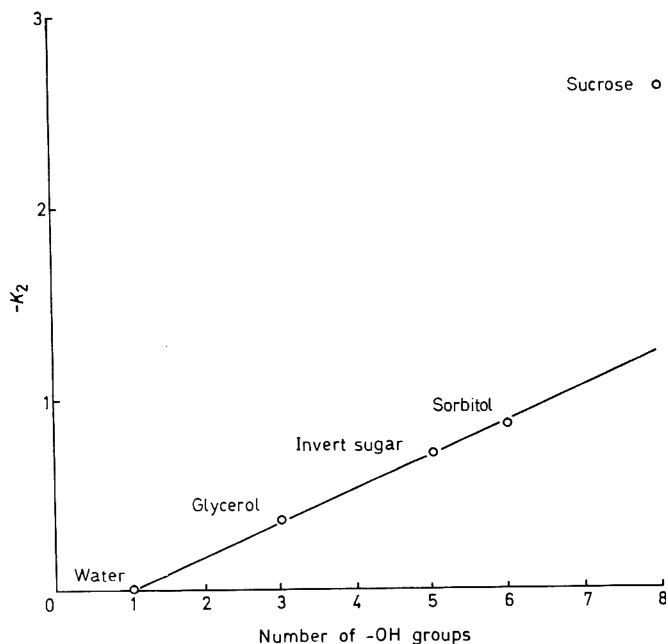

Fig. 8. The dependence of K_2 on the number of hydroxyl groups in the molecule.

It has already been suggested that equations such as the one proposed would be the first step towards a method of predicting e.r.h. values. As an example, from Fig. 8 one would expect erythritol to have a K_2 value midway between that of invert sugar and glycerol, and ribotol to have the same value as invert sugar. Furthermore, it is conceivable that in the case of substances containing effective groups other than hydroxyl, it might be possible to separate the effects of the different groups. For example, a consideration of the homologous series of primary alcohols (which have increasingly positive free energies of mixing with water due to the increasing length of the hydrocarbon chain, but do not obey equation (5)) might lead to a method of predicting values for compounds intermediate between these and the fully hydroxylated compounds.

One advantage of the use of activity coefficients in considering e.r.h. problems has not yet been mentioned. Activity coefficients, and hence e.r.h. values, are in general temperature dependent, and it has been implicit in the discussions above that the temperature has been held constant. The temperature dependence of the activity coefficient is given by:

$$\frac{\partial \ln \gamma_1}{\partial T} = -\frac{\Delta \bar{H}_1}{RT^2} \qquad (11)$$

where $\Delta \bar{H}_1$ is the partial molal heat of mixing. The use of this equation has two advantages; in the first place it is frequently easier to measure $\Delta \bar{H}_1$ than to determine the temperature dependence directly, and secondly, even where the latter course has been chosen, integration of equation (11) on the assumption that over a sufficiently short interval of temperature $\Delta \bar{H}_1$ can be considered constant, leads to the linear correlating equation

$$\ln \gamma_1 = A + \frac{\Delta \bar{H}_1}{RT} \qquad (A \text{ is a constant})$$

so that a plot of $\ln \gamma_1$ versus I/T should be a straight line of slope $\Delta \bar{H}_1/R$. For the substances considered in this paper, the values of $\Delta \bar{H}_1$ are such that the variation of e.r.h. with temperature over the usual range of ambient temperatures is without practical significance.

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Sequential procedures for triangular and paired comparison tasting tests

E. H. STEINER

Summary. The necessary number of tasting tests which must be carried out in the comparison of two samples by triangular tests or paired comparisons is considered in relation to: (1) the risk of stating that the samples are different when they are in fact not distinguishable, (2) the risk of stating the samples are identical when a distinguishable difference really exists, and (3) the degree of 'difference' it is desired to distinguish.

Sequential procedures are put forward which enable a decision to be reached relatively quickly, with 1 in 20 risks of error, when the two samples either are identical or differ by a defined amount. Separate tables are given for difference testing and quality testing. In cases where the sequential test would be lengthy the procedures terminate the tests after an amount of tasting which only slightly increases the risk of error.

Introduction

Both triangular tests and paired comparisons are well-known procedures used for the organoleptic comparison of two different products. When a result from a tasting panel has been obtained it is usual to assess the significance by reference to statistical tables based on the binomial distribution and a result is normally deemed to be significant if the probability of obtaining it by chance when the samples are identical is less than 1 in 20 or 1 in 100. It is not usual, however, to lay down any criterion for the amount of tasting to be done before arriving at a decision. This is of importance when a non-significant result is reached since it affects the chance of this happening when the samples are in fact different. Radkins (1957) has pointed out that in any tasting test there are always two risks of arriving at wrong conclusions. These are: (a) the risk of stating that the samples are different when they are in fact not distinguishable, and (b) the risk of stating the samples are identical when a distinguishable difference really exists.

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The amount of tasting to be carried out before any decision is reached is determined once the risks (a) and (b) and the degree of 'difference' which it is required to distinguish are fixed. This amount of tasting may be achieved either by using a panel of the required size or by a smaller number of individuals who taste more than once. In either event two procedures are available. In one (fixed panel size) the number of tasters can be calculated beforehand and in the other (sequential) it cannot.

The object of a triangular tasting test is to establish whether or not a distinguishable difference exists between two different products A and B. Three samples, two of which are identical (i.e. AAB or ABB) are submitted to a panel of tasters and each taster is required to pick out the odd sample if possible. When tasting is completed the replies can be assessed for significance since $33\frac{1}{3}\%$ correct selections would be expected by chance if there were no difference.

In paired comparison tests two samples, A and B, are presented for tasting. This type of test can be used for two distinct purposes namely: (1) to ascertain whether sample A differs from sample B in respect of some specific quality (e.g. sweetness, smoothness, etc.), and (2) to determine whether an overall preference for A or B exists.

Triangular tests

If the flavour of products A and B are indistinguishable then the proportion of occasions on which the odd sample will be correctly selected by the panel is $\frac{1}{3}$.

If the flavours are distinguishable then the proportion of occasions on which the odd sample will be correctly selected (including chance) will be some other value p_1 greater than $\frac{1}{3}$. Since $\frac{1}{3}$ of all the selections due to chance alone will be expected to be correct, the percent of occasions on which the correct choice is not due to guessing will be $x\%$ where

$$p_1 = \frac{x}{100} + \frac{1}{3}\left(\frac{100 - x}{100}\right) = \frac{1}{3}\left(\frac{100 + 2x}{100}\right) \quad (1)$$

It is possible, for example, to regard the odd sample as being different if, in unlimited tasting, the odd sample should be picked out other than by guess on half of the total number of occasions, i.e. $x = 50\%$ and, hence, $p_1 = \frac{2}{3}$. This definition of 'difference' is arbitrary and other degrees of difference could be postulated by choosing alternative values for x (and p_1). The smaller the stipulated 'difference' the larger will be the amount of tasting required. The presence of correct selections which are not guesses among those which are guesses can arise from the inclusion on the panel of individuals who can pick out a flavour difference which other members cannot and also from variations in a taster's sensitivity on different occasions of tasting.

Examples of panel sizes in triangular tests to allow for different degrees of discrimination are shown in Table 1. The sample size n and the number of correct replies to

TABLE 1. Required panel sizes in triangular tasting tests

Sample 'difference' to be distinguished (% discriminations not due to guess) (x%)	Expected proportion of correct choices in test (including guess) (p_1)	Risks of wrong decisions = 1 in 20	
		No. of tastes required (n)	No. correct for significance (n^1)
50	$\frac{2}{3}$	22	12
25	$\frac{1}{2}$	90	38
10	$\frac{2}{5}$	550	202

reach significance n^1 may be calculated from ordinary sampling theory using the normal approximation to the binomial. Following the treatments given by Davies (1954) and Roessler, Warren & Guynon (1948) we have:

$$n = \left[\frac{u_\alpha\sqrt{(\frac{1}{3} \cdot \frac{2}{3})} + u_\beta\sqrt{[p_1\,(1 - p_1)]}}{p_1 - \frac{1}{3}}\right]^2 \tag{2}$$

and

$$n^1 = \frac{n}{3} + u_\alpha\sqrt{(\tfrac{1}{3} \cdot \tfrac{2}{3}\, n)} + 0{\cdot}5 \tag{3}$$

Here u_α and u_β are values of the normal deviate which cut off single tail areas of α and β. For risks of wrong decisions of 1 in 20, $\alpha = \beta = 0{\cdot}05$ and $u_\alpha = u_\beta = 1{\cdot}64$.

Paired comparisons

Similar considerations to the above apply to paired comparison tests. In comparing products A and B the proportion of occasions on which A will be rated, say, sweeter if there is no distinguishable difference will be $\frac{1}{2}$. Here any 'no difference' replies should be apportioned equally between replies for A sweeter and B sweeter.

If the products are distinguishable then the proportion of occasions on which A will be rated differently from B (including chance) will be some other value p_1. If y denotes the percentage of occasions on which the choice in favour of A is made without guessing, then

$$p_1 = \frac{y}{100} + \frac{1}{2}\left(\frac{100 - y}{100}\right) = \frac{1}{2}\left(\frac{100 + y}{100}\right) \tag{4}$$

As before, a degree of discrimination can be arbitrarily defined such that the number of occasions other than by chance on which sample A is rated higher than sample B is half the total, i.e. $y = 50\%$ and $p_1 = \frac{3}{4}$.

Assuming that there is reason to suppose that A will not be rated lower than B, the purpose of the test is to establish whether A is either better (e.g. sweeter) or the same as B; hence, p_1 will be greater than $\frac{1}{2}$. This is a one-sided test as in the case of the triangular test above and has been discussed previously by Roessler, Baker & Amerine (1956).

Examples of panel sizes for one-sided comparison tests are calculated as before, using the normal approximation, from

$$n = \left[\frac{\frac{1}{2}u_\alpha + u_\beta\sqrt{[p_1(1-p_1)]}}{p_1 - \frac{1}{2}}\right]^2 \quad (5)$$

while the number for significance is

$$n' = \frac{n}{2} + \tfrac{1}{2}u_\alpha\sqrt{n} + 0{\cdot}5 \quad (6)$$

where $u_\alpha = u_\beta = 1{\cdot}64$

Table 2. Required panel sizes in paired comparisons (one-sided)

Sample 'difference' to be distinguished (% ratings in favour of A not due to guess) (y%)	Expected proportion of choices for A in test (including guess) (p_1)	Risks of wrong decisions = 1 in 20: No. of tastes required (n)	No. choices for A for significance (n^1)
50	0·75	38	25
25	0·625	170	96
10	0·55	1080	568

For a two-sided test there is no prior knowledge to indicate whether A should be better or worse than sample B. That is, A may be rated either higher or lower than B in respect of some quality or, in the case of preference, either A may be preferred to B or B preferred to A.

The probability of exceeding either positive or negative deviations from the expected value $n/2$ is of importance when testing the hypothesis that the samples are identical. Hence in equation (5) if double tail values of the normal variable are used $u_\alpha = 1{\cdot}96$.

To refute the hypothesis that the samples have a degree of difference indicated by y%, only results for n^1 in the direction of $n/2$ are of interest and the single-tail values for $\beta = 0{\cdot}05$ i.e. $u_\beta = 1{\cdot}64$ is used.

Table 3 gives examples of panel sizes for two-sided paired comparison tests.

TABLE 3. Required panel sizes in paired comparisons (two-sided)

Sample 'difference' to be distinguished (% rating in favour of either A or B not due to guess) (y%)	Expected proportion of choices for A or B in test (including guess) (p_1)	Risks of wrong decisions = 1 in 20	
		No of tastes required (n)	No. choices for A or B for significance (n^1)
50	0·75	45	30
25	0·625	200	114
10	0·55	1300	680

Sequential tasting

An alternative procedure applicable to both triangular and paired tests, which carries the same risks and degree of discrimination as above, is to make use of sequential sampling theory where the number of tests to arrive at a decision is not predetermined. This subject has been fully discussed by Wald (1947), Davies (1954), Moroney (1951) and Radkins (1958) and in a circular issued by the International Office of Cocoa and Chocolate (1960). The results of each individual tasting are plotted on a chart on which the abscissa represents the total number of tastes and the ordinate represents the number of correct or preferred selections. 'No difference' results should be referred to a table of random numbers. For a triangular test each time the random number is divisible by three plot as 'correct choice', otherwise plot as 'incorrect choice'. For paired comparisons, each time the number is even it is regarded as a preference for A, if odd a preference for B. Tasting is continued until one or other of two parallel straight lines is crossed, indicating the products are either indistinguishable or 'different' respectively. Alternatively a table of two sets of limiting values can be constructed against which the cumulated totals of correct or preferred selections are compared as tasting proceeds. In this case it is convenient to regard each 'no difference' reply as $\frac{1}{3}$ correct or $\frac{1}{2}$ preferred choice (as appropriate).

Limiting values of the totals for risks of 1 in 20 reaching wrong decisions are given in Tables 5–7. These limiting values are calculated from the equations of the straight lines:

$$S = h_0 + sn, D = h_1 + sn \qquad (7)$$

where n = total number of tastes and the constants h_0, h_1 and s depend on the risks of accepting a wrong hypothesis and the degree of difference it is required to distinguish. Values of the constants h_0, h_1 and s are given by the following.

$$s = \log \frac{1-p_0}{1-p_1} \Big/ \log \frac{p_1(1-p_0)}{p_0(1-p_1)} \quad (8)$$

$$h_0 = -\log \frac{1-\alpha}{\beta} \Big/ \log \frac{p_1(1-p_0)}{p_0(1-p_1)} \quad (9)$$

$$h_1 = \log \frac{1-\beta}{\alpha} \Big/ \log \frac{p_1(1-p_0)}{p_0(1-p_1)} \quad (10)$$

In these equations α, β and p_1 have the meaning already attached and p_0 represents the expected proportion of correct or preferred selections if the samples are identical (i.e. $p_0 = \frac{1}{3}$ or $\frac{1}{2}$). In the case of the two-sided paired comparison tests where sample A can be rated either higher or lower than sample B the appropriate value of α to insert in (9) and (10) is 0·025 instead of 0·05.

Values of h_0, h_1 and s for the conditions already considered with fixed sizes of panel are given in Table 4. For the two-sided paired comparison tests two alternative values

TABLE 4. Constants for constructing sequential plans

Types of test	Sample 'difference' to be distinguished (%)	Expected proportions of correct or preferred choices for A	0·05 levels of significance		
			h_0	h_1	s
Triangular	50	2/3	−2·12	2·12	0·500
	25	1/2	−4·24	4·24	0·414
	10	2/5	−10·26	10·26	0·367
Paired comparisons (one-sided)	50	0·75	−2·68	2·68	0·631
	25	0·625	−5·77	5·77	0·562
	10	0·55	−14·7	14·7	0·524
Paired comparisons (two-sided)	±50	0·75	−2·70	3·31	0·631
		0·25	2·70	−3·31	0·368
	±25	0·625	−5·82	7·13	0·562
		0·375	5·82	−7·13	0·437
	±10	0·55	−14·8	18·1	0·524
		0·45	−14·8	−18·1	0·475

of h_0, h_1 and s are obtained for each set of conditions, because in testing the hypothesis that the two samples are indistinguishable the number of preferred choices for sample A may depart from the expected proportion of 0·05 in either an upward or a downward direction. Two sequential tests are here superimposed giving in the graphical plot two regions for 'difference' with a central 'no difference' region. Graphical plots of the regions of acceptance are shown in Fig. 1 in the case of a two-sided paired comparison test where a '50% difference' is to be distinguished. Equations (7) are used to calculate the limiting values given in Tables 5–7.

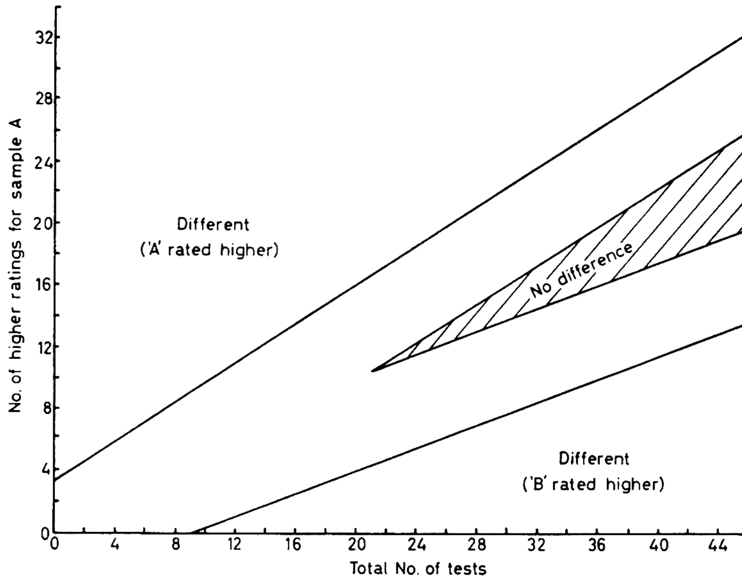

FIG. 1. Sequential tasting procedure for two-sided paired comparison to detect a '50% difference'. $\alpha = 0{\cdot}025$; $\beta = 0{\cdot}05$; $y = 50\%$.

Average number of tastes

The average number of tastes necessary before reaching any decision is generally less in sequential tasting than by fixed sample size (for the same risks). Where differences are large the decision is reached relatively quickly. If the true 'difference' lies in between x or $y = 0$ (no difference) and the value of x or y it is required to discriminate the amount of tasting before coming to a decision may on occasions be greater than that with the equivalent fixed panel.

Average numbers of tastes expected at the 1 in 20 level of probability in order to reach a decision in certain cases are shown in Table 8.

Termination of the sequential procedure

The number of tastes indicated in Table 8 will often be exceeded (although ending

TABLE 5. Sequential triangular test

Total No. of tastes	Sample 'difference' to be distinguished 50% D	50% S	25% D	25% S
5	5	0		
6	6	0		
7	6	1		
8	7	1		
9	7	2		
10	8	2		
11	8	3		
12	9	3	10	0
13	9	4	10	1
14	10	4	11	1
15	10	5	11	1
16	11	5	11	2
17	11	6	12	2
18	12	6	12	3
19	12	7	13	3
20	13	7	13	4
21	13	8	13	4
22	14	8	14	4
23	14	9	14	5
24	15	9	15	5
25	15	10	15	6
26	16	10	16	6
27	16	11	16	6
28	17	11	16	7
29	17	12	17	7
30	18	12	17	8
31	18	13	18	8
32	18	14	18	9
33	18	15	18	9
34	18	16	19	9
35	18	17	19	10

Total No. of tastes	Sample 'difference' to be distinguished 25% D	25% S
40	21	12
45	23	14
50	25	16
55	28	18
60	30	20
65	32	22
70	34	24
75	36	26
80	38	28
85	40	30
90	42	33
95	44	35
100	46	37
105	48	39
110	50	41
115	52	43
120	54	45
125	56	47
130	59	49
135	59	53
140	59	58

Number of correct selections, N, required for a decision in a sequential triangular test (probability level = 0·05). If $N \geqslant D$ samples are different. If $N \leqslant S$ samples are similar If $D > N > S$ continue tasting. Included $\frac{1}{3}$ 'no difference' results with correct selections.

Note: Below dotted lines risks of error rise slightly above 0·05 due to the approaching arbitrary termination of the sequential procedure.

TABLE 6. Sequential paired comparison test (one-sided)

Total No. of tastes	Sample 'difference' to be distinguished			
	50%		25%	
	D	S	D	S
8	8	2		
10	9	3		
12	11	4		
14	12	6	14	2
16	13	7	15	3
18	15	8	16	4
20	16	9	18	5
22	17	11	19	6
24	18	12	20	7
26	20	13	21	8
28	21	14	22	9
30	22	16	23	11
32	23	17	24	12
34	25	18	25	13
36	26	20	27	14
38	27	21	28	15
40	28	22	29	16
42	30	23	30	17
44	31	25	31	18
46	32	26	32	20
48	33	27	33	21
50	35	28	34	22
52	36	30	35	23
54	37	31	37	24
56	38	33	38	25
58	38	35	39	26
60	38	37	40	27

Total No. of tastes	Sample 'difference' to be distinguished	
	25%	
	D	S
70	46	33
80	51	39
90	57	44
100	62	50
110	68	56
120	74	61
130	79	67
140	85	72
150	91	78
160	96	84
170	102	89
180	107	95
190	113	101
200	119	106
210	124	112
220	130	117
230	136	123
240	141	130
250	141	140

Number of higher ratings, N, for sample A required for a decision in a sequential one-sided paired comparison test (probability level = 0·05). If $N \geq D$ sample A is rated higher. If $N \leq S$ samples are similar. If $D > N > S$ continue tasting. Include $\frac{1}{2}$ 'no difference' results with higher ratings.

Note: Below dotted lines risks of error rise slightly above 0·05 due to the approaching arbitrary termination of the sequential procedure.

TABLE 7. Sequential paired comparison test (two-sided)

Total No. of tastes	Sample 'difference' to be distinguished 50% D	50% S	25% D	25% S
10	10	—		
12	11	—		
14	13	—		
16	14	—		
18	15	—		
20	16	—	19	—
22	18	11	20	—
24	19	12	21	—
26	20	13	22	—
28	21	14	23	—
30	23	16	24	—
32	24	17	26	—
34	25	18	27	—
36	27	20	28	—
38	28	21	29	—
40	29	22	30	—
42	30	23	31	—
44	32	25	32	—
46	33	26	33	—
48	34	27	35	—
50	35	28	36	—
52	37	30	37	—
54	38	31	38	—
56	39	32	39	—
58	40	33	40	—
60	42	35	41	—
62	43	36	42	—
64	44	38	44	—
66	45	40	45	—
68	45	42	46	—
70	45	44	47	—

Total No. of tastes	Sample 'difference' to be distinguished 25% D	25% S
80	53	—
90	58	—
100	64	50
110	69	56
120	75	61
130	81	67
140	86	72
150	92	78
160	98	84
170	103	89
180	109	95
190	114	100
200	120	106
210	126	112
220	131	117
230	137	123
240	143	129
250	148	134
260	154	140
270	159	145
280	165	151
290	169	158
300	169	168

Note: Below dotted lines risks of error rise slightly above 0·05 due to the approaching arbitrary termination of the sequential procedure.

Similarity cannot be established until at least twenty-two tastes are made in the procedure to distinguish a 50% difference or 100 tastes in the more selective procedure.

Number of preferences (or higher ratings), N, required for decision in a sequential two-sided paired comparison test (probability level 0·05). If $N \geq D$, where N refers to the sample with the greater number of preferences, the sample is preferred. If $N \leq S$ samples are similar. If $D > N > S$ continue testing. Include $\frac{1}{2}$ 'no difference' results with preferences.

TABLE 8. Average number of tastes expected for different degrees of 'difference' between samples at 0·05 probability level

Types of test	Sample 'difference' to be distinguished (%)	Average number of tastes required when true difference is: 0	$\frac{x}{2}$% (approx.)	x%	100%
Triangular	50	13	18	13	4
	25	52	74	49	7
	10	300	450	310	16
Paired comparisons (one -sided)	50	20	31	22	7
	25	93	135	92	13
	10	610	860	565	31

eventually) since they are only mean values. For practical purposes the test may be arbitrarily terminated after a certain number of tests. The samples are then regarded as similar or not according to whether the number of correct replies falls below or above a point half-way between the two limit values in the sequential test. If the test is arbitrarily terminated in this way the risks of arriving at a wrong decision will be increased.

According to Wald (1947), the probability that a sequential test will continue beyond 2 or 3 times the average sample number is very small and the effect on the operating characteristic of truncating the test after this number of observations is negligible. The expected number of observations, however, is dependent on the actual value of p for the samples under test and the rule adopted here is to truncate the procedure after a number of tastes equal to $1\frac{1}{2}$ times the number required by the non-sequential test with the same risks. Work of the Statistical Research Group of Columbia University (1945) has shown that the application of this rule would not appear to increase the risks in the sequential scheme from 0·05 to more than 0·07 approximately.

When the sequential test is terminated at a certain point the samples are judged to be different if the number of correct results or preferred choices D^1 exceeds $\frac{1}{2}(D + S)$, where D and S are the limit values in the sequential test (i.e. $D^1 = \frac{1}{2}(D + S) + 0{\cdot}5$ or $\frac{1}{2}(D + S) + 1$ according to whether $D + S$ is odd or even). The application of this criterion also affects the limit values just prior to the terminating point since, if the test is terminated after n_0 tastes, the limit value necessary to establish a difference when the number of tastes is less than n_0, cannot exceed D^1. Also, since the limit value

to establish similarity of the samples at n_0 tastes is $(D^1 - 1)$ the limit value cannot be less than $(D^1 - 2)$ at $(n_0 - 1)$ tastes or less than $(D^1 - 3)$ at $(n_0 - 2)$ tastes, etc. In constructing Tables 5–7 the limit values were modified in the light of these considerations and the point at which there is a departure from the strict limits of the sequential scheme is indicated by a dotted line. Decisions on differences or similarity below this line must be regarded as reached with risks of error slightly greater than 0·05.

Discussion

Clearly a sequential scheme is to be preferred over a fixed panel size test as being more economical on average in the number of tastes required to reach a decision. It is quite possible for decisions to be reached in under ten tastings. If a sequential scheme is not considered feasible then the number of tastes must be fixed beforehand in accordance with the requirements of the non-sequential scheme with the same given risks.

Circumstances which might render a strictly sequential procedure impracticable are when the amount of sample available is limited (in case tests should run on) or where preparation of the products for tasting is a lengthy procedure, making it desirable that several tastings are done at a time. The former objection has been met by arbitrarily terminating the procedure when the number of tastes which have been made is equal to about $1\frac{1}{2}$ times that required for a non-sequential test with the same risk. This involves a small increase only in the risks of wrong decisions. To overcome the second objection tasting tests may be presented at one time to several or all members of the tasting panel and the results subsequently taken in random order for assessing by the sequential procedure. The size of panel here may conveniently be some fraction of the number of tastes required in a non-sequential test and repeat tests made if necessary.

As an alternative to randomizing and assessing results individually as tasting proceeds, the total number of correct choices or higher ratings (including $\frac{1}{3}$ or $\frac{1}{2}$, as appropriate, of 'no difference' replies) when the panel has completed its first tasting may be directly compared with the limit values in Tables 5–7. If no decision is reached and a second complete round of tasting is carried out the combined total may be compared with the limits for a sequential test. This procedure, if continued till a decision is reached, requires less labour in examining the results of the tasting test but could lead to more tasting than necessary being performed. If the panel size is not too large, however, the loss in efficiency cannot be great and, in any case, may be offset by the convenience in organizing the tests for all members of a panel at one time. There is no increase in the risks of wrong decisions if this procedure is followed.

Paired comparison tests require about twice as many tastings for a significant result as triangular tests. If in the former case duplicate tests on samples A and B are presented

to each taster at one time, the number of actual tasting tests which need to be arranged is then similar in the two types of test. For paired comparison tests each taster will then be required to taste four samples in all at each sitting instead of three for a triangular test.

In the case of preference tests, if the number of tasters to form a panel of the required size to obtain a decision is not available this deficit cannot logically be remedied by repeated tasting since normally it is the preference of the whole consumer population which is being sought. That is to say the panel is a sample from which a property of the whole population is being estimated. Where the test is for detecting a difference or distinguishing two qualities the panel is used as an instrument to determine a property of the product; hence it is valid to obtain replicate responses from individuals. As a corollary of this, in such cases the panel itself should be composed of those tasters who are most sensitive.

Detecting a degree of difference which can be picked out on 10% of occasions on which the products are tasted requires an excessive amount of tasting. In the Tables given 50% has been adopted as a suitable level of 'difference' to be detected in setting up a tasting procedure for routine examinations and a 25% 'difference' where a more selective discrimination is required. The latter criterion may on occasions require as many as 140 tastes for a difference test or 300 for a preference before a decision can be made whether the samples are similar or not.

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Acid components of fruit and vegetables

I. Production of acids by thermal processing

D. E. C. CREAN

Summary. The breakdown of carbohydrate molecules in acid solutions when heated gives rise to various acids of which two have been identified as glycollic and lactic acids. Evidence has been obtained that this reaction takes place in the sterilization of canned vegetables and is responsible for the drop in pH noted during the sterilization process.

Introduction

It is well known that fruits and vegetables contain a variety of acids and that, as a consequence, their natural pH values can vary widely (Winton & Winton, 1935; Bonner, 1950). These differences are of importance in canning technology; they must be taken into account in choosing the optimum conditions — time and temperature — for sterilization; they can affect the stability of pigments and corrosion of the tinplate during storage as well as having slight but definite effects on flavour and texture.

Apart from acid components naturally present in plant tissues, various observations in these laboratories have led to the conclusion that heat treatment, and perhaps other processing techniques, brings about an increase in the total acid content. Temperatures of 115·5°C or higher are required for the sterilization of the majority of vegetables grown in the United Kingdom which have initial pH values of 4·5 or greater. Adam & Dickinson (1958) found that, under such temperature conditions, a decrease in pH took place. Similar changes were observed following incubation of the canned product at 55°C for bacteriological examination. Further evidence of changes in acid content was obtained in the course of experiments on corrosion using simple mixtures of pure organic acids and sugars (H. A. W. Blundstone, personal communication). This reaction has been noted in vegetables canned in water as well as in those canned with added sugar (e.g. peas).

As a working hypothesis it seemed likely that the change in acidity might correspond

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to that noted in sugar refining (Schlegel, 1963). Alkaline fission of the carbohydrate molecule is a well-known phenomenon which has been extensively investigated over the past 40 years (Lloyd Evans, 1942). Less work has been done on the acid breakdown of sugars though the formation of levulinic and formic acids from the action of hot mineral acid on sugar has been noted (Takahashi, 1944). As the pH of the canned product is of importance in the choice of the time and temperature of sterilization, and also has a marked effect on the stability of chlorophyll, it was decided to investigate further the acid production, both in model systems and in different types of canned vegetables.

Materials and methods

The experiments were carried out in four sections.

1. *The effect of various acids on sucrose*

Sugar solutions were prepared by dissolving sucrose (5 mM) and citric, malic or oxalic acid (5·0 or 0·5 mEq) in 125 ml of water in a 250 ml flask. The contents were rapidly brought to the boil and allowed to boil under reflux for 1 hr. The solution was then allowed to cool, filtered into a 250 ml volumetric flask and made up to volume.

For analysis a 50 ml portion was neutralized with 0·1 N NaOH and taken to dryness on a water bath for chromatographic analysis by the methods outlined below.

2. *The effect of malic acid on glucose, fructose and sucrose*

The solutions used consisted of glucose, fructose or sucrose (5 mM) together with 5 mEq of malic acid made up to 125 ml and treated as before.

3. *The examination of methods for possible artifacts*

To check that no artifacts were produced during the preparation of samples for analysis (neutralization and subsequent heating to dryness) experiments were carried out: (a) by using conditions similar to 1 and 2 above but substituting 5 mEq of NaOH for the malic or other acid added, and (b) by using the sucrose–oxalic acid system and following the general experimental procedure outlined with the omission of the refluxing stage.

4. *Experimental work on peas*

Parallel to the experimental work on model systems, experiments were carried out with various vegetables. In a typical experiment using fresh peas 100 g were blended with 50 ml of water to a smooth purée and 180 g of the same sample of peas together with 116 g water at 88°C were placed in an Al lacquered can (equivalent to 315 ml). Following exhaustion for 6 min at 88°C, the can was hermetically sealed, sterilized following the normal process at 115·5°C for 40 min and then cooled. After 24 hr the can was opened, the contents macerated and sampled as above.

Aliquots of the macerates were extracted from both sterilized and unsterilized material as described by Wager & Isherwood (1961). The pH of the macerates was also measured, using a glass electrode.

Chromatographic analysis

The dry solids were purified on silica gel before chromatography on silica gel using a gradient of n-butanol in chloroform to elute the acids (Wager & Isherwood, 1961; Blundstone & Dickinson, 1964). The acid content of each fraction was estimated by titration with 0·01 N NaOH.

The acids eluted from the column were identified by paper chromatography, having been converted to the hydrogen form by passage down a column of ZeoKarb 225 (H^+ form). The chromatograms were run on Whatman No. 1 paper using n-butyl formate–formic acid–water 10 : 4 : 1 (Blundstone, 1963) and n-propanol–ammonia 7 : 3 (Isherwood & Hanes, 1953) as solvents. The acids were detected by spraying either with 0·5% 2-naphthol and 2% sulphanilamide in 95% ethanol followed by 1% aqueous sodium nitrite (Schmidt, Fischer & McOwen, 1963) or with 0·04% bromophenol blue in 0·2% aqueous citric acid (Kennedy & Barker, 1951).

Results

The histogram in Fig.1 is typical of that obtained in various experiments. In this case it represents the acid pattern obtained by reacting 5 mM of sucrose with 0·5 mEq of malic acid.

Peak C and peak E have been identified as lactic acid (R_F values 0·72 and 0·46 in the solvents used unchanged by admixture with authentic lactic acid) and glycollic acid (R_F values 0·54 and 0·35) respectively. Peaks A and B have been tentatively identified as acetic and formic acid respectively. This identification is based on their volatility in the acid solvents, their R_F in the alkaline solvent (0·37) and the peak elution volumes.

Peak D appeared in such minute traces that not enough was obtained for identification. From its peak elution volume, however, it was thought to be oxalic acid.

1. *The effect of various acids on sucrose*

The quantitative yields of acids produced from sucrose corresponding to peaks A–E are summarized in Table 1.

There is considerable agreement between the sets of experiments especially between those involving two levels of oxalate. At the higher level the considerable excess of oxalate swamped the glycollic acid formed which, in consequence, could not be estimated.

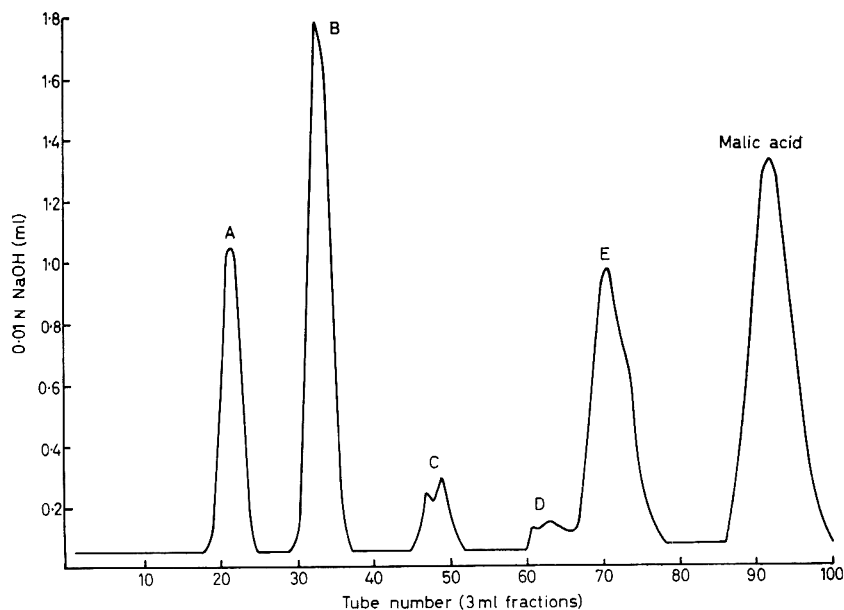

Fig. 1. Histogram of acids eluted from silica column.

Table 1. Acids produced from 1 mM of sucrose and added acid

Acid added to sucrose (mEq)		Acids produced (mEq/50 ml of solution)					
		Peak A	Peak B	Peak C	Peak D	Peak E	Total
Citric	1·0	0·0540	0·0811	0·0167	0·0045	0·0524	0·2087
Malic	1·0	0·0521	0·0605	0·0106	0·0042	0·0547	0·1821
Oxalic	1·0	0·0218	0·0533	0·0089	—	—	—
Citric	0·1	0·0288	0·0543	0·0103	0·0048	0·0518	0·1499
Malic	0·1	0·0311	0·0585	0·0089	0·0049	0·0451	0·1484
Oxalic	0·1	0·0220	0·0529	0·0095	—	0·0507	0·1351
Hydrochloric	1·0	0·0135	0·0139	0·0028	Nil	0·0377	0·0678

The results from using hydrochloric acid indicate that the effect is not due solely to pH effects but that the acid molecule itself may take part in the reaction. This view is supported by the fact that only some 80–90% of the acid is recovered, whereas a more usual recovery by the methods employed is 90–100%. This may be due, however, to the formation of sucrose esters of these acids such as have been isolated by

Australian workers from freeze-dried peach and apricot purée (Anet & Reynolds, 1957).

2. *The effect of malic acid on glucose, fructose and sucrose*

The second set of experiments was designed to investigate the effect of malic acid on the component monosaccharides of the sucrose molecule since it seemed unlikely that this molecule should react as a whole to give rise to the acids formed. The results of these experiments are shown in Table 2.

TABLE 2. Acids produced from 1 mEq of malic acid and 1 mM of added carbohydrate

Carbohydrate added to malic acid	Acids produced (mEq/50 ml of solution)					
	Peak A	Peak B	Peak C	Peak D	Peak E	Total
Glucose	0·0154	0·0348	0·0039	Nil	0·0141	0·0682
Fructose	0·0297	0·0789	0·0088	0·0018	0·0567	0·1759
Sucrose	0·0521	0·0605	0·0106	0·0042	0·0547	0·1821

From the above it appears that the fructose moiety breaks down more readily. The total acidity produced from glucose is only 38% of that produced from fructose and the glycollic acid (peak E) only 25%. The similarity between fructose and sucrose, especially with regard to glycollic acid and total acidity is extremely strongly marked and would indicate that the acids arise from the fructose moiety. This is in accordance with the known chemistry of these sugars.

3. *Possible artifacts*

In view of the known effects of alkali on sugars (Sowden & Schaffer, 1952) it seemed essential to investigate any possible action of the alkali during the neutralization and drying procedures.

TABLE 3. Acids produced from the reaction of NaOH and sucrose and during purification

Reaction conditions	Acids produced (mEq/50 ml of solution)					
	Peak A	Peak B	Peak C	Peak D	Peak E	Total
NaOH + sucrose refluxed	0·0201	0·0037	0·0057	Nil	0·0037	0·0332
Oxalic acid + sucrose (No refluxing)	0·0092	0·0024	0·0011	Nil	0·0091	0·0225

Table 3 shows that when sodium hydroxide was refluxed with sucrose, not only was the total acidity as little as some 20% of that found in a sugar–acid reaction, but the acid pattern was considerably different, peak A contributing some 60% of the total acidity. Where oxalic acid and sucrose were purified without any preliminary refluxing the acidity formed was only 15% of that normally occurring.

It can be inferred, therefore, that no artifacts arise during purification and that the acid patterns previously observed are due to a sugar–acid interaction.

4. *Sugar–acid reactions in canned vegetables*

The results of the experiments with fresh and canned peas are given in Table 4.

TABLE 4. Acids produced in the canning of fresh peas

Peak	Acid	Amount of acid present (mEq/100 g fresh weight)			Percentage increase or decrease (fresh weight basis)
		Fresh peas	Cooked peas	Increase	
—	Fatty acids	0·1032	0·0912	−0·0120	−11·6
A	Acetic	0·9074	1·4333	0·5259	+58·0
B	Glutaric	0·3928	0·1326	−0·2602	−66·2
C	Unknown	0·0686	0·0655	−0·0031	−4·5
C[1]	Unknown	Nil	0·0900	0·0900	—
D	Succinic + lactic	0·5238	0·6080	0·0842	+16·1
E	Glycollic	0·8442	1·1092	0·2650	+31·4
F	Unknown	0·3284	0·3368	0·0084	+2·6
G	Malic	0·9036	0·9609	0·0573	+6·3
H	Citric	0·9383	N.A.	—	—
	Total	4·0720*	4·8275	0·7555	+18·6

* Excluding citric acid.

This shows that significant changes in acetic acid, in the succinic–lactic acid peak and in glycollic acid have taken place. Furthermore, a new peak, here designated C[1], thought to be formic acid was noted. The other acids, with the exception of glutaric acid, remained remarkably constant. The pH of the peas declined from 6·53 to 6·14.

This and similar experiments (Crean, unpublished data) have therefore confirmed previous observations in these laboratories and it appears probable therefore that the sugar degradation reaction is the one primarily responsible for pH changes in vegetables and may also occur in fruits.

Conclusions

It has been shown that, by heating dilute solutions of pure organic acids and sugars, the production of further acids may be induced. The mechanism of the reaction is under investigation and is as yet still a matter for speculation. The reaction products, however, are analogous to those acids obtained during the alkaline decomposition of sugars (Sowden & Schaffer, 1952) and it seems logical to presume that the reaction mechanism in both cases may be similar.

Upon extending the work from model systems to canned vegetables, acid production was again noted. It seems likely that this reaction is a contributory factor to the drop in pH on processing noted in these products.

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Systematic identification of food dyes using paper chromatographic techniques

MAUREEN H. E. GRIFFITHS

Summary. Systematic methods for the separation and identification of (a) red, (b) yellow and orange, and (c) blue, green and black water-soluble food dyes at present in the permitted lists of the U.K. and the U.S.A. and in the E.E.C. approved list have been devised. In addition the two permitted violet food dyes have been separated and identified, and Brown FK separated from Chocolate Brown HT and Chocolate Brown FB. The dyes are separated and identified by a process of elimination using a simple paper chromatographic technique which effectively utilizes a method of 'double-spotting' of the dye samples.

Introduction

The water-soluble food dyes examined in the following schemes are listed in Tables 1–4. It can be a time-consuming operation to identify one of these colours completely even by the use of paper chromatography. However, the work can be reduced considerably by using a step-by-step system which enables each dye to be identified by a system of elimination. A similar scheme was originally described in a report issued by the Association of Public Analysts (1960) but since publication of this report various new solvents for the separation and identification of food dyes have been brought into use. Certain of these, viz. solvents 8, 9 and 11, give more compact spots and a much better separation of dyes than was possible with the solvents used in the original scheme.

The method is not dependent on figures quoted for R_F values as it is now well established that these do not provide reliable information. R_F values are affected by various factors which are not always strictly controllable, and day-to-day variations are inevitable even if the same technique is used. The figures quoted in Tables 1–4 are approximate and must be taken only as a general guide to the behaviour of the dyes with the solvents listed in Table 5.

Bearing these observations in mind, the following schemes for (a) red, (b) yellow

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TABLE 1

Colour	Colour Index number	Approximate R_F values						
		Solvent number (Table 5)						
		5	6	7	8	9	10	11
Ponceau MX	16150	0·5	0·5	0·4	0·5	0·5	0·2	0·1
Ponceau 4R	16255	0·4	0·4	0·2	0·3	0·3	0·5	0·6
Carmoisine	14720	0·5	0·2	0·4	0·3	0·7	0·2	0·1
Amaranth	16185	0·3	0·2	0·1	0·1	0·2	0·03	0·3
Red 10B	17200	0·4	0·4	0·3	0·4	0·5	0·1	0·1
Erythrosine	45430	1·00	0·6	0·6	0·6	1·0	0·6	0·1
Red 2G	18050	0·4	0·4	0·2	0·4	0·5	0·2	0·4
Red 6B	18055	0·3	0·2	0·4	0·2	0·3	0·08	0·0, *0·2*
Red FB	14780	0·4 (S)	0·2 (S)	0·5	0·2 (S)	0·5 (S)	0·2	0·0
Ponceau SX	14700	0·5	0·4	0·4	0·5	0·6	0·2	0·3
Ponceau 3R	16155	0·5	0·5	0·4	0·5	0·5	0·2	0·1
Fast Red E	16045	0·5	0·6	0·5	0·5	0·6	0·2	0·2
Ponceau 6R	16290	—	0·1	—	—	0·1	—	0·6
Scarlet GN	14815	—	0·5	—	—	0·6	—	0·8

S = Streaking of spot.

TABLE 2

Colour	Colour Index number	Approximate R_F values						
		Solvent number (Table 5)						
		5	6	7	8	9	10	11
Tartrazine	19140	0·3	0·2	0·1	0·1	0·2	0·04	0·6
Naphthol Yellow S	10316	0·5	0·6	0·2	0·6	0·7	0·4	0·4
Yellow 2G	18965	0·5	0·4	0·4	0·4	0·6	0·2	0·8
Yellow RFS	13011	*0·4*, 0·5	*0·5*, 0·7	*0·3*, 0·7	*0·4*, 0·7	*0·5*, 0·8	*0·2*, 0·5	0·3, *0·6*
Yellow RY	14330	*0·1*, 0·4	*0·1*, 0·2	*0·2*, 0·5	*0·05*, 0·2	*0·2*, 0·6	*0·00*, 0·13	*0·3*, 0·6 (S)
Acid Yellow (Fast Yellow)	13015	0·3	—	—	0·4	—	—	0·5
Quinoline Yellow	47005	0·5	—	—	0·6	—	—	0·04
Chrysoin	14270	0·5	—	—	0·3	—	—	0·4
Sunset Yellow FCF	15985	0·4	0·5	0·4	0·4	0·5	0·1	0·4
Orange G	16230	0·5	0·5	0·4	0·5	0·5	0·2	0·7
Orange RN	15970	0·4, *0·6*	0·5, *0·8*	0·3, *0·8*	0·4, *0·7*	0·5, *0·8*	0·1, *0·5*	*0·2*, 0·3
Orange GGN	15980	0·4	—	—	0·4	—	—	0·5

S = Streaking of spot.
Figures in italics denote main spot.

TABLE 3

Colour	Colour Index number	Approximate R_F values						
		Solvent number (Table 5)						
		5	6	7	8	9	10	11
Indigo Carmine	73015	0·2	0·2	0·2	0·2	0·3	0·1	0·15 (S)
Blue VRS	42045	0·6	0·7	0·85	0·7	0·7	0·3	0·8
Patent Blue	42051	—	0·6	0·9	0·6	0·7	0·04	1·00
Brilliant Blue FCF	40290	—	0·5	0·7	0·5	0·4	0·14	0·8
Green S	44090	0·5	0·45	0·7	0·5	0·7	0·2	0·9
Guinea Green	42085	—	0·7	1·0	0·7	0·65	0·45	0·9
Light Green SF Yellowish	42095	—	0·5	0·6	0·5	0·4	0·15	0·9
Fast Green FCF	42053	—	0·4	0·6	0·2	0·45	0·15	1·0
Black PN	28440	0·1	0·1	0·1	0·05	0·1	0·05	0·1
Black 7984	—	—	0·03	0·01	0·00	0·1	0·02	0·00

S = Streaking of spot.

TABLE 4

Colour	Colour Index number	Approximate R_F values						
		Solvent number (Table 5)						
		5	6	7	8	9	10	11
Violet BNP	—	0·6	0·7	1·0 (S)	0·7	0·7	0·3	0·4
Violet 6B	42640	0·5	—	—	0·7	0·7	0·4	0·2
Brown FK	—	0·3, 0·5	0·5	0·1 0·5, 0·9	0·1 0·6, 0·7	0·2 0·6, 0·7	0·03 0·2, 0·4	0·01, 0·1
Chocolate Brown FB	—	0·07 (S) 0·2, 0·4	(S)	(S)	(S)	(S)	*0·03*, 0·1	(S)
Chocolate Brown HT	20285	0·07 (S) 0·2, 0·4	(S)	(S)	(S)	(S)	*0·03*, 0·1	(S)

S = Streaking of spot.

and orange, and (c) blue, green and black permitted food dyes have been drawn up. In many cases it will not be necessary to go through every step in the procedure and the schemes are easily broken down to suit each individual problem. The violets and browns have not been included in these groups as they are considered sufficiently different in colour to be easily distinguished from the other food dyes. However, solvents

TABLE 5. Chromatographic solvents

Solvent No.	Composition
5	n-Butanol (20 vol.), water (12 vol.), glacial acetic acid (5 vol.)
6	iso-Butanol (3 vol.), ethanol (2 vol.), water (2 vol.), add 1 ml 0·88 ammonia to 99 ml of this mixture
7	80 g phenol + 20 g water
8	Ethyl-methyl ketone (70 ml), acetone (30 ml), water (30 ml), 0·88 ammonia (0·2 ml)
9	Ethyl-methyl ketone (70 ml), acetone (30 ml), water (30 ml)
10	Ethyl acetate (11 vol.), pyridine (5 vol.), water (4 vol.)
11	Trisodium citrate (2 g), water (95 ml), 0·88 ammonia (5 ml)
—	Hydrochloric acid, specific gravity 1·18 (6·5 ml), water (30 ml)

are given for the separation and identification of the two violet food dyes, and for the separation of Brown FK from Chocolate Brown HT and Chocolate Brown FB.

Methods for separating colours from various foodstuffs and purifying them ready for chromatographic identification are described in the First Report of the Trace Materials (Colour) Committee set up by the British Food Manufacturing Industries Research Association (1963).

Method

The technique known as 'double-spotting' is used to overcome any irregularities—such as impurities derived from foodstuffs—which would otherwise affect the R_F values of the unknown dye. The procedure followed consists in placing a spot of the unknown dye on top of the spots of possible standard dyes so that both will be equally affected by any impurities which may be present. The unknown dye is identified by giving a single spot with the correct standard while with all the other standards double spots to a greater or lesser degree are obtained.

The unknown sample dye is run against a standard control using this technique of 'double-spotting'. A chromatogram would have the following spots:

Unknown   Unknown + Control   Control

After comparing the behaviour of the unknown with the control, the unknown is placed in a smaller limited group of possible dyes. Further chromatograms are then run until by a process of elimination the dye is identified.

Only very simple apparatus is required for the ascending chromatography technique. Whatman Filter Paper No. 1, 20 × 20 cm is used for the chromatograms. A base line

is drawn ¾ in. from and parallel to the bottom of the paper. The dyes are spotted on to this line using a capillary and after drying, the paper is curled into a cylinder and joined at two places with white thread. (N.B. the vertical edges of the cylinder must not touch each other.) The papers are placed in a 2 litre beaker (approximate dimensions: 5 in. diameter × 9 in. tall) containing about 30 ml of solvent. The beaker is first covered with a clock glass, then by a piece of polythene which is held in place by a rubber band.

The chromatogram is left to develop until the solvent front has reached a height of 12 cm from the base line. It is then removed from the beaker and dried.

The total number of solvents needed to identify any one colour is never more than three. The numbering of solvents 5, 6 and 7 follows the convention used by the Association of Public Analysts but solvents 8–11 were numbered purely for convenience in use.

I. Systematic identification of permitted red food dyes[1]

The first step in the procedure is to run a chromatogram in Solvent 9 using Red 2G as the standard control. The following conclusions may be drawn from the behaviour of the unknown sample compared with the standard Red 2G:

[A] Remains behind control—unknown may be Amaranth, Red 6B, Ponceau 4R, or Ponceau 6R.

[B] Moves approximately the same distance as control (i.e. no separation shown) unknown may be: Red 10B, Red FB, Ponceau 3R, Ponceau MX or Red 2G.

[C] Moves ahead of control but behind solvent front—unknown may be Carmoisine, Ponceau SX, Fast Red E, or Scarlet GN.

[D] Moves with the solvent front—unknown is *Erythrosine.*

Group A

Run a chromatogram in Solvent 11 using Amaranth as the standard control.

(a) Moves ahead of control—unknown may be Ponceau 4R or Ponceau 6R.

Run a chromatogram in Solvent 9 using Ponceau 4R as the standard control.

(i) Remains behind control—unknown is *Ponceau 6R.*

(ii) No separation shown—unknown is *Ponceau 4R.*

(b) No separation shown—unknown may be Red 6B or Amaranth.

Run a chromatogram in Solvent 9 for 15 cm using Amaranth as the standard control

(i) Moves ahead of control—unknown is *Red 6B.*

(ii) No separation shown—unknown is *Amaranth.*

Group B

Run a chromatogram in Solvent 11 using Red 2G as the standard control.

(a) No separation shown—unknown is *Red 2G.*

(b) Remains behind control on base line and turns mauve when wet with solvent—unknown is *Red FB.*

(c) Remains behind control—unknown may be Red 10B, Ponceau 3R or Ponaucc MX.

Now run a chromatogram in Solvent 6 using Ponceau MX as the standard control.

(i) Remains behind control—unknown is *Red 10B.*

(ii) No separation shown—unknown may be Ponceau MX or Ponceau 3R.

Finally run a descending chromatogram in Solvent 11 for 4–6 hr using Ponceau MX as the standard control.

(iii) Remains behind control—unknown is *Ponceau 3R.*

(iv) No separation shown—unknown is *Ponceau MX.*

Group C

Run a chromatogram in Solvent 6 using Ponceau SX as the standard control.

(a) Remains behind control—unknown is *Carmoisine.*

(b) No separation shown—unknown is *Ponceau SX.*

(c) Moves ahead of control—unknown may be Fast Red E or Scarlet GN.

Then run a chromatogram in Solvent 11 using Fast Red E as the standard control.

(i) No separation shown—unknown is *Fast Red E.*

(ii) Moves ahead of control—unknown is *Scarlet GN.*

II. Systematic identification of permitted yellow and orange food dyes

The first step in the procedure is to run a chromatogram in Solvent 8 using Yellow 2G as the standard control. The following conclusions may be drawn from the behaviour of the unknown sample compared with the standard Yellow 2G:

[A] Remains behind control—unknown may be Yellow RY, Tartrazine, or Chrysoin.

[B] Moves approximately the same distance as control—unknown may be Sunset Yellow, Orange GGN, Acid Yellow, Yellow RFS or Yellow 2G.

[C] Moves ahead of control—unknown may be Quinoline Yellow, Orange RN*, Naphthol Yellow or Orange G.

* Investigations have shown that Orange RN and the non-permitted dye Orange II (C.I. 15510) have the same R_F values in twenty-seven different solvents (Tilden, 1952). If it is necessary to establish the absence of Orange II, run a chromatogram in a solvent system consisting of equal volumes of ammonia solution, specific gravity 0·88 and 10% sodium chloride solution (Dutt, 1964). Orange RN moves in front of Orange II.

Group A

Run a chromatogram in Solvent 8 using Tartrazine as the standard control.

(a) Moves ahead of control—unknown is *Chrysoin.*

(b) No separation shown—unknown is *Tartrazine.*

(c) Remains behind control—unknown is *Yellow RY.*

Group B

Run a chromatogram in Solvent 11 using Yellow RFS as the standard control.

(a) Moves ahead of control—unknown is *Yellow 2G.*

(b) No separation shown—unknown may be Acid Yellow or Yellow RFS.

Then run a descending chromatogram in Solvent 5 for 19–20 hr (overnight) using Acid Yellow as the standard control.

(i) Moves ahead of control—unknown is *Yellow RFS* (spot turns orange when wet with solvent).

(ii) No separation shown—unknown is *Acid Yellow.*

(c) Remains behind control (orange spot)—unknown may be Sunset Yellow or Orange GGN.

Now run a descending chromatogram in hydrochloric acid for 16–17 hr using Sunset Yellow as the standard control.

(i) No separation shown—unknown is *Sunset Yellow.*

(ii) Remains behind control—unknown is *Orange GGN.*

Group C

Run a chromatogram in Solvent 11 using Naphthol Yellow S as the standard control.

(a) Moves ahead of control—unknown is *Orange G.*

(b) No separation shown—unknown is *Naphthol Yellow S.*

(c) Remains behind control, but above baseline (orange spot)—unknown is *Orange RN.*

(d) Remains behind control on baseline (yellow spot)—unknown is *Quinoline.*

III. Systematic identification of permitted, blue, green and black food dyes

The first step in the procedure is to run a chromatogram in Solvent 8 using Green S as the standard control. The following conclusions may be drawn from the behaviour of the unknown sample compared with the standard Green S:

[A] Remains behind control—unknown may be Black 7984, Black PN, Indigo Carmine or Fast Green FCF (spot turns blue when wet with solvent).

[B] Moves approximately the same distance as control—unknown may be Light Green SF Yellowish (spot fades when wet with solvent), Brilliant Blue or Green S.

[C] Moves ahead of control—unknown may be Patent Blue V, Blue VRS or Guinea Green (spot fades when wet with solvent).

Group A

Run a chromatogram in Solvent 9 using Indigo Carmine as the standard control.

(a) Moves ahead of control—unknown is *Fast Green FCF.*

(b) No separation shown—unknown is *Indigo Carmine.*

(c) Remains behind control—unknown may be Black PN, Black 7984.

Run a chromatogram in Solvent 11 for 18 cm using Black PN as the standard control.

(i) No separation shown—unknown is *Black PN.*

(ii) Remains behind control—unknown is *Black* 7984.

Group B

Run a chromatogram in Solvent 9 using Green S as the standard control.

(a) No separation shown—unknown is *Green S.*

(b) Remains behind control—unknown may be Brilliant Blue or Light Green SF Yellowish.

Then run as ascending chromatogram overnight (16–17 hr) to a solvent front height of 24 cm using Solvent 7, with Brilliant Blue as the standard control.

(i) No separation shown—unknown is *Brilliant Blue.*

(ii) Remains behind control—unknown is *Light Green SF Yellowish.*

Group C

Run a chromatogram in Solvent 6 using Blue RVS as the standard control.

(a) Remains behind control—unknown is *Patent Blue V.*

(b) No separation shown—unknown may be Guinea Green (fades when wet with solvent) or Blue VRS.

Run a chromatogram in Solvent 10 using Guinea Green as the standard control.

(i) No separation shown—unknown is *Guinea Green* (fades when wet with solvent).

(ii) Remains behind control—unknown is *Blue VRS.*

IV. Separation and identification of the permitted violet dyes

Violet 6B and Violet BNP can be separated and identified by running a descending chromatogram in Solvent 11 for 25 cm using Violet BNP as the standard control. Violet 6B is the slower moving spot and remains behind the standard control.

V. Separation of Brown FK from the Chocolate Browns

Brown FK may be separated and identified from Chocolate Brown HT and Chocolate Brown FB by running a chromatogram in Solvent 9 using Brown FK as the standard control. The chocolate browns are slow moving spots and remain behind the standard control.

Separation of a mixture of dyes using two-dimensional chromatography

If the presence of more than one dye is suspected, or definitely shown by separation of the unknown into various bands, one of the following procedures may be used:

1. A chromatogram is prepared by drawing the base line $\frac{3}{4}$ in. from and parallel to the bottom edge of the paper. The unknown dye is spotted on to the base line $\frac{3}{4}$ in. from the left-hand edge of the paper, and the control dye $\frac{3}{4}$ in. from the right-hand edge of the paper. A double spot of unknown + control is also spotted $\frac{3}{4}$ in. from the control dye (Fig. 1). This chromatogram is then run in the solvent which showed separation of the unknown into two or more colours.

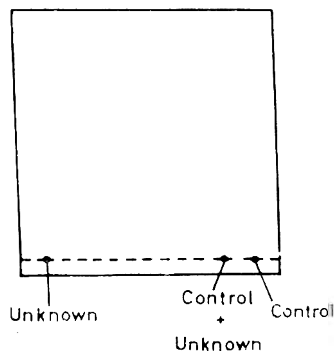

Fig. 1

By their positions relative to the control spot each unknown dye may be placed in a smaller group of possible dyes, and by using the technique of two-dimensional chromatography they can be positively identified.

The paper chromatogram (Fig. 2) which shows separation of the unknown dye is turned through 90° in an anticlockwise direction and a new base line is drawn through the spots formed from the unknown dye—it may be necessary to prepare several of these chromatograms (Fig. 3) in order to identify each unknown.

Each unknown spot is now treated separately and the system of elimination is proceeded with as outlined in the above schemes.

2. Alternatively, a large block of colour (approximately) 1 × 10 cm) of the unknown dye is applied to a paper chromatogram. This chromatogram is then run in the solvent

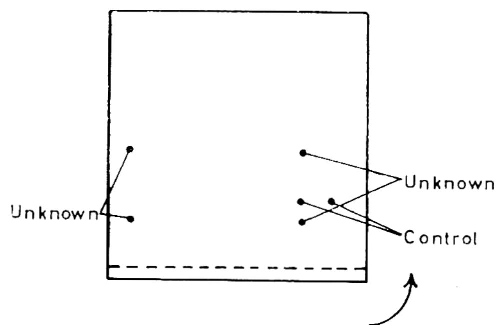

Fig. 2

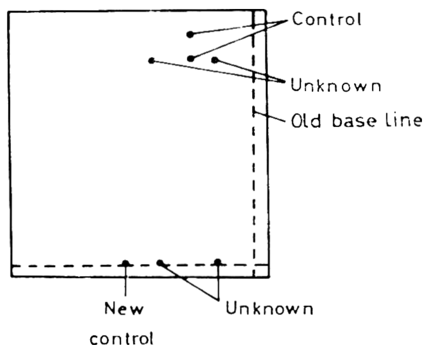

Fig. 3

N.B. When using hydrochloric acid the following procedure must be used after the solvent has reached a suitable level on the paper. The chromatogram is removed from the tank and hung immediately in a neutralizing atmosphere over 0·88 ammonia solution for at least 10 min. It is then dried in the usual manner. If this procedure is carefully observed chromatograms can then be preserved for practically indefinite periods of time and the papers will not become brittle when dry.

which shows separation of the dye into two or more bands of colour. After removal from the solvent and subsequent drying the different bands are cut out from the paper chromatogram and the colours eluted with water. Each dye is then separately identified by the appropriate scheme.

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The glassy state in certain sugar-containing food products*

G. W. WHITE AND S. H. CAKEBREAD

Summary. A review is given of the literature on organic glasses with special reference to sugar glasses. The amorphous sugar glasses in boiled sweets, milk powder, ice cream and certain freeze-dried products are then discussed. Breakdown of these sugar glasses can lead to defects in all four cases.

Introduction

Certain food products contain sugars which are in a vitreous or glassy state, and it is possible to interpret some of the defects associated with these products as a breakdown of this glassy state. The products which will be considered are listed in Table 1.

It can be seen that among the organic glasses involved are glucose, sucrose and lactose in various combinations. Unlike inorganic glasses, these sugar glasses are very unstable in the presence of moisture and this instability, under unfavourable conditions, can lead to various defects.

Before examining these faults, we shall consider some general properties of glasses.

The glassy state — general considerations

Many substances, including alcohols, fats, protein solutions, sugars and sugar solutions can be converted into amorphous glasses. There is some evidence that even water can be vitrified (Pryde & Jones, 1952).

A glass has been described by Jones (1956) as any liquid or super-cooled liquid whose viscosity is greater than 10^{13} P (poises). This definition accords well with the familiar meaning of the term glass, since a liquid whose viscosity is greater than 10^{13} P is capable of supporting its own weight. Modern theories of the liquid and glassy states are based on the observation that the X-ray diffraction patterns given by these consist of one or more diffuse rings. For both liquids and glasses these patterns imply the existence of very small regions characterized by an ordered arrangement of molecules

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* This paper was originally presented at the 1st International Congress of Food Science and Technology, London (1962), but has now been revised and brought up to date.

in contrast with a crystal, which has extended regions of order, and a gas, which has no such regions.

Some of the factors which favour the formation of the glassy state when a liquid is cooled are: (a) a high viscosity in the liquid just above the solidification point, (b) a rapid rate of cooling, (c) a complex molecular structure, and (d) the presence of more than one molecular species. All these factors tend to prevent the molecules taking up the correct positions to form a crystal lattice.

TABLE 1. Food products containing glassy sugars

Product	Method of preparation	Sugars involved	Defects possible on breakdown of glassy state
Boiled sweets	By boiling sucrose-glucose or sucrose-invert sugar syrups and cooling	Sucrose Glucose, etc. Invert sugar	Collapse or distortion, graining (crystallization), stickiness, followed by graining or fluidity
Milk powder	By spray or roller drying	Lactose	Lumpiness or caking
Ice cream	By aeration and rapid freezing of ice cream mix	Sucrose Lactose	Sandiness
Some freeze-dried products	Freezing followed by vacuum sublimation of ice	Various	Partial liquefaction or caking

The transition from a liquid to a glassy state is characterized by discontinuities in certain physical properties, while other properties change in a more or less smooth manner. The nature of these changes is summarized in the diagrams of Fig. 1.

The point T_g on these curves is known as the transformation temperature; this is not a sharply located point but defines the centre of a small region of 20 Centigrade degrees or so over which the transformation takes place. The viscosity at T_g is always of the order of 10^{13} P, but T_g varies greatly from system to system, as shown in Table 2. It should also be noted that, for a given substance, T_g may be somewhat different for different physical properties (Parks, Huffman & Cattoir, 1928).

It can be seen from the table that within a group of like compounds, the transformation temperature increases with molecular weight. It is also evident that the transformation temperatures for sugar solutions are lower than for the dry sugars; in fact, the transformation temperature decreases with increasing water content (Kargin, 1957).

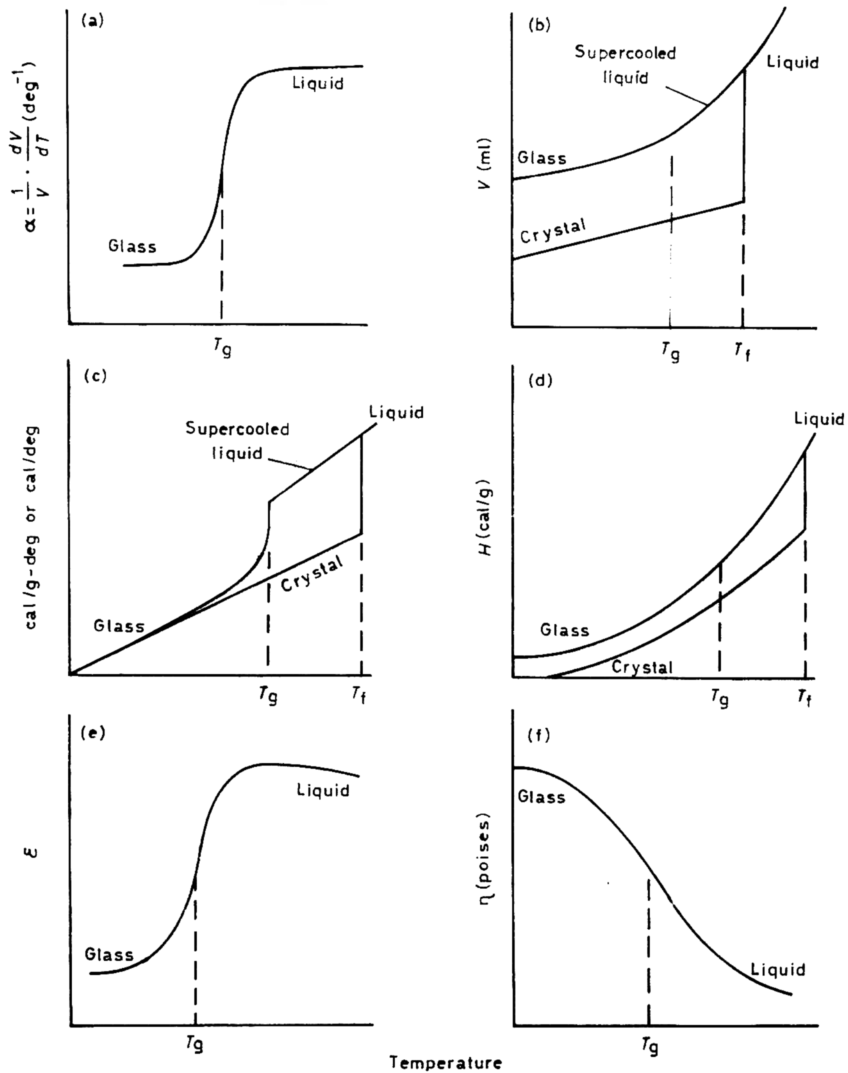

FIG. 1. Characteristics of the transition from the liquid to the glassy state (from Parks & Huffman, 1927; Parks *et al.*, 1928; Parks & Cattoir, 1929; Parks & Gilkey, 1929; Parks, Thomas & Gilkey; 1930; Thomas, 1931; Parks & Thomas, 1934; Kauzmann, 1948; Jones, 1956). (a) Coefficient of expansion, (b) volume, (c) specific or molecular heat, (d) heat content, (e) dielectric constant, and (f) viscosity.

To prove conclusively the existence of the vitreous state in a particular case, the absence of crystals must be shown by X-rays or microscopy, and definite changes in physical properties (e.g. specific heat) must be demonstrated (Smith, 1954).

Methods of making sugar glasses which may be utilized for the study of their properties are: (a) by concentrating a solution at a high temperature and cooling, (b) by

TABLE 2. Glass transformation temperatures

Substance	Transformation temperature (°C)	Molecular weight	References
Water	−150 to −125		(J)
Ethyl alcohol	−183 to −177	46	(C), (D), (H)
n-Propyl alcohol	−172	60	(E)
Isobutyl alcohol	−155	74	(E)
Glycerol	−93 to −83	92	(C), (D), (E)
Glucose (dextrose)	+20 to +35	180	(B), (C), (D), (G), (I)
Sucrose	+67	342	(D), (K)
Lactose	≫20	342	—
Glucose (2 M solution)	−41		(F)
Sucrose (2 M solution)	−32		(F)
Raffinose (1 M solution)	−27		(F)
Polystyrene	+80 to +90		(C), (D)
Silica	1200		(A)

(A), Barrer (1943); (B), Davies & Jones (1953); (C), Jones (1956); (D), Kauzmann (1948); (E), Kobeko, Kuwshinsky & Shishkin (1938); (F), Luyet (1939); (G), Parks *et al.* (1934); (H), Parks & Huffman (1927); (I), Parks *et al.* (1928); (J), Pryde & Jones (1952); (K), Samsoen (1929).

roller, spray or freeze-drying a solution, (c) by extremely rapid freezing of a solution, and (d) by fusion of the crystalline solid and cooling. Not all of these methods can be used in a particular case, e.g. as far as is known it is not possible to make sucrose glasses by fusion of the crystalline material, but they can be prepared by spray drying 20–40% aqueous solutions of sucrose (Makower & Dye, 1956; Palmer, Dye & Black, 1956).

Glucose glasses have been extensively studied by Parks and his collaborators (1927–41), sucrose glasses by Samsoen (1929), Stare (1939), Makower & Dye (1956) and Palmer *et al.* (1956), and lactose glasses by Herrington (1934).

All these glasses are extremely hygroscopic and on picking up moisture, glucose glasses tend to liquefy (Kargin, 1957) whereas sucrose and lactose glasses tend to crystallize.

The glassy state in boiled sweets, milk powder, ice cream and freeze-dried products

(a) *Boiled sweets*

Boiled sweets are sucrose–glucose or sucrose–invert syrups of such high concentration that at ordinary temperatures they are glassy solids. The main defects to which boiled

sweets are liable are shown in Table 3 (Grover, 1947; Money & Born, 1951; Hinton, 1958a; Campbell, 1958a).

Collapse or distortion can occur in dry atmospheres, and is caused by: (1) a low content of glucose syrup (or a high invert sugar content), (2) high residual moisture, or (3) high storage temperatures (Campbell, 1958). All these three factors cause collapse by decreasing the viscosity; in the first two cases the glass transformation temperature is lowered below room temperature, while in the last case the storage temperature is raised above the transformation temperature. The main requirement for stability is $T_g > T_r$ where T_g is the glass transformation temperature and T_r the room temperature.

Graining throughout the mass can also occur in dry atmospheres. It is usually observed when insufficient confectioners' glucose or invert sugar is present; high residual moisture and storage temperatures can also encourage this defect. Control of this defect is again largely a matter of ensuring that the glass transformation temperature is greater than the storage temperature, so that sucrose crystallization cannot occur.

Stickiness can occur in a moist atmosphere whose relative humidity (r.h.) is greater than the relative vapour pressure or equilibrium humidity (e.h.) of the boiled sweet. This is normally the case as the e.h. of boiled sweets lies in the range 10–30% (Campbell, 1958; Grover, 1947; Kelleher, 1956) while the average indoor r.h. in this country is about 65%, varying from below 50% to about 90%. When moisture is

TABLE 3. Defects possible in boiled sweets

In dry atmospheres	In moist atmospheres
Collapse or distortion especially at high temperatures	Stickiness owing to absorption of moisture
Crystallization or 'graining' throughout the mass	Subsequent graining or fluidity

adsorbed on the sweet the transformation temperature of the surface layer rapidly falls below room temperature, the viscosity falls and the surface layer melts. Penetration of water into the sweet follows. When the glucose or invert sugar contents are below certain levels, crystallization of sucrose follows, as in pure sucrose glasses. With larger amounts of glucose (e.g. 40%) the samples become more and more sticky because of the tendency of glucose glasses to liquefy.

Because of the unstable nature of boiled sweet glasses in moist atmospheres, they are normally packed in metal or glass containers or wrapped in 'moisture-proof' film (Grover, 1947; Campbell, 1958; Hinton, 1958b).

A recent book by Matz (1962) devotes a chapter entitled 'Glassy Structured Foods' to consideration of the texture of boiled sweets.

(b) *Milk powder**

Milk dried rapidly to a sufficiently low moisture content, by roller, spray or freeze-drying, contains lactose in a glassy form (Troy & Sharp, 1930; Tuckey, Ruehe & Clark, 1934; Decker & Reid, 1943; Villanova & Ballarin, 1950; Choi, Tatter & O'Malley, 1951; Nickerson, Coulter & Jenness, 1952; King, 1954).

A defect which sometimes develops in stored milk powder is lumpiness or caking. Whole milk powder and skim milk powder contain about 38% and 50% lactose respectively, and the moisture content is usually 3–4% or less. Lea (1947) found that the equilibrium humidities of skim milk powders are directly proportional to the moisture content in the range 3–8% moisture. This explains why so many different relative humidity 'thresholds' have been reported, above which milk powder will adsorb moisture from the air.

When the r.h. of the air exceeds the e.h. of the milk powder, the lactose glass adsorbs moisture and the milk particles become sticky. Adherence of the milk particles to each other follows and when the moisture content reaches about 9% (Lampitt & Bushill, 1931) crystallization of the lactose occurs and solid lumps are formed. This is the cause of caking. The main requirements to prevent caking in milk powders are a moisture content of $\leqslant 4{\cdot}5\%$ in the packaged powder, storage in 'moisture-proof' containers and prevention of damage to these containers during transport.

When crystallization of the lactose occurs in milk powder, α-lactose monohydrate is usually, though not invariably formed, as indicated in another paper from these Laboratories (Bushill *et al.*, 1965).

A comprehensive review on milk powder has recently been published by King (1965).

(c) *Ice cream*

Ice cream is a frozen foam which usually contains 50% by volume of air in the form of minute air cells, embedded in a 'continuous' phase; this latter consists of a concentrated sucrose–lactose syrup or glass distributed in which are fat globules, ice crystals, proteins, etc. After aeration and freezing, ice cream is usually transferred to a hardening room at about −20°F, and Sommer (1944, 1949) has suggested that at this temperature the sucrose and lactose exist in a glassy state.

The evidence for the existence of a sucrose–lactose glass in ice cream is as follows:

(i) X-ray diffraction patterns of freeze-dried ice cream show that the sucrose and lactose are present in a non-crystalline form (Wright, 1961). It is deduced from this that the sucrose and lactose are also non-crystalline in the original ice cream.

* Excluding instant milk powders.

(ii) The glass transformation temperature for a 2 M aqueous solution of sucrose is −25·6°F, and it is known from the amount of ice present in ice cream that the sucrose and lactose concentrations at −20°F are 3 M and 2 M respectively. It is very probable therefore that the transformation temperature of the combined 3 M sucrose–2 M lactose glass is significantly higher than −20°F.

(iii) Lactose crystallization in ice cream rarely occurs below −10°F (Decker, Arbuckle & Reid, 1939; Sommer, 1944, 1949; Nickerson, 1954, 1956, 1957), and it is thought that the lactose molecules are unable to form crystal lattices below this temperature because they are tightly bound in a sucrose–lactose glass.

The combined evidence of (i), (ii) and (iii) suggests that the transformation temperature to a sucrose–lactose glass in ice cream is about −10°F; this is likely to vary somewhat with composition.

The gritty condition known as 'sandiness' in ice cream is caused by the development of lactose crystals. Nickerson (1954, 1956, 1957) has found that: (a) below −10°F sandiness does not develop, (b) between −10 and +2°F lactose crystallization is greatly favoured, and (c) between +2 and +12°F lactose crystallization proceeds rapidly only when nuclei are present. After hardening and transportation, ice cream is usually stored at +6 to +12°F in the vendor's refrigerator; prevention of sandiness during this storage is generally effected by limiting the milk serum solids in the ice cream (Dahle, 1923; Sommer, 1944, 1949). This keeps the transformation temperature, T_g, low and ensures that the danger region, in which lactose crystallization is greatly favoured, is kept well below refrigerator temperature.

(d) *Freeze-dried products*

Much work is now being done in this country on the freeze-drying of food products. This is essentially a process of vacuum sublimation from the ice phase in the frozen product.

Gane (1951) noted that powders obtained by freeze-drying sugar-containing liquids often caked or even partially liquefied on storage, and suggested that because of the rapid rate of freezing the sugars are thrown out of solution in an amorphous form to crystallize later with liberation of water. This is known to happen with *spray-dried* milk powder and Gane (1951) found evidence of similar effects with *freeze-dried* apple juice, tomato purée and milk. Nickerson *et al.* (1952) have confirmed that the lactose in freeze-dried milk is in a glassy form.

It is suspected that in many other freeze-dried products containing sugars, e.g. fruit products, the sugars are present in a glassy form, and we have confirmed this in the case of freeze-dried apple pulp, apricots and orange juice. This may account for many of the problems associated with the freeze-drying, milling, storage and reconstitution of sugar-containing food products.

If the initial freezing of the product is sufficiently rapid, the sugars present may not

crystallize, but set to a glass which coats the ice crystals. This vitreous coating may slow down the freeze-drying process by decreasing the transport of vapour from the ice phase and by binding a certain amount of water.

As the temperature rises during freeze-drying the transformation temperature of the vitreous sugars is often passed at an early stage, particularly if the glasses have a high water content. If this happens the sugar glasses 'melt' and the material expands or puffs, giving a distorted product, and drying is at least partially from the liquid rather than from the frozen state (Rey, 1960, 1962).

If a freeze-dried product containing vitreous sugars is milled to a fine powder, the temperature rise during milling may be sufficient to take the product temperature above the glass transformation temperature, causing stickiness and clumping at or just after the milling stage.

Sugar glasses, particularly when finely divided, or in thin films, are extremely hygroscopic and when present in a freeze-dried product they tend to take up moisture from the air, and may, as already noted, cause the product either to cake or even to liquefy partially on storage.

Another problem with freeze-dried products is that of reconstitution. If proteins and other hydrophilic substances present are embedded in a sugar glass, they will not be able to absorb water until the vitreous shell is dissolved away. This process may take a little time because of the high viscosity of the vitreous sugar matrix. Vitreous sugars, therefore, may slow down reconstitution.

Conclusions

It is clear that there is need for much more work on simple and mixed organic glasses, and in particular on the sugar glasses that exist in certain food products. It would be helpful to demonstrate the discontinuities in physical properties that occur when these products are taken through their transformation temperatures. The physical state of the sugars and other components present in freeze-dried food products would merit study by microscopical, X-ray diffraction and other methods.

Acknowledgments

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A note on ethyl acetate formation in canned 'draught' beer

H. DALLYN AND J. R. EVERTON

Beer canned in packs of 16 oz or less is carbonated up to 2·7 volumes and pasteurized in the sealed can.

In recent years beer canning has been developed in Great Britain to include the packing of 'draught' beer (which is under 1·6 volumes of carbon dioxide) in 4 and 7 pint open-top cans. A few packers are producing a sterile filtered beer aseptically filled into 4 pint cans which are hermetically sealed with standard can ends. However, for most canned 'draught' beer aseptic techniques are not followed, and, since the product is not pasteurized 'in can', only a limited shelf life is expected. This beer is filled into 4 and 7 pint cans fitted with either one or two rubber stoppers inserted in one end by means of which headspace pressure is controlled. One of the rubber stoppers is normally pierced to act as a valve allowing the can to vent at a predetermined pressure. After purchase the beer may be dispensed through one of the bung holes by removing the stoppers.

Spoilage of this draught beer, characterized by a strong ethyl acetate-like odour, in occasional cans fitted with rubber stoppers, has come to our attention. One such can showed the presence of approximately 1% of ethyl acetate, confirmed by gas chromatography. A thick deposit was present in the can. Large numbers of yeasts and small rods were seen on microscopic examination and a number of strains of yeasts and acetobacter type bacteria were recovered in cultures. Among the yeasts isolated was a species of *Hansenula* and, using the Lodder & Kreger-van Rij (1952) classification, this was considered to be a strain of *Hansenula anomala*. All the various organisms isolated, when inoculated into similar 4 pint cans of normally carbonated beer (approximately 1·5 volumes), showed no obvious development and failed to reproduce the 'spoiled' condition even after protracted incubation at room temperature.

Ester production is characteristic of certain yeasts and in this field the formation of ethyl acetate by *H. anomala* has been investigated by several workers, particularly Gray (1949), Davis *et al.* (1951), Peel (1951), Tabachnick & Joslyn (1953) and Smith & Martin (1964). Tabachnick & Joslyn showed that with growing cultures of *H. anomala*, ethyl acetate is a direct product of ethanol oxidation. One condition which they

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demonstrated was the influence of oxygen tension: excess aeration resulted in lower yields of the ester while strictly anaerobic conditions resulted in poor growth of the yeast and no ethyl acetate formation.

In view of the results obtained by these workers and because the yeast isolated was found to be a *Hansenula* species, its behaviour under aerobic conditions in degassed beer was studied. A vessel giving a large surface area to the beer was used so that film formation could be at a maximum. Within 72 hr incubation at 25°C, under these conditions, approximately 0·5% of ethyl acetate had been produced by the yeast.

Failure to reproduce this particular spoilage by inoculation of the *H. anomala* species into similar sound cans of normally carbonated beer, in which conditions are virtually anaerobic, is in accordance with the results of the studies made on the physiology of strains of this yeast by the various workers referred to above. Under normal conditions of packing beer in cans of 4 and 7 pint sizes, spoilage of this type is therefore unlikely as long as carbonation is adequate and oxygen content is at a minimum. However, it is possible to visualize conditions under which the headspace carbon dioxide/oxygen ratio could be changed drastically in the occasional can—such as, for example, by the rubber bungs being insecurely applied or being accidentally dislodged in transit. Under such circumstances if *H. anomala* is present in the beer it could grow out with production of ethyl acetate.

The authors are indebted to their colleague Mr C. J. Barker for carrying out chemical analyses.

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Papers to be published in future issues

Food research in the United Kingdom. By C. L. Cutting.

The spoilage flora of eviscerated chickens stored at different temperatures. By Ella M. Barnes and Margaret J. Thornley.

Brown discoloration in pre-rigor cut fish fillets. By T. R. Kelly and W. T. Little.

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INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY AUTUMN PROGRAMME, 1966

The following programme has been arranged for the autumn period and visitors will be welcome, though it must be remembered that, as the meetings are private, no reports may be sent to the press without prior communication with the Honorary Secretary of the Institute.

Date	*Place*	*Time*	*Subject*	*Speaker*
22nd–27th August	Warsaw	2ND INTERNATIONAL CONGRESS OF FOOD SCIENCE AND TECHNOLOGY		
September	Messrs J. Sainsbury, Basingstoke	Ladies Meeting, Details to be announced		
5th October	Blackpool (Joint with Institute of Meat)	Full Day Symposium	Developments in the production, processing and packaging of meat	Details to be announced; arranged by the North of England Branch
21st October	College of Further Education, Grimsby, Yorks.	Full Day Symposium	Transport of frozen foods	To be announced
27th October	Borough Polytechnic, Borough Road, London, S.E.1	Tea 6.30 for 7 p.m.	Automation in the food industry	L. Simmens, M.SC., F.R.I.C.
6th December	Borough Polytechnic, Borough Road, London, S.E.1	Tea 6.15 for 6.45 p.m.	Annual General Meeting followed by address	W. Rowan Hare (President, F.M.F.)

It is hoped to arrange a joint meeting with the Food Science Students' Society and the University of Strathclyde in November. Arrangements are in hand for joint meetings with Students' Societies at Leeds, Nottingham and Reading, and it is hoped to hold a symposium at Weybridge and Aberdeen and at Carlow, Eire, in the New Year. Other meetings and summer visits have been arranged, and joint meetings with the Food Group of the Society of Chemical Industry, and the Food and Nutrition Group of the Royal Society of Health are being organized.

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

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

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

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

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

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

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