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Harmonization of legislation on foodstuffs, food additives and contaminants in the European Economic Community

R. HAIGH

Summary

Tasks carried out by the Commission of the European Communities find their basis in treaties establishing their existence. The work on the approximation of the laws of the Member States on foodstuffs (including that on additives and contaminants is based on provisions in the Treaty of Rome establishing the European Economic Community (EEC)). The procedures for carrying out this work are also specified in this Treaty.

The present text is designed to explain how these requirements are applied by the Institutions of the Community and their advisory working groups. It is hoped that it will provide a simple guide to those having a particular interest in this sector.

Part I, which appears in this issue, sets out the working procedures, while in Part II some achievements and further programmes will be described.

PART I: WORKING PROCEDURES

Introduction

Over the centuries man has improved his technique of food production, and technological development has allowed the creation of a diet which is today rich in a variety of nutritious foodstuffs. The chemist, the technologist and the nutritionist, indeed scientists in general, have worked together to give these foods better flavour, texture, colour, and nutritional value in forms that can be preserved from attack by disease or deterioration during 'difficult periods' which tend now not to be the long winter months, but rather the long holiday weekend when the housewife, often managing household and profession has to feed her family from her kitchen store – more likely to be a deep freeze than a muslin bag.

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One effect of the evolution in technological progress of food manufacture has been the relatively rapid development of food legislation in recent years. The motivation of this legislation has been to protect the consumer against technological advances which by ignorance or lack of foresight might have led to potential danger to public health or which could make more difficult the choice between products on the market apparently of the same value, without adequate information to enable the consumer to make a proper distinction.

These two major principles of health protection and prevention of fraud underlie the food legislation in all Member States of the European Economic Community.

It is because there is this common basis, because the approaches and techniques each State follows are so similar, and not least, because they have reached a similar stage of development, that it is possible to envisage the close cooperation necessary in the programme of harmonization of their several legislations.

The founders of the EEC were farsighted enough to recognize that a closer union among the peoples of Europe would be a means of removing many of the economic and social ills within the Member States. Continuing differences in the legislative provisions in the Member States governing such details as the nature, composition, manufacturing conditions, handling, packaging and labelling of foodstuffs hamper efforts to create this union and the Treaty of Rome, recognizing this problem, requires that the Community shall remove obstacles to the free movement of goods by working towards the approximation of the laws of the Member States – commonly referred to as 'harmonization of legislation'.

Legal procedure of harmonization

The means to implement these obligations of the Treaty of Rome are also to be found within the Treaty. The founders of the Community created permanent institutions on which real if limited powers have been conferred (Fig. 1). These institutions are:

(1) *The Council*: Nine Ministers each representing Member Governments, the actual Ministers depending on the subject in question.

The Council's main role is to decide on Commission proposals and depending on the circumstances may vote by majority or by unanimous resolution. The Commission is present at Council meetings:

(2) *The European Parliament* (or Assembly): Made up of members which the nine national Parliaments designate from among their own members. The Members will eventually be elected by direct universal suffrage.

The Parliament monitors the work of the Commission and the Council. It has to be consulted on most Commission proposals before the Council can take a decision on them, and its Members can put written or oral questions to the Commission or the Council.

(3) *The Court of Justice:* Composed of nine judges, appointed for a specific period with the consent of Governments, ensures that the implementation of the Treaties is in accordance with the rule of law, and deals with disputes between Member States, between Member States and Community Institutions and between Community Institutions and firms, individuals or Community officials.

(4) *The Commission:* Consists of thirteen members appointed by agreement between Member Governments for a renewable term of office of four years. The Commission is:

- *Independent* of the Member States,
- *A Policy-planning Body* initiating Community action,
- *A Mediator* between Governments steering its policy proposals through the Council and adjusting them if necessary in the light of the discussion,
- *An Executant* taking many detailed decisions,
- *A Watchdog* in the last resort taking Governments or firms to the Court for breaches of Community law (Noel, 1977).

The Treaty also stipulates that the Council and the Commission shall be assisted by the *Economic and Social Committee*, acting in an advisory capacity.

The Economic and Social Committee is composed of Members representing employers' organizations, Trade Unions and other interests (including consumers) in equal numbers.

The Treaty of Rome not only created the institutions but went so far as to give instructions on how these Institutions should cooperate. In a section devoted to the 'Approximation of Laws' (Chapter 3), the Treaty requires that 'the Council shall, acting unanimously on a proposal from the Commission, issue directives for the approximation of such provisions laid down by law, regulation or administrative action in Member States as directly affect the establishment or functioning of the common market. The Assembly and the Economic and Social Committee shall be consulted in the case of directives whose implementation would, in one or more Member States, involve the amendment of legislation' (Article 100). This Article applies to national rules on foodstuffs which hinder trade between Member States and is used in the majority of cases as a basis for Community action.

As most measures are based on the procedures defined in Article 100, the instrument most commonly used for approximation of food laws is the *Directive.*

For foodstuffs of agricultural origin, measures have also been proposed using Article 43 of the Treaty and these have taken the legal form of a *Regulation.* Article 43 governs the establishment and implementation of the common agricultural policy, and is used extensively in the context of setting quality standards for products subject to the Community's rules on the common organization of the market. Its use has met with little success in its application to harmonization.

Directives are binding on the Member States to which they are addressed as regards the result to be achieved, but leave the mode and means to the discretion of the national authorities. The Directive only takes effect after it has been incorporated into the law of the individual Member State, which is given a specified period to put into effect the administrative procedures necessary to comply with its provisions. A *Regulation* is of general application, is binding in every respect, and has direct force of law in every Member State.

Thus the Commission and the Council provide the main day to day impetus in the Community's decision making processes. Without the Commission's proposal no progress can be made, but it is the Council which takes the final decision. Both are essential to the development of Community law.

This is not to underestimate the role of the European Parliament and the Economic and Social Committee, and where a consultation with these bodies results in advice that the Commission's proposal should be revised, it is unlikely that such an opinion will be ignored. The system therefore provides democratic control through the European Parliament and allows the voice of the social interests to be heard in the Economic and Social Committee. In this way the final measure adopted reflects the legitimate interests on all sectors of the Community.

The Court of Justice is the final arbiter as to whether the measure has been properly applied.

Simplified legal procedures for technical adaptations

In this specialized area most, if not all the detailed work is carried out by experts with a particular knowledge of the subject in question. It has become obvious that some technical subjects do not lend themselves to discussion by non-technical participants and for this reason the recognized legal consultative procedures have been simplified in order to process the treatment of such subjects in a more effective manner. For example, directives on purity criteria for food additives are adopted by a procedure which requires only the *unanimous approval by the Council of the proposal submitted by the Commission.* In this way the already heavily engaged European Parliament and Economic and Social Committee have no reason to be needlessly preoccupied with relatively straightforward technical discussions. It has to be stressed that the Directives in question specify the terms in which this simplification is to be applied, and its extension to any new group of additives is not automatic. However it is to be expected that some form of simplified procedure will always be incorporated in future Directives.

The Institution of the *Standing Committee on Foodstuffs* which was created by the Council Decision* of 13 November 1969 (Anon., 1969) was a far more radical simplification. So far its contribution has been minimal but it is potenti-

**Decisions* may be addressed either to a government or to an enterprise or private individual; they are binding in every respect on the party or parties named.

ally a significant factor in the acceleration of the programme on the harmonization of food legislation.

In the period leading up to the institution of the Committee it had become clear that the very complicated technical adaptations to directives would be more easily adopted in a reasonable time if a special procedure could be developed which would avoid the bottleneck which is created by the constraints of the unanimous voting required by Article 100 of the Treaty. The Committee was created to assist the Commission in carrying out tasks which would be specified in the Council Directives on the matter.

The committee is composed of delegates from the Member States and is chaired by a representative of the Commission. It gives opinions on subjects which are referred to it by the Chairman either on his own initiative or at the request of a Member State. The committee gives an opinion of the draft measure, within a time limit set by the Chairman according to the urgency of the matter. The opinion is delivered by a majority voting procedure, the votes of the Member States being weighted according to Article 148 (2) of the Treaty of Rome. The Chairman does not take part in the voting.

Where the measures envisaged by the Commission in its proposal are in accordance with the opinion of the committee, the Commission is empowered to adopt them. Otherwise the proposal is submitted to the Council which itself acts by a qualified majority. In some circumstances where the Council has not acted quickly enough the proposed measures may be adopted by the Commission but this situation is not likely to occur.

This 'committee procedure' can only be applied in situations where the Council delegates its competence to the Commission. This has only occurred in respect of certain sampling procedures and methods of analysis, to make amendments to the annexes of the directive relating to materials and articles intended to come into contact with foodstuffs, and in the application of the so-called 'safeguard clause' (which permits Member States not to apply the Community rules on a particular topic if public health can be shown to be jeopardized by their application).

Working procedures

Consultation Procedures used by the Commission

The Commission is the initiator of Community policy and as such the preparation of the 'first draft' is carried out by its own permanent staff of civil servants recruited in the main from nationals of the Member States, who like the Commission act in full independence both of governments and of the Council. The ideas in this document, which is usually circulated widely, stem from a variety of sources, as will be seen, and provide the impetus for discussion between the Commission Services and the various interests.

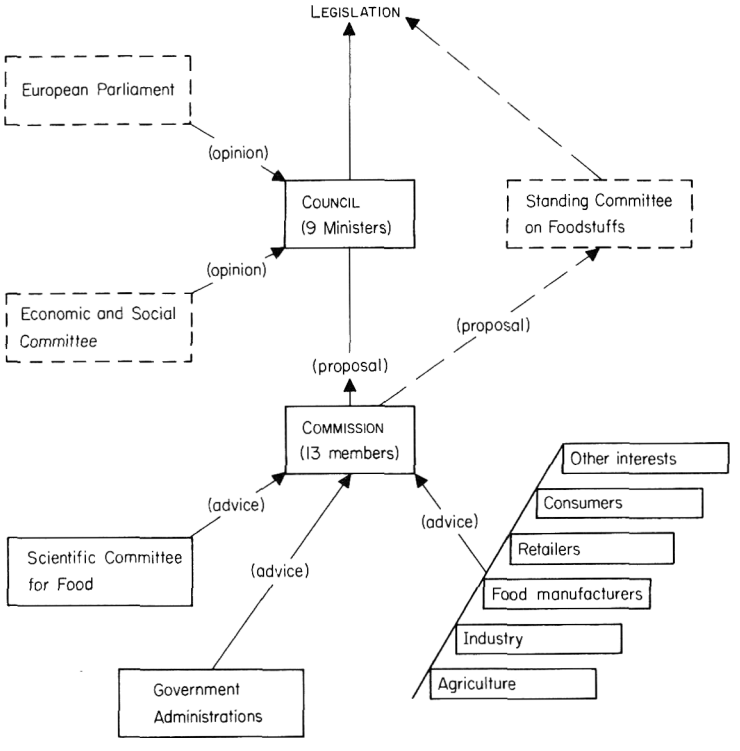

Figure 1. Consultations in the development of Community legislation on food.

If the Commission is to present a credible proposal it follows that it must be extremely well informed on the problems and practices surrounding the subject in question. The consultative procedures used to obtain such information must be flexible but must be designed to avoid bias towards one country, group or industry. For this reason formal contact is made with the European representatives of all national *professional groups* – consumers, manufacturers, food industries, retailers, etc. Detailed meetings take place with *experts of national governments* and the Commission consults the *Scientific Committee for Food* where questions of safety are involved (Fig. 1). For food additives and contaminants the opinions of this latter committee have become a cornerstone of the procedure and indeed in some cases the committee's role is recognized by the Directive on the matter.

The consultation procedures employed by the Commission are described below.

(1) *Consultation with national authorities*

When the working party on foodstuffs legislation met for the first time, it was composed of experts delegated by the Member governments, usually from

food or health ministries, to assist the Commission in the preparation of proposals which were to be submitted to the Council. This working party exists still and meets from time to time chaired by the Commission Services. However, as the working party very quickly became inundated by the number of subjects it had to treat, it designated experts from the national administrations with a specialized knowledge of the subject in question (e.g. additives, labelling, milk products, methods of analysis and sampling, fats and oils, etc.). The nomination of members to these subgroups is left to the discretion of governments, without the intervention of the Commission. If the Commission judges that a meeting is necessary, invitations are sent through the Permanent Representations of the Member States to the Community.

It should be stressed that neither working party nor subgroups have any formal status.

The subgroups also meet under the chairmanship of the Commission Services and they carry out the major part of the preliminary work on the Commission's draft proposal. The role of the participants is to advise the Commission Services on the approach being adopted. They are invited because of their expertise of the subject in question and their knowledge of the likely attitude to be taken by their national governments in the Council. On the basis of the discussion with these experts and other interests it is possible that the draft measure will be modified before being submitted by the Commission to the Council.

(2) *Consultation with the Scientific Committee for Food*

The acceptance of a process of manufacture of a foodstuff or the composition of a food or food ingredient at Community level is dependent upon the assurance that there is no hazard to health or safety of the consumer of such food. Public opinion is becoming, in some cases with justification, more critical of the criteria used by the legislator to justify the absence of harm to public health. It is essential, therefore, that the Commission can have recourse to independent experts, who can give impartial and authoritative advice on the safety of food and who can assess the toxicological acceptability of levels of food additives or residues of contaminants in food, in order that its proposals might be widely supported.

A committee of consultants able to give such advice to the Commission operated very effectively in an unofficial capacity from the time when the first steps were taken in the community programme of harmonization of food legislation. The Committee was given a permanent role in the elaboration of Community legislation on food by its institution on 16 April 1974 (Anon., 1974), as the Scientific Committee for Food. Its fifteen members are independent of the Member States, but chosen from Member States nationals in such a way that the Government authorities can have confidence in their ability. Their expertise ranges from medicine, toxicology, nutrition, to chemistry and biology, and like disciplines. It is quite evident that in the light of present day demands

for assurance which require interpretation of sophisticated data on the safety of food and food additives there will be a need to call increasingly on the services of this committee.

The committee's advice is normally published and it is indicative of the practical nature of its contribution to the programme that the Commission has accepted its advice in principle on every occasion. Indeed, the respect in which this committee is held by the institutions of the Community has been demonstrated by the demands for advice which have been made by Council, Parliament and the Economic and Social Committee. Professional organizations associated with the food sector have also readily accepted its advice.

(3) *Consultation with professional groups*

The Commission's formal contact with *professional organizations* is made only with such groups as can show that their membership is representative of all points of view on the subject in question in the Community. The most frequently found composition of such groups consists of representatives of the national groups on the same subject. Thus to make the point of view of his company known, a manufacturer of a particular product under discussion by the Commission Services would normally make contact with his national association which would submit the views of the national industry to the representatives, sitting on the approved 'European' grouping of this industry where the views of other national associations would also be discussed. It is such a group which is acknowledged by the Commission and with which contact is made.

Although each of these groups is normally represented by one association, many of the detailed questions have to be answered by sectors having a specialized knowledge. For example while the 'Commission of the Agricultural and Food Industries (CIAA)' has overall responsibility for the views of the food processing industries it is not unusual for specific problems to be treated by specialist organizations, as for example the Association of Preserved Milk Manufacturers (ASFALEC) where milk products are concerned, or the Liaison Bureau of the European (EEC) Unions of Aromatic Products on questions relating to flavouring materials.

The *consumers organizations* belong to the Consumers Consultative Committee, the secretariat of which is held by the Commission Services. This committee designates members to the Advisory Committee on Foodstuffs which will be discussed below.

Lists of the recognized non-governmental organizations set up at Commission level are published from time to time by the Commission.

In the past when comment on a particular draft was required it was circulated to the relevant professional organizations which were asked to give their views within a given time. This procedure remains useful but it did not allow the various groups to compare their often divergent approaches. The Commission therefore sets up an *Advisory Committee on Foodstuffs* (Anon., 1975) which all these groups are represented (i.e. agriculture, industry, workers, commerce

and consumers). Each group has two permanent members appointed by the Commission and four experts, who may vary according to the subject under discussion.

The Commission apportioned the seats on the committee equally amongst the following:

For industry:
The Union of Industries of the European Communities (UNICE).

For consumers:
The Consumers' Consultative Committee set up by the Commission Decision of 25 September 1973.

For agriculture:
The Committee of Agricultural Organization of the European Economic Community (COPA), jointly with the General Committee for Agricultural Cooperation in the EEC Countries (COGECA).

For commerce:
The Committee of Commercial Organizations in the EEC Countries (COCCEE).

For workers:
The European Trade Union Confederation (ETUC).

The Committee is asked to comment on Commission draft proposals, and its successful working has permitted a much better mutual understanding of the points of view of the various interests and has allowed the Commission to explain its ideas to a wider circle.

Acceptance of the Commission's proposal

It is unusual for a proposal submitted by the Commission to Council to be acted upon directly by the appropriate Ministers. It is far more likely that working parties of government experts will be set up to determine whether, and how, the Commission's proposal has to be modified to take into account the legitimate requirements of the national authorities. The task of these working parties is to find a formula which is acceptable in each Member State, and also to the Commission, taking into account the opinions of the European Parliament and the Economic and Social Committee. Proposals to be submitted to the Standing Committee on Foodstuffs are treated similarly.

The Commission's proposal is designed to promote European integration and in its elaboration attention is paid to technological practices and questions of safety in use (e.g. of an additive or a process). However, it is inevitable that in many cases not every point of view can be accommodated and depending on the degree of disparity, agreement in Council working parties may be rapid, or slow taking a considerable amount of time in arduous discussion. Agreement on technical matters can usually be obtained in the working parties of experts from the Government departments concerned with the measure, who may, and

do, consult with the national professional association on particular technical or economic problems peculiar to the conditions in that country. Problems which have not been resolved by these groups may be settled by the Committee of the Permanent Representatives (ambassadors) of the Member States to the Communities (COREPER).

COREPER plays an important role in preparing the deliberations of the Council. Only extremely sensitive political issues require the active participation of Ministers – although it is evident that in every case they bear the responsibility of the final compromise agreement.

The Directive thus agreed is introduced into the legislation of the individual Member States according to the specific requirements of their legal systems. Obviously not every 'harmonized' provision requires a change in the law of a Member State in order to be applied. Parts of the Directive will already be included in the national provisions on the matter, but the final result must be a uniform application of the Directive in all the Member States.

The Directive can really only be considered a 'Community measure' after the period of implementation – normally about 2 years.

A large number of topics have been on the programme of the Commission for some years, and considerable success has been achieved in adopting 'Community measures' on several of these. These achievements will be summarized in Part II of this series.

References

Noel, E. (1977) 'Working Together', Office for Official Publications of the European Communities Cat. No. Cl-22.77.P.63-EN-C.

Anon. (1969) Official Journal of the European Communities No. L 291, 19.11.1969, p. 10.

Anon. (1974) Official Journal of the European Communities No. L136, 20.5.1974, p. 1.

Anon. (1975) Official Journal of the European Communities No. L182, 12.7.1975, p. 35.

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Volatiles retention during rehumidification of freeze dried food models

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Summary

Observations of structural collapse during rehumidification of freeze dried substances are used to interpret previously reported data for volatiles loss *v.* time. The results are in accord with a diffusion-based analysis. At low moisture contents the diffusion coefficient is so low as to give only very small losses, while at high moisture contents it is high enough to give complete loss in a relatively short time. The behaviour at intermediate moisture contents depends upon whether or not there is simultaneous structure collapse. If there is no collapse, volatiles-loss data appear to agree well with a slab-diffusion analysis based upon constant diffusivity and unchanging slab thickness – the average thickness of webs within the porous substance. When there is simultaneous collapse, it is necessary to take into account the continuous increase in web thickness as diffusion occurs. A model is derived based upon a linear increase in web thickness with increasing time, and is shown to agree with experimental data for volatiles loss at intermediate moisture contents in several different systems which show structural collapse.

Introduction

Freeze drying can give excellent retention of volatile aroma substances present in liquid foods and related model systems. Nearly all important volatile aroma compounds have quite high relative volatiles in aqueous solution (Bomben *et al.*, 1973); hence in order to explain the high observed retentions it is necessary to postulate a rate limiting mechanism for volatiles loss.

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Two basic mechanisms have been proposed for the interpretation of volatiles loss during freeze drying. One, first put forward by Thijssen and associates (Thijssen & Rulkens, 1968, 1969; Thijssen, 1971), is based upon *selective diffusion*. Through independent measurements of diffusion coefficients of volatiles and water, Thijssen and associates found that diffusion coefficients for both drop markedly as dissolved solids contents increase, but that the diffusion coefficients for volatiles drop to a considerably greater extent. Thus, above a certain dissolved solids content, water is removed by diffusion at a significant rate, but other volatile compounds are not. Freezing prior to freeze drying gives a microscale separation of ice crystals from residual concentrate, as shown in the lower portion of Fig. 1. This increases the dissolved solids content of the residual concentrate and thereby increases the selectivity for transport of water as opposed to volatiles, once the concentrate webs are exposed by the retreating ice front. Furthermore, evaporative drying of the concentrate webs after passage of the ice front increases the selectivity still further. Below the collapse temperature, the concentrate webs are viscous enough so that they will not flow appreciably during evaporative drying. More properly, the volatiles diffusion process should be considered as a ternary system of volatile, water and dissolved solids (Chandrasekaran & King, 1972b), but the basic concept of selective diffusion remains unchanged. The selective diffusion analysis has also been extended to volatiles retention during other dewatering processes, such as air drying (Menting, Hoogstad & Thijssen, 1970b; Chandrasekaran & King, 1972b), spray drying (Rulkens & Thijssen, 1972) and concentration by extraction of water (Kerkhof & Thijssen, 1974).

Flink, Karel and associates (Flink & Karel, 1970a, b, 1972; Chirife & Karel, 1973, 1974a, b; Chirife, Karel & Flink, 1973; Flink, 1974; Kayaert *et al.*, 1975) have interpreted volatiles retention through the concept of *microregion entrapment*, in which volatile compounds are immobilized within cages formed by association of molecules of dissolved solids, such as by hydrogen bonding of carbohydrate molecules. The degree of immobilization would then relate to the water content, freezing conditions and other variables.

Through the selective diffusion concept the directional effects of numerous processing variables on volatiles retention in freezing drying can be explained in terms of the influences of these variables on the dimensionless Fourier group Dt/L^2, and the resulting increase in volatiles loss with increasing values of that group (King, 1970, 1971, 1972). Here D is the diffusion coefficient of the volatile substance in the concentrate webs (Fig. 1), L is the average thickness of the concentrate webs, and t is the time elapsed between passage of the ice front and sufficient drying of the concentrate webs to reduce volatiles loss to a negligible rate. This analysis includes the effects of initial dissolved solids content, freezing rate, specimen thickness and drying rate or ice front temperature. Flink (1974) presents an interpretation of the directional effects of the same processing variables through the microregion-entrapment concept. In fact, it has been noted (King, 1971; Flink, 1974) that in many ways the selective diffusion and microregion entrapment concepts are macro- and microscale

interpretations of the same general phenomenon. The selective diffusion approach is a potentially quantitative, mathematically formulated model based on transport concepts, while the microregion entrapment approach is more qualitative and is based upon molecular concepts. The selective diffusion interpretation has potential utilitarian advantages in that it should be capable of quantitative prediction and analysis, and in that it can be established and calibrated through entirely independent measurements of diffusion coefficients, such as those made by Menting, Hoogstad & Thijssen (1970a) and by Chandrasekaran & King (1972a).

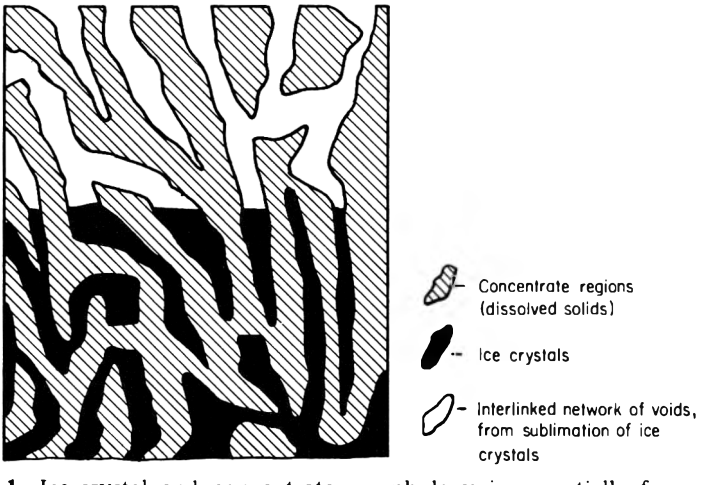

Figure 1. Ice-crystal and concentrate morphology in a partially freeze dried liquid food or model solution.

Many measurements have been made of volatiles retention as a function of time during rehumidification of freeze-dried substances (Flink, 1969; Flink & Karel, 1970a, 1972; Chirife *et al.*, 1973; Chirife & Karel, 1973, 1974a, b; Kayaert *et al.*, 1975). Results typically show that rehumidification at low relative humidities (e.g. below 30%) gives very little volatiles loss, yet volatiles loss is substantially complete over a few hours at high relative humidities (e.g. above 70%). Rehumidification at moderate relative humidities can give substantial, but not total, volatiles loss, with the rate of loss becoming very small and perhaps zero after an initial period of more rapid loss. Volatiles loss during rehumidification occurs more slowly than does water take-up. These results have been interpreted in the references mentioned in terms of the microregion entrapment concept, and the seeming asymptotic retention at longer times for moderate relative humidities has been attributed to break-up of some, but not all, of the microregion cage bonds, releasing some of the molecules of the volatiles material but keeping other molecules of the same compound immobilized.

It is less obvious how the slowing of rate or apparent asymptotic retention

for rehumidification at moderate humidities can be rationalized through the selective diffusion concept. The rate is slowed in many cases to a substantially greater extent than would be predicted by applying a simple slab diffusion model to a concentrate web of a given thickness (King & Massaldi, 1976). A mathematical model where a fraction of the volatile material obeys the simple diffusion model while the remaining fraction is totally immobilized would be able to fit the observed curves. However the distinction of different degrees of mobility for the same solute at different locations runs counter to the usual concepts of diffusion in solutions, except for such extreme situations as partially crystalline polymers. It seems preferable to interpret volatiles loss through a diffusion model without resort to such a concept, if possible.

King & Massaldi (1976) have identified and analysed probable effects of factors which could alter the predictions of a simple diffusion model applied to concentrate webs during rehumidification. These include variations of the diffusion coefficient with water content, temperature and/or volatiles concentration; segregation of volatiles into a second, immiscible phase within the concentrate webs, etc. They concluded that the most likely interpretation involves differences in thickness of the concentrate webs. Thin webs should give more rapid volatiles loss, and thick webs should give much slower percentwise loss; this is the effect of L^2 in the denominator of the Fourier number, Dt/L^2. For all solutions of the diffusion equation the percent removal of a substance increases with increasing values of the group Dt/L^2 (Crank, 1975; Treybal, 1968; etc.). Thicker webs have a lower surface to volume ratio and therefore lose volatiles at a slower rate, per unit volume.

As was noted by King & Massaldi (1976), the disparity in web thicknesses should be particularly pronounced when *collapse* occurs. Collapse is a phenomenon where the webs of semi-solid matter in a porous product flow under the impetus of surface tension and thereby close off some or all of the pores (Bellows & King, 1973). Collapse occurs for freeze drying of food liquids at too high a frozen-core temperature (MacKenzie, 1965. Bellows & King, 1973), as well as for storage of porous, dried products under conditions of too high temperature and/or relative humidity (Tsourouflis, Flink & Karel, 1976).

Partial collapse can form a number of very thick webs and can thereby markedly slow down the rate of volatiles loss from these thicker webs. In an example given by King & Massaldi (1976), a difference of a factor of 10 between web thicknesses within a specimen can give near-asymptotic volatiles retention curves of the sort observed by Flink & Karel (1972) and Chrife *et al.* (1973) for freeze dried maltose and polyvinylpyrrolidone.

From qualitative knowledge of such systems and from observations reported by Flink & Karel (1972, etc.), it seems likely that collapse occurred during some of the experiments on volatiles loss during rehumidification. The purpose of the present work was therefore to investigate experimentally the degree of collapse occurring during rehumidification experiments of the sort for which volatiles losses have been observed, and to relate the rate characteristics of collapse to the rate characteristics of volatiles loss.

Materials and methods

Sample preparation

Solutions in water were prepared, each containing 20% w/w lactose, maltose, polyvinylpyrrolidone (PVP), bovine serum albumin (BSA) or dextran (MW 9×10^4 or 2.2×10^6). Maltose and β-lactose (98%) were supplied by Matheson, Coleman & Bell, Inc. PVP was Type NP-K30, Control No. 10203, GAF Corp. Dextran fractions were OR1212 and 478-1-A (intrinsic viscosities 0.27 and 0.68, respectively) from Pharmacia Uppsala. BSA was Sigma Chemical Corp. A-4503, 96–98% purity. All compounds dissolved at room temperature, except for the dextrans which required heat. The dextran and BSA solutions were quite viscous. BSA dissolved with some difficulty and formed a persistent foam.

15 ml of solution were poured into an open Petri dish, 9 cm in diameter, and were frozen in a cold room at −15°C overnight. The frozen samples were dried in the freezing drying unit described by Bellows & King (1973). Maltose and lactose gave porous freeze dried products in 5 hr at a chamber pressure of 200 μm and a bottom platen temperature of 30°C.

The other substances were freeze dried at 200 μm without applying heat to the platens, and acceptable products were obtained in 8 hr. The freeze dried PVP and dextran products appeared to be less porous than the lactose and maltose products. BSA freeze dried to give two very porous and brittle layers; one was the foam and the other the solution.

Rehumidification effects

Rehumidification was carried out in vacuum desiccators similar to that used by Flink (1969). Saturated salt solution in a Petri dish at the bottom of the desiccator was agitated slowly with a magnetic stirrer to maintain constant relative humidity. Saturated solutions of $KC_2H_3O_2$, $MgCl_2 \cdot 6H_2O_2$, $Mg(NO_3)_2 \cdot 6H_2O$ and NaCl were used to achieve relative humidities of 23, 32, 52 and 75%, respectively (Wexler & Hagesawa, 1954; Labuza *et al.*, 1976). Over the Petri dish was a wire mesh, on top of which were placed watch glasses containing the samples. Aluminium foil was formed into a high wall around the watch glasses to prevent salt solution from splashing into the samples. At the beginning of each experiment the salt solution was degassed by putting the desiccator under vacuum for about 15 min. Then the samples were put into the desiccator, which was again evacuated for 30 min. The measured pressure was always around 1 torr when evacuation was stopped.

The sample was then observed visually at given time intervals, and changes were recorded. After some time, some of the samples would shrink and then change into a glassy transparent mass. The onset of this glassy transparent state was relatively sharp and well defined. The time of onset was recorded as the

collapse time. If there was no visible change after 24 hr, part of the sample was removed and observed under an optical microscope. The appearance of this substance was compared to the original freeze dried sample to see if there was any difference. At the ends of all rehumidification experiments in which no visual changes occurred, the samples were again observed in the microscope. For the microscopic observation, part of the sample was granulated before it was viewed.

Effect of heating

Chirife & Karel (1974a, b) reported the effect of heating on volatiles loss from freeze dried BSA and maltose samples. They reported an asymptotic retention level for maltose at 100°C. Their experiment was repeated in the present work, using a vacuum oven containing activated charcoal and anhydrous calcium sulfate (Drierite). All the six freeze dried samples were put in the vacuum oven, which was controlled at 100 ± 1°C. The samples were observed at 6-hr intervals for 47 hr. The experiment was repeated with the oven at 140 ± 1°C, and the samples were observed at the end of 24 hr.

Experimental results

Table 1 gives results of the observations of collapse during rehumidification of the various freeze dried specimens. Collapse was observed to occur at the time after the start of rehumidification indicated in the right-hand column. NC indicates that no collapse was observed.

Collapse during freeze drying results from closure of pores due to viscous flow (Bellows & King, 1973). It results in much bubbling and spattering, with shrinkage, aberrations in the drying rate and volatiles loss. Collapse during rehumidification produces no spattering, and there were usually no signs of bubbles, since there was no vapour evolution. Incipient collapse was signalled by shrinking, with the sample then becoming a highly viscous and glassy material, in contrast to the opaque and porous appearance of the freeze dried samples before collapse.

In the heating experiments there was no visible change in any of the six samples after 41 hr at 100°C. After 24 hr at 140°C the maltose and lactose samples showed apparent melting and charring, PVP and BSA turned yellow but showed no apparent collapse, and the dextrans remained unchanged in appearance.

Model for volatiles loss during collapse upon rehumidification

A cursive comparison of the results in Table 1 with the previous studies of volatiles loss behaviour upon rehumidification shows that in several cases volatiles loss and collapse occur simultaneously. In cases where a glassy state

Table 1. Results of rehumidification experiments

Sample	Relative humidity (%)	Collapse time (hr)
Maltose	23	230 (NC)
	32	25
	52	2.0
	75	1.2
β-lactose	23	230 (NC)
	52	2.2
	75	1.1 (RX)
Polyvinylpyrrolidone (PVP)	23	230 (NC)
	32	125 (NC)
	52	16
	75	2.5
Dextran (MW = 9×10^4)	23	230 (NC)
	52	48 (NC)
	75	5.0
Dextran (MW = 2.2×10^6)	23	230 (NC)
	52	48 (NC)
	75	8.5
Bovine serum albumin (BSA)	23	230 (NC)
	52	53 (NC)
	75	93 (NC)*

NC = no collapse observed after time indicated.

RX = changed from glassy to white upon standing after experiment (recrystallization).

* Became sticky when removed for microscope viewing.

becomes apparent after a certain time it can be postulated that collapse has been taking place slowly during the previous period of time. The glassy appearance comes once sufficient pores have been eliminated entirely through collapse to remove the light scattering effect caused by individual pores. Since this slow collapse occurs through a viscous flow mechanism, it follows that the thickness of the webs, within which diffusion of volatiles occurs, increase steadily over the same period of time. As time increases, L in Dt/L^2 would thereby increase. This in turn would cause the rate of volatiles loss to slow to a far greater extent at longer times than would be predicted by the simple diffusion model for constant web thicknesses. This is in qualitative agreement with the near-asymptotic retentions shown in previous studies of volatiles loss during rehumidification.

The volatiles loss in such a situation would be described by the partial differential equation

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \tag{1}$$

with the boundary conditions

$$\begin{aligned} C &= C_o && \text{at } t = 0, |x| \leqslant L/2 \\ C &= 0 && \text{at } t > 0, x = \pm L/2 \\ \frac{\partial C}{\partial x} &= 0 && \text{at } t \geqslant 0, x = 0 \\ L &= L_o && \text{at } t = 0. \end{aligned} \tag{2}$$

where $L = f(t)$. Here L is web thickness, t is time, C is volatiles concentration, D is diffusivity, and x is distance from the centre-plane of the web. The subscript o refers to initial conditions. The second boundary condition results from the very high volatilities of the compounds considered.

The time dependence in the boundary conditions can be removed by defining

$$y = x\left(\frac{L_o}{L}\right). \tag{3}$$

The equivalent of eqn (1) is now

$$\frac{\partial C}{\partial t} = D'\frac{\partial^2 C}{\partial y^2} \tag{4}$$

where

$$D' = D\left(\frac{L_o}{L}\right)^2 \tag{5}$$

and the boundary conditions become

$$\begin{aligned} C &= C_o && \text{at } t = 0, |y| \leqslant L_o/2 \\ C &= 0 && \text{at } t = 0, y = \pm L_o/2 \\ \frac{\partial C}{\partial y} &= 0 && \text{at } t = 0, y = 0. \end{aligned} \tag{6}$$

This is then a case of diffusion with the common boundary conditions, but with a diffusion coefficient (D') that is a function of time, through the dependence of L upon t. As is shown by Crank (1975), the solution of eqn (4) with eqns (6) as boundary conditions is given by the constant diffusion coefficient solution for fraction retention (symbolized by F) $v.\ DT/L_o^2$, where D is the value of D' at $t = 0$ (= the molecular diffusion coefficient, eqn (5)) and T is defined by

$$T = \frac{1}{D}\int_0^t D'(t')\,dt'. \tag{7}$$

For the slab the constant-D solution for F $v.\ DT/L_o^2$ is

$$F = \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n-1)^2}\exp\left[-(2n-1)^2\pi^2\ \frac{DT}{L_o^2}\right] \tag{8}$$

(Crank, 1975; Treybal, 1968). Plots of F *v.* DT/L_o^2 corresponding to eqn (8) are given by Treybal (1968), among others. The constant-D solution for F as a function of DT/L_o^2 (eqn (8)) can then be converted to the desired solution (F as a function of Dt/L_o^2) by integrating eqn (7) and determining the value of t corresponding to each value of T. If L does not change with time, T may be directly replaced by t in eqn (8).

What is needed now is an expression for $L = f(t)$, which takes collapse into account where necessary. Since collapse should occur at a more or less steady rate through a viscous-flow mechanism, a reasonable approach is to assume that the web thickness increases linearly with time, in which case

$$L = L_o + bt \tag{9}$$

where b is a constant. Inserting eqn (9) into eqn (5) and the resulting equation into eqn (7) gives

$$T = \int_o^t \frac{L_o^2 \, dt'}{(L_o + bt')^2}$$

$$= \frac{L_o t}{L_o + bt} \tag{10}$$

where D is assumed to be constant.

If F is measured experimentally, the corresponding values of (DT/L_o^2) can be determined as a function of F from a plot of eqn (8). If F is measured at various values of t, we then have values of (DT/L_o^2) corresponding to those values of t. The quantities L_o^2/D and bL_o/D can then be determined from a rearrangement of eqn (10)

$$\frac{t}{(DT/L_o^2)} = \frac{L_o^2}{D} + \frac{bL_o}{D} t \tag{11}$$

as the intercept and slope of a straight line plot of $t/(DT/L_o^2)$ *v.* t. The model can then be tested in two ways – (1) by seeing whether the plot of $t/(DT/L_o^2)$ *v.* t does indeed give a straight line or, equivalently, by the goodness of fit of the model to the experimental volatiles retention data using the fitted values of L_o^2/D and bL_o/D; and (2) by determining whether the fitting values of L_o^2/D and bL_o/D are physically reasonable. The latter test is particularly meaningful if the value of D can be approximated independently, since values of L_o and b can then be calculated and compared with physically reasonable values of the web thickness.

The variable-L model would be expected to break down once collapse has occurred to such an extent that the pores close off. When the pores are gone, the effective thickness for diffusional loss would be the sample thickness, which is much larger than the web thicknesses were. The much larger L would make further changes in volatiles content extremely slow, giving effectively an asymptotic retention.

For either constant L or variable L, the effective value of L must be an appropriate average of the various web thicknesses which exist in the porous specimen.

Interpretation of volatiles-retention data

Measured values of diffusion coefficients for volatile compounds in solutions of sucrose and maltodextrin decrease by orders of magnitude as the moisture content decreases (Menting *et al.*, 1970a; Chandrasekaran & King, 1972a). Presumably, this behaviour extends to all the systems considered in this work.

In the various studies made by Flink, Karel and co-workers very little volatiles loss occurs upon rehumidification to relatively low relative humidities (20% and below). From Table 1 it can also be seen that collapse does not occur at these moisture contents. Apparently, the value of the diffusion coefficient is so low that the quantity Dt/L^2 never becomes large enough to give a significant volatiles loss.

For rehumidification to very high relative humidities (75% and greater), Flink, Karel and co-workers typically find essentially complete loss of volatiles. In these cases collapse also occurs, as shown in Table 1 (except possibly for BSA) and the time required for a glassy appearance to develop (collapse time) is usually less than that reported for complete volatiles loss. Presumably in these cases the volatiles diffusivity is so large that the group Dt/L^2 becomes quite large, even though the web thickness, L, increases due to collapse.

At intermediate relative humidities the behaviour is more complex and will be explored individually for the different substances investigated.

Dextran

Flink (1969) and Flink & Karel (1972) report volatiles loss data for rehumidification of Dextran 10 (MW about 10 000) at relative humidities of 20, 52 and 75%. At 20% humidity there was little or no loss, presumably because of low D. In the present work, no evidence of collapse was found for the dextran samples at 23 and 52% relative humidities, but there was relatively rapid collapse at 75% humidity. Extrapolation of the molecular weight effect observed here suggests that collapse would have been still more rapid at 75% humidity for the 10 000 MW Dextran used by Flink and Karel.

These collapse observations are consistent with the volatiles loss data interpreted through the diffusion analysis, as shown in Figs 2 and 3. In Fig. 2 data for the retention of 2-propanol in Dextran-10 at 52% relative humidity (Flink & Karel, 1972) are plotted as open circles. Curve A, based upon the constant-D, constant-L diffusion model (no increase of L from collapse) fits these data well, using $L^2/D = 570$ hr. This value of L^2/D could correspond, for example, to $L = 50\ \mu$m and $D = 1.2 \times 10^{-11}$ cm^2/sec.

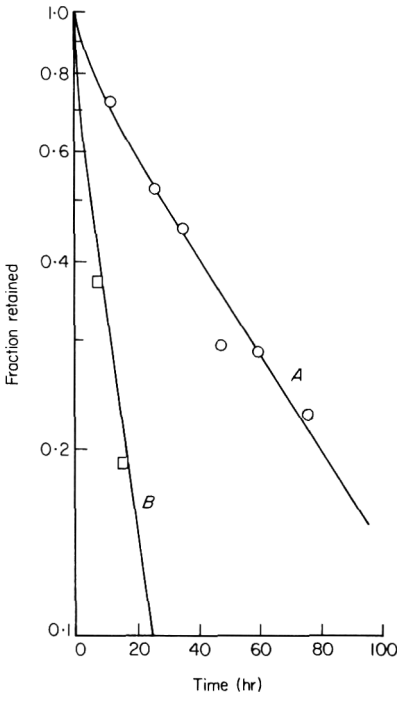

Figure 2. Volatile fraction retained predicted by slab model for constant diffusivity and constant slab thickness, L. *A*, Dextran-10 2-propanol, r.h. 52%; ○ Flink & Karel (1972); *B*, maltose 2-propanol, 100°C; r.h. 0%; □ Chirife & Karel (1974); —— predicted curve.

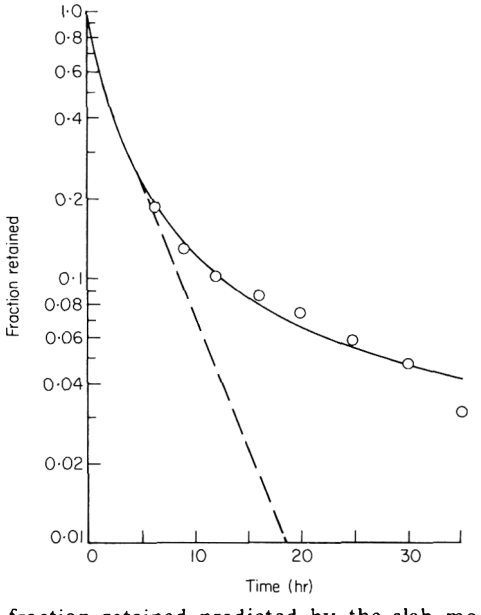

Figure 3. Volatile fraction retained predicted by the slab model for constant diffusivity, D, and varying slab thickness, L. Dextran-10 2-propanol, r.h. 75%; —— predicted curve; ○ Flink & Karel (1972); – – – predicted curve, constant-L.

At 75% relative humidity the two dextran samples in Table 1 did collapse, and it would therefore be expected that the value of L would have increased during the volatiles loss experiment conducted by Flink & Karel (1972) at the same humidity. Their data are plotted in Fig. 3. The curve in that figure comes from the increasing-L model and fits the data well. The curve was derived by fitting the slope and intercept in eqn (11), obtaining L_o^2/D = 28 hr and bL_o/D = 2.5. These values might, for example, correspond to L_o = 50 μm, D = 2.5 × 10^{-10} cm^2/sec, and b = 4.5 μm/hr. From this value of b, the effective average web thickness would increase from L_o = 50 to 185 μm after 30 hr.

The values of D inferred from the volatiles loss curves at both 52 and 75% relative humidity agree closely with values of D measured independently by Menting *et al.* (1970a) for acetone in solutions of maltodextrin (a similar substance) with water concentrations (10 and 17%) in equilibrium with relative humidities of 52 and 75%.

The solution for constant D and constant L ($=L_o$) is shown as the dashed curve. A considerable difference from the variable-L curve is evident.

Maltose

Flink (1969) and Flink & Karel (1972) reported volatiles loss patterns for freeze dried maltose rehumidified to 20% and 61% relative humidities, respectively. In the present work collapse was not found at 23% relative humidity. At 20% relative humidity Flink & Karel found very little volatiles loss, which is presumably attributable to the very low value of D at this moisture content. Signs of relatively rapid collapse were found at 52 and 75% relative humidities in the present work, implying that the Flink and Karel data for 2-propanol-loss at 61% relative humidity should be interpreted in terms of the increasing-L model. Such an analysis is shown by curve B in Fig. 4, which is fitted to the data points shown as squares. The parameters found from the slope and intercept of eqn (11), and used in plotting curve B, are L_o^2/D = 135 hr and bL_o/D = 10. This could correspond to L_o = 50 μm, D = 0.5 × 10^{-10} cm^2/sec, and b = 3.6 μm/hr with L increasing from L_o = 50 μm to 302 μm (probably beyond complete pore closure) at 70 hr.

Chirife & Karel (1974b) report total volatile loss from freeze dried maltose at 75% relative humidity, as the maltose recrystallized. This would be the result of a very high value of D.

Polyvinylpyrrolidone (PVP)

Chirife *et al.* (1973) report retentions of *n*-propanol in freeze dried PVP rehumidified at 11, 32 and 52% relative humidity. At 23 and 32% relative humidities no collapse was found in the present work. At 11% relative humidity, Chirife *et al.* report a very low loss, again probably reflecting the very low value of D at this low moisture content. Chirife *et al.* reported

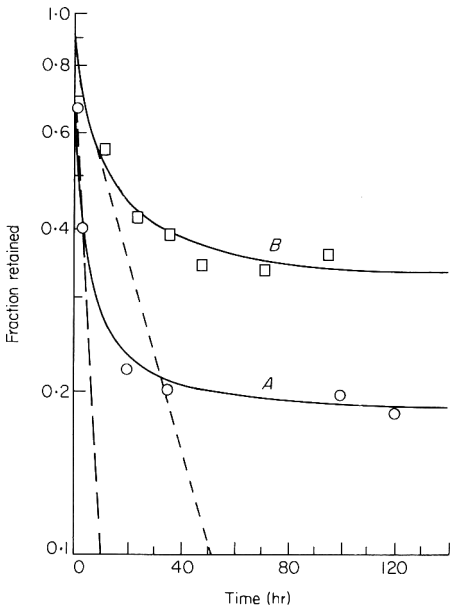

Figure 4. Volatile fraction predicted by the slab model for constant diffusivity, D, and varying slab thickness, L. *A*, polyvinylpyrrolidone (PVP)-*n*-propanol, r.h. 52%; ○ Chirife, Karel & Flink (1973). *B*, maltose 2-propanol, r.h. 61%; □ Flink & Karel (1972); —— predicted curve; – – – predicted curve, constant-L.

measured retentions at only three different times for 32% relative humidity, which precludes making a definitive fit of a model. Collapse was not found at 32% relative humidity in the present work for times up to 125 hr; however, the results of Chirife *et al.* at that humidity suggest that some web thickening was occurring, leading toward a noticeable collapse at some substantially longer time.

At 52% relative humidity the retention data reported by Chirife *et al.* show near-asymptotic behaviour at longer times. The collapse time observed in the present work (16 hr) corresponds rather well to the time at which the retentions reported by Chirife *et al.* begin to flatten out. Figure 4 shows the results (Curve *A*) of the increasing-L model fitted to their data, shown as circles. Again the fit is good. The parameters obtained from eqn (11) and used for the curve are $L_o^2/D = 29$ hr and $bL_o/D = 6.5$. This could correspond to $L_o = 50\ \mu$m, $D = 2.4 \times 10^{-10}$ cm^2/sec and $b = 11.2\ \mu$m/hr, with L increasing from $L_o = 50$ to 274 μm at 20 hr. At some point of time the model would be expected to become inapplicable as the pores close off altogether leading to an asymptotic retention as mentioned above.

Bovine serum albumin (BSA)

Chirife & Karel (1974a) report retentions of 2-propanol in freeze dried BSA rehumidified to 20, 32, 52 and 75% relative humidity. The very low loss

at 20 and 32% relative humidities probably corresponds to a very low value of D, as for the other systems. From Fig. 5 of Chirife & Karel (1974a) it appears that the rate of loss slows considerably at longer times and that the interpretation must be in terms of the increasing-L model with a substantial rate of increase in web thickness. The collapse observations in the present work do not bear this out, with there having been no visible signs of collapse after 53 hr at 52% and 93 hr at 75% relative humidity. However, the sample did become sticky at 75% relative humidity, suggesting some collapse. The collapse properties are probably highly sensitive to impurities and to the prior processing history of the albumin.

Other systems

Flink (1969) and Flink & Karel (1972) report very low loss of 2-propanol at 20% relative humidity and total loss, associated with recrystallization, at 61% relative humidity for freeze dried lactose. The recrystallization phenomenon was also observed in the present work. Presumably these results can be attributed to very low and very high values of D at 20 and 61% relative humidity, respectively.

Chirife & Karel (1973) report retentions of 2-propanol during rehumidification of freeze dried starch suspension at 11, 52 and 75% relative humidity. It would appear that D is very low at 11 and 52% relative humidity, and very large at 75% relative humidity. There was no evidence of retention levelling off at an intermediate value.

Kayaert *et al.* (1975) report volatiles retentions in a freeze dried mixture of various gums during rehumidification at 11, 52 and 75% relative humidities. No collapse observations were made for this system in the present work, but from the results of Kayaert *et al.* it would appear that the system is highly prone to collapse during rehumidification, with the increasing-L model being needed for interpretation of the results at all three relative humidities.

Effect of heating

Chirife & Karel (1974b) measured loss of 1-propanol for freeze dried maltose held in a dry atmosphere at temperatures ranging up to 100°C for 24 hr. There was no observable volatiles loss at temperatures of 82°C and lower, but at 100°C substantial loss was observed. In the present work, collapse was observed for freeze dried maltose held at 140°C, but not for maltose at 100°C. Hence it is appropriate to interpret the data of Chirife & Karel at 100°C in terms of the constant-D, constant-L model. Curve B in Fig. 2 shows the results of such a fit, with L^2/D equal to 94 hr. The fit to the two points is good.

For BSA, Chirife & Karel (1974a) found no appreciable volatiles loss during heating at temperatures up to 100°C in a dry atmosphere. No collapse was

observed for these conditions in the present work, and the low loss presumably reflects low values of D, even at 100°C.

Conclusions

Data reported previously for volatiles loss during rehumidification of freeze dried food models have been interpreted in terms of experiments monitoring structural collapse during the humidification process. The results are in agreement with a diffusion based analysis. At low relative humidities the diffusion coefficient is sufficiently low so that the group Dt/L^2 never becomes large enough to allow substantial loss of volatiles. At very high relative humidities the increased moisture content makes the diffusion coefficient become so large that complete loss occurs relatively rapidly. At intermediate relative humidities, collapse of structure may or may not occur slowly as volatiles loss proceeds. If no collapse occurs, it is appropriate to interpret volatiles loss in terms of a constant-D, constant-L model; this was confirmed from data for Dextran-10 at 52% relative humidity at 25°C and from data for maltose in a dry atmosphere at 100°C. If collapse does occur, it is appropriate to interpret volatiles loss in terms of a model where the web thickness, L, within a sample increases with time. Results for Dextran-10 at 75% relative humidity, for maltose at 52% relative humidity, and for PVP at 61% relative humidity agree well with a quantitative model which allows L to increase linearly with increasing time.

It seems preferably to interpret volatiles loss in this fashion, without introducing the concept of some fraction of the volatiles being totally immobilized, while the remaining fraction is available for loss.

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References

Bellows, R.J. & King, C.J. (1973) *AIChE Symp. Ser.* **69**, (132), 33.

Bomben, J.L., Bruin, S., Thijssen, H.A.C. & Merson, R.L. (1973) In: *Advances in Food Research*, Vol. 20, (Ed. by G.F. Stewart, E. Mrak and C.O. Chichester). Academic Press, New York.

Chirife, J. & Karel, M. (1973) *J. Agr. Food Chem.* **21**, 936.

Chirife, J. & Karel, M. (1974a) *Cryobiology*, **11**, 107.

Chirife, J. & Karel, M. (1974b) *J. Fd Technol.* **9**, 13.

Chirife, J., Karel, M. & Flink, J. (1973) *J. Fd Sci.* **38**, 671.

Chandrasekaran, S.K. & King, C.J. (1972a) *AIChE J.* **18**, 513.

Chandrasekaran, S.K. & King, C.J. (1972b) *AIChE J.* **18**, 520.

Crank, J. (1975) *The Mathematics of Diffusion*, 2nd edn. Oxford University Press, Oxford.
Flink, J. (1969) Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Mass.
Flink, J. (1974) Pres. at Symp. on Flavor Chemistry of Processed Foods, Amer. Chem. Soc. Mtg, Atlantic City, N.J.
Flink, J. & Karel, M. (1970a) *J. Agr. Fd Chem.* 18, 295.
Flink, J. & Karel, M. (1970b) *J. Fd Sci.* 35, 444.
Flink, J. & Karel, M. (1972) *J. Fd Technol.* 7, 199.
Kayaert, G., Tobback, P., Maes, E., Flink, J. & Karel, M. (1975) *J. Fd Technol.* **10**, 11.
Kerkhof, P.J.A.M. & Thijssen, H.A.C. (1974) *J. Fd Technol.* **9**, 415.
King, C. J. (1970) Recent developments in food dehydration technology. *Proc. 3rd Intl. Cong. of Food Sci. & Technol*, Washington, D.C. p. 565.
King, C.J. (1971) *Freeze Drying of Foods*. CRC Press, Chemical Rubber Co., Cleveland, Ohio. Reprint of *CRC Critical Reviews in Food Technology*, **1**, 379 (1970), with new Appendix.
King, C.J. (1972) ch. 6, Freeze Drying. In: *Food Dehydration*, 2nd edn, Vol. 1 (Ed. by W.B. Van Arsdel, M.J. Copley and A.I. Morgan Jr). AVI Publ. Co., Westport, Conn.
King, C.J. & Massaldi, H.A. (1976) *Proc. 4th Intl. Cong. of Food Sci. & Technol. (IUFoST), Madrid*, **4**, 183.
Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J. & McCall, W. (1976) *J. Fd Sci.* **41**, 910.
MacKenzie, A.P. (1965) *Ann. New York Acad. Sci.* **125**, 522.
Menting, L.C., Hoodstad, B. & Thijssen, H.A.C. (1970a) *J. Fd Technol.* **5**, 111.
Menting, L.C., Hoogstad, B. & Thijssen, H.A.C. (1970b) *J. Fd Technol.* **5**, 127.
Rulkens, W.H. & Thijssen, H.A.C. (1972) *J. Fd Technol.* 7, 95.
Thijssen, H.A.C. (1971) *J. Appl. Chem. & Biotechnol.* **21**, 372.
Thijssen, H.A.C. & Rulkens, W.H. (1968) *De Ingenieur*, **80**, (47), 45.
Thijssen, H.A.C. & Rulkens, W.H. (1969) In: *Symp. on Thermodynamic Properties of Freeze-Drying*. Intl. Inst. of Refrig., Lausanne, Switzerland.
Treybal, R.E. (1968) *Mass Transfer Operations*, ch. 4. McGraw-Hill, New York.
Tsourouflís, S., Flink, J. & Karel, M. (1976) *J. Sci. Fd Agric.* 27, 509.
Wexler, A. & Hagesawa, S. (1954) *J. Res. Nat. Bur. Stds.* **53**, 19.

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The inactivation and regeneration of peroxidase in relation to the high temperature–short time processing of vegetables

J. B. ADAMS

Summary

As a result of the high thermal stability of peroxidase and its association with quality deterioration in high temperature–short time processed foods it was decided to investigate some aspects of the kinetics of inactivation of two purified grades of horseradish peroxidase.

The following main conclusions were drawn:

(a) It was shown that regeneration of peroxidase activity took place after partial inactivation at either 70, 90 or 110°C and that the first order plots of regenerated activity were biphasic in each case. Both fast and slow rates of regeneration increased on increasing the holding temperature from 30 to 40°C but no regeneration at all was observed at a temperature of 50°C. It was found that holding for 24 hr at 50°C almost completely prevented regeneration of peroxidase activity on further cooling. The extent of regeneration was found to be governed by the length of time at the inactivation temperature and a maximum was found which coincided with the high rate period of inactivation.

(b) The first order plots of peroxidase inactivation were biphasic. Although the different purities of peroxidase did not give significantly different $D_{121.1°C}$ and z values for the low rate of inactivation over the temperature range 70–160°C, the presence of either pea or green bean purée significantly reduced the $D_{121.1°C}$ and, in the case of pea purée, increased the z value. It was also shown that the proportion of relatively stable enzyme was much lower in the presence of bean purée than in the presence of pea purée at each temperature.

Introduction

Some important relationships for consideration in high temperature–short time (HTST) processing were given by Stumbo (1966) and are graphically illustrated

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in Fig. 1. Here the time–temperature curves for the destruction of *Clostridium botulinum* spores are compared with similar curves for the maintenance of food quality as judged by thiamin and chlorophyll retentions. It can be seen from this figure that although approximately 95% thiamin will be retained by processing to $F = 2.5$ at 121°C, much less than 95% chlorophyll will be retained. On the other hand, processing for a shorter time at 135°C can achieve the same F

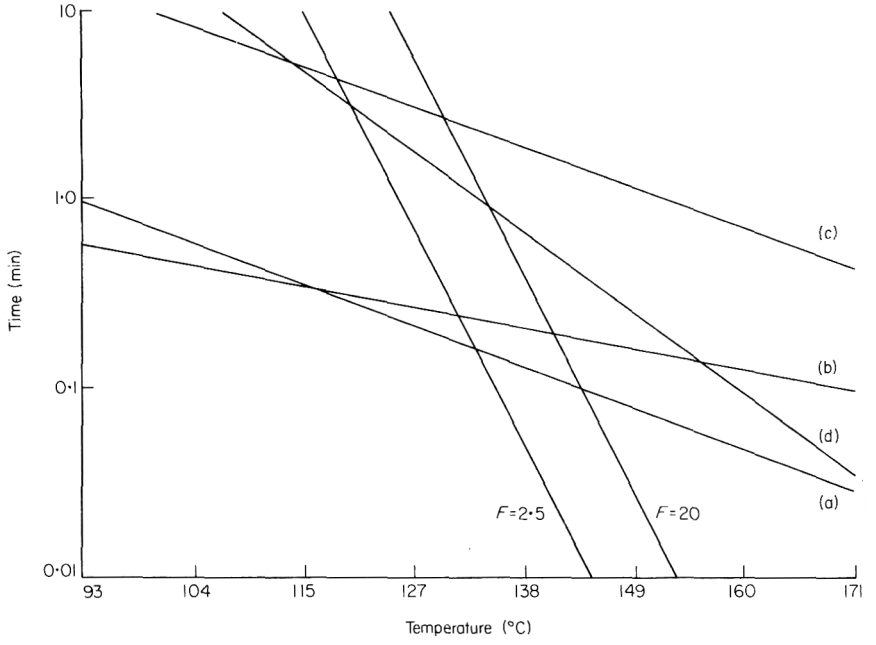

Figure 1. The relative heat resistance of bacterial spores, thiamin, chlorophylls *a* and *b* and green bean peroxidase in the temperature range used in food processing (from Stumbo, 1966). (a) and (b), 95% chlorophyll *a* and *b* retention respectively; (c) 100% inactivation of green bean peroxidase (no regeneration); (d) 95% thiamin retention.

value as well as retaining 95% of chlorophyll *a* and better than 95% of chlorophyll *b* and thiamin. The quality advantages which are possible by the use of HTST processing are therefore clear. However, because of the large D and z values of enzymes such as peroxidase, HTST processes may be less destructive than lower temperature–longer time processes of equal sterilizing efficiency. The results of Resende (1966) and Resende, Francis & Stumbo (1969) showed that F values of 31 and 245 are required at 132 and 143°C respectively to prevent regeneration of peroxidase in green bean purée. These values were considerable overprocesses and led to almost complete loss of colour. In the case of spinach purée, no regeneration of peroxidase was observed even after processing at temperatures as high as 143°C and HTST processing was recommended in order to obtain better quality retention.

The effect of residual and regenerated peroxidase activity on product quality

It has been shown on numerous occasions (Livingston *et al.*, 1957; Joffe *et al.*, 1961a,b; Everson *et al.*, 1964; Luh *et al.*, 1964; Antonakos, 1966; Luh, Antonakos & Daoud, 1969; Daoud, Luh & Seehafer, 1970; Ohlsson, 1975) that the overall quality of HTST sterilized foods immediately after processing is superior to that of the same foods sterilized under conventional conditions. In some cases, however, the higher quality of the HTST processed product is not maintained during storage, the attributes of colour and flavour being most obviously affected.

Colour

Early work showed that peas which had been HTST processed retained a better colour than conventionally processed peas for only 2 weeks at ambient temperature (Adams & Yawger, 1961). After that time the processing conditions originally applied were unimportant and there was no difference in colour between samples. In this work, regeneration of peroxidase was noted in samples which retained a trace of activity immediately after processing but no effort was made to correlate this with colour loss. It was later shown that the rate of fall of pH on storage in HTST processed purées of spinach (Clydesdale, 1966) and green peas (Buckle & Edwards, 1970) was much greater than in conventionally processed purées of the same vegetables. This rapid drop of pH value was suggested as the cause of the loss of green colour as a result of the acid hydrolysis of chlorophyll and chlorophyllide pigments to pheophytins and pheophorbides.

Although it was shown many years ago that the addition of horseradish peroxidase to sterilized green bean purée cause a pH drop of approximately one unit compared with the control (Zoueil & Esselen, 1959), no investigations have been carried out to determine whether the fall in pH of HTST processed green vegetables on storage is caused by enzymic or non-enzymic reactions.

Flavour

It has been demonstrated that canned peas which have been processed to F values in the range 5–12 at 127°C or 3–7 at 121°C develop viny off-flavours within 8 months of storage at ambient temperatures (Guyer & Holmquist, 1954). Processing to higher F values at 121 or 127°C or to similar F values at 115°C did not lead to the development of any off-flavours. It was also found in this work that the intensity of the off-flavour could be correlated with the amount of peroxidase activity which had regenerated on storage. The most viny flavours were found in those samples containing between 0.25 and 0.4% of the original peroxidase activity; those samples which contained 0.1% or less of the

original activity did not have any off-flavours. This work did not investigate whether or not there was a cause and effect relationship between peroxidase activity and off-flavour formation.

More recently it has been shown that the use of 0.1 to 0.15% of sodium metabisulphite in the HTST processing of peas prevents the occurrence of off-flavours although the regeneration of peroxidase activity is only partially reduced (Haisman, 1974). The absence of off-flavour in the presence of peroxidase activity may be explained either by saying that peroxidase does not lead to off-flavour formation at all or that it cannot lead to off-flavour formation in the presence of sulphite. If peroxidase activity and off-flavour formation are not directly related, then the sulphite must be inhibiting other enzymic or non-enzymic systems but if they are directly related then the sulphite must either be inhibiting the peroxidase itself, reacting with the substrates or reacting with the products to prevent off-flavour formation.

Because of the association of residual or regenerated peroxidase enzyme with quality deterioration in various HTST processed foods, it was decided to investigate some important aspects of the kinetics of peroxidase inactivation. Two different purities of horseradish peroxidase were used in this work, the lower purity having an RZ^* = 0.6 and the higher purity having an RZ = 3.0 (± 0.2).

Methods and results

The assay of peroxidase in buffer systems and vegetable purées

(a) *Assay in buffer systems*

The substrate used in this work was a 1:1 mixture of 0.5% (w/v) guaiacol solution and 0.1% (w/w) hydrogen peroxide solution made up in acetate buffer of the desired pH value (usually pH = 5.6). Either 2 ml or 2.5 ml of this mixture was pipetted into a 3 ml cuvette situated in a constant temperature housing in a Pye Unicam SP1800 spectrophotometer and, after temperature equilibration, either 0.5 ml or 0.05 ml (depending on the enzyme activity) of peroxidase in buffer at the same temperature was added. A reference cuvette was prepared in the same way except that 0.5 ml or 0.05 ml of buffer was added. After mixing, the initial rate of production of brown colour was determined by continuously measuring the absorbance of the sample at 420 nm ($A_{420\,nm}$) by means of an AR25 recorder connected to the spectrophotometer. The initial rate of formation of the brown pigment was generally linear and was shown to be directly proportional to the concentration of peroxidase under the conditions

*RZ = Ratio of absorbance at 403 nm to absorbance at 275 nm of peroxidase enzyme in solution; the peroxidase of RZ = 0.6 was purchased from BDH Chemicals Ltd, Poole, Dorset BH12 4NN, whilst the peroxidase of RZ = 3.0 (± 0.2) was purchased from Sigma Chemicals Ltd, Norbiton Station Yard, Kingston-upon-Thames, Surrey KT2 7BH.

employed. Peroxidase activities were expressed in terms of the initial rate of increase of absorbance at 420 nm, i.e. $A_{420\,nm}$ min^{-1}. A temperature of 30°C was used for all routine assays of the enzyme.

Other hydrogen donors were tried instead of guaiacol in the assay and it was found that *o*-phenylenediamine was approximately twenty times as sensitive under otherwise the same conditions. Nevertheless guaiacol was retained for this work as high sensitivity was not normally needed and, in addition, *o*-phenylenediamine suffered from the disadvantage of giving high blank values.

Three different buffers were tried instead of acetate in the assay of peroxidase; these were phthalate-sodium hydroxide, citrate-citric acid and the Universal buffer, all at pH = 5.6. No significant differences in initial rates were observed when compared with acetate at the same pH value. Varying the sodium chloride concentration in the assay mixture appeared to have little or no effect on the initial rate at least in the range 0.1–5.0%.

(b) Assay in vegetable purées

Assays were performed on samples (10–100 mg) of purée weighed directly into the spectrophotometer sample cuvette without prior extraction of the peroxidase. Two ml of acetate buffer (pH 5.60) was then pipetted into the cuvette which was shaken and held for 5 min at 30°C in the constant temperature housing. 0.7 ml of substrate (0.5% guaiacol + 0.1% hydrogen peroxide in acetate buffer, mixed 1:1) was added and the assay carried out with the reference cuvette containing an equal weight of purée shaken in 2.7 ml of acetate buffer. Again, the initial rate of formation of brown colour in the sample was generally linear and peroxidase activities were expressed in terms of the initial rate of increase of absorbance at 420 nm, i.e. $A_{420\,nm}$ min^{-1}.

An investigation of the effect of heat inactivation conditions on the regeneration of peroxidase activity

An investigation of the effect of heat inactivation conditions on the regeneration of peroxidase activity was necessary to ensure that accurate rates of inactivation were obtained. All of the work in this area was carried out in acetate buffer (0.2 M, pH 5.6).

Regeneration after heating at 70° C

Solutions of horseradish peroxidase ($RZ = 0.6$; 10.0 mg/100 ml of 0.2 M acetate buffer, pH 5.60) were heated for 1 hr at 70°C in 15 or 30 ml Pyrex screw-top test tubes and then cooled to the temperature of measurement. In order that reproducible activities were obtained it was necessary to boil the Pyrex tubes for 30 min in the acetate buffer prior to use. First order rate plots of $\log_e [(A_\infty - A_0)/(A_\infty - A_t)]$ *v.* time were made, where A_0 = peroxidase activity as soon as possible after the temperature of measurement had been reached, A_t = peroxidase activity after t min at the temperature of measurement

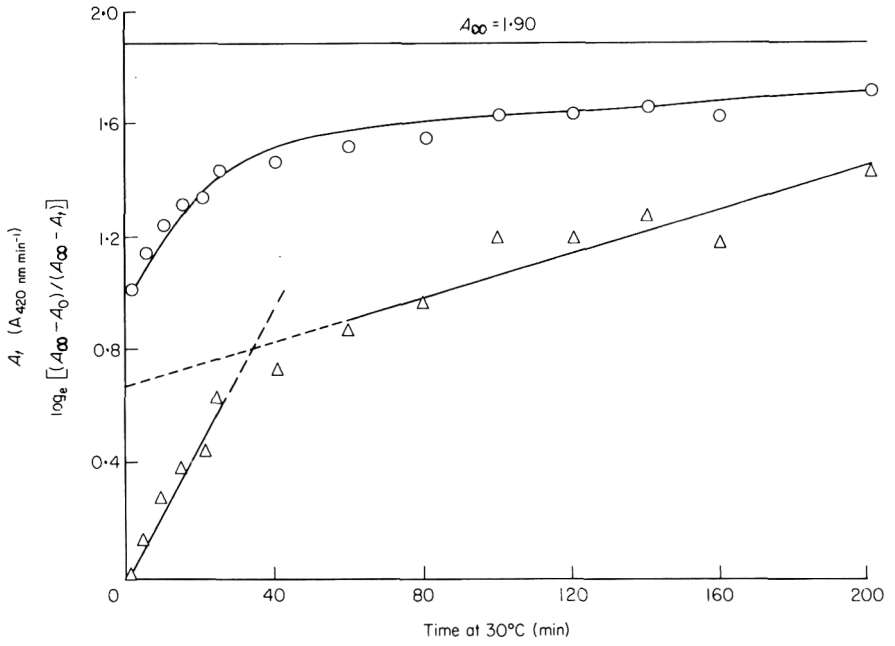

Figure 2. The regeneration of peroxidase activity at 30° C after heating at 70° C. ○, activity, A_t; △, $\log_e [(A_\infty - A_0)/(A_\infty - A_t)]$. Activity of peroxidase (not heated at 70° C) = 3.0.

and A_∞ = the maximum peroxidase activity observed, usually after 20–30 hr at the temperature of measurement. Rate constants (k values) for the regeneration of peroxidase activity were calculated from the gradients of the regression lines as determined by the method of least squares. Solutions of horseradish peroxidase stored at 30° C without prior heating at 70° C showed little or no change in enzyme activity over a 24 hr period.

It was invariably found that values of the log function corresponding to $A_t = A_{20\,min}$ lay outside the 95% confidence limits for the regression line obtained on the remainder of the results and it was shown, by taking measurements at shorter times, that the first order plot was biphasic. A typical activity versus time (at 30° C) curve is shown in Fig. 2 along with the corresponding first order plot. The mean rate constant over four determinations from the steep part of the first order plot was found to be $2.00 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.24 \times 10^{-2}\,min^{-1}$) at 30° C whilst that from the less steep portion was calculated as $4.30 \times 10^{-3}\,min^{-1}$ (standard deviation = $1.00 \times 10^{-3}\,min^{-1}$) at the same temperature. Increases of activity were generally 20–30% of the activity of the unheated enzyme solution.

At 40° C, biphasic first order plots were again observed. The mean rate constants for activity regeneration were correspondingly higher, being $3.39 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.62 \times 10^{-2}\,min^{-1}$) for the steep portion, and $1.37 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.10 \times 10^{-2}\,min^{-1}$) for the less steep

part of the first order plot (three determinations). Increases of activity at 40°C were 30–40% of the activity of the unheated enzyme solution.

At 50°C, no regeneration of peroxidase activity could be observed after first heating at 70°C although it was found that lowering the temperature from 50 to 40°C once again caused the enzyme activity to increase. The higher rate constant at 40°C was significantly reduced when the enzyme was held at 50°C for various times (after heating for a constant time (= 1 hr) at 70°C) approaching the value of the lower rate constant after 5 hr at 50°C. In addition, the A_0 and A_∞ values decreased with increased time at 50°C (see Table 1) and, in fact, A_∞ became nearly equal to A_0 on holding the enzyme solution for 24 hr at this temperature (almost zero regeneration of activity). A plot of $\log(A_\infty^{40°C} - A_0^{40°C})$ against time at 50°C was shown to be linear with a slope of $-0.19\,hr^{-1}$.

Horseradish peroxidase of higher purity ($RZ = 3.2$; 2.5 mg/100 ml of 0.2 M acetate buffer, pH 5.60) was heated for 1 hr at 70°C in a 30 ml tube and then cooled to 30°C. First order plots were again found to be biphasic with a mean rate constant over three determinations from the high rate part of $2.23 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.49 \times 10^{-2}\,min^{-1}$) and a mean rate constant from the low rate part of $3.44 \times 10^{-3}\,min^{-1}$ (standard deviation = $0.14 \times 10^{-3}\,min^{-1}$). Increases of activity were 30–37% of the activity of the unheated enzyme solution.

(b) Regeneration after heating at other temperatures

A solution of horseradish peroxidase ($RZ = 0.6$; 10.0 mg/100 ml of 0.2 M acetate buffer, pH 5.60) was heated for 40 min at 90°C before cooling to 30°C and assaying at regular intervals. The enzyme activity increased with time at 30°C to a final value equal to an increase of about 30% of the activity of the stock peroxidase solution. A biphasic first order plot was observed with mean rate constant (two determinations) of $2.63 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.40 \times 10^{-2}\,min^{-1}$) and $1.16 \times 10^{-2}\,min^{-1}$ (standard deviation = $0.37 \times 10^{-2}\,min^{-1}$).

Table 1. The activities of horseradish peroxidase solutions after heating for 1 hr at 70°C and holding for various times at 50°C

Time held at 50°C (hr)	% Starting activity (at 40°C)		
	At time zero	At time ∞	Amount regenerated
0	10	50	40
0.17	9	37	28
1	8	36	28
1.67	7	34	27
3	7	27	20
5	8	21	13
24	5	6	1

In a separate experiment a solution of horseradish peroxidase ($RZ = 0.6$; 10.0 mg/100 ml of 0.2 M acetate buffer, pH 5.60) was heated for 10 min at 110°C (glass capillary tubes, glycerol bath) before cooling to 30°C and assaying at regular intervals. The enzyme activity increased with time at 30°C to a final value equal to an increase of about 20% of the activity of the stock solution. The first order plot was again biphasic with rate constants of $2.87 \times 10^{-2}\,min^{-1}$ and $4.08 \times 10^{-3}\,min^{-1}$.

It was found by heating horseradish peroxidase ($RZ = 0.6$; 2.0 mg/100 ml of 0.2 M acetate buffer, pH 5.60) at 50°C for various times that, although the enzyme had become partially inactivated, no regeneration of activity could be measured on cooling to 40°C.

(c) The extent of peroxidase regeneration after heating at 70°C

Samples of horseradish peroxidase ($RZ = 3.2$; 2.5 mg/100 ml of acetate buffer, pH 5.60) were heated for various known times at 70°C. Assays were performed immediately on cooling to 30°C (giving an activity, A_0, when a minimum of measurable regeneration had taken place) and after holding approximately 24 hr at 30°C (giving an activity, A_∞, when maximum regeneration had taken place). A graph was then plotted of the increase in activity ($A_\infty - A_0$), as a percentage of the original activity of the enzyme solution before heating (A_s), *v.* time at 70°C. This is shown in Fig. 3 along with the corresponding first order plot of $\log_e A_\infty$ *v.* time at 70°C.

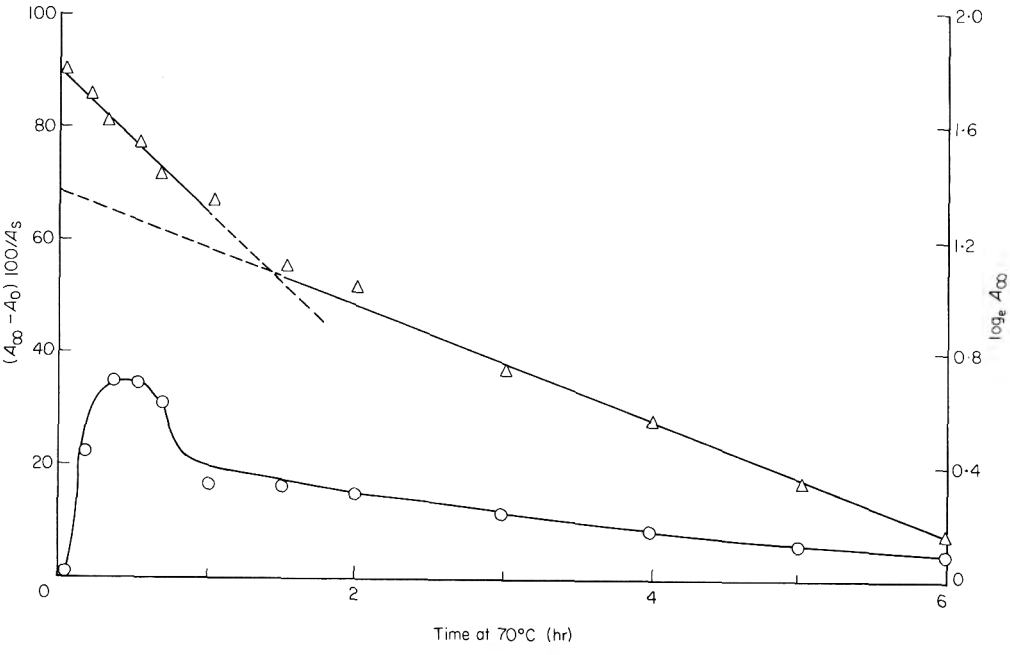

Figure 3. The extent of regeneration of activity after heating peroxidase ($RZ = 3.2$) for various times at 70° C. ○, $(A_\infty - A_0)100/A_s$; △, $\log_e A_\infty$.

Inactivation of peroxidase by heat

High temperature heating (60–160°C) of peroxidase in buffer solutions or vegetable purées was performed in soda glass capillary tubes using a modified form of an apparatus devised by Stern & Proctor (1954). This allowed heating of the tubes for known times in a thermostatic bath containing glycerol followed by their rapid transference to a cooling (water) bath by means of a spring-loaded arm operated by a solenoid and latch mechanism. Modifications of the original apparatus included construction of a protective holder which could be attached to the arm at an angle allowing the tubes to enter the heating and cooling baths horizontally without breaking and incorporation of a safety switch to prevent accidental triggering of the solenoid.

(*a*) *Inactivation in acetate buffer, pH 5.60*

Approximately fifty glass capillary tubes (length = 10 cm) were boiled for 30 min in 0.2 M acetate buffer (pH = 5.60), washed with de-ionized water and oven-dried. After cooling, one end of each tube was carefully sealed in a micro-burner flame so as not to cause a bubble of glass to form and the tubes then filled under vacuum (74 cmHg) with horseradish peroxidase solution (*RZ* = 0.6; 10.0 mg/100 ml or *RZ* = 3.2; 2.5 mg/100 ml of 0.2 M acetate buffer, pH 5.60). Sufficient enzyme solution was removed from each tube, using a microlitre syringe, to give a 1–1½ cm space at the open end. This was then sealed by heating the very end of the capillary tube in a flame in such a way that the solution within remained essentially unheated. Each capillary contained approximately 0.1 ml of enzyme solution. As many as ten capillary tubes could be heated in the glycerol bath for any particular time–temperature combination but it was normally sufficient to use three tubes, the contents of these being pooled in a 5 ml glass phial and stored for 20–30 hr at 30°C to allow maximum regeneration of activity to occur. From the batch of fifty capillary tubes it was possible to obtain fifteen or sixteen points on an activity versus time curve, each activity being the mean of duplicate assays carried out on 50 µl samples of the heated and stored enzyme solution.

Heating times at any given temperature were measured to ± 0.1 sec by stop-watch. The temperature of the thermostatic bath was measured with mercury in glass partial immersion thermometers calibrated by the National Physical Laboratory.

Activity–time curves were obtained at 10°C intervals in the temperature range 60–160°C, duplicate curves being determined at 70, 90, 110, 130 and 150°C. The first order plots were biphasic in the sense of Fig. 3 although the steep part of the graph was usually of such short duration and of such curvature at temperatures greater than 80°C that calculation of a rate constant was not worthwhile. At temperatures higher than 120°C the steep part of the first order graph was replaced by a lag period. It was decided therefore to calculate rate constants (k values) only from the part of the first order graph corresponding

to the slower reaction and these are presented in Tables 2 and 3 along with the thermal death times (D values). The values of $\log_e k$, $\log_e D$ and $\log_e (k/T(\mathrm{K}))$ are also given in these tables as are the temperature functions (°C, °F, and $(\mathrm{K})^{-1} \times 10^3$).

Using the data of Tables 2 and 3, $\log_e D$ was regressed on T(°C) (thermal resistance plot) whilst $\log_e k$ and $\log_e (k/T(\mathrm{K}))$ were regressed on $1/T$ (9 K) (Arrhenius and absolute reaction rate plots respectively). The regression data for the two grades of enzyme are summarized in Tables 4 and 5.

(b) Inactivation in vegetable purées at pH 5.6

Pea purée. A can of HTST processed, aseptically filled pea purée (the purée was made by blending fresh peas with water in the ratio 80:20) was opened

Table 2. The rate constants and thermal resistance parameters of the inactivation of horseradish peroxidase (RZ = 0.6) in the temperature range 60–160°C

T(°C)	k (hr^{-1})	$\log_e k$	$10^3/T$(K) (x 10^3)	$k/T(\mathrm{hr}^{-1}(\mathrm{K})^{-1})$ (x 10^3)	$\log_e\left(\frac{k}{T(\mathrm{K})}\right)$	D (hr)	$\log_e D$	T(°F)
60	0.029	−3.54	3.00	0.09	−9.32	79.41	4.37	140
70	0.27	−1.31	2.91	0.79	−7.15	8.33	2.14	158
70	0.24	−1.43	2.91	0.70	−7.26	9.60	2.26	158
80	0.28	−1.27	2.83	0.79	−7.14	8.22	2.11	176
90	0.77	−0.26	2.75	2.11	−6.16	2.99	1.09	194
90	0.72	−0.33	2.75	1.99	−6.22	3.20	1.16	194
100	1.63	0.49	2.68	4.38	−5.43	1.41	0.35	212
112.1	7.04	1.95	2.60	18.32	−4.00	0.33	−1.11	233.8
112.1	6.95	1.94	2.60	18.13	−4.01	0.33	−1.11	233.8
120	10.12	2.31	2.54	25.73	−3.66	0.23	−1.48	248
130	23.32	3.15	2.48	57.84	−2.85	0.10	−2.30	266
130	23.02	3.13	2.48	57.27	−2.86	0.10	−2.30	266
140	49.91	3.91	2.42	121.24	−2.11	0.05	−3.08	284
150	111.25	4.71	2.36	261.85	−1.34	0.02	−3.86	302
150	95.99	4.56	2.36	227.64	−1.48	0.02	−3.73	302
160	219.4	5.39	2.31	506.6	−0.68	0.01	−4.56	320

Table 3. The rate constants and thermal resistance parameters of the inactivation of horseradish peroxidase (RZ = 3.2) in acetate buffer in the temperature range 70–150°C

T(°C)	k (hr^{-1})	$\log_e k$	$10^3/T$(K)	$10^3 k/T$ $[\mathrm{hr}^{-1}(\mathrm{K})^{-1}]$	$\log_e\left(\frac{10^3 k}{T}\right)$	D (hr)	$\log_e D$	T(°F)
70	0.23	−1.47	2.91	0.67	−0.40	10.01	2.30	158
90	1.44	0.36	2.75	3.96	1.38	1.60	0.47	194
110	3.82	1.34	2.61	9.97	2.30	0.60	−0.51	230
130	18.85	2.94	2.48	46.75	3.84	0.12	−2.10	266
150	97.39	4.58	2.36	229.84	5.44	0.02	−3.75	302

Table 4. Thermal resistance, Arrhenius and absolute reaction rate parameters for the irreversible inactivation of horseradish peroxidase ($RZ = 0.6$) in acetate buffer (pH = 5.60)

Regression parameters	Thermal resistance	Arrhenius	Absolute reaction rate
Correlation coefficients	−0.9923	−0.9933	−0.9928
Number of points	16	16	16
95% Confidence interval	0.70	0.65	0.65
Gradient	$-0.08262\ (^{\circ}C)^{-1}$ $\therefore z = 27.87^{\circ}C$ $(50.17^{\circ}F)$	$-(12.0103 \times 10^3)K$ $\therefore E_a = 23.87$ kcal mole^{-1}	$-(11.6346 \times 10^3)K$ $\therefore \Delta H^{\#} = 23.12$ kcal mole^{-1}
Intercept	8.4819 $[= \log_e D_{(0^{\circ}C)}]$ $\therefore D_{250^{\circ}F} = 13.07$ min	32.9745 $(= \log_e A)$ $\therefore A = 2.08 \times 10^{14}\ hr^{-1}$	26.0450 $\therefore \Delta S^{\#} = +4.54$ cal deg.$^{-1}$ mole^{-1}

Table 5. Thermal resistance, Arrhenius and absolute reaction rate parameters for the irreversible inactivation of horseradish peroxidase ($RZ = 3.2$) in acetate buffer (pH = 5.60)

Regression parameters	Thermal resistance	Arrhenius	Absolute reaction rate
Correlation coefficients	−0.9966	−0.9949	−0.9945
Number of points	5	5	5
95% Confidence interval	0.61	0.74	0.75
Gradient	$-0.07336\ (^{\circ}C)^{-1}$ $\therefore z = 31.39^{\circ}C$ $(56.50^{\circ}F)$	$-(10.6798 \times 10^3)K$ $\therefore E_a = 21.22$ kcal mole^{-1}	$-(10.2864 \times 10^3)K$ $\therefore \Delta H^{\#} = 20.44$ kcal mole^{-1}
Intercept	7.3505 $[= \log_e D\ (0^{\circ}C)]$ $\therefore D_{250^{\circ}F} = 12.94$ min	29.5525 $(= \log_e A)$ $\therefore A = 6.79 \times 10^{12}\ hr^{-1}$	29.4829 $\therefore \Delta S^{\#} = +11.37$ cal deg^{-1} mole^{-1}

and the contents diluted in the ratio 80 g of the canned purée: 20 g de-ionized water. The pH of the resulting purée was then adjusted with hydrochloric acid and sodium hydroxide to pH = 5.6 and horseradish peroxidase ($RZ = 0.6$) added to give a concentration of 1 mg of enzyme in 10 g of the pH adjusted material. Glass capillary tubes were filled with this purée in an identical fashion to that described for filling capillaries with solutions of peroxidase in acetate buffer. After heating the tubes in the modified heating apparatus they were cooled and held at 30°C for 20–30 hr to allow for any regeneration of peroxidase activity to occur. Assays were carried out as described in an earlier

section and it was generally found that the assay of two or three tubes was sufficient to give a reliable activity for each time-temperature combination.

Activity *v.* time-of-heating curves have been obtained in the temperature range 70–150°C (10°C intervals). Rate constants were calculated from the first order plots as described above and are presented in Table 6 along with the thermal death times (*D* values) and various temperature functions. It was found by plotting the thermal resistance, Arrhenius and absolute reaction rate data from Table 6 that the rate functions corresponding to a temperature of 70°C lay outside of the 95% confidence intervals for the remainder of the results. The regression parameters presented in Table 7 were therefore derived only from data in the range 80–150°C.

Table 6. The rate constants and thermal resistance parameters of the inactivation of horseradish peroxidase ($RZ = 0.6$) in pea purée in the temperature range 70–150°C

T(°C)	k (hr^{-1})	$\log_e k$	$\frac{10^3}{T(K)}$	$10^3k/T$ [$hr^{-1}(K)^{-1}$]	$\log_e\left(\frac{10^3k}{T}\right)$	D (hr)	$\log_e D$	T(°F)
70	0.39	−0.94	2.91	1.13	0.12	5.90	1.78	158
70	0.38	−0.97	2.91	1.11	0.10	6.06	1.80	158
80	2.19	0.78	2.83	6.20	1.82	1.05	0.05	176
90	5.52	1.71	2.75	15.21	2.72	0.42	−0.87	194
100	11.66	2.46	2.68	31.26	3.44	0.20	−1.62	212
110	16.01	2.77	2.61	41.80	3.73	0.144	−1.94	230
120	36.25	3.59	2.54	92.22	4.52	0.063	−2.76	248
130	76.16	4.33	2.48	188.95	5.24	0.031	−3.46	266
140	155.29	5.05	2.42	375.98	5.93	0.015	−4.21	284
150	263.15	5.57	2.36	621.97	6.43	0.009	−4.73	302
150	251.10	5.53	2.36	593.60	6.39	0.009	−4.73	302

Table 7. Thermal resistance, Arrhenius and absolute reaction rate parameters for the irreversible inactivation of horseradish peroxidase ($RZ = 0.6$) in pea purée (pH 5.6)

Regression parameters	Thermal resistance	Arrhenius	Absolute reaction rate
Correlation coefficient	−0.9977	−0.9973	−0.9972
Number of points	9	9	9
95% Confidence interval	0.28	0.30	0.29
Gradient	$-0.06687\,(°C)^{-1}$ $\therefore z = 34.44°C$ $(= 61.99°F)$	$-(10.0766 \times 10^3)\,K$ $\therefore E_a = 20.02$ kcal $mole^{-1}$	$-(9.6931 \times 10^3)\,K$ $\therefore \Delta H^{\#} = 19.26$ kcal $mole^{-1}$
Intercept	5.2534 $[= \log_e D_{(0°C)}]$ $\therefore D_{250°F} = 3.49$ min	29.3171 $(= \log_e A)$ $\therefore A = 5.37 \times 10^{12}\,hr^{-1}$	29.2726 $\therefore \Delta S^{\#} = +10.95$ cal deg^{-1} $mole^{-1}$

In addition to the work carried out with HTST processed purée, horseradish peroxidase ($RZ = 0.6$) was also inactivated at 130°C in conventionally processed pea purée using the technique described above and the first order rate constant in this case was found to be equal to 73.47 hr^{-1}.

Green bean purée. 80 g of frozen, unblanched kidney beans were covered with de-ionized water and boiled for 20 min. After decanting the water, 25 ml of de-ionized water was added to the beans which were then macerated to give an homogeneous purée. The pH of the purée was adjusted with sodium hydroxide and hydrochloric acid to pH = 5.6 and horseradish peroxidase ($RZ = 0.6$) was added to give a concentration of 2 mg of the enzyme in 10 g of purée. This was filled into glass capillary tubes which were heated at temperatures in the range 70–110°C (10°C intervals). At each temperature tubes were removed from the heating bath at appropriate time intervals and held at 30°C overnight (to allow for maximum regeneration of activity) prior to assay.

Plots of $\log_e$ (Activity) *v.* time-of-heating were made at each temperature

Table 8. The rate constants and thermal resistance parameters of the inactivation of horseradish peroxidase ($RZ = 0.6$) in green bean purée in the temperature range 70–110°C

T(°C)	k (hr^{-1})	$\log_e k$	$10^3/T$(K)	$10^3k/T$ [hr^{-1}(K)$^{-1}$]	$\log_e (10^3k/T)$	D (hr)	$\log_e D$	T(°F)
70	0.59	−0.53	2.91	1.72	0.54	3.90	1.36	158
80	0.99	−0.01	2.83	2.80	1.03	2.33	0.85	176
90	3.21	1.17	2.75	8.84	2.18	0.72	−0.33	194
100	7.75	2.05	2.68	20.78	3.03	0.30	−1.20	212
100	5.04	1.62	2.68	13.51	2.60	0.46	−0.78	212
110	9.41	2.24	2.61	24.57	3.20	0.24	−1.43	230

Table 9. Thermal resistance, Arrhenius and absolute reaction rate parameters for the inactivation of horseradish peroxidase ($RZ = 0.6$) in green bean purée (pH 5.6)

Regression parameters	Thermal resistance	Arrhenius	Absolute reaction rate
Correlation coefficient	−0.9806	−0.9812	−0.9800
Number of points	6	6	6
95% Confidence interval	0.61	0.60	0.60
Gradient	$-0.07528\,(°C)^{-1}$ $\therefore z = 30.59°C$ (= 55.07°F)	$-(9.8674 \times 10^3)$ K $\therefore E_a = 19.61$ kcal $mole^{-1}$	$-(9.5007 \times 10^3)$ K $\therefore \Delta H^{\#} = 18.88$ kcal $mole^{-1}$
Intercept	6.6454 $\therefore D_{250°F} = 5.07$ min	28.1862 (= $\log_e A$) $\therefore A = 1.734 \times 10^{12}\,hr^{-1}$	28.1855 $\therefore \Delta S^{\#} = +8.79$ cal deg^{-1} $mole^{-1}$

and were invariably found to be biphasic. The first phase was extremely rapid and accounted for the inactivation of most of the peroxidase whilst the second phase involved a relatively slow loss of the remaining activity. Rate constants were calculated for the slow phase of inactivation and are presented in Table 8 along with the thermal death times (*D* values) and various temperature functions. Thermal resistance, Arrhenius and absolute reaction rate plots were made from the data in Table 8 and the technique of linear regression was used to obtain the gradients and intercepts of these plots which are summarized in Table 9.

Other vegetable purées. Inactivation of horseradish peroxidase (*RZ* = 0.6) in other vegetable systems has, so far, only been carried out in a commercial mixed-vegetable purée and in puréed canned potatoes at a single temperature and pH value. The experimental technique was the same as that described for pea purée. The first order rate constant found for the commercial purée was 48.59 hr^{-1} at 130°C whilst that for the puréed potatoes was 108.43 hr^{-1} at the same temperature.

Discussion and conclusions

Regeneration of peroxidase activity

Regeneration as a function of inactivation temperature. Partial inactivation of horseradish peroxidase at 70, 90 and 110°C was followed by regeneration of activity on storage of the enzyme solutions at 30°C. First order plots of regeneration were biphasic in each case and the increase in activity was in the range of 20–37% of the original activity prior to heating depending on the enzyme purity (or *RZ* value) and also, presumably, on the extent of inactivation (see below).

This work, in general terms, confirms that of Resende (1966) and Resende *et al.* (1969) who showed that regeneration of peroxidase activity in green beans took place after heat treatments in the range 99–177°C.

The effect of storage temperature on peroxidase regeneration. Solutions of horseradish peroxidase were heated at 70°C and then held at 30, 40 or 50°C. The mean rate constant of regeneration for the high rate part of the first order plot was 1.7 times greater at 40°C than it was at 30°C whilst the corresponding ratio from the low rate parts was 3.2. Increases in activity at 40°C were 30–40% of the activity prior to heating. At 50°C no regeneration of peroxidase activity was observed although it was found that lowering the temperature from 50 to 40°C once again caused the enzyme activity to increase. After holding for 24 hr at 50°C, however, it was shown that little or no regeneration took place on cooling to 40°C, i.e. the reversibly inactivated forms of the enzyme had become permanently inactivated after this time.

The observation that holding for 24 hr at 50°C almost completely prevented regeneration of peroxidase activity on further cooling may have some practical

application in those foods where regeneration is a problem. Of course, the beneficial effect of storing for a short time at 50°C would have to be balanced against the possible detrimental effects of non-enzymic reactions on organoleptic and nutritional quality.

The effect of heating time on the extent of peroxidase regeneration. It is clear from Fig. 3 that the extent of regeneration is governed by the length of time at the inactivation temperature. A maximum of regenerated activity occurred when the sample had been heated for approximately 30 min at 70°C which was near the end of the period of high rate of inactivation. The extent of regeneration gradually diminished after this time and presumably could have been completely prevented by a sufficiently long heat treatment as others have indeed found (Guyer & Holmquist, 1954; Zoueil & Esselsen, 1959; Resende *et al.*, 1969).

Inactivation of peroxidase by heat

The effect of enzyme purity and vegetable purée on the kinetics of peroxidase inactivation over a wide range of temperature. The thermal resistance and Arrhenius data obtained from this work is summarized in Table 10. It can be seen from this that although the different grades of peroxidase did not generally give different results, the presence of either pea or bean purée significantly reduced the $D_{121.1°C}$ and Arrhenius 'A' values and pea purée significantly increased the z value (decreased the activation energy). This means that in order to calculate peroxidase levels in vegetable products which have had known heat treatments, the $D_{121.1°C}$ and z values must have already been determined for the vegetable under investigation. It is not sufficient to use data for one vegetable in calculating peroxidase levels in another.

Table 10. Thermal resistance and Arrhenius parameters for the irreversible inactivation of horseradish peroxidase in various media at pH 5.6

Buffer or vegetable purée	Horseradish peroxidase *RZ* value	z (°C)	E_a (kcal mole^{-1})	$D_{121.1°C}$ (min)	Arrhenius 'A' (min^{-1})
Acetate buffer	0.6	27.87[a]	23.87[b]	13.07[cd]	3.47×10^{12}[ghi]
Acetate buffer	3.2	31.39	21.22	12.94[ef]	1.13×10^{11}[h]
Pea purée	0.6	34.44[a]	20.02[b]	3.49[ce]	8.95×10^{10}[g]
Green bean purée	0.6	30.59	19.61	5.07[df]	2.89×10^{10}[i]

Values with letters in common are significantly different ($P = 0.05$).

Table 11. The effect of vegetable medium and temperature on the amount of peroxidase in a relatively stable form

Temperature (°C)	Amount of peroxidase in the relatively stable form (as percentage of total)	
	Pea purée	Green bean purée
70	77.2	39.1
80	55.1	12.4
100	77.8	10.3
110	57.9	6.0

Another outcome of this work, as shown in Table 11, is the fact that the proportion of relatively stable enzyme was much lower when peroxidase was added to bean purée than when it was added to pea purée and whereas the proportion of the 'stable' form evidently decreased when the temperature of the bean purée was increased it showed no particular trend with the temperature of the pea purée. Thus it appears that the initial activity of the relatively stable fraction of peroxidase may vary as a result of differences in composition and/or temperature of the vegetable product. This means that computation of the final activity of the enzyme after known heat treatments requires a prior determination of the proportion of enzyme in the 'stable' form and how this proportion varies with temperature.

The treatment of thermal inactivation data. The rate constants presented in the various tables have been calculated from the least steep part of the first order plots and therefore represent the rate of inactivation of the most heat resistant form of peroxidase and its isozymes. The correlation coefficients of the thermal resistance, Arrhenius and absolute reaction rate plots were all highly significant ($P < 0.001$). In order to determine whether the E_a, $\Delta H^{\#}$ or z values were independent of temperature, the data of Table 2 were divided into eleven groups of six and E_a, $\Delta H^{\#}$ and z determined for each group. These values were then plotted against the group mean reciprocal temperature (for E_a and $\Delta H^{\#}$ values) or against the group mean temperature (for z values). The result, however, was a broad scatter of points in all three cases so that it was not possible to say which of the parameters E_a, $\Delta H^{\#}$ or z was truly independent of temperature. More accurate rate and temperature data are necessary to settle this point.

As a result of the empirical nature of the thermal resistance and Arrhenius equations it has been proposed that the absolute reaction rate equation should be used in the treatment of thermal rate data (Warren, 1973). The significance of the activation parameters ($\Delta H^{\#}$, $\Delta S^{\#}$) has been questioned, however, on the grounds that derivation of the absolute reaction rate equation is based on simple reactions occurring in the gas phase (Banks, Damjanovic & Vernon, 1972). These parameters may therefore be no more meaningful than the z and D values of the thermal resistance equation or the E_a and A values of the

Arrhenius equation. In addition, it has been shown in this present work that the rate data gives correlation coefficients of high significance no matter which equation is used. It is therefore suggested that future thermal rate data should be taken over as wide a temperature range as possible and evaluated by the thermal resistance, Arrhenius and absolute reaction rate methods to determine which gives the best linear correlation coefficients in any particular case.

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References

Adams, H.W. & Yawger, E.S. (1961) *Fd Technol., Champaign,* **15**, 314.
Antonakos, J. (1966) M.Sc. thesis, University of California, U.S.A.
Banks, B.E.C., Damjanovic, V. & Vernon, C.A. (1972) *Nature, London,* **240**, 147.
Buckle, K.A. & Edwards, R.A. (1970) *J. Fd Technol.* **5**, 173.
Clydesdale, F.M. (1966) Ph.D. thesis, University of Massachusetts, U.S.A.
Daoud, H.N., Luh, B.S. & Seehafer, M.E. (1970) *Confructa,* **15**, 4.
Everson, G.J., Chang, J., Leonard, S., Luh, B.S. & Simone, M. (1964) *Fd Technol., Champaign,* **18**, 84.
Guyer, R.B. & Holmquist, J.W. (1954) *Fd Technol., Champaign,* **8**, 547.
Haisman, D.R. (1974) *J. Sci. Fd Agric.* **25**, 803.
Joffe, F.M., Stier, E.F., Bonzolan, D.C., Epstein, A.I. & Ball, C.O. (1961a) *Fd Technol., Champaign,* **15**, 507.
Joffe, F.M., Stier, E.F., Gustafson, L.L., Epstein, A.I., Hayskawa, K. & Ball, C.O. (1961b) *Fd Technol., Champaign,* **11**, 510.
Livingston, G.E., Esselem, W.B., Feliciotti, E., Westcott, D.E. & Baldauf, M.P. (1957) *Fd Technol., Champaign,* **11**, 1.
Luh, B.S., Leonard, S., Simone, M. & Villareal, F. (1964) *Fd Technol., Champaign,* **18**, 363.
Luh, B.S., Antonakos, J. & Daoud, H.N. (1969) *Fd Technol., Champaign,* **23**, 377.
Ohlsson, T. (1975) SIK, The Swedish Food Institute, Rapport No. 379.
Resende, R. (1966) Ph.D. thesis, University of Massachusetts, U.S.A.
Resende, R., Francis, F.J. & Stumbo, C.R. (1969) *Fd Technol., Champaign,* **23**, 63.
Stern, J.A. & Procter, B.E. (1954) *Fd Technol., Champaign,* **8**, 139–43.
Stumbo, C.R. (1966) *Proceedings of the 2nd International Congress on Food Science,* Warsaw, p. 171.
Warren, D.S. (1973) *J. Fd Technol.* **8**, 247.
Zoueil, M.E. & Esselen, W.B. (1959) *Fd Res.* **24**, 119.

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The effects of simple processing on the cyanide content of cassava chips

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Summary

The changes in concentration of free cyanide (non-glycosidic) and bound cyanide (cyanogenic glucosides) in fresh cassava chips during dehydration, boiling or soaking in water were studied. All of these processes rapidly removed free cyanide from the chips, but only 8–12% of the total cyanide is present as free cyanide. Air-drying at four different temperatures showed that 29% of the bound cyanide was removed by drying at 46.5°C; smaller losses were recorded at the higher temperatures. Boiling chips for 25 min removed 55% of the bound cyanide, all of which could be accounted for in the boiling water. Stirring in cold water was ineffective (< 5% loss after 4 hr) for short periods, but cyanide losses increased after longer periods (50% loss after 18 hr) probably because of the onset of fermentation. These decreases in total cyanide content are smaller than indicated by earlier workers.

Introduction

Cassava (*Manihot esculenta* Crantz) roots are an important staple food for about 200 or 300 million people in tropical areas (Nestel, 1973). Cassava contains the cyanogenic glucosides linamarin and lotaustralin which may be hydrolysed to hydrogen cyanide by endogenous linamarase (EC 3.2.1.21, linamarin β-D glucoside glucohydrolase) on tissue damage (Conn, 1969). Chronic and acute cyanide toxicity in humans and animals on cassava diets are well recognized problems, but the relative roles of free cyanide (non-glycosidic) and bound cyanide (cyanogenic glucosides) in this toxicity are unknown (Montgomery, 1969).

Cassava roots are traditionally processed by a wide variety of different methods to improve their palatability and reduce their toxicity. These methods

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comprise different combinations of drying, soaking, boiling and fermentation of whole or fragmented roots; all of these processes reduce the total cyanide contents of the cassava, but there is little published quantitation of these effects (Coursey, 1973). Furthermore, Zitnak (1973) has indicated many shortcomings in the analytical techniques used to obtain these earlier data. The present paper evaluates the cyanide losses incurred by three of the physical processes involved in traditional methods, i.e. dehydration, soaking in water (leaching) and boiling. The differential effects on free and bound cyanide were determined using a sensitive enzymatic assay described earlier (Cooke, 1978).

Materials and methods

Dehydration experiments

Cassava roots from 1-year-old plants (Nigerian cultivar 60444) were peeled by hand within 1 hr of harvest and chipped using a 9 inch vegetable slicer fitted with a Julienne plate attached to a Hobart A200 mixer (Hobart Manufacturing Co., Troy, Ohio 45373). The batches of chips (5–8 kg) were sieved to remove chip fragments and processed within 30 min of preparation. The mean and standard deviation (in parentheses) of the dimensions of 105 chips were: 40(12) mm × 8.2(1.0) mm × 6.8(1.2) mm. These chips were dried at four different temperatures by distributing them evenly on the shelves of a Gallenkamp moisture extraction oven OV-440 (in which the air is changed about 3× per min). The progress of drying was monitored by periodically removing two batches (~25 g) of chips and drying (100°C) to constant weight.

The cyanide concentrations remaining at various times were determined by adding duplicate samples of the chips to 0.1 M *o*-phosphoric acid, allowing 30 min for rehydration and softening (in sealed beakers) followed by homogenization and extraction as described earlier (Cooke, 1978). The approximate ratio of solvent:chips (dry weight basis) was maintained by decreasing the weight of chips sampled as drying progressed: 60 g chips to 160 ml acid if the dry weight basis (dwb) of chips is ⩽40%; 40 g to 160 ml if dwb ⩽ 65%; 25 g to 200 ml if dwb > 65%. This procedure was adopted to facilitate filtration of the extracts, but the yield of cyanide was independent of the ratio within wide limits (Cooke, 1978): duplicate extractions of 10 and 20 g of dried material gave the same cyanide content. The extracts were assayed in duplicate for free and bound cyanide.

Cold water leaching and boiling experiments

Fresh chips, prepared as described above, were dispensed into five wire cages (size 70 × 60 × 140 mm, fabricated in aluminium insect-wire screening of 18 × 16 mesh), such that each contained 80 (±1) g of chips. These cages were

supported in an outer wire container (190 × 180 × 140 mm, fabricated in the same screening) about 10 mm from the bottom of an open aluminium pan containing 3 litres of tap water. The water was boiled and the five lots of chips completely immersed in the boiling water at zero time. Periodically a cage was removed, drained for 30 sec (into the pan) and 60 g of the chips weighed for cyanide determination (as above). The filtration rate of these boiled cassava extracts was much less than that of fresh or dried cassava material and consequently the extracts were centrifuged in some cases (Cooke, 1978). The remainder of the chips in each cage were used for dry matter determination. A sample of the boiling water (10 ml added to 20 ml of 0.1 M *o*-phosphoric acid) was taken at the same intervals. The volume of the water in the pan had decreased by 10–15% after the 25 min boiling, and the total quantity of cyanide in the water was calculated allowing for this evaporation. Experiments were done in triplicate and the rates of loss of cyanide were within 5–8% of the mean of the three experiments.

The effect of stirring in cold water was done with the same mesh cages immersed in 4 litres of tap water in a plastic bucket. The water was stirred with a Gallenkamp magnetic stirrer (on speed 5) with a 60 mm stirrer bar. The dry matter and cyanide contents of the chips were determined as described above.

Results

Dehydration of cassava chips

The average total cyanide content of six batches (5–8 kg) of fresh cassava chips was 6.8 mg per 100 g fresh weight (i.e. 27 mg/100 g dry weight), and between 8 and 12% of this was free cyanide. The sampling method used in these experiments was evaluated by analysing six samples (60 g) of one batch of chips; this gave a mean total cyanide content of 6.69 mg/100 g fresh weight with a standard deviation of 0.45. The standard error of the mean of the two samples taken in the dehydration experiments should therefore be about 5%. The effects of drying cassava chips at four different temperatures are shown in Figs 1 and 2. The losses in free cyanide were in all cases very marked. The behaviour at the two lower temperatures were similar (Fig. 1(a) and (b)): 29 and 26% of the bound cyanide were lost after drying at 46.5 and 60°C respectively for 18 hr. The marginally greater loss at 46.5°C reflects the longer time spent in the intermediate moisture content range (under conditions in which the enzyme is active). Joachim & Panditteseker (1944) reported that cassava linamarase is relatively stable up to 72°C. The corresponding losses of free cyanide were 82.5 and 83%; the free cyanide decreased rapidly at first, followed by a slight increase before a continued decrease (Fig. 1). The reversal in the rate of loss of free cyanide is presumably related to linamarase activity during drying at these mild temperatures. This was not observed at the higher temperatures (Fig. 2(a) and (b)) but there was a decrease in the rate of loss of

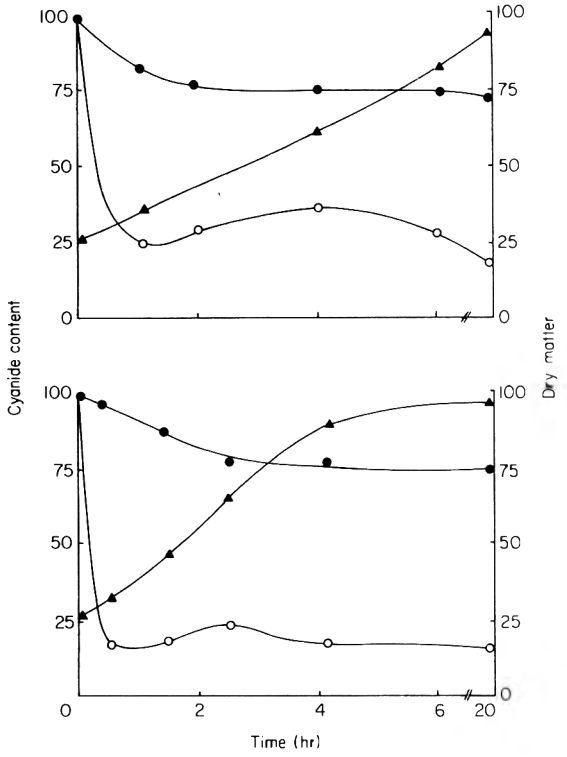

Figure 1. The effect of drying cassava chips (a) at 46.5°C, (b) at 60°C on the cyanide concentrations in the chips (dry weight basis). Free (non-glycosidic) cyanide (○ percentage of initial concentration in the chips); bound cyanide (●); dry matter content (▲).

free cyanide which may be caused by the cyanohydrin component of the free cyanide, cyanohydrins being relatively non-volatile.

The faster drying rate of 80°C (Fig. 2(a)) is accompanied by a loss of only 10% of the bound cyanide (but 96% of the free cyanide) after 18 hr. A similar loss in free cyanide occurred at 100°C, but the bound cyanide loss increased to 15%. This increased loss may be linked to the greater degree of non-enzymic browning (McWeeny, Knowles & Hearne, 1974) at 100°C. This is indicated by the absorbances at 400 nm of the acid extracts of the cassava chips dried at 46.5, 60, 80 and 100°C for 18 hr which were 0.04, 0.04, 0.14 and 0.76 respectively.

Soaking (leaching) and boiling of fresh cassava chips

The free cyanide was rapidly removed in boiling water (over 90% removed within 15 min, Fig. 3); the bound cyanide was removed at a slower rate. The chips were thoroughly cooked after 25 min, the dry matter content had

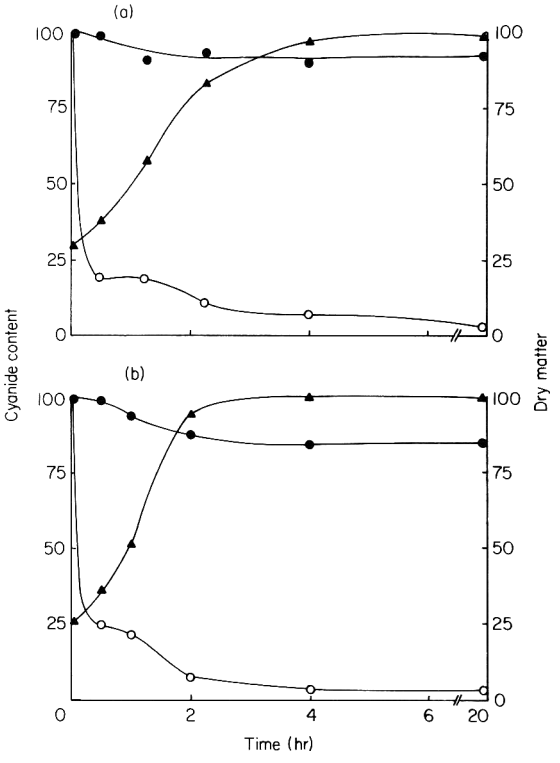

Figure 2. The effect of drying cassava chips (a) at 80°C (b) at 100°C; the symbols are as described in Fig. 1.

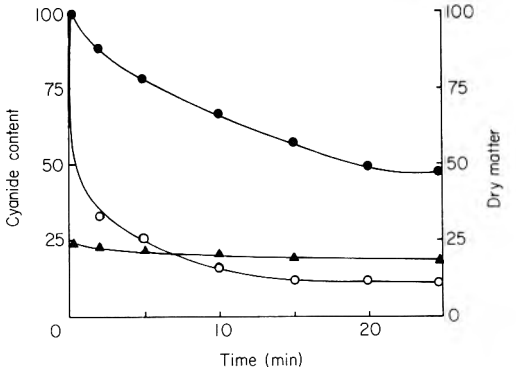

Figure 3. The effect of boiling cassava chips in water; the symbols are as described in Fig. 1.

decreased from about 25 to 19% and 55% of the bound cyanide content had been removed. This loss in bound cyanide was paralleled (to within 10%) by an increase in the water used for boiling the chips (Fig. 4). The free cyanide content of the water increased initially, and then decreased because of the volatility of the free cyanide.

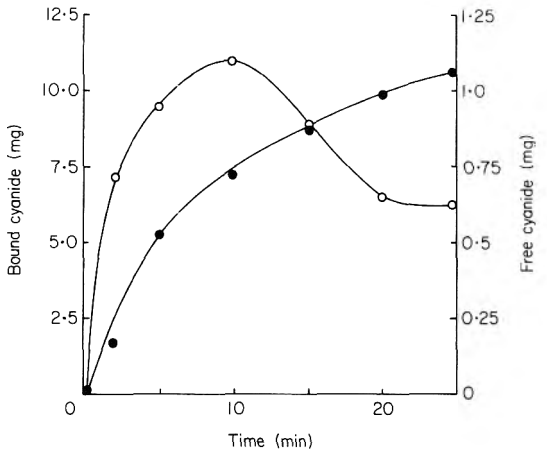

Figure 4. The appearance of free and bound cyanide in the water during boiling of chips (symbols as described in Fig. 1).

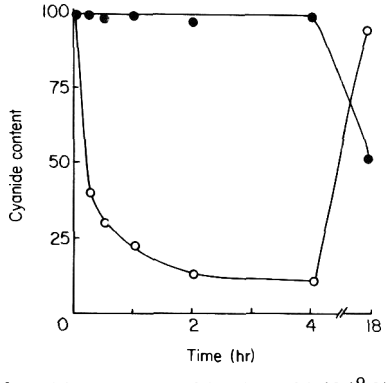

Figure 5. The effect of soaking cassava chips in cold (24°C) water on the cyanide concentrations in the chips (symbols as described in Fig. 1.)

Rapid magnetic stirring in cold water produced a negligible decrease in bound cyanide content after 4 hr (Fig. 5), but 90% of the free cyanide was removed and most of this could be accounted for in the water. Stirring the chips overnight (18 hr) caused a marked decrease in bound cyanide accompanied by a sour smell indicating the onset of fermentation (Akinrele, 1964). The free cyanide content had returned to about the initial concentration, perhaps due to endogenous linamarase activity following cellular disintegration, or to microbial glucosidase activity.

Discussion

The cyanide losses on processing reported in this paper are generally smaller than those reported by other workers. Earlier reports are often contradictory,

probably because of problems with assay methodologies (Zitnak, 1973; Cooke, 1978). Important process parameters such as cassava piece size, dehydration rates, temperatures, etc. were often not specified, and no attempt was made to differentiate between free and bound cyanide. These different forms of cyanide respond differently to processing as shown above, and have different toxicities (Montgomery, 1969).

Paula & Rangel (1939) reported an 85% loss of cyanide on oven drying and 56% on sun-drying; Charavanapavan (1944) indicated that drying sliced or rasped root at 60°C removed up to 90% of the cyanide and that drying at higher temperatures was less effective. Joachim & Panditteseker (1944) reported lower losses: 26–33% at 60°C and 18–21% at 80°C. The average cyanide losses on sun-drying small pieces of cassava roots (twenty cultivars tested) for 3 days was 73% (Correia, 1947); similar losses on sun-drying were reported by de Bruijn (1971) and Razafimahery (1953). Tewe, Gomez & Maner (1978) reported losses of 43 and 94% on oven drying sweet and bitter grated cassava samples. There are reports that soaking sliced cassava prior to drying increases the cyanide loss (Joachin & Panditteseker, 1944; Gondwe, 1974). Jansz *et al.* (1974) claimed a 95% reduction in total cyanide content after a three stage dry-soak (9–12 hr)-dry procedure, but the product appearance was poor. The losses in soluble solids, the changes in nutritional value and the process costs need to be evaluated before such a procedure could be adopted.

The literature data for cyanide losses on boiling are also very variable. Losses of 95–100% (Raymond, Jojo & Nicodemus, 1941; Paula & Rangel, 1939); 50–80% (Joachin & Panditteseker, 1944; Gondwe, 1974; Pieris, Jansz & Kandage, 1974) and 10% (de Bruijn, 1971; who boiled tuber halves for 30 min) have been reported.

The present study shows that all the processes tested rapidly remove free cyanide from fresh cassava chips. Bound cyanide is negligibly affected by drying at high temperatures and drying at 46.5°C only removes about a third of the bound cyanide. Preliminary studies on the dehydration of Malaysia cassava chips (R. H. Booth and R. D. Cooke, unpublished observations) indicated similar losses of bound cyanide on oven-drying. Greater losses occurred on sun-drying, presumably because of the longer drying times at moisture contents and temperatures at which endogenous linamarase is active. Stirring chips in cold water is ineffective (Fig. 5) until the onset of fermentation. Cassava is commonly fermented in West Africa (Akinrele, 1964), but this is accompanied by a decrease in crude protein and carbohydrate and an increase in crude fibre and ash (Oke, 1966). Further studies are planned to investigate detoxication on fermentation and on sun-drying. Boiling the chips in water removed over 50% of the bound cyanide and further experiments are planned to evaluate the effects of the different cassava piece sizes and different cassava cultivars encountered in traditional processing. These results further emphasize the need to investigate the chronic toxicity implications of bound cyanide in cassava based foods.

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References

Akinrele, I.A. (1964) *J. Sci. Fd Agric.* **15**, 589.
Bruijn, G.H., de (1971) *Mededelingen Landbouwhogeschool (Wageningen)*, **71**, 1.
Charavanapavan, C. (1944) *Trop Agriculturist*, **100**, 164.
Conn, E.E. (1969) *J. Agric. Fd Chem.* **17**, 519.
Cooke, R.D. (1978) *J. Sci. Fd Agric.* 29, 345.
Correia, F.A. (1947) *Bragantia*, 7, 15.
Coursey, D.G. (1973) In: *Chronic Cassava Toxicity* (Ed. by B.L. Nestel and R. MacIntyre) p. 27. International Development Research Centre, Ottawa, Canada.
Gondwe, A.T.D. (1974) *E. Afr. agric. For. J.* **40**, 161.
Jansz, E.R., Pieris, N., Jeyaraj, E.E. & Abeyratne, D.J. (1974) *J. Natn. Sci. Coun. Sri Lanka*, **2**, 129.
Joachim, A.W.R. & Panditteseker e, D.G. (1944) *Trop. Agriculturist*, **100**, 150.
McWeeny, D.J., Knowles, M.E. & Hearne, J.F. (1974) *J. Sci. Fd Agric.* **25**, 735.
Montgomery, R.D. (1969) In: *Toxic Constituents of Plant Foodstuffs* (Ed. by I.E. Liener) p. 143. Academic Press, New York.
Nestel, B.L. (1973) In: *Chronic Cassava Toxicity* (Ed. by B.L. Nestel and R. MacIntyre). International Development Research Centre, Ottawa, Canada.
Oke, O.L. (1966) *Trop. Sci.* 8, 23.
Paula, R.D. de G. & Rangel, J. (1939) *Rev. Alimentar*, 3, 215.
Pieris, N., Jansz, E.R. & Kandage, R. (1974) *J. Natn. Sci. Coun. Sri Lanka*, **2**, 67.
Raymond, W.D., Jojo, W. & Nicodemus, Z. (1941) *E. Afr. Agric. J.* **6**, 154.
Razafimahery, R. (1953) *Bull. Acad. Malgache (Ser Nouvelle)*, **31**, 71.
Tewe, O.O., Gomez, G.G. & Maner, J.H. (1978) *J. Sci. Fd Agric.* (In press).
Zitnak, A. (1973) In: *Chronic Cassava Toxicity* (Ed. by B.L. Nestel and R. MacIntyre) p. 89. International Development Research Centre, Ottawa, Canada.

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Taste panel assessment of textural properties of fish minces from Australian species

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Summary

The relationship between taste panel scores for texture (toughness and moisture), flesh pH and salt extractable protein for sixteen Australian species of fish has been investigated using an approach outlined by Cowie & Little (1966, 1967) for frozen stored cod.

By graphing their data, Cowie & Little (1967) showed that a line could be drawn which divided the results into those from samples with soft flesh and those from samples with tough flesh. This dividing line was found to fit the present data, accumulated in the course of a series of experiments on frozen stored minced fish flesh.

This approach emphasizes the underlying relationship of toughness with pH and salt extractable protein.

Introduction

An investigation into the properties of the separated flesh of a wide variety of Australian fish species (Bremner, 1977a) presented an opportunity to examine the resulting data for underlying relationships between pH, salt extractable protein and toughness and moisture scores as measured by a taste panel.

The relationship between the decrease in extractability of fish muscle proteins in saline solutions and organoleptic toughness was first shown by Dyer (1951). Fourteen years later Little (1965) reported a correlation between low pH and toughness in frozen cod muscle and this relationship was subsequently confirmed on both fresh and frozen cod muscle (Kelly *et al.*, 1966; Connell, 1968a; Kelly, 1969; Love, 1968; MacCallum *et al.*, 1968). Little's (1965) original observations were extended by Cowie & Little (1966)

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who employed a toughness–moisture matrix in their taste panel work on frozen stored cod. They also found (Cowie & Little, 1967) that, when the values for the protein extractability of a number of frozen stored cod fillets were plotted against the flesh pH of the fillets on a scatter diagram, the plotted points could be separated by a straight line into two groups. Samples with acceptable toughness scores formed a set on one side of the line and those with unacceptable toughness scores formed the set on the other side. Bosund & Beckeman (1972) reported that the negative correlation between toughness and pH obtained on samples stored at $-30°C$ was higher than that obtained on samples stored at $-10°C$ where other factors have a greater effect. For example, cod forms formaldehyde more rapidly at $-10°C$ than at $-30°C$ and this in turn appears to lead to rapid denaturation and inextractability of the proteins (Sikorski, Olley & Kostuch, 1976). The present study extends the approach outlined by Cowie & Little (1966, 1967) and demonstrates that their concept of a dividing line between tough and soft cod fillets, applies to the stored minces and fish fingers made from sixteen different Australian species of fish despite considerable difference in properties between the species (Bremner, 1978). Using the parameters pH and salt extractable protein, 46% of the variance in toughness was accounted for; the further incorporation of moisture (taste panel) increased the variance explained to 71%.

In view of the importance of the underlying concepts, it seems worthwhile to describe the data in detail.

Materials and methods

Materials

The methods of processing, storage, sampling and taste panel evaluation have been reported previously (Bremner, 1977a–c) but for completeness some details are included here. Minced fish was produced from a variety of Australian fish species caught by mid-water trawling. The different species, their identifying symbols and their common and scientific names are listed in Table 1. The minced fish was stored at $-18°C$ in the form of small (650 g) frozen blocks (approx. $40 \times 100 \times 130$ mm) for periods of up to 1 year. Blocks were withdrawn from store at intervals, thawed, sub-divided and mixed. Two portions were used for taste panel evaluation and a third portion for chemical analyses. Fish fingers were made from similar blocks and the results presented in this paper are restricted to those fish fingers which had been freshly prepared even though the mince from which they were made had often been stored for considerable periods.

Chemical methods

Saline extractable protein (g/100 g flesh) was determined by the method of Anderson & Ravesi (1968) involving extraction of three 10 g samples of flesh

with saline solution (ionic strength 0.8) and estimation of the extractable protein in the supernatant liquor of the centrifuged extract by the biuret method. Inextractable protein was calculated as total protein (N × 6.25, Kjeldahl) minus the extractable protein determined by the biuret method.

Protein extractability (%) was calculated as 100× saline extractable protein/ total protein. Non-protein nitrogen was not determined and hence the protein extractability figures are low by about 10% of the value shown.

Taste panel

The minces were cooked by heating a 200 g portion for 1 hr in a covered vessel on a water bath set at 60°C. Individual portions were served in pre-heated glass jars marked with abstract symbols. Fish fingers were heated for 45 min in an oven set at 180°C and were identified with abstract symbols marked on the individual serving trays. The same taste panel, consisting of ten members of staff, evaluated the minces and the fish fingers, although for practical reasons fish fingers and minces were never presented at the same session or on the same day.

Each sample was tasted once, at each of two sessions by the ten panellists and the means of these twenty observations are referred to as taste panel mean scores.

The texture section of the score sheet is shown in Table 2. It was divided into two components; terms relating to the behaviour of the material under stress and strain (tough–soft) and terms relating to mouthfeel characteristics (wet–dry). This is in line with Jowitt (1974). The mid point of the toughness scale (5) was designated as preferred texture, while the mid point (5) of the moisture scale was designated as normal moisture. This is consistent with the definition of moist as 'possessing the textural property producing the sensation of immediate increase in the free fluids of the oral cavity' (Jowitt, 1974). Samples which 'possessed the textural property of producing the sensation of a reduction of free fluids in the oral cavity' (Jowitt, 1974) were classed as dry. In fish muscle tasting, the situation occurs where the sample may release moisture immediately on chewing to leave a dry wad in the mouth. This is known as the wet–dry phenomenon and the panel was instructed to mark these samples, also, as dry. Samples were classed as wet if they produced the sensation of an increase in free fluids in the oral cavity without rapidly leaving the dry wad. This is not in agreement with the proposed definitions of Jowitt (1974) in which provision for the wet–dry phenomenon is not made. The panellists were also required to assess overall acceptability (i.e. not just texture acceptability) on a nine point scale from very poor (1) to very good (9).

Statistical methods

All calculations were done using the GENSTAT computer package (Nelder *et al.*, 1975). Analysis of variance was done on the individual toughness and

moisture scores. Main effects only were included in the analysis because of the non-orthogonal nature of the data – obtained from a number of separate, but related, experiments. Hence the residual mean square includes interactions, random (uncontrolled) variation and a contribution from the fact that the taste panel scores were integers whereas analysis of variance assumes measurement on a continuous scale. Snell (1964) discusses the use of analysis of variance on sensory scores obtained on integer scales. An estimate of the variation associated with each mean sample score was calculated from the relationship: Variation = Residual mean square/No. of tasters × No. of sessions (Table 3).

Stepwise multiple regressions maximizing 'percentage variance in toughness accounted for' were calculated from the relationship: percentage variance accounted for $\equiv 100\,(1 - \sigma_e^2/\sigma_y^2)$, where σ_y^2 is the variance of the toughness scores and σ_e^2 is the mean square residual error after fitting a regression model. An estimate of the error in the developed equations was obtained from the square root of the mean square error (Snedecor & Cochran, 1967), although this error contains a substantial between species component whenever residuals within the same species are correlated.

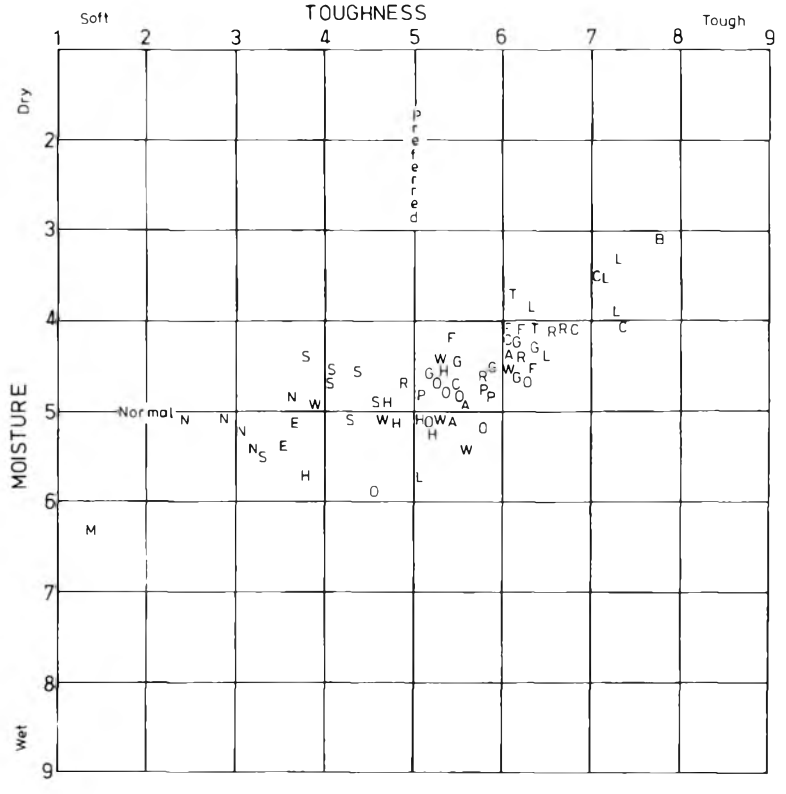

Figure 1. Taste panel mean scores displayed as a toughness–moisture diagram, for the minces from sixteen species of fish caught in Australian waters, and stored for varying times up to a year at $-18°C$. Each species is marked with a symbol (Table 1).

Results and discussion

Toughness–moisture diagram

The toughness–moisture results given by the taste panel for each sampling of each individual fish species are displayed as a scatter diagram in Fig. 1 identified by the symbols in Table 1. Attention is drawn to those species which were judged to have a soft texture even though their moisture was judged normal, i.e. nannygai, shark and silver trevally.

The Cowie and Little line

Taste panel toughness scores are shown in Fig. 2 plotted with pH and protein extractability percentage (g extractable protein/100 g total protein) as axes to provide a comparison with Fig. 5 in the paper by Cowie & Little (1967).

The taste panel used by Cowie & Little (1966, 1967) was trained and was tasting fillets of a well known species, cod, whereas the panel results reported here were obtained from an untrained panel tasting minced fish from sixteen mostly unfamiliar species. Furthermore, the former panel used a five point scale while the latter used a nine point scale, making direct comparison difficult. Nevertheless, when the Cowie & Little (1967) dividing line is drawn on the scatter diagram of the present data, it again serves to divide the points into two groups, tough and soft.

Total protein (N × 6.25) content of the flesh of the various species ranged from 22% (tuna) to 15.3% (ocean perch); however when the scatter diagram

Table 1. Key to species

Symbol	Common name	Generic name
A	Australian salmon	*Arripis trutta esper*
B	Barracouta	*Leionura atun*
C	Cucumber fish	*Chlorophthalmus nigripinnis*
E	Saw shark	*Pristiophorus cirratus*
G	Gemfish (hake or king barracouta)	*Rexea solandri*
L	Ling	*Genypterus papillosus*
M	Morwong (jackass fish)	*Cheilodactylus macropterus*
N	Nannygai (redfish)	*Centroberyx affinis*
O	Ocean perch	*Helicolenus papillosus*
P	Perch*	
R	Red gurnard	*Currupiscis kumu*
S	Silver trevally	*Usacaranx nobilis*
H	Spiny or deep sea flathead	*Hoplichthys haswelli*
T	Tuna	*Katsuwonus pelamis*
F	Tiger flathead	*Neoplatycephalus richardsoni*
W	Blue grenadier (whiptail)	*Macruronus novaezelandiae*

* Generic name not identified

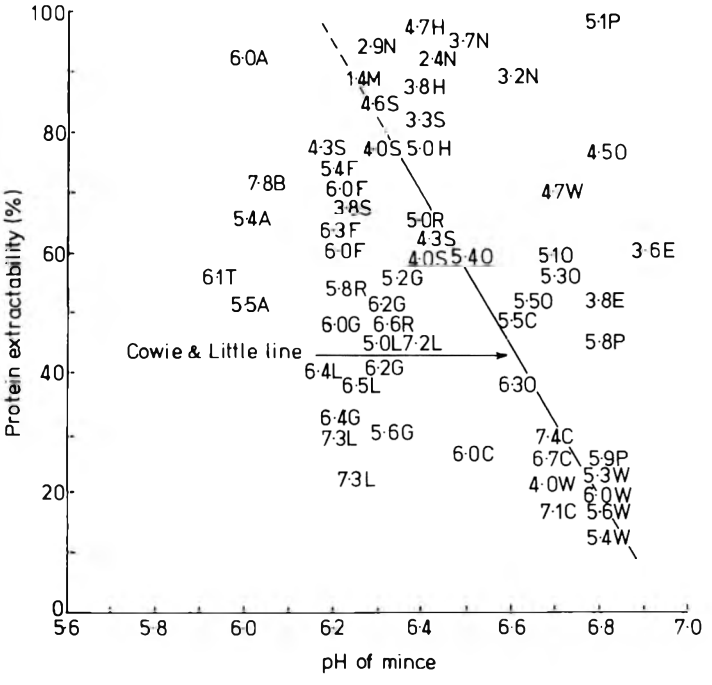

Figure 2. The relationship between taste panel score for toughness (shown in diagram against symbol for each species) for fish minces and the pH and percentage of salt extractable protein. The Cowie and Little line is taken from Fig. 5 in their paper (Cowie & Little, 1967) – where it divides the results of tough from acceptable fillets. It has been drawn here as a solid line where it encompasses the range of their data. See legend to Fig. 1.

is plotted using the amount of extractable protein (g/100 g flesh) as the y-axis a similar diagram results in which a dividing line between soft and tough samples can again be drawn. This points to the general applicability of the concept and its practical use since saline extractable protein g/100 g flesh, is more readily, and more commonly, determined than percentage protein extractability.

It is evident from Fig. 2 that the nannygai, for example, are softer than would be expected from their pH and extractable protein levels; even so, they are still on the appropriate side of the Cowie and Little dividing line. The data for fish fingers have not been shown graphically because the smaller number of tastings provided fewer data for plotting, and the presentation to the panel of the fish mince in this breaded and battered form resulted in fewer toughness scores above 5 (cf. Bremner, 1977c). The Cowie and Little line again separated the tough from the soft fish fingers.

Robustness of the dividing line

There is no single suitable statistical method for drawing dividing lines for a continuum of data such as presented here. One method is that of discriminant

analysis and this first requires an arbitrary judgement on the criteria for classification into soft and tough, taken here as a toughness score of 5.0. Another method involves solving the relationship derived from a regression model (discussed later) between pH, extractable protein and toughness scores, at a toughness score of 5.0.

No better dividing line than the Cowie and Little line was found that minimized the number of seriously misclassified points, viz. toughness scores above 6.0 or below 4.0.

Subsequent to the completion of this work investigations on the minced flesh of six tropical Malaysian species have given results which lie on the toughness–moisture diagram and which are differentiated by the Cowie & Little (1967) line – one point out of six misclassified (Bremner & Snell, 1978). Shaw & Botta (1977) expressed surprise at the good textural properties of capelin (*Mallotus villosus*) stored at $-23°C$ for up to 2 years, however, this is readily explained in terms of this present concept, in that their pH was high (range 7.2 to 6.7) and their extractable protein did not decrease sufficiently to place them below the Cowie and Little line.

Relationship between variables

The correlation coefficients relating texture scores with pH, protein extractability and moisture for the fish minces are shown in Table 4: values for fish fingers, based on smaller numbers of samples, are shown for comparison.

To take into account the fact that there were missing values due to taster absenteeism (unavoidable over the near 2 year period of the experiments) the variance associated with each mean sample score was calculated using the average number of tasters present (8.3). The resulting figure for the variance is 0.081 which represents 5% of the total variation in mean sample scores and hence the upper meaningful limit to the percentage variation in toughness accounted for is thus an estimated 95%. Multiple linear regression analysis showed that pH and extractable protein (*EP*) could account for 46% of the variance in toughness (*T*) of the minces; the regression equation obtained (1) has an associated error of 0.98 scale units estimated from the square root of the mean square error.

$$T = 21.4 - 0.21EP - 2.18\,\text{pH}. \qquad (1)$$

Replacing the extractable protein in eqn (1) with inextractable protein (highly negatively correlated with *EP*, Table 4) or by percent protein extractability (*PE*, eqn (2)) resulted in the same percentage variance accounted for (46%) and, as stated, extractable protein (g/100 g flesh) is the more commonly and more readily determined parameter.

$$T = 18.5 - 0.04PE - 1.73\,\text{pH}. \qquad (2)$$

Table 2. Taste panel score sheet for texture

Panel score	Toughness	Moisture
9	Tough	Very wet
7	Slightly tough	Wet
5	Preferred texture	Normal moisture
3	Slightly soft	Dry
1	Soft	Very dry

Table 3. Analysis of variance of toughness scores of sixty-one samples of cooked fish minces tasted by ten tasters at each of two sessions

Source	Degrees of freedom	Sum of squares	Mean square	*F*-ratio
Sessions	1	1.3	1.3	1.0
Tasters	9	154	17.1	12.7***
Samples	60	2068	34.4	25.7***
Residual	946	1270	1.34	
Total	1016	3493		

*** Significant at 0.1% level.

If the period of storage of the mince before tasting is considered as a predictor variable in eqn (1), 44% of the variance could be accounted for, thus changes in toughness with time of storage seem to be explained by changes in pH and extractable protein.

Incorporation of taste panel moisture scores (M) in eqn (1) as a sensory measurement of water holding capacity – influenced by both pH and protein extractability (Hamm, 1960) – increased the variance in toughness accounted for to 71%.

$$T = 16.2 - 0.12EP - 0.59\,\text{pH} - 1.27M. \tag{3}$$

This suggests that an objective measurement related to water holding capacity would be valuable in conjunction with pH and saline extractable protein – as a predictor of texture.

Inclusion of species identity as a variable in eqn (1) raised the variance accounted for to 18%. The toughness score of a mince from species i ($i = 1 \ldots 16$) would be given by

$$T = 0.27\,\text{pH} - 0.10EP + S_i \tag{4}$$

where S_i is a constant which varies from species to species. The coefficients for pH and EP in eqn (4) are different from those in eqn (1) since part of the variation of toughness due to variation in pH and extractable protein has been absorbed in S_i. Equations (3) and (4) are of course irrelevant for predicting the

Table 4. Correlation matrix between variables for fish minces and fish fingers†

	Fish minces (d.f. = 59)				Fish fingers (d.f. = 20)			
	Toughness	pH	Extractable protein g/100 g flesh	Inextractable protein g/100 g flesh	Toughness	pH	Extractable protein g/100 g flesh	Inextractable protein g/100 g flesh
pH	−0.16				−0.21			
Extractable protein	−0.57***	0.37**			−0.42*	0.26		
Inextractable protein	0.63***	0.19	−0.94***		0.50*	−0.08	−0.91***	
Taste panel moisture	−0.78***	0.32*	0.34**	−0.46***	−0.74***	0.36	0.33	−0.50*

*, **, *** Significant at the 5, 1 and 0.1% level respectively.

† Note the similarity between the correlation coefficients between variables for fish minces and fish fingers.

toughness score of an untested species. The inclusion of taste panel moisture scores in eqn (4) increased the variance accounted for to 90%. Most of the variability in toughness which can be accounted for by species identity but not moisture is due to those samples of normal moisture which were soft (Fig. 2).

It is impossible to ascertain whether a better correlation exists for one species, cod, than for the variety of species reported here, since Cowie & Little (1967) did not include toughness scores on their figure; neither did they make use of their toughness–moisture matrix as an aid to explaining the variability in their results.

Robustness of the relationships

A number of common sources of variability in raw material are taken into account in the relationship ((1) and (2)). Variables such as season, feed, fishing ground, catching and handling techniques all affect either pH or level of salt extractable protein, or both. These in turn affect water holding capacity – measured here sensorily as taste panel moisture.

Moreover, where fish continue to toughen even after their level of salt extractable protein has dropped almost to zero (Connell, 1968b) then, this too, is reflected in the strong negative correlation (Table 4) between toughness and taste panel moisture, implying that, at least as far as the senses are concerned, fish tend to become drier as they toughen (see also Fig. 1).

The complex influence of the state of rigor in which fish are frozen (Amlacher, 1961) is not directly taken into account; neither will the relationship hold where fish soften markedly in frozen storage due to catheptic activity.

Texture and acceptability

Cowie & Little (1967) equated a lower toughness score with greater acceptability, without considering that acceptability may decrease with fish of too soft a texture. Scoring on the scale used for the present work precludes a linear relationship between acceptability and the texture variables toughness and moisture, since peak acceptability should occur at the middle of the scales and decrease as samples are scored either tough or soft, or wet or dry respectively. The effects of the texture scores on acceptability and their interrelationship with other sensory parameters has been investigated further (Laslett & Bremner, unpublished) and the results indicate that the attributes of flavour and off-flavour are more important determinants of acceptability than are the texture attributes particularly in the case of fish fingers. This agrees with Kelly (1969) who considered that flavour changes rather than texture changes were of importance in determining the acceptability of fish with a high pH.

While toughness may not be the prime determinant of acceptability of cooked minces and fish fingers, this is not so for fish sausages and heat gelled

fish products, where quality depends on correct pH and high protein extractability to provide the emulsifying and water holding capacities necessary to obtain a product with suitable rheological properties (Sadowska & Sikorski, 1977).

Conclusions

The relationship between toughness, pH, salt extractable protein and moisture explored by Cowie & Little (1966, 1967) on frozen stored cod has been re-examined and extended to results on a random mixture of sixty-two samples of mince from sixteen species of fish. The striking similarity in the results obtained shows the validity of the underlying concept of the relationship of toughness with pH and salt extractable protein and the interrelated phenomenon of moisture in the flesh.

As traditional stocks become over-fished and new species enter the trade, this information may be of considerable value, particularly in framing investigations on newer species.

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References

Amlacher, E. (1961) *Fish as Food* (Ed. by G. Borgstrom), Vol. 1, p. 385. Academic Press, New York.

Anderson, M.L. & Ravesi, E.M. (1968) *J. Fish. Res. Bd Can.* **25**, 2059.

Bosund, I. & Beckeman, M. (1972) *Annexe 1972–2, Bull.* IIR p. 29. Commissions C2 & D1, Warsaw.

Bremner, H.A. (1977a) *Proc. Fish Expo '76 Seminar*, p. 319. Aust. Govt. Publ. Serv., Canberra.

Bremner, H.A. (1977b) *Fd. Technol. Aust.* **29**, 89.

Bremner, H.A. (1977c) *Fd Technol. Aust.* **29**, 183.

Bremner, H.A. & Snell, P.J.I. (1978) *Proceedings of the Indo-Pacific Fisheries Commission, Symposium on Fish Utilization Technology and Marketing in the IPFC Region, Manila.* IPFC/78/SYMP/17.

Connell, J.J. (1968a) *Freezing and Irradiation of Fish*, (Ed. by R. Kreuzer), p. 323. Fishing News (Books), London.

Connell, J.J. (1968b) *Low Temperature Biology of Foodstuffs*, (Ed. by J. Hawthorn and E.J. Rolfe). *Recent Adv. Food Sci.* **4**, 333. Pergamon Press, London.

Cowie, W.P. & Little, W.T. (1966) *J. Fd Technol.* **1**, 335.

Cowie, W.P. & Little, W.T. (1967) *J. Fd Technol.* **2**, 217.

Dyer, W.J. (1951) *Fd Res.* **16**, 522.
Hamm, R. (1960) *Adv. Fd Res.* **10**, 355.
Jowitt, R. (1974) *J. Texture Studies*, **5**, 351.
Kelly, K.O., Jones, N.R., Love, R.M. & Olley, J. (1966) *J. Fd Technol.* **1**, 9.
Kelly, T.R. (1969) *J. Fd Technol.* **4**, 95.
Little, W.T. (1965) *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 136. Fishing News (Books), London.
Love, R.M. (1968) *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 40. Fishing News (Books), London.
MacCallum, W.A., Jaffray, J.I., Idler, D.R. & Churchill, D.M. (1968) *Freezing and Irradiation of Fish*, (Ed. by R. Kreuzer), p. 56. Fishing News (Books), London.
Nelder, J.A. & Members of the Rothamsted Statistics Department (1975) *Genstat Reference Manual, Inter-university/Research Councils Series*, Report No. 3, 3rd edn, Program Library Unit, Edinburgh Regional Computing Centre, Edinburgh.
Sadowska, M. & Sikorski, Z.E. (1977) *Lebensm.-Wiss. u-Technol.* **10**, 239.
Shaw, D.H. & Botta, J.R. (1977) *J. Fish Res. Bd Can.* **34**, 209.
Sikorski, Z.E., Olley, J. & Kostuch, S. (1976) *Crit. Rev. Fd Sci. Nutr.* **8**, 97.
Snedecor, G.W. & Cochran, W.G. (1967) *Statistical Methods*, 6th edn, p. 385. Iowa State University Press, Iowa.
Snell, E.J. (1964) *Biometrics*, **20**, 592.

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Equations for fitting water sorption isotherms of foods

II. Evaluation of various two-parameter models

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Summary

The objective of this study was to evaluate the capacity of eight published two-parameter equations in describing water sorption isotherms of various types of foods. Thirty-nine food isotherms obtained from literature and corresponding to fruits, meats, milk products, proteins, starchy foods and vegetables, were used to evaluate the fitting abilities of the various equations.

The results obtained made it possible to propose the best equations to fit the experimental sorption data for each group of foods.

Introduction

In Part 1 of this work, Chirife & Iglesias (1978) have compiled and discussed most of the isotherm equations which have been reported in the literature for fitting water sorption isotherms of foods. Twenty-three equations were compiled by Chirife & Iglesias (1978), who suggested that an 'over-all' evaluation of that large number of equations was needed in order to have a more precise (and quantitative) definition on its fitting abilities as applied to different foods. This is now done in the present work, where a statistical analysis is made on the goodness of fit of various of the above isotherm equations.

Labuza (1968) noted that the usefulness of a sorption model will depend on the desired objectives of the user. For instance, for the prediction of drying times or shelf life of packaged dried foods, the user is interested in an equation which fits as closely as possible the experimental data, rather than in the correctness of the theory. The other important factor in selecting a sorption model is the simplicity (i.e. less number of parameters) which improves the usability of the equation for engineering calculations. With this in mind, only two-parameter equations, among those compiled by Chirife & Iglesias (1978),

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will be evaluated here. These authors compiled fourteen two-parameter equations; however, only eight of them will be considered. These are the Bradley (Bradley, 1936), Caurie (Caurie, 1970), Halsey (Iglesias, Chirife & Lombardi, 1975), Henderson (Henderson, 1952), Kuhn (Labuza, Mizrahi & Karel, 1972), Iglesias & Chirife (Iglesias & Chirife, 1976a), Mizrahi (Mizrahi, Labuza & Karel, 1970) and Oswin (Oswin, 1946) equations. The rest of the two-parameter equations are not evaluated here because of the following reasons (Chirife & Iglesias, 1978). Some of them are mathematically equivalent, and with others the limitations for fitting purposes in a reasonably wide range of water activity are already well known. The latter is the case of B.E.T. (Brunauer, Emmet & Teller, 1938), Smith (Smith, 1947), Harkins–Jura (Harkins & Jura, 1940) and linear equations.

Results and discussions

There are two basic facts which should be properly established before any evaluation of the goodness of fit of the various models is made. The first one refers to the specification of the range of water activity in which the equations will be tested. This is very important, because as it was previously noted (Chirife & Iglesias, 1978), the depression of water activity in foods is due to a combination of mechanisms each of which may be predominant in a given range of water activity. For this reason, a fixed range of 0.10 to 0.80 water activity will be utilized in the evaluation. This range appears to be the one of most practical interest. As a matter of fact, at higher water activity microbial growth may occur leading to lack of reliability in the experimental sorption data. The second fact consists in that the moisture sorption isotherms of foods represent the integrated hygroscopic properties of various constituents, like proteins, sugars, starch, cellulose, and so on. For this reason, the experimental sorption data will be grouped according to their main constituents, and the goodness of fit of the various equations will be evaluated separately on each group of foods.

Thirty-nine experimental water sorption isotherms obtained from the literature will be used to establish the fitting abilities of the models. Most of these isotherms correspond to room or near room temperature and were grouped in the following form:

(a) Fruits, (b) Meats, (c) Milk products, (d) Proteins, (e) Starchy foods, and (f) Vegetables.

Table 1 shows the list of products utilized, specifications and source of data.

The isotherm equations that will be evaluated are the following: (in all cases a_w refers to water activity and M to moisture content, dry basis)

Bradley equation

$$\ln 1/a_w = K_2 K_1^M \quad (1)$$

where, K_2 and K_1 are constants.

Table 1. Experimental water sorption isotherms utilized for the evaluation of the various isotherm equations

Isotherm no	Product	Specifications	Range of a_w	Reference
Fruits				
1	Banana	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
2	Grapefruit	45°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
3	Peach	20°C, Ads.	0.10–0.80	Saravacos & Stinchfield (1965)
4	Pear	25°C, Des.	0.20–0.80	Wolf *et al.* (1973)
5	Pear	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
6	Pineapple	45°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
7	Strawberry	25°C, Ads.	0.10–0.80	Lafuente y Piñaga (1966)
Meats				
8	Beef	30°C, Ads.	0.10–0.80	Iglesias & Chirife (1976c)
9	Beef	Room, Ads.	0.10–0.80	MacKenzie & Luyet (1967)
10	Chicken, raw	45°C, Des.	0.10–0.80	Wolf *et al.* (1973)
11	Cod	30°C, Ads.	0.10–0.75	Jason (1958)
12	Pork, raw	19.5°C, Des.	0.10–0.75	Taylor (1961)
13	Salmon	37°C, Ads.	0.10–0.80	Martinez & Labuza (1968)
14	Trout, cooked	45°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
15	Trout, raw	45°C, Des.	0.10–0.80	Wolf *et al.* (1973)
Milk products				
16	Cheese, Edam	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
17	Cheese, Emmental	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
18	Yoghurt	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
Proteins				
19	Egg albumin	25°C, Ads.	0.10–0.80	Bull (1944)
20	Egg albumin, heat coag.	25°C, Ads.	0.10–0.80	Benson & Richardson (1955)
21	Fish protein conc.	25°C, Ads.	0.10–0.80	Rasekh *et al.* (1971)
22	Gelatin	25°C, Ads.	0.10–0.80	Bull (1944)
23	Lactoglobulin	25°C, Ads.	0.10–0.80	Bull (1944)
24	Serum albumin (horse)	25°C, Ads.	0.10–0.80	Bull (1944)
Starchy foods				
25	Corn	30°C, Des.	0.10–0.80	Chen & Clayton (1971)
26	Potato	19.5°C, Des.	0.10–0.80	Taylor (1961)
27	Rice, cooked	19.5°C, Des.	0.10–0.80	Taylor (1961)
28	Sorghum	21.1°C, Ads.	0.15–0.74	Fenton (1941)
29	Wheat	25°C, Des.	0.09–0.747	Becker & Sallans (1956)
30	Wheat flour	30.1°C, Ads.	0.13–0.78	Bushuk & Winkler (1957)
Vegetables				
31	Beans	25°C, Ads.	0.10–0.80	Lafuente y Piñaga (1966)
32	Celery	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
33	Chives	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
34	Green peas	19.5°C, Des.	0.10–0.80	Taylor (1961)
35	Laurel	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
36	Lentil	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
37	Paranut	25°C, Ads.	0.10–0.80	Wolf *et al.* (1973)
38	Peas	25°C, Ads.	0.10–0.80	Lafuente y Piñaga (1966)
39	Spinach	37°C, Ads.	0.10–0.75	Makower & Dahority (1943)

Caurie equation

$$\ln M = \ln A - r \cdot a_w \quad (2)$$

where A and r are constants.

Halsey equation

$$a_w = \exp(-a''/M^r) \quad (3)$$

where a'' and r are constants.

Henderson equation

$$1 - a_w = \exp -(k \cdot M^n) \quad (4)$$

where k and n are constants

Iglesias & Chirife equation

$$\ln (M + \sqrt{M^2 + M_{0.5}}) = b\, a_w + p \quad (5)$$

where b and p are constants and $M_{0.5}$ is the moisture content at $a_w = 0.5$.

Kuhn equation

$$M = \frac{a}{\ln a_w} + b \quad (6)$$

where a and b are constants.

Mizrahi equation

$$a_w = \frac{a + M}{b + M} \quad (7)$$

where a and b are constants.

Oswin equation

$$M = a\left[\frac{a_w}{1 - a_w}\right]^n \quad (8)$$

where a and n are constants.

Analysis of data

All the isotherm equations (eqn (1) to (8)) were linearized and grouped in the following symbolic forms,

$M = \alpha + \beta\, f(a_w)$: Bradley and Kuhn equations (A)

$\ln M = \alpha + \beta\, f(a_w)$: Caurie, Halsey, Henderson and Oswin equations (B)

$\ln f(M, M_{0.5}) = \alpha + \beta\, a_w$: Iglesias & Chirife equation (C)

$M\, f(a_w) = \alpha + \beta\, a_w$: Mizrahi equation (D)

where f denotes a functional relationship.

In order to find the weighted least squares parameters, α^* and β^*, which give the best fit of experimental data, a criterion of minimum,

$$\sum_{i=1}^{n} [M_i - \varphi(\alpha, \beta, a_{w_i})]^2$$

was used. This criterion implies equal weight for each experimental point. In the case of expressions (B) to (D) an equivalent criterion was used, demanding

$$\sum_{i=1}^{n} \left[\frac{g(M_i, a_{w_i}) - \alpha - \beta\, h(a_{w_i})}{\left(\frac{\partial g}{\partial M_i}\right)} \right]^2$$

to be a minimum.

In order to evaluate the goodness of fit of the various equations as applied to the experimental sorption data, two values were computed:

A root mean square of the deviations, S,

$$S = \sqrt{\frac{1}{n} \sum_{i=1}^{n} [M_i - \varphi(\alpha^*, \beta^*, a_{w_i})]^2} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \epsilon_i^2}$$

and,

A mean relative percentage deviation in modulus, P,

$$P = \frac{100}{n} \sum_{i=1}^{n} \frac{|\epsilon_i|}{M_i}.$$

The S value is convenient to compare the fitting abilities of the different models when applied to the same experimental data. However, it does not allow a direct visualization of the goodness of fit from a technological (or practical) point of view. It was thought that the P value is somewhat better to describe the behaviour as a whole of a particular equation. This selection yields a minimum of the sum of square deviations, as it was previously demanded, in addition to a reasonable P value which is an independent condition. For this reason, the P values are the ones which are reported to describe the goodness of fit of each particular equation, although the S values were also calculated.

The results are shown on Tables 2–7 which indicate the goodness of fit of the various isotherm equations as applied to the different food isotherms. In view of the large number of equations and experimental isotherms analysed, the parameters α^* and β^* are not reported here. All the results are summarized in Table 8 which shows the average of the P values for each group of foods.

Conclusions

The following conclusions can be drawn about the fitting abilities of the eight isotherm equations studied, as applied to the various groups of foods in the range of water activity 0.10–0.80.

Fruits: The best equation for correlating the data is that of Iglesias & Chirife (1976a). Henderson's (1952) equation also gives a reasonably good representation of the isotherms. The rest of the equations fail to describe the experimental data accurately.

Table 2. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in fruits

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
1	96.4	20.9	55.8	2.2	5.9	16.7	27.3	22.6
2	100.5	23.4	49.2	5.1	6.8	7.8	17.5	18.0
3	19.9	12.4	22.7	3.6	7.1	21.2	24.4	9.5
4	21.1	13.0	14.4	4.3	0.5	9.9	11.6	6.2
5	42.4	20.4	20.7	8.7	2.0	11.8	16.4	4.9
6	47 1	20.8	39.9	4.9	4.6	15.8	23.3	14.6
7	35.8	19.5	17.2	8.7	1.6	11.8	15.7	3.5

Table 3. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in meats

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
8	13.3	17.0	1.8	10.7	6.2	6.8	8.5	5.3
9	15.5	17.5	3.1	10.4	5.1	7.7	9.5	4.7
10	20.4	18.8	6.2	10.8	5.4	9.4	12.0	3.0
11	10.1	11.7	3.5	6.7	3.8	7.1	8.8	2.4
12	6.5	9.3	2.9	5.3	1.6	6.6	8.0	2.0
13	16.3	18.5	5.7	12.1	8.4	8.1	10.2	6.2
14	14.6	16.6	3.7	10.1	5.3	8.8	10.8	3.8
15	23.3	20.2	4.5	12.2	7.1	6.9	9.4	4.5

Table 4. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in milk products

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
16	29.3	24.6	3.6	16.3	12.1	3.7	5.1	9.2
17	20.6	20.4	2.4	13.1	8.6	5.9	8.3	6.2
18	29.7	24.1	2.8	15.7	10.8	2.8	5.2	8.2

Table 5. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in proteins

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
19	3.2	7.5	9.3	2.0	4.4	15.2	16.5	3.2
20	4.8	9.4	8.2	3.7	5.2	14.4	15.9	2.2
21	3.0	8.1	2.9	4.5	3.4	8.5	9.3	1.6
22	3.2	7.5	6.7	3.2	4.1	12.7	13.8	2.0
23	3.4	7.8	10.9	1.9	4.8	16.6	18.1	4.3
24	4.5	9.7	6.5	4.9	4.7	12.5	13.9	2.2

Table 6. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in starchy foods

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
25	3.4	5.4	6.4	3.7	5.7	12.1	13.0	3.7
26	4.8	9.7	3.1	5.6	3.2	8.8	9.8	2.2
27	1.7	6.0	5.7	2.3	2.7	11.3	12.1	2.1
28	2.8	6.0	4.1	3.4	2.0	7.7	8.3	2.6
29	1.2	4.6	6.5	1.5	5.5	10.7	11.9	2.3
30	3.0	8.2	2.5	4.6	0.9	7.6	8.3	1.6

Table 7. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption data in vegetables

Experimental isotherm no.	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
31	16.6	18.5	2.1	11.4	6.2	6.4	8.2	5.4
32	20.4	17.8	9.4	9.1	3.7	11.7	14.6	1.6
33	15.5	22.8	2.1	14.5	8.9	4.2	6.3	7.3
34	19.4	23.9	4.9	16.8	11.4	1.4	1.6	11.1
35	9.7	13.4	4.1	7.7	3.8	10.2	11.8	2.9
36	3.6	8.8	5.7	3.9	3.1	11.9	13.1	0.75
37	8.1	13.3	1.7	8.1	4.9	7.7	9.0	3.7
38	20.4	22.2	3.1	14.6	9.2	3.0	4.8	8.4
39	22.9	21.7	4.1	15.5	9.2	2.7	1.8	10.4

Table 8. Average goodness of fit of the various equations for each group of foods, expressed as the average P value for the total number of experimental isotherms comprising each group

	Bradley eqn (1)	Caurie eqn (2)	Halsey eqn (3)	Henderson eqn (4)	Iglesias & Chirife eqn (5)	Kuhn eqn (6)	Mizrahi eqn (7)	Oswin eqn (8)
Fruits	51.9	18.6	31.4	5.4	4.0	13.6	19.5	11.3
Meats	15.0	16.2	3.9	9.8	5.4	7.7	9.7	4.0
Milk products	26.5	23.0	2.9	15.0	10.5	4.1	6.2	7.9
Proteins	3.7	8.3	7.4	3.4	4.4	13.3	14.6	2.6
Starchy foods	2.8	6.7	4.7	3.5	3.3	9.7	10.6	2.4
Vegetables	15.2	18.0	4.1	11.3	6.7	6.6	7.9	5.7

Meats: The Halsey (Iglesias *et al.*, 1975) equation is able to give the best fit for meats followed closely by the Oswin (1946) one. The Iglesias & Chirife (1976a) equation and the Kuhn (Labuza *et al.*, 1972) one have also some merit for describing the equilibrium moisture content data.

Milk products: The Halsey (Iglesias *et al.*, 1975) equation gives by far the best description of the experimental data followed by the Kuhn (Labuza *et al.*, 1972) one. The Mizrahi (Mizrahi *et al.*, 1970) equation gives a fair representation of this group of foods.

Proteins: The Oswin (1946) equation is able to fit the data better than the other equations. A fairly good description of the isotherms is also possible with the Henderson (1952), Bradley (1936) and Iglesias & Chirife (1976a) equations (in this order).

Starchy foods: The Oswin (1946) and Bradley (1936) equations (in this order) fit the data better than the other equations, although the Iglesias & Chirife (1976a), Henderson (1952), and Halsey (Iglesias *et al.*, 1975) also perform adequately.

Vegetables: The Halsey (Iglesias *et al.*, 1975) equation gives the best fit in this group of foods. The Oswin (1946), Kuhn (Labuza *et al.*, 1972) and Iglesias & Chirife (1976a) equations also show reasonably good fitting abilities.

There are some additional comments which can be made about the results here obtained. The most versatile equations are the Halsey (Iglesias *et al.*, 1975) and the Oswin (1946) ones. The former was developed by Halsey (1948) on theoretical ground as a criticism of the B.E.T. theory, while the latter is a purely empirical equation. The very good performance of Halsey's equation is not unexpected in view of the extensive testing of this equation made by Iglesias *et al.* (1975) and Iglesias & Chirife (1976b). The also very good behaviour of Oswin's equation may be considered somewhat surprising in view of the relatively little use of this equation reported in the literature. The Iglesias & Chirife (1976a) equation, which was originally proposed for fruits and related high-sugar foods,

may be also used to describe reasonably well the sorption behaviour of other foods. The Caurie (1970) equation, which was proposed as an isotherm equation for most types of foods, showed poor fitting abilities in most cases. The Mizrahi (Mizrahi *et al.*, 1970) equation showed only moderate fitting abilities; however, it has to be recognized that Mizrahi *et al.* (1970) did not propose their equation as a general food isotherm one, but rather used it to fit sorption data in a particular food (cabbage).

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References

Becker, H.A. & Sallans, H.R. (1956) *Cereal Chem.* **33**, 79.
Benson, S.W. & Richardson, R.L. (1955) *J. Am. Chem. Soc.* 77, 2585.
Bradley, R.S. (1936) *J. Chem. Soc.* 1467.
Brunauer, S., Emmet, P.H. & Teller, E. (1938) *J. Am. Chem. Soc.* **60**, 309.
Bull, H.B. (1944) *J. Am. Chem. Soc.* **66**, 14.
Bushuk, W. & Winkler, C.A. (1957) *Cereal Chem.* **34**, 73.
Caurie, M. (1970) *J. Fd Technol.* 5, 301.
Chen, C.S. & Clayton, J.T. (1971) *Trans. of the ASAE*, **14**, 927.
Chirife, J. & Iglesias, H.A. (1978) *J. Fd Technol.* **13**, 159.
Fenton, F.C. (1941) *Agric. Engng*, **22**, 185.
Halsey, G. (1948) *J. Chem. Physics*, **16**, 931.
Harkins, W.D. & Jura, G. (1944) *J. Am. Chem. Soc.* **66**, 1366.
Henderson, S.M. (1952) *Agric. Engng*, **33**, 20.
Iglesias, H.A., Chirife, J. & Lombardi, J.L. (1975) *J. Fd Technol.* **10**, 385.
Iglesias, H.A. & Chirife, J. (1976a) *Can. Inst. Food Sci. & Technol. J.* **11**, 12.
Iglesias, H.A. & Chirife, J. (1976b) *J. Fd Technol.* **11**, 565.
Iglesias, H.A. & Chirife, J. (1976c) *J. Fd Sci*, **41**, 984.
Jason, A.C. (1958) In: *Fund. Aspects of the Dehydration of Foodstuffs.* Soc. Chem. Ind., 103.
Labuza, T.P. (1968) *Fd Technol., Champaign*, **22**, 263.
Labuza, T.P., Mizrahi, S. & Karel, M. (1972) *Trans. of the ASAE*, **15**, 150.
Lafuente, B. y Piñaga, F. (1966) *Rev. Agroq. y Tecnol. de Alimentos*, **6**, 113.
MacKenzie, A.P. & Luyet, B.J. (1967) *Cryobiology*, **3**, 341.
Makower, B. & Dahority, G.L. (1943) *Ind. Engng. Chem.* **35**, 193.
Martinez, F. & Labuza, T.P. (1968) *J. Fd Sci.* **33**, 241.
Mizrahi, S., Labuza, T.P. & Karel, M. (1970) *J. Fd Sci.* **35**, 799.
Oswin, C.R. (1946) *J. Chem. Ind. (London)*, **65**, 419.
Rasekh, J.G., Stillings, B.R. & Dubrow, D.L. (1971) *J. Fd Sci.* **36**, 705.
Saravacos, G.D. & Stinchfield, R.M. (1965) *J. Fd Sci.* **30**, 779.
Smith, S.E. (1947) *J. Am. Chem. Soc.* **69**, 646.
Taylor, A.A. (1961) *Fd Technol., Champaign*, **15**, 536.
Wolf, W., Spiess, W.E.L. & Jung, G. (1973) *Lebensm.-Wiss. u. -Technol.* **6**, 94.

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Protein recovery from blood plasma by precipitation with polyuronates

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Summary

Bovine serum albumin (BSA) and blood plasma formed insoluble complexes with alginate, pectate and carboxymethyl cellulose (CMC) under appropriate conditions of pH. More than 90% of the BSA could be recovered by all three polysaccharides at ionic strengths of <0.001 and at the optimum pH for recovery and a ratio of protein to polysaccharide of about 5:1. At low ratios of protein to polysaccharide, alginate and CMC appeared to resolubilize the complex. When the ionic strength was increased to 0.2 almost complete recovery could still be obtained with alginate and pectate at protein:polysaccharide ratios of 5:1 and 3:1 respectively but the efficiency of precipitation was reduced and the range of the ratios for optimum recovery narrowed. The results for precipitation from blood plasma were very similar to those obtained in the BSA studies. A crude pectate preparation obtained from orange peel gave results similar to pure pectate except that three times as much was required to precipitate a given amount of protein.

Introduction

Increasing concern about pollution of the environment and the world shortage of animal proteins suitable for human consumption has resulted in an upsurge of interest in techniques for recovering proteins from food industry effluent streams. One method of achieving this is to precipitate the protein from solution using another polyelectrolyte. For example, it has been shown that protein in cheese whey can be recovered using any one of a number of materials including sodium alginate, carboxymethyl cellulose (CMC) and polyacrylic acid (Shank & Cunningham, 1968; Hansen, Hidalgo & Gould, 1971; Sternberg, Chiang & Eberts, 1976). The interaction of CMC with whey proteins

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has been extensively investigated and it has been reported that the amount of protein precipitated is strongly dependent on the pH, ionic strength and degree of substitution of the cellulose derivative. Under favourable conditions more than 90% of the protein can be removed from solution (Hansen *et al.* 1971; Hill & Zadow, 1974) and the protein extracted by this method has excellent functional properties (Morr, Swenson & Richter, 1973).

Polyelectrolytes have been used to precipitate other proteins from solution including soya, casein, edestin and yeast protein and sunflower seed albumins (Smith *et al.*, 1962; Tolstoguzov *et al.*, 1974; Schwenke *et al.*, 1977); however little attempt has been made to employ polyelectrolytes to recover blood proteins. Other methods, such as chemical fractionation (Tybor, Dill & Landmann, 1973), ultrafiltration or diafiltration (Delaney, 1977a) have been proposed as ways of utilizing the large quantities of blood which are available annually as a meat industry byproduct. Although the use of red cells in human food is limited because of their colour and poor nutritional quality (Delaney, 1977b), plasma proteins have excellent functional and nutritional properties (Tybor, Dill & Landmann, 1975). In this paper the use of the polyuronates sodium alginate and sodium pectate to recover plasma protein is considered. In addition, because of its potential low cost and wide availability, a crude pectate preparation obtained from orange peel was also examined. These polysaccharides were chosen because they possess the ability to form thermostable gels and it seems possible that this property could be utilized to fabricate the precipitated complexes into meat analogues as has been described for some protein–alginate mixtures (Tolstoguzov *et al.*, 1972).

The interaction of bovine serum albumin (BSA) and myoglobin with some anionic polysaccharides at pH 6.0 has been previously investigated (Imeson, Ledward & Mitchell, 1977). This interaction was shown to be electrostatic in nature and increased with a decrease in pH leading to precipitation of a protein–polysaccharide complex. In order to define the optimum conditions for protein precipitation initial studies were carried out on solutions of BSA; the conditions were then adapted for blood plasma.

In order to compare the present work with previous findings CMC was also studied.

Materials and methods

Preparation of plasma

Porcine blood was obtained immediately post slaughter from a local abattoir. An anti-coagulant of sodium citrate solution (10% w/v) was added to the blood at a concentration of about 10% v/v. The blood was centrifuged at 22 000 *g* for 20 min to separate the plasma from the cellular components. The plasma was blast frozen and stored at −20°C until required since freezing and thawing has no adverse effect on plasma proteins (Brooks & Radcliff, 1959). All deter-

minations of protein recovery were made on a single batch of plasma which had a protein content of 6.0% as determined by Biuret analysis.

Proteins and polysaccharides

Bovine serum albumin (Lot 17C-8025) and sodium pectate (Lot 34C-3030) were obtained from Sigma Chemical Co. The pectate had a small residual degree of esterification of 4% as measured by the G.L.C. method of Krop, Pilnik & Faddegon (1974) and a polygalacturonic acid content of 70% as determined by titration. The intrinsic viscosity in 0.10 M NaCl at 20°C was measured as 2.8 dl g^{-1}. Sodium alginate (type Manucol DM, Lot 265611, donated by Alginate Industries Ltd) had an intrinsic viscosity of 7.0 dl g^{-1}. Sodium carboxymethyl cellulose (CMC) (type Edifas B50, Lot DUM/S 834) was obtained from Imperial Chemical Industries Ltd. The degree of substitution was 0.51 as determined by titration.

Pectate pulp

Pectate pulp was prepared by de-esterifying the pectins in orange peel albedo as described by Baier & Wilson (1941). The treated peel was water washed and dried at 70°C. This material contained 25% polygalacturonic acid with a degree of esterification of 15%.

Crude pectate solutions were produced by dispersing the finely ground pectate pulp and sodium orthophosphate (peel:orthophosphate ratio 3:1) in water at 80°C.

Measurement of protein recovery

Aliquots (5 ml) of polysaccharide of the appropriate concentration were mixed with 2.0 ml of aqueous solutions of BSA (1.0%) or blood plasma. Where high ionic strengths were required 1.0 ml sodium chloride solution was added. In all cases the desired pH was obtained with 0.5 M HCl and the sample made up to 10.0 ml with distilled water. Mixtures were left to equilibrate at room temperature for approximately 30 min, the pH rechecked, and the solution centrifuged at 25 000 *g* for 20 min. The protein content of the supernatant was determined and the results expressed as percentage protein recovery, i.e. the amount of protein precipitated as a percentage of the total protein.

Protein determination

Protein concentrations in the blood plasma systems were made by the Biuret method of Gornall, Bardawill & David (1949). For the BSA studies the method

was modified as described by Diamond & Denman (1973) to increase its sensitivity.

Samples containing pectate, pectate pulp and alginate became turbid after the addition of Biuret reagent but centrifugation at 2500*g* for 5 min clarified the solutions.

A correction was made for the yellow colouration in treated citrus peel samples with a blank of sodium hydroxide solution (3%) in place of the Biuret reagent.

Results

Recovery from BSA solutions

Figure 1 shows that, at low ionic strength and a ratio of protein to polysaccharide of 4:1, the relationship between protein recovery and pH is similar for both alginate and pectate, although the former appears to be a more efficient precipitant over a wider pH range. In both cases maximum recovery was around pH 3.8. CMC under the same conditions gave maximum recovery at pH 4.0 to 4.2.

As well as being pH dependent protein recovery was greatly affected by the

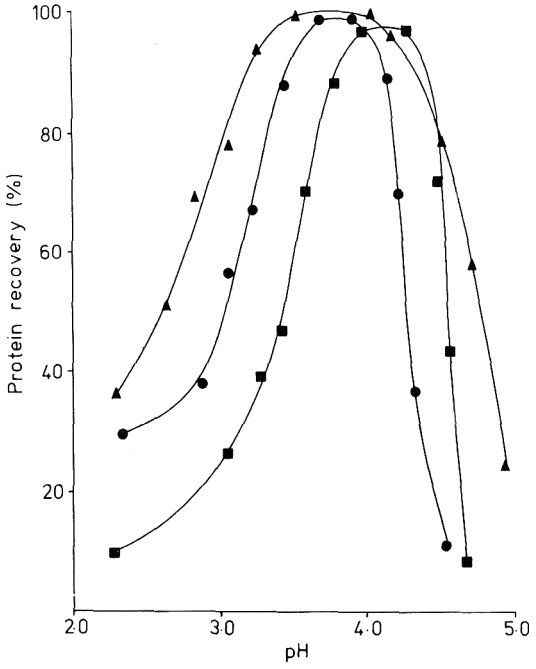

Figure 1. The effect of pH on the recovery of BSA by sodium alginate (▲), sodium pectate (●) and CMC (■) at an ionic strength of < 0.001. Protein concentration 0.20%; polysaccharide concentration 0.05%.

ratio of protein to polysaccharide. Under pH conditions for optimum precipitation all three polysaccharides gave 90% protein recovery at ratios of protein to polysaccharide of about 5:1 (Fig. 2). Precipitation by pectate remains at this high level at lower ratios while alginate, at ratios of less than 2.4 : 1, and CMC, at ratios of less than 2 : 1, appear to resolubilize the protein–polysaccharide complex and hence lower protein recovery.

The conditions for maximum precipitation were strongly dependent on the

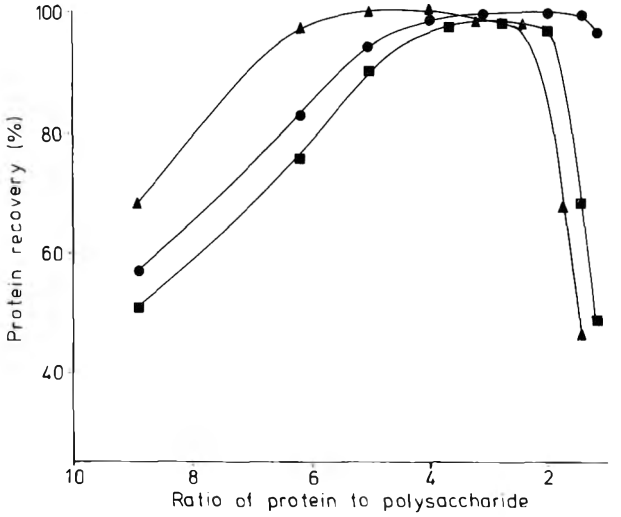

Figure 2. The recovery of BSA at different ratios of protein to polysaccharide by sodium alginate at pH 3.8 (▲), sodium pectate at pH 3.8 (●) and CMC at pH 4.1 (■) at an ionic strength of < 0.001. Protein concentration 0.20%.

ionic strength. In Fig. 3 it is seen that when the ionic strength is increased to 0.2 the optimum pH for protein recovery at a ratio of protein to polysaccharide of 4:1 is moved to more acid values. Under these conditions it is only possible to recover about 75% of the protein when CMC is used as the precipitant and about 80% of the protein when sodium pectate is employed although over a narrow pH range almost complete recovery can still be obtained with sodium alginate. However, if the ratio of protein to polysaccharide is reduced below 4:1 almost total recovery can also be obtained with pectate although with CMC, even at low ratios of protein to polysaccharide, it is not possible to obtain more than 85% recovery at high ionic strengths (Fig. 4).

From Figs 2 and 4 it is apparent that the range of the ratio of protein to polysaccharide for optimum recovery narrows as the ionic strength is increased. For example, in the case of sodium alginate optimum ratios change from between 7:1 and 2.4:1 to 5:1 and 2.8:1 on raising the ionic strength from < 0.001 to 0.2 although at high ionic strengths there is a reduction in the resolubilization of the protein–polysaccharide complex by alginate and CMC at low ratios of protein to polysaccharide.

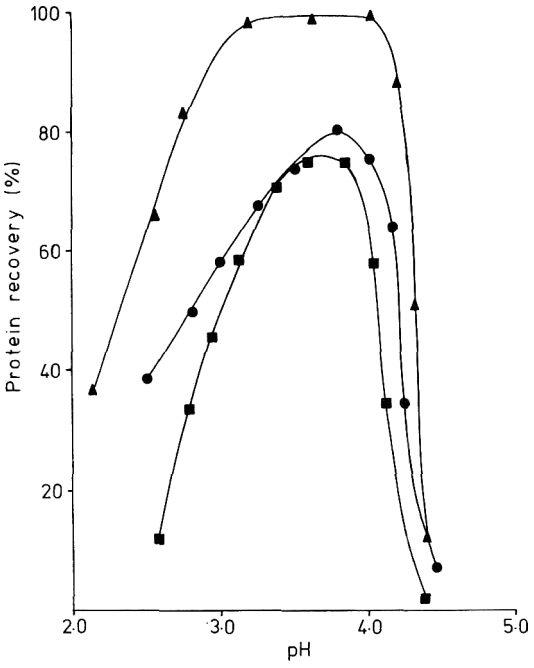

Figure 3. The effect of pH on the recovery of BSA by sodium alginate (▲), sodium pectate (●) and CMC (■) at an ionic strength of 0.20. Protein concentration 0.20%; polysaccharide concentration 0.05%.

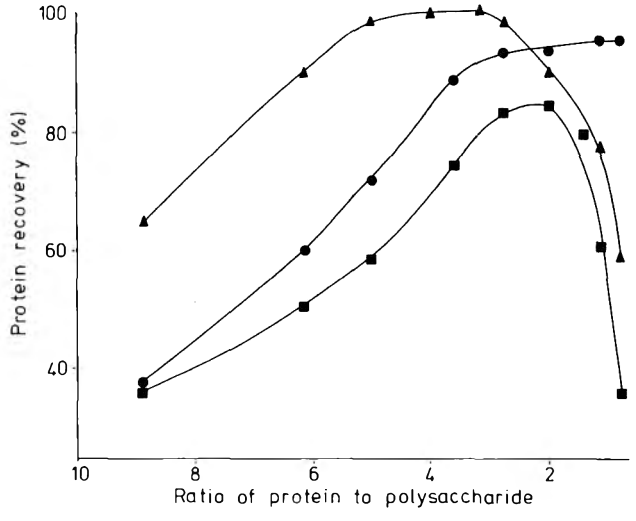

Figure 4. The recovery of BSA at different ratios of protein to polysaccharide by sodium alginate (▲), sodium pectate (●) and CMC (■) at pH 3.8 and at an ionic strength of 0.20. Protein concentration 0.20%.

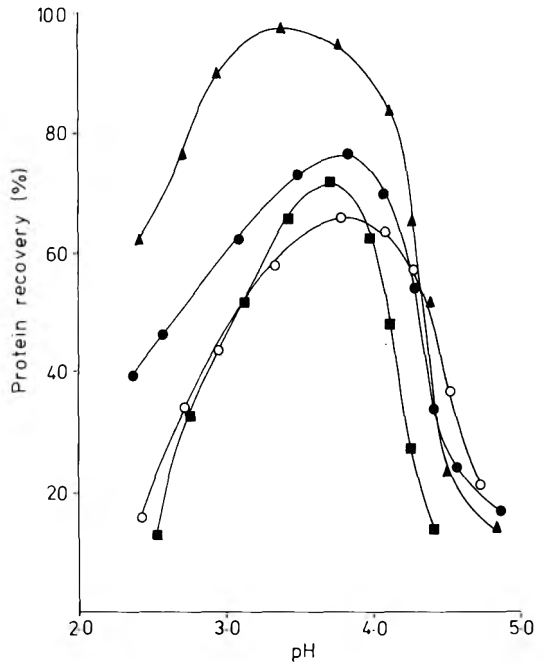

Figure 5. The effect of pH on the recovery of blood plasma by sodium alginate (▲), sodium pectate (●), pectate pulp (○) and CMC (■) at an ionic strength of 0.16. Protein concentration 1.2%; polysaccharide concentration 0.4%; pectate pulp concentration 1.2%.

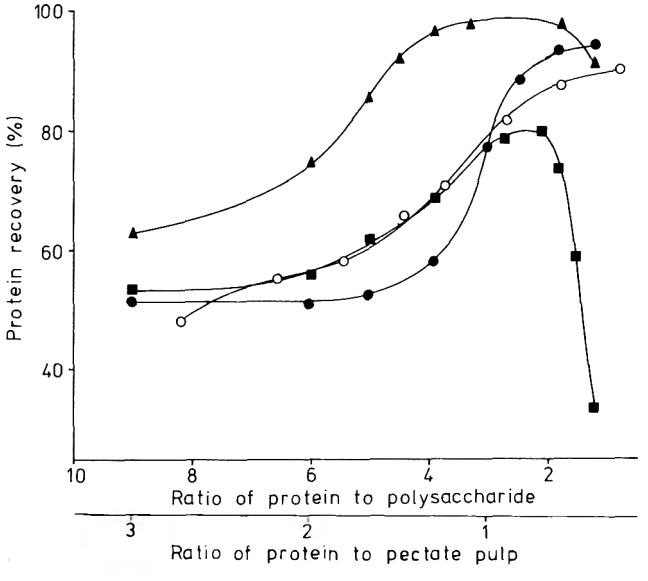

Figure 6. The recovery of blood plasma at different ratios of protein to polysaccharide by sodium alginate at pH 3.4 (▲), sodium pectate at pH 3.8 (●), pectate pulp at pH 3.8 (○), and CMC at pH 3.7 (■) at an ionic strength of 0.16. Protein concentration 1.2%.

Recovery from blood plasma

The ionic strength of the solution after all reagents had been added was 0.16 as this is the ionic strength of undiluted plasma. The results for pH dependence of protein precipitation from blood plasma and a ratio of protein to polysaccharide of 3:1 are shown in Fig. 5. The curves show great similarity to those obtained on BSA solutions at high ionic strengths. Protein recovery by pectate peel at the same ionic strength but at a protein:peel ratio of 1:1 closely follows the precipitation curve obtained with purified sodium pectate.

Figure 6 shows that under conditions of optimum pH for recovery the dependence of plasma recovery on pectate and CMC concentration is very similar to the BSA system whereas recovery with alginate is shifted to lower ratios of protein to polysaccharide. The precipitation curve obtained with pectate peel was similar to that found for sodium pectate at recoveries up to 85% except that three times as much peel was required to precipitate a given amount of protein compared with the purified material.

At very low ratios of protein to polysaccharide, alginate and CMC again show some resolubilization of the precipitated complex: this effect is especially marked in the case of CMC.

Discussion

The pH for optimum recovery of protein from BSA-CMC systems is higher than that reported by Zadow & Hill (1975). At an ionic strength of 0.1 and a ratio of protein to polysaccharide of 2.5:1 these workers obtained maximum precipitation at a pH of 2.8 which compares with our values of 4.1 and 3.7 at ionic strengths of <0.001 and 0.2 respectively and a ratio of protein to polysaccharide of 4:1. This discrepancy cannot be explained by differences in the ratio of protein to polysaccharide as Zadow & Hill found that at a ratio of 2.5:1 there was minimal precipitation at pH 4 whereas we obtained more than 85% recovery under these conditions. The only apparent significant difference between the two systems is that the degree of substitution of the CMC employed in this study was 0.51 compared to 0.8 in the previous investigation. It has been suggested that maximum precipitation occurs at a minimal *net* charge (Hill & Zadow, 1974) and as the optimum ratio of protein to polysaccharide is similar in both systems then this point will inevitably occur at a high pH in our system. Thus the results are not necessarily inconsistent although Hidalgo & Hansen (1971) did find that BSA could be selectively precipitated from diluted whey at a pH of 4.0 with CMC of similar degree of substitution to that used by Zadow & Hill.

Even at high ionic strength, in both the BSA and blood plasma systems high levels of recovery could be obtained with alginate and pectate at a pH of 3.8. This contrasts with the work of Shank & Cunningham (1968) who claim that maximum precipitation of whey protein occurs below pH 3 with alginate. However at this pH alginate would be predominantly in the insoluble acid form so it

would appear that their mechanism for precipitation is different from ours. The precipitation of protein by pectate was not inhibited by high ionic strength to the same extent as found by Schwenke *et al.* (1977) with sunflower seed albumins though this may be related to the fact that the pectate used in the present study had a degree of esterification of only 4% whereas their sample was a low methoxyl pectin with a higher degree of esterification.

From this investigation into the use of anionic polysaccharides for protein precipitation it appears that sodium alginate is both the most efficient and the least affected by increasing salt concentration. The higher charge per unit residue for alginate compared with pectate and CMC may contribute to the greater protein recovery obtained at high ratios of protein to alginate. In addition steric factors may contribute to the differences between alginate and the other polymers. Pectate contains some neutral sugars which include 2–0 (α-D-galactopyranosyluronic acid)-L-rhamnose which introduces 'kinks' into the pectate molecule (Rees & Wight, 1971) and may reduce the number of freely accessible carboxyl groups available for participation in the protein-pectate interaction as they become 'buried' by the conformation of the polysaccharide chain. In the case of CMC all the carboxyl groups are easily accessible although the linear rigid anhydroglucose chain probably restricts the number of acid groups capable of interacting with globular proteins such as BSA. Alginates are composed of three kinds of polymer segments: blocks of D-mannuronic acid, blocks of L-guluronic acid and segments of alternating D-mannuronic acid of L-guluronic acid residues. The two former segments have been shown to be ribbon-like and extended (Atkins, Mackie & Smolko, 1970) and this may allow participation of the carboxyl groups in the protein interaction while the alternating segments may permit sufficient flexibility in the chain to maximize the number and strength of such interactions.

The present results show that at low ratios of protein to polysaccharide, pectate is more effective than either alginate or CMC in precipitating protein. This phenomenon is probably due to the low stability of concentrated pectate solutions, especially under conditions of high ionic strength. Consequently the effect of re-solubilization of the protein–alginate or protein–CMC complexes under conditions of excess of these polysaccharides is not observed with pectate solutions.

Where pectate pulp is used as the protein precipitant about three times as much is required to recover a given amount of protein compared to the purified material. There is also a factor of three difference in the galacturonic acid residues found in the two preparations indicating that only pectate in the citrus sample is able to precipitate the protein.

Protein recovery from plasma using polyuronates has some advantages over other systems studied previously. The relatively high content of good quality protein (6–7%) in plasma is the major constituent of this waste liquid and consequently its removal will leave an innocuous solution with a low BOD value. In these respects blood plasma contrasts with whey which contains only 0.8% protein and a relatively high concentration of lactose (4%) which will not

be removed by the polyuronate. In addition, texturization of the plasma–polyuronate complexes is being undertaken in these laboratories by redissolving the precipitates at neutral pH and spinning or extruding the mixtures to produce novel protein foods.

Orange peel is available in large quantities as a byproduct of the orange juice industry. Since the preparation of pectate pulp only involves a pH adjustment of the peel, followed by washing and drying stages, it is expected that this material could be manufactured very cheaply. This work suggests that this material could possibly have application for removal of proteins from solution whether the main objective is to recover protein or to reduce the BOD of the effluent.

Acknowledgment

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References

Atkins, E.D.T., Mackie, W. & Smolko, E.E. (1970) *Nature, London,* **225**, 626.
Baier, W.E. & Wilson, C.W. (1941) *Ind. Engng Chem.* **33**, 287.
Brooks, J. & Radcliff, P.W. (1959) *J. Sci. Fd Agric.* **10**, 486.
Delaney, R.A.M. (1977a) *J. Fd Technol.* **12**, 339.
Delaney, R.A.M. (1977b) *J. Fd Technol.* **12**, 355.
Diamond, P.S. & Denman, R.F. (1973) *Laboratory Techniques in Chemistry and Biochemistry,* 2nd edn, p. 452. Butterworths, London.
Gornall, A.G., Bardawill, C.S. & David, M.M. (1949) *J. biol. Chem.* **177**, 751.
Hansen, P.M.T., Hidalgo, J. & Gould, I.A. (1971) *J. Dairy Sci.* **54**, 830.
Hidalgo, J. & Hansen, P.M.T. (1971) *J. Dairy Sci.* **54**, 1270.
Hill, R.D. & Zadow, J.G. (1974) *J. Dairy Res.* **41**, 373.
Imeson, A.P., Ledward, D.A. & Mitchell, J.R. (1977) *J. Sci. Fd Agric.* **28**, 661.
Krop, J.J.P., Pilnik, W. & Faddegon, J.M. (1974) *Lebensm.-Wiss. u.-Technol.* **7**, 50.
Morr, C.V., Swenson, P.E. & Richter, R.L. (1973) *J. Fd Sci.* **38**, 324.
Rees, D.A. & Wight, A.W. (1971) *J. Chem. Soc. B,* 1366.
Schwenke, K.D., Kracht, E., Mieth, G. & Freimuth, U. (1977) *Nährung,* **21**, 395.
Shank, J.L., Cunningham, W.H. (1968) *U.S. Patent,* 3404141.
Smith, A.K., Nash, A.M., Eldridge, A.C. & Wolf, W.J. (1962) *J. agric. Fd Chem.* **10**, 302.
Sternberg, M., Chiang, J.P. & Eberts, N.J. (1976) *J. Dairy Sci.* **59**, 1042.
Tolstoguzov, V.B., Mzhelsky, A.I., Chekhovskaya, V.T. & Mikeeva, N.V. (1972) *French Patent,* 2090572.
Tolstoguzov, V.B., Vainerman, E.S., Rogoshin, S.V., Kurskaya, E.A. & Kalissanov, S.E. (1974) *Nährung,* **18**, 355.
Tybor, P.J., Dill, C.W. & Landmann, W.A. (1973) *J. Fd Sci.* **38**, 4.
Tybor, P.T., Dill, C.W. & Landmann, W.A. (1975) *J. Fd Sci.* **40**, 155.
Zadow, J.G. & Hill, R.D. (1975) *J. Dairy Res.* **42**, 267.

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The control of hygroscopicity, browning and fermentation in glucose syrups

M. W. KEARSLEY

Summary

Hygroscopicity, browning (Maillard) and fermentation reactions of glucose syrups in foods may be desirable (as in cakes, bread and beer respectively) or undesirable (as in sweets, canned foods and sugar confectionary respectively). The effects of these reactions may be reduced by using lower D.E. glucose syrups in a product but in this case other, desirable properties (e.g. sweetness) of the syrup may be lost. The undesirable properties can be conveniently controlled by hydrogenation of the parent glucose syrup whilst other properties (sweetness, viscosity) are unaffected. The degree of hydrogenation governs the reduction and hence the control of these properties. Browning and fermentation can be controlled by hydrogenation more effectively than hygroscopicity, which is affected only above about 70% r.h.

Introduction

Glucose syrups are added to a very wide variety of food products which utilize the properties (bodying effect, viscosity, osmotic pressure) of the syrup to a greater or lesser extent. The hygroscopic, browning and fermentable properties of these syrups may or may not be the main reason for their inclusion in a food product and if these effects are undesirable they must be controlled or eliminated. The following examples illustrate this. High D.E. glucose syrups are used in cake formulations where the syrups' hygroscopic tendencies are utilized to keep the cake fresh and prevent moisture loss. The use of the same syrup in boiled sweets however, would produce undesirable, sticky products. Similarly, brown crusty loaves are desirable, but brown boiled sweets are not; and highly fermentable carbohydrates are required in brewing but the same carbohydrates

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taken orally appear to increase the incidence of dental caries (Ostrom & Koulourides, 1976; Koulourides *et al.* 1976). Hygroscopicity, browning and fermentation all increase with increase in D.E. of the syrup and thus all the abovementioned properties may be controlled to a certain extent by using a lower D.E. glucose syrup in the product. Often, however, other functional properties are also reduced or lost.

Sorbitol, the hydrogenated form of glucose (100 D.E.) is known to be less hygroscopic (above 70% r.h.), cause less browning and be non-fermentable in comparison with glucose itself (British Patent 1,169,538, 1966; Robertson, 1966; Phillips, 1963). Thus by hydrogenating glucose syrups a series of compounds should be produced with somewhat similar properties. It has already been shown that low D.E. glucose syrups are less fermentable when hydrogenated (Canadian Patent 899,143, 1972) but little work has been directed to the study of this effect over the whole range of D.E.

When glucose syrups are hydrogenated they can lose all or part of their reducing capacity depending on the degree of hydrogenation, yet physical properties such as viscosity and osmotic pressure remain unchanged, as does sweetness (Kearsley & Birch, 1977). Since the reducing power of a syrup governs its D.E., the degree of hydrogenation can be conveniently determined by measuring the D.E. of the syrup before and after hydrogenation. The differences in properties before and after hydrogenation would obviously be greatest in the higher D.E. syrups where a greater number of potential reducing glucose residues are present. This is exemplified in specific rotational measurements (Kearsley & Birch, 1977).

Materials

Commercial glucose syrups were provided by Corn Products Ltd, Manchester, England (courtesy of Dr D. Howling) and hydrogenated glucose syrups were prepared by catalytic hydrogenation of these parent syrups as previously described (Kearsley & Birch, 1977). When the starting syrup had a D.E. of less than 50 it was possible to dry the hydrogenated products to form solid samples but when the D.E. of the starting syrup was greater than 50 drying proved impossible and the hydrogenated products were stored as syrups (about 80% w/v solids).

Methods and results

Hygroscopicity. The hygroscopicity of glucose syrups and glucose syrup fractions produced by reverse osmosis has previously been reported (Kearsley & Birch, 1975). Hygroscopicity was shown to increase with increase in D.E. and the physical state of the sample was shown to govern the absorption rate. Since some of the hydrogenated samples could only be prepared as syrups, a

modification of the method previously used to determine moisture uptake was necessary. Thus, instead of 5 g, about 1 g of each dried sample (1.2 g in the case of syrups) was weighed into a tared nickel dish (diameter 3 inches, depth 1 inch) containing about 20 g of sand. About 10 ml of distilled water was added to the dish and the sample dissolved in the case of dried samples or dispersed in the case of syrups. The dish was placed in a 100°C oven for 4 hr with frequent stirring and then placed in an oven at 80°C under reduced pressure for a further 16 hr. After cooling in a desiccator (P_2O_5) the dish was weighed accurately and the weight of the sample noted. Using this procedure it was shown that dry samples of the otherwise undryable syrups could be produced. The dish was then placed in a 75% r.h. atmosphere and weighed every day for 14 days. Moisture gain of the sample was expressed as:

$$\frac{\text{Gain in weight of sample}}{\text{Dry weight of sample}} \times 100\%.$$

Although moisture uptake in this method was more rapid than in the previous method, final moisture contents of the samples before and after hydrogenation, where measured, were not significantly different. (cf. moisture absorption curves for spray dried and oven dried glucose syrups; Kearsley & Birch, 1975.)

21, 31, 43, 50, 65, 84 and 100 D.E. glucose syrups were used in this study both before and after hydrogenation. Maltose and maltitol were also included. The results are shown in Table 1 as the moisture contents of the samples after 14 days at 75% r.h. As expected, statistical analysis revealed no significant difference between the final moisture contents before or after hydrogenation.

The work was then repeated at 100% r.h. using the same procedure and immediately problems became apparent in that the frequent removal of the dishes from the 100% r.h. atmosphere for weighing affected the moisture uptake of the sample. This is shown in Fig. 1 for the parent 21 and 50 D.E.

Table 1. Moisture contents of glucose syrups, before and after hydrogenation, after 14 days at 75% r.h.

	Moisture absorption (% w/w)	
D.E.	Before hydrogenation	After hydrogenation
21	17.51	17.05
31	19.97	20.45
43	21.35	22.23
50	25.29	25.29
65	28.42	28.83
84	33.80	32.84
100	14.56	38.61
Maltose	24.92	26.48

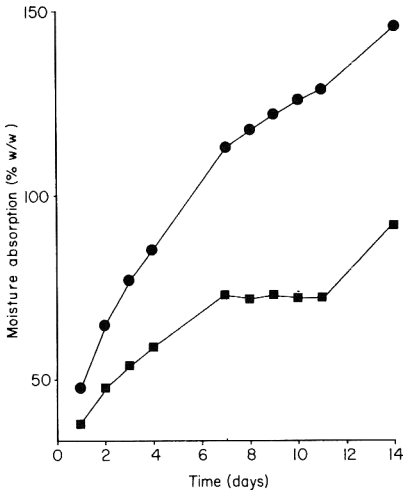

Figure 1. Moisture absorption curves for 50 (●) and 21 (■) D.E. glucose syrups at 100% r.h.

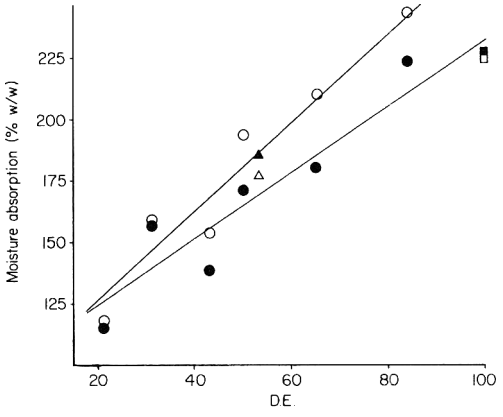

Figure 2. Moisture absorption before (open symbols) and after (filled symbols) hydrogenation for commercial glucose syrups (○, ●), glucose (□, ■) and maltose (△, ▲).

glucose syrups by the large increases above those expected after 3 days (during which time the dishes were not removed to be weighed). To overcome this problem the dishes were only weighed weekly and the study extended from 14 to 21 days. Table 2 gives the results for each sample on a weekly basis and excluding glucose (100 D.E.) and maltose (about 53 D.E.) shows that there was a reduction in moisture uptake after hygrogenation. Figure 2 shows the final moisture contents (21 days) and the best fit regression lines (x upon y) for each set of data. 100 D.E. and maltose, before and after hydrogenation, were not included in the analysis to determine the regression lines since the individual components of glucose syrups are known not to follow trends

Table 2. Moisture contents of glucose syrups, before and after hydrogenation, after 7, 14 and 21 days at 100% r.h.

Carbohydrate	Time (days)		
	7	14	21
21 B	88.94	105.35	117.96
21 A	87.73	102.04	115.20
31 B	112.38	136.29	156.52
31 A	113.60	136.94	158.05
43 B	101.20	132.20	153.33
43 A	90.57	119.30	138.29
50 B	124.79	167.21	193.30
50 A	108.07	145.18	171.32
65 B	135.56	180.77	210.02
65 A	115.14	153.01	179.83
84 B	152.44	208.23	244.00
84 A	139.68	189.78	223.66
100 B	135.50	184.85	224.50
100 A	139.60	189.80	228.52
Maltose B	113.01	149.22	177.12
Maltose A	120.34	157.36	185.09

B, before hydrogenation.
A, after hydrogenation.

expected by their calculated D.E. (glucose, 100 D.E.; maltose, about 53 D.E.). They are, however, shown for reference purposes. Statistical analysis of the data (excluding glucose and maltose) shows a significant decrease ($P \leqslant 0.05$) in hygroscopicity after hydrogenation and thus hydrogenation can be used to control the moisture uptake of glucose syrups at elevated humidities.

Browning. The interactions of carbohydrates and proteins/amino acids are very complex and the formation of the familiar brown colour represents only one feature of these diverse reactions. The literature describes many of the mechanisms of Maillard browning and it is not the purpose of this study to agree or disagree with these findings. It is accepted that browning occurs in certain instances between carbohydrates and proteins and that in some cases it must be controlled to avoid undesirable food products. In some instances this brown colour is highly desirable, for example, in the baking of bread where a brown crusty loaf is acceptable.

Initially work was directed to determine which amino acids produced the most intense coloration with glucose (100 D.E.) so that these could be used in a later part of the study in conjunction with other glucose syrups. Amino acids were used throughout the study as the 'protein source'. A 50% solution of

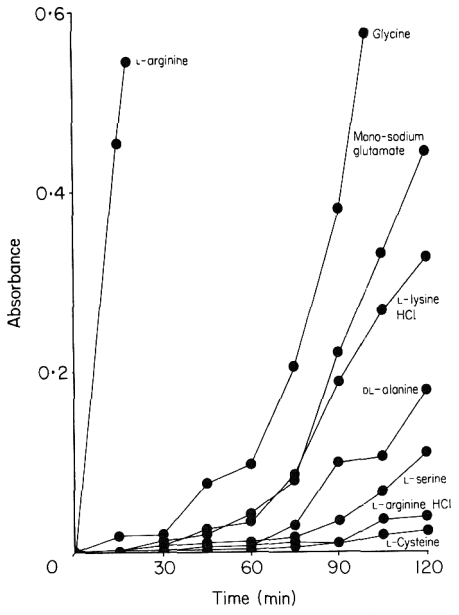

Figure 3. Colour development upon heating glucose with different amino acids.

glucose and 10% solutions of eight amino acids were prepared. 3 ml of carbohydrate solution and 2 ml of each amino acid solution were mixed and the product heated at 100°C for 2 hr. A small condenser in the neck of the heating tube prevented loss of water vapour during this heating. Optical density was determined every 15 min at 490 nm and the development of the brown colour (Maillard browning) followed. The results are shown in Fig. 3. As a result of this, L-arginine, DL-alanine, glycine and monosodium glutamate (MSG) were selected for browning studies using the glucose syrups. It was previously stated that browning can be controlled by the use of lower D.E. glucose syrups in a product and this is illustrated in Fig. 4 where a series of glucose syrups (3 ml of 50% solution) were heated with DL-alanine and MSG (2 ml of 10% solutions).

A series of partially hydrogenated glucose syrups were then heated with glycine and arginine to show the effect of hydrogenation at a particular D.E. Figure 5 shows the results for 100 D.E. and 65 D.E. samples heated with glycine with the remaining syrups showing similar trends. The values shown on each graph indicate the D.E. of the syrup after hydrogenation. Thus in the case of the 100 D.E. sample reduced to 76 D.E., this indicates that the apparent D.E. of the syrup has fallen from 100 D.E. to 76 D.E. owing to the hydrogenation and the sample is therefore 24% hydrogenated. Hydrogenation is thus shown to cause a decrease in the degree of browning and browning may be controlled in a glucose syrup by adjustment of the degree of hydrogenation.

Fermentability. Fermentable carbohydrates have many applications commercially, not the least of which is in the brewing industry, where the conversion of these sugars to alcohol by yeast forms the basis of many alcoholic beverages. Vinegar production also requires a preliminary fermentation step prior to

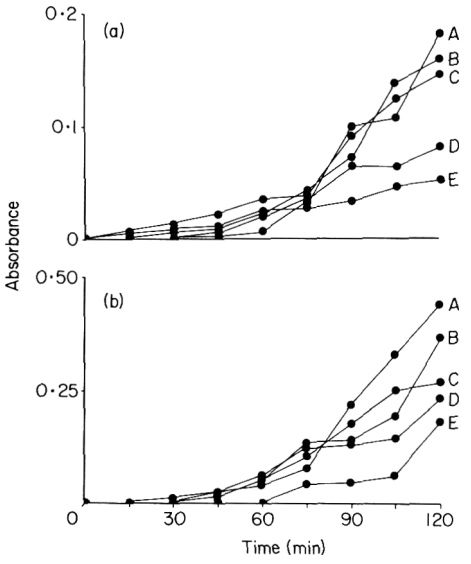

Figure 4. Colour development upon heating different D.E. glucose syrups with DL-alanine and monosodium glutamate. (a) Glucose syrups + DL-alanine, (b) glucose syrups + monosodium glutamate. A, 100 D.E.; B, 84 D.E.; C, 65 D.E.; D, 43 D.E.; E, 21 D.E.

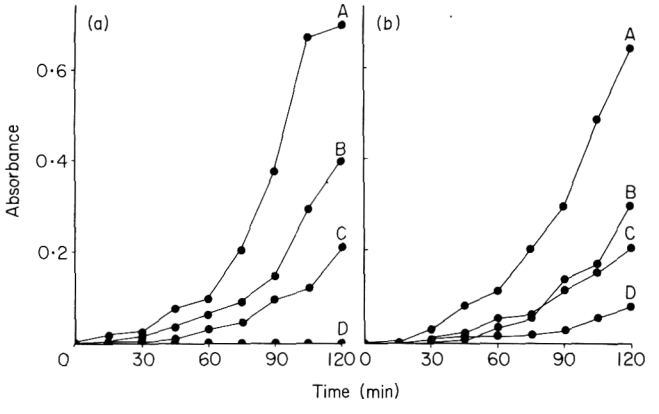

Figure 5. Effect of hydrogenation on colour development between glucose syrups and glycine. (a) 100 D.E. sample (A), 100 D.E. reduced to – 76 D.E. (B), 38 D.E. (C), 0 D.E. (D). (b) 65 D.E. sample (A), 65 D.E. sample reduced to – 51 D.E. (B), 35 D.E. (C), 12 D.E. (D).

acetification of the alcohol to acetic acid. In recent years, however, fermentable carbohydrates in the diet have come under attack from dentists in connection with dental caries. This subject has received special attention concerning the consumption of soft drinks by young people. The carbohydrates in soft drinks now form a substantial part of their diet and the increase in dental caries owing to these fermentable carbohydrates has risen sharply. Sorbitol is non-fermentable and the fermentable properties of glucose syrups should also be

reduced as the degree of hydrogenation is increased. The fermentable sugars in glucose syrups before and after hydrogenation were thus determined. The method of the Corn Industries Research Foundation (1963) was used with a slight modification.

Reagents:

Yeast suspension – 45 g dried bakers yeast made up to a total volume of 190 ml with distilled water.

Nutrient solution – 32 g K_2HPO_4, 40 g $NH_4H_2PO_4$ and 20 g yeast extract made up to 1 litre.

50% solution of each carbohydrate.

25 ml yeast suspension, 10 ml carbohydrate solution, 10 ml nutrient solution and 5 ml water were placed in a 250 ml flask. The soluble solids were determined by refractometer after filtering a portion and the flask transferred to a shaking water bath at 35° C. Further samples were taken at 30 min intervals and the soluble solids determined as above. From these results the percentage of fermented material was calculated and the results are shown in Fig. 6 for a series of commercial glucose syrups. Although the method does not take into account the volume of yeast cells in each flask, and is thus only comparative, not absolute, it shows nevertheless that the percentage of fermentable sugars in each glucose syrup corresponds to the D.E. of that syrup. Thus, as D.E. decreases, so the percentage of fermentable sugars decreases and a means of controlling fermentability is thus offered.

Glucose syrups which had been partially hydrogenated and completely hydrogenated were then treated in a similar manner to show the effect of hydrogenation at a particular D.E. Soluble solids were determined by drying a

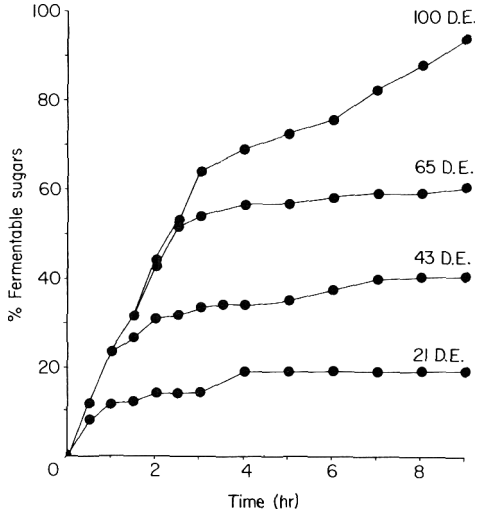

Figure 6. Percentage of fermentable sugars *v.* D.E.

Table 3. Effect of hydrogenation of glucose syrups on the percentage of fermentable sugars

D.E. of starting material	D.E. of sample	% fermentable sugars
21	21	25.3
21	10	17.8
21	0	16.8
43	43	39.6
43	23	17.7
43	0	16.4
65	65	65.9
65	35	24.4
65	12	17.9
84	84	82.0
84	43	42.8
84	0	0.0
100	100	99.3
100	38	36.2
100	0	0.0

filtered portion of the reaction mixture. The results are shown in Table 3, where the final value for the percentage of fermentable carbohydrates at each D.E. is given after 6 hr at 35°C. As expected, the percentage of fermentable sugars decreases as the degree of hydrogenation increases. With the lower D.E. samples (21 and 43 D.E.), although no reducing power was detected by boiling the sample with Fehlings solution, the syrups were nevertheless fermented, which appears to contradict the theory behind the experiment. The yeast may have possessed some amylase activity and hydrolysed the longer chain oligomers but this explanation is not entirely satisfactory since the totally hydrogenated 84 D.E. sample gave no fermentable sugars, as expected. Overall, however, a decrease in percentage fermentable sugars was observed and thus a second means of controlling the fermentability of glucose syrups is offered with the advantage that the other functional properties of a glucose syrup can be retained.

Discussion

Hydrogenated glucose syrups are less hygroscopic than their unmodified counterparts at 100% r.h. which presumably would make them more useful as humectants where resistance to change in moisture content is important. More work is obviously required to fully understand how individual components of glucose syrups behave when placed in controlled humidity environments since

anomalies appear to exist concerning hydrogenated glucose and maltose compared with glucose syrups. The change in browning capacity is more dramatic and significant reductions can be achieved by the use of hydrogenated glucose syrups. A similar pattern is also shown concerning the percentage of fermentable sugars after hydrogenation.

Since these modified glucose syrups are relatively easy to prepare, their use in the food industry could provide significant benefits to the consumer.

Acknowledgments

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References

British Patent (1966) 1,169,538.
Canadian Patent (1972) 899,143.
Corn Industries Research Foundation (1963) Standard Analytical Methods of the Member Companies, Washington D.C.
Kearsley, M.W. & Birch, G.G. (1975) *J. Fd Technol.* **10**, 625.
Kearsley, M.W. & Birch, G.G. (1977) *Die Stärke*, 29 (12), 425.
Koulourides, T., Bodden, R., Keller, S., Manson-Hing, L., Lastra, J. & Housch, T. (1976) *Caries Res.* **10**, 427.
Ostrom, C.A. & Koulourides, T. (1976) *Caries Res.* **10**, 442.
Phillips, M.A. (1963) *Br. Chem. Engng*, **8**, 767.
Robertson, J. (1966) *Ind. Chem.* 39, 233.

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The effect of curing salts on the growth of *Clostridium perfringens (welchii)* in a laboratory medium

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Summary

Strains of *Clostridium perfringens* freshly isolated from bacon or mud, and others from food poisoning incidents, showed a similar spectrum of tolerance to NaCl as strains from a culture collection. Representative strains were grown in a laboratory medium (pH 6.0) with mixtures of NaCl (3–6% w/v) and $NaNO_2$ (0–300 μg/ml) and the tolerance of these curing salts was similar to that of *Cl. botulinum*.

Introduction

Clostridium perfringens is a common cause of food-poisoning in the U.K., on average some 1300–2770 cases occurring annually (Vernon, 1977). These outbreaks are usually associated with fresh meat which has been lightly cooked, permitting survival of *Cl. perfringens* spores, and subsequently stored under poor refrigeration when the surviving spores germinate and grow rapidly. Such meat is often reheated, but inadequately, and ingestion of the relatively large numbers of cells present can result in food poisoning. Fresh meat is frequently contaminated with *Cl. perfringens* (Hobbs *et al.*, 1953; Sylvester & Green, 1961; Hall & Angelotti, 1965) and the organism is present at similar levels in bacon (Roberts & Smart, 1976). The rarity of *Cl. perfringens* food poisoning associated with cured meats suggests that the organism may grow poorly in the presence of salt and/or nitrite.

Mead (1969) reported that four strains of *Cl. perfringens* (two food poisoning and two type A) grew in VL medium (Beerens *et al.*, 1963) at pH 7.0–7.2 in the presence of 6% (w/v) NaCl, but at 7% NaCl only one type A grew and only when a large inoculum (10^5–10^6/ml) was used. Gough & Alford (1965) tested eighteen unspecified strains in fluid thioglycollate medium of unstated pH. Nine grew in 6% w/v NaCl and one grew 'slightly' in 8% w/v. Two food poisoning

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strains from a culture collection, (NCTC 8359 and NCTC 8797) tested by Spencer (1970) required 6.5% w/v NaCl at pH 6.0–6.2 to inhibit growth.

More information is required on the factors which combine to prevent the growth of *Cl. perfringens*, since there are trends towards lower salt concentrations ('milder' cures) and to reduce the sodium nitrite to the minimum, to minimize the occurrence of nitrosamines (Wolff & Wasserman, 1972; Sebranek & Cassens, 1973). Additionally, the frequency of high pH pork has increased, and pH is known to be an important factor in controlling bacterial growth, particularly where sodium nitrite is present (Castellani & Niven, 1955). The growth of bacteria in cured meats is controlled by the interaction of several factors including pH, salt concentration (or water activity), sodium nitrite and storage temperature (Roberts & Ingram, 1973; Roberts, Jarvis & Rhodes, 1976). It is important to understand more fully the relative roles of these factors if traditional curing practices are to be changed without increased microbial hazard.

Materials and methods

Organisms

The following strains of *Cl. perfringens* were used:

'Classical' strains type A NCTC 8237; type B NCTC 3110; type C NCTC 3181.

Food-poisoning strains (all type A) NCTC 8238; NCTC 8359; NCTC 8678; NCTC 8797; NCTC 9851; NCTC 10240. The following food-poisoning strains were kindly supplied by the Food Hygiene Laboratory, Colindale Ave., London, NW9 5HT; FH 611/72; FH 1415/72; FH 1785/72; FH 2619/72; FH 3199/72; FH 6160/72.

The following strains were isolated at the MRI from the source indicated: (bacon) 4e7; 4e18; 4e21; 4e25; (soil) 3H; (estuary mud) E3.

Media

(a) Cooked meat medium (CMM) was obtained from Southern Group Laboratory, Hither Green Hospital, London, SE13.

(b) TPYGC broth contained (% w/v): Trypticase (BBL), 2; Bacto-peptone (Difco), 0.5; yeast extract (Difco), 0.2; glucose, 0.4; cysteine hydrochloride (BDH), 0.05. The basal medium was sterilized by autoclaving at 121°C/15 min with the required concentration of sodium chloride. Glucose was autoclaved separately (115°C/10 min) as a 20% w/v solution and added prior to inoculation. Cysteine HCl and sodium nitrite were added as filter sterilized solutions (Millipore 0.22 μm) immediately before inoculation.

Method

Duplicate 28 ml screw-capped bottles of TPYGC broth containing appropriate concentrations of salt and/or nitrite were inoculated with two drops (each

1/70 ml) of an overnight CMM culture of *Cl. perfringens* and incubated at 35°C for at least 2 months. A positive result was recorded if one or more replicates showed visible turbidity.

Results and discussion

A total of twenty-one strains of *Cl. perfringens* have been tested in this study in TPYGC broth at pH 6.0 (Table 1). Of three 'classical' strains only one grew in 6% NaCl after incubation at 35°C for 3 months. Two of six food-poisoning strains obtained from a culture collection grew in 6% w/v NaCl, and none in 7% w/v. Of strains isolated from food-poisoning incidents three grew in 6% w/v NaCl and one in 7% w/v. Four isolates from bacon grew in 6% w/v NaCl but none in 7% w/v. A single soil isolate grew in 5% w/v NaCl and a single estuary isolate in 6% w/v. Hence, strains from a culture collection were of the same order of resistance as those isolated from food-poisoning incidents and from bacon and soil.

Table 1. The effect of sodium chloride on the growth of *Cl. perfringens* in TPYGC (pH 6.0)

Strain	NaCl (% w/v)				
	3	4	5	6	7
NCTC 8238	+	+	+	+	–
8359	+	+	+	+	–
8678	+	+	–	–	–
8797	+	+	+	–	–
9851	+	+	+	–	–
10240	+	+	+	–	–
8237	+	+	+	–	–
3110	+	+	+	–	–
3181	+	+	+	+	–
FH 611	+	+	+	–	–
1415	+	+	+	+	–
1785	+	+	+	+	–
2619	+	+	+	+	+
3199	+	+	+	–	–
6160	+	+	+	–	–
Bacon isolates 4e7	+	+	+	+	–
4e18	+	+	+	+	–
4e21	+	+	+	+	–
4e25	+	+	+	+	–
Soil isolate 3H	+	+	+	–	–
Estuary isolate 3	+	+	+	+	–
% Strains +ve	100	100	95.2	52.4	4.8

+ = Turbidity in at least one duplicate culture after incubation at 35°C for 3 months.

The effect of different combinations of salt and nitrite on growth of selected strains of *Cl. perfringens* is shown in Table 2. Again strains from different sources were of the same order of resistance. A total of 10 strains was tested in combinations of NaCl and unheated $NaNO_2$ (added as a filter sterilized solution). The data, summarized in Table 2, show clearly the expected interaction of NaCl and $NaNO_2$. The resistance pattern is similar to that of *Cl. botulinum* type B (Roberts & Ingram, 1973) although *Cl. perfringens* is slightly more sensitive to combinations of these salts. With reducing nitrite concentrations more salt was required to prevent growth at 35°C. For example, at 300 μg/ml $NaNO_2$, 3% NaCl (w/v) inhibited growth; at 200 μg/ml 4% was required, and at 50 μg/ml 5%.

There may be other controlling factors which have minimized the incidence of *Cl. perfringens* food poisoning. Kafel & Ayres (1969) reported true antagonistic action by enterococci on selected species of clostridia in a canned ham; and nearly 50% of commercially canned hams were found to contain enterococci. Lactobacilli can also cause inhibition by production of acid or antibiotic substances.

The similarity of these twenty-one strains of *Cl. perfringens* in their tolerance

Table 2. The effect of sodium chloride with sodium nitrite (unheated) on the growth of *Cl. perfringens* in TPYGC (pH 6.0)

	Strains growing / Strains tested					
NaCl (% w/v)	$NaNO_2$ added (μg/ml)					
	0	25	50	100	200	300
3	ND	8/8	8/8	8/9	3/6	0/3
4	9/9	8/9	4/8	2/9	0/3	ND
5	9/9	3/7	0/7	0/7	ND	ND
6	4/9	0/2	ND	ND	ND	ND

ND = not done.

Strains growing = turbidity in at least one of duplicate broth cultures of TPYGC at pH 6.0. Incubation at 35° for at least 2 months.

Cl. perfringens strains

Food poisoning, type A: NCTC 8359, NCTC 10240.

Food poisoning strains supplied by Food Hygiene Laboratory: FH 611/72, FH 1415, FH 1785, FH 3199, FH 6160.

Bacon isolates: 4e7, 4e25.

Estuary mud isolate: E3.

Classical type C: NCTC 3181.

of curing salts suggests that further study of any strain would be relevant to a fuller understanding of the failure of *Cl. perfringens* to grow in cured meat products. Whatever the precise mechanism of inhibition of growth of *Cl. perfringens*, the recent trends to lower salt and nitrite concentrations and the increasing frequency of high pH products seem likely to increase the possibility of its growth in cured meats stored under inadequate refrigeration.

References

Beerens, H., Schaffner, Y., Guillaume, J. & Castel, M.M. (1963) *Annls Inst. Pasteur, Lille*, **14**, 5.

Castellani, A.G. & Niven, C.F. Jr, (1955) *Appl. Microbiol.* **3**, 154.

Gough, B.J. & Alford, J.A. (1965) *J. Fd Science*, **30**, 1025.

Hall, H.E. & Angelotti, R. (1965) *Appl. Microbiol.* **13**, 352.

Hobbs, Betty C., Smith, Muriel E., Oakley, C.L., Warrack, G. Harriet & Cruickshank, J.C. (1953) *J. Hyg., Camb.* **51**, 75.

Kafel, S. & Ayres, J.C. (1969) *J. appl. Bact.* **32**, 217.

Mead, G.C. (1969) *J. appl. Bact.* **32**, 468.

Roberts, T.A. & Ingram, M. (1973) *J. Fd Technol.* **8**, 467.

Roberts, T.A., Jarvis, B. & Rhodes, Annette (1976) *J. Fd Technol.* **11**, 25.

Roberts, T.A. & Smart, J.L. (1976) *J. Fd Technol.* **11**, 229.

Sebranek, J.G. & Cassens, R.G. (1973) *J. Milk Fd Technol.* **36**, 76.

Spencer, R. (1970) *Factors affecting the survival and growth of food poisoning clostridia in cured foods.* Ph.D. thesis, University of Reading.

Sylvester, P.K. & Green, J. (1961) *Med. Offr.* **105**, 289.

Vernon, Enid (1977) *Publ. Hlth, Lond.* **91**, 225.

Wolff, I.A. & Wasserman, A.E. (1972) *Science, N.Y.* **177**, 15.

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Technical note: Fatty acid composition of tree nut oils

L. R. BEUCHAT AND R. E. WORTHINGTON

Introduction

Tree nuts are an important part of the diets of many people throughout the world (Woodroof, 1978). While information has been published concerning the fatty acid composition of most tree nuts, it is often difficult to compare data from various sources because of a lack of uniformity of analytical techniques used for quantitation.

Changes in agronomic practices and the introduction of new cultivars in recent years have been shown to affect tree nut composition. For example, Tkhagushev, Grienenko & Merzhanian (1971) demonstrated that irrigation retarded oleic acid synthesis in filberts (hazel nuts) and reduced its proportion at all stages of ripening. Heaton, Marion & Woodroof (1966) showed that nitrogen application to pecan trees caused oleic acid to decrease and linoleic acid to increase in nut kernels. Heaton, Worthington & Shewfelt (1975) reported that variations in fatty acid composition of pecan nuts were associated with cultivar and year of production, and Shokraii (1977) showed that fatty acid composition of pistachio nuts may be cultivar dependent. Fatty acid profiles in macadamia nuts vary according to species (Saleeb *et al.* 1973). These variations in fatty acid composition may influence the nutritional value and storage stability of tree nuts.

In light of this knowledge, thirteen different high-quality commercial nut meats were examined for oil and fatty acid composition using a single analytical procedure.

Materials and methods

Kernels of almond, butternut, Brazil, cashew (Indian), chestnut (Chinese), filbert, hickory, macadamia, pecan, pignolia (pine nut), pistachio, and walnut (black and English) were analysed. Tree nuts had received no additional proces-

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sing beyond normal cracking and shelling operations. Kernels (6 g) which had been chopped and passed through a No. 4 mesh screen were extracted with diethyl ether using a Goldfisch oil extractor for 12 hr. The nut pieces were then ground to a meal consistency and returned to the reflux column for an additional 8 hr; samples were again thoroughly mixed and subjected to a final 8-hr extraction treatment. Ether was evaporated from the tared beakers used to collect the extract and the percentage of kernel dry weight attributed to oil was calculated.

Fatty acid methyl esters were prepared by treating duplicate samples (c. 30 mg) of each oil with a solution of methanol-benzene (2.1) containing 3% sulphuric acid. The transesterification reaction was carried out in teflon-capped tubes as described previously (Worthington, Boggess & Heaton, 1972).

Methyl esters prepared from duplicate samples were analysed on a gas chromatograph equipped with dual flame ionization detectors and 1.86 m × 4 mm (i.d.) glass columns packed with 10% stabilized diethylene glycol succinate and 3% OV225 on 80/100 mesh Chromosorb W(AW)(DMCS). Column temperature was programmed from 185 to 230°C at 2°C/min with a helium flow rate of 100 ml/min. Detector and injection port temperatures were maintained at 300 and 275°C, respectively. Each methyl ester preparation was analysed in duplicate; peak areas were measured with an electronic integrator and fatty acid values are reported as weight percentage of total fatty acids.

Results and discussion

Listed in Table 1 are the total oil content and fatty acid composition of thirteen types of tree nut kernels. Data for oil contents agree closely with those reported by Adams (1975). Levels of fatty acids in several tree nuts differ somewhat from those reported by other researchers. For example, Garcia Olmedo & Marcos Garcia (1971) reported that filbert oil contained 12% linoleic (18:2) acid and Spell (1976) reported that Brazil kernel oil contained 36.1% linoleic and 39.1% oleic (18:1) acids as compared to 46.2 and 29.0%, respectively, listed here. On the other hand our data are generally consistent with those of Seidemann (1973) who observed that oils of almonds, pistachios, and cashews had similar composition.

If the degree of unsaturation gives any indication of susceptibility of tree nut kernels to oxidation and consequent flavour deterioration, butternuts should be least stable and Brazil nuts should be most stable during prolonged storage. While the unsaturated:saturated fatty acid ratio may be of value in predicting the likelihood of kernels to undergo hydrolytic or oxidative rancidity, other factors such as moisture content, non-oil constituents, and temperature also influence changes in organoleptic qualities of oils in tree nuts upon storage.

In addition to the effects of cultivar and agronomic practices on oil content and fatty acid composition of tree nuts, the state of maturity may have an influence. Mature pecan kernels contain a higher percentage of oil accompanied

Table 1. Oil fatty acid composition of tree nuts

Common name	Botanical name	Oil (%)	Fatty acid (%)													Trace and unidentified	Unsaturated: saturated ratio
			16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0		
Almond	*Prunus amygdalus*	52.7	6.7	0.52	T*	T	1.2	66.3	22.3	T	T	T	T	–†	–	3.0	11.3
Butternut	*Juglans cinerea*	60.0	1.6	T	T	T	0.78	19.0	61.9	16.0	–	–	–	–	–	0.7	40.7
Brazil	*Berthrolletia myrtaceae*	68.7	14.1	0.32	T	T	8.6	29.0	46.6	T	0.32	T	T	–	–	1.1	3.3
Cashew	*Anacardium occidentale*	47.7	10.2	0.41	T	T	8.5	60.9	18.3	–	0.76	T	T	–	–	0.9	4.1
Chestnut	*Castanea mollisima*	2.5	14.6	0.73	T	T	1.1	54.0	24.9	2.7	–	1.0	0.20	–	–	0.8	5.2
Filbert	*Corylus avellana*	62.3	4.7	0.19	T	T	1.6	76.4	16.3	0.15	0.12	0.16	–	–	–	0.4	14.5
Hickory	*Caryaovata ovata*	70.4	8.8	0.45	T	T	2.3	52.0	33.5	1.7	0.22	T	–	–	–	1.0	7.7
Macadamia	*Macadamia tetraphylla*	73.2	8.3	21.8	T	0.14	2.1	56.4	2.8	–	2.4	3.1	0.79	0.34	0.48	1.3	6.0
Pecan	*Carya illioensis*	70.3	5.7	0.11	T	T	2.2	66.9	22.1	1.1	0.21	0.39	–	–	–	1.3	11.2
Pignolia	*Pinus* sp.	48.1	5.8	0.26	T	T	3.8	38.1	46.4	0.82	0.66	0.90	–	–	–	3.3	8.4
Pistachio	*Pistachia vera*	57.0	8.6	0.72	T	T	2.3	68.8	17.8	0.32	0.26	0.56	T	–	–	0.6	8.1
Walnut, black	*Juglans nigra*	59.0	3.1	T	T	T	2.6	29.1	58.3	4.9	T	T	T	–	T	2.0	16.6
Walnut, English	*Juglans regia*	67.4	7.3	0.17	T	T	2.3	19.1	57.4	13.1	T	–	–	–	–	0.6	9.4

* Trace. † None detected.

by a higher degree of saturation than do immature nuts (Heaton *et al.* 1977). Oil from mature cashews also has been noted to be more saturated than that from immature fruits (Maia *et al.* 1975). Pecan trees having a heavy crop are likely to yield nuts that are smaller in size, lower in oil content, and higher in unsaturated:saturated fatty acid ratio (Heaton *et al.* 1977). Barroso *et al.* (1973), however, reported that while there were some differences in the fatty acid values of three kernel sizes of Brazilian cashew nuts, the trends were not uniform.

In summary, we have presented for the first time, to the authors' knowledge, data showing the total oil content and fatty acid composition of thirteen tree nuts as determined by a single analytical procedure. Values may vary slightly from those found in previous reports due to culture, maturity, size, or other factors, but comparisons among tree nuts examined and reported here can be made.

References

Adams, C.F. (1975) *Nutritive Value of American Foods, A.R.S., U.S.D.A., Agr. Handbook No. 456,* Washington, D.C., U.S.A.

Barroso, M.A.T., Whiting, F.M., Brown, W.H. & Stull, J.W. (1973) *HortScience,* **8**, 99.

Garcia Olmedo, R. & Marcos Garcia, M.A. (1971) *Anales Bromatol.* **2**, 233. (In Spanish).

Heaton, E.K., Marion, J.E. & Woodroof, J.G. (1966) *Peanut J. Nut World,* **45**, 36.

Heaton, E.K., Shewfelt, A.L., Badenhop, A.E. & Beuchat, L.R. (1977) *Pecans: Handling, Storage, Processing & Utilization, Univ. Georgia Res. Bull. 197.* Athens, Georgia, U.S.A., 77 pp.

Heaton, E.K., Worthington, R.E. & Shewfelt, A.L. (1975) *J. Fd Sci.* **40**, 1260.

Maia, G.A., Brown, W.H., Whiting, F.M. & Stull, J.W. (1975) *HortScience,* **10**, 233.

Saleeb, W.F., Yermanos, D.M., Huszar, C.K., Storey, W.B. & Labanauskas, C.K. (1973) *J. Am. Soc. Hort. Sci.* **98**, 453.

Seidemann, J. (1973) *Seifen-Ole-Fette-Wachse,* **99**, 302. (In German).

Shokraii, E.H. (1977) *J. Fd Sci.* **43**, 244.

Spell, E. (1976) *Gordian,* **76**, 341. (In German).

Tkhagushev, N.A., Grinenko, V.V. & Merzhanian, Y.A. (1971) *Izvestiya Vysshikh Uchebnykh Zavedenii Pishchevaya Tekhnologiya* **2**, 39. (In Russian).

Woodroof, J.G. (1978) *Tree Nuts. Production, Processing, Products,* 2nd edn. AVI Publ. Co., Westport, Connecticut, U.S.A.

Worthington, R.E., Boggess, T.S. & Heaton, E.K. (1972) *J. Fish. Res. Bd Can.* **29**, 113.

(Received 29 November 1977)

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Technical note: The relationship between parameters used as composition indices of cooked ham

G. A. GARDNER

Introduction

The most common indices used to assess the composition of cooked ham are meat content (MC), protein on a fat-free basis (PFF) and water-protein ratio (WPR). Traditionally cooked hams were prepared from cured pork legs which had been drained and matured for 2–3 weeks before cooking. In the last 15–20 years, technological innovations have led to the more rapid production of cured meat by machine brine injection, massaging or tumbling prior to cooking. Processors of this 'modern' ham can add considerably more brine to the product and with the widespread use of refrigeration produce a milder and more succulent product, which in effect contains less meat that the 'traditional' ham. Such 'modern' hams, including imports, constitute 35% of the U.K. market in 1973, and this proportion is probably increasing every year.

There is currently much concern over the description and composition of cooked hams offered for sale in this country. Hence a survey was conducted to evaluate the use of the indices listed above to differentiate between 'modern' and 'traditional' hams, and the relationships between the indices are described in this note.

Materials and methods

Both 'modern' and 'traditional' cooked hams, all open-pack, were obtained from eight manufacturers. Cross-section samples were minced and analyses performed on the homogenate. The contents of moisture, protein, fat and ash were determined by BSI methods (BS 4401, parts 1, 2, 3 and 5).

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Calculations:

$$\text{PFF (protein, fat-free)} = \frac{\text{Protein (\%)} \times 100}{100 - \text{fat (\%)}}$$

$$\text{WPR (water/protein ratio)} = \frac{\text{Water (\%)}}{\text{Protein (\%)}}$$

$$\text{MC (meat content)} = \text{Lean (\%)}^* + \text{Fat (\%)}$$

The results were analysed by the statistical methods of Bishop (1974).

Results and conclusions

The PFF, WPR and MC values for both 'modern' and 'traditional' cooked hams are given in Table 1.

Table 1. Fat-free protein (PFF), water protein ratio (WPR) and meat content (MC) of modern and traditional cooked ham

Quality index	Type of ham	No. of samples	Mean	Standard deviation	Range	*t* value
PFF	Modern	57	19.17	1.10	16.23–21.79	9.164
	Traditional	24	24.14	2.56	19.70–29.00	
WPR	Modern	57	4.011	0.276	3.471–4.822	12.718
	Traditional	24	2.911	0.384	2.304–3.745	
MC	Modern	57	89.28	4.88	75.77–101.03	9.608
	Traditional	24	111.17	10.70	90.17–132.43	

By all three methods highly significant differences were found between the two types of ham, and on the basis of the *t* value water protein ratio was the best index to differentiate the types. There were differences between some manufacturers in both types of ham, and these may be attributed to differences in production procedure (e.g. amount of brine used in cure, cooking losses, etc.).

The correlation coefficient (*r*) between MC and PFF was 0.993. This very closely correlated situation arises directly from the calculations in that both are based on the protein and fat contents of the ham. The *r* value for WPR and PFF was 0.963 and for WPR and MC 0.966. These again are highly correlated, so that both sets of indices, i.e. MC, PFF and WPR, are measuring the same quality in the ham, namely the amount by which the ham has been diluted by brine.

*Calculated by Stubbs & Moore method using the factor of 3.45 (Pearson, 1976).

Regression equations were calculated to enable the conversion of MC to PFF and *vice versa* to be made. Because of the lower correlation, the conversion of PFF values to WPR or MC to WPR or *vice versa* is not so accurate. These formulae are given below:

Meat content and fat-free protein

$$MC = 4.328\,(PFF) + 6.416$$
$$PFF = 0.228\,(MC) - 1.160$$

Meat content and water/protein ratio

$$MC = 169.515 - 20.011\,(WPR)$$
$$WPR = 8.151 - 0.047\,(MC)$$

Fat-free protein and water/protein ratio

$$PFF = 37.511 - 4.577\,(WPR)$$
$$WPR = 7.870 - 0.203\,(PFF)$$

Acknowledgment

The technical assistance of Miss A. McMillan is gratefully acknowledged.

References

Bishop, O.N. (1974) *Statistics for Biology*, 2nd edn. Longman, London.

Pearson, D. (1976) *The Chemical Analysis of Foods*, 7th edn. Churchill Livingstone, Edinburgh.

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J. Fd Technol. (1978) **13**, 363–366

Book reviews

New International Dictionary of Refrigeration. International Institute of Refrigeration, Paris, 1976. Pp. xxxvii + 560. Price FF 300.

The first edition of the dictionary published by the I.I.R. has now been revised and brought up to date. In the process it has gone from 278 pages to 560: from 1500 expressions of which 400 were defined in French and English to 2400 expressions all defined in these two languages: from six languages to seven, Norwegian having been added: from a weight of about 1 kg to just under 2 kg: and from a price of FF 40 to FF 300.

To those familiar with the earlier work these figures will give some idea of the extent of the revision. For those readers not so acquainted it should be said that the first part of the book consists of parallel columns containing equivalent terms in English, French, Russian, German, Spanish, Italian and Norwegian. Definitions are given in English and French on the left hand pages, the right hand pages containing the equivalents, without definitions, in the other languages. This first part is divided into fifteen sections, expanded from ten in the original edition, with separate sections now for cold rooms, etc., food industry, freeze drying and biological applications, and cryology, amongst others.

One may browse through these sections in order to acquire some of the specialist vocabulary in another language. To identify a particular term in any language one has to turn to the alphabetical indexes in the second part of the book, one index for each language, in which can be sought the reference number of that term by which it can be found in the first part of the book.

A choice which has to be made by all lexicographers is whether the terms included and their definitions should merely reflect current usage, even when that usage may be bad, or whether they should attempt to introduce some standardization of the usage. In this dictionary a middle course is taken. Most terms in common use are given, but ones which are not recommended are indicated by a typographical sign. For example, instead of the older terms 'total heat' and 'heat content' the term 'enthalpy' is preferred.

Attempts are made in places to impose distinctions between the meanings of closely related words. For example in Section A, Basic Data and Instrumentation, the authors introduce a distinction between 'dehydration' defined as 'removal of water' and 'desiccation' defined as 'complete removal of water'. This distinction is blurred in Section L, Food Industry, however, where we are told that 'freezer burn' is 'damage produced by excessive desiccation'.

A firm attempt is made to limit the meaning of the prefix 'cryo-' in section P, Cryology. This is defined as 'the ensemble of all scientific and technological applications dealing with cryotemperatures (i.e. below about 120°K)'. This

limitation does not seem to be entirely acceptable to the cryobiologists however who have a sub-section, Cryobiology under M, Freeze Drying and Medical Applications, which seems to admit terms relevant to much higher temperatures.

One can differ with the authors over some of the definitions provided. A 'thermodynamic system' is not usually understood as a region of space but as a collection of matter, exactly as in mechanics. A defined region of space is nowadays called, following Prandtl, a 'control space' or 'control volume'. 'Cold box' is not synonymous with 'gas separation unit' since cold boxes are to be found in many plants which do not separate gases. Users of hydrometers will know that the term 'specific gravity' in this country usually implies the ratio of the density of a liquid to that of water at the same temperature, not at 4°C. And it is unfortunate that 'supersaturated air' is correctly defined but with a rider implying that the additional water substance is in the form of fog, i.e. a two-component two-phase mixture, thus leaving us without a term for air containing supersaturated water vapour, i.e. vapour in the metastable state.

Users of this book will find few omissions of important terms. A few surprising ones perhaps are the names of the various cellular insulating materials now available, and the expression 'Freon blown' insulants: translated terms for the various grades such as 'high density' and 'fire retarding' would also have been useful. In connection with frozen food the term 'high quality life' is not admitted: and in the section on humidity the 'thermodynamic wet bulb temperature' or 'temperature of adiabatic saturation' is not given. The 'maximum operating pressure' (MOP) of an expansion valve is mentioned under another definition but is not indexed. 'Polytropic efficiency' of turbocompressors is not to be found.

Inconsistencies and omissions are bound to creep into a work prepared by many specialists who have given freely of their time to produce what is certainly a useful and authoritative book. They are to be congratulated on their achievement.

This reviewer's only criticism of the work as a whole concerns its size, weight and price. The result of the layout adopted is that much of the book is simply blank paper, particularly on the right hand pages where space has to be left to accommodate the definitions on the left hand pages. The two-column layout of the alphabetical indexes also causes much wasted space. Consequently the book is not one to take on travels abroad, especially by air, where it would account for 10% of the standard baggage allowance!

W. B. Gosney

Sausage Products Technology: Food Technology Review No. 39. By Endel Karmas. New Jersey: Noyes Data Corporation, 1977. Pp. xi + 316. US $39.

This reviewer's first reaction on seeing No. 39 of this series was to seek for some grounds to regard it as different in principle from any of the others: he

sought and found none. Experienced readers, either of Noyes Data Reviews or of reviews of them in this Journal, may stop here.

The book is a compilation from the United States patent literature in the period 1960–1976 and is the third of a sub-series within the Noyes Data range, following 'Fresh Meat Technology' (No. 23, 1975) and 'Processed Meat Technology' (No. 33, 1976). Some 8% of the patents reviewed were also reviewed, in similar words, in 'Processed Meat Technology', but there is no overlap with 'Fresh Meat Technology'. Its strengths and weaknesses may most simply be described by direct quotation from the opinions of some recent reviewers of previous volumes in the series.

'The author has set out to review each patent and record the relevant technical details without including the legal jargon and this has certainly been achieved. However, it is a pity that in such a useful work the review is merely presented and no critical assessment is made of the subject matter of the patent.' M. R. Hewitt; *J. Fd Technol.* (1976) **11**, 198.

'There are one or two minor complaints such as the varying depths of critical assessment, which is never very deep, of the processes, also why do we have grams, ounces, Farenheit and Centigrade in the same text?' 'Those wishing to become acquainted with (sausage) processing would do well to acquire basic knowledge before consulting this (book).' J. D. Henshall; *J. Fd Technol.* (1977) 12, 560.

'In spite of the criticisms . . . a useful reference work . . . especially (for) those concerned with new product innovation and development.' 'Would be much improved by an adequate subject index . . . the table of contents is not an adequate substitute.' N. L. Thomas; *J. Fd Technol.* (1977) 12, 201.

M. D. Ranken

Books Received

Safety in Biological Laboratories. Ed. by E. Hartree and V. Booth. London: The Biochemical Society, 1977. Pp. xii + 68. £1.60.

A most useful short publication on safety precautions. The chapter headings are: Precautions for All Laboratory Workers; Electrical Hazards, Fire and Explosions; The Role of the Safety Officer; The Animal House; Microbiological Hazards; Carcinogens, Mutagens and Teratogens; Radiation Hazards.

Salmonella. The Food Poisoner.
London: The British Association for the Advancement of Science, 1978. Pp. i + 51. £1.00.

This short report by an expert committee covers questions of salmonellosis as it affects man and his domestic animals, the problems of the farmer, legislation and control means and the safeguard against salmonella food poisoning.

Animal Feed from Waste Materials. Food Technology Review No 46. By M. T. Gillies.
New Jersey: Noyes Data Corporation, 1978. Pp. x + 346. US $39.

A survey of mainly the U.S. patent literature on the utilization of waste materials as animal feed.

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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, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (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)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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

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Page proofs will be submitted to the contributors for minor corrections and should be returned to the Editor within 3 days. Major alterations to the text cannot be accepted.

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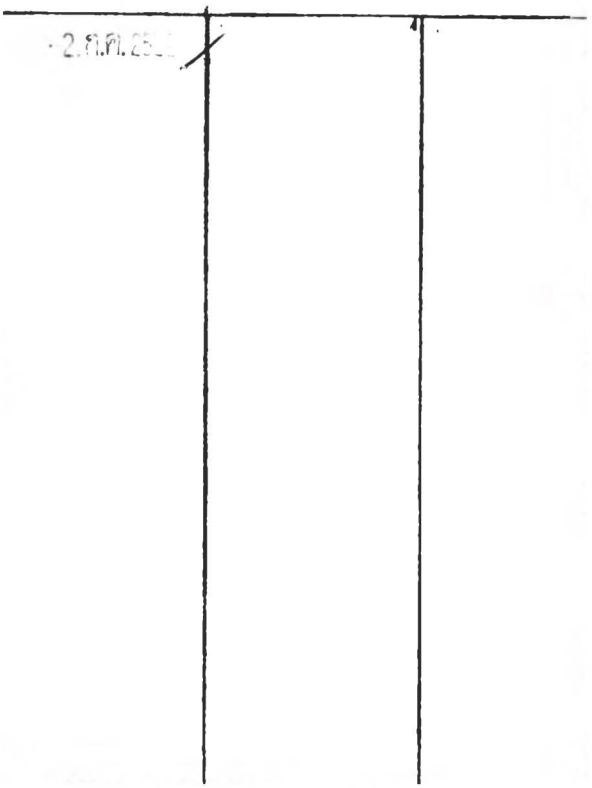

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