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The continuous chilling of poultry in relation to EEC requirements

N. L. THOMAS

Summary

The development of commercially viable techniques for the continuous chilling of poultry processed on high speed mechanized lines has attracted considerable research effort throughout Europe subsequent to the introduction of an EEC Directive concerned with the hygienic production, processing and distribution of fresh, chilled and frozen poultry and poultry meat.

The Directive included a qualified ban of the so-called 'spin chiller' system of chilling which had reportedly been used under unhygienic conditions in certain processing plants.

The reason for this ban and possible alternative chilling systems are reviewed.

Introduction

Modern high speed poultry slaughterhouses all operate in basically similar ways although the mechanical details vary considerably as a result of differing approaches to mechanization and automation. The basic stages of the process are shown schematically in the flow diagram (Fig. 1).

The main technical differences between factories both in the U.K. and internationally are in the scalding operation, in the use of either manual or automatic evisceration and in the methods used to chill the final product prior to packaging. Scalding temperatures may range from 50 to 65°C with immersion times ranging from 200 sec down to 30 sec at higher temperatures. Spray scalding systems either alone or combined with the plucking operation are also under development. The time/temperature relationship is dictated by the extent to which the processor wishes to remove the outer layers of the epidermis and to facilitate complete mechanical feather removal. Generally the more severe scalding conditions are preferred for birds which are subsequently sold in the frozen state whereas milder scalding conditions, although necessitating some

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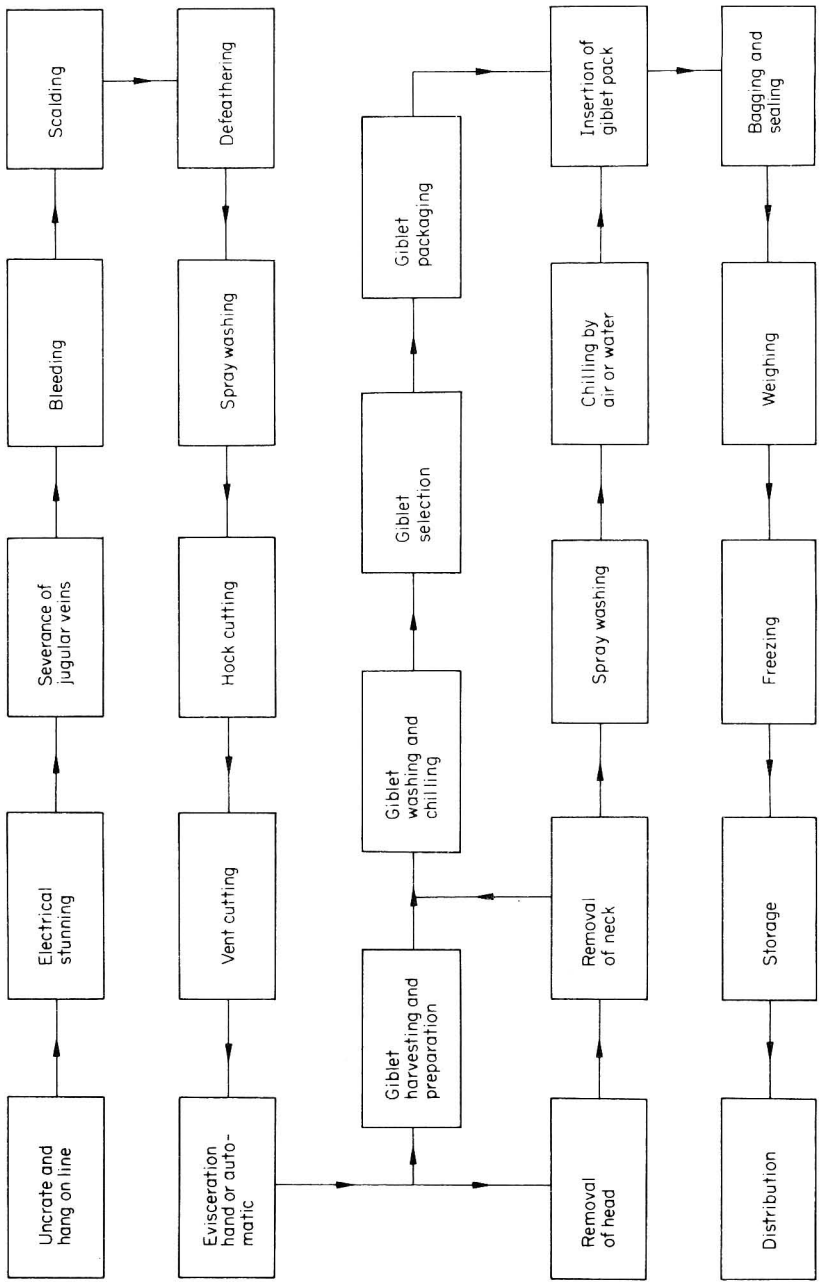

Figure 1. Basic stages in a modern high speed poultry slaughterhouse.

hand finishing after plucking, are preferred for birds which are intended for 'fresh' outlets and for air chilling.

Directive 71/118/EEC dated 15 February 1971 (OJ 1971) is concerned with the hygienic production, processing and distribution of fresh poultry and poultry meat. In the Directive, fresh poultry and poultry meat is defined as all those parts of the bird which are normally fit for human consumption and have not undergone any processes of preservation other than chilling and/or freezing.

Amongst its many proposals for the technical regulation of the industry Article 14 prohibits the use, in Member States, of the so called 'spin chiller' system for chilling poultry. Article 14 was to be effective from 1 January 1976 qualified by the proviso that extension of the time limit to 1 January 1977 would follow if the Council had not received a proposal from the Commission by 1 January 1972 confirming that one or more commercially applicable alternative chilling methods had been developed. It was proposed at that time that the spray-chilling method under development in Germany be designated as an appropriate chilling process and that the many technical and economic problems relating to the use of the spray-chilling process would be overcome by 1 January 1976. However the Council rejected this proposal and subsequent detailed investigations at various Research Institutes in the Member States and by the Industry showed that at the time the proposed process was neither economically viable nor technically efficient and that no practical commercially operated plant existed.

Introduction of Directive 71/118/EEC in 1971 resulted in a very considerable increase in the volume of research carried out on various chilling systems and on the hygienic problems associated with their use. By 1973/1974 the number of research papers published had risen from the pre-1970 average of perhaps eight to ten, to between thirty-five and forty per annum. Additionally major sections at five International Symposia (London 1972, Roskilde 1973, Brookings 1973, New Orleans 1974 and Oosterbeek 1975) were devoted to specialist presentations and discussions by scientists and industrial management to evaluate current research and to exchange views on methods of cleansing and chilling poultry which would overcome the hygiene problems associated with the poorly operated 'spin-chiller' systems which had been reported earlier.

As a result of these discussions and further meetings between representatives of the major poultry processing companies and research workers a European Code of Practice relating to the hygienic operation of immersion chilling systems was prepared and accepted by the Industry (Anon 1975). This Code of Practice and its application to certain chilling systems is further discussed later.

On 10 July 1975 the Council issued Directive 75/431/EEC (OJ 1975) amending certain provisions of the original Directive 71/118/EEC including substitution of the original Article 14 by the following:

'1. Member States shall prohibit the "spin-chiller" poultry chilling process currently in use. This prohibition shall have effect only 18 months following submission of the report referred to in paragraph 2 and not later than 1 January 1978.

2. After consulting the Member States within the Standing Veterinary Committee the Commission shall submit to the Council before the 1 July 1976 a report on the chilling processes which are not covered by the prohibition in paragraph 1.'

As a result of this proposal an EEC Working Group was established consisting of scientists from various National Research Institutes with known expertise in the hygienic problems associated with the commercial production of fresh poultry meat. This group proposed that a programme of research be carried out in a number of commercial situations in each of the Member States to establish the operating parameters of the various chilling systems available to the industry and to compare their hygienic status and their conditions of commercial operation. This programme has now been completed and a report summarizing the results of this work has been prepared for the Commission.

Reasons for chilling poultry

In a well managed plant the time elapsing between slaughter and the completion of plucking, evisceration and post-evisceration washing is very short and the carcase has not cooled appreciably. At this stage rapid cooling of the carcase is necessary to delay the multiplication of psychrophylic organisms associated with the deterioration and subsequent spoilage of poultry meat and to prevent the multiplication of any microorganisms of Public Health significance during the subsequent stages of grading, weighing, packaging and final chilling or freezing.

During this post-evisceration cooling process it is generally considered desirable to reduce the temperature of the carcase to 10°C or below (Barnes, 1973). Under these conditions multiplication of microorganisms of concern to the processor is very slow and has no significant effect on a microbiological quality of a carcase during the relatively short time between the end of post-evisceration cooling and final chilling or freezing. Under final chill conditions the microbiological life of a carcase is extended to many days whilst under frozen conditions multiplication of all microorganisms naturally ceases.

Objections to the classical 'spin-chilling' process

Before considering operating parameters for commercial processes for chilling poultry and discussing systems which might provide viable alternatives to the classical 'spin-chilling' system which has been criticized it is necessary to consider the events which led to the original prohibition.

The term 'spin-chilling' was originally derived from a manufacturer's name for a particular model of continuous automatic poultry chiller (Gordon-Johnson, Stephens Co.) using water and ice but was rapidly adopted as a generic

term for many types of continuous water chillers in which the carcases were immersed for varying times and at various temperatures. In many instances (Peric, Rossmanith & Leistner, 1971; Lillard, 1971; Grossklaus, 1972) it was shown that certain methods of operation led to an increase in microbial counts on the carcase and these undesirable processes became known as classical 'spin-chiller' systems in contrast to the controlled continuous immersion chilling processes which many workers have shown to improve carcase bacteriology and at the same time effectively chill the carcase (Surkiewicz *et al.*, 1969; Brant, 1973; Mead & Thomas, 1973).

In addition the Council undoubtedly considered suggestions made at that time, that the immersion chilling processes generally used in North America, loosely referred to as 'spin-chilling' but in fact very different in technical content and operated under rigorous government control, were to be discouraged. It has been confirmed that these suggestions were and are incorrect (Brant, 1973). All available information from recent research and from discussion at international meetings indicate that throughout the world controlled immersion chilling processes are accepted and endorsed as technically satisfactory methods for the hygienic chilling of poultry carcases without prejudice to public health or the interests of the ultimate consumer.

The fundamental objections to the classical 'spin-chiller' process as used at the time of the original proposal to prohibit its use may be summarized as: (a) unhygienic in operation; (b) leading to possible cross-contamination within the chilling system; (c) allowing the uptake of excessive quantities of grossly contaminated water. Furthermore the Commission stated that any alternative chilling system proposed must be capable of commercial operation and hence, by implication, must be of commercially acceptable capital and operating cost.

Considering now each of these essential parameters in turn.

Process hygiene

In its initial report the Commission stated that the use of the classical 'spin-chiller' was to be restricted by the Council because it had been found to be unhygienic. Reports produced by workers at that time indicated that this was indeed so in certain instances, (e.g. Lillard, 1971; Peric *et al.*, 1971; Grossklaus, 1972) and that on occasions the chilling systems then in use resulted in an increase in the surface contamination of the carcases during processing with undesirable spoilage organisms and with microorganisms of public health significance especially salmonellae and other enterobacteriaceae. From reports made at that time it is clear that the Commission desired that a chilling system should be developed and adopted which could be operated in such a way that the overall extent of bacterial contamination of the carcase could be reduced progressively throughout the processing operation thus presenting the ultimate consumer with a safe hygienic end product. It is evident that, subject to compliance with other essential parameters, those processes which yield the greatest

overall reduction in bacterial numbers are those most desirable from the point of view of hygiene, public health and the consumer.

Cross contamination

Advocates of certain alternative systems of chilling poultry carcases continue to place great emphasis on the possibility that cross contamination between carcases during immersion chilling may occur, and that microorganisms present on one carcase may be transferred wholly or in part to an adjoining carcase (Leistner, Rossnanith & Waltersdorf, 1972; van Schothurst, Notermans & Kampelmacher, 1972).

Whilst it is obvious that in any manufacturing operation a limited transfer of microorganisms from one carcase to another may occur in many ways, the hygienic significance of this transfer must be evaluated in relation to the overall extent to which avoidable transfer of microorganisms between birds and carcases is inevitable in other stages of the production cycle. Thus:

(a) In the modern poultry industry, prior to slaughter, the birds are reared in large groups derived from common ancestry. There is little evidence to suggest that any significant difference in the microbiological status of individual birds exists within a group unless a specific disease condition occurs in a small number of birds and the crop is slaughtered before horizontal transmission of micro-organisms to the common environment of the birds and hence to the gut and external surfaces of the remaining birds in the group has occurred. Experimentally it has been shown that the introduction of, for example, salmonellae to growing birds either at time of hatch or in the early stages of growth as a result of vertical transmission from breeding stock or as a food contaminant results in the rapid development of a contaminated environment and the resultant surface and gut contamination of virtually the entire crop of birds without necessarily producing a condition where individual birds are capable of producing a significant increase in the contamination of other members of the group during processing.

(*b*) During catching, crating and transport the birds are handled, crated and carried in communal containers and vehicles where unavoidable contact between live birds occurs and where, within a population, there is undoubtedly a high degree of uniformity of bacteriological condition on arrival at the processing factory.

(*c*) During processing birds share a common path through entry to slaughter, scalding, plucking, evisceration, chilling, grading, packing and distribution during which opportunities occur for contact and the transfer of microorganisms from one carcase to another. Thus it is unrealistic to suggest that commercially viable techniques are likely to be developed in the foreseeable future which would adequately eliminate all possible methods of transfer of microorganisms from one carcase to another. The limited transfer of microorganisms which may occur in any particular chiller system should be considered in this context.

(*d*) In real terms it is likely that any cross-contamination which may occur is of little practical significance. Thus spoilage organisms brought into the plant with the birds or introduced to the processing environment are only of concern in the context of final numbers on the processed carcase and that overall process which leads to the greatest reduction in count is obviously the most hygienic and most desirable. At the same time as the total numbers of spoilage organisms are reduced any organisms which could be of public health significance are also removed from the carcase and a comparable reduction in these numbers also occurs. It is unlikely that the interchange of small numbers of pathogens between carcases is of great significance since in the majority of cases the numbers are very low and in any event only the final numbers of pathogenic bacteria present are of any importance to the consumer.

Water uptake

In many discussions concerning the use of spray and immersion chilling systems attention has been drawn to the fact that water is absorbed by the carcase at this time and that this absorbed water could be detrimental to the consumer's interest both as a result of excessive quantity and as a result of its contamination with microorganisms.

It is generally accepted that during the washing of any food product some of the washing water is inevitably retained and absorbed by the product. Indeed this may, in many cases, be advantageous to the consumer and a proposed Council regulation (COM(75) 593 dated 3 December 1975) relating to the total water content of frozen and deep frozen poultry recognizes this need.

The explanatory memorandum forming part of the proposal submitted by the Commission states 'The deep-freezing, freezing and storage of carcases results in the dehydration of the product which is detrimental to its organoleptic qualities. The introduction of water during the preparation of the poultry thus constitutes an improvement in the quality of the end product, provided that the quantity added is not excessive. The desired result is obtained by the addition of a quantity of water representing 6–8% of the weight of the carcase'.

It has been suggested that the fact that the carcase is rinsed to some degree during both spray and immersion chilling operations results in the 'clean and wholesome water' used in the process becoming non-potable as a result of material removed from the carcases. Whilst fundamentally there is some justification for this argument on a strict legalistic basis, it would appear to lack justification in terms of poultry hygiene and consumer acceptability. It is inevitable that some water absorption will occur during the earlier stages of scalding, plucking, evisceration and especially during the very thorough washing and cleansing which must precede any chilling process. It is also inevitable that the water absorbed during these early stages when the carcase is less well cleansed will also not accord with the strictest definition of potable quality. Carried to its logical conclusion proponents of the 'potable water' argument could suggest that a similar ban be applied to all processes in which food is

washed prior to consumption! Many workers now consider that it is therefore somewhat illogical to object to a chilling process on these grounds and that a more helpful criterion is the effect of the process on the overall bacteriological condition of the carcase. Thus although the main function of any chiller is to reduce the temperature of carcases passed through it, the additional benefit of a final rinse following thorough washing prior to the chilling operation can only be advantageous.

Considerations relating to the selection of a suitable chilling method

Before outlining the criteria which must be met by any system used for the chilling of mechanically plucked poultry on a continuous line basis one must consider the fundamental requirement of all chilling processes which is to chill the poultry rapidly and economically to a temperature which is acceptable from both a hygienic and a public health point of view.

It is generally accepted that the carcase and in particular the inner and outer surfaces and any cut surfaces of the carcase should be cooled as rapidly as possible to a temperature of 10°C or below and thereafter either further chilled to between 0 and 4°C, or frozen or deep frozen shortly after final preparation and packaging. Cooling rates of the order of 0.5°C per minute or better have been suggested. Under these conditions it has been found that there is no multiplication of microorganisms of public health significance and the rate of growth of potential spoilage organisms which might affect carcase quality is acceptably low.

Essential factors to be considered in relation to any alternative chilling systems are:

(*a*) it must satisfactorily chill poultry carcases in a hygienic manner and in particular must show a significant hygienic improvement in comparison with the classical 'spin-chiller' process which it is to replace.

(*b*) In view of the general concern which has been expressed about the possibility of cross-contamination which has been referred to earlier in this paper it is appropriate to consider various alternatives with special regard to this factor.

(*c*) Any uptake of water which occurs during the chilling process must be controllable within any limits the Commission may indicate that it finds acceptable and further the bacteriological condition of the carcases should not be impaired by the absorption of grossly contaminated wash waters.

(*d*) Both the Commission and the Industry are concerned that alternative methods for chilling poultry must be commercially applicable to continuous throughput production lines, must be efficient in their utilization of both energy and water resources and must be capable of installation and operation without entailing prohibitive additional production costs.

Possible alternatives to the classical 'spin chiller' process

Careful consideration of the scientific and technical literature and the proceedings of recent symposia in which chilling techniques have been discussed in

depth suggest that the following systems might be considered: (a), air chilling; (b), cryogenic chilling; (c), evaporative chilling; (d), spray chilling; (e), controlled continuous immersion chilling. Each of these methods is therefore considered in relation to the criteria for acceptability already outlined.

Air chilling

Processors in some Member States chill poultry by hanging the birds on racks transported mechanically through air chilling tunnels. These usually consist of an initial section where the birds pass through dry circulating relatively warm air which removes surface moisture from the carcase thus reducing the possibility of excessive bacterial growth occurring during the air chilling cycle and subsequent packaging and distribution. Following the drying section the birds pass through the air chiller proper where they are cooled by circulating air at or below 0°C for up to 1 hr. Poultry which is to be chilled must be scalded at lower temperatures than usual prior to mechanical plucking to prevent unacceptable discoloration during the chilling process.

Hygiene. Many workers (Mulder, 1971; Mead, 1975; Thompson *et al.*, 1975) have compared surface counts of air chilled birds with similar carcases chilled by a variety of immersion methods. Generally the counts have been subsequently higher indicating the hygienic superiority of the latter process.

It has been thought that air chilled birds, having a drier surface should have a longer store life at chill temperatures due to the reduction in water activity on the surface of the carcase caused by the drying which occurs during the air chilling process. Others have expressed preference for water chilled birds for prolonged storage at chill temperatures. This is, perhaps, due to the higher bacterial counts on the air chilled birds which may more than off-set any initial inhibition of growth as a result of a reduced water activity. It is also likely that the reduced surface water activity soon returns to normal as a result of migration of moisture from within the carcases when the birds are in impervious packaging or when storage conditions are poor.

Cross contamination. Precautions can be taken to prevent contamination with drip-water from incoming birds. However, the birds are hung from racks which have been in contact with other birds and in commercial tunnels dictates of air flow and economy result in systems where lateral contact between carcases is unavoidable. Additionally it has been suggested that since the use of air chilling processes requires the use of soft-scalded carcases such cross contamination which does occur might be of more significance since there is less thermal destruction of microorganisms during scalding.

Water uptake during air chilling. There is no water uptake and indeed a degree of dehydration occurs. Those most concerned with the marketing of poultry have expressed considerable concern that many consumers might find both the texture and appearance of the final product less acceptable than that of the familiar and widely available water chilled and frozen product.

Commercial acceptability. Many Member States have extensive experience

of air chilling systems especially in the United Kingdom and the Netherlands. The process is only used commercially for soft-scalded birds which are destined for 'fresh' chilled markets. It is not commercially acceptable for frozen or deep frozen poultry due to the drying effect and problems with colour if used with birds scalded at the higher temperatures used for frozen and deep-frozen production.

In North America where about 90% of the birds are sold fresh, the use of air chilling, as an alternative to immersion chilling, has been considered but has been largely rejected except in a few cases where it is used to dry and further cool birds which have already passed through a controlled immersion chiller system (Anon, 1968; Timmons, 1971). Recent estimates (Simpson & Thomas, 1973) suggest that the process is generally much more expensive in operation than are immersion chilling methods.

Cryogenic chilling

Chilling methods using liquid nitrogen, fluorcarbons and solid carbon dioxide as contact coolants have specialized application in many parts of the food industry. Possible extension of one or more of these techniques to poultry has been suggested but no industrial application has developed apart from the use of solid carbon dioxide snow as a secondary means of crust freezing poultry and hence super-cooling packed fresh birds to aid long distance shipment for the chilled trade (Thompson, Whitehead & Mercuri, 1974).

Cross contamination. In the absence of commercial experience the effects of any continuous cryogenic process, which might be developed in the future, on general carcase bacteriology and its cross-contamination potential cannot be clearly defined. However since the process would require some form of mechanical transport through a tunnel within which the coolant would be applied and in the absence of any possible rinsing effect it is likely that the end product would be comparable to that produced by air chilling.

Water uptake. It is likely that water balance within the carcase would remain unchanged although some surface condensation might occur under conditions of high humidity immediately after chilling.

Commercial acceptability. None of the cryogenic processes are available in a form suitable for incorporation in a continuous production line producing whole carcases. Recent estimates suggest that coolant costs would be very high, a figure of 2.75p per kg. (Sture Astron, 1972) has been calculated for a theoretical system using liquid nitrogen.

It is also thought that the effective use of any cryogenic method would require surface freezing of the carcase which would then necessarily be held for a period to allow thermal stabilization and hence thawing of the already frozen surface before the carcase could be finally trussed and packaged. This would appear to conflict with the requirements of a proposed Marketing Directive, which does not permit the rethawing of frozen poultry for packaging.

Evaporative chilling

As an alternative to cryogenic chilling methods, evaporative cooling by the vacuum evaporation of moisture from the carcase has been suggested (Klose, 1975). The technique has been tried only under laboratory conditions when dehydration of the order of 5% occurred. Generally the method appears unlikely to gain commercial acceptance in the foreseeable future.

Spray chilling

Various proposals for the spray chilling of poultry have been made (Leistner *et al.*, 1972; Veerkamp, Mulder & Gerrits, 1972) including the use of refrigerated water, combinations of ordinary water and refrigerated water, the incorporation of forced air circulation and finally the use of an initial spray system followed by either air cooling or by direct freezing.

The only industrial spray chilling system which had been installed was subsequently discarded because of the very high water requirement (12 litres per bird) which was found to be uneconomical and ecologically unacceptable (Leistner *et al.*, 1972). Other variants have been tested experimentally but no commercial operating plant is known to exist.

Hygiene. Experimental work with a true spray chilling installation using very large volumes of refrigerated water has shown bacteriological results comparable with those obtained in well operated controlled immersion chilling systems. However when the rate of water usage is reduced in an endeavour to develop a commercially viable process especially in association with subsequent air-cooling it is likely that this rinsing effect will be significantly reduced. Additionally under these conditions it has been suggested, from experimental data, that the birds leaving the spray-chilling system will be cooled to no more than 18°C and that it will be at this temperature that the birds will be graded, weighed and packaged prior to further chilling. Under practical conditions these operations entail a significant time lag during which cross contact between the warm birds will occur with risk of considerable bacterial multiplication and cross contamination. From a hygienic point of view this situation appears to be totally unacceptable.

A further limitation, common to all spray chilling systems, is that they do not provide for a proper rinse of the body cavity of any carcase which may be soiled from the visceration process. Furthermore unless the carcases are suspended by the wings, which is not easy under continuous production conditions, there may be an accumulation of contaminated liquid in the body cavity leading to decreased keeping quality in birds intended for the fresh market. If the carcases are hung from the wing in this manner cleansing of the body cavity in a spray system is likely to be even less effective.

Cross contamination. It is reported that the original spray chilling system examined by Peric, Rossmanith & Leistner (1974) showed an acceptably low

level of cross contamination of approximately 4%. This was in a system using forced air circulation and approximately 12 litres of water per bird at very low pressure. This process can no longer be advocated on economic and ecological grounds. A careful comparison by Notermans *et al.*, (1973) between an immersion chilling system and a spray chiller system operating at high and low pressures in which a proportion of carcases were contaminated at varying levels with an identifiable strain of *E.coli* gave the following results:

Level of contamination	10^5	10^6	10^7	10^8	10^9
	% Contaminated carcases after chillers				
'Spin-chiller'	8	14	47	44	81
High pressure spray chiller	0	14	31	56	83
Low pressure spray chiller	–	–	8	42	58

It is evident from these results that cross contamination can occur in both systems but is only of significance at very high levels of initial contamination which are unlikely to occur under normal commercial conditions. It was concluded that cross contamination in the chiller system was of far less importance than adequate cleansing of the carcases prior to chilling.

Pietzsch and Levetzow (1974) carried out tests on spray chilling system using carcases with known levels of contamination with salmonellae.

% Contaminated before chilling	% Contaminated after chilling
6	7
31	52
23	15

The authors concluded that the instruction of spray chilling systems would not significantly improve the hygienic state of the final product.

Water uptake. Various workers (Woltersdorf, 1971; Veerkamp *et al.*, 1972) have confirmed that there is an absorption of water as a result of a spray chilling system. Proponents of the system have suggested that this absorbed water is of potable quality. However, the water absorbed is obviously that which is on the surface of the bird and within the cavity and will contain the bacteria which the washing effect of the system has removed from the carcase. Within the cavity the water is likely to be highly contaminated due to the relatively ineffective cavity rinsing which occurs with this system.

Commercial acceptability. In the absence of commercially operating chillers based on the spray principle either alone or in combination with air chilling there is little information available on operating costs which can give any indication of commercial acceptability. However, since it is known that the systems proposed require large volumes of water under pressure and may also incorporate either refrigerated water supplies or an air chilling stage, it is evident that both capital and running costs will be high (Simpson & Thomas, 1973). Additionally the contaminated water resulting from the chilling process will add substantially to the loading on effluent treatment plants if unacceptable ecological impact is not to occur.

It must be emphasized that spray chilling processes using very moderate amounts of water in an attempt to reduce cost and ecological impact are really washing processes and not chilling systems. Birds leave these low flow processes with an internal temperature of perhaps 18°C or above with the result that undesirable bacterial multiplication is still occurring after the process and continues during grading, weighing and packing, prior to final cooling.

Controlled continuous immersion chilling

Earlier work had indicated that the problems were primarily associated with the use of certain types of 'spin-chiller' which were operated in an unsuitable and unhygienic manner with inadequate water flow, little, if any, ice resulting in excessively high operating temperatures and of inadequate capacity. Additionally it appears certain that the bacteriological condition of the birds entering the chilling system was unsatisfactory due to inadequate cleansing following plucking and evisceration.

With a view to assisting poultry processors to improve existing installations and as a guide line for the development of more satisfactory continuous immersion chilling systems which could replace the classical 'spin-chiller', representatives of the poultry industry from Member States and scientists from National Research Institutes cooperated in incorporating the results of the latest research into a Code of Practice (Anon, 1975; Simpson, 1975; Mulder *et al.*, 1976) which provided that any continuous immersion process used for the chilling of mechanically plucked poultry in a continuous processing operation should: (a) use only fresh, clean and wholesome water; (b) incorporate an effective system of cleansing the poultry after evisceration and before entry into the chiller system; (c) maintain a temperature of not more than 4°C in the chiller or in the last stage of a multi-unit chiller system; (d) utilize at least 2.5 litres of fresh, clean and wholesome water for each kg of carcase passing through the system and in a multi-stage system allow at least 0.5 litres of overflow from the last unit for each kg of carcase passing through the system; (e) whenever the continuous passage of carcases through the chilling system is stopped for any reason so that birds are unavoidably delayed within the system then the system should be such that no agitation occurs and further the time of the

passage through the chiller system should be controlled; and (f) water absorption resulting from the passage of carcases through the chilling system should be controlled.

On the basis of extensive practical experience and as a result of experiments and trials carried out it appears that two controlled immersion chilling processes accord with the parameters which have been established.

(1) *Continuous counter flow systems* are systems in which the carcases and water flow in opposite directions in a controlled manner so that the carcases are continuously cooled and rinsed by passage through increasingly cool water which enters the system at the point at which the carcases are discharged thus providing a final clean water rinse immediately prior to discharge. Ice is normally introduced with the water thus maintaining an appropriate temperature gradient throughout the system.

(2) *Cascade systems of 'ideal mixers'* within which the temperature of the water used for chilling the product is maintained at the same temperature as the overflow as a result of thorough mixing of the water phase which have recently been described by Dutch workers. In this process the product is handled by mechanical or hydraulic systems, the temperature of the cooling water being maintained as necessary by the addition of ice or by the introduction of chilled water.

Both these chiller systems have been developed and operated in accordance with the proposals put forward in the Code of Practice (Simpson, 1975, Mulder *et al.*, 1976) and have shown themselves capable of adequate control and satisfactory chilling rates under continuous production line conditions.

Hygiene. It has been well demonstrated that properly managed continuous immersion chiller systems, as described, can be operated in a hygienic manner and will rapidly cool carcases on a continuous basis to acceptable temperatures. At the same time passage through the chilling system has a useful rinsing effect. Generally it has been found that the passage of carcases through continuous immersion chillers under controlled conditions results in a reduction of the organisms still capable of being rinsed from the carcase by up to 90% (Mead & Thomas, 1973; Simpson, 1975). A brief post-chill rinse with high pressure water sprays can be incorporated into either of the systems with some advantage.

Cross contamination. As with spray chilling systems cross contamination between heavily soiled carcases during immersion chilling has been demonstrated. In well supervised processing plants where carcases have been adequately cleansed prior to introduction into the chilling system there is a substantial reduction in the total numbers of organisms on the carcase leaving the chilling system and presumably in any pathogens which might be present.

Water uptake. In controlled continuous immersion chilling systems water uptake which occurs can be maintained at the desired level with no detrimental effect on the overall bacteriological quality of the carcase.

Commercial acceptability. Sufficient commercial experience of various sys-

tems of immersion chilling exists to suggest that either of the two systems which have been proposed will be commercially feasible and commercially acceptable. In each system water and energy is used efficiently with minimum wastage and existing continuous production systems can be economically adapted to incorporate either chilling system. Consumer acceptability is well established as evidenced by the wide demand for the product both in the Member States and elsewhere. Ecological impact is minimal and excessive demands on potable water resources and effluent treatment facilities are avoided.

Conclusion

Although various alternatives to the classical 'spin-chilling' technique for cooling poultry have been proposed only carefully controlled continuous immersion chilling and direct air chilling appear to be commercially acceptable. The former requires the use of appropriate quantities of fresh water cooled either by the addition of ice or by refrigeration and can be used for carcases intended for marketing either in a fresh or frozen condition. Air chilling is frequently used for birds intended for the 'fresh' market. Lower scald temperatures are used and very thorough cleansing of the carcases prior to chilling is necessary.

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Thermal death kinetics of *Bacillus stearothermophilus* spores at ultra high temperatures

I. Laboratory determination of temperature coefficients

F. L. DAVIES, H. M. UNDERWOOD, A. G. PERKIN AND H. BURTON

Summary

A study was initiated into the inconsistencies reported to occur between (a) estimations of sterilizing efficiency (of UHT plants) based on experiments in which bacterial spores are heated in capillary tubes at sub-UHT temperatures and (b) determinations of sterilizing efficiency made by heating spores at UHT temperatures in the plants themselves. Capillary tube experiments were extended to 160°C with the aim of obtaining data suitable for direct comparison, i.e. obviating any need for extrapolation, with those obtained by plant inoculation. As heat-treatment temperatures were increased from 120 to 160°C the shape of the survivor curves became increasingly sigmoidal, possibly due to the greater significance of heat transfer times at high temperatures.

Temperature coefficients for spores suspended in water were constant ($Q_{10} = 23.5$) up to 132.5°C and for spores suspended in milk were constant ($Q_{10} = 13.2$) up to 142.5°C; beyond these temperatures, Q_{10} values gradually diminished. Such an effect could not be explained by Arrhenius kinetics and again may reflect the physical limitations of the capillary tube system. Its importance is discussed in relation to the value of capillary tube experiments for predicting the sterilizing efficiency of UHT plants.

Introduction

The sterilizing efficiency of ultra high temperature (UHT) processes may be assessed by either of two methods. The first of these involves direct experimental determination in which a UHT plant, operating at normal processing temperatures (130–150°C), is used to process a quantity of product seeded with high known concentrations (at least 10^5/ml) of resistant bacterial spores. Expressions of sterilizing efficiency are then derived from the proportion of spores surviving. The hazards of contamination often preclude the use of such a method from a commercial environment and, furthermore the large scale

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spore preparation required is not only laborious but may introduce problems of spore ageing and batch consistency (Franklin, 1970). Consequently the second approach to assessment of sterilizing efficiency is often favoured. Here, small volumes of spore suspension sealed in capillary tubes or ampoules are heated by immersion in a heating fluid, much in the manner of the classic experiments of Bigelow & Esty (1920) and again the proportion of surviving spores determined.

Because of the very short heating times involved at higher temperatures such laboratory experiments are not normally conducted at temperatures exceeding 125°C so that the results must be extrapolated into the UHT range to enable estimates of sterilizing efficiency at appropriate times and temperatures to be made. Such a practice assumes that temperature coefficients (values of Q_{10} or z) for thermal death of spores are constant over the whole temperature range involved.

Unfortunately, sterilization data obtained by these two methods are not always in accord (Burton, 1970; Cerf & Hermier, 1973) and various explanations have been suggested to account for this discrepancy. For instance Hermier, Begue & Cerf (1975) claimed that heating spores by injection into steam in a UHT plant 'has some particular effect on their heat resistance' so that at lower temperatures spores are more resistant in ampoules than in the plant, whereas at UHT temperatures the converse is true.

Burton (1970), on the other hand, showed that the sterilizing effect obtained using direct inoculation of plant was much lower than that estimated by calculation from laboratory data obtained at lower temperatures. He further suggested that the apparently higher Q_{10} for inactivation of *B.stearothermophilus* TH24 spores suspended in milk obtained using a UHT plant compared to that extrapolated from lower temperature capillary experiments was due in part to the known (Franklin, 1970) inhibitory nature of the milk after UHT processing, so that with the more severe UHT treatments giving fewer survivors and requiring lower dilutions in the plating medium the inhibitory effect was greater, resulting in an overestimate of thermal death. However, results obtained using a laboratory scale UHT plant (Perkin, 1974) which could be seeded with higher initial numbers of spores resulting in lower residual milk levels on plating, indicated that the Q_{10} value for thermal destruction of *B.stearothermophilus* TH24 spores was similar to that quoted by Burton (1970) for the large UHT plant. It therefore seems improbable that this inhibitory effect of the milk was responsible for the difference in Q_{10} values noted by the latter author, since the inconsistency between estimated and experimentally determined sterilizing effects still existed.

A third possibility to explain the above discrepancy is that the Q_{10} value does actually fall with increasing temperature as suggested by Miller & Kandler (1967). Since thermal death is usually regarded as a first order reaction, the death rate constant would be expected to obey the Arrhenius equation which indicates a linear relationship between the log of reaction rate and the reciprocal of absolute temperature. From such kinetics, the relationship between log decimal reduction time and temperature would, over a wide temperature range,

be described by a curve rather than a straight line and the Q_{10} would fall with increasing temperatures, though there would be a close approximation to linearity over narrow temperature ranges. Extrapolation of thermal death data on the assumption of a constant temperature coefficient may therefore be invalid (Rahn, 1945; Jonsson, 1974) though it is not certain whether the magnitude of such an effect would be sufficient to explain the discrepancies between capillary tube and plant data referred to above and it would be quite insufficient to explain the apparent lowering of Q_{10} with increasing temperature found by Miller & Kandler (1967).

It is important that the relationship between death rate and temperature be clarified if laboratory data obtained at lower temperatures are to be used with any certainty to predict the efficiency of plant processes operating at higher temperatures. Accordingly a detailed study using capillary tube and plant experiments has been carried out over a wide temperature range and reported in this and the following two papers (Perkin *et al.*, 1977; Burton *et al.*, 1977). In the initial work reported here the feasibility of conducting capillary tube experiments over a wide range (120–160°C) was examined so that a large number of data might be available for a single heating system up to temperatures used in UHT processes, so obviating the need for extrapolation in evaluating the sterilizing effect of such systems.

Spores of *B.stearothermophilus* TH24 were used throughout this study since they had previously been shown (Franklin *et al.*, 1970) to have high thermal resistance, making them very suitable for such work and also to facilitate comparisons between current and previous data.

Materials and methods

Spore suspensions

Spores of *B.stearothermophilus* TH24 (NCDO 1096) were grown on Bacillus Spore Agar (BSA) (Franklin *et al.*, 1970) using 3% agar in Roux bottles, a spore inoculum and incubation at 60°C for 7 days. Harvested spores were washed six times in cold sterile distilled water and stored for at least six months at 4°C before use, preliminary experiments having shown that less consistent results were obtained with freshly harvested spores. Twelve batches of such spores were prepared, bulked, washed a further twice and stored as an aqueous suspension of approximately 10^9/ml. All the experiments were carried out with this same bulked suspension.

Heat treatment

Glass capillary tubes (melting point tubes, C.E. Payne & Sons Ltd, G.A. 1724) 100 mm long, 0.9–1.0 mm i.d. and 0.15 mm wall thickness were sealed at one

end and sterilized by autoclaving. Spore suspension (0.01 ml of the bulked suspension either in aqueous form or after replacement of the water with whole raw milk to give the same spore concentration) was introduced into capillary tubes using an Agla (Wellcome) micrometer syringe and a specially made needle of fine stainless steel tube; the open ends of the capillary tubes were drawn out and sealed giving a final length of 65–75 mm. Ten such capillary tubes (0.1 ml spore suspension) were subjected to each heat treatment, using either a holder with which they could be heated individually or one (Franklin *et al.*, 1959) with which all ten could be treated simultaneously (see Fig. 1). This dual approach was used since the momentary fall in heating-bath temperature caused by the introduction of the multiple tube holder was considered unacceptable when heating times were very short. With longer heating times this fall was correspondingly less significant and use of the multiple-holder obviated the impracticably long aggregate times which would otherwise have resulted. An arbitrary lower limit of 1 min was selected for use of the multiple-holder, the single tube holder being used for all treatments shorter than this.

To further minimize cooling of the treatment bath fluid, capillary tubes were preheated by immersion in a preheat bath at 100 ± 1°C for 5 sec before rapid transfer to and immersion in the treatment bath at the required temperature. Furthermore the fluid in the treatment bath was vigorously agitated to dissipate any local cooling effects which might occur. Both heating baths contained a polyalkylene glycol heating fluid (Union Carbide HTF 14) and the temperature of the treatment bath was maintained to within ± 0.15°C according to a

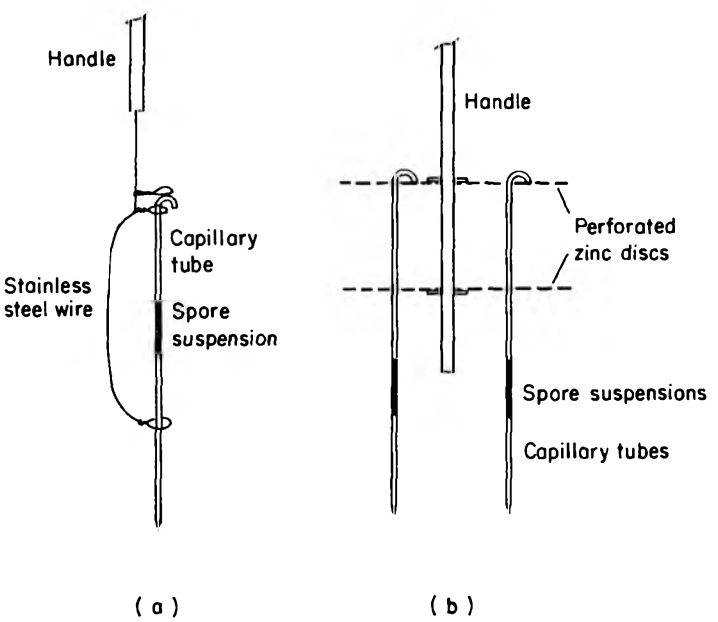


Figure 1. Devices used to suspend capillary tubes in heating fluids; (a) single capillary tube holder, (b) multiple capillary tube holder.

calibrated thermocouple placed in the bath to continuously record temperature during treatment. After heating, capillaries were cooled by immediate transfer to a cold water bath. Transfers to and from the heating bath were performed manually and were accomplished within 0.2 sec as measured by miniature thermocouples attached to capillaries and connected through an amplifier to a high speed recorder. A very high level of accuracy and reproducibility was achieved by operators after some practice.

Activation of spores for control counts was performed by steaming spore suspension, sealed in capillaries as above, at 100°C for 30 min.

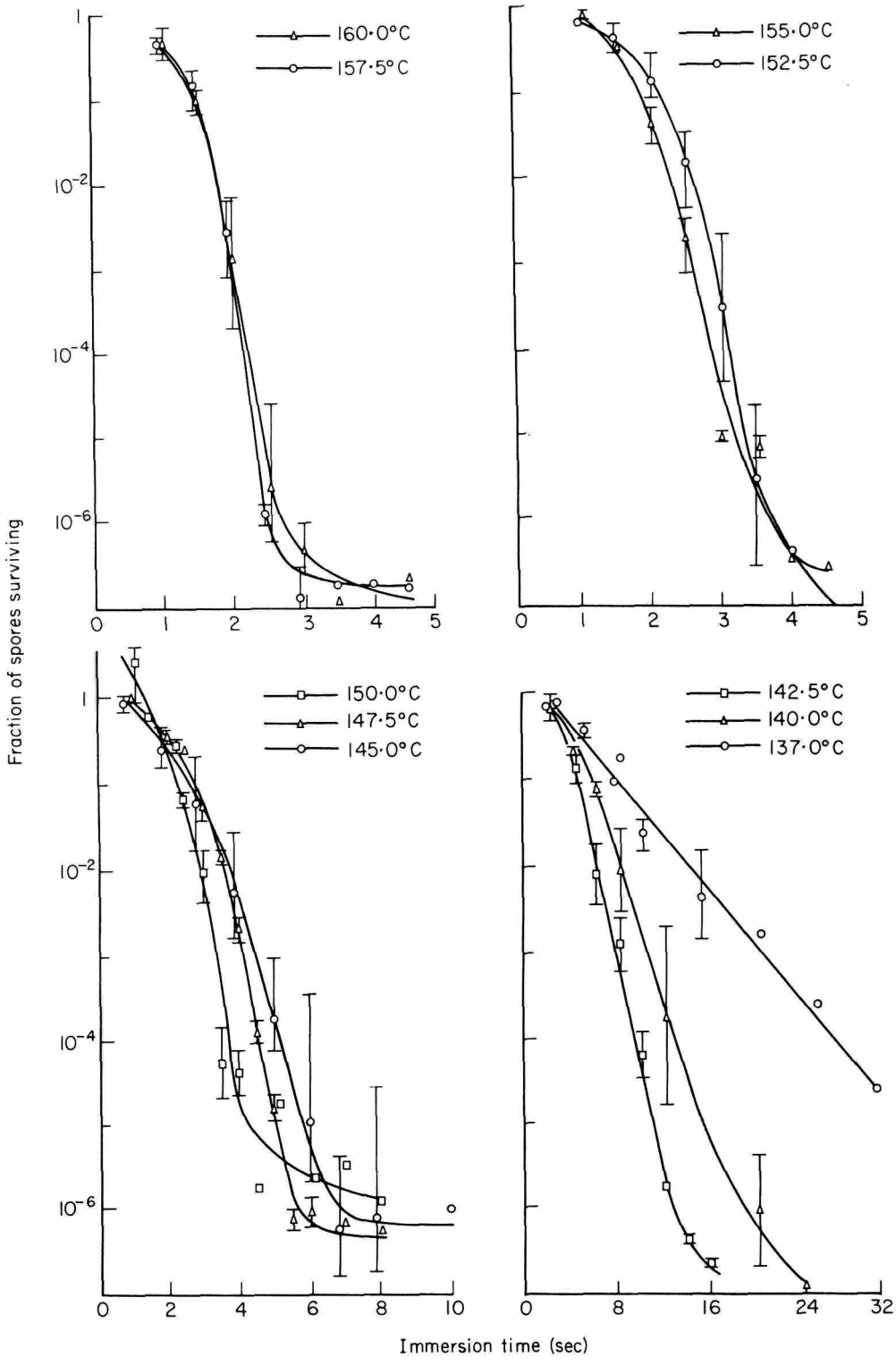
Recovery of heated spores

Immersion of capillaries in the cooling bath was sufficient to remove the heating fluid due to its detergent nature. Their outer surfaces were treated with sodium hypochlorite (10–12% available Cl_2) for at least 10 min then washed with six changes of sterile distilled water. Each set of ten capillaries corresponding to each heat treatment was transferred to 1oz sterile Universal bottles and shaken with glass beads and 10 ml sterile distilled water using a wrist action (Gallenkamp) shaker until all capillaries were ground to a coarse powder and the heat treated spores released. This system was adopted only after preliminary comparative experiments in which it was established that the crushing and shaking techniques gave similar results to more laborious extensive 'wash-out' methods, i.e. its abrasive effect caused no apparent reduction in survivor levels. The resulting suspension, after suitable decimal dilutions, was plated in BSA, the plates dried for 30–45 min at 37°C and incubated at 55°C for 48 hr before counting colonies.

Results and discussion

Shape of survivor curves

Spores suspended in whole raw milk or water were heated in capillaries at temperature intervals of 2.5°C between 120 and 160°C for times selected to give measurable numbers of survivors. Some variation was apparent in the results of replicate experiments and survival curves were drawn by inspection through their mean values (Figs 2 and 3). There was a gradual change in the shape of both sets of curves from high to low temperatures; those below 137.5°C most closely approaching linearity, i.e. indicating an exponential mode of death, though there was some evidence of tailing. At higher temperatures, especially above 142.5°C, the curves assumed a more sigmoidal shape through the appearance of definite shoulders in addition to tailing. Such deviations from the exponential are not uncommon and have been discussed by several authors, e.g. Vas & Proszt (1957), Roberts & Hitchins (1969) and Russel (1971).


160·0°C
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137·0°C
Fraction of spores surviving
Immersion time (sec)

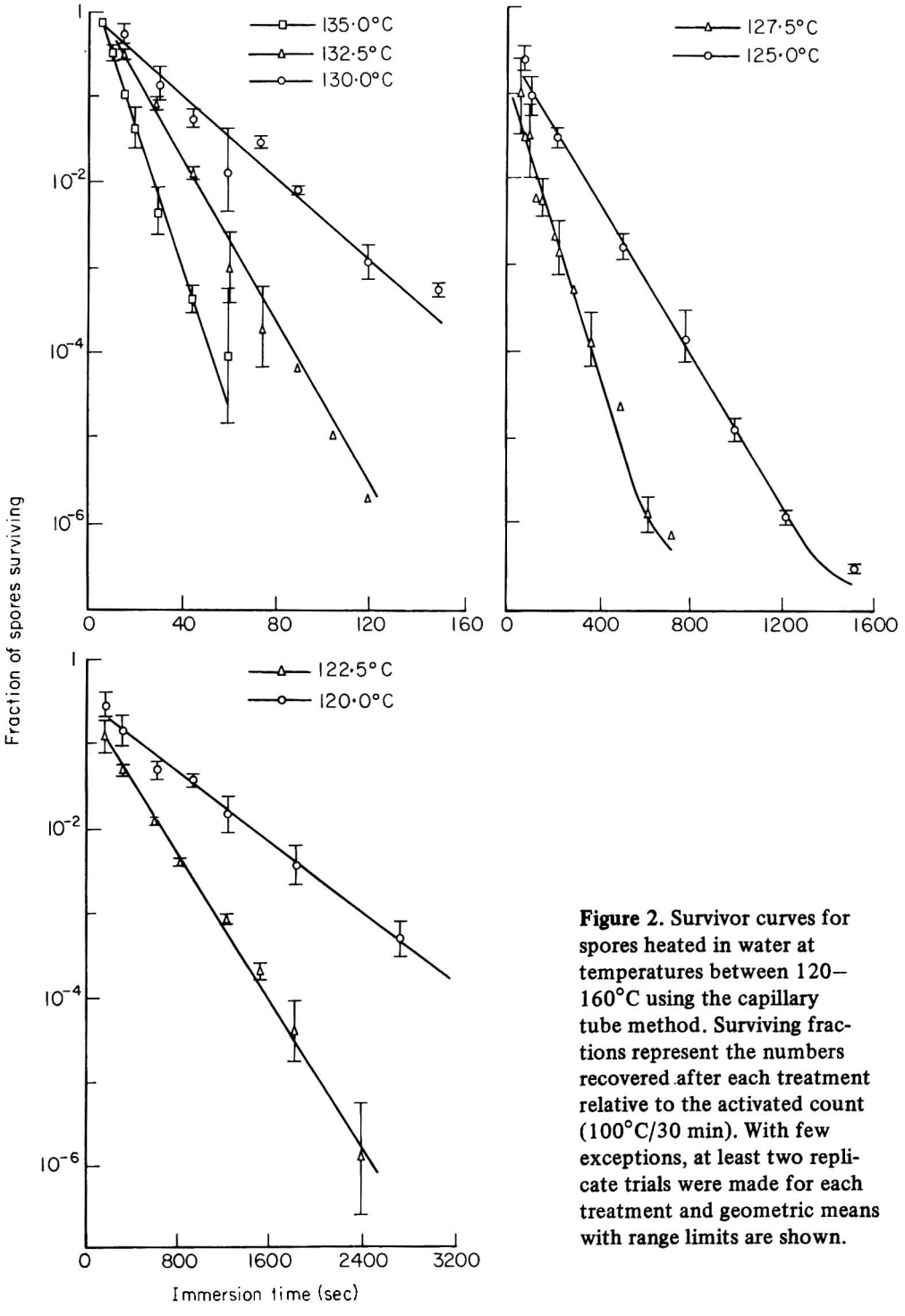
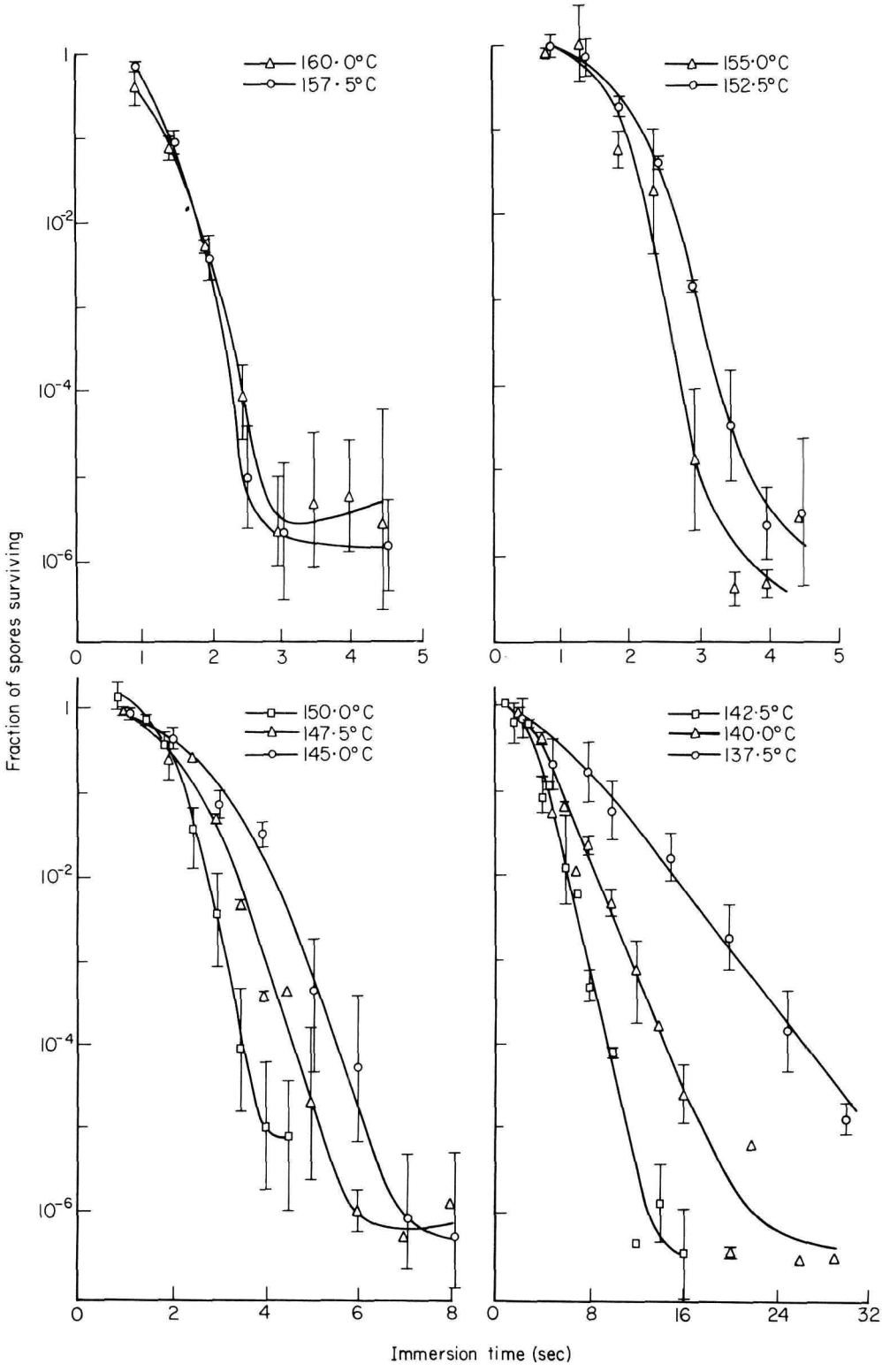


Figure 2. Survivor curves for spores heated in water at temperatures between 120–160°C using the capillary tube method. Surviving fractions represent the numbers recovered after each treatment relative to the activated count (100°C/30 min). With few exceptions, at least two replicate trials were made for each treatment and geometric means with range limits are shown.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Fraction of spores surviving
Immersion time (sec)

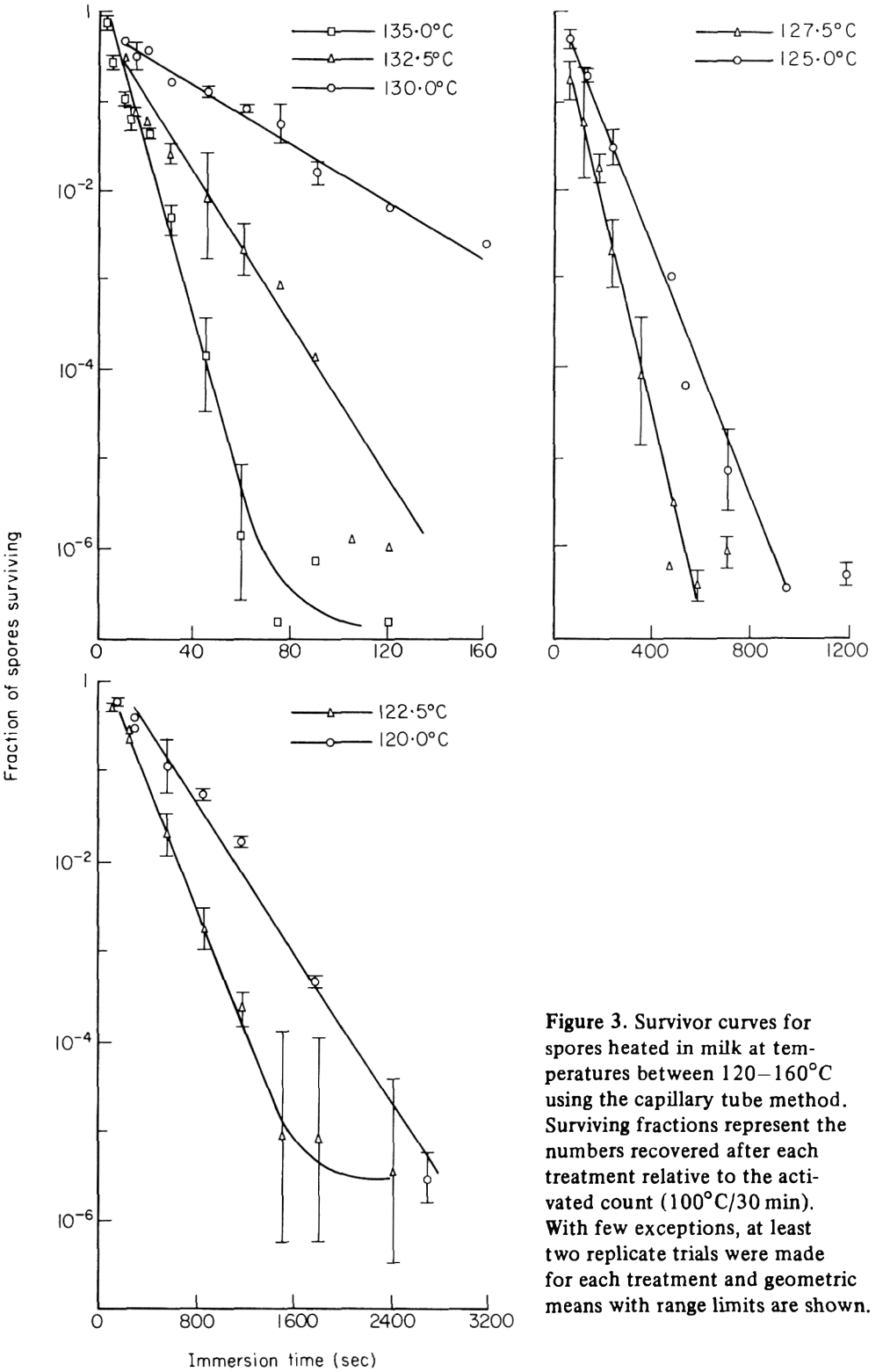

Figure 3. Survivor curves for spores heated in milk at temperatures between 120–160°C using the capillary tube method. Surviving fractions represent the numbers recovered after each treatment relative to the activated count (100°C/30 min). With few exceptions, at least two replicate trials were made for each treatment and geometric means with range limits are shown.

The most commonly accepted explanation for the tailing effect is that there exists in spore populations a small fraction (1 in 10^6–10^8) with resistance appreciably higher than the rest. Where tailing was apparent here, it occurred below survivor ratios of 1 in 10^5 and usually below 1 in 10^6, the precise points being difficult to locate since at these levels surviving numbers of spores were very low (less than 10 colonies per plate) and enumeration therefore, inherently less accurate. The apparent slight rises in the tails of some of these curves is probably an artifact also resulting from inaccuracy at very low survival levels, though the possibility of secondary activation of a resistant fraction cannot be excluded.

In an attempt to determine whether survivors of severe heat treatment, i.e. those normally responsible for tailing, would, after further propogation, produce progeny which also show elevated resistance, a spore crop was prepared from survivors after heating at 145°C for 18 sec and compared with spores of the original bulked suspension in a series of heat treatments at 145°C. No evidence was obtained of selection for elevated heat resistance, both suspensions giving similar survivor curves in accordance with the findings of Vas & Proszt (1957) and Moats, Dabbah & Edwards (1971). Recent work by Han (1975) showed clearly that concave survival curves for heated *B.cereus* spores were due to heterogeneity in heat resistance of the spore population though it was not possible to determine whether this reflected the innate characteristics of the spore population before heating or acquisition of heat resistance as a result of heating. It is likely that the tailing effects observed here can be similarly explained, though from the shape of the survivor curves a less normal distribution of resistance seems probable.

Shoulders on survivor curves are usually regarded as indicating activation of spores to an extent greater than that resulting from the activation treatment used to obtain the control or initial count, in this case 100°C for 30 min. An alternative explanation, favoured by Vas & Proszt (1957) ascribes the sigmoid shape of the whole survival curve to a distribution of heat resistance among members of a spore population. The results obtained here cannot be entirely explained by either of these theories since shoulders were evident only at higher temperatures. In the absence of precise data concerning relative energy requirements for thermal death and for activation, it is not possible to predict whether such a range in degree of activation would be expected at different time/temperature combinations which result in similar thermal death levels. Neither is there supporting evidence available to suggest that the distribution of resistances within a spore population varies at different temperatures as would be implied by the results here if the second theory were accepted.

A third, and more likely possibility to at least partially explain our results, is that the rate of heat transfer from the oil bath to the spore suspension becomes more significant at higher temperatures. Thus at 120°C, with long heating times in the order of minutes, heat transfer time must be insignificant and the spore suspension will be in equilibrium with the heating fluid temperature for almost the entire measured heating period; at 160°C on the other hand with treatment

times as short as 1–5 sec, the heating times are more likely to constitute an appreciable part of the treatment period. A further investigation of this possibility is reported in paper II (Perkin *et al.*, 1977) of this series.

Thermal resistance of B. stearothermophilus *TH24 spores*

Decimal reduction times or D values can readily be calculated from the straight line survival plots for lower temperatures. At temperatures of 145°C and above, however, the sigmoidal character of the survivor curve makes this more difficult so that the common practice of ignoring the shoulder and tail and drawing, by inspection, a best fitting straight line through the remainder of the points was adopted. D values so obtained are shown in Table 1. At temperatures of 120–125°C decimal reduction times were much longer for spores in water than in milk whereas at higher temperatures there was little consistent difference between the two. In experiments at temperatures of 110–120°C, Franklin *et al.* (1959) obtained for spores of this same strain of *B.stearothermophilus* higher D values in water than in milk and an examination of these latter data along with results obtained here suggest that the D values for spores heated in these two menstrua become increasingly divergent with lowering temperatures, indicative of differing Q_{10} values.

Table 1. D values from straight lines drawn by inspection through survivor curves for *B.stearothermophilus* TH24 heated in water and in milk

Treatment temperature (°C)	D value (sec)	
	Water	Milk
120.0	1000.0	468.0
122.5	461.0	265.0
125.0	216.0	140.0
127.5	95.0	100.0
130.0	42.6	62.7
132.5	21.6	23.8
135.0	12.5	10.8
137.5	6.16	5.65
140.0	2.45	3.02
142.5	1.60	1.73
145.0	0.73	0.73
147.5	0.53	0.58
150.0	0.46	0.40
152.5	0.32	0.32
155.0	0.33	0.23
157.5	0.18	0.24
160.0	0.22	0.32

Table 2. Heat resistance of spores prepared under different conditions

Inoculum	Sporulation temperature (°C)	*D* value at 120°C (sec)	
		Grown from 7 yr old spore suspension*	Grown from 0.5 yr old spore suspension
Vegetative cells	55	660	735
Vegetative cells	60	690	705
Spores	55	705	820
Spores	60	900	990

*Current *D* value at 120°C = 350 sec.

The absolute values of *D* obtained by Franklin *et al.* (1959) were considerably shorter at 120°C (water *c.* 382 sec, milk *c.* 220 sec) than those reported here, suggesting that spore crops of *B.stearothermophilus* TH24 prepared for the present study were more resistant than those used previously. The only obvious differences in the manner of their preparation were the higher sporulation temperature (60°C as opposed to 55°C) and use of spores rather than vegetative cells to inoculate the Roux bottles in the current work since this inexplicably gave higher spore yields. In order to test whether either of these two factors were responsible, fresh spore crops were prepared at the two sporulation temperatures and using both types of inocula. The spore inocula were taken from the 6-month-old bulk suspension used throughout this work and also from a 7-year-old suspension which had been prepared using vegetative inoculum and sporulation temperature of 55°C and was found to currently possess a *D* value in water of 350 sec at 120°C (i.e. consistent with the value reported by Franklin *et al.*, 1959); vegetative inocula were also prepared from these two suspensions. *D* values obtained by the methods described above, at 120°C for the resulting eight suspensions are shown in Table 2 and while they suggest that increased resistance is probably favoured by the higher sporulation temperature and spore inoculum, they are all substantially higher than either the values of Franklin *et al.* (1959) or those of the 7-year-old suspension. Neither these two factors, nor genetic selection for increased resistance are therefore sufficient to account for the higher *D* values obtained in this work and some other undefined change(s) in methodology or media composition must be responsible.

Temperature coefficients for spore inactivation

In order to determine whether Q_{10} was constant over the temperature range studied, the relationship between log *D* values from 120 to 160°C was examined (Fig. 4). For spores heated both in water and in milk this relationship was linear, i.e. the Q_{10} was constant, at lower temperatures but deviated from

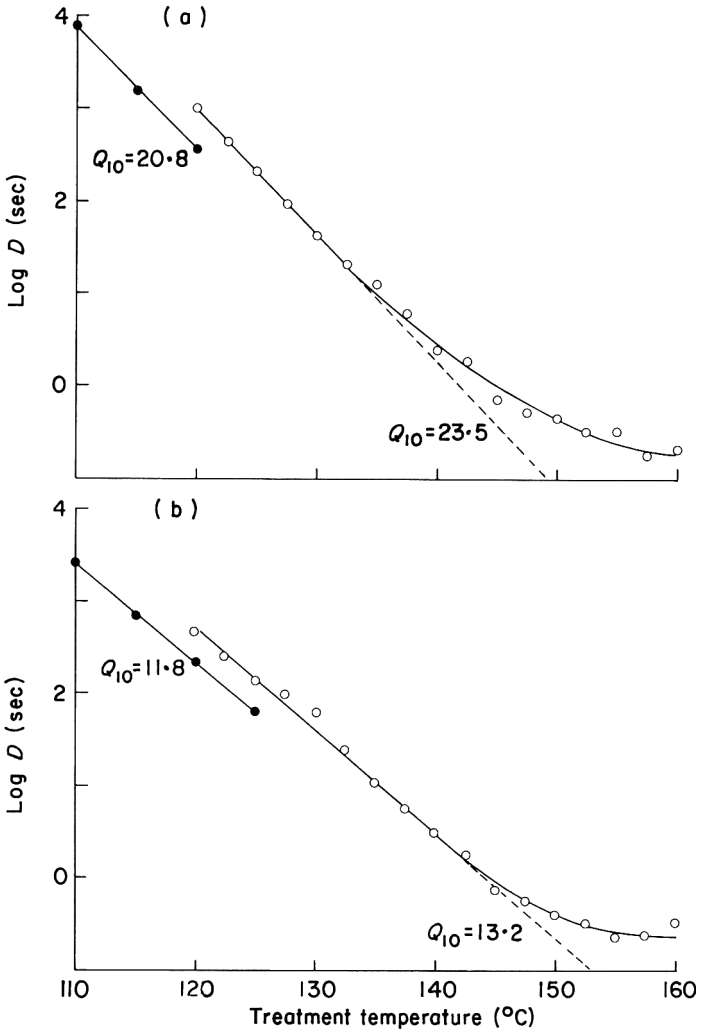

Figure 4. The relationship between log D and temperature for spore inactivation over the range 110–160°C; (a) spores heated in water, (b) spores heated in milk. ○——○ results obtained in current study; ●——● results calculated from Franklin *et al.* (1959); – – – – – extrapolation of linear relationship obtained at lower temperature.

linearity showing a progressive decrease in Q_{10} above about 132.5°C in the case of water and 142.5°C in the case of milk. The scatter of results, especially for spores in milk, makes it difficult to determine precisely the points at which linearity fails and illustrates well how even small deviations in such data can influence appreciably the derivation of Q_{10} values; clearly however, the departure from linearity is maximal at high temperatures and short heating times. The actual Q_{10} values over the linear portions of the curves were different for milk (13.2) and water (23.5) but in agreement with the values calculated from data previously reported (Franklin *et al.*, 1959) for spores of this organism between 110–125°C despite the discrepancy in actual heat resistance found on the two occasions (see above and Fig. 4 where essentially parallel curves resulted).

Although the points at which the coefficients depart from linearity cannot be deduced with absolute precision it is clear that the data do show such an effect and agree with those of Miller & Kandler (1967) who reported a decreasing Q_{10} for spores of *B.stearothermophilus* NCA 1518 when heated over about 120°C. It is not certain, however, whether such an effect is real or an artifact, again resulting from heat transfer effects which, as described above, become more critical as higher temperatures and shorter times are reached. It has been pointed out that the linear relationship of death rate constants is simply an approximation of Arrhenius behaviour valid only over a narrow temperature range (Rahn, 1945) so that a curvilinear relationship might be expected over the range studied here (120–160°C); it seems unlikely, however, that the shape of the curves in Fig. 4, with substantial linear portions and pronounced curvature

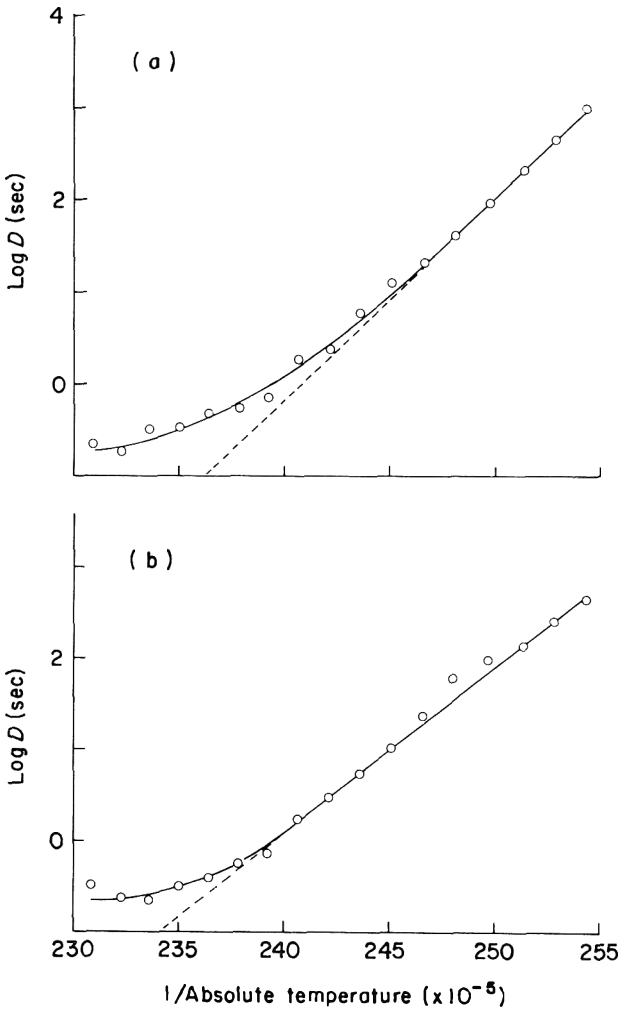

Figure 5. The relationship between log D and the reciprocal of absolute temperature for spore inactivation over the range 110–160°C; (a) spores heated in water, (b) spores heated in milk. – – – – extrapolation of relationship obtained at lower temperature.

(indicating Q_{10} values unrealistically diminishing to unity) at only one end of the temperature scale could be explained by such thermodynamics. This is confirmed by Fig. 5 where the same results are expressed as Log D against the reciprocal of absolute temperature; the curvilinearity at higher temperatures is not reduced so that it cannot be attributed to Arrhenius kinetics.

Before such data can be confidently used in comparison with those from UHT plants or used to predict the sporicidal efficiency of the latter, it is necessary to determine the reason for the departure from linearity, e.g. does it result from the physical limitations of the capillary tube system and is it possible to compensate for this effect? This possibility will be discussed in detail in the next paper of this series (Perkin *et al.*, 1977).

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Thermal death kinetics of *Bacillus stearothermophilus* spores at ultra high temperatures

II. Effect of heating period on experimental results

A. G. PERKIN, H. BURTON, H. M. UNDERWOOD AND F. L. DAVIES

Summary

The effect of the heating period on the death of bacterial spores when heated in capillary tubes was investigated. Theoretical equations for the calculation of an equivalent heating time if there were no heating up period were derived and a computer programme developed to carry out the necessary analysis. The temperature distribution within a capillary tube during heating was determined by experiment so that the parameters for the analysis could be obtained.

Results showed that the heating period has a negligible effect for treatment temperatures up to 135°C. Above this temperature the heating period becomes increasingly more significant and analysis of this kind can be used to obtain more reliable data. It was shown that thermal death rates defined by semi-logarithmic curves increase sensibly in accordance with Arrhenius kinetics up to temperatures of the order of 150°C.

Introduction

The first paper in this series (Davies *et al.*, 1977) described a method for determining thermal death curves of organisms using bacterial spore suspensions sealed in glass capillary tubes and heated by immersion in a temperature-controlled liquid bath. It was suggested in that paper that as the treatment temperature was raised and the immersion time correspondingly reduced to substantially less than one minute, the heating period for the spore suspension would become a progressively more important part of the heat treatment and could possibly account for the shoulders on the survivor curves.

The present paper investigates the effect of the heating period on the death of the bacterial spores and shows how a correction can be applied so that more reliable thermal death data can be derived even when the lethal effect of the heating period becomes a significant proportion of the total effect.

When a capillary containing spore suspension is placed in a heating bath the

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temperature within the suspension varies both with position within the capillary and with time after immersion. Because of the lag in temperature rise the effective holding time will be less than the actual immersion time, the effect becoming more important with increase in temperature and with reduction in immersion time. There will also be a similar effect during cooling. In the canning industry these effects are recognized and dealt with by calculating effective heating times for the slowest heating point in the can (Jacobs, Kempe & Miloar, 1973). This is satisfactory when minimum safe processing conditions are being determined, but it is not suitable for the correction of laboratory thermal death data. In this case it is necessary to integrate the sporicidal effect throughout the whole volume of suspension for all temperatures and all times and use the integrated effect to modify the experimental holding times by deriving a correction.

Equations for calculating lethal effect

Consider a suspension of spores sealed in a capillary as a cylinder of length l and radius R which has been inoculated with a total number of spores N. The suspension is preheated to a temperature θ_p and then placed in a heating bath, maintained at a temperature θ_s, for a total time T. For the capillaries used, the length of a cylinder of suspension is very much greater than the radius so that the transfer of heat can be considered radial, with a negligible temperature gradient along the length of the cylinder. Figure 1 shows an annular element formed between an inner cylinder of radius r and an outer cylinder of radius $r + dr$. If dr is very small, then all the suspension contained within this annular element can be assumed to be at the same temperature at any time.

The volume of the annular element = $2\pi r\, dr\, l$.
If it is assumed that there is an even distribution of spores within the suspension then the initial number of spores in this volume

$$= \frac{\text{Volume of element} \times \text{Total number of spores}}{\text{Total volume}}$$

$$= \frac{2r\,N\,dr}{R^2}$$

If the thermal death of the organism is logarithmic (Burton, 1958) then the change in the number of surviving organisms with time t, at temperature θ_t, is given by

$$dL_t = -K_{\theta_t}\, dt$$

where L_t = log (proportion of survivors at time t),

K_{θ_t} = slope of thermal death curve at temperature θ_t, and

θ_t = temperature at time t, which will increase steadily towards θ_s as $t \to \infty$.

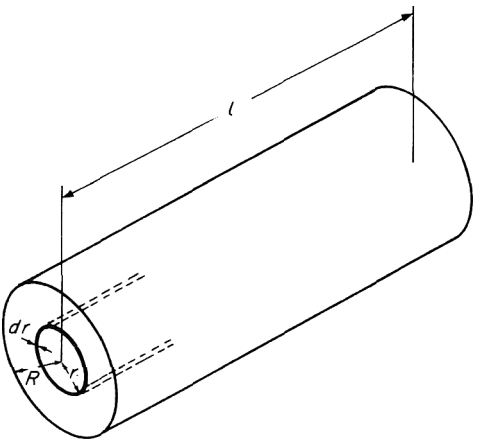

Figure 1. Sketch showing annular element.

As the temperature rises through a total heating time T, the final proportion of survivors

$$L_T = \int_0^T -K_{\theta_t}\, dt$$

where K_{θ_t} varies with time as the temperature varies with time.

The proportion of survivors = 10^{L_T}.

The final number of spores in this volume after heating

= Original number spores x Proportion of survivors

$$= \frac{2rN\, dr}{R^2} \times 10^{L_T}.$$

Then the total number of survivors in the whole volume

$$= \int_0^R \frac{2rN\, 10^{L_T}}{R^2}\, dr$$

$$= \frac{2N}{R^2} \int_0^R r\, 10^{L_T}\, dr.$$

The proportion of survivors = $\dfrac{\text{Number of survivors}}{\text{Original number of spores}}$

$$= \frac{2}{R^2} \int_0^R r\, 10^{L_T}\, dr. \qquad (2)$$

Let T_a be the time required to give this proportion of survivors if the suspension had been heated instantaneously to the bath temperature θ_s and cooled instantaneously from it. Thus T_a is the equivalent time if there were no heating up or cooling down periods.

Then $L = -K_{\theta_s} T_a$

and the proportion of survivors $= 10^{-K_{\theta_s} T_a}$. (3)

Then, since this proportion is the same as that given by equation (2)

$$10^{-K_{\theta_s} T_a} = \frac{2}{R^2} \int_0^R r\, 10^{L_T}\, dr$$

$$\therefore\ T_a = -\frac{1}{K_{\theta_s}} \log\left[\frac{2}{R^2} \int_0^R r\, 10^{L_T}\, dr\right]. \tag{4}$$

If the value of L_T obtained from equation (1), which is a function of the radius r, is substituted in equation (4) then the equivalent holding time for the suspension can be calculated. This involves a double integration, one over distance and the other over time.

To perform the integration, it is necessary to know the temperature distribution within the suspension both in space and time, and the way in which the rate of thermal death (as defined by the Decimal Reduction Time D, or the slope of the thermal death curve, K) varies with temperature. Since the variation with temperature can only be found with accuracy after the original thermal death curves have been corrected for thermal lags as described, an iterative procedure is needed which gives a progression towards the true values. The way this is done will be clear from the description of the procedure.

Theoretical analysis

A complete theoretical analysis of temperature variations during heating is not possible as certain data are not available. For example the specific heat, conductivity and density of spore suspensions at high temperatures (100–160°C) are not known. Neither is it easy to obtain heat transfer coefficients for the transfer of heat from the heating medium to the glass, through the glass, from the glass to the suspension or through the suspension. However, a combination of theoretical analysis with temperature measurement can be used to give the necessary data.

In capillaries of small diameter in relation to their length, such as those used in these experiments, heat transfer by convection will be negligible and it can

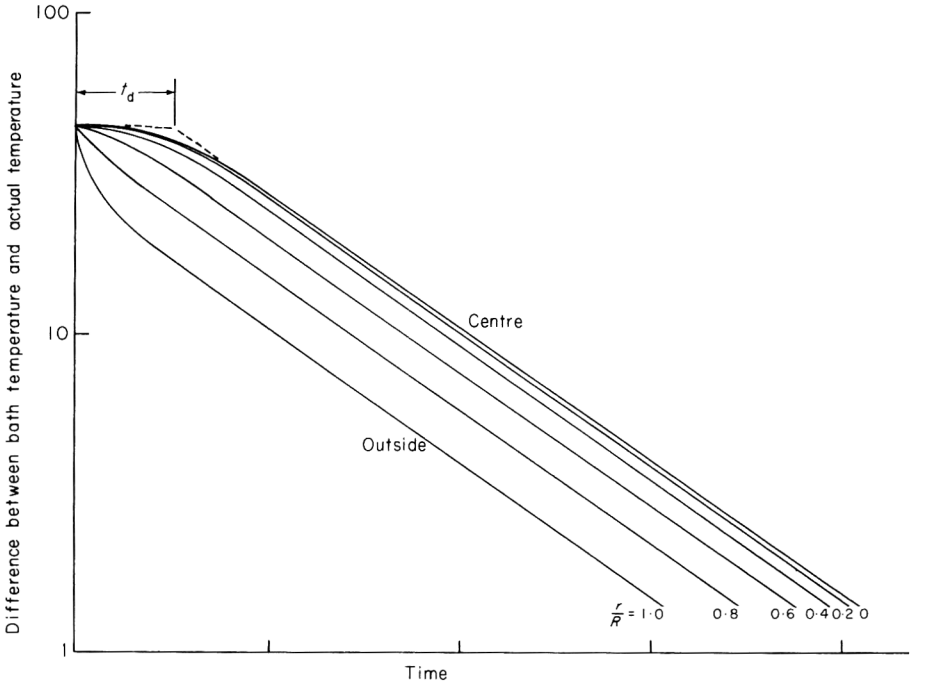

Figure 2. Temperature change at different radii.

be assumed that heating is by radial conduction only and end effects can be ignored.

The transfer of heat from the heating liquid through the glass wall to the suspension can be represented by a single heat transfer coefficient. This form of heating has been intensively studied by many workers (Olson & Jackson, 1942; Carslaw & Jaeger, 1947; Jakob, 1949; Kreith, 1958) and the temperature distribution throughout the body can be found using published data. Figure 2 shows the type of result obtained when a solid cylinder is heated by means of a sudden environmental temperature rise, as would be expected by plunging the cylinder into a well-agitated, heated bath (adapted from Jakob, 1949). The difference between the bath temperature and the temperature at radius r is plotted on a logarithmic scale against the time after immersion, for various positions inside the cylinder as represented by the ratio of radius r divided by the outside radius R. The time scale is a function of linear dimensions and thermal diffusivity, and therefore is given no units in this general example. After a short initial period the response is linear, indicating that this form of heating can be modelled by a first order system of equations, the solution of which is an exponential. The slight deviation from a straight line during the initial period of time can be accommodated as shown by the dashed line in Fig. 2 where the temperature at the centre of the cylinder remains constant for a period of time t_d before rising exponentially. It can be seen that any error introduced in predicting the temperature variation is only small and is confined to the short initial period of heating where the effect on rate of thermal death

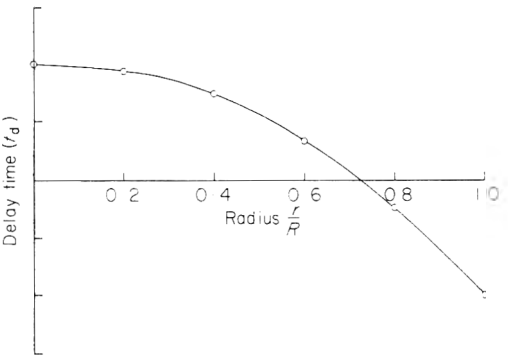

Figure 3. Delay time at different radii.

of organisms is in any case negligibly small. The delay before the temperature rises will be a function of position (radius) within the cylinder and for this ideal case will be negative for positions towards the outside of the cylinder.

Figure 3 shows the way in which the delay time t_d varies with radius of the cylinder for this ideal case: as in Fig. 2, no scale is given for t_d. For the exponential rise in temperature,

$$\theta_t = A - B\, e^{-t/\tau} \tag{5}$$

where θ_t = temperature at time t, A and B are constants and τ is the time constant.

The time constant τ, is the time during which the temperature changes by 63.2% of the difference between the initial and final temperatures and can be found from the slope of the lines in Fig. 2. Since all the lines in Fig. 2 are parallel, it follows that the time constant τ will itself have a constant value which is independent of the initial and final temperatures. It appears therefore that, for the case of a cylinder heated by conduction only, the temperature at any point and any time can be found by assuming a short delay period which is a function of radius, followed by an exponential rise in temperature characterized by a constant time constant. However, the magnitudes of the constant time constant and the delays which are a function of radius, cannot be determined theoretically and must be derived from measurements. It is shown below that the derived temperature variations are as given in equations (7) and (8).

Experimental determination of temperature distribution

Due to the small size of the capillary tubes (100 mm x 1 mm i.d.) it was not possible to accurately measure the temperature distribution throughout the suspension. However it was possible, using a microminiature thermocouple, to measure the temperature changes at certain points and from these data to derive the characteristics which define the distribution of temperature.

Method of temperature measurement. A copper-constantan thermocouple (type TCC-ES-50, BLH Electronics Inc., Waltham, Massachusetts) was used to measure the temperature within the capillary. The thermocouple as supplied

consisted of 0.02 mm wire contained within a 0.34 mm o.d. stainless steel tube, with the junction exposed beyond the tube. The stainless steel tube was held in a 2 mm o.d. ceramic mounting. For the experimental measurements, the stainless steel tube was inserted into the capillary until the thermocouple junction was 12 mm from the end and the ceramic mounting was sealed to the end of the capillary with epoxy resin. A larger stainless steel tube, 3.3 mm o.d. and 60 mm long, was cemented round the ceramic mounting with epoxy resin so that the whole assembly could be conveniently manipulated by hand. The construction is shown in Fig. 4. Distilled water was inserted into the capillary tube with a microsyringe and the tube sealed as in the bacteriological tests (Davies *et al.*, 1977).

The thermocouple was connected to a cold junction held in ice and the signal after amplification passed to an ultra-violet recording oscillograph (Type 5-127, Bell & Howell Ltd, Basingstoke, Hants.). The couple was calibrated by placing the capillary tube in the heating bath and, after the temperature had stabilized to the bath temperature, comparing the recorder reading with the temperature indicated by a mercury-in-glass thermometer placed next to the capillary tube. A second bare thermocouple was positioned outside the capillary tube in order to indicate the instant of immersion in the heating fluid. The time constant of the bare thermocouples was measured and found to be 40 msec, which was negligible in relation to the rates of temperature change to be measured.

The capillary and thermocouple assembly was manipulated into and out of the heating liquid as in the bacteriological tests (Davies *et al.*, 1977) and recordings of the temperature changes at the thermocouple junction were obtained. The delay and time constant are independent of the bath temperature and all measurements were therefore made at the same temperature, 145°C.

The radial position of the thermocouple junction within one capillary was fixed. It was determined by a jig during assembly and confirmed by microscopic examination after curing of the resin. To obtain temperature curves at different radii, different assemblies were constructed using the same thermocouple.

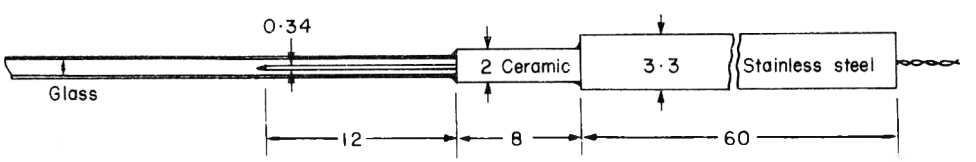

Figure 4. Assembly of thermocouple and capillary tube (thermocouple wire ≙ 0.02 mm diameter copper-constantan); dimensions in mm.

Results of temperature measurements. Figure 5 shows a typical temperature record, in this example with the thermocouple junction positioned on the centre line of the capillary tube. The general shape of the curve obtained was independent of the position of the thermocouple junction within the capillary tube. Fig. 6 shows the same data plotted to a logarithmic scale of temperature

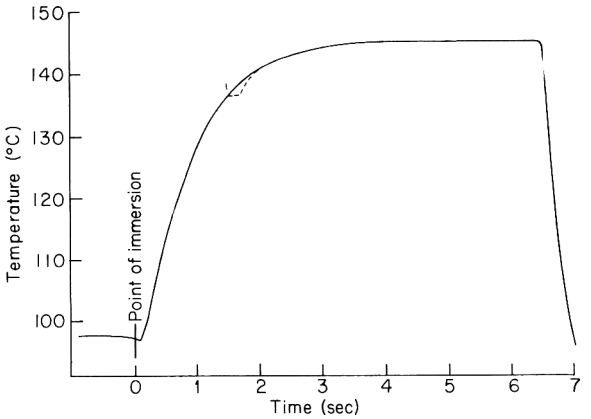

Figure 5. Typical temperature record with the thermocouple on the centre line of the capillary tube.

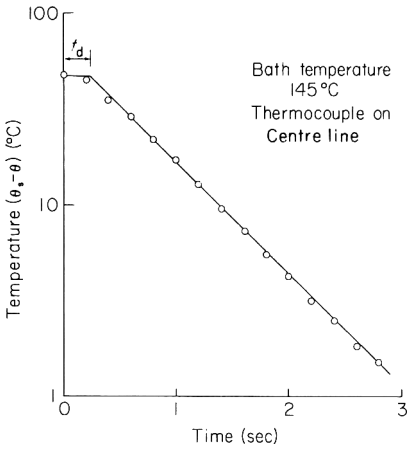

Figure 6. Typical temperature record plotted with logarithmic scale of temperature deficit.

deficit. This is of the theoretical form shown in Fig. 2 and demonstrates that the rise of temperature found in practice can in fact be represented with very little error by a short delay period followed by an exponential temperature rise. For each experimentally determined temperature record, the experimental results were plotted as shown in Fig. 6 and the best fitting straight line for the results between 1 and 3 sec was calculated using the method of least squares and the time constant was calculated from this straight line. The delay period was taken as the time at which this straight line when extended below 1 sec reached the preheat temperature. Fig. 7 shows the values of the calculated time constant, and Fig. 8 the values of time delay, for all the temperature records obtained, plotted against the radial position of the thermocouple junction within the capillary.

There is a large scatter of experimental results and it would be difficult to justify choosing the lines drawn through the points in Figs 7 and 8 if it were not for the fact that the shape of the curves had been predicted theoretically.

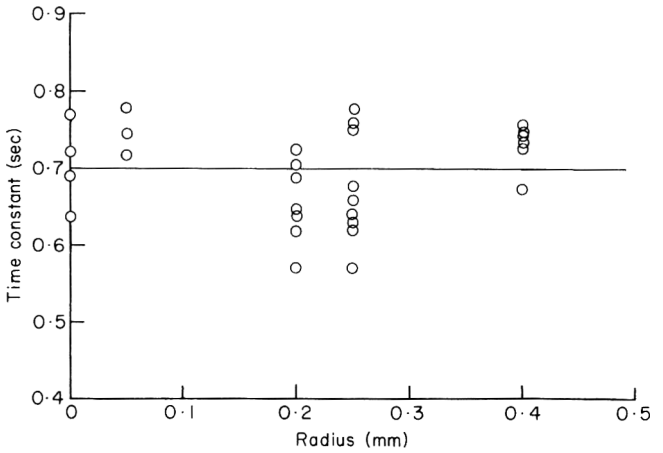

Figure 7. Calculated time constants.

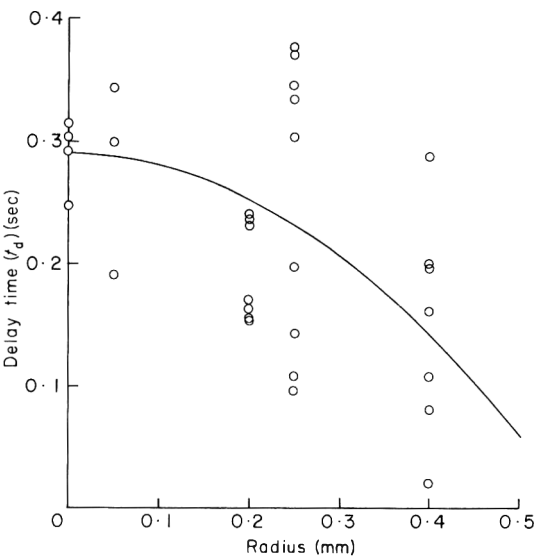

Figure 8. Delay times.

Limitation in the experimental procedure would be expected to account for variations in the results.

Sources of error in the temperature measurements. The capillary tubes were sealed by heat fusion at the end of each tube. This procedure could not guarantee that the length, and thus the enclosed volume, of each sealed capillary was exactly the same. During the sealing process the air within the tube was heated and the resultant expansion expelled some air from the tube. After sealing and cooling to room temperature the pressure within the tube was less than atmospheric and almost certainly varied from tube to tube. When a capillary tube containing an aqueous suspension is heated in the bath to a temperature above the boiling point corresponding to the pressure in the tube, some of the liquid must evaporate to increase the internal pressure to that corresponding to the

final temperature. For the capillary tubes used, the amount of heat required in evaporating sufficient liquid to prevent boiling on heating to 145°C is about 4% of the total heat required to raise the capillary and liquid to the final temperature. This amount depends on the initial volume and pressure within the capillary. It appears therefore that the size of capillary tube and the method of sealing will affect the heat load of the capillary tube and hence the shape of the heating curve. In approximately 10% of the tests performed a small kink appeared in the ultra-violet recorder curves as shown by the dotted line on Fig. 5. It is possible that this was due to sudden boiling of the liquid. In tests with spore suspensions it was also observed that the column of liquid occasionally broke up into a number of separate segments during the heating process, which again could be caused by sudden boiling of the liquid, with consequent temporary modification of the heat transfer conditions.

Measurement of the glass capillaries showed that there was a 12% variation in diameter and for temperature measurement tests the capillaries were specially selected so that they all had the same outside diameter. There is no certainty that all the tubes had the same wall thickness, so there is still the possibility that the rate of heat transfer through the glass varied for different tubes.

The bath used to heat the capillary tubes had a very large volume of heating liquid as compared to the volume of the capillary tube and the liquid was stirred vigorously. The general movement of liquid, however, was across the capillary tube rather than along its length, and this resulted in non-uniform radial heating. One capillary tube with a thermocouple inserted into the tube and positioned close to the outside wall, was heated in the bath with the tube positioned so that the thermocouple was towards the general direction of flow of heating liquid. This test was repeated with the thermocouple positioned to the left, to the right and away from the general direction of flow. The time taken for the temperature indicated by the thermocouple to change by a fixed amount was measured and the results are shown in Table 1. These results indi-

Table 1. Time for temperature to change 95% of the initial difference in temperature between the spore suspension and the heating bath

	Position on thermocouple relative to the general flow of heating liquid			
	Towards (sec)	Left side (sec)	Right side (sec)	Away from (sec)
	2.04	2.37	2.35	2.39
	2.23	2.49	2.28	2.44
	2.17	2.42	2.26	2.34
	2.31	2.32	2.23	2.40
Average	2.19	2.40	2.28	2.39

cate that the experimental temperature measurements are dependent on the direction of impact of the heating liquid on a capillary, even when the liquid is very thoroughly agitated.

Parameters defining rise in temperature

It was shown above that at any point within the capillary, the rise of temperature towards the bath temperature can be defined by two parameters, the time constant of the temperature–time curve, τ, and the time delay t_d, before an exponential temperature curve is established. The time constant τ is theoretically independent of position. The experimentally determined variation of time constant with radius, given in Fig. 7, confirms this and the mean time constant τ, was taken as 0.70 sec for water as the liquid being heated.

For liquids other than water, the time constant will be slightly different, since it is inversely dependent on the thermal diffusivity of the medium, $\alpha = k/c\rho$ where k = thermal conductivity, c = specific heat, ρ = density. For water, in the range 100–150°C, $k = 0.69$ J/sec m °C, $c = 4.25$ kJ/kg °C, $\rho = 940$ kg/m^3 (Ede, 1967). Corresponding values for milk in this temperature range are uncertain, but they can be estimated as $k = 0.67$ J/sec m °C, $c = 3.99$ kJ/kg °C (Perkin & Burton, 1970) and $\rho = 930$ kg/m^3. The thermal diffusivities for water and milk are therefore 1.73×10^{-7} and 1.81×10^{-7} m^2/sec respectively. Consequently a time constant of 0.70 sec for water implies a time constant of 0.67 sec for milk, and this value has been used for calculations relating to spore suspensions in milk.

The delay time t_d varies with radius r, for an assumed constant Biot number ($\bar{h}R/k$, $\bar{h}$ = surface heat transfer coefficient) as shown for example in Fig. 3. If $\bar{h}$ were known, t_d could be calculated for any value of r. However, it is impossible to know the effective values of $\bar{h}$ at the heating medium–glass–suspension interfaces. The variation of t_d with r can, however, be derived from the results given in Fig. 8, guided by the fact that the variation in Fig. 3 can be closely represented by the equation

$$t_d = P - Qr^2$$

where P and Q are constants.

The best-fitting curve of this form was therefore fitted to the results of Fig. 8, giving the relation

$$t_d = 0.29 - 0.918r^2 \quad (r \text{ in mm}). \tag{6}$$

The theoretical curve of Fig. 3 shows the time delay t_d, becoming negative at $r/R \simeq 0.73$. This effect does not appear in Fig. 8. The reason for this is that, while Fig. 3 refers to a simple cylinder, the practical results in Fig. 8 refer to a compound cylinder with the outer layer formed by the glass of the capillary.

The range of radii in Fig. 8 therefore constitutes only the smaller ratios of r/R in Fig. 3.

The variation in temperature at radius r within a capillary can therefore be described by

$$\theta_t = \theta_p \text{ for } t \leqslant t_d \tag{7}$$

where t_d is defined by equation (6)

$$\theta_t = \theta_s - (\theta_s - \theta_p) \exp[-(t - t_d)/\tau] \text{ for } t > t_d \tag{8}$$

where t_d is defined by equation (6), and $\tau = 0.7$ sec for water or 0.67 sec for milk.

As the drop of temperature on removal of the capillary from the bath is extremely rapid (see Fig. 5), no correction has been applied for the cooling period.

Calculation of equivalent heating time and analysis of thermal death data

The equivalent heating time for each individual combination of temperature and time used in the experiments was calculated from equation (4)

$$T_a = -\frac{1}{K_{\theta_s}} \log\left[\frac{2}{R^2}\int_0^R r\, 10^{L_T}\, dr\right]$$

where L_T is a function of r and is given by equation (1)

$$L_T = -\int_0^T K_{\theta_t}\, dt.$$

The temperature θ_t is a function both of radius and time as given by equations (6), (7) and (8).

The relationship between K_{θ_t} and θ_t is most generally described by the Arrhenius equation which can be expressed as

$$\log(K_{\theta_t}) = \frac{U}{\theta_t + 273} + V$$

where $(\theta_t + 273)$ is the absolute temperature corresponding to θ_t °C and U and V are constants.

Since the slope of the thermal death curve (K_{θ_t}) is required in order to calculate the corrected times and the values of the constants U and V required to find K_{θ_t} can only be derived with accuracy after correction of the raw data, it is necessary to adopt an iterative technique using a computer to carry out the analysis. A flow chart of the procedure is given in Fig. 9.

From inspection of the raw data, approximate values for the constants U and V were assumed. These were chosen to give a satisfactory visual fit to the

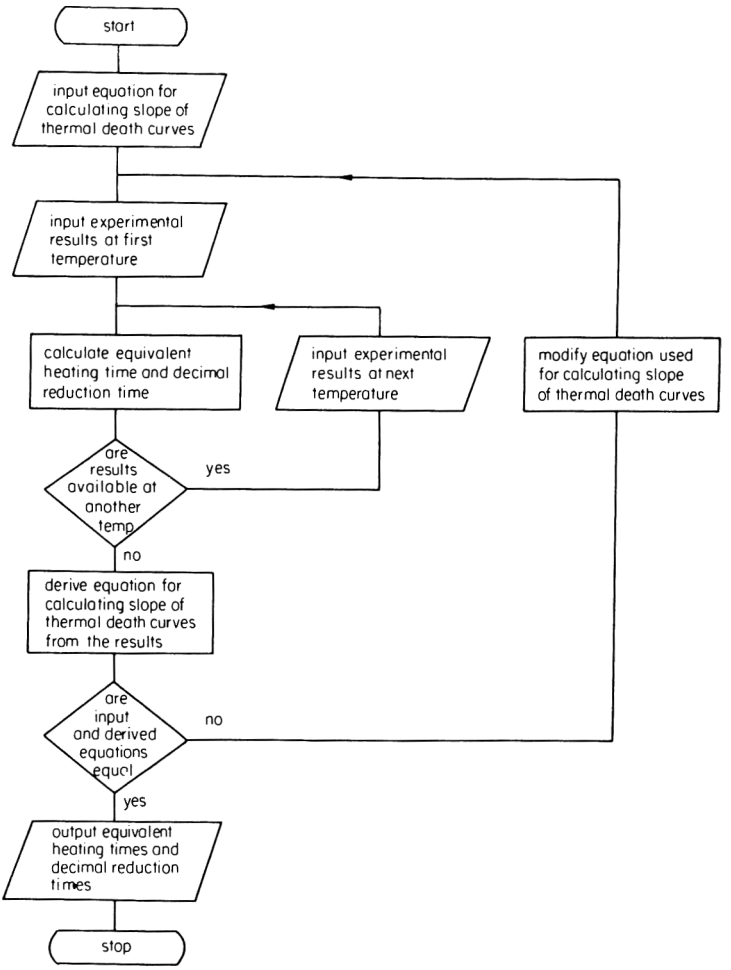

Figure 9. Flow chart for calculating equivalent heating times and decimal reduction times.

linear (low temperature) part of the uncorrected Arrhenius relation. The experimental heating times were then corrected using these assumed values, and new K_{θ_t} values were derived for each temperature by linear regression analysis of the thermal death curves. From the new values, the linear Arrhenius relation could be extended to include experimental results at higher temperatures, giving new and more reliable values of U and V, which could be used in a second stage of correction of the heating times. This process was continued until the change brought about by each successive cycle of correction was less than 0.005 in the K_{θ_t} value at any temperature up to 150°C. The limit of 150°C was set because it was felt that the potential errors above that temperature were too great for correction to be justified.

The results were finally expressed at each experimental temperature in terms of decimal reduction time (D), which is numerically equal to the inverse of the slope of the thermal death curve (K).

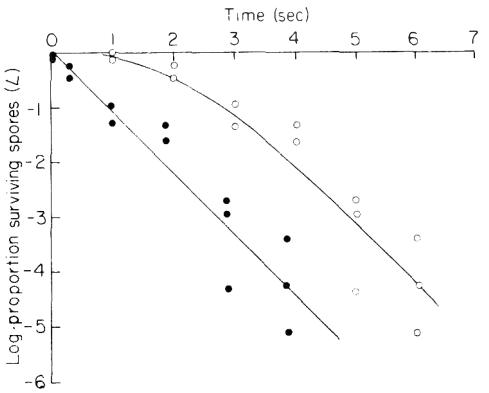

Figure 10. Effect of adjusting times on slope of line. Bath temperature 145°C; ○ calculated, ● experimental times.

The effect of correction of the heating times on the interpretation of the thermal death data can be seen in Fig. 10 which shows the corrected and uncorrected results for spores suspended in milk with the capillaries immersed in a heating bath at 145°C. The curvature shown by the raw, uncorrected data makes it difficult to derive a true slope of the thermal death curve (K) or a decimal reduction time ($D = 1/K$), particularly at the higher temperatures, for useful comparison with values obtained at other temperatures. With corrected times, however, the thermal death line can be more confidently obtained using the method of least squares.

The tailing effect at very low survivor levels is not important in a consideration of the kinetics of inactivation. Any disturbing influence of tailing has been avoided by visual inspection of the data and exclusion of any result which appeared to lie within this region.

Results and discussion

The variations of log (decimal reduction time) with the inverse of absolute temperature between 120 and 160°C are shown in Fig. 11 for spores of *Bacillus stearothermophilus* suspended in water and milk. A linear variation exists at temperatures up to about 150°C which is in agreement with the Arrhenius equation, the deviation of the individual points from the straight line being insignificant in relation to the inevitable variation in the individual bacteriological determinations. At temperatures above about 150°C there are indications of a deviation from linearity. However, the immersion times at these temperatures are very short and are almost entirely contained within the heating period of the suspension. Experimental errors in the measurement of immersion times and the correction of heating times are thus becoming increasingly more significant. The apparent deviation at temperatures above 150°C should therefore be

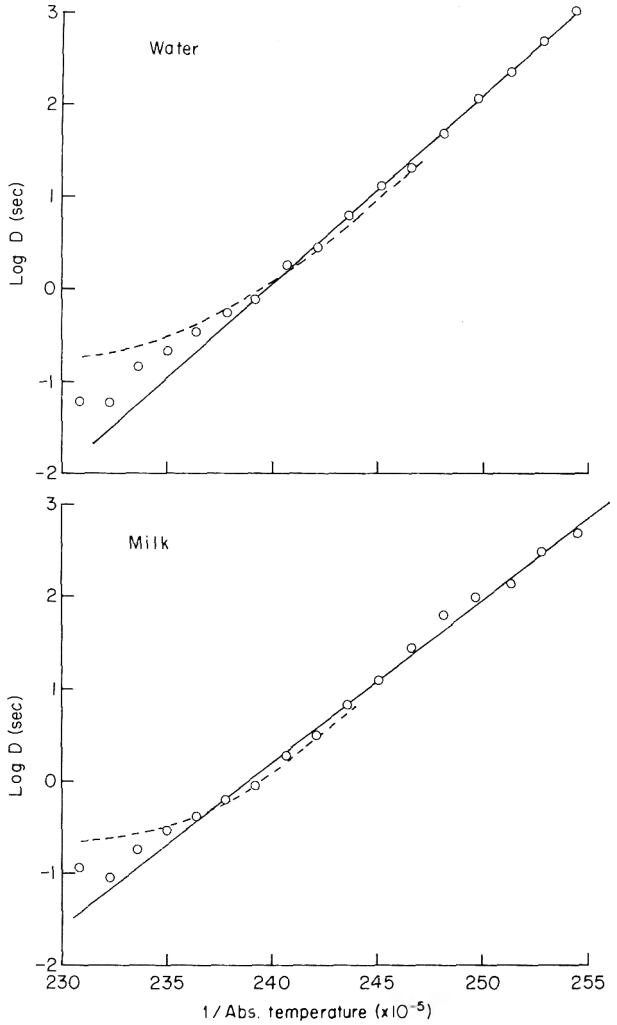

Figure 11. Variation of log D with the inverse of absolute temperature.

treated with reserve. Practical UHT processes do not usually involve treatment temperatures above 150°C.

The lines drawn on Fig. 11 indicate a fall of Q_{10} from 19 at a temperature of 120°C to 13 at a temperature of 150°C for spores suspended in water, and 14 at a temperature of 120°C to 10 at a temperature of 150°C for spores suspended in milk.

The dotted lines on Fig. 11 indicate the deviation from the straight line for the uncorrected data. Correcting the heating times has extended the linear portion of the curve allowing the line to be determined with more confidence.

The corrections to be applied to the immersion times to give the equivalent holding times, as calculated with the computer for a series of heating bath temperatures and immersion times, are summarized in Fig. 12. The required

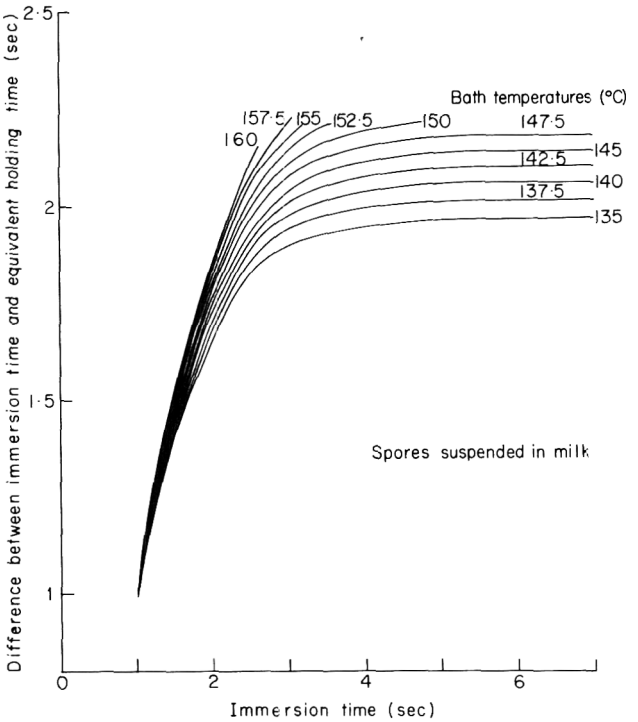

Figure 12. Correction times at different bath temperatures and immersion times.

correction tends to a constant value as the immersion time increases, and for practical purposes can be considered constant for times over 5 sec. It follows that the heating period in capillaries of the size used in these experiments has a negligible effect on the determination of the slope of a thermal death curve (the K or D values) as long as the minimum immersion time used in deriving the curve is 5 sec or above. In the present experiments, this applied to all bath temperatures below 135°C. There will be a slight displacement of the thermal death curve along the time axis, but this will become negligible as the correction (of the order of 2 sec) becomes an insignificant proportion of the immersion times used.

A thermal death curve in which any immersion time is less than about 5 sec will show a false non-linearity caused by the changes in the required correction. Interpretation of such curves requires a detailed analysis of the type presented in this paper. The analysis shows that the thermal death curves for the organisms used continued to be semi-logarithmic over a range of proportion of survivors of six log cycles as the temperature increased and that the thermal death rates defined by the semi-logarithmic curves increased sensibly in accordance with Arrhenius kinetics up to temperatures of the order of 150°C.

This would suggest that thermal death data at ultra high temperatures could be obtained by extraplolation from data obtained at lower temperatures. However, the lower-temperature data need to be extremely accurate if the extrapolation is to be reliable. Even in the present experiments, extrapolation on the

basis of lower-temperature data would have produced significant errors in estimated thermal death rates at ultra high temperatures and it is doubtful whether the data available for microorganisms at lower temperatures, particularly for *Clostridium botulinum* spores, is sufficiently accurate or could be obtained with sufficient accuracy for values to be obtained by extrapolation in the range 140–150°C with any reliability. Direct determinations at the higher temperatures should therefore be made, using suitable techniques.

The comparison between the laboratory results reported in this and the previous paper (Davies *et al.*, 1977), and the sterilization effects determined experimentally for a continuous 1100 l/hr UHT sterilizer and for a laboratory UHT sterilizer will be presented in paper III of this series (Burton *et al.*, 1977).

Notation

θ = general symbol for temperature;
θ_p = preheat temperature;
θ_s = temperature of heating bath;
θ_t = temperature at time t;
t = general symbol for time;
t_d = delay time;
T = total measured time of immersion in heating bath;
T_a = equivalent heating time;
τ = time constant;
R = outside radius;
r = general symbol for radius;
dr = small incremental radius;
l = length;
N = total number of bacterial spores;
L_t = log (proportion of surviving spores) at time t;
K = slope of a thermal death line;
K_{θ_t} = slope of thermal death line at temperature θ_t;
D = decimal reduction time;
α = thermal diffusivity;
k = thermal conductivity;
c = specific heat;
ρ = density.

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Thermal death kinetics of *Bacillus stearothermophilus* spores at ultra high temperatures

III. Relationship between data from capillary tube experiments and from UHT sterilizers

H. BURTON, A. G. PERKIN, F. L. DAVIES AND H. M. UNDERWOOD

Summary

Thermal death data were obtained for *B. stearothermophilus* spores heated in (a) a laboratory scale, direct heating UHT plant and (b) a larger scale direct heating UHT plant. These were compared with the corrected data from experiments in which spores of the same batch were heated in capillary tubes. Temperature coefficients (Q_{10} values) for inactivation of spores in UHT plants were higher than those for spores in capillary tubes but agreement was closer when spores were suspended in water than in milk. For aqueous spore suspensions, the discrepancy between plants and capillaries was least for the laboratory scale plant. The divergence always became greater at lower temperatures and the D values were coincident at 145°C. Possible reasons for these various effects are discussed.

Introduction

The purpose of the work described in this series of papers was to explain, if possible, the fact that less satisfactory sterilizing effects are frequently obtained in practical UHT sterilizers than are expected from laboratory capillary tube experiments on the organisms involved. By the methods described in the first two papers (Davies *et al.*, 1977; Perkin *et al.*, 1977) it was possible to extend capillary tube thermal death data to temperatures within the practical UHT range. The data could then be applied directly without the uncertainties of extrapolation.

The final part of this work, therefore, was to compare the corrected capillary tube results for thermal death with the results given by practical UHT sterilizers.

Two sterilizers were used for this comparison. The first was an experimental 1100 l/h sterilizer capable of being used either as a direct or as an indirect plant (Burton & Perkin, 1970). This sterilizer was one in which the practical

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sterilizing effect was found to be less than that expected from the distribution of temperatures and flow times in the process (Franklin *et al.*, 1970; Burton, 1970). The second sterilizer was a laboratory-scale plant of the infusion type for processing small batches of material (Perkin, 1974). Preliminary experiments had shown that the two sterilizers gave very similar bacteriological results for the same processing temperatures.

Although data for both these sterilizers have been published previously (Franklin *et al.*, 1970; Perkin, 1974), experience in the present experiments showed that it was unwise to draw conclusions from experimental results obtained at different times and under different experimental conditions. The sterilization experiments were therefore repeated, using the same stock suspension of spores and the same counting methods as used in the capillary tube experiments.

Interpretation of sterilization data

It was shown in the second paper of this series (Perkin *et al.*, 1977) that the Arrhenius relationship satisfactorily describes the variation of thermal death rate with temperature. As it is also theoretically more acceptable than either a constant Q_{10} or a constant z-value, the Arrhenius relationship has been used as the basis for comparison of the sterilizing performance of the sterilizers.

If it can be assumed that in the sterilizing plants the sterilizing temperature θ is reached instantaneously, is maintained for a holding time t, and that the temperature then falls instantaneously, the slope of the thermal death curve K_θ at temperature θ can be derived from

$$L = -K_\theta t \qquad (1)$$

where $L = \log_{10}$ (proportion of surviving spores); t = holding time; K_θ = slope of thermal death curve at temperature θ, equal to $1/D_\theta$, where D_θ is the decimal reduction time.

Following Hermier, Begue & Cerf (1975), log K_θ and log D_θ should be linearly related to $1/\theta$, where θ is the absolute temperature. Differences between sterilizing processes can be inferred from any differences between the linear relationships: sterilizing processes which are exactly the same in their mechanisms will give the same line.

The straight line relating log D_θ to the increase of absolute temperature for the capillary tube experiments was derived in the second paper of this series, and is believed to be valid for the temperatures used in practical UHT processes. The purpose of the present work was to confirm that the practical sterilizers gave the same line within the limits of experimental error, and therefore that the same sterilization mechanisms were involved.

To obtain the corresponding relationship with the experimental UHT equipments, it is necessary to know (a) the sterilizing effect L, from bacteriological experiments; (b) the treatment temperature θ; and (c) the holding time t.

Determination of sterilizing effect

The stock suspension of spores of *Bacillus stearothermophilus* TH24 (NCDO 1096) was prepared as described in the first paper of this series (Davies *et al.*, 1977). The methods used for determining the sterilizing effect, defined as log (proportion of surviving organisms), at a series of operating temperatures were the same as those described in the previous papers dealing with the two sterilizers (Franklin *et al.*, 1970; Perkin, 1974). A larger number of experiments was performed than in the previous series, in order to improve the derived relationship between sterilizing effect and temperature. Experiments were performed with both milk and water as operating media, in both sterilizers.

Determination of treatment temperature

To eliminate as far as possible the risk that the temperatures measured for the different sterilization systems might not be equivalent, the same copper-constantan thermocouple wire was used to determine the processing temperature in the continuous and the batch UHT sterilizers. The thermocouple was mounted directly into the holding section of each plant. The same high speed potentiometric recorder (Type Electronik 194, Honeywell Controls Ltd, Brentford, Middlesex) was used to record the temperatures as was used in the capillary tube experiments.

The thermocouple was calibrated in an oil bath against a sub-standard mercury-in-glass thermometer before each series of experiments.

Determination of heating and holding times

The determination of an effective holding time for the experimental UHT plant is of prime importance in the interpretation of the results. The validity of any conclusions depends on the accuracy with which the holding times were determined. Since the holding times in both sterilizers were not the same for every particle of liquid processed, it was necessary to investigate the holding time distribution, and the sporicidal implications of the distribution.

Furthermore, the heating time might also be important. The continuous 1100 l/hr sterilizer (Burton & Perkin, 1970) was used in its steam-injection direct-heating mode. The laboratory batch sterilizer (Perkin, 1974) operates on the infusion heating system, by the injection of the experimental liquid into a steam vessel. In both sterilizers, therefore, the heating was rapid and might be considered as instantaneous for most purposes. It could not, however, be considered instantaneous for these experiments without proof.

The methods for studying the heating time varied with the two sterilizers. The method for measuring holding time distribution was, however, the same.

Holding time distributions were obtained by an NaCl-injection method with detection by change in electrical conductivity. Salt solution was injected into the product flow with a special syringe driven by a strong spring (Holding time test set, The A.P.V. Co. Ltd, Crawley, Sussex), and the injection time was always < 0.1 sec. The arrival of the salt at the end of the holding tube was detected by an electrode system of suitable form.

The electrode was connected into one arm of a bridge system supplied with alternating current at 250 Hz. The output of the bridge was amplified by a high gain solid-state amplifier and applied to the galvanometer of a high speed ultraviolet recorder (Type 5–127, with galvanometer type 319, Bell & Howell Ltd, Basingstoke). The capacitance of the electrode system when in place in an aqueous medium was compensated by capacitance in the opposite arm of the bridge.

The output recorded on the galvanometer was not linearly related to the salt concentration at the electrode. Each electrode system was therefore calibrated in place in the flow line by operating the equipment continuously with successive batches of salt solution obtained by serial dilution from the strongest solution.

A microswitch was mechanically operated by the salt-injection syringe and applied a signal to a second galvanometer in the recorder to indicate the time of injection.

This method of determining holding time distributions could not be used for experiments with milk, because the natural electrical conductivity of milk is too high. The distributions were therefore obtained with water, and it was assumed that they also applied to milk.

The bacteriologically effective mean holding time, which is not the same as the simple mean holding time, was derived from the holding time distribution as described later.

Investigations on the batch infusion sterilizer

The sterilizing section of this sterilizer is shown diagrammatically in Fig. 1. The product is injected into the heating chamber through a nozzle at A. It falls to the bottom of the chamber and at some stage of its fall it reaches the true holding temperature.

Measurements with a stop watch showed that the mean time between opening of the inlet valve and the arrival of liquid at the sight glass was 0.82 sec when the nozzle was in its normal position 36.5 cm above the bottom of the heating vessel. The nozzle was modified so that it could be lowered in stages to positions such as B. While the nozzle was lowered, the temperature of the heated product was measured by a thermocouple. The temperature did not fall until the nozzle was only 4.5 cm above the bottom of the heating vessel, i.e. the heating period is only the first 4.5 cm of fall of product. If the normal fall time is 0.82 sec, the heating time is therefore $0.82 \times (4.5/36.5) = 0.1$ sec. Further tests with the nozzle removed from the heating chamber showed that the

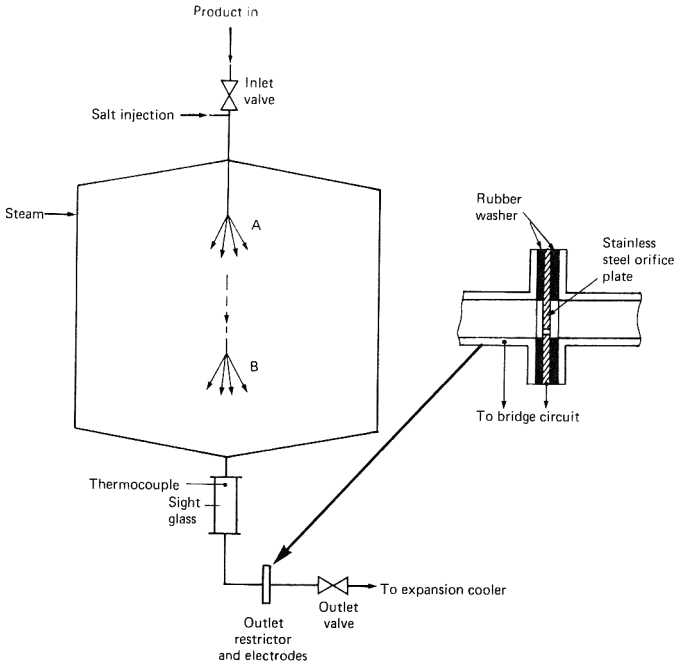

Figure 1. Schematic diagram of heating and holding sections of batch infusion sterilizer.

time of 0.82 sec was almost entirely accounted for in flow times across the base of the vessel into the sight glass, and the fall time through 36.5 cm was not measurable by stop watch. The true heating time is therefore negligible and was ignored in calculation.

Electrodes for detection of the salt solution, because of the small size of the flow passages, were combined with the outlet restrictor as shown in Fig. 1. The stainless steel orifice plate was insulated from the adjacent pipework by thin rubber washers at each side and the assembly was held in a non-conducting clamp. The upstream pipe section and the orifice plate were used as the electrodes and were connected to the bridge circuit.

It was necessary to extend the entry pipework between the inlet valve and the nozzle in order to accommodate the salt injection system. The additional volume added was 8 cm^3, and with a mean inlet flow rate of 12 cm^3/sec an additional time of 0.67 sec mean was spent in the inlet tube which was deducted from all the holding time determinations. The distribution of holding times derived from the recorded output of the bridge, after deduction of 0.67 sec, was as shown in Fig. 2. The arithmetic mean holding time calculated by integration of this curve was 3.26 sec.

The distribution of spores surviving the process is not, however, the same as the holding time distribution or distribution of total spores. The holding time distribution function indicates the proportion of total spores taking a certain time to pass through the holder. At each time, therefore, the number of surviving spores is equal to the proportional number of total spores, multiplied by

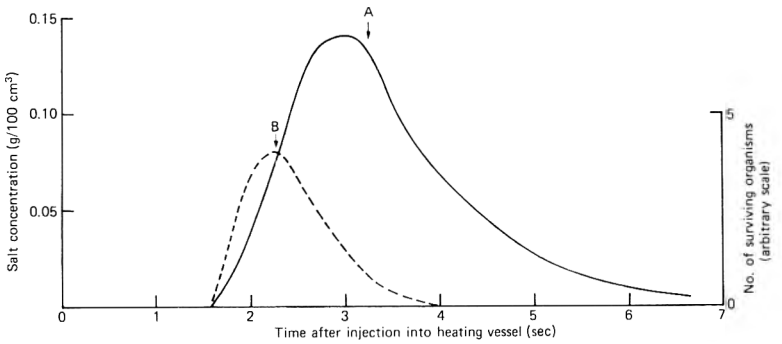

Figure 2. Holding time distribution for batch infusion sterilizer operating at 145°C. ——— Measured holding time distribution, A = mean holding time, 3.26 sec; – – – – – distribution of surviving microorganisms, B = bacteriologically-effective mean holding time, 2.31 sec.

the proportion of survivors for that holding time and that temperature. This can be expressed as

$$S(t) = H(t)\ 10^{-K_\theta t} \text{ (Burton, 1958)} \qquad (2)$$

where $S(t)$ = survivor distribution function at time t; $H(t)$ = holding time distribution function at time t; t = holding time; K_θ = slope of thermal death curve at temperature θ, = $1/D_\theta$, and θ = operating temperature. For each temperature of operation, and for each suspension medium, the holding time distribution was therefore converted to the survivor distribution by using the values of K_θ at different temperatures derived in Part II of this series from the capillary tube data. As an example, the survivor distribution at a processing temperature of 145°C with spores suspended in water is also shown in Fig. 2. The bacteriologically effective mean holding time was calculated from the survivor distribution by integration using Simpson's rule. For the example given in Fig. 2, the effective holding time is 2.31 sec, as compared with the arithmetic mean holding time of 3.26 sec. The effective mean holding time falls with increasing processing temperature, because of the change of D_θ with temperature, and it is also different for suspensions in water and in milk. The bacteriologically effective holding times for the batch UHT plant are given in Fig. 3.

For each experiment in which a sterilizing effect had been determined, the slope of the thermal death curve K_θ was calculated from Eqn 1 using the value of holding time obtained from Fig. 3 corresponding to the processing temperature and according to whether the experiment had been performed with suspensions in water or milk. The decimal reduction time D_θ was derived from K_θ.

Investigations on the continuous steam-injection sterilizer

During the experiments it appeared that discrepancies between the capillary tube results and the plant results were greater with the continuous sterilizer

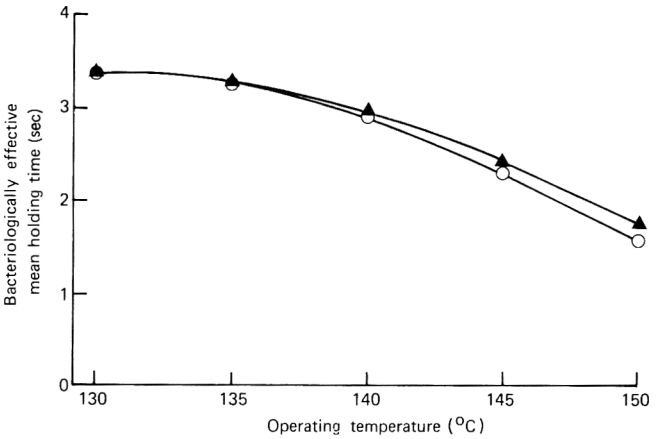

Figure 3. Variation of bacteriologically effective mean holding time with temperature, for batch infusion sterilizer. ○ Water suspensions; ▲ milk suspensions.

than with the batch infusion sterilizer. It seemed that inadequate characterization of the holding time in this much larger sterilizer was the probable cause, so the holding time distribution was studied in greater detail than with the infuser.

The sterilizing section of the sterilizer is shown diagrammatically in Fig. 4. To determine the heating time and the true start of the holding period, a thermocouple was fitted into a gland in the bend at the outlet of the steam injector so that it could be moved axially into the injector. From a position well beyond the outlet of the injector, the thermocouple was slowly moved downwards and its output was recorded. There was no drop in output until the thermocouple junction reached a point 8 cm above the last hole in the injector.

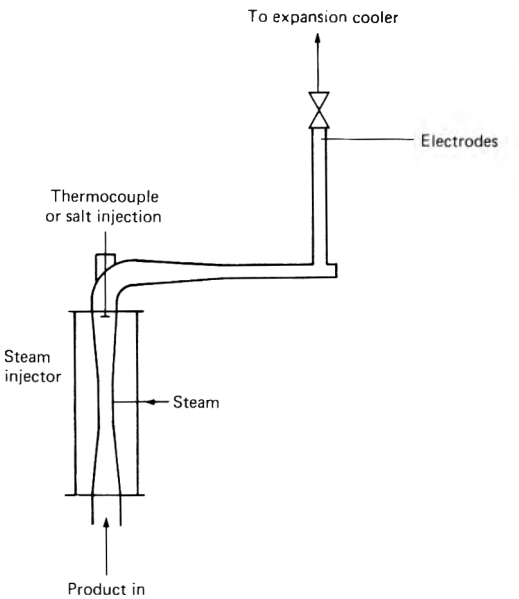

Figure 4. Schematic diagram of heating and holding section of steam injection sterilizer.

This point was therefore taken to be the start of the holding period, and in studies of the holding time distribution, salt was injected at this point.

From the volume of the steam injector between the plane of the first steam orifice and the plane at which the full temperature was reached, the mean heating time was calculated to be 0.9 sec. It was impracticable to measure the heating curve within the steam injector because of the rapid cyclic temperature changes and the probability of damage to the thermocouple caused by the violent agitation within the injector. It can be assumed that the temperature rise is linear, and in such a situation any lethal effect will be concentrated at the end of the injector within a very small proportion of the overall heating time. Thermal death during heating in the injector was therefore ignored in comparison with that occurring during holding at the full temperature.

In order to investigate variations in the holding time distributions at different radii within the holding tube, an electrode system was constructed consisting of four pairs of electrodes in a common transverse plane. Each pair of electrodes consisted of two stainless steel wires, 0.8 mm diameter at 2.5 mm centres, projecting radially into the tube. The wires were insulated with epoxy resin except for the end 2 mm of each wire, and each pair was mounted in a gland in the wall of a standard pipe section which could be mounted at the end of the holding tube in the position normally occupied by the expansion valve. The pairs of electrodes were mounted at 90° intervals round the pipe section, with their inner ends 0 mm, 5 mm, 10 mm and 15 mm from the axis of the 36 mm internal diameter tube section (Fig. 5). Each electrode was independently connected to a bridge circuit, solid-state amplifier and u.v. recorder galvanometer, and calibrated at the series of processing temperatures to be used by the processing of serially diluted salt solutions, as described above.

The product flow rate, and hence the holding time, is a function of processing temperature, since approximately 10% of the original product volume is added as condensed steam, with the added volume increasing with processing temperature. Holding time distributions were therefore measured at 130, 135, 140, 145 and 150°C. At each temperature three separate experiments were performed; the holding time distributions for one radius of measurement varied very little between replicates, and the replicates were averaged.

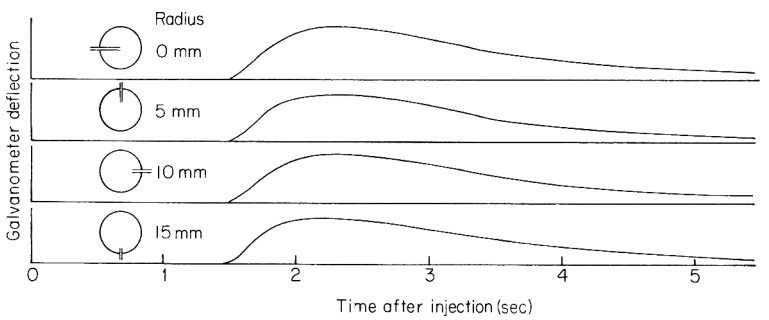

Figure 5. Holding time distributions at different radii in holding tube, operating temperature 145°C.

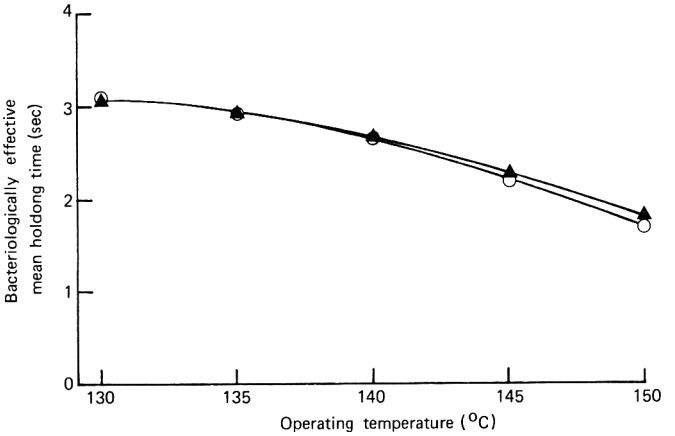

Figure 6. Variation of bacteriologically effective mean holding time with temperature, for continuous steam-injection sterilizer. ○ Water suspension; ▲ milk suspensions.

At each temperature, there was little difference between the four radii in the measured holding time distribution. This is shown in Fig. 5, which gives the results for an operating temperature of 145°C. This implies completely turbulent flow in the holding tube and a Reynolds number calculated to be in excess of 5000 confirms this. The holding time distribution was therefore assumed to be uniform over the whole cross-section of the holding tube.

At each processing temperature, the measured holding time distribution was used to calculate a bacteriologically equivalent mean holding time, using the capillary tube data for milk and water as described above. The variation of the equivalent holding time with temperature for the continuous steam-injection equipment is shown in Fig. 6.

These data were used as described above to calculate the thermal death rate and decimal reduction time for each sterilizing effect determined for the sterilizer.

Results and discussion

The variation of log (decimal reduction time) with the inverse of absolute temperature is shown for the batch infusion sterilizer in Fig. 7 and for the continuous steam injection sterilizer in Fig. 8. For each system, the corresponding experimental points and regression for the capillary tube experiments is shown; this is a part of the curve for suspensions in either water or milk derived in the second paper of this series (Perkin *et al.*, 1977, Fig. 11).

If thermal death follows Arrhenius kinetics at ultra-high temperatures (allowing the assumption of constant Q_{10} or z-values over limited temperature ranges), the relationship in Figs 7 and 8 should be linear. This is found to be true to a high degree.

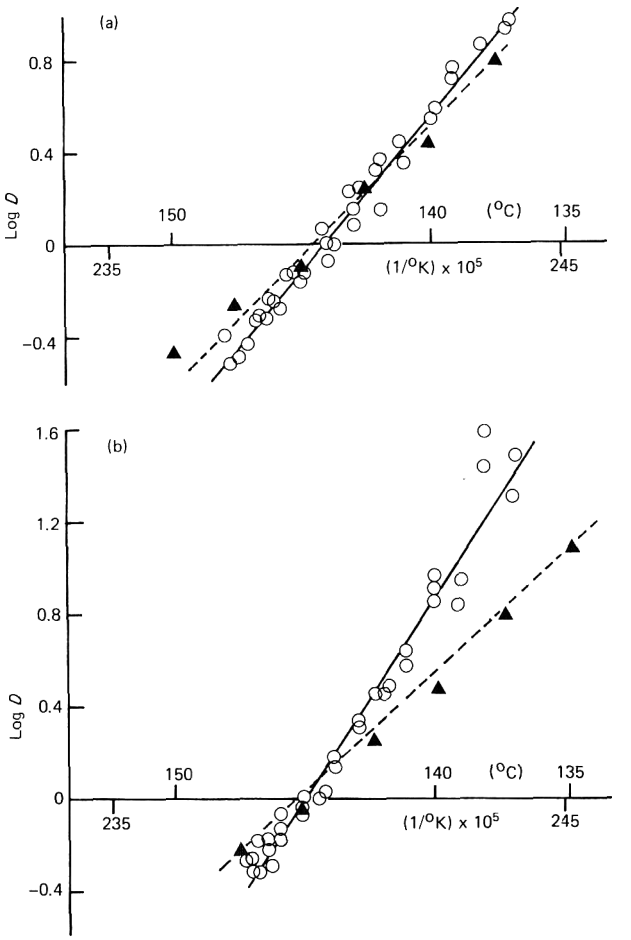

Figure 7. Comparison between thermal death data from batch infusion sterilizer and capillary tube experiments. (a) Suspensions in water, O———O sterilizer results and linear regression from 34 points; ▲– – – –▲ capillary tube linear regression with points in range; (b) suspensions in milk, O———O sterilizer results and linear regression from 34 points; ▲– – – –▲ capillary tube linear regression with points in range.

If the capillary tube results and the sterilizer results are in complete agreement, the pairs of regression lines should be coincident. This is not generally true, and the practical sterilizers show a greater change of rate of thermal death with temperature than would be expected from the capillary tube experiments.

The results are more satisfactory for *B. stearothermophilus* spores suspended in water than for those in milk. In fact the agreement for the batch infusion sterilizer is almost complete (Fig. 7(a)), while for the continuous injection sterilizer there is a slight divergence as the temperature falls (Fig. 8(a)), and at a temperature of 137°C the rate of thermal death in the sterilizer is half that found in the capillary tube experiments.

For suspensions of spores in milk the agreement is considerably poorer (Figs 7(b) and 8(b)) and the rate of thermal death changes much more rapidly in the

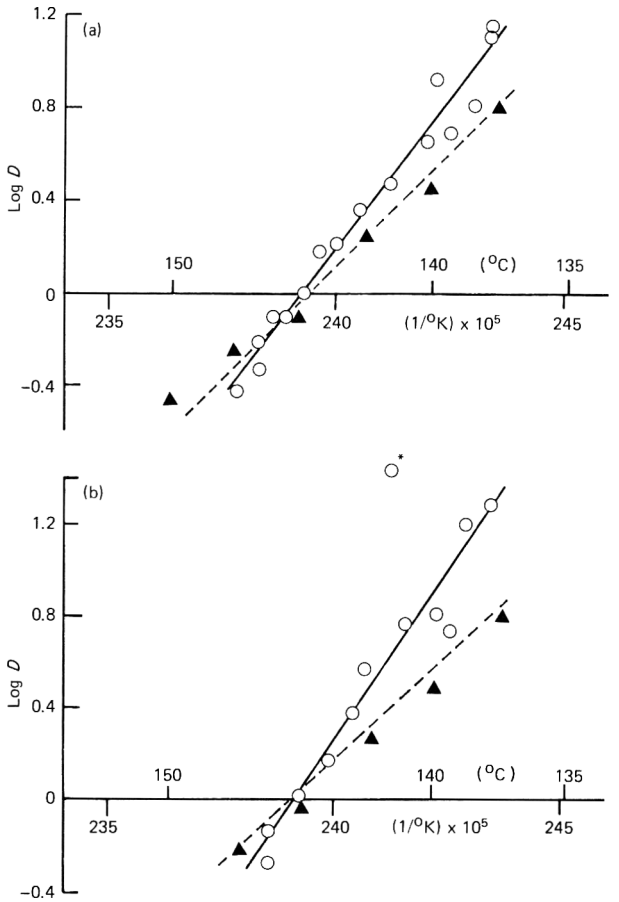

Figure 8. Comparison between thermal death data from continuous steam injection sterilizer and capillary tube experiments. (a) Suspensions in water, ○———○ sterilizer results and linear regression from 16 points; ▲– – – – –▲ capillary tube linear regression with points in range; (b) suspensions in milk, ○———○ sterilizer results and linear regression from 11 points (*point omitted); ▲– – – – –▲ capillary tube linear regression with points in range.

practical sterilizers than would be expected from the capillary tube data. It is surprising, however, that the agreement is reasonable in the higher range of ultra-high temperatures (e.g. above 140°C) and the discrepancy takes the form of an unexpectedly low thermal death rate for the practical sterilizers at low temperatures.

It could be argued that the divergence only becomes marked at temperatures which are undesirably low for direct UHT processing, and therefore is of itself unimportant. However, it is clearly present, and so should be explained if possible. There is no reason to doubt the capillary tube results, since they form a small part of a series of results which is self-consistent over a wide temperature range. It therefore seems that the explanation must be either in a failure to characterize the plant performances correctly in time or temperature, or in

some fundamental difference between spore destruction in capillary tubes and spore destruction in bulk in a processing plant, perhaps brought about by the close proximity of surfaces in the capillaries.

It is undesirable to invoke a fundamental effect if there is any possibility of an undisclosed technical factor which might be responsible. The experimental sterilizers were controlled closely and characterized in considerable detail so as to eliminate technical uncertainties as far as possible. Since the results with milk were consistently poorer than those with water, some factor concerned with milk might be responsible. The holding time distributions were obtained for water, since the conductivity method cannot be used with milk because of its already high conductivity. It is possible that the higher viscosity of milk modifies the holding time distribution from that measured. This would be through a lowering of the Reynold's number, and would give a spreading in time of the distribution. Since the first part of the distribution tends to control the bacteriological mean holding time, as shown in Fig. 2, any spread of the distribution would lead to a lowering of the effective time which would influence the results. However, this effect would become greater with increasing temperature, and we need an effect which is greater at lower processing temperatures.

The heat capacity of milk is slightly lower than that of water. In processing milk, therefore, less steam will be needed than for water at the same temperature, and the lower volume of condensate will lead to longer holding times than expected. However, the change from this cause is so slight (about 0.4% change in mean holding time) as to be imperceptible, and in any case the correction of such an effect would increase the discrepancy rather than decrease it.

We are therefore unable to explain why the capillary tube results and the experimental sterilizer results only coincide at one temperature, approximately 145°C. The greater variation of death rate with temperature in the practical sterilizer as compared with that given by the capillary tube data, and the greater variation for suspensions in milk as compared with those in water, cause discrepancies at both higher and lower processing temperatures. It seems to be coincidental that the errors are small in the centre of the practical range of UHT processing temperatures.

These discrepancies, however, account for only a small part of the inconsistencies which we set out to explain in this work, i.e. those between sterilizing effects determined experimentally on practical UHT sterilizers, and estimated from laboratory data obtained at lower temperatures. The substantial over estimate of sterilizing effect in our own earlier work (Burton, 1970) now appears to have arisen not so much by errors in extrapolation, as by increase in the absolute resistance of the spores to thermal processing at lower temperatures (cf. Fig. 4 of the first paper of this series, Davies *et al.*, 1977). The results of the laboratory experiments appear to indicate that extrapolation of thermal death data to ultra high temperatures is valid.

There are however, serious objections to this conclusion. First, the basis of extrapolation is uncertain. We have expressed our results in terms of the Arrhenius relation, with a constant activation energy, which leads to a continu-

ously varying Q_{10} or z-value. The assumption of a constant Q_{10} or z-value for extrapolation would lead to significant errors.

Secondly, it is very unlikely that thermal death data have ever been obtained with such precision at low temperatures that extrapolation over a 20–30°C range can be performed accurately. Single points at one temperature normally represent a series of replicates with a scatter of results. When several such points are taken together, there is uncertainty about the temperature coefficient which they indicate, so that inaccuracies can easily arise. Small inaccuracies in the rate of change of thermal death rate with temperature determined at low temperatures will lead to substantial errors in the ultra-high-temperature range, causing large over or under estimates of the sterilizing effect to be expected. The only way to avoid these errors is to perform a large number of experiments over the widest possible range of temperatures.

We therefore suggest that any estimates of sterilizing effects at ultra high temperatures should be treated with very great caution until the thermal death data have been extended into the required temperature range so that they can be used without extrapolation. We believe that the methods outlined in these papers will enable data at ultra high temperatures to be obtained for other and perhaps more-practically-important organisms, e.g. *Clostridium botulinum*, than that which we have used.

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Biochemical changes in experimental soy sauce Koji

F. M. YONG* AND B. J. B. WOOD

Summary

Changes in the levels of major enzymes and in amounts of principal groups of compounds, during the growth of three strains of the mould *Aspergillus oryzae* on a mixture of soy beans and wheat flour, under laboratory conditions designed to approximate as closely as possible those found in practical soy sauce production, are described. Evidence for the presence of lipase and sucrase is presented. Protease and α-amylase were also assayed. Tyrosinase could not be detected. Changes in the concentrations of reducing sugars, total soluble organic nitrogen, amino-nitrogen and ammonia are described. Two soy sauce moulds of Chinese origin behaved in a similar fashion to each other, but a mould from Singapore had a rather different pattern of enzymes. Practical significance and physiological implications of the results are discussed.

Introduction

In a previous paper (Yong & Wood, 1976) we described the results of studies on the succession of microbes which occurs in the course of the soy sauce fermentation.

The fermentation is divided into two stages; first mould is grown on soy beans coated with wheat flour. This stage is called the *Koji*. After 72 hours of fermentation, the moulded mixture is immersed in brine, where a bacterial and yeast fermentation ensues, this is called the *Moromi* or soy mash. Upon completion of the second stage, the mixture is filtered to give the liquid soy sauce of commerce. In the present paper we first describe the changes which we observed in the Koji, then discuss them and compare them with the results of Japanese studies.

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Materials and methods

Purity and source of materials

All inorganic chemicals used were of British Drug House 'Analar' grade. Organic compounds were of the highest grade readily available, 'Analar' wherever possible. Soy beans (*Glycine max*) were an oilseed grade, donated to us by Clyde Oil Products Ltd, of Glasgow. Wheat flour was a soft or biscuit grade purchased from a commercial miller. Spectrophotometric work was carried out on a Unicam SP 600 Series 2 Spectrophotometer.

Microorganisms

Three fungi were examined:

(i) *Aspergillus oryzae* strains NRRL 1988 and 1989. These organisms had been isolated from soy sauce Koji obtained from China (Hesseltine *et al.*, 1966) and were kindly donated to us by Dr C.W. Hesseltine.

(ii) *Aspergillus oryzae* strain A01, originally isolated from tempeh obtained in Singapore. The precise identity of this fungus is still doubtful but S.T. Tan (personal communication, cited in Yong, 1971) has used it to produce good quality soy sauce.

Media and culture methods, sterilization, preparation of Koji, sampling procedure and pH measurement.

These were described in our previous paper (Yong & Wood, 1976).

Analytical procedures

Moisture content of sample. The moisture content was determined by the method of Jacobs (1951). Approximately 2 g duplicated portions of sample were accurately weighed into a tared dish, then dried in an oven for 5 hr at 100°C, removed from the oven, cooled in a desiccator, reweighed and the drying and cooling procedure repeated until constant weight was achieved.

Preparation of extracts. Similar procedures were followed in preparing extracts for extracellular enzyme assay and extracts for chemical analysis, except that in the former case extraction was with an appropriate buffer, in the latter it was with distilled water.

An approximately 5 g sample was weighed into a tared 100 ml Erlenmeyer flask and appropriate extracting fluid was added, 5 ml if an enzyme was to be assayed, 10 ml if for chemical analysis. The sample was then crushed with a glass rod, and additional extractant (10 ml) added. The flask was then stoppered

and shaken on a Griffin flask shaker operated at medium speed for 14 min at 10°C. Enzyme extracts were then transferred to centrifuge tubes, centrifuged at 11 000 r.p.m. on an M.S.E. High Speed 18 Centrifuge at 5°C for 30 min, the supernatant decanted, the residue suspended in extracting buffer (7 ml) and re-centrifuged; the supernatants were combined and made up to 25 ml. Extracts for chemical analysis were filtered through Whatman No. 12 fluted filter papers, the residue washed with water and the combined filtrate made to 50 ml.

Assays and analyses were carried out on duplicate fermentations, and for each sample two determinations were made of the item concerned.

α-Amylase assay. The extracting and diluting buffer was molar potassium hydrogen phosphate, pH 6.0. The assay was a modified Wohlgemuth (1908) procedure suggested by Associated British Maltsters Industrial Products Ltd (ABMIP Ltd). The assay measured hydrolysis of a solution of starch (0.2%) in buffer (diluted 1 : 10 with water) by the enzyme. Pre-warmed starch solution (50 ml) was mixed with enzyme extract (5 ml) and the mixture was incubated at 30 ± 1°C. At intervals, 1 ml aliquots of this mixture were withdrawn and mixed with 5 ml portions of a solution containing iodine (0.088 g) and potassium iodide (40.0 g) in water (1 litre). Initially this gave the typical purple colour obtained when starch absorbs iodine, but as hydrolysis proceeded, the colour produced gradually changed to the red-brown obtained with dextrin. By measuring the optical density at 617.5 nm of these treated sub-samples, it was possible to determine graphically the time at which the optical density reached 0.80, using a 10 mm-path-length cell. This optical density is called the 'achroic' point, and the dilution of enzyme extract used in the determination was such that this achroic point was reached 10–25 min after starting the hydrolysis.

The unit of enzyme activity employed was an arbitrary one, defined as follows: 'The α-amylase has an activity of one x unit per gram, when 25.0 mg of that preparation reacts on 1 g dry weight of starch in a total volume of 55 ml, a temperature of 30°C and a pH of 6.0, so that the achroic point is reached in 15.0 minutes'.

Thus the strength of a particular sample was calculated according to the equation $-x$ units per g = $15/2tc$, where t = time to the achroic point (min); c = concentration of the extract in g per 100 ml.

Protease assay. The extracting and diluting buffer was 0.1 M sodium hydrogen phosphate pH 6.5. A casein hydrolysis was selected as the assay because *Aspergillus oryzae* and *A. sojae* are known to produce several enzymes of varying specificity, pH optimum, etc., active against proteins and peptides. For the purposes of the present study, we decided that an overall estimate of protein hydrolysis would give the most useful information. The method selected was supplied by A.B.M. Industrial Products Limited and involved hydrolysis of Hammersten casein (10.0 ml of 2% solution) by the enzyme preparation (5.0 ml) at 35.0°C for 30 min. The reaction was terminated by adding trichloroacetic acid (10.0 ml of a 10% solution), the mixture filtered through a Whatman No. 12 filter paper and the optical density of the filtrate measured at

275 nm. This assay can be criticized in that it depends on the presence of particular amino acids, such as those containing aromatic rings, in the filtrate, but Rosenthal & Sobieszczanska (1970) and O'Sullivan & Mathison (1970) have shown that reducing sugars, which will be present in all soy bean extracts, interfere with the colorimetric method of Lowry *et al.*, (1951) using the Folin phenol reagent. Similar problems may arise with other colorimetric assays.

Enzyme activity was expressed in terms of an arbitrary unit used by A.B.M.I.P. Ltd, called an XS unit, and defined thus: 'An enzyme, a solution of which containing 1.5 g per litre, which, under the stated experimental conditions produced a filtrate with an optical density of 0.500 when measured in a 10 mm path length cell, had a strength of 36 XS units per gram'.

Sucrase assay. The extracting and diluting buffer was 0.68 M sodium hydrogen phosphate, pH 5.5. Sucrase activity was assayed by measuring the increase in reducing sugar level which occurred when equal volumes of a 5% sucrose solution and enzyme extract were mixed and incubated at 30°C for 10 min. Reducing sugars were measured by Sumner's (1925) method. The unit of enzyme activity was that amount of enzyme which would liberate 1 mg reducing sugar (expressed as glucose) per minute when incubated under the specified conditions (Bacon, 1955).

Tyrosinase assay. The extracting and diluting buffer was 0.1 M sodium hydrogen phosphate, pH 6.0. The assay procedure was that of Horowitz *et al.* (1960), in which the oxidation of 3,4-dihydroxy-DL-phenylalanine is examined spectrophotometrically at 475 nm after 5 min incubation at 30°C.

Lipase assay. The extracting and diluting buffer was a mixture of 0.1 M sodium acetate solution (90.0 ml) and 0.1 M acetic acid (10.0 ml), and had a pH of 5.5.

The assay procedure was supplied by A.B.M.I.P. Ltd, and involved hydrolysis of glycerol trioleate (20.0 g) emulsified in a solution of sodium taurocholate (8.0 g), acetate buffer (120 ml) and 2.2% calcium chloride (20 ml). The emulsion (10.0 ml) was mixed with enzyme extract (5.0 ml) and incubated at 35°C for 60 min, with constant gentle shaking. The reaction was terminated by adding industrial ethanol (40 ml). The mixture was then titrated with alcoholic 0.02 N potassium hydroxide against phenolphthalein. Conditions were arranged so that the titre used in the calculation lay between 7 and 20 ml. The unit of enzyme activity is that amount of enzyme which liberates 1.0 mg of oleic acid per min under the specified conditions.

Total nitrogen content of soybean, wheatflour, Koji and Moromi estimated by the Kjeldahl method. For soybean and wheatflour the macro-method described in Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.) (1965) Section 2.004 was used. For Koji and Moromi, Jacobs' (1951) modification of the method was used. Protein content of wheatflour was obtained by multiplying the nitrogen content by 5.70 (A.O.A.C., 1965). The factor used for soybeans was 6.25 (Lillevik, 1970).

Amino-nitrogen. The Sorensen formol titration method as given in A.O.A.C. (1965) was used. Both amino and ammonia nitrogen are determined by this

method, but results given in this paper are corrected for ammonia, which was determined separately (Yokotsuka, 1960).

Ammonia. Determined by the Conway diffusion method (Conway, 1957).

Reducing sugars. Determined by Sumner's (1925) method, as employed in the analysis of soy sauce by Yoshino and Takano (1954) who discuss reasons for preferring this method over other techniques for assaying reducing sugars in soy sauce. The problems of analysing for reducing sugars in soy sauce have also been discussed by Yamada & Ishimaru (1930), Kobayashi & Tabuchi (1954) and Yokotsuka (1960).

Results

Changes in reducing sugar level

Figure 1 shows how the reducing sugar level changed as the fermentation progressed. All strains gave a very rapid increase at the very early stages of growth, with strain 1988 giving the highest level. This was followed by a decrease, then toward the end of the fermentation there was a second increase, with the tempeh strain A01 showing both the earliest increase and the highest level of reducing sugar. Thereafter, levels in all cases remained fairly steady until the end of the fermentation. Strains 1988 and 1989 gave very similar changes to each other, which were distinctly different from those seen in strain A01.

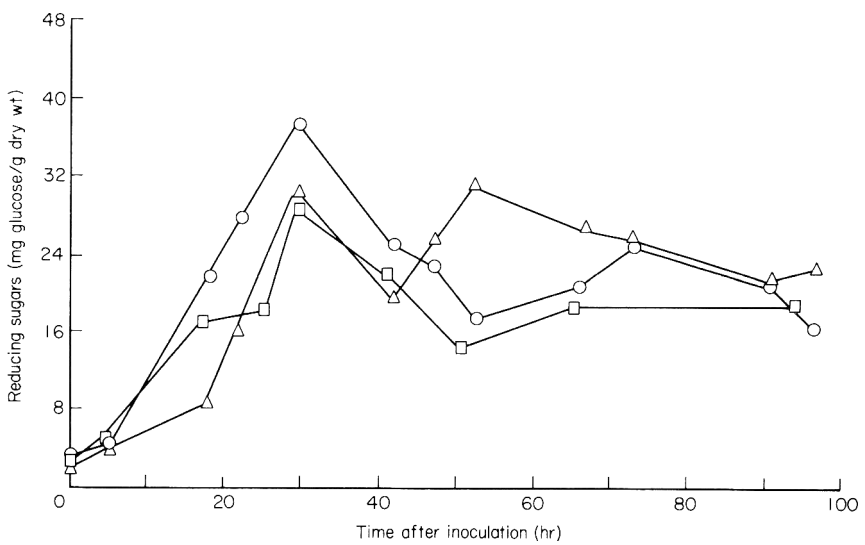

Figure 1. Reducing sugars in Koji. △ *A. oryzae* strain A01; □ *A. oryzae* strain 1988; ○ *A. oryzae* strain 1989.

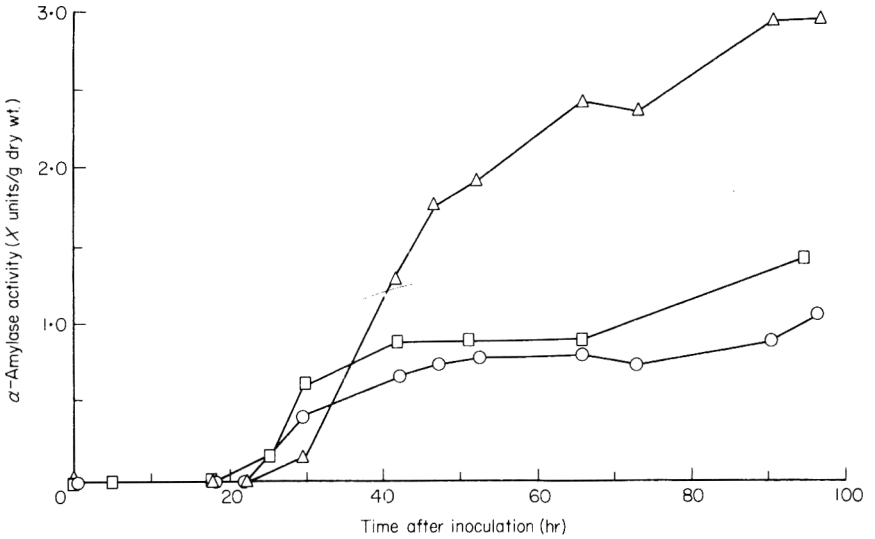

Figure 2. α-Amylase in Koji. Key as in Fig. 1.

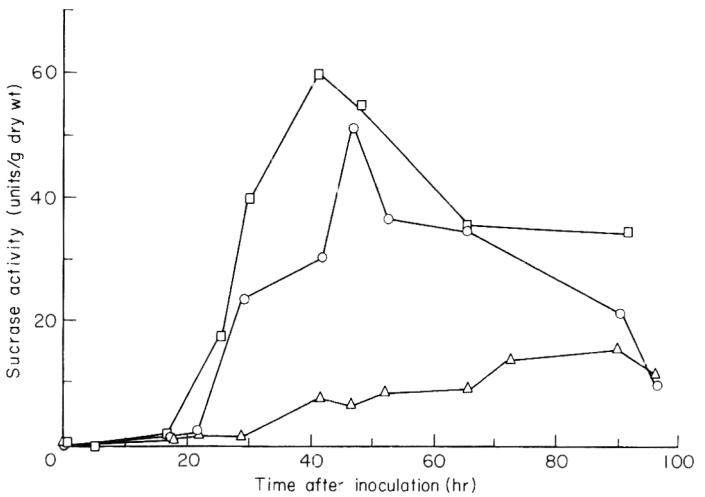

Figure 3. Sucrase in Koji. Key as in Fig. 1.

Changes in α-amylase and sucrase levels

These are shown in Figs 2 and 3. In α-amylase production, strain A01 is distinctly different from the other two, which gave broadly similar patterns of change. The enzyme concentration in the strain A01 Koji was more than twice that found in the other two Kojis, even after 94 to 96 hr incubation, and after 72 hr, when a Koji would normally be terminated, the level produced by A01 was more than three times that seen in the other Kojis.

It is noteworthy that the amylase level in all cases continued to increase overall between the 70th and 96th hr of incubation, although the reducing

sugars showed a general tendency to remain steady or even decline slightly during that period, when active sporulation was taking place.

It will be noticed that the reducing-sugar level increases rapidly during the first 20 hr of incubation, but that α-amylase activity only becomes detectable after 20 hr. This caused us to investigate other means of producing reducing sugars in the Koji, and we demonstrated the presence of sucrose hydrolysing activity at a very early stage, work which we have described in a preliminary report (Yong & Wood, 1975). Since beans contain 15 to 19% invert sugar on a dry weight basis (Yokotsuka, 1960) it is not too surprising that the mould should produce enzymes capable of hydrolysing the carbohydrates (principally sucrose) constituting this fraction. Since beans constitute 50% of the Koji on an original weight basis, if we accept a median figure of 17% for 'invert sugars', and assume this to be all sucrose, it would yield about 90 mg reducing sugar per g dry weight of Koji, more than sufficient to account for the observed levels of 30–38 mg reducing sugar per g dry weight of Koji at the peak of the first increase in reducing sugar.

When sucrase levels are measured in Koji (Fig. 3) we see that *Aspergillus* strains 1988 and 1989 again show generally similar patterns, with rapid increases in enzyme level for about the first 40 to 45 hr, and thereafter a decrease in enzyme levels. Strain A01 gives a much slower increase, which however continues through almost the entire fermentation. Even the levels of enzyme observed after 18 hr incubation (1 to 1.8 units/g weight of Koji) should be sufficient to hydrolyse all the sucrose present in the Koji, although the latter's relative dryness may impose some practical restraints. Strain A01 gave a first maximum of reducing sugar at the same time as, and at a level mid-way between the other two strains, despite its markedly lower levels of both sucrase and α-amylase at that time. Indeed the levels of sucrase seem disproportionately high in strains 1988 and 1989, when compared with the amount of substrate present in the Koji.

Lipase. Although the soybean is an oil-rich seed, and free fatty acids would

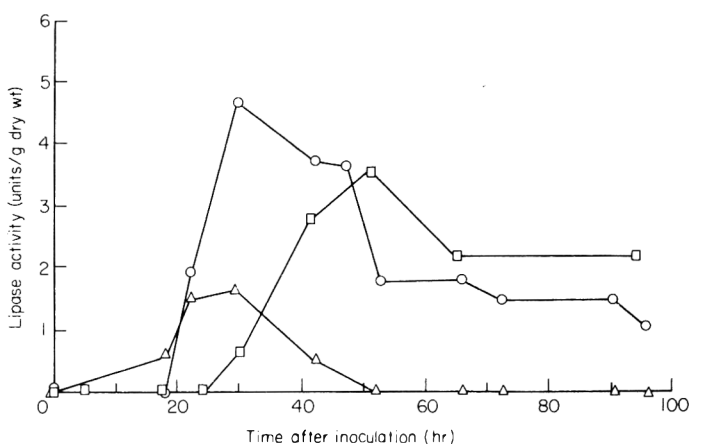

Figure 4. Lipase in Koji. Key as in Fig. 1.

seem to be an undesirable class of compound to have present to any great extent, both because of their own taste and because of the danger of rancidity developing, little attention seems to have been paid to lipase levels in Koji. If these views as to the practical significance of lipase levels are correct, then it would appear that strain A01 has an advantage over the two soy sauce strains (Fig. 4). Although the enzyme was detectable somewhat earlier in the A01 Koji than in the other two, it reached a maximum level in A01 Koji less than half that in the other Kojis, and disappeared from the Koji again much more rapidly and completely.

Tyrosinase. Surprisingly, this enzyme, which is widespread among the *Aspergilli*, could not be detected in any of our fermentations. In this respect our findings are in agreement with those of Crewther & Lennox (1953) who used liquid cultures. On the other hand, Oba (1974) has reported the presence of tyrosinase in rice Koji, where it reached a maximum level after 45 hr incubation; a moist Koji had a lower level of the enzyme. He found that the extent of browning occurring in the Koji correlated with the tyrosinase level.

Protease. The extent of protein hydrolysis is generally acknowledged to be the most important factor governing the quality of soy sauce, since flavour, palate-fullness and nutritional value all depend on the extent to which the wheat and soybean proteins are brought into stable solution. Consequently the complex of protein and peptide hydrolysing enzymes produced by Koji moulds has been subjected to particular scrutiny by Oriental workers. We decided that for the purposes of the present work, the best approach would be to use a single assay to test overall protein hydrolysing capability at a pH approximating to that of Koji, rather than examine individually the acid, alkaline and neutral proteases (Yamamoto, Yangida & Suminoe, 1972) and the peptidases which may be assumed to be present by analogy with other members of the genus *Aspergillus*.

Protease activity was detectable (albeit at a low level) right from the start of the fermentation, despite the spores having been washed three times to prevent carry-over of enzymes from the old mycelium (Fig. 5). No other enzymes were detectable at that early a stage in the fermentation. A rapid increase in protease activity between the 20th and 30th hr of incubation, with the activity reaching a maximum level at between 40 and 50 hr, was then followed by a decline in activity and a fluctuating level for the rest of the fermentation, Yokotsuka (1972) and Yamamoto, Yangida & Suminoe (1972) also conclude that around 50 hr is an optimal cultivation time for enzyme production.

Changes in soluble, amino and ammonia nitrogen levels

Total soluble nitrogen increased in all three types of Koji, from about the 20th hr of incubation, when a rather more rapid initial increase occurred, followed by a steady overall increase until about 70 hr had elapsed, thereafter remaining steady until the fermentation was terminated (Fig. 6). These results

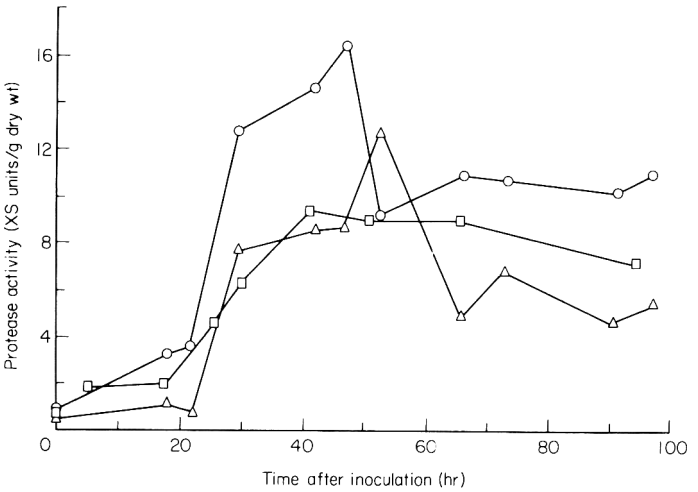

Figure 5. Protease in Koji. Key as in Fig. 1.

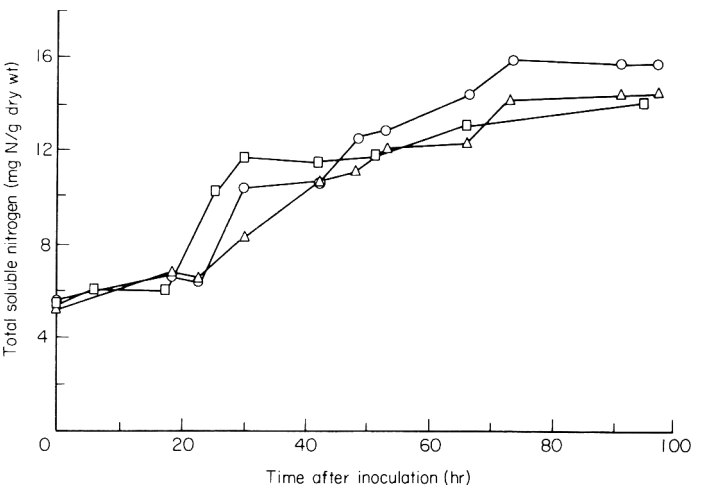

Figure 6. Total soluble nitrogen in Koji. Key as in Fig. 1.

are consistent with the proteinase levels observed in the Koji, with strain 1989 giving both the highest final proteinase concentration and a slightly higher final total soluble nitrogen level than did the other two strains. Amino-nitrogen levels in the three types of Koji (corrected for ammonia present in it) were markedly different however (Fig. 7). They fluctuated considerably during the course of the fermentation, showing no simple or clear-cut correlation with either the total soluble nitrogen or the proteinase levels. All strains gave a rapid increase in proteinase in the Kojis. The increase was relatively much greater than that in total soluble nitrogen, since it began from a much lower initial level, although in absolute terms it was less. Thereafter, the amino-nitrogen first declined rapidly then fluctuated considerably during the rest of the fermentation.

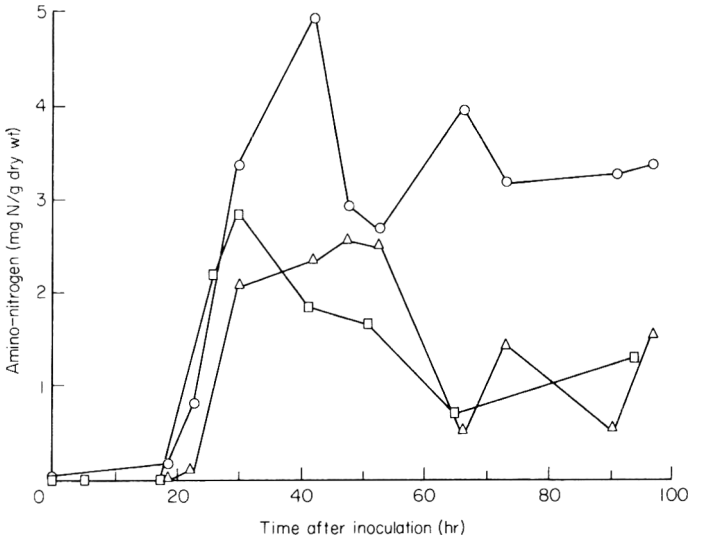

Figure 7. Amino-nitrogen in Koji. Key as in Fig. 1.

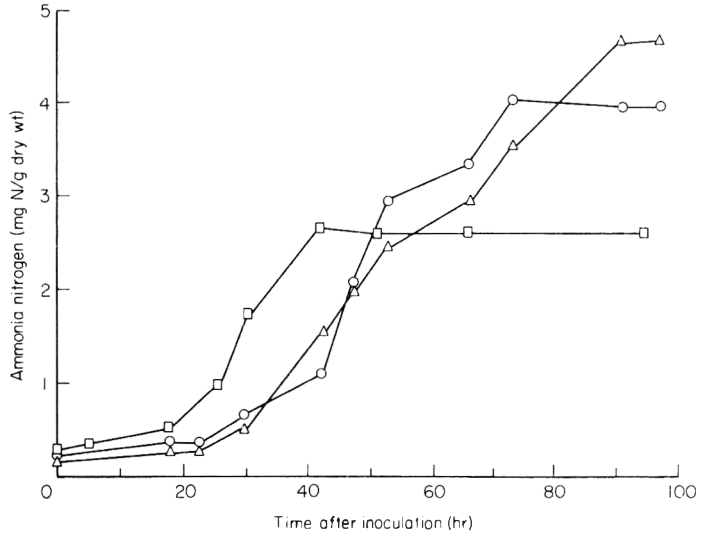

Figure 8. Ammonia nitrogen in Koji. Key as in Fig. 1.

The production of ammonia is of considerable technical importance, since a high level is unacceptable to the manufacturer and consumer of soy sauce, and normally ammonia nitrogen should constitute about 10–15% of the total soluble nitrogen in the finished product. Ammonia in the Kojis made from strains A01 and 1989 increased rapidly from about the 30th hr of incubation until fairly late in the incubation period (Fig. 8). In strain 1988, on the other hand, all the increase in ammonia took place between the 20th and 40th hr of incubation, and the level thereafter remained steady at rather more than half the maximum value reached by the other two strains.

Discussion

It is clear that the three *Aspergillus* strains examined behaved rather differently in Koji, with strain 1989 being the best for soy sauce production according to the criteria employed by Japanese workers, since it gave the highest levels of proteinase, total soluble nitrogen and amino-nitrogen in the finished 72 hr Koji. The much higher levels of amino-nitrogen found, imply a greater extent of protein hydrolysis to amino acids and small peptides. Time did not permit an examination of the relative extent of peptidase formation by the three strains, but such a study might be expected to yield interesting and useful information.

In respect of the other criteria examined, it is unlikely that the rather small differences between strains 1988 and 1989 would be of much practical significance in soy sauce production, although the markedly lower ammonia level in the strain 1988 Koji is interesting. On the other hand, the much higher α-amylase and lower lipase levels found in strain A01 might have considerable effect on the quality of soy sauce produced by this strain, since experimental evidence (Yong, 1971) is consistent with the view that mould hydrolytic enzymes continue to operate in the soy mash or Moromi fermentation.

Our results agree with those of Yokotsuka (1972) and of Yamamoto, Yangida & Suminoe (1972) in showing that most of the important enzymes had reached their maximum level after about 50 hr incubation. We also know that the ammonia concentration is still comparatively low at that time. Reducing sugar levels do not show a consistent pattern of change when the incubation is prolonged beyond 50 hr. On the other hand the important total soluble nitrogen increased substantially after the 50 hr mark. In commercial practice the soy sauce maker traditionally permits the Koji to develop for around 72 hr, the precise timing being adjusted by experienced operatives to allow for variations between batches of Koji. If the Koji ages excessively, with considerable sporulation occurring, then the resulting soy sauce will have a mouldy off-flavour and an excessive ammonia content. In our experiments it was very noticeable that as the Koji aged beyond the 72 hr mark, profuse sporulation developed, and the mild, slightly musty smell of the young Koji was replaced by a harsh, mouldy smell.

Turning now to more specific points arising from this study, we selected enzymes and analyses which we considered to be relevant to the process of soy sauce manufacture while covering a reasonable range of properties within the confines imposed by time and manpower. In a study with some similarities to ours, Yamamoto, Yangida & Suminoe (1972) investigated the activity of alkaline and neutral proteinases, α and β amylases, and phosphatase. It is difficult to see what role phosphatase would play in the development of soy sauce; on the other hand it is interesting that it should be present, and that it showed most of its increase in the first 40 hr of the fermentation. The importance of β-amylase in producing fermentable carbohydrate is evident. Their observation that this enzyme, like α-amylase, reached its maximum activity after 48 hr incubation is fairly consistent with our own observations on α-amylase and reduc-

ing sugar levels. Obya, Shimizu & Morita (1966) have reported finding a glucoamylase in *Aspergillus oryzae* and it would be interesting to know if this type of enzyme is produced in soy sauce Koji.

In some way it seems rather surprising that the mould should produce a sucrose-hydrolysing extracellular enzyme, but it is clear that the production of this enzyme by the mould is increased by the presence of sucrose or related sugars such as raffinose in the medium (Goel, 1974). If, as this might suggest, the mould cannot take up the intact disaccharide satisfactorily, the importance of the enzyme's production at the very beginning of the growth cycle, thus rapidly making the sucrose present in the beans available to the mould, will be readily apparent.

The increase in ammonia content with time, is a very puzzling aspect of Koji development. Liquid cultures of *Aspergilli* on defined media normally do not commence sporulation until the ammonia content has decreased to a very low level and it can be halted by the addition of fresh ammonia. Our analyses seem to indicate that there is ample assimilable carbohydrate in the Koji. We have no direct evidence that the mould produces ammonia by deaminating amino acids, but this seems to be the only likely route for its production in the quantities which we have observed. Thus we are left with several questions: (1) what causes the mould to commence sporulation in the presence of an apparently ample supply of nutrients? (2) Why does ammonia production occur? (3) By what mechanism is it formed? (4) Why do these rather high levels of ammonia not inhibit sporulation as they would do in liquid culture?

The least implausible answer to the first of the above questions may well be that sporulation occurs in response to the loss of moisture which the maturing Koji experiences (Yong & Wood, 1976), but at present neither we nor colleagues studying *Aspergillus* physiology are able to offer satisfactory answers to the other questions. We consider that these questions are not only of importance in the realm of fungal physiology, but may also have practical importance in that their answers could lead to ways of regulating Koji development so as to permit more complete solubilization of the proteins and carbohydrates present by restricting sporulation while (or, and so) permitting increased enzyme production.

Acknowledgments

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Functional properties of proteins for foods – water vapour sorption

A. -M. HERMANSSON*

Summary

Sorption isotherms of proteins of different origins and heat treated proteins of the same origin were studied at 5, 15 and 25°C. Differences in sorption were related to differences in swelling and solubility. A soy protein isolate, a sodium caseinate and a whey protein concentrate were studied. It was found that sorption at low and intermediate water activities (a_w) increased with the ability to swell and take up liquid water. At high a_w where the solvation process starts, differences in sorption could be related to swelling as well as solubility. The sorption increased with temperature or was temperature independent in the a_w range 0.4–0.6. In this a_w range, pronounced minima in sorption rates were found.

Introduction

Work on sorption isotherms of proteins for foods are generally related to storage stability and drying processing problems. Few studies have been made of the relationships between sorption of water and functional properties depending on water-protein interactions, such as solubility and water binding (Berlin, 1975). The latter is very important for the use of proteins in meat systems. Hagenmaier (1972) suggested that sorption data at a water activity (a_w) of 0.84 could be used as a measure of the water binding ability of proteins in meat systems. Puski (1975), on the other hand, found a poor correlation between sorption data at $a_w = 0.84$ and the water binding properties of meat systems. Hermansson (1973a, 1975) showed that swelling, when measured as the spontaneous uptake of water, was highly correlated with water binding of proteins in meat systems.

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The aim of this study was to see whether sorption isotherms of proteins show differences that can be related to differences in functional properties. Interest is focused on two functional properties, namely solubility and swelling. The latter is measured as the ability to take up liquid water (Houwink, 1949; Hermans, 1949; Hermansson, 1972). A review of the concept of sorption and swelling has been made by Hermans (1949).

Three protein systems with different solubility and swelling properties have been used in this study; soy protein isolate, caseinate and whey protein concentrate (WPC). As the three protein systems differ both with regard to chemical composition and physical nature it was of interest to compare isotherms of different proteins as well as differently treated proteins of the same origin. The soy protein isolate used, Promine-D, has a good swelling ability and a relatively low solubility. Both caseinate and WPC are highly soluble but caseinate has a high initial swelling, whereas WPC has a poor swelling ability (Hermansson, 1972). Processing has a great impact on the structure and may strongly influence solubility as well as swelling. As soy proteins and whey proteins, but not caseinate, are sensitive to moderate heat treatment, sorption isotherms of variously heat-treated soy protein isolates and WPC were studied.

Materials

Soy protein isolate. A commercially available sodium soybean proteinate, Promine-D (Central Soya). Analysis (dry weight): protein (N x 5.69) 86.8%, ash 4.7%.

Sodium caseinate. A commercially available sodium caseinate (A/S Lidano). Analysis: protein (N x 6.38) 91.5%, ash 4.6% and fat 1.1%.

Whey protein concentrate (WPC). The WPC used was isolated by gel filtration in industrial scale. Analysis (dry weight): protein (N x 6.38) 76.8%, ash 5.4%, fat 5.2% and lactose 8.3%.

Methods

All samples to be tested were sieved through a 40 DIN screen. Heat pretreatment was done by heating 10% dispersions at 80 or 100°C for 30 min. After cooling the samples were freeze dried and ground. The samples formed gels on heat treatment (Hermansson, 1972). Water sorption was measured gravimetrically with the Cahn RG recorder balance installed in a high vacuum system. A similar procedure to that described by Berlin, Anderson & Pallansch (1968) was used. Sorption measurements were made at 25, 15 and 5°C. Duplicates were made. The water activity (a_w) is measured as p/p_0, where p_0 refers to the vapour pressure of pure water at the isotherm temperature.

Swelling was measured as the spontaneous uptake of water at 20°C. The method has previously been described (Hermansson, 1972). The solubilities

of 1% dispersions were determined as percent extractable nitrogen after centrifugation at 40 000 x *g* (Hermansson, 1973b).

Results

Effect of changes in the physical nature

Both soy and whey proteins have the ability to form gels on heat treatment. The formation of a three-dimensional network induced a reduced solubility and an increased swelling ability in the freeze dried gel samples. The solubility and swelling data of the tested samples are shown in Table 1.

Figure 1 shows the effect of heat treatment on the isotherms of WPC at 15°C. Heat treatment resulted in higher sorption values at low and intermediate water activities. The curves cross each other at a_w around 0.7, above which the untreated sample shows the highest sorption values. Isotherms of WPC at 5 and 25°C gave similar results. At 25°C the curves crossed at $a_w = 0.75$. At 5°C, however, only the curve of the sample treated at 100°C crossed the curve of the untreated sample. The sample treated at 80°C showed higher sorption values in the entire a_w range.

Figure 2 shows the effect of heat treatment on the isotherm of Promine-D at 15°C. The same phenomenon as for WPC is observed. Heat treatment caused higher sorption at low and intermediate a_w. Above $a_w = 0.75$ the untreated sample has the highest sorption. Although the differences are small, they are significant. Sorption studies at 5 and 25°C gave similar results. The isotherms of the heat treated samples crossed that of the untreated sample at 25 but not at 5°C.

The results show that changes in the physical nature of a protein sample effect the sorption behaviour. The differences are significant in the a_w range

Table 1. Swelling and solubility data

Protein product		Solubility % extractable nitrogen	Spontaneous uptake of water (ml/g)*
Promine-D	untreated	52.9	9.6
	treated at 80°C	15.4	20.0
	treated at 100°C	23.7	14.2
WPC	untreated	78.3	1.8
	treated at 80°C	28.7	4.1
	treated at 100°C	27.8	4.4
Caseinate	untreated	80.8	7.5

*In the case of unlimited swelling maximum values are given (see Hermansson, 1972).

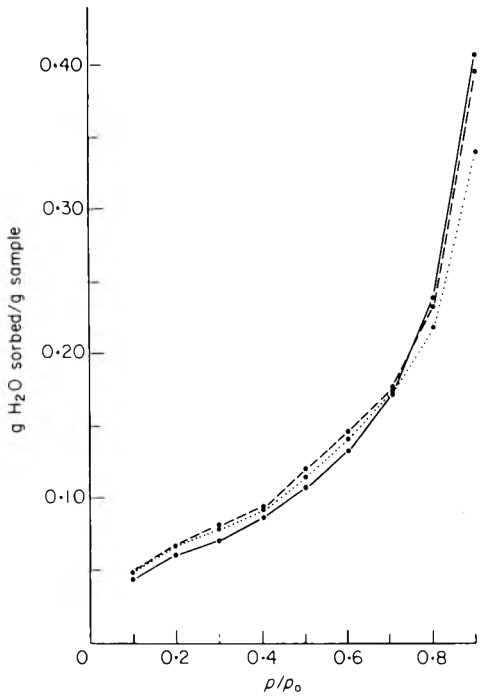

Figure 1. Water vapour sorption isotherms (15°C) for WPC. —— untreated, – – – pretreated at 80°C, ... pretreated at 100°C.

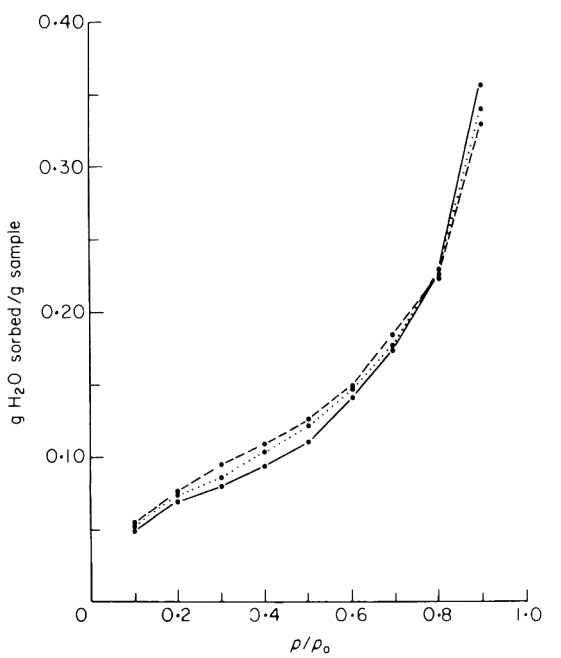

Figure 2. Water vapour sorption isotherms (15°C) for Promine-D. —— untreated, – – – pretreated at 80°C, ... pretreated at 100°C.

0.3–0.6. From Table 1 it can be seen that heat treatment induced a considerable increase in swelling ability and a decrease in solubility. The increase in sorption in the a_w range 0.3–0.6 seems to be positively correlated with swelling. At higher a_w the crosslinking shown by the decrease in solubility seems to have a restricting effect on the sorption.

Comparison between proteins of different origins

The protein products under investigation are crude and contain small amounts of lipids as well as carbohydrates. The presence of fat may for example depress the sorption ability of a protein sample (Berlin, Anderson & Pallansch, 1970). Storage may further change the composition of protein products. Therefore, comparisons of sorption isotherms with regard to protein composition and structure must be made with caution. The purpose of this work was, however, to see whether solubility and swelling, defined as the spontaneous uptake of water, was correlated with the sorption behaviour. It was then of interest to see whether the trends observed due to changes in the structure caused by heat treatment could also be found when proteins of different origins were compared.

The three protein products, WPC, caseinate and Promine-D were studied at 5, 15 and 25°C. The isotherms at 15°C are shown in Fig. 3. The solubility and

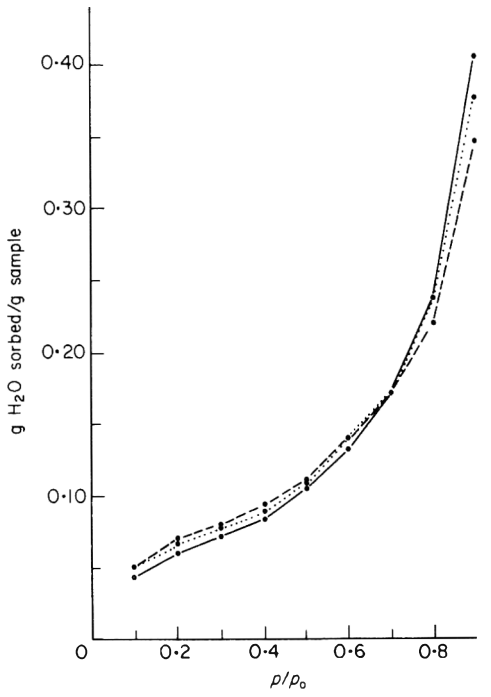

Figure 3. Water vapour sorption isotherms (15°C) for —— WPC, – – – Promine-D, ... sodium caseinate.

swelling data are shown in Table 1. The solubility is higher for caseinate and WPC than for Promine-D. Both Promine-D and caseinate show high swelling ability. However, Promine-D has a limited type of swelling restricted by cross-links, whereas caseinate has an unlimited type of swelling, which results in solvation (Hermansson, 1972). The value for caseinate in Table 1 is the maximum water uptake before the protein lost its ability to hold water due to solvation.

Caseinate and Promine-D show higher sorption than WPC at low and intermediate water activity. The isotherms cross each other at $a_w = 0.7$, above which the caseinate and the WPC with high solubility and unlimited type of swelling shows higher sorption values than Promine-D. The sorption isotherms at 5 and 25°C showed similar trends but the curves crossed at $a_w = 0.6$ and 0.85 respectively.

Effect of the sorption temperature

Sorption data from several temperatures are often used to compute the isosteric heat of adsorption by application of the Clausius–Clapeyron equation. The amount of adsorbed water at each a_w should then decrease with increasing temperature. As has been pointed out by Bettleheim, Block & Kaufmann (1970) the Clausius–Clapeyron equation does not hold for proteins, since it refers to the equilibrium of reversible processes, whereas proteins swell during sorption, undergoing structural changes and consequently exhibit hysteresis. This was substantiated by the results from the present protein products. When the effect of sorption temperature was investigated, a positive temperature dependence was observed at $a_w = 0.4$ for all the WPC samples, as can be seen in Fig. 4.

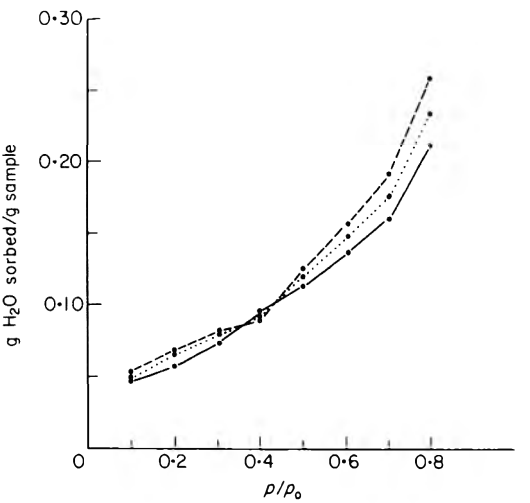

Figure 4. Water vapour sorption isotherms at —— 25°C, ... 15°C and – – – 5°C for WPC pretreated at 80°C.

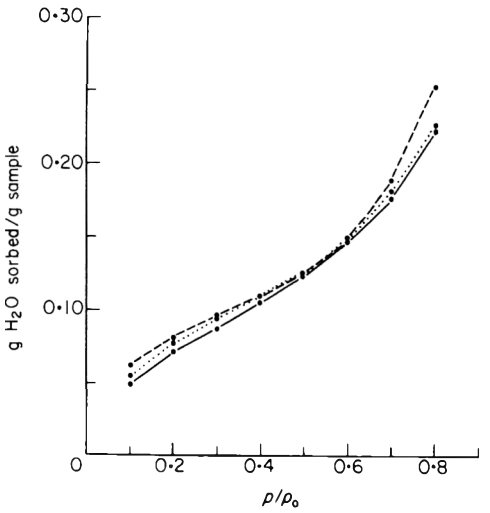

Figure 5. Water vapour sorption isotherms at —— 25°C, ... 15°C and – – – 5°C for Promine-D pretreated at 80°C.

For the Promine-D samples a positive or zero temperature dependence was observed in the a_w range 0.4–0.6. An example is given in Fig. 5.

A positive temperature dependence has previously been reported for milk powders by Berlin *et al.*, (1970) and for hydrophobic surfaces by Zettlemoyer (1968). Zettlemoyer discussed the thermodynamic conditions necessary for this unusual property of hydrophobic surfaces. For proteins, the process involves not only sorption but also structural changes of the protein matrix due to swelling. To evaluate the thermodynamics, calorimetric heats of sorption would have to be measured.

Kinetics of sorption

It has generally been observed that equilibrium is reached very rapidly in the central part of the isotherm, somewhat more slowly at low relative pressure and much more slowly as the saturation pressure is approached (Berlin, 1975). As can be seen from Figs 6–8 this was not the case in the present study. In the figures, the time required to reach 75% saturation ($t_{3/4}$) at each a_w is plotted against a_w.

Figure 6 shows the effect of pretreatment on the 15°C isotherms of WPC and corresponds to the sorption isotherms in Fig. 1. First, there is a pronounced maximum in $t_{3/4}$ at a_w 0.4–0.5. Secondly there is an increase in $t_{3/4}$ with heat pretreatment. Figure 7 shows the kinetics of the same WPC samples at 5°C. The same trends are observed at 5 as at 15°C but a considerable increase in $t_{3/4}$ was caused by the decrease in the sorption temperature.

A similar temperature effect was observed for Promine-D, and $t_{3/4}$ increased with decreasing sorption temperature, as can be seen from Fig. 8. The

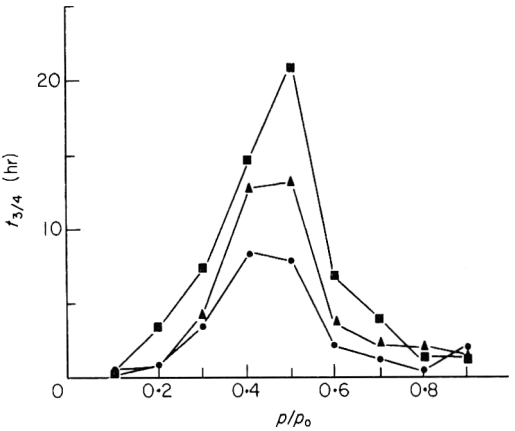

Figure 6. Time of 75% saturation as a function of relative vapour pressure at 15°C for WPC. ● untreated, ▲ pretreated at 80°C, ■ pretreated at 100°C.

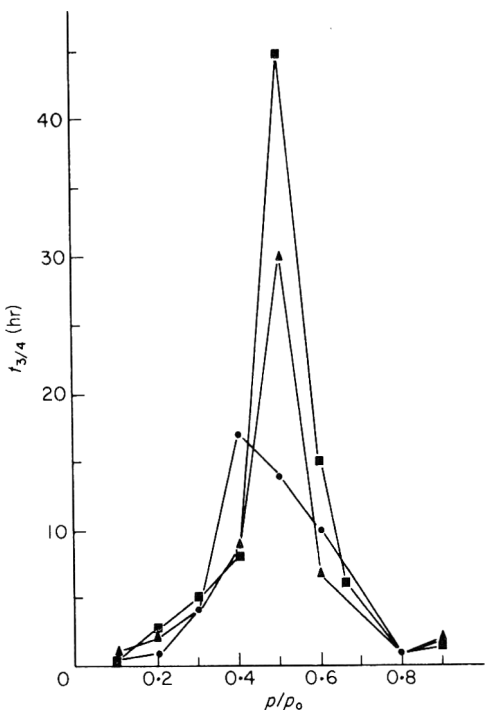

Figure 7. Time of 75% saturation as a function of relative vapour pressure at 5°C for WPC. ● untreated, ▲ pretreated at 80°C, ■ pretreated at 100°C.

maximum in $t_{3/4}$ was determined for sorption isotherms at 5, 15 and 25°C with various degrees of pretreatment and was found to be in the a_w range 0.4–0.5 for WPC and in the a_w range 0.5–0.6 for Promine-D and caseinate. The results for caseinate are in accordance with the results of Rüegg, Luscher & Blanc (1974) on $t_{1/2}$. They found a maximum in $t_{1/2}$ in the vicinity of a_w 0.5

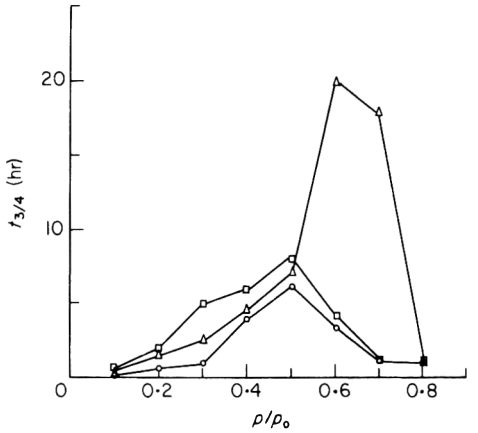

Figure 8. Time of 75% saturation as a function of relative vapour pressure at ○ 25°C, □ 15°C and △ 5°C for Promine-D pretreated at 80°C.

for native casein micelles and rennet treated casein and referred this to structural changes within the sorbant.

The data presented for $t_{3/4}$ are only valid when the water activity is changed in steps of 0.1. Complementary experiments showed that the saturation time was considerably decreased when the driving force was increased, i.e. changes in a_w of 0.2 (0–0.2, 0.2–0.4, etc.).

Discussion

The change in physical nature by heat treatment of WPC and soy protein isolate involved gelling and formation of a three-dimensional network in an aqueous solution. The ability of the dried samples to take up water showed a good correlation with the gel strength before the freeze drying process, although new junction points might have been formed during the drying (Hermansson, 1972). Sorption of water at low and intermediate a_w also increased with the ability to swell and take up liquid water.

In a review on protein hydration, Kuntz & Kauzmann (1974) pointed out that voids created between molecules by drying would give rise to enormous unsatisfied intermolecular forces, which could cause structural rearrangement of the proteins in order to reduce the surfaces of these voids. The dominating driving force at low a_w would then be to remove the strains in the molecules due to the unfavourable surface free energy. Unfolding and crosslinking in the gelling process means an increase in exposed interfaces after removal of water. This would increase the driving force for water sorption at lower a_w, and then capillary and osmotic forces would lead to swelling, which at higher a_w can either be restricted by the presence of intermolecular bonds or can continue until the protein is fully solvated. An interesting similarity has been observed in a work by Gregor & Frederich (1953) on ion exchange resins with

varying degrees of crosslinking. At low and intermediate a_w, the linear polyelectrolyte and those with a low degree of crosslinking showed lower sorption values than those with higher degrees of crosslinking, and at higher a_w the restraints of crosslinking made itself clear.

The observed differences in sorption isotherms, as well as in the kinetics and temperature dependance show the existence of structural changes and swelling of the protein matrix. The differences in sorption are quite small but considerable differences can be found in the kinetics. Pronounced maxima in saturation times were found in the a_w range 0.4–0.6. At low and high a_w, $t_{3/4}$ values were in general less than 2 hr but in the a_w range 0.4–0.6 $t_{3/4}$ maxima of more than 20 hr were observed in several cases.

The difference in the kinetics is probably related to swelling of the protein matrix, which may explain the unusual temperature dependance. The lower the isotherm temperature, the lower was the sorption rate and the higher the $t_{3/4}$. This would mean that a lowering of the temperature has the effect of reducing the swelling of the protein matrix. If the swelling is temperature dependent the sorption will increase with increasing sorption temperature, contrary to what is expected from a reversible sorption process.

From the studies made on the kinetics, no correlation can be made with the ability to take up water. In some case $t_{3/4}$ increased with the ability to take up liquid water but it seems more probable that $t_{3/4}$ is a measure of the tightness of the structure. Further studies on the kinetics involved in sorption of water vapour will be made.

The results of this study show that differences in swelling properties when measured as the spontaneous uptake of liquid water are positively correlated with sorption values in the a_w range 0.2–0.6. At higher a_w the solvation process starts and the differences in sorption data reflect swelling as well as solubility. The restricted swelling has previously been shown to have a positive correlation with the water binding and texture of meat systems (Hermansson, 1975), whereas solubility which is a consequence of unlimited swelling, has a negative correlation. As the effect of solubility in most of the observed cases dominated the effect of restricted swelling on the sorption at high a_w, sorption at $a_w = 0.84$ is not a suitable measure of water binding. Sorption values at $a_w = 0.5$ would give a safer prediction. The observed differences were, however, very small and the ability to take up liquid water is better as a measure of water binding.

Acknowledgment

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Technical note: Vitamin C content of some fruits grown in Nigeria

S. R. MUDAMBI AND M. V. RAJAGOPAL

Introduction

There are few reports on the vitamin C content of fruits grown in Nigeria. According to a FAO report (Woot-Tsuen Wu Leung, Busson & Jardin, 1968) the findings for African foods are scanty and often the methods are not clearly specified; of the 208 fruits investigated only forty-five were analysed for their ascorbic acid content. To provide some data in this area, the vitamin C content of fruits commonly grown in Eastern Nigeria has been reported in this note.

Materials and methods

Fresh grapefruit, lime and sweet oranges were collected from the Nsukka market every morning. Pawpaw, guava and pomegranate were collected from the trees growing in the campus gardens. Tomatoes were collected from the University farm. Cashew apples were collected from two cashew plantations, one about ninety miles and the other about five miles from Nsukka. Fruits which had to be brought from long distances were refrigerated when received until they were taken for analysis. As far as possible, fruits were analysed as soon as they were brought into the laboratory. Vitamin C was estimated in the juice of grapefruit, lime and sweet orange. Juice was expressed from the fruits by a hand juice extractor. In other fruits the vitamin C was estimated in the total fruit. If the juice was used, it was rendered acidic by the addition of metaphosphoric acid so that the final percentage of metaphosphoric acid was 3%. If the whole fruit was taken for analysis, the fruit was blended with equivalent quantities of metaphosphoric acid so that the final concentration of metaphosphoric acid in the mixture was 3%. Both juices and fruit extracts were filtered and made to volume so that there was a 1 : 1 dilution.

Ascorbic acid was estimated in the extracts in duplicate by titrating against a standardized solution of 2,6,dichlorophenol, indophenol (Association of Vitamin Chemists, 1966).

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Table 1. Vitamin C content of some tropical fruits

Fruit	Botanical name	No. of samples analysed	Vit. C (mg/100 g with S.D.)	Range	Vit. C from FAO tables (mg/100 g)
Cashew apple (red)	*Anacardium occidentale*	20	229 ± 6	222 to 236	252*†
Cashew apple (yellow)	*Anacardium occidentale*	20	183 ± 5	175 to 196	–
Guava (red pulp)	*Psidium guajava*	20	80 ± 3	75 to 91	–
Grapefruit juice	*Citrus paradisi*	20	56 ± 3	50 to 61	43 (29 to 61)‡
Pawpaw	*Carica papaya*	20	53 ± 2	48 to 57	52 (22 to 78)‡
Lime juice	*Citrus aurantifolia*	20	36 ± 2	32 to 38	40†
Sweet orange juice	*Citrus sinensis*	20	31 ± 3	28 to 35	44 (37 to 54)‡
Tomato	*Lycopersicon esculentum*	10	27 ± 2	26 to 30	26 (8 to 31)‡
Pomegranate	*Punica granatum*	5	7 ± 0.5	6.5 to 7.8	–

* Red or yellow variety not stated.
† No range given.
‡ Figures in parentheses refer to range in FAO tables.

Results and conclusions

The Vitamin C content of cashew apple, guava, grapefruit juice, pawpaw, lime juice, sweet orange juice, tomato and pomegranate are presented in Table 1. The results obtained compare with those obtained by other workers in Africa. (Woot Tsuen Wu Leung *et al.*, 1968). Cashew apple contains three to four times more ascorbic acid than the citrus fruits such as grapefruit and sweet oranges. Though cashew apple is consumed to a considerable extent in eastern Nigeria, large quantities of cashew apple are allowed to go to waste because the plantation owners are more interested in the kernel which fetches a good price. It would be worthwhile to consider ways and means of preserving the juice of the

cashew apple which is such a good source of Vitamin C. The guavas grown in eastern Nigeria are not as good a source of Vitamin C as those varieties which have been developed in other tropical countries. For example Mangalam Mudambi (1972) have reported values as high as 307 mg/100 g for varieties of guava grown in India. Varieties rich in Vitamin C could be grown in Nigeria as well, and the fruit consumed, either raw or processed, in view of its high ascorbic acid content. Grapefruit could also be processed as concentrate and then used for manufacturing drinks rich in Vitamin C. Pawpaw could be used as one of the components of tropical fruit cocktail or could be packed as jam in view of its moderate Vitamin C content. Sweet oranges and tomatoes are moderate sources of ascorbic acid and as they are consumed in considerable quantities, contribute a fair share of ascorbic acid to the Nigerian diet.

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(Received 5 July 1976)

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Technical note: The determination of chloride in vegetables, fruits and juices with an ion-selective electrode

G. J. MOODY AND J. D. R. THOMAS

Introduction

During the past six years ion-selective electrodes have been used to determine many ions in vegetation, vegetables, fruits and juices (Moody & Thomas, 1976). The Mohr titration procedure has been frequently employed to determine chloride in plant tissue but the end-point is difficult and the charcoal used to clear solutions constitutes a source of extraneous chloride. LaCroix, Keeney & Walsh (1970) devised an alternative, less tedious method based on the Orion 94-17 chloride ion-selective electrode. The chloride content of plants can reach 10% but 0.5–2.5% is more usual. This is well within the linear response range ($\sim 5 \times 10^{-5} - 10^{-1}$ mol/dm^3) of the sensor electrode and without interference from sulphate or nitrate. The rapid analyses for alfalfa tissues by potentiometric titration (relative s.d. 2.1%) closely matched those by the tedious Mohr titration (relative s.d. 1.8%); up to 150 samples per day could be individually processed (LaCroix, Keeney & Walsh, 1970). Similar trends are reported by Cantliffe, MacDonald & Peck (1970) for mature table beet petioles using the same solid-state ion-selective model, whereas erroneous results obtained with the Orion 92-17 liquid ion exchanger chloride electrode are probably due to interference from sulphate ($k^{pot}_{ClSO_4} \sim 0.14$) and particularly nitrate ($k^{pot}_{ClNO_3}$ 1.7–5.9) since the nitrate levels can attain 24 000 mg/kg in petioles (Moody & Thomas, 1976).

The performance parameters of the Orion 96-17 combination and the single 94-17 chloride ion-selective electrodes are essentially identical but the combination model is more convenient. This note reports the chloride assays of apples, potatoes, cucumbers, oranges, carrots and several fruit juices with the 96-17 chloride ion-selective based on known addition potentiometric techniques (Moody & Thomas, 1973; Craggs, Moody & Thomas, 1974).

Experimental

The pH and pCl values of the juices, fruit/vegetable extracts and AR potassium chloride standards were measured with an Orion 801 ion meter at 25 ± 0.1°C.

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	pH	Potential (mV) for sample (V_0) and first spike (V_1)		C_S (mol/dm^3)	V_S (cm^3)	Ethanol content (mg-weight or volume-pe calculated fr Gran's data*				
		V_0 (10 cm^3)	V_1 (0.1cm^3)			Calibra-tion	Equa-tion (4)	Equa-tion (3)	Gran's Plot (= $C_S V_e/V_0$)	
	4.18	154.4	143.6	1.0	0.17	2	18.1	17.6	17	602 (51, raw tomato)
	3.32	150.6	137.0	1.0	0.16	1	13.5	13.2	16	56.7 (38.3)
	2.88	154.9	150.1	0.1	0.42	2	4.63	4.38	4.2	14.9 –
	3.33	167.2	159.8	0.1	0.28	5	2.83	2.73	2.8	9.9 (1.2)
	3.31	164.2	160.0	0.1	0.24	5	5.35	5.03	2.4	8.5 (1.2)
	3.84	153.0‡		–	–	0.4				
		186.2	158.6	0.1	0.07	0.5	0.48	0.47	0.7	12.9 –
	5.19	89.0‡				5				
		139.6	134.8	0.1	0.43	6	4.53	4.38	4.3	98.9 (78.5)
	3.2	226.0‡		–	–	0.25				
		221.0	203.3	0.02	0.11	0.3	0.19	0.18	0.22	0.9 (1–2)
	5.49	130.0‡		–	–	2				
		160.9	149.3	0.1	0.24	3	1.75	1.61	2.4	25.5 (24.5)
	5.98	103.0‡				3				
		150.1	143.3	0.1	0.31	4	2.97	2.85	3.1	123 (68.5)
dward potato	6.2	77.0‡				6				
		147.4	140.0	0.1	0.31	2.5	2.83	2.73	3.1	96.4 (78.5)
	6.16	138.5	134.1	0.1	0.45	4	5.05	4.76	4.5	100 (78.5)

Juices were assayed directly without dilution except for a ten-fold dilution of tomato juice (Table 1). Chloride was extracted from known weights of freshly peeled fruit/vegetable samples, for example, 85.5 g of apple, with de-ionised water in a Kenwood mixer and finally made up to a fixed volume, for example, 100 cm^3 (Table 2). In each case 10 cm^3 aliquots were taken for multiple stage spiking with standard chloride (Table 1).

Table 2. Fruits and vegetables analysed for chloride with a chloride ion-selective electrode: preparation of extracts

Fruit/Vegetable	Weight (g)	Final volume of extract (cm^3)
Orange	48.0	250
Banana	30.8	200
Apple	85.5	100
Cucumber	50.1	150
Old carrot	22.4	250
Old King Edward potato	28.5	250
	39.9	250

Results and discussion

The pH values of samples all lie within the operational range of the chloride ion-selective electrode and no buffering control was employed. The single potentials recorded for each sample can be translated into their respective chloride content from a linear potential-chloride concentration graph which needs daily checking to control the drift of several mV in the constant term of equation 1. However, these chloride values will usually be in error due to the uncertainty of ionic strengths. Nonetheless, they are convenient as approximate values to set the choice of the chloride concentration spikes necessary for the subsequent single and multiple stage known addition procedures. The single stage known addition method estimates the concentration C_0, of primary chloride ion in a sample of volume V_0, whose potential E_0 is given by:

$$E_0 = \text{Constant} - S \log x_0 \gamma_0 C_0 \qquad (1)$$

and where S, x_0 and γ_0 are the electrode calibration slopes, fraction of uncomplexed chloride and its activity coefficient, respectively. Next the potential, E_1, following a single spiking with chloride of concentration C_s (where $C_s \sim 10^2\, C_0$) and volume V_1 (where $V_0 = 10^2\, V_1$) is measured when

$$E_1 = \text{Constant} - S \log x_1 \gamma_1 \left[\frac{V_0 C_0 + V_1 C_s}{V_0 + V_1}\right] \qquad (2)$$

where x_1 and γ_1 are the new free fraction and activity coefficient of uncomplexed chloride, respectively. The assumption that $\gamma_0 \approx \gamma_1$ and $x_0 \approx x_1$ is generally valid when it can be shown that

$$C_0 = C_s \Big/ \left[10^{(E_1 - E_0)/S}\left(1 + \frac{V_0}{V_1}\right) - \frac{V_0}{V_1}\right]. \tag{3}$$

An alternative version of equation (3),

$$C_0 = V_1 C_s / [V_0(10^{(E_1 - E_0)/S} - 1] \tag{4}$$

is based on the fact that $V_0 \gg V_1$ but gives slightly higher chloride values (Table 1).

Gran's plots

The presentation of Gran's data in linear form using semi-antilog plots constitutes an extended version of simple known addition and is based on the general antilog version of equation (2).

$$(V_0 + V_s)\, 10^{-E/S} = x\gamma 10^{-\text{Constant}/S}[V_0 C_0 + V_s C_s] \tag{5}$$

where V_s is the spike volume. The tedious evaluation of the $(V_0 + V_s)\, 10^{-E/S}$ function necessary for point-by-point plotting on the ordinate versus V_s may be avoided by using the semi-antilog Orion Gran's Plot Paper (Orion Research Inc., 1970). This paper is volume-corrected for dilution by spike volume units of 0.01 parts of V_0 up to 0.1 parts of V_0 and it is only necessary to plot the e.m.f. readings on the ordinate for the appropriate V_s values. The Paper is set up with calibrations for Nernstian response of electrodes for univalent (and bivalent) ions and the plot of the e.m.f. reading corresponding to the sample solution (when $V_s = 0$) should be made at a point on the ordinate towards the bottom left-hand corner of the Paper. Subsequent points for e.m.f.s corresponding to the various V_s values will then lie on a line pointing to the top right-hand corner, while extrapolation of this line back to the abscissa (the V_s axis) will give a V_s value corresponding to $-V_e$ where

$$C_0 V_0 = -C_s V_e \tag{6}$$

and from which C_0 may be calculated.

The chloride values in these materials are normally quoted on a mg-weight or volume-percent basis (McCance & Widdowson, 1969). Hence the values have been so presented in the final column of Table 1. For juices these are the product of the Gran's C_0 values (mol/dm^3) and 3.546 respectively. In the case of the fruits/vegetables, e.g. apple, the chloride content is given by (0.22 x 35.46

x 0.1) x 100/85.5, that is, by the general relationship given below.

$$\frac{C_0 \text{ (from Gran's plot) x } 35.46 \text{ x Final volume of extract in dm}^3 \text{ (from final column of Table 2)}}{\text{Weight of sample (g) taken for extract (from second column of Table 2)}} \text{ x } 100$$

Each Gran assay in Table 1 is based on ten successive spiking steps, taking about 10 min in all. Although chloride levels can be quickly found from just a single spiking step, Gran's plots are recommended as they are based on a larger set of data points.

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Book Reviews

Intermediate Moisture Foods. Ed. by R. Davies, G. G. Birch and K. J. Parker. London: Applied Science Publishers, 1976. Pp. xii + 306. £15.

This well produced book contains the nineteen papers and discussions presented at a Symposium held at the National College of Food Technology, Weybridge, in April 1976.

The first of four papers are introductory in nature, outlining the scientific and commercial concepts behind Intermediate Moisture Foods (IMF) and briefly reviewing the present technology involved in the manufacture of IMF's for human and petfood consumption. In the excellent review by M. Karel (Paper 2) the readers' attention is also drawn to the areas in which future research and development should be directed if new IMF's are to become commercially viable for mass marketing for human consumption.

Paper 5 deals with the concept of water activity in IMF's while Paper 7 discusses the techniques available for the measurement of water activity. The significance of water, hydration and aqueous systems in IMF's is discussed in Paper 6 which also outlines how a combination of nuclear magnetic and dielectric relaxation measurements may be used to determine the extent of hydration of humectants with less uncertainty and ambiguity than is usual.

Papers 8 and 9 are both concerned with the chemical and non-enzymic changes which may occur in IMF's although the paper by R. B. Duckworth *et al.* (Paper 8) deals primarily with the effect of the nature of the aqueous environment on these reactions while the review by Williams (Paper 9) gives a more general review of the potential chemical (and nutritive) changes in IMF's. Papers 10–19 are all concerned with various microbial aspects of IMF's.

As would be anticipated by the breakdown given above, the book is an excellent, authorative reference to the microbial aspects of IMF's and also serves as a useful introductory text for anyone entering the IMF field. However, for those interested in the effect of storage on the chemical, organoleptic or nutritional quality of such foods the book leaves much to be desired as, in the space available, the pertinent contributions can do little more than highlight some of the problems. This is rather unfortunate as an understanding, and control of, the chemical changes undergone during storage is of prime importance if new IMF's are to be developed and accepted for human consumption. Another obvious omission is the limited discussion on the current search for new, or improved, humectants with sensory and nutritional properties suitable for use in human foods, for as Karel states, this is one of the chief areas in which further research and development is necessary. The reader expecting the Chapter by A. J. Sinskey on 'New Developments in IMF's:

Humectants' to cover this aspect of the problem would be disappointed as it deals almost exclusively with the ability of some aliphatic diols to control microbial proliferation in IMF.

As is inevitable with contributed papers there is some repetition, but to see the identical Table on pages 8 and 38 and identical Figure on pages 47 and 265 was, to this reader, rather irritating and left the impression that the book would have benefited from more positive editing with a concomitant reduction in size and cost.

In conclusion, I would certainly recommend this volume to anyone interested in the microbiology of IMF's and also to readers wishing to be introduced to the science and technology of IMF but, at a cost of £15, would have great reservations about recommending it to a wider audience.

D. A. Ledward

People and Food Tomorrow. Ed. by D. Hollingsworth and E. Morse. London: Applied Science Publishers, 1976. Pp. xii+173. £10.

This is the published proceedings of the second conference on food and nutrition held by the British Nutrition Foundation in Cambridge in April 1976. Among its most interesting chapters is that contributed by Dr Kenneth Blaxter on the development of agriculture and the state of nutrition in the People's Republic of China. Here, in a land previously notorious for its periodic famines, a population fifteen times that of the United Kingdom, greater than that of the Soviet Union and the United States combined, with an area of cropped land per person little different from ours, has succeeded in supplying adequate food for the community's needs. All this has been done without purchasing grain from the world's market, without loans or aid and without advice which Western experts, assured of the axiomatic superiority of their thinking, are always eager to disseminate.

A second excellent chapter is that of Prof. W. Brass. 'What', so many concerned nutritionists have been accustomed to write, 'are we' – that is, enlightened 'we' contrasted with unenlightened 'them' – 'to do about the fecundity of developing nations whose fruitfulness threatens not merely to replenish the earth but over-crowd it?' Professor Brass draws attention to the abruptness with which that most nebulous but potent of human attributes, an idea, can change events. When prosperity came at the end of the Industrial Revolution to parts of Great Britain and family size dropped, the fashion for few children quickly spread nationwide. And the idea is spreading. Between 1966 and 1973 the mean completed family size in Mauritius dropped 44% from 5.4 to 3.0. The same thing has happened in predominantly Catholic French-Canadian Quebec. Blaxter points to the family norm of two in China brought about by the puritan ideas of citizens of the resurgent nation. Ideas have up till now enjoyed all too little space in our computer programs. When we give them their due weight, according to Brass, 'a plausible case can be made

that the pace of change will lead to considerably lower population sizes in the year 2050 than have previously been anticipated'.

Other chapters of this book are less memorable. J. McKenzie contrasts foods for nutrition with foods for fun; J. B. M. Coppock, A. T. James and D. W. Yarbey discuss some of the problems of food manufacturers on the world stage; J. Mauron considers a variety of things from algae to potatoes as sources of protein; H. C. Pereira reviews what some of the international research centres – CIMMYT, CIAT, CIP, IITA, ILRAD, ILCA, ICRISAT, IRRI – strung around the world have achieved; and N. W. Pirie surveys some of the theoretical limitations on food supply.

Perhaps the last words should be those of Dr C. Gopalan, Director General of the Indian Council of Medical Research. In a short chapter on growing populations and rising aspirations he says several things to which Western readers should attend. One of these is that there would be very little malnutrition in India today if all the food available in India could be equitably distributed in accordance with physiological need. Another is that population growth may actually generate a movement – an idea – to reduce economic disparities, poverty and unemployment.

This is a mixed book, as reports of conferences often are, and among the 173 pages are some very good ones.

Magnus Pyke

Commercial Processing of Poultry. Food Technology Review No. 31. By G. H. Weiss.
New Jersey: Noyes Data Corporation, 1976. Pp. ix + 254. US$32.

To the student, poultry technologist, poultry meat inspector or processor unfamiliar with the style and content of the Food Technology Reviews from NDC, this latest volume in this series could prove a disappointment due to the misleading title.

The present volume follows the usual pattern of previous NDC reviews of the U.S. patent literature describing ninety-three patents relating to poultry processing dating from 1960 to early 1975. The coverage is said to include 'practically' all those issued on the subject in the U.S. during this time. No reference is made to the extensive developments in poultry technology which have occurred in other countries during the period under review.

The reviews are divided into sections in approximate chronological order consisting of chapters covering preservation, chilling and freezing and improvements in palatability followed by sections dealing with a range of consumer products based on poultry and specialized cooking techniques. The final section covers poultry concentrates and synthetic flavouring agents. No reference is made to developments in stunning, slaughter, plucking and evisceration techniques. Drawings and explanatory sketches based on the original patent drawings are good and the author's introductory paragraphs to

each chapter, although occasionally rather brief, are very helpful and readable as is the author's general style of presentation. The volume would be much improved by an adequate subject index in addition to the indexes covering companies, inventors names and patent numbers. For many purposes the table of contents is not an adequate substitute.

In spite of the criticisms the volume provides a useful reference work for workers engaged in the processing of poultry and poultry based products especially those concerned with new product innovation and development.

N. L. Thomas

Books received

Theory and Practice of Emulsion Technology. A. L. Smith (Ed.)
London: Academic Press, 1976. Pp. vi + 352. £14.50.

Proceedings of a symposium held in 1974. Although not of direct interest to those concerned with food, the papers are useful as background reading.

Starch Production Technology. J. A. Radley (Ed.)
London: Applied Science Publishers, 1976. Pp. viii + 587. £35.

Industrial Uses of Starch and its Derivatives. J. A. Radley (Ed.)
London: Applied Science Publishers, 1976. Pp. vi + 268. £15.

Examination and Analysis of Starch and Starch Products. J. A. Radley (Ed.)
London: Applied Science Publishers, 1976. Pp. vi + 220. £15.

The three volumes attempt to survey the whole area of starch technology. The chapters on the food industry take up ninety-six pages.

Food, Man and Society. D. N. Walcher, N. Kretchmer and H. L. Barnett (Eds)
New York: Plenum Press, 1976. Pp. xv + 288. £27.

A multidisciplinary approach to problems of food production and human nutrition.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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

SI UNITS

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

NON SI UNITS

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

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

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