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Computer aided design in the food processing industry.
I. Overview of process flowsheeting

J. N. PETERSEN* AND D. C. DROWN†

Summary

A review of process flowsheeting as applied to the food processing industry is presented. Included is a history of process flowsheeting, an introduction to how this tool is used by the process engineer, limitations of the tool in the food processing industry, a discussion of ongoing research relating to process flowsheeting, and a discussion of research needs in order to make this tool applicable to the food processing industry.

Introduction

Process flowsheeting is a tool which has been used in the petrochemical and pulp and paper industries for many years. This tool utilizes the computer to perform steady state material and energy balance simulations of the operation of an entire processing plant. These simulations are then used to facilitate either process design, or process evaluation. This tool is now becoming available to the food processing industry (Part II, Nelson & Drown, 1984; Hsu, Okos & Reklaitis, 1983; Shah, Okos & Reklaitis, 1984). This paper describes this technology as it relates to the food processing industry. It provides a history of process flowsheeting, discussions of the situations in which it should be used and of its limitations. In addition, ongoing research in order to improve the robustness of the calculations is described, as well as a discussion of the limitations of the technique in the food processing industry and research needs in order to make it more useful for the food technologist.

History of process flowsheeting

In the late 50s and early 60s, computer gurus in each of the major oil and petrochemical companies were developing process specific computer programs to perform material and energy balance calculations around the major unit operations found in this industry (Kesler & Kessler, 1958; Nagiev, 1957). These unit operations included distillation columns, heat exchangers, chemical reactors, etc. These simulators did a very good job of providing information about the individual unit operations and how the operation of each would be affected if its operating conditions were changed.

However, the personnel using these programs soon learned that a systems approach in which the interactions between the various operations could be considered was

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necessary in order to completely understand the operation of the plant. In other words, it is often necessary to determine the effects of changing the operating conditions in one process upon the operation of the rest of the plant. When the simulations were performed on a single unit operation, the person performing the simulation was required to manually put the data produced by that simulation into the simulation of another process. He could then, after much trial and tribulation, determine the effects of changing the operating conditions in one unit operation upon the entire system, if he were patient enough.

Because persons performing process design and evaluation are not patient, as a rule, the concept of modular simulators was developed. In this case, the entire process flow sheet is simulated at one time (Rosen, 1962; Vela, 1961). The output from a distillation column is automatically fed into the input to a reactor, for example. This allowed the person performing the simulation to spend more time analysing the results to determine whether they were realistic and also to decide what to do with the results presented.

Several of these process simulators were developed in the late to mid 60s through to the mid 70s. The majority of them, however, are specific to the petrochemical industry because these industries have the money necessary to develop such systems, and (more importantly because these industries typically employ personnel who are more likely to use the computer) to analyse problems encountered in the plant than do most other industries.

PACER was the first flowsheeting program to be developed. It was developed in the early 60s by P. T. Shannon (1962). In late 1964, the Monsanto company formed a team of six to attempt to define and implement what has now become the FLOWTRAN system. The FLOWTRAN system is commercially available on time-shared computers to perform material and energy balances for the petrochemical industry (Rosen & Pauls, 1977).

Simultaneous to the development of FLOWTRAN at Monsanto, most of the other petrochemical companies were also developing their own process simulators. In addition, independent software houses were beginning to develop simulators for the entire industry.

For example, Simulation Sciences has developed a process simulator which they titled PROCESS. PROCESS is a very well written simulator containing an extensive thermodynamics package, many calculational blocks, and the capability to perform optimization (Brannock, Verneuil & Wang, 1979). Optimization lets the computer select the most economic design or operating conditions.

In the late 70s, the oil crisis hit, and many people thought the way out of this crisis was through the development of natural oil reserves, such as oil shale. Because of the interest in oil shale development, the U.S. government spent about 5.7 million dollars to develop the ASPEN system for material and energy balances, on processes specific to the oil shale and coal processing industries.

One of the additional problems which ASPEN addressed which few of the previous simulators had considered was how to handle solids in the processing streams. In the oil shale industry, solids handling is one of the major problems encountered. Most previous simulators had not addressed this problem because in the petrochemical industry, where most of the other simulators had been developed, there was no need for solids handling capability.

In the food processing industry, however, solids handling is one of the major concerns. The separation of solids from fluid components, or water from solids constitute some of the most important unit operations.

One other industry has the same sort of problems as the food processing industry. In the pulp and paper industry pulp solids, rather than food solids, must be considered in any viable simulation package. In the early 70s, a group of researchers at the University of Idaho began development of a flow sheet simulator specifically adapted to the problems encountered in the pulp and paper industry (Edwards & Baldus, 1972). This package was named GEMS and is used worldwide for pulp and paper plant simulations.

The thermodynamics package contained within GEMS is not nearly as extensive as is that contained within packages developed for the petrochemical industry. However, the need for thermodynamic properties of more than a few compounds does not, in general, exist in the pulp and paper industry, nor, in many cases, in the food processing industry.

With this brief introduction to the history of process flowsheeting, we will now discuss why this technology has gained so much acceptance in the past few years.

Advantages of process flowsheeting

In this section of the paper, we will discuss the potential benefits of using process flowsheeting to help in the design of new processes and to help in the evaluation of how existing processes can be operated more efficiently. In order to help identify ways that the program can be used, we will look at some questions which could be answered by using this tool in the food processing industry.

Utility of process flowsheeting

Process flowsheeting is used in the modelling of the steady state operation of a processing facility. Two types of problems are encountered in which modelling can prove beneficial: performance calculation and design calculation. A plant is calculated in the performance mode if from the known input stream parameters and data specifying the performance of the unit operations, the profiles of dependent variables and/or the output stream information are calculated. This information can then be used to test different operating conditions and operation strategies, in order to determine the conditions and strategies where the product can be produced while optimizing some cost function.

An example of a calculation in the performance mode would be testing to determine how well a dryer which, for example, was designed to dry 3/4 inch square french fries would operate when 3/8 inch by 1 inch steak fry potatoes were to be processed. Alternatively, the study might be to determine how well the current dryer would perform on the current product when the operating temperature or air flow rate are changed to new values. The dryer would currently exist; the study would simply indicate how well it would perform under different operating conditions.

On the other hand, a complex plant is calculated in the design mode if from the desired performance specifications on output and input streams and parameters concerning the units, the unknown set of missing parameters, such as equipment sizes, is calculated.

An illustration of the use of a model in the design mode would include the initial design of a dryer to dry instant rice. The size of dryer needed in order to produce the specified product from the feed stream would not be known before the calculations were started. Instead, the model would predict the size of equipment needed to perform the operation.

The question then is, how would such a program help in either of these situations. In

the performance mode, the program would help the process engineer determine whether the equipment could be used for the new operation or how the existing equipment could be operated more efficiently. In the design mode, the program would be used to determine the configuration and the size of equipment which must be specified and the conditions at which the equipment should be operated in order to obtain the desired product. In either case, the calculations could be performed by hand given a sufficient amount of time and an engineer who is very experienced. However, using a flowsheeting program, it is possible to perform the calculations very quickly, easily, and without worry about possible computational errors. This would leave the engineer to the task at which he should do best: describing the problem, setting the parameters in the problem, and then interpreting the results once they are obtained.

In addition, because it is much easier to make changes in the input data for a program than it is to perform, by hand, a set of calculations to modify a process, the engineer is much more likely to perform the calculations when he has access to a computer aided design package. This will make it possible for the engineer to investigate many more process alternatives when he is using a simulation package than he would be willing to do if he had to perform the calculations by hand. This will lead to more innovative designs and better operation of the plant once it is brought on stream.

People are not prone to go through iterative calculations, especially when the loop time may be several days of calculations. Instead, the average engineer will find a design that works and use it, not even trying to find a better design if the process is complex. What sort of things make it difficult for an engineer to manually perform simulations of a complex plant? The most important are (Halvacek, 1977):

(i) existence of recycle loop(s);
(ii) calculation of operating parameters in unit operations in which iterative methods must be used;
(iii) use of counter-current configuration in the plant (e.g. a counter-current cascade of blanchers);
(iv) the specification of internatal and/or exit streams with unknown input streams.

When any of these complications are present, the solution of the material and energy balance equations becomes a tedious, iterative procedure. Thus, a flowsheeting program provides a valuable tool for the food process engineer.

Applications to the food processing industry

In the food processing industry, the use of computer aided design programs is in its infancy. The potential for growth is tremendous because we are feeling the need for more efficient processes to produce products with the same or higher quality at much lower costs in energy and raw materials. A flowsheeting system could be used to calculate the design that produces product using the least amount of energy and raw materials, or to determine conditions at which current processes should be operated in order to minimize costs. In addition, research is ongoing which will allow the computer to also predict product quality.

Questions which such a program will answer are:

(i) What are the effects of recycle on energy costs?
(ii) What are the effects of recycle on product quality?
(iii) How can we better operate the plant as it is now configured?
(iv) Will recycle introduce bio-hazards about which we do not currently worry?
(v) Is there any way we can reconfigure the plant so that the same product is

produced at less cost?

(vi) Are there any by-proudcts which could be economically recovered from any of the process streams?

In addition, your imagination will lead you to specific questions which you can ask and have answered much more easily if you had access to such programs tailored for our industry.

It is necessary to keep in mind the limitations of the technology. Because foods are of biological origin, and as such have properties of unstable complex mixtures that need to be acknowledged at every stage of the design, the simulation may not give a complete picture of the process operation. Current approaches can provide an approximation to reality, and significant improvements to present processes, but they will seldom provide a complete answer. Chemical reactions must be accounted for at every stage of the design. Because of this complexity, dynamic simulators hold great promise for the food processing industry.

Future directions in process flowsheeting

In this section, we will discuss the direction that research in process flowsheeting is taking. We will look at different types of computational strategies used to obtain the solution of the problem, and the advantages and disadvantages of each. In particular we will discuss sequential modular, equation oriented, and simultaneous modular methods for obtaining steady state solutions of process flowsheets. We will also discuss dynamic systems used to investigate the dynamic responses of flowsheets to process upsets. In addition, the new area of process synthesis will be briefly described.

Sequential modular

So far, all the systems we have discussed have been of the sequential, modular type. This means, that for any given process, the flow diagram is broken down into modular blocks representing the unit operations in the process. These blocks are then solved sequentially. Unit calculations are performed by procedures, or modules, which calculate output streams from a unit given values for the input streams, and sufficient information to define the performance of the unit. To calculate a flowsheet of interconnected units, calculations are made in a sequence determined by the process of topography. If recycles are present, then an iterative calculation is performed where recycle streams are 'torn', and a convergent sequence of guesses for these streams is produced by standard numerical algorithms. Although this procedure is reliable, easy to assemble and usually robust, it often lacks the flexibility to perform design and optimization tasks. The main situation where the inflexibility is encountered involve the use of the programs for coupled flowsheets that have nested recycle loops where the designer wishes to impose design specifications that lead to deviations from the normal information flow. A network with each module calculating its output, given the output of the previous module, is perhaps the most efficient way of simulating a process, provided no iterative calculations are required. However, the recycle streams and design specifications, the sequential modular mode can be very inefficient, because some level of iteration is required for its solution. (Biegler, 1984).

Equation oriented

Equation oriented (or simultaneous or global) process flowsheeting is based on representing units as sets of equations. To calculate a flowsheet, the equations de-

scribing all units are assembled, and solved interactively, as a set, using standard numerical methods.

The advantage of the equation oriented approach is that it more efficiently handles problems in which a number of design constraints are imposed and it is well suited to the solution of optimization problems. These results are due directly to the fact that the flowsheeting program has the complete set of equations available on which to work. Derivatives can be calculated more easily, making higher order convergence techniques, such as Newton's method, available while sequential modular programs can easily use only first order techniques.

In addition, again because derivatives can be more easily calculated, process optimization can more easily be used in equation oriented flowsheeting packages. In optimizing a process it is necessary to evaluate several partial derivatives of the objective function with respect to the variables which can be varied. These derivatives then point the way to the optimal solution.

With these advantages to equation oriented flowsheeting, it would not seem logical to use anything but an equation oriented flowsheeting package. However, the technique does have significant disadvantages. Perkins (1984) has outlined the disadvantages given for using equation oriented techniques. He has, in his paper, addressed each of these problems and the interested reader is directed to his discussion. Suffice it to say that several disadvantages exist for the equation oriented approach. Possibly the most important of these problems are: (i) when the solution fails the user is left with little useful information about what caused the failure; and (ii) the engineer using the package must determine the set of equations to be used to describe his system. The package only solves the equations and provides the thermodynamic information to him. However, in order to debug his data set, the engineer using a package needs to be able to easily determine where a problem occurred in the simulation. In addition, he must be able to simply use 'canned' routines and not be required to set up his own equations for each particular simulation.

Simultaneous modular

A third type of approach which is gaining popularity is the simultaneous modular approach. This approach is very good at handling steady state simulation problems where feed streams to the process and equipment parameters for all units are specified, although even in this case there are potentially three nested levels of iteration in the solution procedure (Perkins, 1984), i.e. the physical properties calculation, the module calculations, and the convergence of torn streams. This type of calculational procedure is more efficient than the sequential modular approach at solving design problems where some of this information is not given by the user, being replaced either by specifications on other variables in the process, or by some objective function to be optimized. Broadly speaking, the simultaneous modular approach can be defined as 'the art of flexibly solving simulation problems made of black box process modules' (Biegler, 1984).

In this approach, the user again connects black boxes together to form a flowsheet. He then tells his program the input and output streams for each of his black box processes. The executive program then decides the calculational scheme to be used to solve the particular problems used in the simulation, however, the equations are usually solved as a complete set rather than sequentially. Derivatives can be easily calculated because the entire set of equations is available. Thus optimization can easily be undertaken. This approach is, to some extent the best of both worlds: it allows the ease

of use provided by the sequential modular approach while still providing the power of the equation oriented approach.

Summary for steady state simulators

In summary, there is no question that an equation-based approach is theoretically superior to the sequential modular strategy. In practice, sequential modular simulators will continue to be used because:

(i) they are much easier to construct and understand;
(ii) they presently require less core storage and thus can be extended to much larger simulation problems;
(iii) they allow incorporation of new modules or complex versions for unit operations without changing the overall solution strategy;
(iv) process simulations are currently easier to program and debug in the sequential modular mode;
(v) they are available, they work, and they are most familiar to engineers.

Dynamic simulators

With the growth of interest in control system design, hazard analysis, and operability studies, there has developed a need for dynamic as well as steady state process simulation packages. Dynamic simulators have developed independently of the activity in steady state flowsheeting because the roots for dynamic simulations is in digital simulation of analogue computers. This forced the model forms and the data requirements for the two systems to be quite different.

This is obviously a great inconvenience for the user, since much of the basic information is required by both systems, in spite of differences in use and differences in level of sophistication of the models employed.

Thus, it would be advantageous if a system were developed which could be used for both steady state and dynamic analysis of a process. In recent years, several computer languages have been developed for representing and solving ordinary differential equation systems characterizing various physical processes. The most widely used of these languages are ACSL, CSSL IV and CSMP III. Each of these languages was developed primarily to model electrical, mechanical and aerospace systems.

In modelling these systems, it is only necessary to solve the differential equation systems for known initial conditions and parameter values to study the behaviour of the physical process as a function of time. However, none of these systems have the physical properties of various compounds built into the simulator, nor do they have standard blocks to represent standard unit operations already developed and ready to be used by the engineer desiring a quick solution to a complex dynamic problem.

In recent years, however, some progress has been made in developing dynamic process flowsheet simulators. Perkins & Sargent (1982) describe a flowsheeting system which they named SPEEDUP. This program is essentially an equation oriented flowsheeting system with the added capability of having some of the system equations be dynamic. Thus this program is able to perform, to some extent, dynamic simulations of processes based upon the flowsheet of the system. To date, however, no system has been described which will use simultaneous modular logic to set up a dynamic flowsheeting system. This would be the next logical step in this process of developing easy to use dynamic simulators. This would allow the average engineer to use standard subroutines to simulate the dynamics of a process without having to be so familiar with

computer programming and process simulation to write the defining differential equations himself.

Process synthesis

Process design has been described as a succession of alternating steps of synthesis and analysis (Umeda, 1983). Analysis typically involves calculating the outputs from a known process, given input conditions. Synthesis, on the other hand, requires the conception of a process which will transform given inputs into given outputs. Analysis characteristically involves deductive logic, while synthesis utilizes inductive logic. Alternative steps of synthesis and analysis imply first conceiving a process, then evaluating its capacities and cost requirements, then using the information gained plus new ideas to generate a new process, then evaluating that process, and so on.

Process synthesis is an act of determining the optimal interconnection of processing units as well as the optimal type and design of the units. The task is to select a particular system out of a large number of alternatives which meet the specified performance.

Because of ongoing research in artificial intelligence, it is becoming possible to expect the computer to not only calculate material and energy balances which will help the engineer to synthesize a process, but to also perform the actual process synthesis. Currently, the computer aided process synthesis technology is such that the computer will provide a limited number of alternative processes which the engineer should then evaluate in detail in order to determine the best system.

Flowsheeting needs in the food processing industry

In this section, we will describe the needs, as we perceive them, in the food processing industry in order to make this powerful tool available to the entire food processing industry just as it is now available in other industries. Once this tool is available, it will be possible to see great reductions in the cost of designing, operating and studying alternative operating strategies for food processing plants.

The main needs before acceptance of these techniques will be widespread are five-fold:

(i) Predictive unit operation models must be developed. In the petrochemical industry, it would have been impossible to develop a program to simulate a refinery without a distillation model. In a similar manner, it is impossible to develop a model to simulate a vegetable processing plant without a good blancher model. Thus, rigorous, predictive models of each of the major unit operations in the food processing industry must be developed.

(ii) Thermodynamic data and good correlations to predict these data must be developed. In simulators such as PROCESS, at least 50% of the calculational time is spent in calculating thermodynamic data. In the food processing industry, this figure will probably not be nearly as high because we do not, in a typical processing facility, work with the wide variety of compounds as are encountered in the petrochemical facilities. However, good thermodynamic data to calculate physical properties of food products are not generally available. These data must be developed before viable simulations can be performed.

(iii) Foods are of biological origin and as such they have properties of unstable

complex mixtures that need to be acknowledged at every stage of the design. The kinetics describing these chemical reactions must be characterized so that they can be included in the process simulation. Without this information, the simulation will provide only an approximation of reality but will seldom, in itself, provide a complete answer.

(iv) Personnel working in the food processing industry must be trained to use tools such as these. It is important that they understand the capabilities and, more importantly, the limitations of the programs. If the capability to perform calculations is introduced without training to be able to interpret and use the results of the calculations, a great disservice is done to the plant engineer. He must be able to understand how accurate the calculations are and use only the viable part of the results.

(v) Process synthesis work should be begun for the food processing industry. With the application of artificial intelligence to this industry, the design of processes will be greatly facilitated. Again, this design must be tempered by the fact that personnel must be trained to be able to interpret and analyse the computer generated design.

The last point to remember is that being one of the later industries to see the utility of having flowsheet simulators is not necessarily a disadvantage. We can build on the mistakes made by others as they were developing simulators for other industries. The petrochemical industry has spent millions of dollars developing simulators which now do not have to be spent again in order to determine the type of system to be used to design a good flowsheet simulator for the food processing industry. We should begin to capitalize on the knowledge they have gained as soon as possible.

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Computer aided design in the food processing industry.
2. Applications of process flowsheeting

D. C. DROWN* AND J. N. PETERSEN†

Summary

Applications of the GEMS computer flowsheet simulation program to identify and evaluate cost saving opportunities in the food processing industry are described. Examples include modification of existing peel-oil recovery during orange juice processing, energy reduction and sugar control in potato blanching, and design improvements in process equipment for cross flow air tunnel dryers.

Introduction

Mass and energy balance calculations are required as part of any economic analysis. Application of a computer flowsheet calculation system for steady state mass and energy balances applied to food processing operations will be described.

It is advantageous to use a modular or building block approach to computer calculations of mass and energy balances because all food processing operations consist of the same basic unit operations (e.g. mixing, flow splitting, heating, cooking, drying, etc.). Food processes vary primarily in the way these basic unit operations are connected and in specific operating conditions used in the unit operation. This suggests that if computer blocks are written to represent the material and energy balances for the basic unit operations and if the computer system allows these blocks to be connected in alternative configurations, then a process engineer can compute material and energy balances for different operating systems without writing new programs.

The petroleum and petrochemical industries have used a modular approach to computing material and energy balances for over 20 years. The pulp and paper industry has been using a modular approach for the past 12 years. The most widely used modular computer system for mass and energy balance calculations in the pulp and paper industry is GEMS (Edwards & Baldus, 1972). GEMS is an acronym for General Energy and Material Balance Systems. It has been under development at the University of Idaho for over 15 years. GEMS consists of an executive program to connect the blocks and keep appropriate records plus a large number of blocks describing the various unit operations in pulp and paper mills. GEMS has been expanded over the past three years to include unit operations common to the food processing industry (Drown & Petersen, 1983). Significant advances are being made in the areas of modelling blanching and drying operations.

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The following sections of this paper will concentrate on applications of GEMS to food processing problems. The objective will be to show how GEMS or another similar program can be used to help identify economically attractive methods of operation for existing processes as well as to aid the design of new process equipment.

The first application to be described is the recovery of peel-oil from oranges during juice extraction. Alternative flowsheets will be analyzed for improvement in oil recovery and to minimize operating problems in existing equipment. The second application is concerned with optimizing the performance of a potato blanching process for controlling sugar content while minimizing energy consumption. The third application deals with analyzing drying operations to minimize energy consumption, increase production capacity, and improve product uniformity.

Orange peel-oil recovery systems simulation

The objective of this simulation is to develop process alternatives to the present system that are more economically feasible and operationally reliable. The process flowsheet of the oil recovery system is shown in Fig. 1. The desludger and polisher shown are both centrifuges that separate the oil emulsion into solids, water, and oil. The aqueous stream from the desludger is recycled back to the extractor.

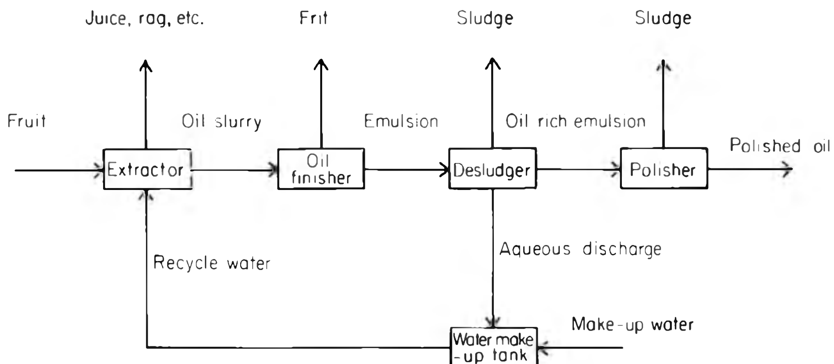

Figure 1. Oil recovery system material balance base case.

The oil recovery from such a system is good but there are operating problems. Typical oil recovery is 56.8% from existing process conditions. The recycle stream is fairly high in suspended solids content and these solids clog the holes in the spray rings of the extractor on a regular basis. A successful filtering scheme is yet to be developed to solve this problem. The results are an abundance of down time and clogged spray rings. Therefore a non-recycle system is preferred by many operators. The non-recycle system produces 10% less oil or only 45.4% oil recovery. The recycle system was used as a base case and the computer simulation calculational block diagram is shown as Fig. 2.

The alternative case proposed and simulated recycled the aqueous discharge to the desludger instead of the extractors as shown in Fig. 3. The desludging centrifuges are not being operated at nearly their design capacity, which is also their optimum operating conditions. So the aqueous discharge is recycled to the desludger. This brings the feed rate up to the design capacity of the unit, allowing it to operate at optimum

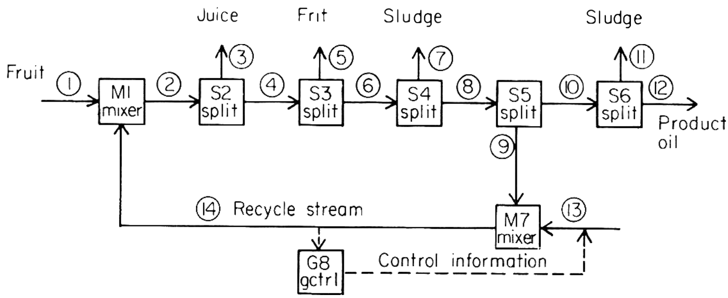

Figure 2. GEMS base case block diagram of oil recovery system.

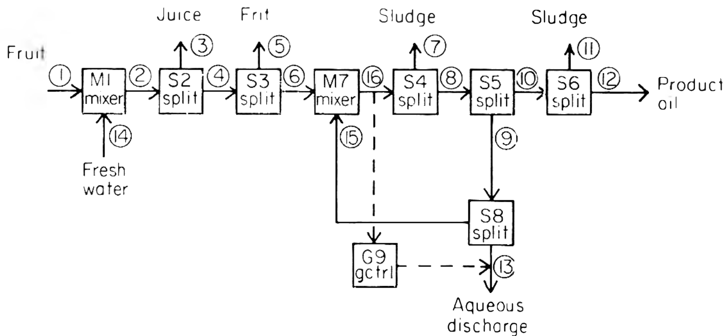

Figure 3. GEMS alternative case block diagram of oil recovery system.

efficiency. Fresh water can then be used in the spray rings, eliminating the plugging problem. This equipment flowsheet arrangement is shown in Fig. 4 and the result is a 4% loss in oil recovered compared with the base case recycle system, but the savings in down time and maintenance could easily make up the lost oil difference. A reduction of 1 hr in down time per 24 hr of operating time would easily make up the loss not including the reduced maintenance cost. Table 1 summarizes the results of the three alternative cases.

Thus, by analyzing various flowsheet arrangements an alternate process configuration that obtained 52% oil yield compared to the 56.8% base case, while eliminating maintenance and operating problems of the base case was achieved. It should be noted that while the computer simulation consists of 8 or 9 calculational blocks, these blocks consist of three basic unit operations: mixers, splitters, and controllers. This process was simulated without any computer programming requirement, using equipment unit operation models that had been developed for the pulp and paper industries. Once the base case was simulated and compared to existing process operating data, the process engineer could rapidly evaluate alternative flowsheet configurations with only minor input modifications to the data file. Steady state material balance calculations were performed for an iterative recycle problem; if done by hand, these calculations are time consuming and tedious, but were obtained in a matter of seconds on the computer. Thus the process engineer has more time available for conceiving new configurations and analyzing the performance of these configurations to develop improved processes.

Table 1. Comparison of peel-oil recovery alternatives

	Alternative	Oil recovery (%)
(i)	Design base case High maintenance time Highest oil recovery	56.8
(ii)	Non-recycle system Minimum maintenance Lowest oil recovery	45.4
(iii)	Proposed recycle system Low maintenance time Good oil recovery	52.0

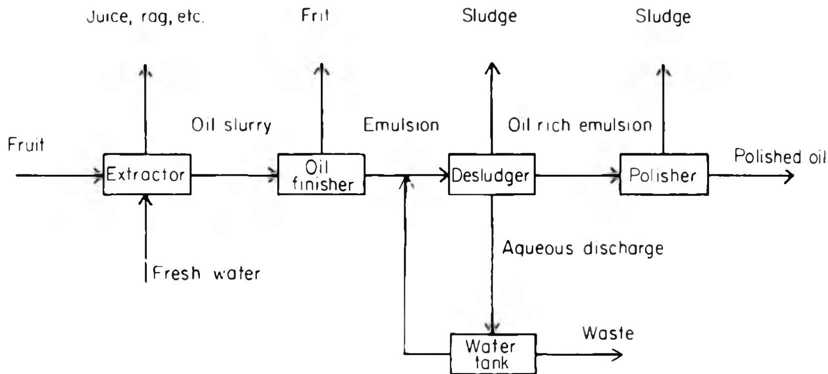

Figure 4. Oil recovery system alternate equipment flowsheet.

Potato blanching

There are numerous phenomena that take place during the blanching of vegetables, which typically consists of immersing vegetables in a hot water bath. Four identifiable results of blanching are: leaching to remove specific components, enzyme inactivation via temperature inhibition, food sterilization, and texture changes. One specific goal of blanching potatoes cut for French frying in the Par-Fry process is to remove sugar for product color control during subsequent frying. A typical potato blancher system configuration is shown in Fig. 5. The blanching operation consists of submerging raw potatoes in hot water, which is recirculated through a heat exchanger to provide the energy to heat the raw potatoes from ambient conditions to blanch temperature. Sugar is leached from the potatoes into the blanch water. Water flush is added to the blancher and overflows, controlling sugar content in the system. Water is also lost by evaporation from the top of the vessel. There are heat losses by radiation and natural convection to the surroundings. The computational potato blancher block diagram is shown in Fig. 6 and consists of two blocks, one for the leaching blancher and one for the heat exchanger. A predictive potato blancher model has been developed and is based on the bench top experiments of CIDCA (Califano & Calvelo, 1983) and on the pilot plant work of the USDA (Kozempel, Sullivan & Craig, 1981; Kozempel *et al.*, 1982).

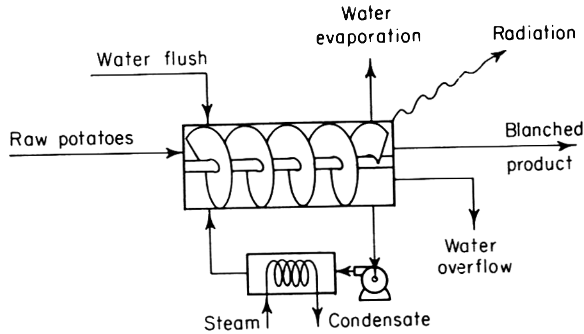

Figure 5. Potato blancher system configuration.

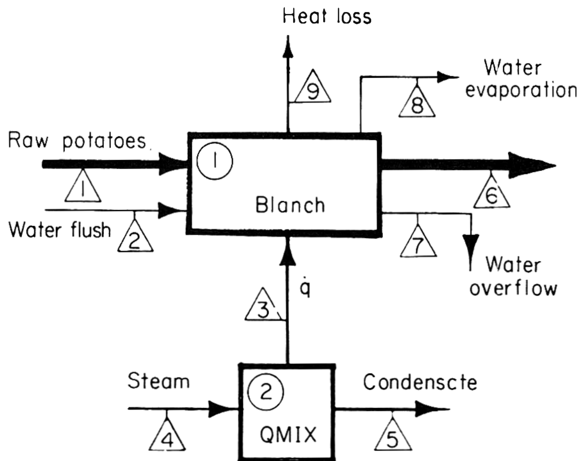

Figure 6. Potato blancher GEMS block diagram.

Due to the large water recirculation rates relative to the throughput rates in the system, a commercial blancher can be modelled as a continuous stirred tank reactor. The rate controlling step for leaching sugar from the potato is diffusion within the potato slab. The solute concentration from the slab is given by Fick's Law of Diffusion. In addition the transient conduction of heat within the potato pieces can be analysed. Evaluating the centerline temperature of the potato is necessary to ensure that adequate blanching and enzyme degradation has been achieved. The differential equations for mass and energy have been integrated analytically and solved to predict the outlet concentrations and energy requirements of the blancher.

Commercial process data was utilized to determine 'effective' sugar diffusion coefficients and validate the model (Nelson & Drown, 1984). In addition to sugar, pilot plant work of the USDA (Kozempel *et al.*, 1982) indicates that minerals and vitamins could also be predicted with a similar model, provided that the inlet concentrations and the effective diffusion coefficients are known. Thermo-labile effects could be included in the model if sufficient data were available to determine reaction rate constants and temperature dependency.

In analysing the energy inputs and outputs of the blancher, the water evaporation, while a very small water mass loss, was shown to be a significant energy requirement. Significant energy savings can be achieved in commercial blanchers by minimizing the surface area of hot water exposed to the ambient atmosphere. In one instance, an 8% energy reduction was possible on a £30 000 per hr process line. This resulted in a steam savings in excess of $10 000 per year for an individual blancher, and was achieved by covering the previously open blancher vessel to reduce evaporation losses, while having no impact on product quality. Thus the program was used to identify areas where improvement opportunities existed in order to maximize profit.

Using the predictive leaching model it is possible to predict the effects of residence time, operating temperature and water flush rates on product sugar content and energy consumption in order to achieve optimum operating conditions to produce a desired product with minimum energy input.

Energy consumption in drying operations

To identify economically attractive methods of operation for existing dryers as well as to aid the design of new dryers, it is necessary to develop an understanding of the energy utilization within the dryer. The purpose of this work was to develop a computational tool to evaluate alternative methods to improve energy utilization in dryer systems. More efficient energy utilization will result in significant cost savings.

The largest production volume product line in many potato processing plants is frozen french fries, commonly termed Par Fry. The basic Par Fry dryer consists of a cross-flow air tunnel as illustrated schematically in Fig. 7. The potatoes travel horizontally on a moving screen through the dryer while air is recirculated in either up-flow or down-flow vertically across the bed of potatoes. A steam coil or other heat source supplies the energy requirements to evaporate the water from the potatoes. Dryers utilized in Instant Rice processing and for chocolate nib drying after dutching have analogous equipment configurations and have also been simulated using GEMS. The first step in conducting a GEMS simulation was to construct a calculational block diagram of the process.

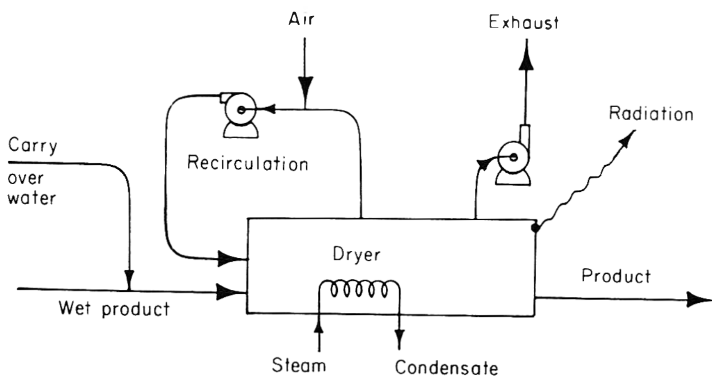

Figure 7. Potato dryer system configuration.

Figure 8. GEMS block diagram for potato dryer system.

Existing dryer energy utilization analysis

A GEMS block diagram for energy audit calculations around the dryer is shown in Fig. 8. The modular diagram consists of adding the wet product potato stream with surface water or water carryover from the slurry transport system to form the actual feed stream to the dryer. Calculationally, a heat exchanger is then used to adjust the feed temperature to the operating temperature of the dryer, then separate or split the water from the potatoes. The dried potatoes then exit the dryer, while the water is vaporized from liquid to steam and then mixed with the air which is exhausted from the dryer. The PSYDRY block is an information control calculation which adjusts the inlet air to achieve a water balance. Several options are available depending upon which process streams have been measured and which are the unknowns; the PSYDRY block will adjust one of the following streams: the exhaust air flow rate, the water carryover inlet rate, or the moisture content of the dry product stream, while holding the other streams constant until a water balance is achieved. Once the water balance has been converged, the QMIX block sums the energy requirements and determines the steam or energy used by the process. From the block diagram, the data file for the GEMS computer program is constructed.

The utility of the GEMS program for energy audit simulation is rapid and accurate data reduction from the process data. These particular calculations take about 2 sec on an IBM 4341 or 1–2 min on an Apple II. The energy audit results provide a means of comparing existing processes to determine the most energy efficient operating conditions. By analysing the steam consumption in parallel product lines at one process plant, an 8% energy reduction was achieved by adjusting the air recirculation rate to increase the humidity of the exhaust stream, and thus match the operating conditions within the dryer to a parallel production line dryer.

With an effective energy audit simulation it is now practical to implement optimization procedures such as EVOP (Box & Draper, 1969; Lowe, 1967) to steadily improve the performance of the process. EVOP is a technique for accelerating the natural evolution of process improvements by systematically making small perturbations in the primary process variables and evaluating the response of the process. Another type of

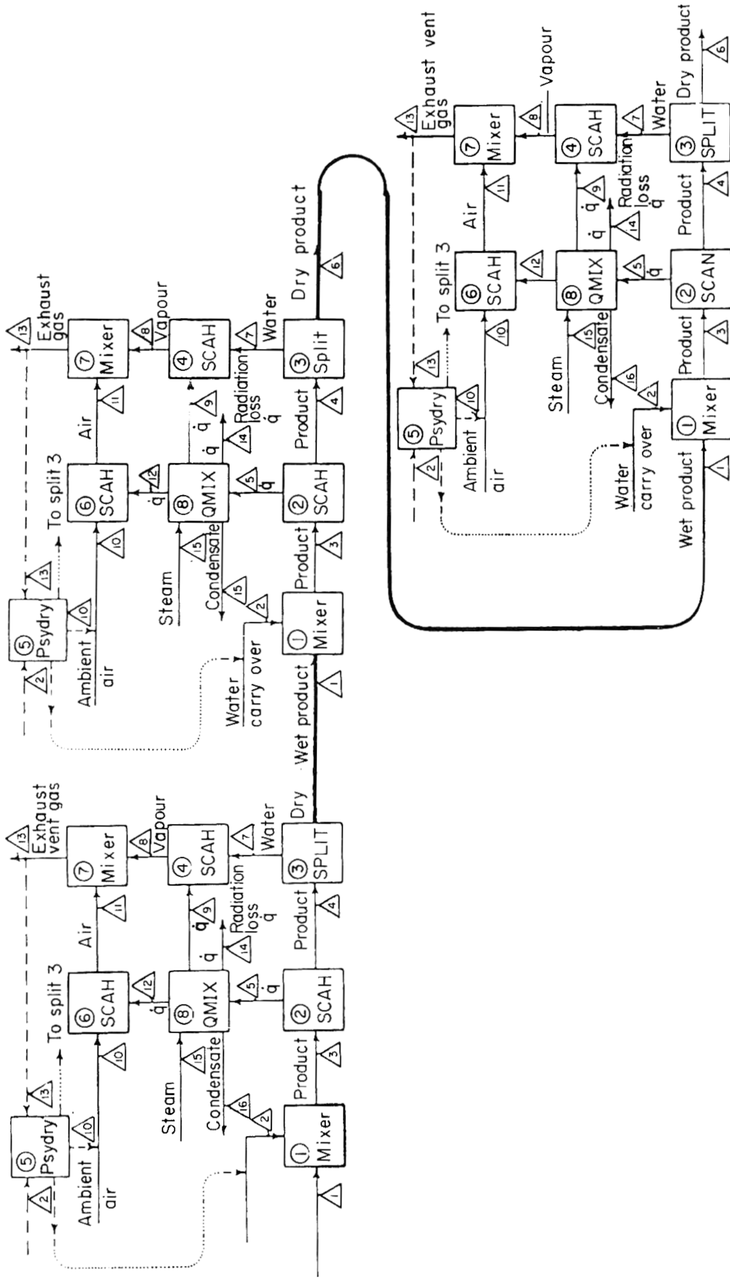

Figure 9. Multiple zone dryers, and modular diagram repetition.

analysis that can be conducted with an energy audit simulation program is to simulate major perturbations in the operating conditions and assume the process would behave as predicted; the results will indicate the effect on energy consumption and indicate if the variable and the direction it was perturbed would be a likely candidate for experimental optimization with EVOP.

Once a basic process configuration is simulated, it is a simple matter to adapt the simulation to alternative process configurations. The dryer we have dealt with up to this point was a single zone system; however, many commercial dryers are multiple zone configurations with independent operating conditions in each zone. Without any additional computer programming it is a simple matter of modifying the data file to replicate the basic dryer zone as shown in Fig. 9 to simulate a triple zone dryer. Each zone has the identical block diagram with the product from the first zone being the feed to the second zone.

Predictive dryer modelling

In order to evaluate the economic potential of the proposed alternatives, a basic understanding of the dryer operation is required. The ability to predict mass transfer effects in the dryer is needed to predict performance as a function of inlet operating conditions. Increasing the inlet air temperature by preheating will provide a direct reduction in dryer energy requirements by increasing the saturation vapour pressure, and thus increasing the mass transfer driving force. However, increasing the humidity of the air entering the dryer will increase the approach to saturation within the dryer, and thus decrease the mass transfer driving force. Typical dryers operate at various degrees of saturation, with 15–90% relative humidity in the exhaust gas being common. Therefore, it is necessary to develop a model to predict dryer performance by combining theoretical heat and mass transfer phenomena with actual operating experience. This will result in a realistic predictive simulation which can be tuned to individual dryer operations by adjusting correlations for transport coefficients.

Many sophisticated models have been described in the literature to calculate moisture and temperature profiles in various drying processes (Hall, 1980; Rossen & Hayakawa, 1977; Bakker-Arkema *et al.*, 1974; Kayihan, Stanish & Schajer, 1984). However, much of the theoretical work that has been done is too complex to be easily used for design and engineering analysis. Our current research is attempting to develop and apply a simple, robust dryer model that can be used to examine energy usage and dryer air recycle alternatives with respect to energy savings and increases in production. Based on published literature for modelling drying operations, primarily in the pulp and paper industry and other agricultural crop drying such as corn, wheat and hops, we have derived an appropriate form of the heat and mass transport differential equations to describe a cross-flow air tunnel dryer. Using a modification of standard finite difference techniques for numerically solving this type of simultaneous differential equation set, a model has been developed which will predict the solid temperature and moisture content, and the air temperature and humidity at any segment in the dryer. The accuracy of this model depends upon the ability to measure or accurately predict convective heat transfer coefficients, mass transfer diffusivity coefficients, and solid properties such as thermal conductivity and heat capacity.

Preliminary results obtained from commercial potato, rice, and chocolate dryers indicate that energy consumption in the drying operation is significantly affected by humidity in the exhaust gases and temperature in the dryer. Increasing air recycle

causes the humidity to increase; however, this has minimal effect on drying rates found in commercial dryers, and significantly reduces the volume of exhaust required to vent the same amount of moisture. Reduced exhaust volumes result in lower energy requirements to heat the carrier air and thus lowers energy costs. Slight increases in dryer temperature exponentially increase the amount of water that can be carried in the air at the same relative humidity. Therefore, increased drying temperatures also result in reduced exhaust volumes and hence, lower energy requirements.

The simulation program was used to identify significant variables and their effect on dryer operating costs and product quality. Sensitivity studies with these variables indicate that many commercial dryers have significant potential for improved operation. The simulation results are being utilized by operating supervisors to guide selection of operating conditions to increase production capacity, reduce energy costs, and improve product quality uniformity. The results are also being utilized by design engineers to improve the configuration of drying equipment.

Overall summary and conclusions

The economic effectiveness of the modular computer system for calculating mass and energy balances for food processing operations has been demonstrated. Computer simulation helps process engineers identify and evaluate many alternate methods of operating existing equipment, and the design engineer to evaluate multiple design options. This can result in significant savings in both capital and operating costs. Utilizing a computerized flowsheet material and energy balance simulation as a calculational tool reduces the drudgery of tedious hand calculations of iterative recycle systems. This allows the process engineer to concentrate his efforts on understanding the chemical and physical phenomena that are going on within the process, and on conceiving and evaluating the cost effectiveness of alternatives.

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Gas chromatographic study of sorption of vinylchloride by unplasticized polyvinylchloride: Effect of concentration, temperature and polymer particle size

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E. K. VOUDOURIS

Summary

The interaction of vinylchloride monomer with unplasticized polyvinylchloride is studied by inverse gas chromatography. The effect of monomer concentration, temperature and polymer particle size are particularly examined. The specific retention volume ($V°g$) of VCM was taken as the parameter of the PVC/VCM interaction and was found to be dependent upon monomer concentration, polymer particle size and temperature. $V°g$ increased exponentially at very low monomer concentrations, increased with decreasing resin particle size and decreased with increasing temperature. For a given resin particle size the effect of temperature on $V°g$ is a lot more pronounced than the effect of particle size for a given temperature.

Values for thermodynamic parameters calculated and peak shapes recorded are indicative of an exothermic, spontaneous interaction between PVC/VCM, and support the 'active site' hypothesis which limits potential migration of the monomer from the polymer into a food contacting phase.

Introduction

Polyvinylchloride (PVC) is widely used in food packaging applications both in the flexible and rigid form. Like all polymers, the finished PVC product contains, among other commercial additives, unpolymerized vinylchloride (Crompton, 1979) the amount of which depends on the method of polymerization and other conditions of manufacture. Numerous studies on laboratory animals, several of which are still in progress, have shown vinyl chloride monomer (VCM) to be carcinogenic even in concentrations below 10 ppm (Hefner, Watanabe & Gehring, 1975; Maltoni & Lefemine, 1974; 1975a; Fed. Reg., 1975a; Maltoni, 1980); thus the use of PVC in food packaging applications has been an issue of great controversy over the past several years (Daniels & Proctor, 1975; Figge, 1972; Gilbert, 1976; Kontominas, Gupta & Gilbert, 1982; Gilbert *et al.*, 1982; Bellobono, Marcandalli & Selli, 1981).

The migration of VCM from a PVC package into a food contacting phase depends on (i) the concentration of residual VCM in the polymer (Morano, Giacin & Gilbert, 1975); (ii) the chemical affinity of the monomer to the contacting food phase (Daniels & Proctor, 1975); (iii) the environmental conditions such as temperature, relative

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humidity etc.; and (iv) the composition (plasticizer content) and form, (film, bottle etc.) of the packaging material (Morano *et al.*, 1975; Morano, 1975; Kontominas & Voudouris, 1982). Previous studies (Kashtock, 1977; Kinigakis, 1979; Kontominas & Voudouris, 1982) on the migration of VCM from both unplasticized and plasticized PVC resins showed a non linear relationship between the monomer concentration in the polymer and the partition coefficient of the PVC/VCM interaction in favour of the polymer. These results were in accordance with the active site hypothesis (Gilbert, 1976; Gilbert, Miltz & Giacin, 1980) which assumes certain irregularities (active sites) within the polymer matrix which tend to bind the monomer, thus decreasing the possibility of its transfer from the polymer into a food contacting phase.

In this paper, the thermodynamic parameters, ΔG, ΔH, ΔS and γ of the PVC/VCM interaction are determined in relation to temperature and polymer particle size with the objectives:
(i) to further elucidate the nature of the PVC/VCM interaction; and (ii) to further test the active site hypothesis. The study was carried out using Inverse gas chromatography (Kontominas & Voudouris, 1982; Gilbert, 1984).

Calculation of thermodynamic parameters from gas chromatographic data

The partial molar enthalpy of solution of vapour in the column stationary phase (ΔH_s) is related to the specific retention volume of the monomer in the column ($V°g$), through the Clausius–Claperyon equation (Kiselev & Yashin, 1969):

$$\frac{d(\ln V°g)}{dT} = \frac{\Delta H_s}{RT^2}. \qquad (1)$$

The specific retention volume is given by equation (2)

$$V°g = \frac{J\dot{V}(t_r - t_f)}{w_s} \times \frac{273}{T}, \qquad (2)$$

where:

J = compression factor, taking into account the pressure drop along the column length,
$\dot{V}$ = flow rate of carrier gas (ml/sec),
t_r = retention time of compound under study (monomer),
t_r = retention time of unadsorbed species (air),
w_s = weight of stationary phase (9).

The partial molar enthalpy and entropy are related to the column capacity coefficient (k) through equation (3)

$$\ln k = \ln f + \frac{\Delta H}{RT} - \frac{\Delta S}{R} \text{ (Knox \& Vasvari, 1973)}, \qquad (3)$$

where:

ΔH = partial molar enthalpy of solute when transferring from the stationary to the mobile phase (apparently $\Delta H_s = -\Delta H$) (4),
ΔS = partial molar entropy of solute when transferring from the stationary to the mobile phase ($\Delta S_s = -\Delta S$) (5),
f = ratio of volume of stationary phase to mobile phase,
k = column capacity coefficient, defined as the ratio $V°g/V_o$ where V_o is the dead volume of the column, associated with 1g of stationary phase.

Combination of equations (3), (4) and (5) give: $\Delta S_s = R(\ln k - \ln f) + \Delta H_s/T$ (6).

Equations (1) and (6) provide the ΔH_s and ΔS_s values necessary to calculate Gibb's free energy (ΔG_s) of the solute-solvent interaction using equation (7).

$$\Delta G_s = \Delta H_s - T\Delta S_s. \quad (7)$$

If we define as 'excess' free energy mixing (ΔG_{xs}) for a real solution (ΔG_s) in comparison to an ideal solution ΔGi then:

$$\Delta G_{xs} = \Delta G_s - \Delta Gi, \quad (8)$$

$$\Delta G_s = RT \ln \gamma Pi^\circ \quad (9)$$

$$\text{and } \Delta Gi = RT \ln Pi^\circ, \quad (10)$$

where:
γ = activity coefficient
Pi° = vapour pressure of pure solute.
Combination of equations (8), (9) and (10) give:

$$\Delta G_{xs} = RT \ln \gamma. \quad (11)$$

Equations (1), (6), (7), (8) and (11) were used to calculate the thermodynamic parameters of the PVC/VCM interaction.

Materials and methods

Three different columns, containing unplasticized PVC resin (VC 47 BE-1, Borden Chem. Div., N. Andover Mass. U.S.A.) of mesh size 60–80, 100–120 and 150–200 respectively were prepared. The resin was stripped of its residual monomer using a well established method (Morano, Giacin & Gilbert, 1977). The columns were weighed before and after filling with the PVC resin to determine the weight of the polymer in each one. A series of standard VCM samples were prepared by direct injection of a known volume of VCM gas (99.99% pure, Matheson Gas Co.) in glass serum vials of 70 and 120 ml capacity. The columns were connected to the gas chromatograph and known amounts of VCM were injected into the instrument. Temperatures used were: 15, 22, 30 and 40°C. Gas chromatograph operational conditions were as follows: instrument: 3700 gas chromatograph equipped with a dual FID; columns: 5×¼ in O.D. st.steel; T detector: 200°C; T inject. port: 30°C; flow rate of carrier gas: N_2–30 ml/min.

Measurement of retention times were accurate to ±0.05 sec. The retention time of air was taken as the retention time of the unadsorbed species.

Results and discussion

The effect of concentration of VCM in the polymer, at equilibrium, on the specific retention volume, for the three columns at four different temperatures is shown in Figs 1–3 respectively.

In these Figures the point of intersection of the curves with the $V^\circ g$ axis, represents the $V^\circ g$ values corresponding to infinite dilution of the monomer in the polymer. These values are shown in Table 1.

Figures 1–3 show that for all three resins used, at all four temperatures (below Tg = 81°C) the specific retention volume is dependent upon: the concentration of VCM, particularly at very low levels, resin particle size and temperature.

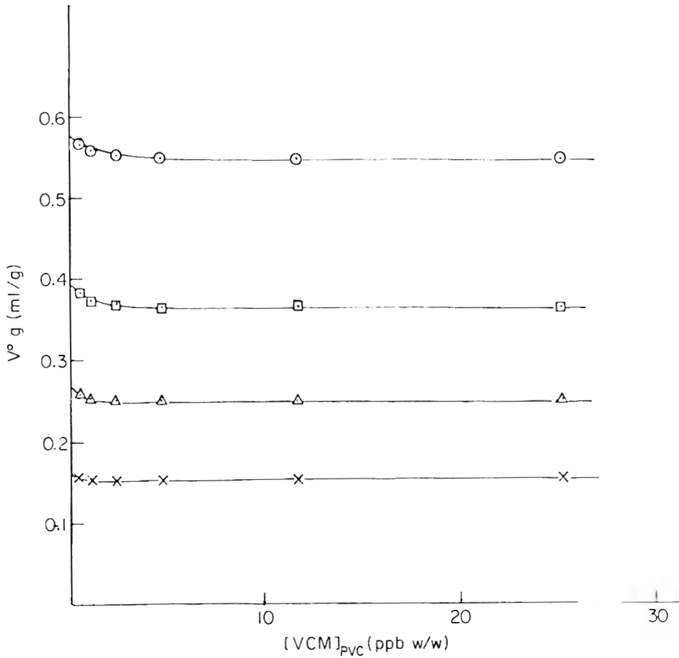

Figure 1. Specific retention volume as a function of concentration of VCM injected, into the PVC (60–80 mesh); column at temperatures 15(O), 22(⊡), 30(△) and 40°C(×). The part of curves in Figs 1–3 corresponding to $[VCM]_{PVC}$ between 0.1 and 2 ppb was constructed using six consecutive points only three of which are shown on the plots.

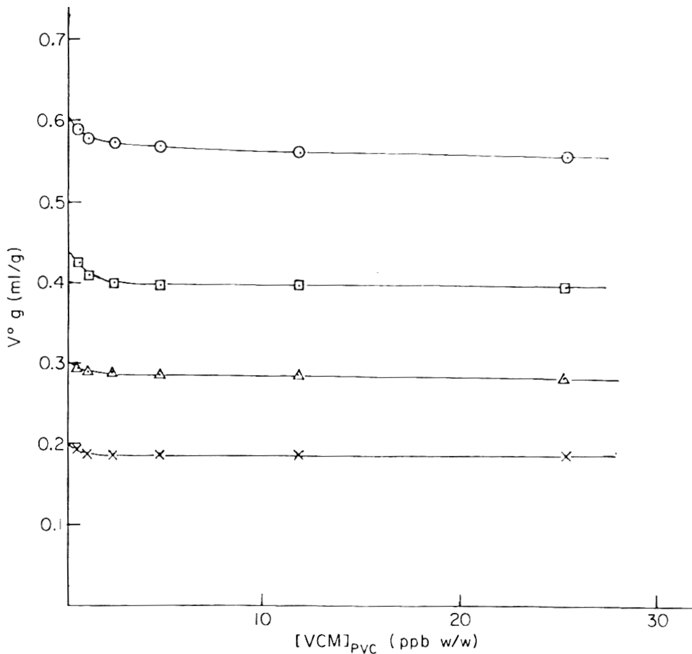

Figure 2. Specific retention volume as a function of concentration of VCM injected, into the PVC (100–120 mesh); column at temperatures 15, 22, 30 and 40°C.

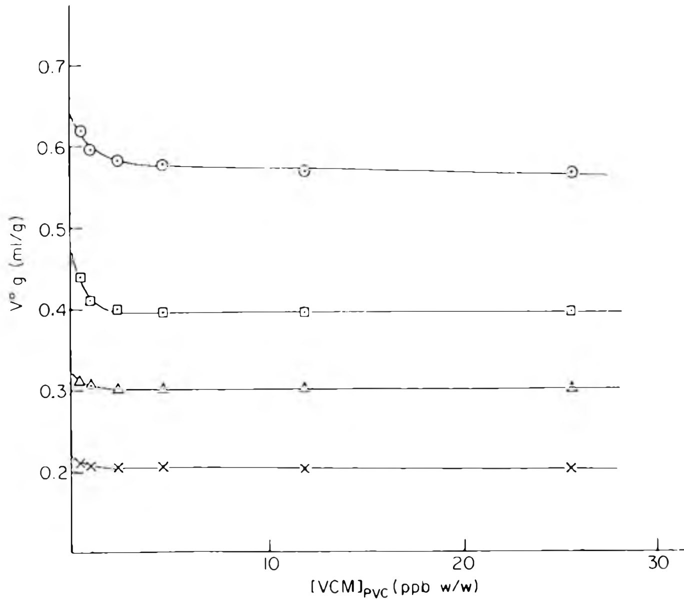

Figure 3. Specific retention volume as a function of concentration of VCM injected, into the PVC (150–200 mesh); column at temperatures 15, 22, 30 and 40°C.

Table 1. Specific retention volume values at infinite dilution (V°g^x) for the Three PVC Resins at 15, 22, 30 and 40°C

60–80 mesh resin		100–200 M		150–200 M	
V°g (ml/g)	T (°C)	V°g (ml/g)	T (°C)	V°g (ml/g)	T (°C)
0.573	15	0.601	15	0.640	15
0.398	22	0.440	22	0.472	22
0.270	30	0.305	30	0.328	30
0.158	40	0.196	40	0.218	40

More specifically it is shown that for a given temperature and resin particle size, V°g increases exponentially with decreasing concentration of VCM. It is postulated that at very low VCM concentrations, the monomer is reversibly bound onto 'active sites' in the polymer thus V°g increases, while at higher VCM concentrations additional monomer molecules are loosely retained by the polymer and thus V°g decreases.

This hypothesis is supported by results of the effect of resin particle size on V°g. It is shown in Figs 1 and 3 that for a given temperature e.g. 22°C and VCM concentration e.g. 0.5 ppb, V°g increases from 0.38 ml/g to 0.44 ml/g, when the resin particle size decreases from 60–80 mesh to 150–200 mesh. Increase in the total surface area of the polymer uncovers new active sites which reversibly bind additional monomer molecules.

Temperature, of course, is the critical factor in the retention of the monomer by the polymer. Increase in temperature results in increase of the kinetic energy of the monomer molecules which in turn antagonizes thermodynamic binding of VCM onto active sites in the polymer. It should also be stressed that for a given resin particle size the effect of temperature on V°g is more pronounced than the effect of particle size for a given temperature.

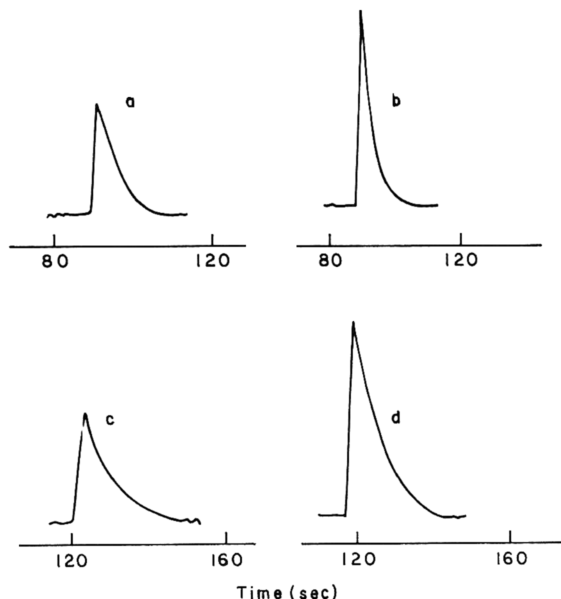

Figure 4. Effect of monomer concentration and resin particle size on chromatographic peak shapes (15°C). (a) 60–80 mesh 0.5 ppb W/W; (b) 60–80 mesh 25 ppb W/W; (c) 150–200 mesh 0.5 ppb W/W; (d) 150–200 mesh 25 ppb W/W.

Further support of the active site hypothesis is provided by chromatographic peak shapes shown in Fig. 4. For a given particle size and temperature e.g. 60–80 mesh, 15°C, the retention pattern of the monomer shifts from a more asymmetric (tailing of peak) to a more symmetric peak shape (a *v*. b). Analogous results are observed when increasing the particle size for a given temperature and monomer concentration (case c *v*.a and d *v*.b). Asymmetric tailing of the peak is indicative of active site binding of the monomer while a symmetrical peak suggests that simple dissolution of VCM is the predominant mode of interaction of the monomer with the resin (Kiselev & Yashin, 1969).

Plots of ln $V°g$ v. $1/T$ for the three different columns and for infinite dilution and very low but finite concentration (0.49 ppb w/w) are shown in Figs 5–7. The slope of these straight lines equals $-\Delta H_s/R$. Thus ΔH_s values were determined graphically from the above figures.

Values for partial molar enthalpy of sorption (ΔH_s), partial molar entropy of sorption (ΔH_s), partial molar free energy of sorption (ΔG_s) excess molar free energy (ΔH_{xs}) and activity coefficient (γ) corresponding to sorption of VCM by the three different resins at 15, 22, 30 and 40°C are shown in Table 2. These values are characteristic of an exothermic and spontaneous interaction between PVC and VCM ($\Delta H < 0$, $\Delta G < 0$) and indicate the presence of strong attractive forces between the polymer and monomer ($\gamma \ll 1$).

It is shown that for a given temperature, ΔG_s, ΔG_{xs} and γ decrease when particle size decreases. Thus the PVC/VCM interaction becomes more spontaneous and attractive forces increase between the polymer and monomer. Likewise for a given particle size ΔG_s and γ values decrease when temperature decreases.

Negative ΔS_s values are related to the high degree of order of the monomer

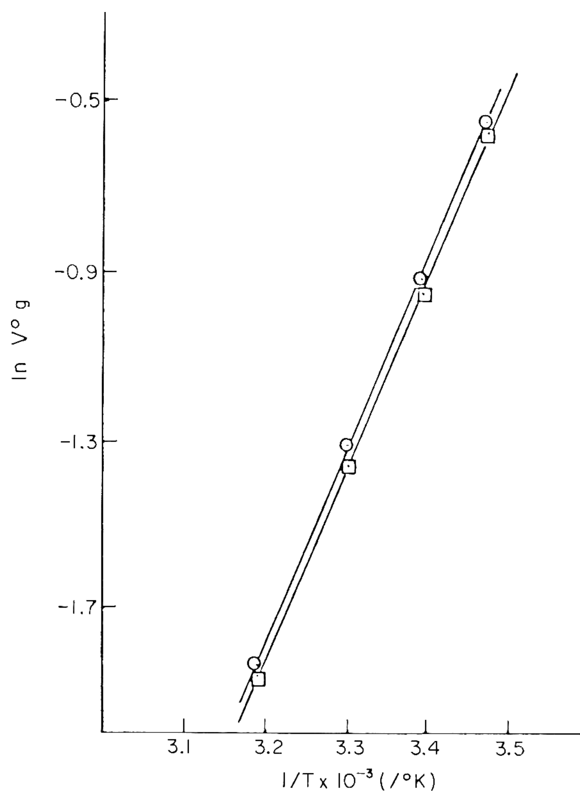

Figure 5. Ln V°g as a function of reciprocal temperature at (a) ⊙ infinite dilution and (b) ⊡ 0.49 ppb for the PVC (60–80 mesh) column.

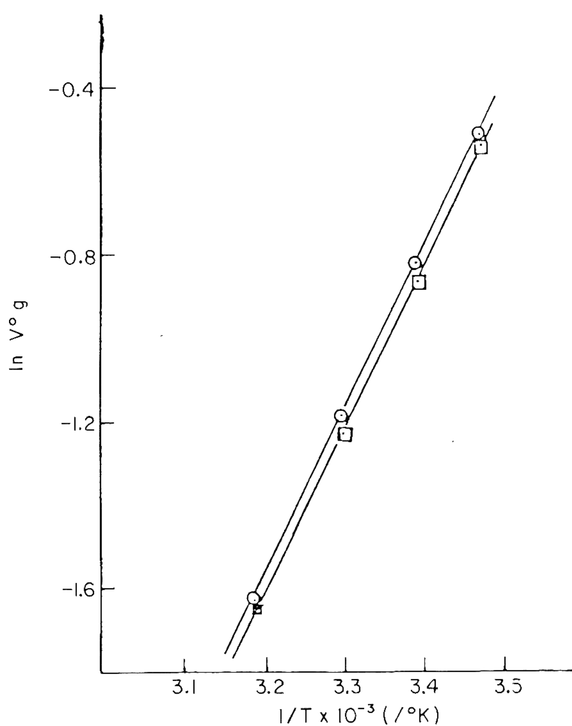

Figure 6. Ln V°g as a function of reciprocal temperature at (a) infinite dilution and (b) 0.49 ppb for the PVC (100–120 mesh) column.

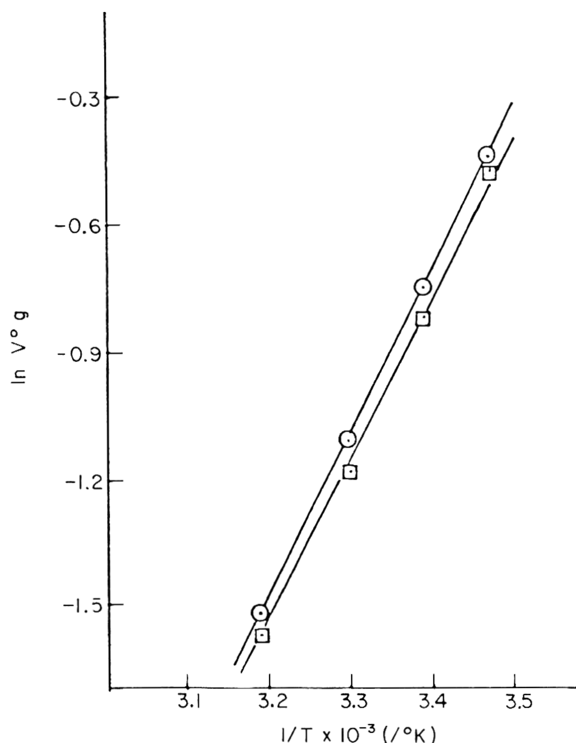

Figure 7. Ln V°g as a function of reciprocal temperature at (a) infinite dilution and (b) 0.49 ppb for the PVC (150–200 mesh) column.

Table 2. Changes in partial molar enthalpy (ΔH_s), partial molar entropy (ΔS_s), partial molar free energy (ΔG_s), excess molar free energy (ΔG_{xs}) and activity coefficient (γ) of sorption of vinylchloride by the three PVC resins at 15, 22, 30 and 40°C

PVC resin	ΔH_s (Kcal/mol)	ΔS_s (cal/mol)	ΔG_s (Kcal/mol)	ΔG_{xs} (Kcal/mol)	$\gamma \times 10^{-3}$
15°C					
60–80 mesh	−9.0	−22.8	−2.4	−3.0	5.0
100–120 mesh	−7.9	−18.7	−2.5	−3.1	4.1
150–200 mesh	−7.5	−16.4	−2.7	−3.3	3.0
22°C					
60–80 mesh	−9.0	−23.5	−2.1	−2.8	8.1
100–120 mesh	−7.9	−19.3	−2.3	−3.0	6.0
150–200 mesh	−7.5	−17.1	−2.4	−3.2	4.5
30°C					
60–80 mesh	−9.0	−24.2	−1.7	−2.6	13.3
100–120 mesh	−7.9	−19.9	−1.9	−2.8	9.4
150–200 mesh	−7.5	−17.6	−2.1	−3.0	6.4
40°C					
60–80 mesh	−9.0	−25.1	−1.2	−2.2	25.1
100–120 mesh	−7.9	−20.5	−1.6	−2.7	13.9
150–200 mesh	−7.5	−18.2	−1.8	−2.9	9.4

molecules in the sorbed state. ΔS_s does not change considerably during sorption at temperatures below Tg, because there is basically no change in the structure of the polymer in this temperature region, thus the probability for sorption of monomer molecules onto active sites does not change statistically. Thermodynamic data provide additional support of the active site hypothesis.

It should be noted however that specific conditions in this work differ to some extent from conditions experienced in practice, (unplasticized PVC resin, absence of liquid contacting phase etc.). Present experimental conditions were chosen as part of a model food simulating system under study by our group and others (Diachenko *et al.*, 1977) and provide data of considerable value on the nature of the polymer/monomer interaction. The effect of a liquid contacting phase has been studied by HPLC and sorption by classical partition (Kontominas, Gupta & Gilbert, 1985; Demertzis & Kontominas, 1985).

In conclusion all the above results support the active site hypothesis (Gilbert, 1976; Kontominas & Gilbert, 1979) according to which the monomer, at significantly low concentrations (< 1 ppm) and temperatures is reversibly bound onto active sites in the polymer thus limiting the potential for monomer migration into a food contacting phase.

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Composition of flesh from different edible parts of cod

R. McLAY

Summary

Cod fillet, trimmings from the fillet, and tissue recovered from the skeleton after filleting were assayed over a 12 month period for nitrogen, moisture, hydroxyproline and water holding capacity. No significant differences were found in nitrogen or moisture content. However, the mean hydroxyproline contents were significantly different in all three tissues. Water holding capacity was significantly less in tissues recovered from the skeleton than in fillet or fillet trimmings. A seasonal trend was observed in moisture and nitrogen content. Soluble and myofibrillar protein nitrogen and non-protein nitrogen were also measured on the three tissue types. No significant differences were found.

Introduction

The value at present in use by public analysts, for the nitrogen content of cod muscle was determined on skinless fillets using the method recommended by the Analytical Methods Committee (1966). With the increasing utilization of tissue from trimmings and frame in products, either with fillets, or alone, it was thought necessary to determine the nitrogen content of these different parts of the fish. The term 'frame' is used to describe the remainder of the fish after the head, guts and fillets are removed; and 'trimmings' is used to describe the belly flaps.

In order to gain further information on the chemical composition of various tissue types that might prove useful in predicting their processing properties, the investigation was extended to include the protein fractionation of fillet, trimmings and frame tissue. Hydroxyproline content was measured as an indication of the connective tissue content.

Materials and methods

Raw material

One iced, gutted cod (*Gadus morhua*) 50 cm in length was obtained from Aberdeen fish market at approximately 10 day intervals for twelve months, a total of thirty fish. One large batch of cod was caught and frozen whole on the Station's research vessel and stored at −30°C until required.

Procedure

Fish from the market were hand filleted and skinned, and the belly flap (trimmings) was removed. The head was discarded; the remaining frame was hand scraped to separate as much non-bone material, including kidney tissue and swim bladder, as possible. The three separate parts of the fish, fillets, trimmings and frame, were passed twice through a bench mincer with 3 mm holes, and thoroughly mixed. The batch from the research vessel was air thawed and divided into two aliquots. The first aliquot was

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processed, as below, immediately; the second was stored in ice for 10 days and then similarly treated.

The fish were beheaded and gutted; these were called 'nobbed' fish. Some were passed through a Baader 694 bone separator, to give 'nobbed mince'. The remainder were filleted (skin-on), the pin bones removed by a V-cut, the belly flaps removed, and the frames divided at the end of the gut into anterior and posterior portions. This subdivision of the frame permitted the effect of swim bladder and kidney tissue on chemical composition to be studied. Each portion was passed separately through a Baader 694 to give five separate minces, namely nobbed, fillet, trimmings, and anterior and posterior frame minces. The yields from the hand operations were not compared with that from the meat/bone separator.

Nitrogen and moisture

Nitrogen by Kjeldahl and moisture by drying to constant weight at 105°C contents were determined as recommended by the Analytical Methods Committee (1979).

Hydroxyproline

Hydroxyproline was determined using the method described by Leach (1960).

Protein fractionation

Protein fractions were solubilized by 0.1 M KCl and 1% sodium dodecyl sulphate (SDS) in a variation of the method, used by Webb *et al.* (1976) as described below. Five g of minced fish tissue were extracted with 90 ml of 0.1 M KCl with gentle stirring at 4°C to remove the water soluble sacroplasmic proteins (SP) and the non-protein nitrogenous components (NPN). The extraction mixture was centrifuged at 10 000×**g** for 30 min at 4°C, the supernatant made up to 100 ml, and 50 ml taken for nitrogen determination (water soluble N). NPN was determined on the remaining 50 ml of supernatant by adding an equal volume of 15% trichloroacetic acid (TCA) and stirring at 4°C overnight. The mixture was filtered through Whatman No. 1 filter paper and a 50 ml aliquot of filtrate was analysed for nitrogen content. The residue from the 0.1 M KCl extraction was gently stirred overnight in 90 ml 1% SDS at 25°C and subsequently centrifuged at 15 000×**g** at 25°C for 30 min. The supernatant was made up to 100 ml and a 50 ml aliquot taken for nitrogen determination to give myofibrillar protein nitrogen.

Water-holding capacity

Frame mince appears much more 'liquid' than fillet mince. To test this observation water-holding was determined by the centrifugal method of Wierbicki, Kunel & Weatherage (1957) which measures cook liquor loss. Ground samples of 25 g were heated for 30 min at 70°C and centrifuged for 10 min at 250×**g**. The fluid released as a percentage of total water was calculated. Triplicate determinations were made.

Results

The data presented in Table 1 for thirty fish collected over 1 year, indicate that the hydroxyproline content was significantly higher in trimmings than in fillets and significantly higher in frame tissue than in trimmings, but that there were no significant differences in total nitrogen or water content between the three fractions. Table 2 presents results from the large batch of frozen fish analysed after thawing, and after a further 10 days in ice. Each tissue type was analysed in duplicate. The period of storage

Table 1. Means, ranges and standard deviations (s.d.) of nitrogen, moisture and hydroxyproline contents, based on the analyses of thirty fish

	Moisture (%)			Nitrogen (%)			Hydroxyproline (mg/100 g)		
Tissue	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.
Fillet	80.90	78.54–82.93	0.99	2.76	2.46–3.40	0.19	46.47	25.92–68.82	11.15
Trimmings	80.85	78.85–83.53	1.23	2.78	2.43–3.46	0.20	105.20	39.42–131.75	54.04
Frames	80.74	78.01–82.97	1.14	2.78	2.50–3.29	0.23	270.96	116.79–373.83	68.85

No significant difference was found in moisture and nitrogen contents.
The hydroxyproline contents of fillet, trimmings and frames were significantly different at the 99% level.

Table 2. Mean contents of nitrogen, moisture and hydroxyproline in machine minced fractions from 300 cod

Mince	Moisture (%)	Nitrogen (%)	Hydroxyproline (mg/100 g)
Nobbed	80.96	3.14	66.61
Fillet	81.19	2.98	34.36
Trimmings	81.51	2.98	49.36
Posterior frame	81.73	2.86	82.85
Anterior frame	81.20	2.92	210.32

There were no significant differences between moisture and nitrogen in any of the fractions.

The hydroxyproline content of posterior frame and anterior frame were significantly different at the 95% level.

did not affect the results and the values in Table 2 are means of the two treatments. Again the only obvious differences occur in the hydroxyproline content which was highest in frame tissue, particularly in mince from the anterior section. Storage of fish in ice causes an increase in water content. This effect was presumably masked by the machine mincing process which uses running water as a lubricant. It was not possible to compare the results in Table 1 with Table 2 since the fish were from a different source. What was significant was the effect of hand *versus* machine mincing on the hydroxyproline contents.

The nitrogen contents of the seasonally collected fish in the various protein fractions are shown in Table 3 for the three tissue types. They are similar to those determined on Croaker by Webb *et al.* (1976). SDS was chosen for the myofibrillar protein extraction since 0.6 M KCl, the more usual extractant, caused gel formation as reported also by Ravesi & Anderson (1969). The extractant solutions were not filtered. In spite of the use of SDS as an extractant small amounts of gel were precipitated with the stromal residue, rendering a direct measurement of stromal nitrogen impracticable. The column in Fig. 3 headed 'residual nitrogen' is the calculated residue. There were no significant differences between the fractions from fillet, trimmings and frame tissues.

Table 4 shows the amounts of moisture lost by fish tissues after heating to 70°C for 30 min and is a measure of the water-holding capacity on cooking, which is an indirect measure of water-holding capacity. There was no difference between fillet and trimmings tissue, but frame tissue was significantly lower in its water-holding capacity. The

Table 3. Means, ranges and standard deviations (s.d.) for nitrogen (N) content of total and extractable fractions of fish muscle tissue, based on seasonal analyses of thirty fish

					g N/100 g tissue									
		Total N (%)			Water soluble N			Non-protein N			Myofibrillar N			Residual N (% TN)
Tissue	No.	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.	Mean	Range	s.d.	
Fillet	30	2.76	2.46–3.40	0.19	0.80	0.51–1.19	0.22	0.26	0.12–0.34	0.05	1.91	1.58–2.35	0.24	1.81
Trimmings	30	2.78	2.43–3.46	0.20	0.79	0.52–1.12	0.19	0.25	0.17–0.32	0.05	1.84	1.24–2.24	0.28	5.39
Frame	30	2.78	2.50–3.29	0.23	0.79	0.48–1.12	0.20	0.28	0.18–0.37	0.06	1.82	1.29–2.15	0.25	6.11

No significant difference was found within columns.
Residual N = Total N − water soluble + myofibrillar N) × 100.

Table 4. Means and standard deviations of eight samples of water-holding capacity of fish tissues

Tissue	% Loss of moisture	s.d.
Fillet	19.99	5.34
Trimmings	19.97	4.83
Frames	24.35	3.05

Frames were significantly different at 95% probability level.

measurements reported in Table 4 were carried out on the single batch of fish processed by the Baader 694.

Figures 1 and 2 show the variation of nitrogen and moisture with season for the three flesh types. A fall in nitrogen from a maximum in November to a minimum between March and June and a corresponding rise in moisture content are just discernible in fillets, trimmings and frame tissues. This is in agreement with the findings of Dambergs (1964) and the Analytical Methods Committee (1966). The mean nitrogen content of cod fillet was 2.75% compared to 2.87% reported by the Analytical Methods Committee (1966), which is used to determine the fish content of fish products.

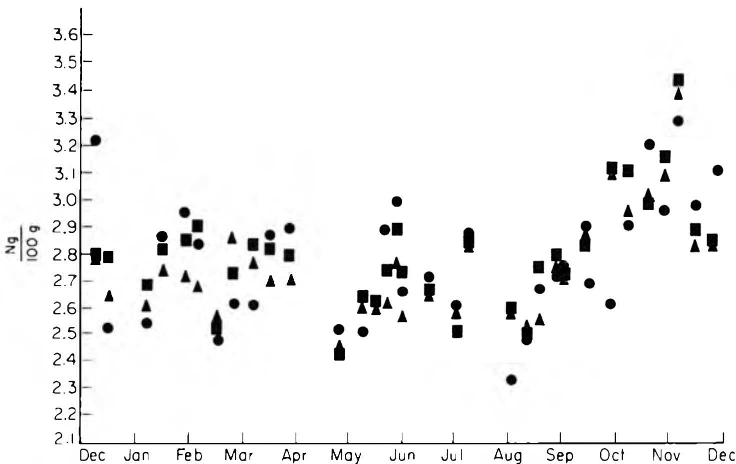

Figure 1. Seasonal variation in nitrogen contents of fillets (▲), trimmings (■) and frames (●).

Figures 3 and 4 demonstrate that there is no obvious relationship between season and the ratios of soluble and myofibrillar protein nitrogen to total nitrogen in any of the tissue types examined. Fig. 5 shows no trend in the hydroxyproline content of fish tissues with season.

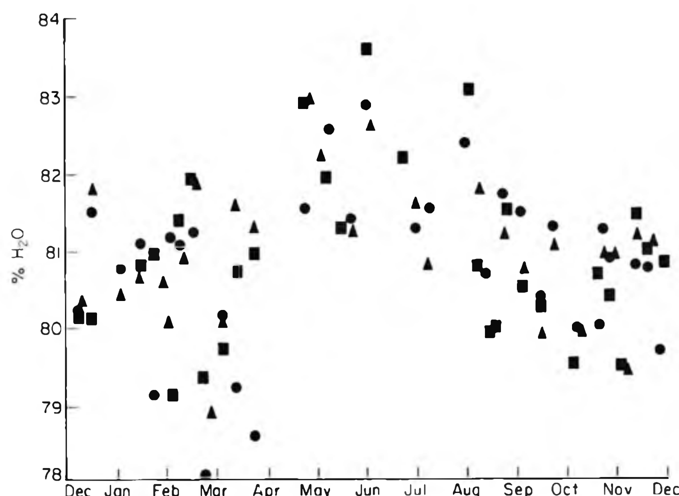

Figure 2. Seasonal variation in moisture contents of fillets (▲), trimmings (■) and frames (●).

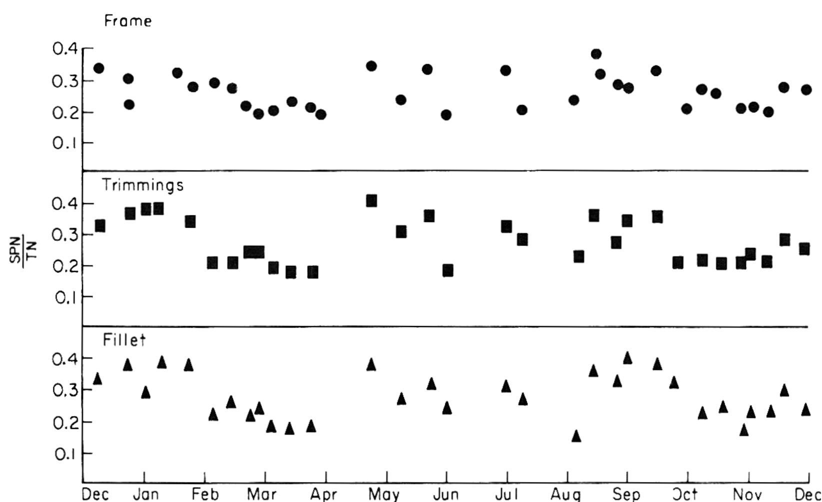

Figure 3. Seasonal variation in soluble protein nitrogen/total nitrogen ratios.

Discussion and conclusions

This work was undertaken primarily to provide basic information on the proximate chemical composition of those parts of cod which are used in the preparation of minced fish blocks. It was hoped that such information would be useful in differentiating between fish blocks made from different sources. There were no significant differences in the chemical composition of fillet, trimmings and frame material apart from hydroxyproline content and water-holding capacity of heated flesh.

Hydroxyproline contents of fillet, trimmings and frame tissue were significantly different. There was however considerable scatter in the results. This scatter may be due to the fact that the flesh was hand separated and passed through a domestic mincer:

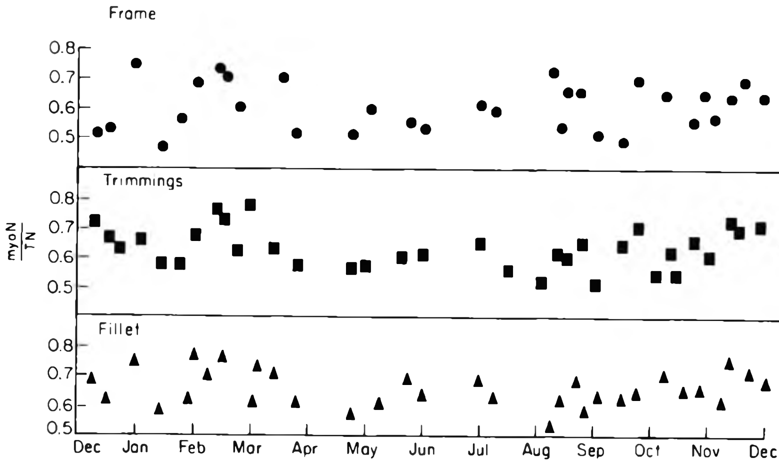

Figure 4. Seasonal variation in myofibrillar nitrogen/total nitrogen ratios.

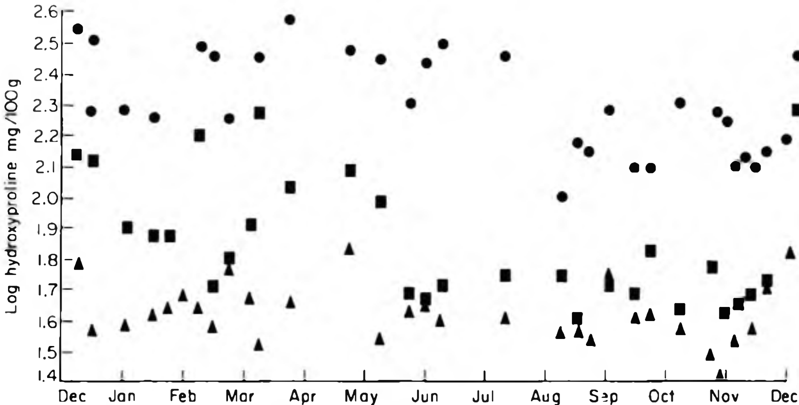

Figure 5. Seasonal variation in Log hydroxyproline contents of fillets (▲), trimmings (■) and frames (●).

as a result the composition of the fractions was variable, especially that of the frame tissue.

The conversion of hydroxyproline to collagen gives values of 0.34 g, 0.76 g and 2.0 g collagen per 100 g of fillet, trimmings and frame respectively. There is no correlation between collagen content and the calculated residual nitrogen shown in Table 3 underlining the imprecision of the extraction procedure. The values in Figs 1–4 were not subject to an analysis of variance. Given the differences between the collagen content of the three portions it would appear to be the most promising line for further investigation.

In view of the small number of seasonally collected fish in the present study, the agreement of mean total fillet nitrogen content, 2.76%, with the value reported by the Analytical Methods Committee (1966), 2.87%, is satisfactory and the latter should continue to be used as the mean value. Since no significant difference was observed

between fillet and other tissues, it is likely that the presently recommended mean value of 2.85% for fillet is valid for the other tissues.

A seasonal variation in nitrogen and moisture contents of cod flesh has been confirmed. The periods were more in line with those reported by Dambergs (1966) than those observed by the Analytical Methods Committee (1966). There was no obvious 'starving' during the winter. If the depletion of nitrogen is due to utilization of protein (Love, 1958) prior to spawning it is interesting to note that 'sarcoplasmic' and 'myofibrillar' proteins are depleted simultaneously and that no increase in connective tissue is observed.

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Studies of the iced storage characteristics and composition of a variety of Bolivian freshwater fish. 1. Altiplano fish

N. H. POULTER AND LINDA NICOLAIDES

Summary

The iced storage characteristics of three Bolivian freshwater fish species from the Altiplano region were monitored using chemical, microbiological and sensorial methods. Fish studied were: pejerrey (*Basilichthys bonariensis*) captured in two locations, Lake Poopo and Lake Angostura; trout (*Salmo gairdneri*) from Lake Titicaca and carp (*Cyprinus carpio*) from Lake Angostura. The proximate composition and physical nature of these fish are presented. The shelf lives of these species, as determined by both sensory and microbiological evaluations, were found to be: pejerrey from Lake Poopo: 20–21 days, pejerrey from Lake Angostura: 15–19 days, carp from Lake Angostura 17–20 days and trout from Lake Titicaca: 15–18 days. The value of the various methods used to monitor quality changes are discussed.

Introduction

In Bolivia fish are caught in a very wide range of environments. ranging from cold clear mountain lakes at 10°C or less, to warm slow moving muddy rivers at temperatures closer to 30°C. Previous studies with tropical and temperate water fish species from other regions of the world have indicated that the storage lives of fish in ice are closely related to the temperature and type of environment from which they are caught (Disney, Cole & Jones, 1974; Shewan, 1977; Lima dos Santos, 1981).

It is generally apparent that tropical warm water fish have much longer shelf lives in ice than similar types of temperate cold water fish (Disney, 1976). Thus, many species of tropical fish from warm waters have been reported to have storage lives in ice of 3–4 weeks compared with about 2 weeks for many species from temperate cold water. This difference has mostly been explained in terms of the types of microflora found on tropical fish. The suggestion is that the flora found on tropical species will be adapted to live at higher ambient temperatures and be mesophilic in nature, whereas the bacteria which cause spoilage of fish in ice are known to be psychrotrophic in nature (Disney, 1976; Shewan, 1977; Liston, 1979). Furthermore, it has been observed that certain physical and chemical characteristics, including the shape, size and fat contents can all combine to influence the duration of iced storage (Disney *et al.* 1974; Shewan, 1977). It has been reported for marine fish species that flatfish remain acceptable longer than round-shaped fish, red-fleshed fish longer than white-fleshed fish and low fat fish longer than high fat fish (Liston, 1979; Lima dos Santos, 1981). The precise reasons for these observed differences in storage lives have been considered at length by research workers and various explanations have been put forward. Such explanations have

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included the possible anti-microbial properties of fish muscle and/or slime (Watanabe, 1965–1966), the production of inhibitory substances by spoilage bacteria and low postmortem pH of muscle which may inhibit bacterial growth (Shewan, 1977; Liston, 1979).

The majority of investigations concerned with iced storage lives of tropical and temperate fish have been made using marine species. However, the small amount of

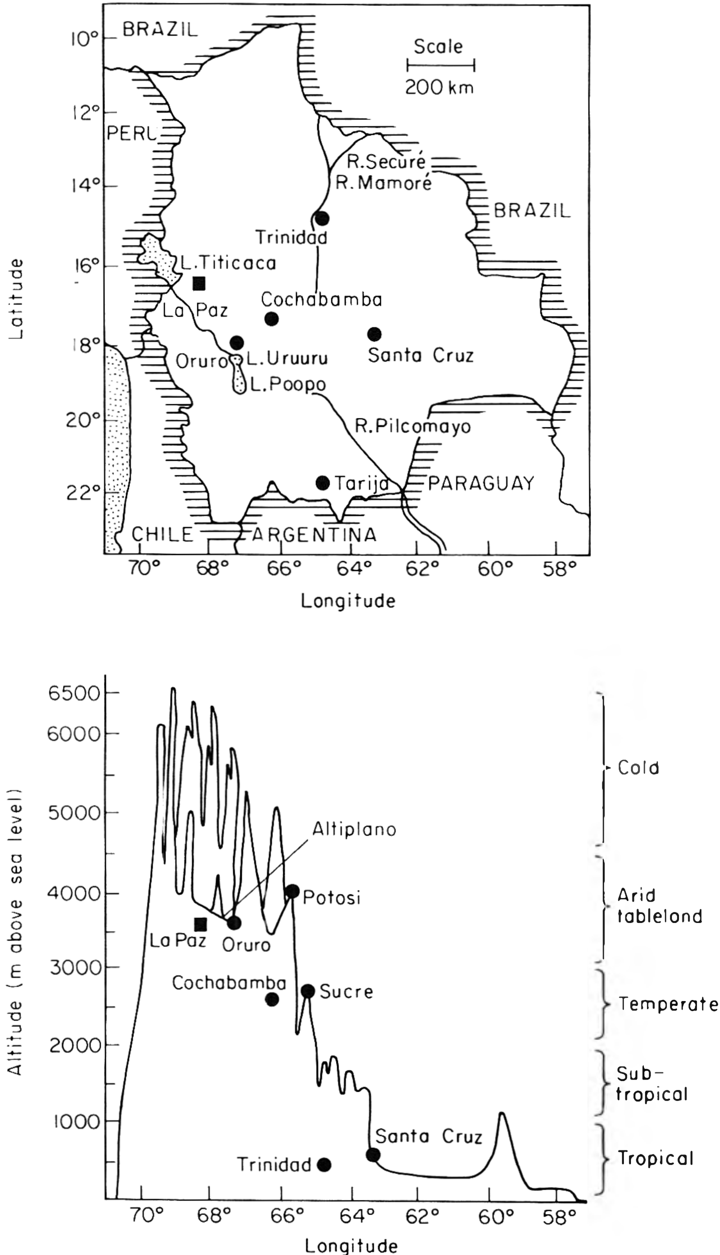

Figure 1. Map and longitudinal profile of Bolivia (redrawn from: Bolivia Red Vial 1982. Ministerio de Transportes y comunicaciones servicio Nacional de Caminos).

information that is available on the iced storage characteristics of freshwater fish does indicate that patterns of spoilage are similar to those of marine species (Shewan, 1977), but that their storage lives generally appear to be longer (Bramstedt & Auerbach, 1961).

This paper describes the work carried out on three species of fish captured from the Altiplano basin, which is one of the major fisheries in Bolivia (Fig. 1). The main basin of the Altiplano region consists of a series of high altitude glacial lakes, of which Lake Titicaca is the largest. Lake Titicaca drains via the River Desaguadero into the delta north of Lake Poopo. In this study fish were collected from Lake Titicaca, close to La Paz, Lake Poopo, near to Oruro and Lake Angostura, close to Cochabama (Fig. 1).

Materials and methods

Fish collection and storage

The species of fish collected for investigation were:

(i) pejerrey, *Basilichthys bonariensis* (Fig. 2a);
(ii) trout, *Salmo gairdneri* (Fig. 2b);
(iii) carp, *Cyprinus carpio* (Fig. 2c).

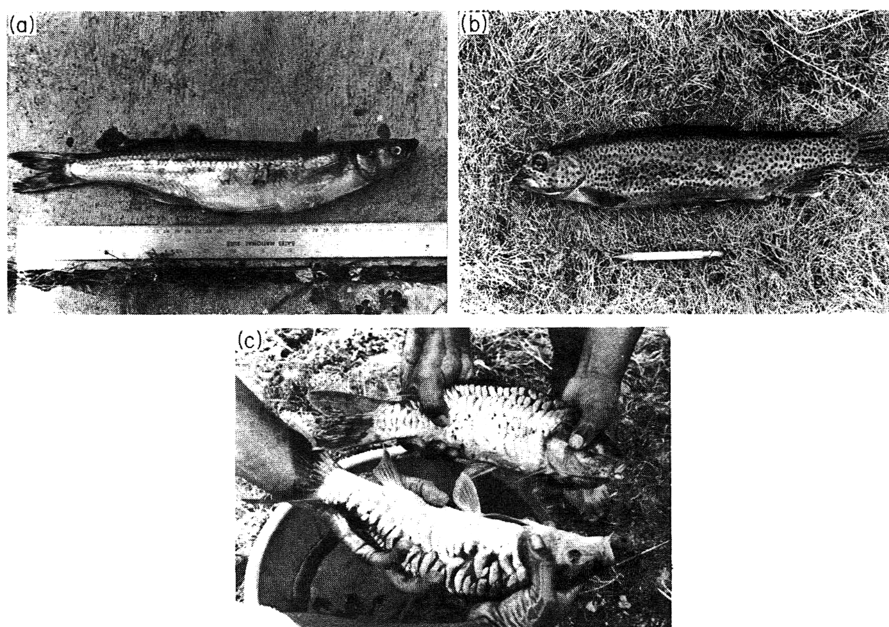

Figure 2. (a) Pejerrey (*Basilichthys bonariensis*); (b) trout (*Salmo gairdneri*); (c) carp (*Cyprinus carpio*).

Pejerrey were collected from Lake Poopo and Angostura, carp from Lake Angostura and trout from Lake Titicaca in late June 1983. The trout, unlike the pejerrey and carp, were not feral and were collected from a Fish-culture Station where they were held in open culture-food supplemented cages. Fish immediately after capture and preliminary evaluations were packed whole into crushed block ice held in insulated ice boxes. Fish were packed so as not to be in contact with one another and at least two parts crushed ice to one part fish was used at all times. All ice boxes had drain holes to allow the escape of

melt water. Fish were sampled every 2–3 days and completely re-iced approximately every 8 days. Sampling was continued until the fish had become spoilt and were determined to be no longer acceptable for human consumption. The day of fish capture was designated 'day 0' for the purpose of these trials.

Carcase evaluation and proximate chemical composition

Carcase evaluations were made by removal of the head, gills and guts from ten fish followed by the cutting of single skinless fillets to leave the skeletons (frame). Yields of each component were calculated on a percentage basis relative to the total weights of the whole fish. The skinless fillets from these ten fish were subsequently ground coarsely through a hand mincer and the minces mixed thoroughly. The mixed minces were used to determine the proximate chemical composition using standard techniques. All proximate chemical determinations were made in triplicate. Moisture was determined by drying to constant weight at 105°C in a vacuum oven. The weight loss was assumed to be entirely due to the evaporation of water. Crude fat was determined on previously dried samples by the Soxhlet method using petroleum spirit, bp 40–60°C, as solvent. Total crude protein was determined using the Kjeldahl method (AOAC, 1975a). A nitrogen factor of 6.25 was used throughout. Ash was determined by heating samples in a muffle furnace at 525°C (AOAC, 1975b).

G.R. Torrymeter evaluations

At each sampling time sixteen fish were removed from the ice and one reading taken from the left-hand side of each fish, just above the lateral line and posterior to the gill operculum. These readings were averaged to obtain a single figure and this is presented together with the range of values obtained.

Visual and olfactory characteristics

The same sixteen fish evaluated above were examined at each sampling time to assess the condition of the eyes, gills, body cavity, skin and muscle.

Following these evaluations the fish were returned to their insulated ice boxes and re-packed in original ice. All remaining analyses which are described below, utilize only two fish at each sampling time. The sampling of such a restricted number of fish was imposed by the constraints of time and availability of fish.

Bacteriological assessments

At each sampling time two individual fish were assessed bacteriologically for aerobic counts on the skin and in the muscle. A known area of skin (10 cm^2) or weight of muscle (10 g) was aseptically removed and placed into a sterile atomix unit together with 100 ml (skin) or 90 ml (muscle) 0.1% peptone diluent. The contents of the atomix were homogenized for 2 min. From this, the initial dilution, ten-fold dilution series were prepared and used to inoculate sets of pre-poured agar plates. The spread plate method (ICMSF, 1978a) was used with plate count agar (Oxoid) as the culture medium. Duplicate plates were prepared for each dilution. After inoculation, plates were dried and incubated at 25°C. The number of colony forming units (c.f.u.) were counted after 3 days and the weighted means for the total counts per cm^2 of skin or per g of muscle were determined (Farmiloe *et al.*, 1954).

Following the removal of samples for bacteriological assessments, fillets were taken from the same two fish and used for the determinations of pH, total volatile bases (TVB) and for sensory evaluations (taste panel assessments).

Sensory evaluations

Sensory evaluations were conducted using a panel of trained personnel. Skinless fillets were cut into approximately 15 g portions and placed in plastic bags which were sealed. The bags were immersed in boiling water and cooked for 20 min. The taste panel (5–7 members), recorded their opinions of colour, flavour, texture and overall acceptability on a hedonic scale: 10 = good, 0 = bad and 4 = just unacceptable.

The muscle remaining from cut skinless fillets was pooled, minced coarsely through a hand mincer and used to prepare extracts for the following analyses.

pH

Triplicate 2 g portions of minced pooled muscle were homogenized in 10 ml distilled water. The pH of the extracts was immediately measured at room temperature using a pH meter with a glass electrode.

Total volatile bases (TVB)

Duplicate 3 g samples of minced pooled muscle were homogenized in 20 ml ice cold 5% (w/v) perchloric acid. These extracts were then centrifuged at 3000 rpm for 10 min using a laboratory centrifuge. Extracts were subsequently stored frozen at −30°C until analysis. Analysis of TVB were made using a procedure based on the micro-diffusion method of Conway (Beatty & Gibbons, 1937), using sulphuric acid to absorb the liberated bases. The analyses were carried out in duplicate on each extract and mean values calculated as mgN (TVB)/100 g muscle.

Results

Carcase evaluation and proximate chemical composition

Table 1 summarizes the basic data for freshly captured fish. All the fish species were found to be very small. Little difference in the weights and sizes of pejerrey caught from the two different lakes was found and they were all very small (overall average weight = 55.30 g). The proximate composition of the muscle of these Altiplano fish were fairly similar in most respects. However, the pejerrey coming from Lake Poopo were found to have one of the highest muscle fat contents whilst pejerrey from Lake Angostura the lowest. Trout showed the highest crude protein contents which may have been influenced by the type of artificial diet that they had received (Table 1).

The small range in the weight and sizes of these Altiplano fish meant that few large differences in the yields of the various carcase constituents were noted (Table 1).

Physical and sensory evaluations

Very fresh fish from the Altiplano were found to possess those characteristics typical of high quality. From visual and olfactory examinations made during the first few days of storage it was found that all the fish studied had eyes which were convex and bright, gills which were red with a sweet/weedy/neutral odour, and firm flesh. Sensory evaluations (taste panels) provided values for colour, flavour, texture and overall acceptability which were all high, indicating a high degree of acceptability. However, inherent differences between species were apparent. Trout from Lake Titicaca and pejerrey from Lake Poopo, both high altitude lakes, had exceptionally darkly coloured skins. The trout and carp also naturally possessed large amounts of mucus on the skin whilst the pejerreys were virtually mucus free when very fresh. Further, the sensory characteristics of the muscle of fish when fresh differed significantly, particularly in

Table 1. Basic data for freshly captured Altiplano fish species used in iced storage trials

	Fish species (and location)			
	Pejerrey (L. Angostura, Cochabamba)	Pejerrey (L. Poopo, Oruro)	Carp (L. Angostura, Cochabamba)	Trout (L. Titicaca, La Paz)
Average total weight (g)	55.32	51.27	132.06	90.13
Range	32.5–77.5	27.8–93.5	70.8–198.1	63.2–122.9
Average stand. length (cm)	16.85	17.04	17.53	17.38
Range	13.6–19.1	15.0–21.7	14.2–22.1	15.4–19.5
Average total length (cm)	19.91	20.74	21.97	20.25
Range	17.0–24.3	18.0–26.1	18.0–27.5	18.2–23.0
Average Torrymeter (day 0)	13.22	11.10	13.06	13.59
Range	10–16	8–12	12–14	13–14
Carcase analysis (%):				
Fillets	55.2	56.5	49.9	63.0
Muscle	47.7	47.1	45.2	53.4
Skin	7.7	9.6	4.7	9.6
Head	21.4	18.0	20.5	11.6
Skeleton	10.6	13.4	16.3	11.8
Viscera	7.9	7.6	8.5	10.4
Gills	4.7	4.3	4.8	3.2
Proximate analysis of muscle (%):				
Moisture	80.00	79.70	81.64	76.50
Protein (N×6.25)	17.32	17.86	15.95	20.52
Fat	0.65	3.62	2.09	3.51
Ash	0.76	0.78	0.92	1.06

terms of colour and texture. Thus, for example, the flesh of carp contained a great deal of red muscle and blood whilst that of the pejerreys was creamy-white and very firm. Trout had a finer and softer textured flesh and was coloured pink.

The G.R. Torrymeter provided readings from very fresh fish which were very similar for all the Altiplano fish species studied. The overall averaged readings obtained using the values for all the different species on either day 0 or day 1 was calculated to be 13.36 (ranges in individual values 8–16) (Table 1).

The results of the physical and sensory evaluations showed a gradual deterioration on storage of the fish in ice. Pejerrey from Lake Poopo and Angostura showed similar rates of decline in their G.R. Torrymeter readings with time throughout the duration of the trials (Fig. 3). The taste panel results for general acceptability (Fig. 4) also showed similar rates of deterioration for about the first 11 days, during which time the fish were in very good condition. Thereafter, however, the changes in the visual and olfactory characteristics of these two lots of fish differed significantly. Rupturing of the body cavity and liquefaction of gut contents (belly burst) was evident after only 8 days of storage with pejerrey from Lake Angostura, while belly burst did not occur with pejerrey from Lake Poopo. This particular physical deterioration could possibly have

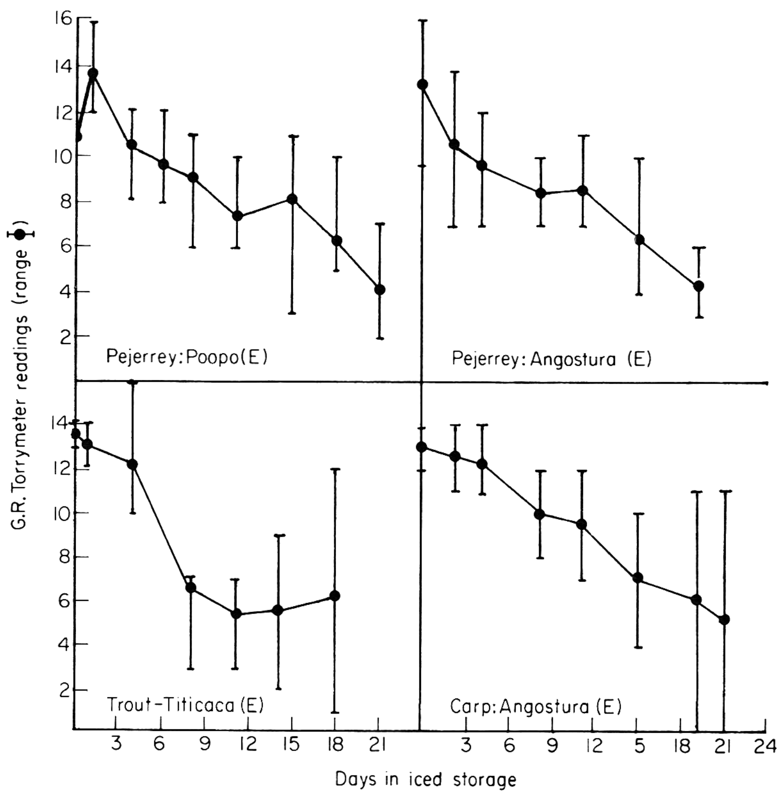

Figure 3. G.R. Torrymeter readings of Altiplano fish stored in ice. E = entire.

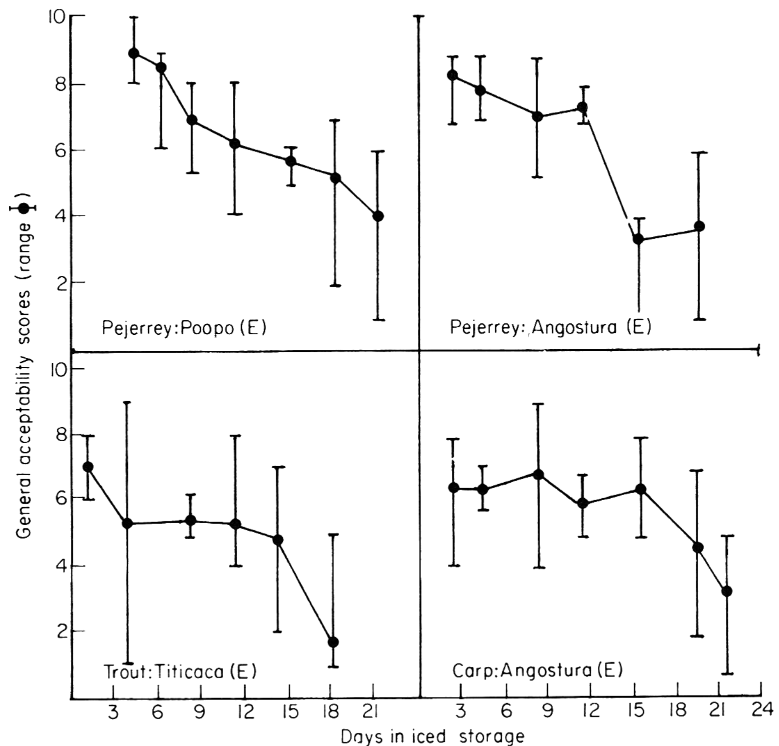

Figure 4. Sensory evaluation (general acceptability scores) of Altiplano fish stored in ice. E = entire.

resulted in a more rapid decline in the sensory qualities of pejerrey from Lake Angostura (Fig. 4). From the values obtained by taste panel and using the score of 4 as that value which indicates the fish to be just unacceptable, then storage lives in ice for pejerreys from Lakes Angostura and Poopo differed significantly and would be 15 and 21 days, respectively.

The use of the G.R. Torrymeter with carp from Lake Angostura provided readings which declined fairly smoothly with storage time (Fig. 3). This was not, however, the case with the results of the sensory evaluations. The mean scores for the overall acceptability of fish remained high and in the range 6–7 for the first 15 days (Fig. 4). At this time, like pejerrey from the same lake, these fish started to show signs of belly burst and this coincided with a rapid fall in acceptability scores. Using the acceptability scores as a guideline to possible storage life then about 20 days was recorded.

The results of the G.R. Torrymeter with trout from Lake Titicaca differed from all of the other fish species studied from the Altiplano. It was observed that a decline in meter readings was only obtained for the first 8 days of storage (Fig. 3). Thereafter, and for the rest of their storage life, mean readings remained constant (6.04±s.d. 0.56). The converse, however, was true for the overall acceptability scores during storage (Fig. 4). With those evaluations it was clear that the scores remained fairly constant for the first 12 days of storage and then rapidly declined. Once again, as with carp and pejerrey from Lake Angostura, the trout showed very advanced signs of belly burst at this time. The overall acceptability scores would indicate a possible storage life for trout held in crushed ice of 15–16 days.

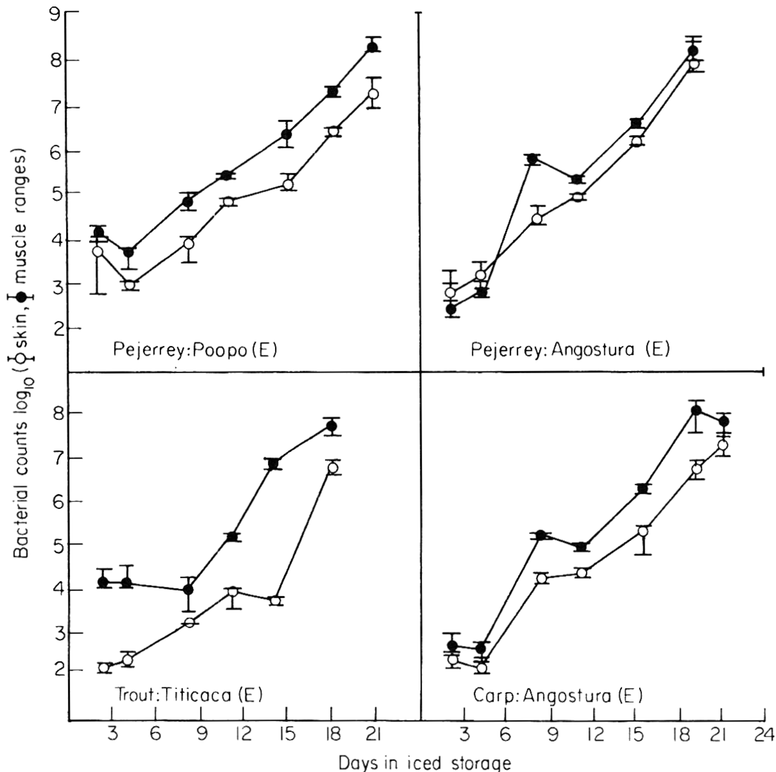

Figure 5. Bacterial counts at 25°C of Altiplano fish stored in ice. E = entire.

Bacteriological assessments

Changes in bacterial counts are presented in Fig. 5. The three species of fish showed initial bacterial loads of between 10^2 and 10^4 c.f.u./g of muscle on day 1. These levels rose to exceed 10^7 c.f.u./g between 17–20 days of iced storage. This is the maximum microbiological limit for fresh fish recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 1978b). Therefore, the results obtained indicated that the three species of Altiplano fish had storage lives in ice of between 17–20 days. Similar changes in bacterial counts were noted from analyses of the skin though the bacterial numbers per gram of muscle were found to be consistently higher than those per cm^2 skin.

Chemical analyses

The results of chemical analyses of the pH and total volatile bases (TVB) for these Altiplano fish are shown in Figs 6 and 7 respectively.

The values obtained for the pH of the fish muscles changed with duration of storage. The initial pH of the muscle of most fish was in the range of 6.3–6.4. On storage the pH tended to rise, often erratically and with pejerrey reached about 7.1 after 15 days (Fig. 6). The values obtained for trout, however, were exceptionally erratic and would not provide a useful index of fish freshness with this species.

The results of the analysis for TVB of fish muscles indicate that all the fish had initial values of between 4–7 mg N/100 g (Fig. 7). The TVB contents increased gradually on storage in ice and was more rapid with the pejerreys than with carp or trout. A level of

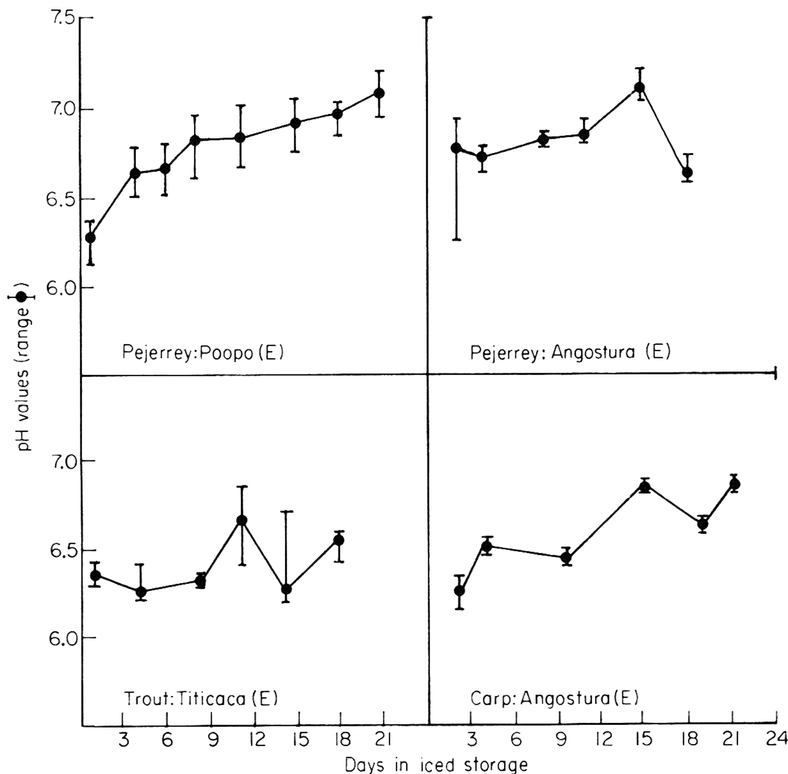

Figure 6. pH of Altiplano fish stored in ice. E = entire.

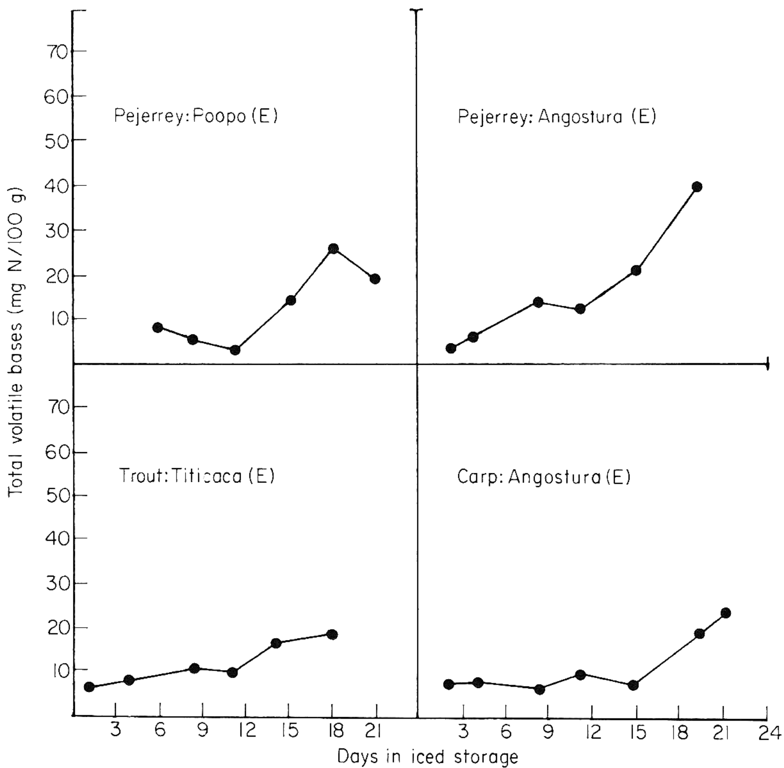

Figure 7. Total volatile base content of muscle of Altiplano fish stored in ice. (N.B. Ranges not presented due to loss of data.)

about 40 mg N/100 g was attained by pejerrey from Lake Angostura after 19 days, whilst trout and carp contained only 18–22 mg N/100 g after the same length of storage.

Discussion and conclusions

The lake environments from which these fish were collected may be described as being 'temperate' or 'cold water', by reason of their altitude on the Altiplano (Fig. 1). Fish were collected during the winter months (May to October) from waters which are reported to range in temperature from about 5 to 13°C. The waters of Lake Poopo, from which one batch of pejerrey were collected, are also reported as being very saline and as having lower average winter temperatures than either Lake Titicaca or Lake Angostura (M. Beveridge, pers. comm., 1983).

The fish from the Altiplano were all found to be very small and to have relatively low fat contents. All these factors may, therefore, have influenced the shelf lives of these fish when stored in ice. Table 2 presents the shelf lives of all the fish species studied following interpretation of the results of both the sensory evaluations and bacteriological assessments. It may be seen that the storage lives of pejerrey from the two sources differed significantly if the results of the sensory evaluations were used as the index. The pejerrey from the colder water of Lake Poopo showed a greater shelf life (21 days) than those from the relatively warmer waters of Lake Angostura (15 days) despite the former fish having higher fat contents. A fairly apparent reason for the shorter shelf life of Lake Angostura pejerrey was the occurrence of burst belly. The incidence of this

Table 2. Storage lives of fish species studied as determined by sensory evaluations (taste panel acceptability scores) and bacteriological assessments

	Storage life (days)	
Fish species and location	Sensory evaluations	Bacteriological assessments
Pejerrey (E), L. Poopo	21	20
Pejerrey (E), L. Angostura	15	18–19
Carp (E), L. Angostura	20	17–18
Trout (E), L. Titicaca	15–16	17–18

E = Entire.

phenomenon appeared to precede a rapid decline in taste panel acceptability scores with these fish. The decline in acceptability was principally due to off-flavours and softness of the flesh which could have been brought about by the contamination of these small fish by their broken gut contents. The reasons for the occurrence of burst belly in pejerrey from Lake Angostura but not from Lake Poopo can only be surmised, but a possible and likely explanation could be differences in the diet of these fish and hence proteolytic enzyme activity. However, using the microbiological guidelines recommended by the ICMSF (1978b) then the two batches of pejerrey had similar shelf lives of between 18–19 days Lake Angostura and 20 days Lake Poopo. Although initial counts on pejerrey from Lake Angostura were lower than those found on pejerrey from Lake Poopo, bacteria on fish from Lake Angostura reached levels of 10^7 or higher 1–2 days before those on the fish from Lake Poopo. This also could have been due to the spread of the microorganisms from the intestines following belly burst.

The observation that bacterial numbers per gram of muscle were consistently higher than those per cm^2 of skin may be explained, at least in part, by the washing action of the melting ice (Curran, de B. Crammond & Nicolaides, 1981).

Consideration of other analyses made upon the pejerreys, such as the G.R. Torrymeter evaluations and total volatile base (TVB) contents, provide conflicting evidence supporting the exact storage life of the fish from different sources. The values obtained using the G.R. Torrymeter showed very similar rates of decline with time with both lots of pejerrey. However, TVB contents differed during the last few days of storage. Connell (1975) has suggested that for marine species of fish such as cod, TVB values of 30–40 mg N/100 g flesh may be taken as an indication that the fish has reached its limit of acceptability. In these investigations pejerrey from Lake Poopo were found never to reach these levels whilst pejerreys from Lake Angostura exceeded 30 mg N/100 g after 17 days. This result would seem to confirm the acceptability score as representing the more appropriate index of storage life for this species. Nonetheless, it must be noted that the 30–40 mg N/100 g limit has been suggested for a marine fish species. However, freshwater fish may differ significantly from their salt water counterparts in that they often contain little or no trimethylamine oxide (TMAO) (Hebard, Flick & Martin, 1982). Trimethylamine, the degradation product of TMAO, was not studied in the present investigation.

Thus, dependent upon the index used, the storage lives of the pejerreys from different lakes were found to differ significantly (Table 2). However, the sensory

characteristics of fish must in the final event determine whether the consumer accepts or rejects the product. Consequently, the acceptable storage life for pejerrey from Lake Angostura must be taken to be 15 days.

Trout from Lake Titicaca, like pejerrey from Lake Angostura, showed different storage lives dependent upon the index used for its estimation (Table 2). Sensory evaluations indicated a possible storage life of 15–16 days whilst microbiological results indicated 17–18 days. Belly burst also occurred with this species, starting around the eighth day of storage and being well advanced after 14 days. It is possible, therefore, that the quality and shelf life of both trout and pejerrey would benefit from the iced storage of gutted rather than whole fish.

Belly burst was also found to occur with carp from Lake Angostura, but this only started between day 15 and day 19, and again this phenomenon was associated with a more rapid decline in sensory assessments. In support of this, both the limit of acceptability and the recommended microbiological limit were reached after 20 and 17–18 days of storage, respectively (Table 2).

Throughout the investigation with these Altiplano fish species the rates of decline in meter readings, as discussed, did not change during the later stages of storage as quickly as those obtained from sensory assessments. In particular the readings provided by the meter were not affected to any degree by the incidence of burst belly. Thus, the G.R. Torrymeter did not provide an unequivocal index of fish quality but could, in conjunction with visual and olfactory observations, provide the basis of an inspection system with these Altiplano fish species.

The use of TVB as an index of fish quality does not appear to be appropriate with these Altiplano species. Although values did increase on storage of the fish in ice, the changes were often small and irregular. Similar irregular changes in the pH of the muscle of fish on storage were noted and although this value did tend to rise from about 6.3 to 7.0 over the periods of storage, their interpretation in the absence of other chemical and physical evaluations must be limited.

Microbial action has been shown to play a large part in the spoilage of fish (Reay & Shewan, 1949; Shewan & Jones, 1957; Shewan, 1977). Therefore, by monitoring the bacterial load the quality of a particular fish species can be indicated. However, because the standard plate count has a time lapse of 3 days before the number of bacteria in a sample can be assessed this method cannot be considered as a rapid index of fish quality (Poulter, Nicolaides & Hector, 1978).

Acknowledgments

See Part 2.

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Studies of the iced storage characteristics and composition of a variety of Bolivian freshwater fish. 2. Parana and Amazon Basins fish

N. H. POULTER AND LINDA NICOLAIDES

Summary

A selection of fish from the warm lowland waters of the Parana and Amazonian Basins were studied to determine their shelf lives and spoilage characteristics when stored in crushed ice. The proximate composition and physical nature of these fish species was also determined. A shelf life of 25 days was estimated for sabalo (*Prochilodus platensis*) from the Parana Basin regardless of whether the fish were stored entire or eviscerated. The larger fish from the Amazonian Basin, pacu (*Colossoma macropomum*), chincuiña (*Pseudoplatystoma tigrinum*) and tambaqui (*Colossoma branchypomum*) exhibited shelf lives of greater than 40 days. The smaller fish from this location had shelf lives of over 30 days for corvina (*Plagioscion squamosissimus*) and 25 days for bagré (*Ageneiosus* spp.). An evaluation of the methods used to assess fish quality was made. The assertion that tropical fish have longer shelf lives than temperate species when stored in crushed ice is discussed.

Introduction

This paper concludes the survey of a variety of Bolivian fish species of commercial and semi-commercial importance with data collected for fish captured from the warm lowland waters of the Parana and Amazon Basins (Fig. 1, Part 1).

The Pilcomayo and Bermejo Rivers are major tributaries of the Parana system that lies in the south-west corner of Bolivia. Of the two rivers, the Pilcomayo is considered the more important fishery, and it is from here that the breeding migrations of sabalo (*Prochilodus platensis*) are exploited during the Bolivian spring and summer. These fish, at the appropriate times of year, are sold in large quantities in major towns and cities.

The Amazonian Basin lies to the north-east of Bolivia. The predominant species caught from this area are the pacu (*Colossoma macropomum*), the tambaqui (*Colossoma brachypomum*) and the catfishes surubi and chincuiña (*Pseudoplatystoma fasciatum* and *P. tigrinum*). However, many more fish species are abundant and available to the fishery. ORSTOM (1983) have to date identified over 300 species from this area. Many of these fish are organoleptically acceptable and yet are rejected by commercial fishermen. Several of these fish were studied in order to assess their acceptability to the consumer and to determine their shelf lives stored in ice.

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Materials and methods

Fish collection and storage

The species of fish collected for this iced storage study were:

(i) sabalo, *Prochilodus platensis* (Fig. 1a);
(ii) pacu, *Colossoma macropomum* (Fig. 1b);
(iii) tambaqui, *Colossoma brachypomum* (Fig. 1c);
(iv) chincuiña, *Pseudoplatystoma tigrinum* (Fig. 1d);
(v) corvina, *Plagioscion squamosissimus* (Fig. 1e);
(vi) bagré, *Ageneiosus* spp. (Fig. 1f).

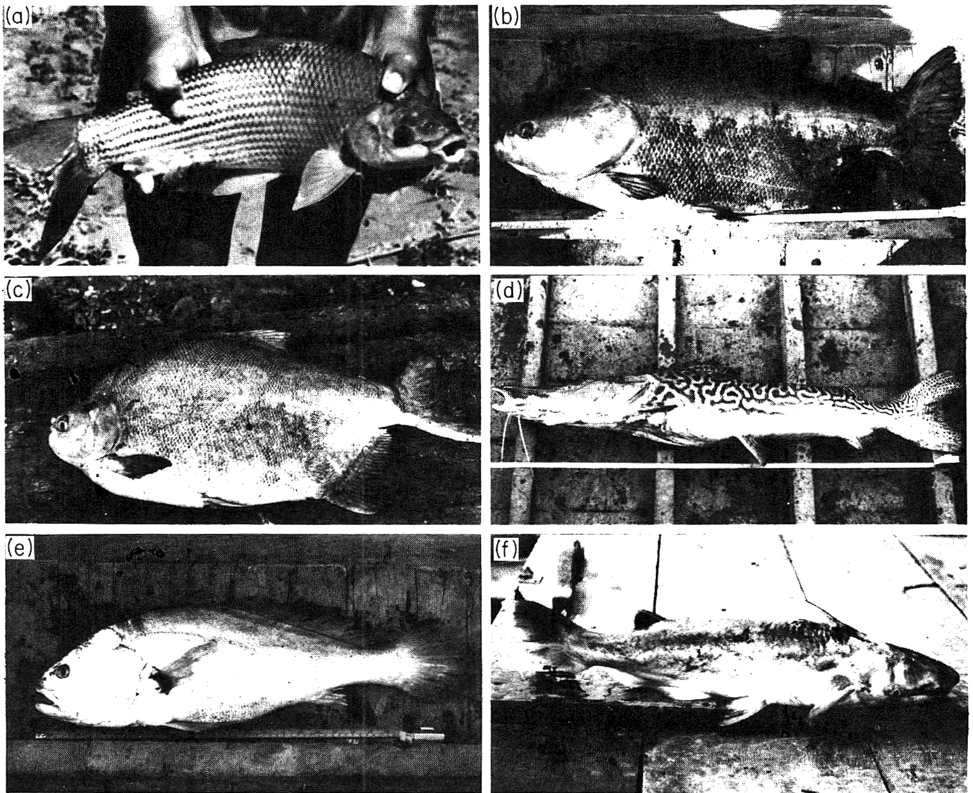

Figure 1. (a) Sabalo (*Prochilodus platensis*); (b) pacu (*Colossoma macropomum*); (c) tambaqui (*Colossoma branchypomum*); (d) chincuiña (*Pseudoplatystoma tigrinum*); (e) corvina (*Plagioscion squamosissimus*); (f) bagré (*Ageneiosus spp.*).
Abbreviations on all Figs E = entire; EV = eviscerated; HG = headed and gutted.

Of the fish species collected only one, sabalo, was from the River Pilcomayo in the Parana Basin. This fish was stored both whole and with guts and gills removed (eviscerated), in crushed ice as described in Part 1 of this study. All the remaining fish were collected from the River Mamoré or its tributary, the River Securé, in the Amazon Basin near the town of Trinidad (Fig. 1, Part 1). All the fish species studied were collected in early July 1983.

The commercial fish species: pacu, tambaqui and chincuiña were particularly large. For reasons of saving ice box space and to comply with the traditional handling

techniques of the Amazon fishermen, these three fish species were all headed and gutted prior to storage in ice. However, fishermen occasionally store the somewhat smaller tambaqui whole and an additional trial was conducted with such fish. Corvina and bagré, which were the appreciably smaller and semi-commercial fish species studied, were stored whole in crushed ice as described in Part 1.

Fish sampling and analysis

Fish were sampled every 3–6 days and evaluated for their qualities using various physical, chemical and bacteriological techniques as have been described in Part 1 of this study. These techniques were modified in only two respects for this part of the study. The first modification concerned the sampling of the larger fish, namely pacu, tambaqui and chincuiña. In the case of these fish the analyses of the carcase, proximate chemical composition, G.R. Torrymeter and the visual and olfactory characteristics were made using only four fish, rather than ten or sixteen. The remaining analyses involving the bacteriological assessments, sensory evaluations and measurement of pH and TVB employed only one fish, rather than two. The second modification involved the sampling for the bacteriological assessments. For these three large fish species only, samples of 30 g of muscle, rather than 10 g and 25 cm^2 of skin, rather than 10 cm^2 were taken and prepared in the same manner as described previously.

Results

Parana Basin fish

Carcase evaluation and proximate chemical composition. Sabalo were found to have an average weight of just over 1 kg and when eviscerated lost a total of about 13% of their original weights (Table 1). The percentage yield of skin-on-fillets recorded from whole fish was 55.1%.

Sabalo is regarded by most Bolivian consumers as being a 'fatty fish'. The proximate analyses carried out upon the muscles of these fish (Table 1), do not fully confirm this assumption and they may be regarded as having a medium fat content. They were, however, found to have relatively high crude protein and low moisture content.

Physical and sensory evaluations. No sensory evaluations were conducted with these fish during the course of the iced storage trial. The muscle of the fish is initially grey–white in colour and contains many fine intramuscular bones. Very fresh fish were silver–green in colour and possess large firm scales. They showed the usual appearance characteristics and olfactory qualities associated with fresh fish. On storage in ice these characteristics started to diminish after 5–6 days. At this time the eyes started to become sunken and cloudy and mucus was produced upon the skin and gills. There were few noticeable differences in the rates at which these changes occurred between entire sabalo and eviscerated sabalo. By the end of the trial (32 days) the fish showed large quantities of malodorous yellow slime on the skin surfaces, the eyes were sunken and cloudy–bloody and their muscles were pulpy.

The G.R. Torrymeter readings obtained with this fish species declined gradually and fairly linearly over the period of the storage trial (Fig. 2). The mean values declined from 11 to about 1 over 32 days and the technique would appear to provide a useful index of fish freshness. The effects of evisceration upon the meter readings and their rates of decline appeared to be minimal (Fig. 2).

Table 1. Basic data for freshly captured fish species used in iced storage trials

	Fish species (and location)					
	Sabalo (R. Pilcomayo, Tarija)	Pacu (R. Securé, Trinidad)	Tambaqui (R. Securé, Trinidad)	Chincuiña (R. Securé, Trinidad)	Corvina (R. Mamoré, Trinidad)	Bagré (R. Mamoré, Trinidad)
Average total weight (kg)	1.02	8.93	3.88	14.53	1.02	0.67
Range	0.60–1.95	7.2–12.5	2.0–6.0	13.7–15.3	1.00–1.03	0.34–1.03
Average stand length (cm)	34.54	66.54	52.63	1015	36.36	26.63
Range	26.4–42.4	46–74	47–58	975–1150	29.5–44	21–32
Average total length (cm)	42.44	75.21	57.31	1172	50.71	37.13
Range	36.4–52.0	57–87	48–66	1152–1191	43–60	32–42
Average Torrymeter (day 0)	11.00	11.57	10.81	11.80	12.11	10.83
Range	10–12	10–12	9–13	10–13	10–13	10–12
Carcase analysis (%):						
Fillets	55.1	52.3	48.4	—	56.5	52.02
Muscle	45.4	44.2	39.4	50.5	49.5	48.7
Skin	7.7	8.1	9.3	10.5	7.0	7.3
Head	17.7	20.7	16.6	11.7	23.8	30.1
Skeleton	16.5	17.4	16.6	15.2	11.3	10.9
Viscera	10.3	5.3	7.3*	7.8	5.9	4.0
Gills	3.2	4.3	2.2	4.5	2.6	3.1
Proximate analysis of flesh (%):						
Moisture	67.00	67.09	69.27	70.75	67.89	78.98
Protein ($n \times 6.25$)	23.38	14.11	15.84	18.89	21.69	14.77
Fat	4.26	18.02	15.61	8.85	5.90	3.66
Ash	1.46	0.86	0.98	1.01	0.77	0.53

*Tambaqui possessed particularly large fat glands associated with the viscera averaging 8.3% of total body weight.

Bacteriological assessments. Changes in bacterial numbers in the muscle and on the skin of sabalo are given in Fig. 2. The counts were found to increase from about 10^4 colony forming units (c.f.u.)/g or /cm^2 after 5 days of storage to 10^6–10^7 c.f.u. after about 25 days. The bacterial numbers per g of muscle were consistently higher throughout the trials than those numbers found per cm^2 of skin. No notable differences in the absolute values or rates of increases in bacterial numbers were observed between fish stored whole or eviscerated. The results for sabalo when compared with those increases in bacterial numbers found for Altiplano fish (Part 1) show more gradual increases resulting in curves with considerably reduced gradients. This reduced rate of bacterial growth indicates that psychrotrophic bacteria may not be present in very high numbers during the initial stages of storage.

Chemical analyses. The values for pH obtained for both whole and eviscerated sabalo increased with length of storage to become close to 7 after about 25 days (Fig. 3). Eviscerated fish showed appreciably lower initial values than fish which had been stored entire. However, these differences were reduced with length of storage and were minimal after about 17 days.

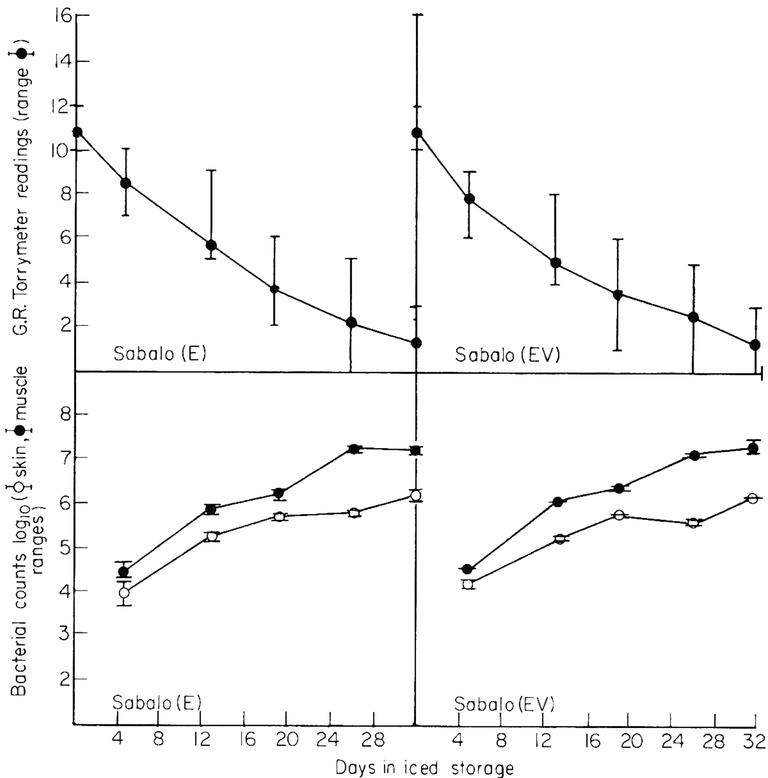

Figure 2. G.R. Torrymeter readings and bacterial counts of Parana Basin fish stored in ice.

The TVB content of muscle from sabalo were found to be very low initially (2.64–2.85 mg N/100 g) (Fig. 3). On storage of both whole and eviscerated fish these values increased very slightly and after about 10 days were in the range 10–15 mg N/100 g. This range of values was maintained from this time on for a further 16 days when values again started to rise near the end of the trial. After 32 days of storage the final TVB content of whole and eviscerated sabalo were found to be 26 and 45 mg N/100 g, respectively.

Amazon Basin fish

Carcase evaluation and proximate chemical composition. The three fish which form the basis of the commercial fishery in this region of Bolivia are pacu, tambaqui and the catfish known locally as chincuiña. The fish were all large and showed ranges in average weights of from 3.9 to 15.5 kg and average total lengths of from 57 to 1172 cm (Table 1). The removal of head, gills and guts from these fish was found to result in losses of total weight of between 24.0% (chincuiña) and 30.3% (pacu) (Table 1). The greatest contribution to these losses came from the heads. In all these fish large fat glands were found to be associated with the gut tissue and were often retained by fishermen for rendering into oil.

Yields of muscle from these fish ranged from 39.4% (tambaqui) to 50.5% (chincuiña) (Table 1). In all three fish, but particularly in chincuiña, large desposits of adipose tissue were found immediately beneath the skin. The muscles themselves also

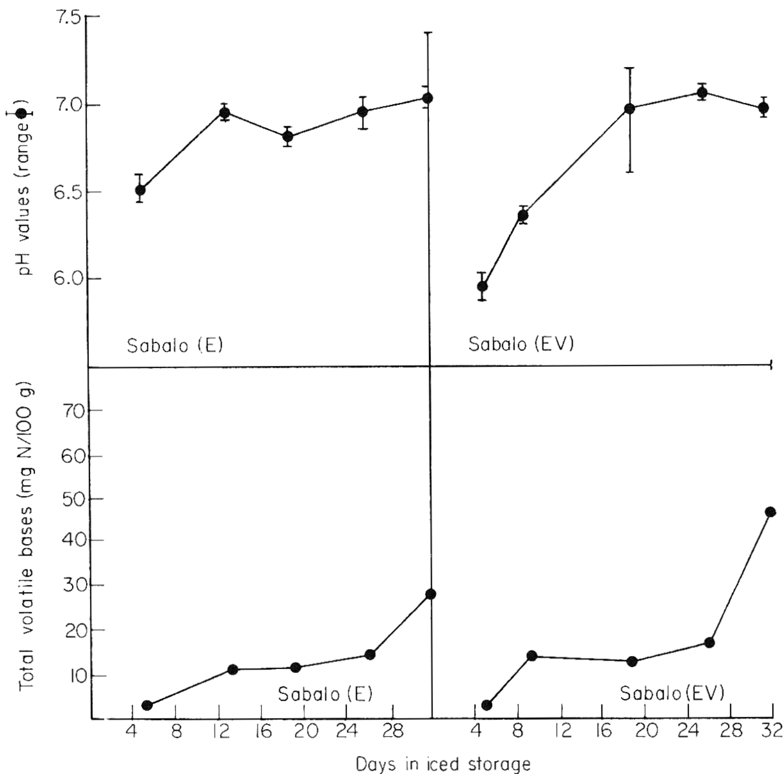

Figure 3. pH and total volatile bases content of Parana Basin fish stored in ice. (N.B. Ranges for TVB not presented due to loss of data.)

showed very high fat contents ranging from 8.85% (chincuiña) to 18.02% (pacu) (Table 1). There also appeared to be an inverse relationship between fat and crude protein contents with pacu showing the lowest protein content (14.11%) and chincuiña the highest (18.89%). The sum of these two components was found to be fairly similar for all three species (pacu: 32.13%; tambaqui: 31.45%; chincuiña: 27.74%).

The two semi-commercial species collected from the Amazon were both smaller and had lower fat contents (Table 1).

Physical and sensory evaluations. All three of the larger commercial fish species investigated were stored in the headed and gutted form. Additionally, tambaqui was stored whole, as is the practice of some fishermen. The visual and olfactory characteristics of the fish gradually changed with length of storage. In particular it was noticeable with the fish stored headed and gutted that the skin and body cavity started to be coated with large quantities of yellow malodourous slime after about 16 days. At this time the belly flaps and other tissue which had become exposed during heading and gutting became very soft; almost liquefied. The muscles also started to soften. By the end of the storage periods the production of yellow slime and liquefaction of the outer surfaces was advanced giving a penetrating putrid odour.

The scales of the pacu and tambaqui were by the end of the trial loosened and the black markings of chincuiña could be easily rubbed off. Similar changes were also found

for the smaller fish species investigated, although the changes were found to occur more rapidly. With these smaller fish, which were all stored whole, the eyes were found to become cloudy and sunken quite rapidly (5 days) and the gills changed in colour from being red through pink or brown to white. The gills also produced mucus with a sweet but strongly penetrating odour. The changes described above could be used to form the basis of a fish inspection system if required.

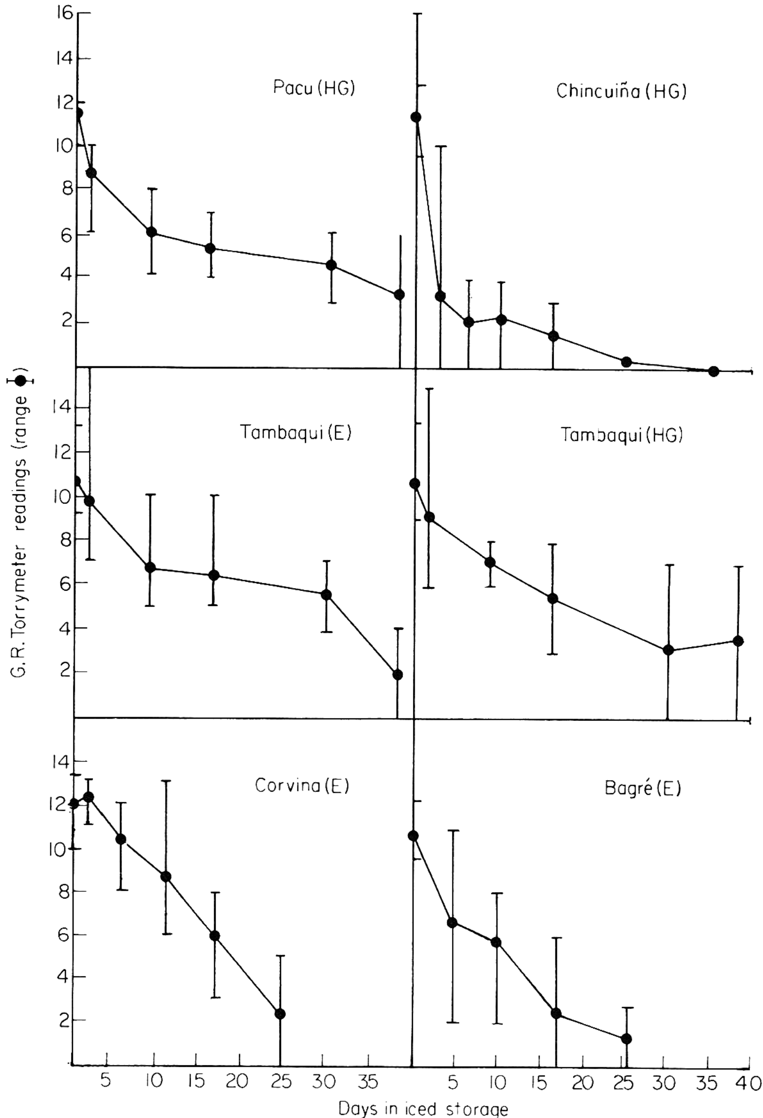

Figure 4. G.R. Torrymeter readings of Amazon fish stored in ice.

The results of the G.R. Torrymeter evaluations are given in Fig. 4. It was found for chincuiña, a catfish with a reasonably high fat content, that the meter readings fell very rapidly during the first 5 days of storage, from values of 11–12 to 2–3, and thereafter declined more slowly over the remaining storage period to zero. The fish species corvina

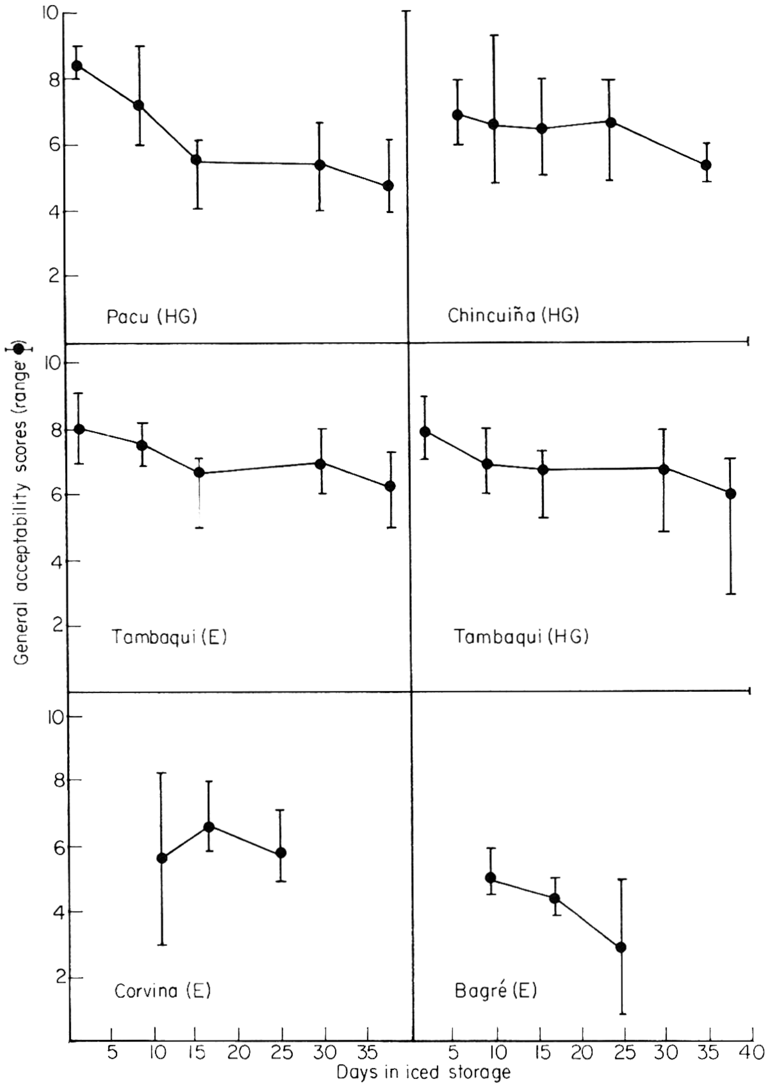

Figure 5. Sensory evaluation (general acceptability scores) of Amazon fish stored in ice.

and bagré showed more linear declines in meter readings over the storage periods; values falling from being in the range 11–12 to 1–2 over 25 days. The two large and scaled commercial fish, pacu and tambaqui, which are species of the same genus (*Colossoma* spp.), showed similar changes in their G.R. Torrymeter readings during storage (Fig. 4). The method of storage of tambaqui, namely whole or headed and gutted, appeared to have little effect on the rates of changes of Torrymeter readings.

The sensory evaluations (taste panels) conducted with these fish and the general acceptability scores obtained are given in Fig. 5. Interestingly, the scores obtained over the period of the trials did not fall rapidly. For example, in the case of both whole and gutted tambaqui the average scores remained in the range of 6.2–8.0 for the total period of the 38 day trial. These scores, therefore, never indicated that these fish were unacceptable to taste panellists. It appeared therefore, that the fish were still acceptable

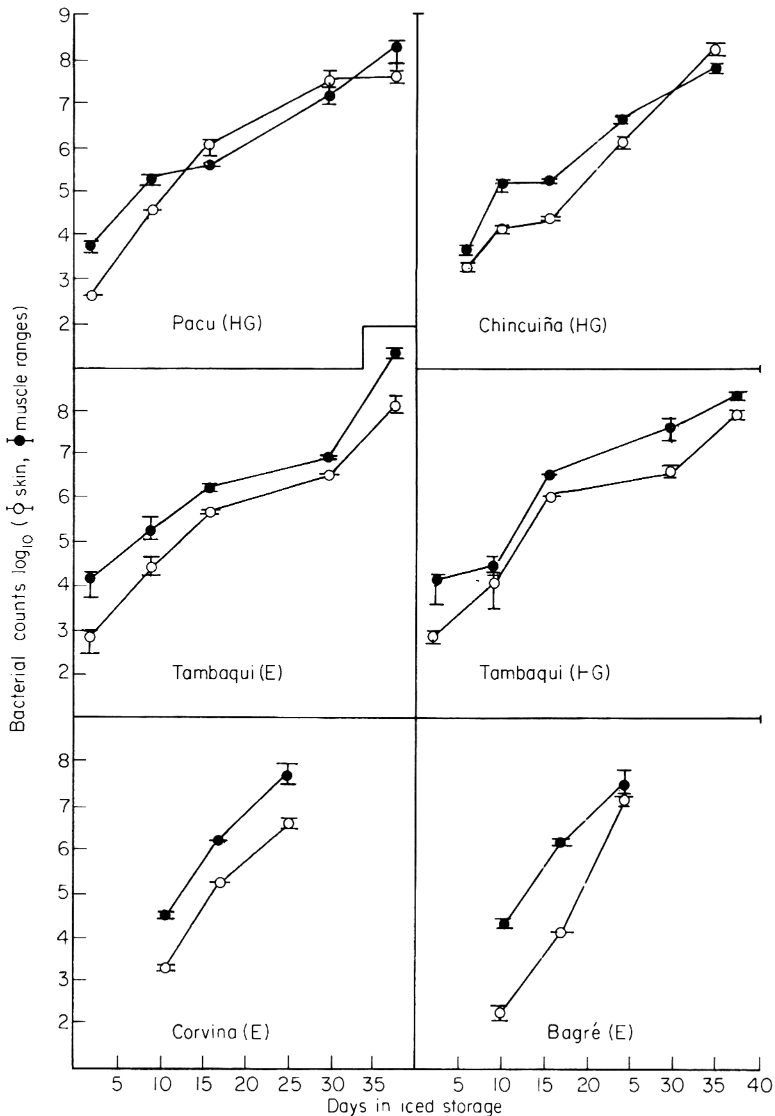

Figure 6. Bacterial counts at 25°C of Amazon fish stored in ice

despite the very low G.R. Torrymeter readings and extremely poor visual and olfactory characteristics found near the end of the trials. A similar result was found for pacu, chincuiña and corvina although with these fish an initial decline over the first 10–15 days was apparent followed by a plateau with scores in the region of 5–7.

Bacteriological assessment. The changes in bacterial counts of the Amazon fish species studied are given in Fig. 6. The large and commercial species pacu and tambaqui, showed signs of bacterial invasion after only 2 days of iced storage. At this time the muscles of pacu were found to have 10^3 c.f.u./g while tambaqui had values of 10^4 c.f.u./g. The bacterial contamination of the muscle, in terms of the numbers of c.f.u./g, tended to be higher than that of the skin (c.f.u./cm^2) for all the Amazon fish studied.

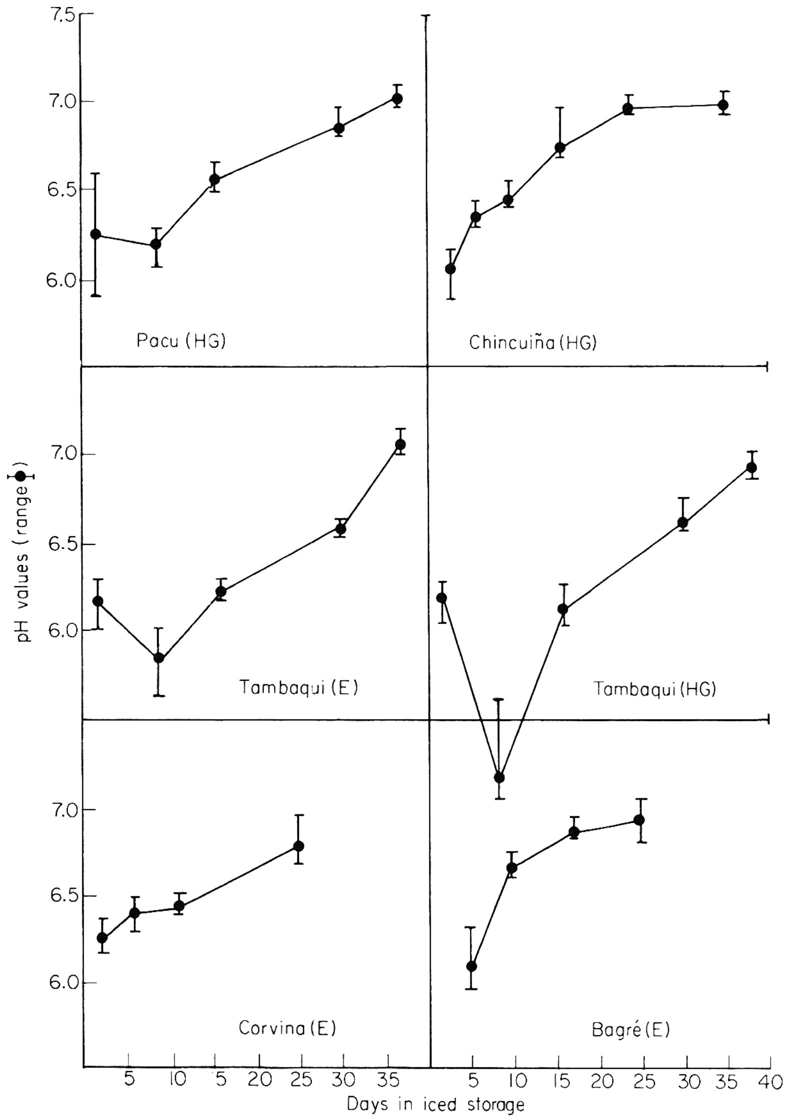

Figure 7. pH of Amazon fish stored in ice.

The bacterial numbers of these large Amazon fish were bound to increase steadily with storage time in ice and had reached about 10^7 c.f.u./g muscle after 30–36 days. There appeared to be no obvious differences between tambaqui stored entire or headed and gutted in their rates of spoilage and their storage lives using ICMSF recommended bacteriological guidelines (ICMSF, 1978) were determined to be 35–36 days. This level (10^7 c.f.u./g) however, was more rapidly reached by the small, semi-commercial fish species studied. Thus, both corvina and bagré were found to have storage lives as determined using this bacteriological guideline of approximately 25 days.

Chemical analyses. The results of the chemical analyses of pH and total volatile base (TVB) content are given in Figs 7 and 8. The initial pH values obtained for all the fish

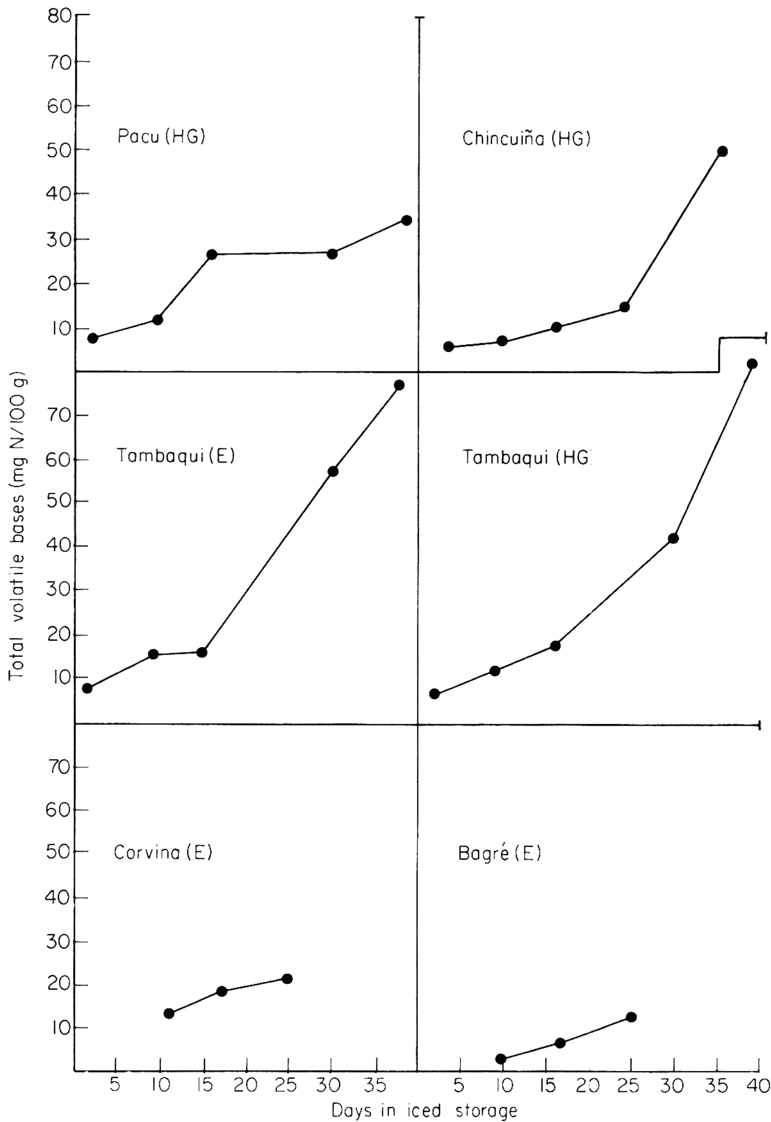

Figure 8. Total volatile bases content of muscle of Amazon fish stored in ice. (N.B. Ranges not presented due to loss of data.)

studied during the first few days of storage were in the range 6.10–6.25. On subsequent storage there was a general tendency for pH to increase to around pH 7.

The results of analyses for the TVB content of fish showed that most had initial values in the range of 5–7 mg N/100 g. These levels increased gradually for the following 15–20 days of storage and then more rapidly for the remaining period. Tambaqui, both in the whole and headed/gutted form, showed similar relative changes and after 38 days of iced storage had values exceeding 75 mg N/100 g, whilst headed and gutted pacu contained only 34 mg N/100 g after the same period (Fig. 8)

Discussion and conclusions

Fish caught in the Parana and Amazonian Basins are classified as 'lowland tropical' or

'warm water' fish. The waters of the River Pilcomayo had temperatures of 25°C whilst those of the Amazonian tributaries were around 30°C.

Sabalo from the River Pilcomayo were found to have low–medium muscle fat contents. However, large fat deposits were found to be associated with the gut tissue. During the breeding migration of this fish from the lowland swamps in Paraguay to the headwaters of this river they apparently do not feed. Undoubtedly, therefore, during their upstream mass migrations they utilize these body fat stores for energy and as a result their muscle fat contents are likely to fluctuate. Sabalos were stored in ice in both the whole and eviscerated forms. However, few notable differences in the absolute values or rates of changes of any of the analyses conducted upon these fish were found. Using the microbiological guidelines of acceptability recommended by the ICMSF (1978) then a storage life of about 25 days was indicated for both whole and eviscerated sabalo.

Sensory evaluations were not conducted with this fish but it was apparent from the visual and olfactory observations at storage times in excess of about 25 days that the fish were of extremely poor quality. A previous study has indicated an acceptable iced storage life for the sabalo from the same river, based on taste panel analysis, of slightly less than 24 days (R.R. Coutts, pers. comm., 1982).

The use of the G.R. Torrymeter as an appropriate index of freshness of sabalo seems justified from this study. Mean values fell from about 11 to 1 over the 32 day trial and had come to reach a value of 2–3 after 25 days of storage. The decline over this period was reasonably linear. Conversely, values obtained for the total volatile base (TVB) content of the muscle of sabalo over the period of storage showed three phases. The second of these represented a plateau from days 10–26 when values in the range 10–15 mg N/100 g were obtained. However, the third and upward phase did not bring TVB values in the range 30–40 mg N/100 g, i.e. that limit of acceptability for the marine fish, cod, described by Connell (1975), until 28–33 days of storage. The use of TVB as a chemical monitor of quality with sabalo does not therefore appear appropriate.

The results given in Table 2 which indicate the larger Amazon fish species to have storage lives of about 40 days confirm claims made by fishermen for the longevity of their product. Certainly, from sensory evaluations the fish are still acceptable after

Table 2. Storage lives of fish species studied as determined by sensory evaluations (taste panel acceptability scores) and bacteriological assessments

Fish species and location	Storage life (days)	
	Sensory evaluations	Bacteriological assessments
Sabalo (E), R. Pilcomayo	—	25
Sabalo (EV), R. Pilcomayo	—	25
Chincuiña (HG), R. Securé	> 40	30–31
Pacu (HG), R. Securé	> 40	36
Tambaqui (E), R. Securé	> 40	33–34
Tambaqui (HG), R. Securé	> 40	35–36
Corvina (E), R. Mamoré	30	25
Bagré (E), R. Mamoré	19	25

E = entire; EV = eviscerated; HG = headed and gutted.

about 40 days. However, all other indicators of acceptability, most particularly bacterial numbers point to significantly shorter storage lives. It is, however, possible that the inadequate use (if at all) of ice and the poor knowledge of basic hygiene and lack of facilities used by fishermen, distributors and in the market places, have resulted in the consumer becoming accustomed by necessity to buying and eating poor quality fish.

Fish of over 40 days storage although still acceptable to consumers, may carry a certain risk. For example the high levels of bacteria present may produce toxins, or contribute towards the formation of other toxic chemical constituents such as histamine.

The fish studied in this investigation ranged very widely in their shapes, sizes, weights and chemical compositions. In general it may be summarized that fish from the cold waters of the Altiplano had low fat contents and were small (Part 1) whilst fish from warmer waters of the lowland rivers were larger and had considerably higher fat contents. It is also evident that fish from the warmer lowland rivers of the Parana Basin (River Pilcomayo) and the Amazon (Rivers Mamoré and Securé) had considerably longer storage lives in ice than fish from the colder waters of the high altitude Altiplano lakes.

However, apart from a clear environmental effect upon the length of storage of the different fish species, their size played a significant role. Thus, for example, the smaller tropical fish species collected from the Amazon such as corvina and bagré, and sabalo from the River Pilcomayo, showed significantly shorter storage lives than larger species of fish collected from the same areas. It should also be noted that the fish from the Altiplano were all very small, even when compared with these smaller fish species collected from lowland locations. The principle that has already been demonstrated in other studies (Disney, 1976; Shewan, 1977), where indications are given that fish from tropical warm waters tend to have longer shelf lives in ice than similar types of fish from temperate cold waters, is consistent with the results of this study.

The muscles of marine fish after capture have been reported to be sterile (Poulter, Nicolaides & Hector, 1978; Curran *et al.*, 1980; Curran, Nicolaides & Al-Alawi, 1981; ICMSF, 1980), unless the flesh has been invaded by marine parasites (Curran *et al.*, 1981). Although current opinion is that the muscle of healthy freshwater fish is sterile at death, the information available from the few publications on this topic indicate that tropical freshwater fish may have an intrinsic bacterial flora (Balakrishnan Nair, Tharamani & Lahiry, 1971; Maltchewsky & Partmann, 1951; Moorjani *et al.*, 1958). Maltchewsky (1955) reported that the numbers and types of bacteria present may be influenced by the environment from which the fish are captured and the types of food that have formed their diet. In the present study with Bolivian freshwater fish, it was not possible to sample directly after death, and so observations on the initial sterility of the muscle cannot be made.

The assessments of sensory characteristics conducted in this investigation do perhaps represent one of the more relevant methods for the determination of fish quality since it is on the basis of such evalutions that the consumer ultimately accepts or rejects the product. They do not, however, provide an unequivocal measure of quality since by definition they depend on the senses and judgements of individuals and can, therefore, be subject to personal prejudices. This bias was clearly seen in the present investigation with fish from the Amazon. The results showed that many of the fish were still highly acceptable, for over 40 days despite poor visual and olfactory characteristics and high bacterial contamination. Possible reasons for such results were mentioned earlier in this discussion but it is noteworthy here, since the question arises of whether

fish should be considered to have reached their maximum storage lives when, (i) the consumers reject the fish or (ii) when other physical, chemical and bacteriological analyses indicate rejection. Such a question cannot be easily answered in a country such as Bolivia in which food is often limited and where American and European bases of quality and hygiene have no real grounding. Should option (i) be taken as the most appropriate index of storage life then it must be made clear to all concerned that should the analyses conducted in option (ii) indicate earlier rejection that such indications have been made for reasons of their increased potential toxicity. Consequently, the vendor and consumer must be made aware of the possible increased health hazard and implications of preparing and consuming such fish.

The G.R. Torrymeter has been developed and tested extensively with cold water species and has been found to give quick, reliable indications of the quality of fresh fish. In recent years several studies have been conducted to determine its use with tropical fish and these have been reviewed by Curran (1982). The use of the meter in this investigation was found to provide a useful index of fish quality with many of the species studied. This was so with pejerreys and carp from the Altiplano, (Part 1), sabalo from the River Pilcomayo and pacu, tambaqui, corvina and bagré from the Amazon. However, the use of the G.R. Torrymeter with a species of catfish from the Amazon, chincuiña, was not of any value since the readings declined very rapidly during the first few days of storage. The possible reasons for the poor results obtained with the meter on these fish may be related to their thick and leathery skins which are often associated with large deposits of underlying adipose tissue. These catfish were also found to have high muscle fat contents. All these factors are known to affect the readings made by the Torrymeter (Curran, 1982).

In conclusion, therefore, the determination of visual and olfactory characteristics in conjunction with taste panel evaluations could provide an adequate system for the assessment of freshness and quality of all the fish species investigated here. However, use of the G.R. Torrymeter with certain species would also provide rapid back-up information to support and monitor these assessments. Results have indicated that total volatile bases and pH values could not be used on their own to give any accurate assessment of the quality of fish. The application of bacteriological assessments has been shown to be of greatest value from these studies but the obvious delay in obtaining results, due to incubation times, would mean that such analyses could not be used for rapid screening purposes.

This study has represented the first effort to evaluate the storage lives and quality characteristics of Bolivia's fish species. As such, the study provides basic information of vital importance to future projects concerned with fisheries development in Bolivia. It is hoped that this will enable optimum distribution and marketing networks for fresh fish to be established.

Acknowledgments

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Potential value of the *Limulus* lysate assay for the measurement of meat spoilage

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Summary

Using a microtitre plate method the *Limulus* lysate endotoxin assay was shown to be capable of detecting *Pseudomonas* spp. at viable cell concentrations of 10^2–10^3/ml. The changes in endotoxin concentration, microbial flora, pH and ERV of laboratory-produced minced beef and minced pork stored at 4°C were monitored. The endotoxin concentration was shown to increase with the onset of spoilage. Factors were derived enabling an estimate of AMC to be made from the endotoxin concentration determined by the *Limulus* lysate assay. The use of the microtitre plate system significantly reduces the cost of the *Limulus* lysate assay.

Introduction

The discovery by Levin & Bang (1964) of the ability of *Limulus* amoebocyte lysate to clot in the presence of lipopolysaccharide (LPS)/endotoxin from cells of Gram negative bacteria has led to the development of an extremely sensitive method for the detection of these organisms. The haeomocyte lysate from the horseshoe crab *Limulus polyphemus*) contains a pro-clotting enzyme which is transformed, in the presence of LPS, into an active clotting enzyme. This enzyme coagulates a clottable protein (coagulogen) contained within the lysate (Sullivan & Watson, 1975; Liu *et al.*, 1979; Liang, Sakamar & Liu, 1980). Wildfeuer *et al.* (1974; 1975) have demonstrated that peptidoglycans elicit a *Limulus* lysate reaction but that they are 1000–40 000 times less reactive than *Escherichia coli* LPS. Fine *et al.* (1977) have also suggested that there might be some cross reaction with lipoteichoic acids; however, the lysate was less sensitive to these compounds compared with LPS for which the *Limulus* lysate for all practical purposes is specific.

Typically the assay involves rehydrating a lyophilized amoebocyte lysate with a known volume of pyrogen-free water (PFW). The lysate (0.1 ml) is then incubated for 1 hr at 37°C with an equivalent volume of suitably diluted sample or standard. Following the incubation period the test tubes are inverted and the tube highest in the dilution series with a stable clot is noted as the titration end point. Reference to the end point of a titration of standard endotoxin against lysate enables an estimate to be made of the concentration of LPS in a test sample. The drawbacks of the method are primarily the cost (£36 per 2.5 ml lysate, Sigma Chemicals, 1985) and poor end-point determination. Recent research has centred upon alleviating these problems.

Prior & Spagna (1979; 1980) have compared the standard tube method with a microdilution method and found the latter more sensitive whilst using only 20–50 μl of

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lysate per dilution. Sudi *et al.* (1981) reconstituted lysate and dispensed it into U-bottomed pyrogen-free microtitre plates which were stored lyophilized until required. A modification of this method was used in the study below. Many microscope slide methods have been developed (Goto, Watanabe & Nakamura, 1977; Okuguchi, 1978; Flowers, 1979) but have done little to improve end point detection. More recently the use of chromogenic substrates has been advocated (Nakamura *et al.*, 1977; Iwanaga *et al.*, 1978; Scully *et al.*, 1980; Webster, 1980; Thomas *et al.*, 1981; Aune *et al.*, 1982). The activity of an LPS activated amidase contained within the clot is measured using peptides linked to p-nitroanilide (PNA) (usually tert-butoxycarbonyl-valine-leucine-glycine-arginine-PNA). The quantity of PNA released by the action of the amidase is determined spectrophotometrically (OD_{405}) and is proportional to LPS concentration. However, the chromogenic substrates are expensive (*circa* £89 per 25 mg) although sensitivity is improved by an order of magnitude.

In food and related areas of microbiology the *Limulus* lysate method has been used to estimate the accumulative Gram negative load of foods (Hansen, 1982; and Hansen, Mikkelsen & Moller-Madsen, 1982), for the detection of LPS in UHT treated milk (Sudi *et al.*, 1981) and for the detection of LPS in water (Watson *et al.*, 1977). Sullivan *et al.* (1983) working on lean fish quality have shown that a good relationship ($P < 0.001$) exists between the *Limulus* lysate titre and the total volatile bases present. The majority of the work relating *Limulus* lysate titres to the microbiological status of meat has been performed by Jay (1977); Jay & Margitic (1979); Jay *et al.* (1979) and Jay (1981).

The importance of *Pseudomonas* spp. in low temperature spoilage of aerobically packed meat has been noted by many authors (for review see McMeekin, 1982). The determination of the sensitivity of the *Limulus* lysate assay to cells of *Pseudomonas* spp. is important if the assay is to be used for the assessment of incipient spoilage. The work described below was aimed at evaluating the *Limulus* lysate assay for the assessment of meat spoilage using a microtitre plate system.

Materials and methods

Meat samples

Boned pork shoulder and flank of beef were obtained from local meat producing plants approximately 24 hr after slaughter. The surfaces of the meat were scraped clean and diced using sterile knives. The diced pieces were passed twice through a sterile mincer. 250 g amounts were packed into plastic trays, overwrapped with oxygen permeable film and stored at 4°C. Individual packs were removed from storage daily for analysis.

Physico chemical analysis

Extract release volume (ERV). Twenty-five g meat sample was homogenized in 100 ml distilled water in an Ato-Mix blender (MSE Ltd.) for 2 min. The volume of filtrate passing through a Whatman No. 1 filter paper, folded according to the method of Jay (1964), in 15 min was determined and reported as the ERV.

pH value. A 10 g meat sample was homogenized in 100 ml distilled water as above and filtered. The pH was determined in 20 ml of filtrate using a Russell pH electrode attached to a Data Scientific Digital Water Analyser.

Organoleptic assessment

A six member panel, familiar with meat products, was used to assess the acceptability of the stored meat. The panel was requested, daily, to determine whether the meat was acceptable or unacceptable using appearance and odour of the raw meat as criteria.

Microbiological analysis of meat samples

A sample of meat (10 g) was homogenized in 90 ml peptone saline diluent for 2.5 min using a Colworth Stomacher. Following ten-fold serial dilution in peptone saline the aerobic mesophilic count (AMC) was determined by spread plating 0.1 ml of diluted sample onto plate count agar (PCA) (Oxoid Ltd.). The plates were incubated at 22°C for 3 days. Enterobacteriaceae were enumerated in 1.0 ml of a suitable dilution of meat sample using violet red bile agar (glucose) (VRBG) overlayed on PCA. In addition Enterobacteriaceae were enumerated using the bile-amphotericin-cycloheximide (PBAC) medium and method described by Cyzeska *et al.* (1981). Both media were incubated at 37°C for 48 hr. *Pseudomonas* spp. were enumerated using the cetrimide-fucidin-cephaloridine (CFC) medium of Mead & Adams (1977).

Bacterial cultures

Organisms used in pure culture studies were *Pseudomonas fragi* (ATCC 4973), *Pseudomonas aeruginosa* (ATCC 10145), *Pseudomonas putida* (NCIB 9494) and *Pseudomonas fluorescens* (NCIB 9046). Bacteria were grown in nutrient broth for 48 hr at 22°C except *P. aeruginosa* which was incubated at 37°C. Washed cell suspensions were prepared by centrifuging nutrient broth cultures and washing three times with, and resuspending the pellet in, pyrogen-free water (PFW) (Ivex Ltd., Larne, Co. Antrim). The *Pseudomonas* cells were enumerated using the methods described above for AMC.

Limulus *lysate assay*

Pyrogen free glassware. Glassware used in the assay was rendered free of pyrogen (LPS/endotoxin) by heating to 140°C for 4 hr. Plastic disposable ware was found to be pyrogen free although this was not guaranteed by the manufacturers.

Assay procedure. PFW (2.5 ml) was added aseptically to lyophilized *Limulus* lysate (Sigma Chemicals Ltd.) and mixed gently to avoid frothing. The batches of *Limulus* lysate used were sensitive to 0.05–0.2 ng/ml of FDA *Escherichia coli* lot EC2 (Sigma Chemicals Ltd.) standard reference toxin. The reconstituted lysate was maintained on ice throughout the assay procedure. The sensitivity of the lysate to endotoxin was determined for each assay by titration against doubling dilutions of standard endotoxin of *Shigella flexneri* (Sigma Chemicals Ltd.). Meat samples were diluted ten-fold (10 g meat, 90 ml PFW), homogenized in a Colworth Stomacher and stored frozen (−20°C) until assayed. The *Limulus* lysate assay was performed in U-bottomed microtitre plates fitted with lids (Sterilin Ltd.). Forty μl of suitable dilutions in PFW of sample or standard endotoxin were pipetted into the wells to which was added 40 μl of reconstituted lysate. Positive (2.0 ng endotoxin/ml) and negative (PFW) controls were included in each assay. The plates were sealed with Parafilm and incubated at 37°C for 1 hr in a static water bath. Following incubation 40 μl of 0.005% crystal violet was carefully added to each well. The plate was observed obliquely from below: a well which

was blue throughout its depth was scored a negative reaction, one with a blue layer upon an unstained gel was scored a positive reaction. The well of the highest dilution showing a positive reaction was recorded as the end-point titre. Reference to the standard endotoxin titre and calculation from the dilution factor enabled the quantity of endotoxin in a sample to be determined.

Results

The sensitivity of the Limulus *lysate assay to* Pseudomonas *spp.*

Preliminary experiments confirmed the findings of Prior & Spagna (1979) that the microtitre plate method was at least as sensitive as the standard tube method described by Jay (1977). The sensitivity of the *Limulus* lysate to the washed cell suspensions of *Pseudomonas* spp. is shown in Table 1. The highest concentration of endotoxin was produced by the *P. fluorescens* culture which also had the highest cell density. The dilution of a washed cell suspension of *P. fragi* demonstrated that there was a direct relationship between *P. fragi* cell numbers and endotoxin concentration.

Table 1. The sensitivity of *Limulus* lysate to washed cell suspensions of *Pseudomonas* spp

	Log_{10} CFU/ml	Endpoint titre	Endotoxin concentration (μg/ml)	CFU/ng endotoxin
P. fragi	8.9	10^5	25.0	31 773
	6.9	10^3	0.25	31 773
	4.9	10^1	0.0025	31 773
	2.9	ND*	ND	
P. putida	9.1	10^6	250	5035
P. aeruginosa	8.9	10^6	250	3177
P. fluorescens	9.4	10^7	2500	1004

*ND = not detected.

Pre-treatment of *P. fragi* cells with Tris/HCl buffer (0.1 M, pH 8.0) and 0.25 mM EDTA for 2 hr or boiling them (10 min) did not increase the sensitivity of the lysate to this organism. Preliminary results suggest that treatment of the cells with ultrasound (sonication 40 μA, 2 min) may increase the sensitivity of the lysate to *P. fragi* by an order of magnitude (Table 2).

Table 2. Effects of various pretreatments upon the sensitivity of *Limulus* lysate to cells of *P. fragi*

	Nutrient broth culture	PFW washed cells	EDTA/ Tris/HCl treated	Boiled cells	Sonicated cells
Initial viable count (/ml)	1.1×10^9	8.0×10^8	8.0×10^8	8.0×10^8	8.0×10^8
Endpoint titre	10^7	10^5	10^5	10^5	10^6
Endotoxin (μg/ml)	2500	24.0	25.0	25.0	250
CFU/ng Endotoxin ratio	440	32 000	32 000	32 000	3200

The results indicated that the detection limit using the *Limulus* lysate assay was of the order of 10^3 CFU/ml for *P. fragi* and *P. putida* and 10^2 CFU/ml for *P. aeruginosa* and

P. fluorescens. Inspection of the CFU/ng endotoxin ratio (Table 1) shows that at least ten-fold more washed *P. fragi* cells were required to produce 1 ng endotoxin compared with *P. aeruginosa*, *P. fluorescens* and *P. putida* cell suspensions. Furthermore results presented in Table 2 show that considerably more endotoxin was present in the original nutrient broth culture of *P. fragi* than in the washed cell suspension. The CFU/ng endotoxin ratio of the broth culture was also much lower than that of washed cell suspension (Table 2). These results show that much of the endotoxin was not bound to the cells of *P. fragi* but free within the nutrient broth suspension.

Changes in endotoxin content of minced beef and minced pork and the relationship with low temperature spoilage

The initial aerobic mesophilic counts (AMC) of both the minced beef (Fig. 1) and the minced pork (Fig. 2) were low, in the order of 10^5 and 10^3/g respectively. A short lag phase was noted in both samples before the bacteria entered the logarithmic phase of growth attaining an AMC of 10^9/g in both meats after 6 days storage at 4°C. Analysis of variance showed that there was no statistical difference ($P = 0.05$) between counts of Enterobacteriaceae enumerated using either the VRBG overlay method or PBAC

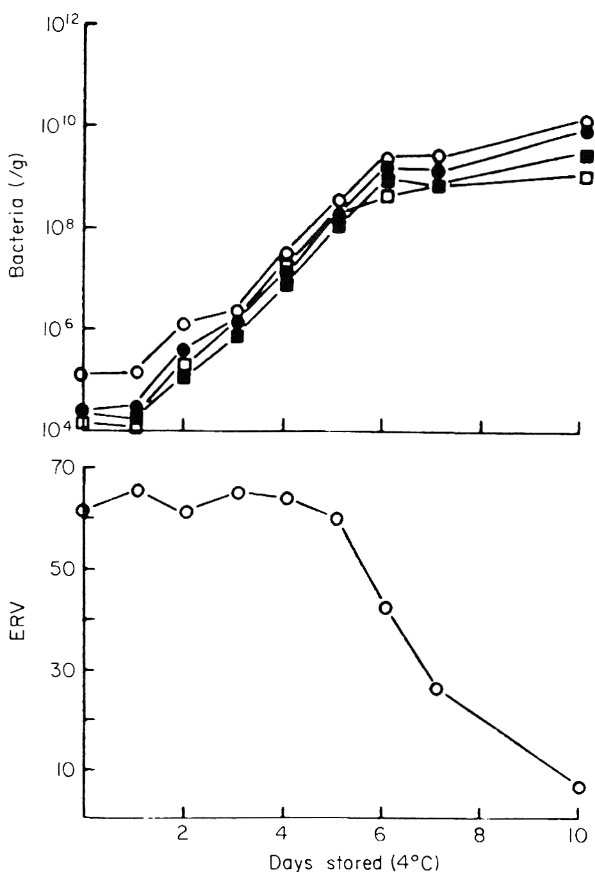

Figure 1. The changes in ERV and bacterial numbers of laboratory produced minced beef stored at 4°C; bacteria enumerated on. plate count agar (PCA) (O); bile-amphotericin-cycloheximide (PBAC) (□); VRBG (■); both selective for Enterobacteriaceae and *Pseudomonas* selective cetrimide-fucidin-cephaloridine agar (CFC) (●).

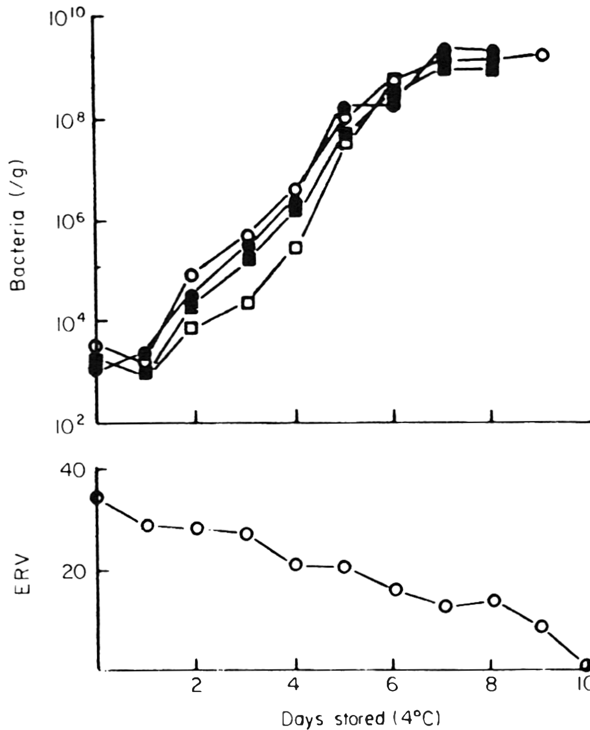

Figure 2. The changes in ERV and bacterial numbers of laboratory produced pork mince stored at 4°C; bacteria enumerated on PCA (O); PBAC agar (□); VRBG agar (■); and CFC agar (●).

agar. Organoleptic assessment indicated that both the pork and the beef mince was unacceptable after 5 days storage.

The changes in both ERV and pH were somewhat different in the two meat samples; however, the same general relationship existed between these parameters within the two meat products (Fig. 3). The ERV values for the minced beef (Fig. 1) fluctuated about 60 from days 0–5, only after 6 days storage when the meat was deemed unacceptable did the value fall to 40. In contrast the pork mince (Fig. 2) showed a steady decline in ERV from an initial value of 34 to 16 after 6 days storage. The pH value of the minced beef increased from 5.6 (0–4 days) to 6.2 after 6 days storage. This value increased to pH 7.0 after 10 days at 4°C. The initial value of the pork mince was higher (pH 6.3–6.7) and fluctuated about this value for the duration of the experiment.

The changes in the endotoxin content of the minced beef and minced pork are shown in Fig. 4. The initial endotoxin content of the minced beef was less than 2.5 ng/g and attained a maximum value of 2500 μg/g after 6 days storage. Although the initial AMC for the pork mince was lower than that for the minced beef the endotoxin content was higher (5.0 ng/g). The maximum concentration, reached after 6 days storage, was 500 μg/g five-fold lower than that of the minced beef; however, the AMC was also lower than that of minced beef after the same storage period at 4°C. Despite apparent differences in the AMC: endotoxin relationship, particularly at the lower endotoxin concentrations, a similar linear relationship existed between AMC and endotoxin content for both the pork and the beef mince (Fig. 5).

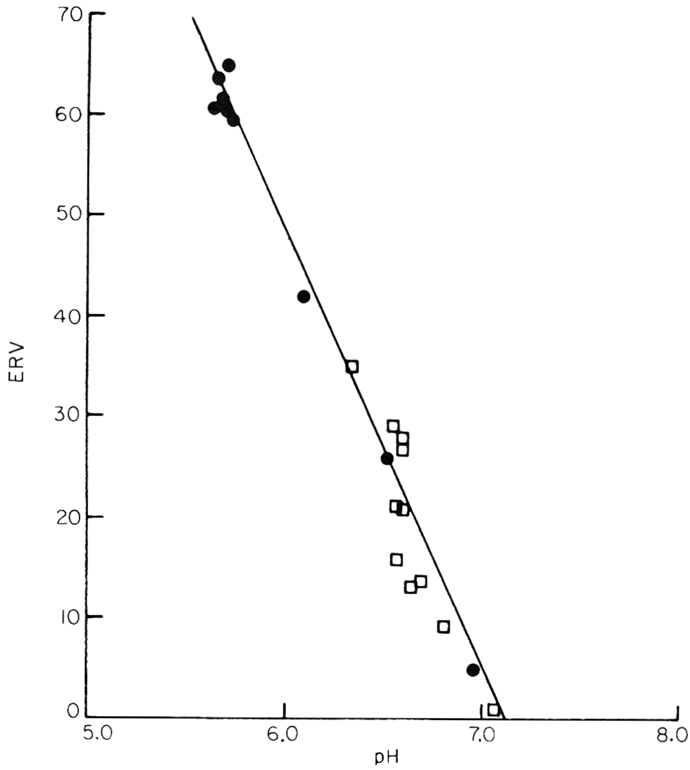

Figure 3. The relationship between extract release volume (ERV) and pH for minced beef (●) and minced pork (□) stored at 4°C, showing best fit regression line ($y = 314-44x$, $r^2 = -0.97$, $n = 20$).

Discussion

The use of microtitre plate method for the *Limulus* lysate assay, similar to that devised by Prior & Spagna (1979) and Sudi *et al.* (1981) reduced the volume of lysate required and the use of the dye assisted end point detection. The microtitre method was as sensitive as the standard tube method and was capable of detecting 10^2-10^3 cells/ml of various *Pseudomonas* spp. This was somewhat lower than the minimum number of cells reported by Jay (1977) to produce a positive titre. The lowest value recorded by this worker was 3.5×10^6/ml for *Enterobacter aerogenes* and the mean was 2.5×10^6/ml which was also similar to that recorded for the *Pseudomonas* spp. assayed. The reasons for these differences are not clear. However, Jay (1977) did note that the values he obtained for the minimum number of cells required to give a positive titre were approximately ten-fold higher than those reported by other workers.

Coates (1977) using a spectrophotometric method found that treatment with EDTA/Tris/HCl increased the sensitivity of the *Limulus* assay to LPS. The results presented here showed no enhancing effect of EDTA treatment. However, the end point detection was probably less sensitive than that used by Coates (1977) and only treatments causing a change in sensitivity of at least a factor of ten would be apparent. An increase of this nature was apparent after sonication which improved the sensitivity of the assay to cells of *P. putida* and *P. fragi* by an order of magnitude.

Jay (1977) suggested that a negative *Limulus* titre at 10^{-3} sample dilution should be

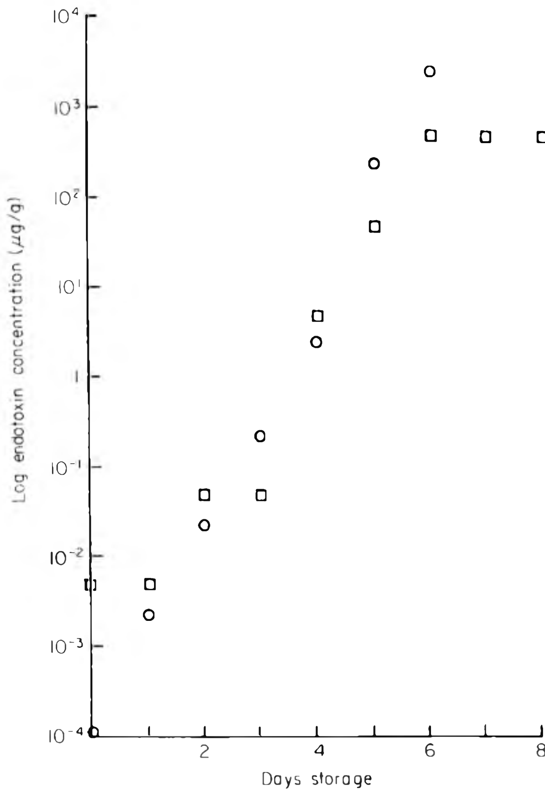

Figure 4. The changes in the *Limulus* lysate determined endotoxin concentration in minced beef (□) and minced pork (O) stored at 4°C. The batches of *Limulus* lysate used for assaying the endotoxin content of the minced beef and minced pork were sensitive to 0.25 and 0.5 ng/ml respectively of *Shigella flexneri* standard reference endotoxin (Sigma Chemicals Ltd.).

considered indicative of a minced beef sample of excellent microbial quality. Furthermore, he suggested a positive titre at this dilution implied that the sample is in a state of incipient or real spoilage with an aerobic plate count (APC) of about 10^7-10^8/g. Results of experiments reported here show that positive titres were recorded for minced beef at 10^{-1} dilution (AMC 1.5×10^5/g) after 1 day's storage at 4°C and for minced pork at a 10^{-1} dilution (AMC 5.5×10^3/g) after 0 days. Clearly from these results a negative or positive titre at a 10^{-3} dilution should not be considered a 'cut off' point for microbial acceptability.

The relationship between ERV and *Limulus* titre is equivocal. Jay (1977) reported good agreement between the two parameters suggesting that an ERV of 25 was the lowest value for acceptable minced beef. However, Jay *et al.* (1979) subsequently reported that there was no good agreement between the two parameters. He further suggested that ERV was mainly suitable as a quality determinant for minced beef in which a psychrotrophic Gram negative flora had actually developed rather than for fresh meats where a sufficient amount of low temperature growth had not occurred. The results of the study reported here show that ERV's of 20 and 25 for minced pork and minced beef respectively were associated with high endotoxin concentrations and organoleptically unacceptable material. These findings are in general agreement with those of Jay (1977).

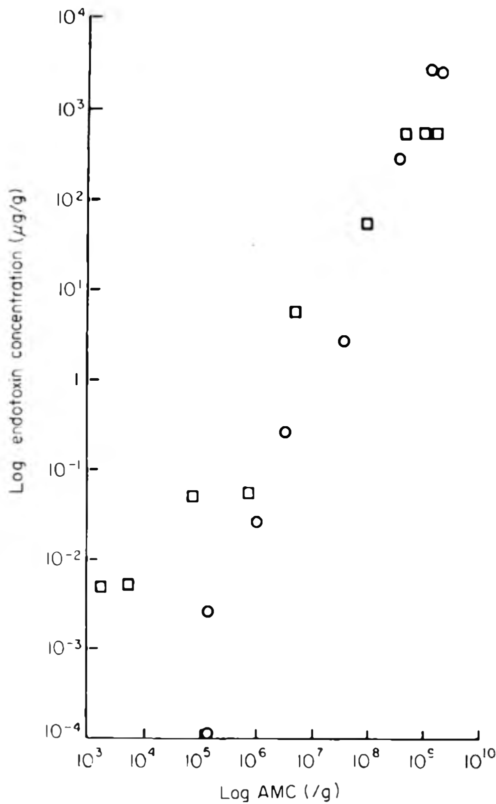

Figure 5. The relationship between endotoxin concentration and the aerobic mesophilic count (AMC) in minced beef (□) and minced pork (O) stored at 4°C.

A method of estimating the aerobic plate count (APC) from the endotoxin concentration was recently presented by Jay (1981). He enumerated Gram negative bacteria (GNB) in ground beef samples purchased from supermarkets using a VRBG overlay technique. The aerobic plate count, enumerated using plate count agar incubated at 30°C, and the endotoxin concentration were also determined. GNB counts were then grouped into half log cycles and the mean APC:GNB and GNB:ng endotoxin ratios calculated for each half log cycle. Since the *Limulus* lysate reacts only with GNB the number of GNB in a sample may be estimated from the endotoxin concentration using a predetermined fixed value for the GNB:ng endotoxin ratio;

$$GNB_{estimated} = \text{Endotoxin (ng/g)} \times \text{GNB:ng endotoxin}.$$

Jay (1981) calculated the mean GNB:ng endotoxin ratio as 1900, using this value and the APC:GNB ratio, $APC_{estimated}$ can then be calculated:

$$APC_{estimated} = GNB_{estimated} \times \text{APC:GNB}.$$

However, the use of VRBG to enumerate the GNB excludes the *Pseudomonas* population from the determination of the total GNB population which might be expected to influence the APC:GNB and GNB:ng endotoxin ratios.

Using an approach similar to that of Jay (1981) mean values for the AMC (APC): GNB and GNB: ng endotoxin ratios were calculated for the minced beef and the minced pork stored at 4°C. Summation of the colony counts from VRBG and *Pseudomonas* selective agar (CFC) and PBAC and CFC yielded two values for the total GNB population in the two minced products. PBAC was developed by Cyseka *et al.* (1981) who reported increased recovery rates of Enterobacteriaceae using this media compared with VRBG. However, in our hands no significant increase in recovery was identified.

Table 3. Mean values and 95% confidence limits of AMC: colony count on selective medium (SM) ratio and the colony count on selective medium: ng endotoxin ratio. The ratios calculated from minced beef and minced pork stored at 4°C

Selective medium (SM)	Minced beef		Minced pork	
	AMC: SM count	SM: ng endotoxin	AMC: SM count	SM: ng endotoxin
VRBG + CFC	1.38 ± 0.84	14 900 ± 10 700	0.77 ± 0.21	6261 ± 4721
PBAC + CFC	1.79 ± 1.25	16 026 ± 10 925	0.92 ± 0.39	5828 ± 3645
CFC	3.42 ± 2.63	7859 ± 7418	1.45 ± 0.67	3873 ± 3052
PBAC	3.9 ± 2.43	6888 ± 4210	6.23 ± 5.28	1956 ± 1541
VRBG	3.24 ± 1.37	7570 ± 4389	1.87 ± 0.44	1466 ± 1000

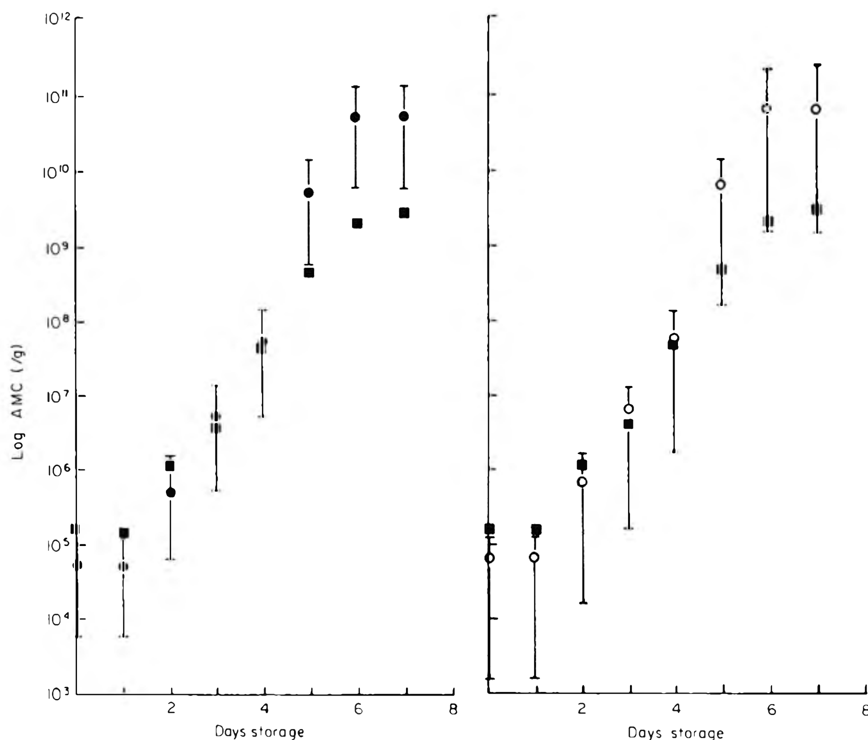

Figure 6. The aerobic mesophilic count (AMC) of minced beef, enumerated by spread plating on PCA (■) and estimated, within 95% confidence limits, from the endotoxin concentration and using factors derived (see Table 3) from plate counts on VRBG + CFC (●) and VRBG (O) media.

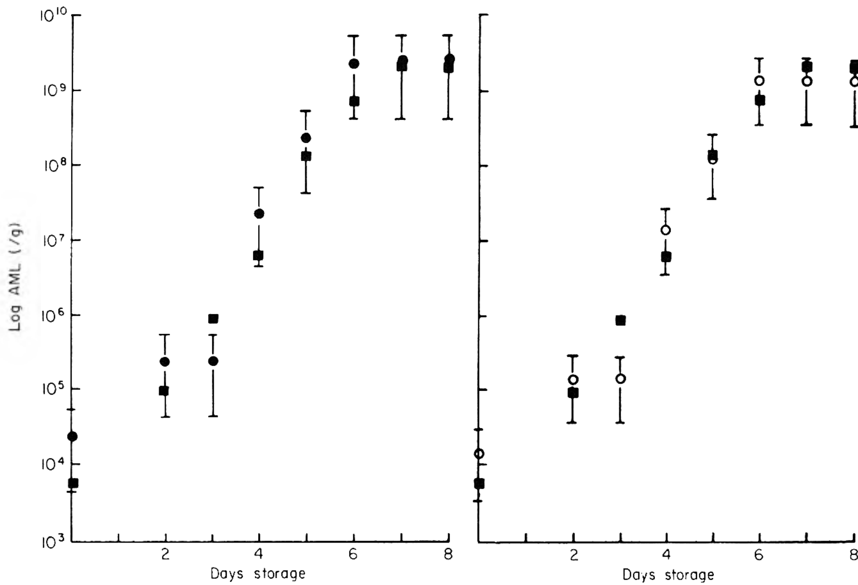

Figure 7. The aerobic mesophilic count of minced pork stored at 4°C, enumerated by spread plating on PCA (■) and estimated, within 95% confidence limits, from the endotoxin concentration and using ratios derived (see Table 3) from plate counts on VRBG+CFC (●) and VRBG (O) media.

The mean values, within 95% confidence limits, of the ratio of AMC:colony count on selective medium (SM) and the SM : ng endotoxin ratio for both the minced beef and pork are given on Table 3. These ratios varied from 1.38 (AMC:VRBG+CFC) to 3.9 (AMC:PBAC) for the minced beef and from 0.77 (AMC:VRBG+CFC) to 6.23 (AMC:PBAC) for the minced pork. The values for the AMC:VRBG ratios for both the minced beef and the minced pork were within the range (APC:VRB) obtained by Jay (1981) for ground beef samples within the same half log cycle ranges reported here. In contrast differences were apparent in the mean GNB : ng endotoxin ratios for the two minced meats which ranges from 14900 (VRBG+CFC:ng endotoxin) to 1466 (VRBG:ng endotoxin) compared with a mean ratio of 1900±2200 (VRB:ng endotoxin) reported by Jay (1981). The ratios for CFC:ng endotoxin determined for the minced meats were similar to those obtained for the pure cultures of *P. putida* and *P. aeruginosa* (Table 1). These results suggest that the GNB : ng endotoxin ratio depends upon the selective media used to enumerate the total GNB population of the sample.

$AMC_{estimated}$ was calculated for the minced beef and minced pork using either the ratios determined using VRBG+CFC or VRBG counts (see Table 3). The values of $AMC_{estimated}$ calculated using these ratios, are compared with the actual AMC values determined by spread plating on PCA for minced beef and minced pork in Figs 6 and 7 respectively. In general there was good agreement between the $AMC_{estimated}$ values and the actual AMC values determined by counting colonies on PCA. There was a particularly good correlation between the two values when the bacteria within the minced beef were in the exponential growth phase. The 'fit' was not as good at AMC values greater than 10^9/g. In contrast, whilst mean AMC values were within the confidence limits of the $AMC_{estimated}$ values in the exponential growth phase for pork mince, the agreement between the estimated and experimentally determined values was better at

high AMC values (> 10^9/g). The 95% confidence limits of $AMC_{estimated}$ for the minced beef were larger when the estimate was based solely upon VRBG ratios. The confidence limits were, however, similar for both $AMC_{estimated}$ values for the pork mince.

Conclusions

The development of the microtitre *Limulus* lysate assay significantly reduces the cost associated with the method enabling sixty-two individual assays to be performed from 2.5 ml of lysate compared with only twenty-five assays using the standard tube technique. The microtitre end point was easily determined using 0.005% crystal violet and the assay was able to detect 10^2-10^3 viable *Pseudomonas* cells/ml. A method pioneered by Jay (1981) was shown to be useful in relating endotoxin concentration to the AMC of minced beef and minced pork although care must be exercised since the theoretical values were compared with the values of the experiment from which the model was developed. However, the results are sufficiently promising to warrant further experiment to validate the method.

Acknowledgments

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Investigation into the use of a carbocyanine dye for the rapid detection of lipopolysaccharide associated with the low temperature spoilage of meat

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Summary

The possibility for the use of a carbocyanine dye assay for the detection of lipopolysaccharide (LPS) associated with low temperature bacterial spoilage of meat was evaluated. The assay was only capable of detecting *Pseudomonas* spp. when the viable count exceeded 10^8 colony of forming units (CFU) per ml or per g. Pre-treatment of cell suspensions with EDTA, dimethylsulphoxide or by sonication with the aim of releasing LPS failed to increase the sensitivity of the assay. It was hence concluded that the carbocyanine dye assay is of little use for the detection of incipient meat spoilage.

Introduction

The spectral shift of the carbocyanine dye 1-ethyl-2-(3-(1-ethyl naphtho (1,2d) thiazolin-2-ylidene)-2-methyl propenyl) naphtho (1,2d) thiazolium bromide brought about by anionic polymers was first described by Kay, Walwick & Gifford (1964). They concluded that the spectral shift in the presence of polyanions was due to increased aggregation of the dye monomers forming new molecular species with new absorbance maxima. The reaction was subsequently shown to occur also in the presence of acidic polysaccharides by Edstrom (1969), the wavelength of the absorbance maxima increasing from 510 to 600–650 nm. New absorbance maxima for the dye (430–485 nm) in the presence of endotoxins and long chain aliphatic sulphates were determined by Janda (1971). A technique for the colorimetric estimation of lipopolysaccharide (LPS) present in cultures and culture filtrates of *Escherichia coli* was reported by Janda & Work (1971). Clinical studies were carried out by Gray & Miller (1978) in attempts to detect LPS in suspected cases of bacteriuric urine using the spectral shift of the carbocyanine dye. The method was found to be as sensitive as the *Limulus* lysate assay and capable of yielding a positive reaction to 10^3 bacteria/ml urine. More recently McMeekin (1982) suggested that the dye reaction might similarly be of use in the detection of incipient meat spoilage. Since *Pseudomonas* spp. are predominant organisms during low temperature spoilage (McMeekin, 1982) work was undertaken to determine the sensitivity of the carbocyanine dye to these organisms and to assess the value of the dye as a rapid non-cultural method for the determination of incipient spoilage of meat.

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Materials and methods

Bacterial cultures

The organisms used in this study were *Pseudomonas fragi* (ATCC 4973), *Pseudomonas aeruginosa* (ATCC 10145), *Pseudomonas putida* (NCIB 9494) and *Pseudomonas fluorescens* (NCIB 9046). The bacteria were grown in nutrient broth media (Oxoid Ltd.) reconstituted with pyrogen-free water (PFW) (Ivex Ltd., Larne, Co. Antrim). The cultures were incubated for 48 hr at 22°C except for *P. aeruginosa* which was incubated at 37°C.

Preparation of bacterial cells for the carbocyanine dye assay

All glassware used in the assay procedure was rendered pyrogen free by heating to 140°C for 4 hr. Preliminary experiments showed that the nutrient broth contained polyanions which caused a spectral shift of the dye to longer wavelengths. Bacterial cells were therefore centrifuged, washed three times with, and resuspended in, sterile PFW. In an attempt to improve the sensitivity of the dye assay by destruction of cell integrity, bacterial cells were also resuspended in Tris/HCl buffer (0.1 M, pH 8.0) containing 0.25 mM EDTA and incubated at 20°C for 1 and 2 hr prior to dye assay. Washed bacterial cells suspended in PFW were also heated in a boiling water bath for 5, 10 and 30 min prior to performing the dye assay. In addition washed PFW cell suspensions were exposed to ultra sound (sonicated) at 40 μA for 2 min. Serial ten-fold and doubling dilutions of the various bacterial cell suspensions were prepared in PFW. However, cells to be treated with dimethylsulphoxide (DMSO 5 and 10% in PFW), to improve dye permeability, were suspended in PFW and serially diluted with the respective concentrations of DMSO. Viable bacterial cells in the initial suspensions were enumerated following ten-fold serial dilution in peptone saline on plate count agar after incubation at 22°C for 3 days, except for plate counts of *P. aeruginosa* which were incubated at 37°C for 2 days.

Preparation of meat samples

Flank of beef was obtained from a local meat plant 24 hr after slaughter. The surface of the meat was scraped clean and diced using a sterile knife. The diced pieces were passed twice through a sterile mincer. 250 g amounts were packed into plastic trays, overwrapped with oxygen permeable plastic film and stored at 4°C. Individual packs were removed from storage daily for analysis, as described below.

A 10 g meat sample was homogenized in 90 ml of sterile peptone saline diluent using a Colworth Stomacher (2.5 min). Homogenates were serially diluted in sterile peptone saline and the aerobic mesophilic count (AMC) obtained on plate count agar (PCA) following incubation at 22°C for 3 days. A presumptive *Pseudomonas* count was performed using the cetrimide-fucidin-cephaloridine (CFC) agar of Mead & Adams (1977). A further 10 g of meat was homogenized, centrifuged (2000 **g**, 10 sec), and diluted in PFW and used for subsequent LPS assay using the carbocyanine dye.

Carbocyanine dye assay procedure

A 3.6×10^{-4} M solution of the carbocyanine dye 1-ethyl-2-(3-(1-ethyl naphtho (1,2d) thiazolin-2-ylidene)-2-methyl propenyl) naphtho (1,2d) thiazolium bromide (Eastman Kodak Ltd.) was freshly prepared before each assay by dissolving 10 mg in 50 ml ethanol AR. The dye solution was maintained in a dark bottle on ice throughout the assay procedure. 0.8 ml of sample or standard was pipetted into a polystyrene micro-

cuvette (2.9 ml, Ultra-Vu Ltd.) to which was added 0.1 ml acetate buffer (0.2 M, pH 4.0) and 0.1 ml carbocyanine dye solution. The samples were mixed and allowed to stand in the dark for 5 min. The absorbance spectra were recorded using a Pye Unicam SP 8000 scanning spectrophotometer. The absorbance at 515 and 472 nm of samples/standards were measured routinely using a Pye Unicam SP 6500 spectrophotometer. Samples and standards were read against blanks containing no dye reagent but with 0.1 ml ethanol to account for non-specific absorbance.

LPS assay using Limulus *lysate*

The assay of LPS using a *Limulus* lysate microtitre plate assay was used as previously described (Fallowfield & Patterson, 1985).

Organoleptic assessment

A six member panel, familiar with meat products, was used to assess the acceptability of the stored minced beef. The panel was requested to determine whether the meat was acceptable or unacceptable using appearance and odour as criteria.

Results

The spectral shift of the dye from 515 nm in the absence of LPS to 472 nm in the presence of LPS derived from PFW washed cells of *P. putida* (1.19×10^9/ml) is shown in Fig. 1. The relationship between the cell count of *P. fragi, P. putida, P. aeruginosa* and *P. fluorescens* in PFW and the absorbance of the carbocyanine dye at 472 nm is shown in Fig. 2. The absorbance at 472 nm increased only when the viable count rose above 1×10^8 CFU/ml. The sensitivity of the dye was, however, strain dependent. Similar viable counts of *P. fragi* and *P. aeruginosa* (8.0×10^8 CFU/ml) gave absorbances (OD_{472}) of 0.5 and 0.35 respectively. Overall the results of the experiment suggested that the carbocyanine dye was only sensitive to viable counts in excess of 10^8/ml.

Centrifuging bacterial suspensions which had been reacted with dye showed that the dye was bound to the bacterial cells with a negligible amount reacting with 'free' LPS

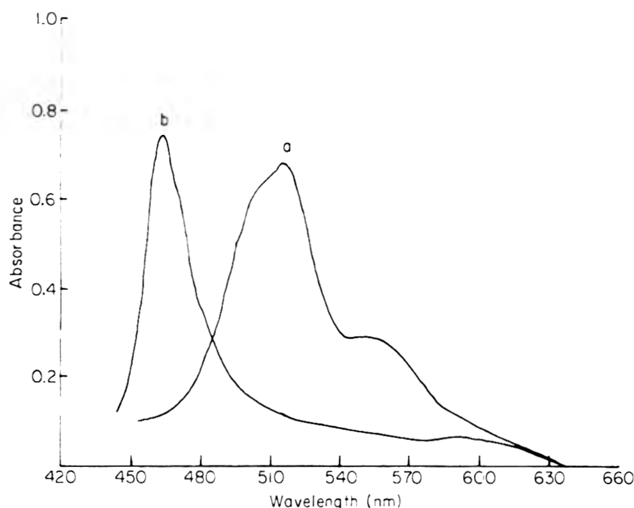

Figure 1. The absorbance maxima of the carbocyanine dye (a) in ethanol and (b) in the presence of lipopolysaccharide from cells of *Pseudomonas putida*.

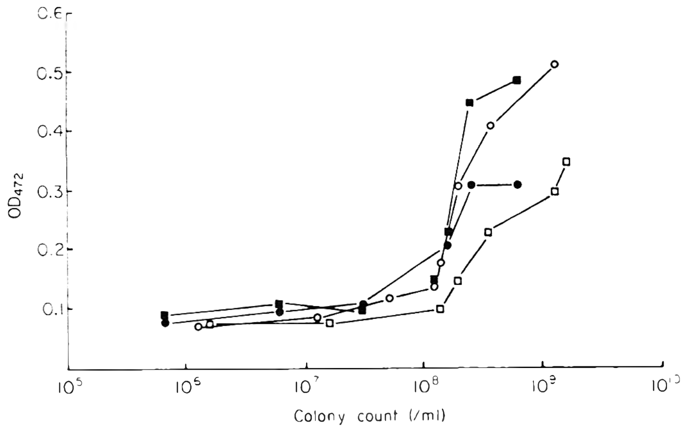

Figure 2. The relationship between OD_{472} of the carbocyanine dye and washed cell suspensions of *Pseudomonas aeruginosa* (■), *Pseudomonas fluorescens* (□), *Pseudomonas fragi* (●), and *Pseudomonas putida* (○).

within the supernatant liquid. Two approaches were investigated in an attempt to improve the sensitivity of the dye to LPS from *Pseudomonas* spp. Firstly, DMSO treated cells were assayed to determine whether pre-treatment improved the permeability of the cells to the carbocyanine dye. Secondly, cells were disrupted by treating with 0.25 mM EDTA in Tris/HCl buffer and by sonication to make more LPS available for dye reaction.

The results of two experiments where cells of *P. fragi* and *P. putida* were boiled and pretreated with DMSO are shown in Table 1. The only enhancement of dye sensitivity recorded was when cells of *P. putida* (3.5×10^7 CFU/ml) were boiled for 10 min. In general these treatments did not increase the sensitivity of the carbocyanine dye to *Pseudomonas* LPS. Similarly, sonication for 2 min also failed to increase the sensitivity of the dye to cells of *P. putida* (Fig. 3).

Table 1. The effect on the sensitivity of the carbocyanine dye reaction of boiling and pre-treatment of *Pseudomonas fragi* and *Pseudomonas putida* with 5 and 10% DMSO*

Treatment	*P. fragi* OD_{472}		*P. putida* OD_{472}	
Boiled cells:				
Washed PFW cell suspension	0.098	0.152	0.086	0.102
PFW cells boiled (min)				
5	0.100	0.143	0.068	0.078
10	0.090	0.141	0.119	0.124
30	0.098	0.128	0.088	0.102
Initial viable count (per ml)	8.0×10^6	8.0×10^7	3.6×10^6	3.5×10^7
DMSO treated cells:				
Washed PFW cell suspension	0.058	0.081	0.053	0.057
DMSO 5%	0.059	0.079	0.058	0.044
10%	0.057	0.057	0.047	0.057
Initial viable count (per ml)	9.0×10^6	9.0×10^7	4.2×10^6	4.2×10^7

*Dimethylsulphoxide.

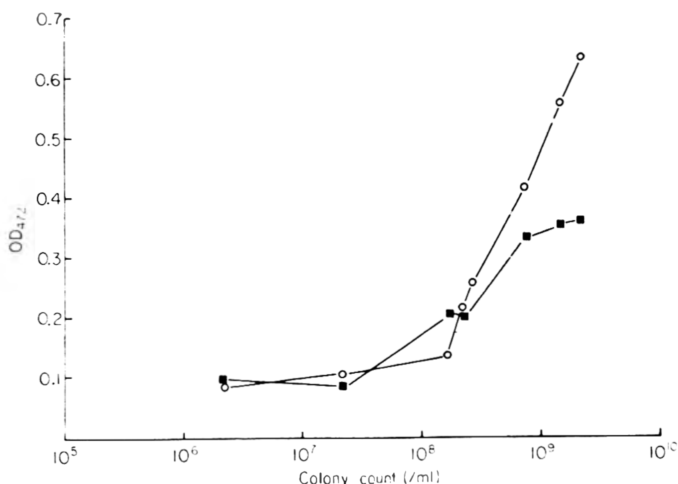

Figure 3. The effect of sonication (40 μA, 2 min) on the sensitivity of the carbocyanine dye to cells of *Pseudomonas putida*: cells washed and suspended in pyrogen free water (o), cells sonicated in pyrogen free water (■).

The effect of pre-treatment with 0.25 mM EDTA Tris/HCl buffer (0.1 M, pH 8.0) for 1 and 2 hr at 20°C on the reaction of the dye to *P. fragi* together with the *Limulus* lysate determined endotoxin/LPS titre is shown in Fig. 4. Pre-treatment with EDTA reduced the sensitivity of the dye to cells of *P. fragi* compared with the washed PFW cell suspension. The endotoxin/LPS concentration, determined using *Limulus* lysate, was apparently linear from 25.0 μg/ml (7.9×10^8 CFU/ml) to 0.25 μg/ml (7.9×10^6 CFU/ml). However, a positive *Limulus* titre equivalent to an LPS concentration of 2.5 ng/ml was also recorded at a *P. fragi* viable count of 7.9×10^4 ml.

Reaction of the carbocyanine dye with dilutions of *E. coli* 026:B6 LPS (Difco) in PFW showed that the dye response was only linear over the range 0.5–6.0 μg LPS/ml (Fig. 5). Inspection of Figs 4 and 5 shows that an OD_{472} of 0.2, associated with about 10^8 *Pseudomonas* CFU/ml, was equivalent to an endotoxin/LPS concentration of approxi-

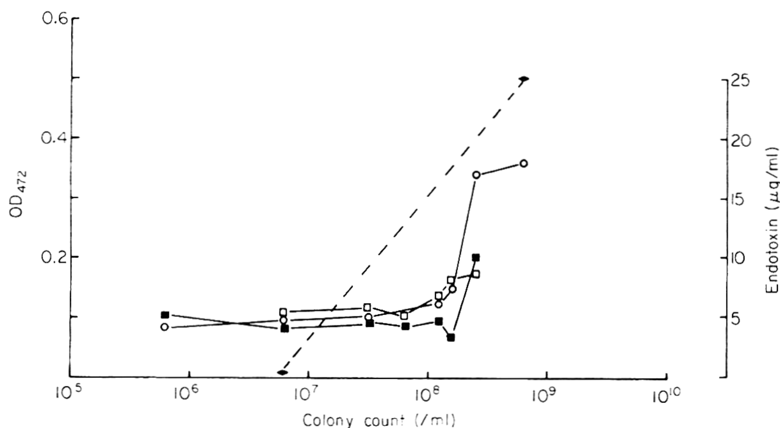

Figure 4. The effect of pre-treatment with 0.25 mM EDTA/Tris/HCl (0.1 M, pH 8.0) on the sensitivity of the carbocyanine dye to washed cells of *Pseudomonas fragi*: pre-treated at 20°C for 1 hr (■), 2 hr (□), pyrogen free water cell suspension (o) and the *Limulus* lysate determined endotoxin concentration of the pyrogen free water cell suspension (◆)

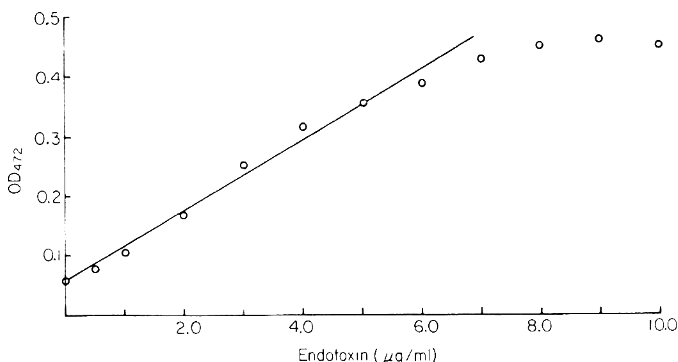

Figure 5. The relationship between the absorbance at 472 nm of the carbocyanine dye in the presence of various concentrations of endotoxin from *E. coli* (026:B6).

mately 2.4 μg/ml, assuming that there was little difference in the relative sensitivity of the dye to *E. coli* and *Pseudomonas* LPS. However, the *Limulus* lysate assay, at the same colony count, gave an endotoxin concentration of about 15 μg/ml (Fig. 4).

Minced beef was produced with a low initial AMC of 7.9×10^5/g. A positive dye reaction (OD_{472} = 0.22) was only recorded at an AMC of 4.75×10^8/g and when the presumptive *Pseudomonas* population had reached 2.5×10^8 CFU/g. This occurred after 5 days storage at 4°C when the meat was still deemed organoleptically acceptable, although after a further day's storage the meat was unacceptable using the same criteria. Similar to the findings for the pure culture work, differences in the endotoxin/LPS concentration determined by the dye and by the *Limulus* lysate assay procedures were evident. After storage for 5 days the endotoxin content of the meat determined using the *Limulus* assay was estimated as 250 μg/g compared with a carbocyanine dye determination of 3.0 μg/g. These results taken together suggest that the carbocyanine dye assay severely underestimated the endotoxin/LPS concentration of samples tested.

Discussion

The sensitivity of the carbocyanine dye 1-ethyl-2-(3-ethyl(1-ethyl naphtho (1,2d) thiazolin-2-ylidene)-2-methyl propenyl) naphtho (1,2d) thiazolium bromide to washed cell suspensions of *Pseudomonas* spp. was investigated. The dye assay was found only to be sensitive to cell concentrations in excess of 10^8 ml. The dye was found to be associated with the pellet after centrifuging dye-reacted cell suspensions. This indicated that the dye was bound to the bacterial cell surface. Results of work by Janda (1971) and Janda & Work (1971) showed that the dye was more sensitive to 'free' LPS most likely to be present in culture filtrates. Pre-treatments likely to affect cell integrity, EDTA/tris/HCl, boiling or sonication, together with those which might increase cell permeability (DMSO), all failed to increase the sensitivity of the carbocyanine dye to the species of *Pseudomonas* tested. Pre-treatment with other solubilizing agents e.g. Triton X, Soluene and NCS were abandoned when they were found to cause major spectral shifts of the dye to longer wavelengths.

Similar to the findings for the pure culture studies, minced beef produced with a low initial aerobic mesophilic count only recorded a positive dye reaction when the aerobic mesophilic count and the presumptive pseudomonad count exceeded 10^8 g. Furthermore, the endotoxin content determined by the dye reaction was almost 100 times lower

than that determined by the *Limulus* lysate assay. These results suggest that the carbocyanine dye assay severely underestimates the endotoxin concentration and that it is therefore of little use as a rapid method for the detection of lower numbers of bacterial cells in meat.

Conclusions

The inability of the dye to detect LPS at viable counts below 10^8 CFU/g or /ml suggests that the dye is of little use for the detection of incipient spoilage of meat.

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Functional aspects of blood plasma proteins 4. Elucidation of the mechanism of gelation of plasma and egg albumen proteins

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Summary

The mechanism of gelation of plasma and egg albumen proteins in a cake-type model system was elucidated by chemical modification, polyacrylamide gel electrophoresis and electron microscopy. It was demonstrated by chemical modification that gelation via disulphide bonds was predominant in all the plasma and egg albumen proteins. Hydrophobic and hydrogen bonds also prevailed in the plasma protein gels. In contrast the breakdown of hydrophobic and hydrogen bonds increased the strength of egg albumen gels. Electrophoretic characterization confirmed that most of the component proteins of plasma and egg albumen were modified chemically. Electron micrographs of the gels of plasma, egg albumen and a mixture of these proteins revealed a generally similar type of network structure, and indicated the compatibility of these proteins. The plasma gels had a more dense network, however, than the egg albumen gels and this was reflected in their correspondingly greater gel strength.

Introduction

The gelation of blood plasma proteins and the interaction of these with other proteins such as egg albumen and whey in a cake-type model system has been reported by Howell & Lawrie (1984a,b). It is generally believed that the gelling of proteins occurs as a result of the unfolding of the polypeptide chains on heating and the exposure of the reactive groups which then link up to give a three dimensional network of molecules. This association involves disulphide, hydrogen, hydrophobic and ionic bonds (Joly, 1965; Brandts, 1967).

Only a limited amount of information exists on the mechanism of gelation of the proteins of plasma and egg albumen (Tombs, 1970, 1974; Hospelhorn & Jensen, 1954; Tanford, 1980; Hegg & Lofquist, 1974).

It was considered that chemical reagents which block the formation of these bonds would provide the means of testing which of the bonds are involved in gel formation and in protein-protein interactions. Thus, for example cysteine hydrochloride reduces disulphide bonds, urea breaks hydrogen bonds between the NH and CO groups and sodium dodecyl sulphate (SDS) breaks hydrophobic bonds.

The effect of these chemicals on the gelation of the proteins of plasma, serum and porcine plasma fractions I, II and III (Howell & Lawrie, 1983) and egg albumen was

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examined by measuring the changes in the gel strength and breaking strength of gels before and after chemical treatment.

The chemically modified proteins were characterized by polyacrylamide gel electrophoresis to ascertain which of the component proteins were modified. Furthermore, other types of reducing agents, namely, sodium nitrite, sodium sulphite, ascorbic acid and the protease enzyme, trypsin, were selected to study the gelling behaviour of commercial plasma and egg albumen proteins. A brief study of porcine plasma and egg albumen proteins by electron microscopy was also undertaken in the hope of further elucidating the mechanism of gelation.

Materials and methods

Proteins. Those used were as follows: commercial porcine plasma, Regalbumin (Regal Food Ltd., Dublin); porcine serum (Howell & Lawrie, 1983); porcine plasma ion exchange fractions I, II and III to be referred to as Fr I, Fr II and Fr III (Howell & Lawrie, 1983); and spray-dried egg albumen.

Chemicals. These were from various sources: cysteine hydrochloride; urea; sodium dodecyl sulphate (SDS), sodium nitrite; sodium sulphite; β-mercaptoethanol; ascorbic acid and trypsin.

Chemical and enzymic modification and gelation

Each of the chemical reagent, (namely 1% (w/w) cysteine hydrochloride, 4 M urea and 3% (w/w) SDS) was added to the proteins in 45% (w/w) sucrose solutions. In these, the concentrations of commercial porcine plasma, porcine serum, porcine plasma fractions, or egg albumen was 6% (w/w). The mixture of plasma protein and egg albumen contained 3% (w/w) of each. The protein solutions containing the reagent were kept at 20°C for 24 hr to enable maximum modification of the proteins prior to gelation at 95°C for 20 min in tubes according to the method by Howell (1981). The gels were tested on the Instron Tester as described previously (Howell & Lawrie, 1983). Control experiments using protein solutions without the reagents were also performed. Eight replicates were used for each test. Changes in the gel strength and breaking strength brought about the reagent were expressed as an interaction index i.e.

$$\frac{\text{Value without the reagent} - \text{Value with the reagent}}{\text{Value without the reagent}} \times 100.$$

The effect of other types of reducing agents: namely, 1% (w/w) sodium nitrite, 1% (w/w) sodium sulphite, 1% (w/v) β-mercaptoethanol, 1% (w/w) ascorbic acid and 1% (w/w) trypsin which breaks peptide bonds, on the gelling behaviour of 6% (w/w) commercial plasma, 6% (w/w) egg albumen proteins and a mixture of the two proteins (3% + 3% w/w) all in 45% sucrose solutions, was also investigated.

Characterization of porcine plasma and egg albumen proteins by polyacrylamide gel electrophoresis

The native proteins and modified proteins of porcine plasma and egg albumen were characterized by polyacrylamide gel electrophoresis on 7% polyacrylamide gel rods as described by Howell & Lawrie (1983).

Electron microscopy of porcine plasma and egg albumen protein gels

Gels were prepared from either 6% (w/w) porcine plasma. 6% (w/w) egg albumen or mixtures of 3% (w/w) porcine plasma and 3% (w/w) egg albumen which had been heated at either 85 or 95°C for 15 min. These were examined by electron microscopy.

Small pieces (approximately 1 mm^3) of the gel were fixed in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer for 1 hr. The fixed gel pieces were washed in distilled water and then dehydrated using ethanol, starting at 70% and finishing with absolute ethanol dried over sodium sulphate. Samples were infiltrated with freshly prepared Transmit resin overnight, embedded in Transmit and cured at 60°C.

Sections (approximately 100 nm) were cut on a Cambridge Huxley Mk I microtome and collected on copper grids. They were then stained with 4% (w/v) uranyl acetate at 60°C for 30 min followed by 0.2% (w/v) lead citrate in 0.5% (v/v) sodium hydroxide for 2 min. The stained sections were washed, dried and viewed in an Hitachi HS 75 transmission electron microscope operating at 50 kV and photographed using a magnification of ×9000.

Results

Cysteine hydrochloride (1% w/w), 4 M urea and 3% (w/w) SDS, all reduced the gel strength (GS) of porcine plasma and porcine plasma/egg albumen protein gels (Table

Table 1. Gel strength of chemically modified porcine plasma and egg proteins heated at 95°C for 20 min

	Gel strength of additive (g)			
6% (w/w) Protein in 45% sucrose solution	None	1% Cysteine hydrochloride	4 M urea	3% SDS
Egg albumen (E)	210	64 (−69)	288 (+84)	348 (+66)
Porcine plasma (P)	707	402 (−43)	460 (−35)	318 (−55)
P+E (3%+3%)	582	352 (−39)	453 (−22)	530 (−9)
Porcine serum (S)	448	690 (+54)	337 (−24)	No gel
S+E (3%+3%)	525	483 (−8)	480 (−8)	Very weak gel
Porcine Fr I	572	Broke at 4 mm	477 (−16)	No gel
Fr I+E (3%+3%)	390	350 (−10)	480 (+23)	No gel
Porcine Fr II	240	310 (+29)	500 (+108)	No gel
Fr II+E (3%+3%)	375	503 (+34)	905 (+141)	No gel
Porcine Fr III	545	325 (−40)	Broke at 4 mm	No gel
Fr III+E (3%+3%)	256	253 (−1)	550 (+114)	Broke at 4 mm

Figures in brackets refer to percent change in strength compared to the control.

Table 2. Breaking strength of chemically modified porcine plasma and egg proteins heated at 95°C for 20 min

6% (w/w) Protein in 45% sucrose solution	Breaking strength of additive (g)			
	None	1% (w/w) Cysteine hydrochloride	4 M urea	3% (w/w) SDS
Egg albumen (E)	222	64 (−71)	1024 (+361)	496 (+123)
Porcine plasma (P)	1850	421 (−77)	3083 Break not clear	341 (−81)
P+E (3%+3%)	1393	352 (−74)	2450 (+75)	1590 (+14)
Porcine serum (S)	920	963 (+4)	920 (0)	No gel
S+E (3%+3%)	1140	483 (−57)	1200 (+5)	Very weak gel
Porcine Fr I	899	292 (−67)	1929 Break not clear	No gel
Fr I (3%+3%)	997	337 (−66)	1277 Break not clear	No gel
Porcine Fr II	240	385 (+60)	400 (+66)	No gel
Fr II+E (3%+3%)	875	573 (−34)	1340 (+53)	No gel
Porcine Fr III	622	380 (−39)	165 (−73)	No gel
Fr III+E (3%+3%)	1006	253 (−76)	1320 (+22)	216 (−80)

Figures in brackets refer to percent change in strength compared to the control.

1). The breaking strength (BS) of the gels was also reduced, except with 4 M urea (Table 2). With 4 M urea, the gel was very sticky which resulted in compression into a flat disc rather than the production of a clean break point. In contrast the GS and BS of egg albumen proteins increased with the addition of 4 M urea and 3% (w/w) SDS, but decreased with 1% (w/w) cysteine hydrochloride ($P < 0.005$) (Tables 1 and 2).

Cysteine hydrochloride (1% w/w) increased the GS of 6% (w/w) porcine serum gels but did not affect the BS (Table 2). Urea (4 M) changed the GS and BS of serum and serum/egg albumen protein gels only slightly. But 3% (w/w) SDS prevented the gel formation of serum and mixtures of serum and egg albumen proteins. SDS also prevented the gel formation of fractions I, II and III and Fr I/egg albumen and Fr II/egg albumen proteins (Tables 1 and 2). Cysteine hydrochloride (1% w/w) reduced the GS and BS of gels prepared from Fr I, Fr I/egg albumen, Fr III and Fr III/egg albumen proteins ($P < 0.005$) (Tables 1 and 2). In contrast the GS and BS of Fr II gels were slightly increased by 1% cysteine hydrochloride. The GS of Fr II/egg albumen mixture was increased but the BS reduced (Tables 1 and 2).

4 M urea reduced slightly the GS of Fr I and Fr III gels. Gels of Fr II and mixtures of all fractions with egg albumen proteins increased in GS. Sticky gels were produced with 4 M urea making the breaking strength difficult to determine (Table 1). With the exception of 1% sodium sulphite and 1% (v/w) β-mercaptoethanol+4 M urea the GS and BS of egg albumen gels increased in the presence of 1% (w/w) sodium nitrite, 1%

Table 3. Gel strength of chemically modified porcine plasma and egg albumen protein gels

6% (w/w) Protein in 45% (w/w) sucrose solution	Gel strength of additive (g)						
	No additive	1% (w/w) Sodium sulphite	1% (w/w) Sodium sulphite +4 M urea	1% (v/w) β-mercaptoethanol +4 M urea	1% (w/w) Sodium nitrite	1% (w/w) Ascorbic acid	1% (w/w) Trypsin
Egg albumen (E)	210	204	248	105	398	401	300
% change		(−3)	(+18)	(−49)	(+89)	(+90)	(+43)
Porcine plasma (P)	707	785	386	127	657	171	53
% change		(+11)	(−45)	(−82)	(−7)	(−76)	(−92)
P+E (3%+3%)	582	628	512	225	600	218	325
% change		(+8)	(−12)	(−61)	(+3)	(−62)	(−44)

Figures in brackets refer to percent change in strength compared to the control.

Table 4. Breaking strength (g) of chemically modified porcine plasma and egg albumen protein gels

6% (w/w) Protein in 45% (w/w) sucrose solution	Breaking strength of additive (g)						
	No additive	1% (w/w) Sodium sulphite	1%(w/w) Sodium sulphite +4 M urea	1% (v/w) β-mercaptoethanol +4 M urea	1% (w/w) Sodium nitrite	1% (w/w) Ascorbic acid	1% (w/w) Trypsin
Egg albumen (E)	222	204	273	106	720	491	420
% change		(−8)	(+23)	(−52)	(+224)	(+121)	(+89)
Porcine plasma (P)	1850	2121	(3243)	127	1510	171	53
% change		(+10)	(+75)	(−93)	(−18)	(−91)	(−97)
P+E (3%+3%)	1393	886	856	225	1243	218	328
% change		(−38)	(−38)	(−83)	(−5)	(−84)	(−77)

Figures in brackets refer to percent change in strength compared to the control.

(w/w) ascorbic acid and 1% (w/w) trypsin (Tables 3 and 4). All these reagents reduced the GS and BS of procine plasma and procine plasma/egg albumen gels. However, sodium sulphite 1% (w/w) and 4 M urea increased BS of plasma protein gels.

Densitograms of the electrophoretic characterization of plasma and egg albumen proteins are shown in Fig. 1. Electrophoretic characterization of chemically modified egg proteins shows that conalbumin is the main peak which was reduced by the addition of all the chemical reagents (Table 5). The high molecular weight (MW) peak (peak 1) increased presumably due to the increase in denatured protein. The presence of 4 M urea, 1% (w/w) cysteine hydrochloride, 1% (v/w) β-mercaptoethanol and 1% (w/w) sodium sulphite+4 M urea reduced the conalbumin peak by over 60%. With these reagents the globulin peak 3 was also reduced. β-Mercaptoethanol 1% (v/w) reduced all the peaks. However 1% (w/w) cysteine hydrochloride and 1% (w/w) sodium sulphite increased peaks 3–6. Ovalbumin was affected to a small extent with the addition of 1% (v/w) β-mercaptoethanol, 1% (w/w) ascorbic acid and 1% (w/w) trypsin.

In Table 6, the effect of these chemicals on the porcine plasma proteins is given. It is evident that, although all the reagents reduced most of the peak heights by about

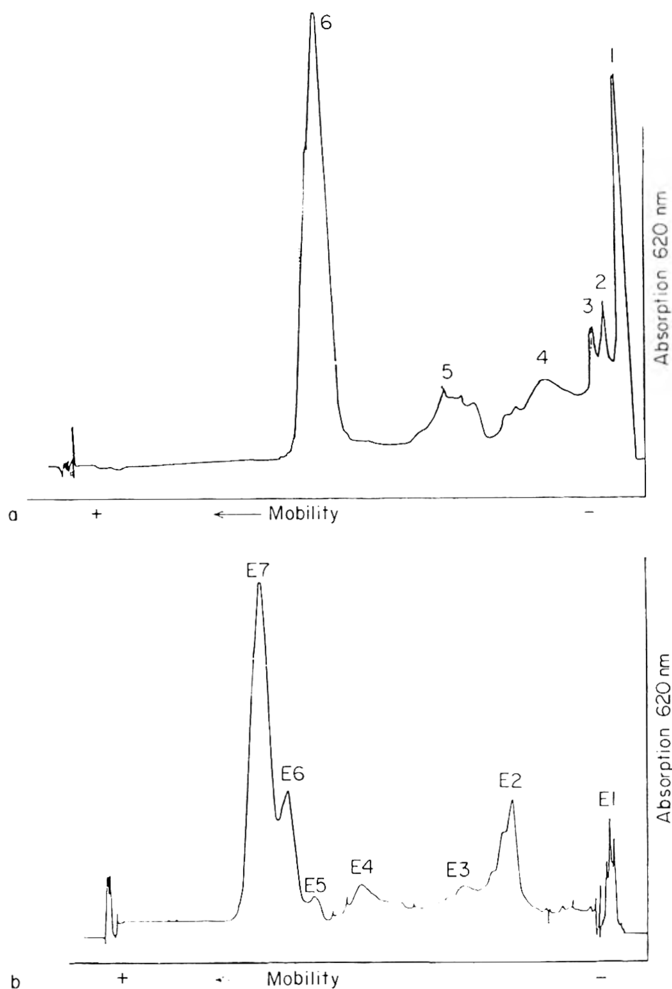

Figure 1. Densitogram of the electrophoretic separation of (a) 3% (w/w) porcine plasma proteins; and (b) 6% (w/w) egg albumen proteins.

5–20%, 1% (v/w) β-mercaptoethanol, and 4 M urea+1% (w/w) sodium sulphite and 1% (w/w) trypsin reduced the heights of peaks 1–6, by about 40–100%.

Table 7 indicates that 4 M urea, 3% (w/w) SDS and 1% (w/w) cysteine hydrochloride reduced the peak heights of most of the globulins in Fr I. The high MW peak (No. 1) was higher than that in the unmodified Fr I due to the aggregation of denatured protein. Fraction II was affected mainly by 3% (w/w) SDS and a little by 1% (w/w) cysteine hydrochloride; but not very much by 4 M urea except for peak 5 (Table 8). The peak height of albumin, the main protein in fraction III, was reduced by 3% (w/w) SDS and to a lesser extent by 1% (w/w) cysteine hydrochloride and 4 M urea (Table 9). The serum protein peak heights were reduced by all three reagents. Peak 7 in particular was changed by 3% (w/w) SDS and peaks 2 and 7 by 1% (w/w) cysteine hydrochloride (Table 10).

Table 5. Electrophoretic characterization of chemically modified egg albumen proteins

Chemical added to 6% (w/w) egg albumen in 45% (w/w) sucrose solution	Peak height (mm) of peak no. High MW protein	Conalbumin	Globulins			Ovalbumin	
	E1*	E2*	E3*	E4*	E5*	E6*	E7*
No additive	57	45	15	14	8	45	128
s.d. ±	8.0	3.2	1.0	1.0	2.0	7.0	14
4 M Urea	98	17	7	12	8	48	135
s.d. ±	12.0	0.5	0.5	0	0.8	1.5	4.0
% change	+72.2	−61.1	−50.0	−14.2	+5.2	−8.2	+7.0
3% (w/w) SDS	72	33	14	12	7	45	120
s.d. ±	9.8	4.0	1.1	1.5	2.0	8.1	11.0
% change	+26.3	−26.6	−6.6	−10.7	0	0	−6.2
1% (w/w) Cysteine hydrochloride	83	62	18	187	112	48.7	127
s.d±	3.9	0.5	5.0	2.1	3.0	2.5	8
% change	+45.6	−86.2	+20.0	+25.1	+47.3	+8.2	−0.8
1% (v/w) β-mercaptoethanol	65	1	4	9	7	40	118
s.d. ±	12.0	0.9	1.5	1.4	1.8	8.2	6.6
% change	+14.0	−97.3	−72.0	−90.0	−5.2	−11.1	−7.8
1% (w/w) Sodium sulphite	10	2	18	33	20	85	140
s.d. ±	1.4	0.5	3.0	7.0	2.1	5.5	10.5
% change	+78.9	−48.8	+20.0	+137	+163.1	+88.8	+13
1% Sodium sulphite + 4 M urea	108	3	5	14	8	40	122
s.d. ±	19.0	1.5	0	4.0	2.0	11.1	12.2
% change	+89.4	−94.0	−66.6	0	+5.2	−11.1	−4.7
1% (w/w) Sodium nitrite	89	40	13	13	5	41	114
s.d. ±	10.6	7.4	1.1	1.3	0.8	2.8	7.1
% change	+56.1	−10.0	−13.3	−8.5	−27.6	−8.8	−10.9
1% (w/w) Ascorbic acid	85	30	12	12	7	36	103
s.d. ±	5.0	4.9	0	0	0	3.3	3.5
% change	+49.1	−33.3	−20.0	−14.2	−7.8	−19.5	−19.5
1% (w/w) Trypsin	41	42	11	10	6	40	111
s.d. ±	8.6	6.4	1.7	0.5	0.5	3.4	6.2
% change	−27.1	−5.5	−28.6	−30.7	−14.4	−11.1	−13.1

* Peak number.
Figures refer to mean values based on eight replicates.
s.d., Standard deviation.
Compare Fig. 1b.

Table 6. Electrophoretic characterization of chemically modified commercial porcine plasma proteins

Chemical added to 6% (w/w) porcine plasma in 45% (w/w) sucrose solution	Peak height (mm) of peak no.					
	High MW protein	α-, β-, γ-globulins				Albumin
	1	2	3	4	5	6
No additive	134	51	50	28	20	152
s.d. ±	13.0	11.1	9.1	6.2	2.0	3.0
4 M Urea	118	72	45	24	20	134
s.d. ±	11.2	6.1	2.0	2.0	2.0	11.3
% change	−11.9	−26.3	−10	−14.2	0	−1.5
3% (w/w) SDS	141	55	48	31	22	12
s.d. ±	9.2	11.3	8.2	6.1	4.0	7.1
% change	−5.2	−0.35	−4.0	+10.7	+10	−9.0
1% (w/w) Cysteine hydrochloride	136	48	49	31	20	136
s.d. ±	2.6	9.3	2.5	6.3	0.4	4.1
% change	+1.4	−5.7	−2.0	−10.7	0	3.0
1% (v/w) β-mercaptoethanol	82	16	15	2	2	3
s.d. ±	12.0	2.1	1.0	0.1	0.5	1.2
% change	−38.4	−71.7	−70.0	−92.8	−90.0	−98.4
4 M urea + 1% (w/w) sodium sulphite	> 140	17	16	9	8	26
s.d. ±		0.9	1.4	0.4	2.0	6.2
% change		−70.1	−68.0	−67.1	−60.0	−79.9
1% (w/w) Sodium nitrite	136	61	50	33	23	130
s.d. ±	14.0	6.9	4.7	3.5	2.6	5.7
% change	+1.5	+7.0	0	+15.1	+15.2	−15.0
1% (w/w) Ascorbic acid	123	42	40	19	14	128
s.d. ±	16.0	5.3	4.0	1.0	6.5	8.1
% change	−8.2	−26.3	−20.0	−32.0	−30.0	−3.0
1% (w/w) Trypsin	20	13	13	13	106	55
s.d. ±	1.0	0.5	0.8	0.8	0.8	0.9
% change	−84.7	−77.2	−74.0	−53.5	−70.0	−58.3

Figures refer to mean values based in five replicates.
s.d., Standard deviation.
Compare Fig. 1a.

Table 7. Electrophoretic characterization of chemically modified porcine plasma Fraction I proteins

Chemical added to 6% (w/w) Fr I in 45% sucrose solution	Peak height (mm) of peak no.								
	1	2	3	4	5	6	7	8	9
No additive	66.6	96.2	60.0	55.0	50.0	15.0	14.7	8.5	40.0
s.d. ±	7.6	11.0	12.0	9.4	11.4	4.0	3.7	2.5	6.5
4 M urea	90.2	53.0	37.0	42.0	34.7	12.0	5.5	5.5	36
s.d. ±	4.0	9.0	4.9	9.0	6.9	2.5	0.5	0.5	3.0
% change	+36.6	−44.9	−38.3	−23.6	−30.6	−20	−62.5	−94.1	−10.0
3% (w/w) SDS	108.0	81.7	56.0	34.0	37.0	17.0	9.0	52.0	36.5
s.d. ±	9.0	7.0	2.0	1.1	1.9	0.5	0.8	0.5	2.3
% change	+63.6	−15.0	−6.6	−38.1	−26	+13.3	−38.7	−38.8	+29.2
1% (w/w) Cysteine hydrochloride	64.5	69.0	51.0	55.0	50.0	19.0	11.0	9.0	47.0
s.d. ±	4.2	7.1	3.3	2.0	1.7	1.4	1.1	2.0	0.5
% change	−2.2	−28.2	−15.0	0	0	+2.6	−25.1	−5.5	+17.5

Figures refer to mean values based on five replicates.
s.d., Standard deviation.
Compare Howell & Lawrie, 1983: Fig. 5a.

Table 8. Electrophoretic characterization of chemically modified porcine plasma Fraction II proteins

Chemical added to 6% Fr II	Peak height (mm) of peak no.							
	1	2	3	4	5	6	7	8
No additive	52.0	18.5	18.0	18.0	35.0	6.7	7.0	125.0
s.d. ±	2.5	0.5	1.5	1.1	4.0	2.8	2.0	1.5
4 M urea	52.0	18.0	17.0	18.0	28.0	6.2	7.5	125
s.d. ±	0.9	2.6	0.9	0.9	2.7	0.5	0.5	2.5
% change	0	−2.7	−5.5	0	−20.0	−7.4	+7.1	0
3% (w/w) SDS	43.0	13.0	17.5	17.5	17.5	4.0	8.0	71.1
s.d. ±	6.0	0.8	1.2	1.7	1.7	3.0	0.2	2.5
% change	−17.2	−29.7	−2.7	−2.7	−50.0	−40.0	+14.2	−55.9
1% (w/w) Cysteine hydrochloride	50.0	14.5	19.1	20.0	35.2	7.0	9.0	125.0
s.d. ±	7.0	0.5	0.9	1.6	3.8	2.2	2.2	2.5
% change	−3.8	−21.6	+5.5	+11.0	0	+4.4	+28.0	0

Figures represent mean values based on five replicates.
s.d., Standard deviation.
Compare Howell & Lawrie, 1983: Fig. 5b.

Table 9. Electrophoretic characterization of chemically modified porcine plasma Fraction III proteins

Chemical added to 6% (w/w) Fr III in 45% (w/w) sucrose solution	Peak height (mm) of peak no.	
	High MW protein 1	Albumin 2
No additive	2.0	143
s.d. ±	0.5	4.0
4 M urea	18.0	130
s.d. ±	0.5	5.0
% change	−10.0	−9.0
3% (w/w) SDS	19.0	120
s.d. ±	3.3	5.0
% change	−5.0	−16.0
1% (w/w) Cysteine hydrochloride	20.0	127
s.d. ±	4.0	2.4
% change	0	−11.0

Figures refer to mean values based on five replicates.
s.d., Standard deviation.
Compare Howell & Lawrie, 1983: Fig. 5c.

Table 10. Electrophoretic characterization of chemically modified proteins of porcine serum proteins

Chemical added to 6% serum	Peak height (mm) of peak no.							
	1	2	3	4	5	6	7	8
No additive	122.5	75.0	34.0	32.0	31.0	11.0	11.5	195.0
s.d. ±	2.8	0.8	1.4	1.4	0.9	1.0	0.5	5.7
4 M urea	115.5	60.5	30.5	35.0	32.0	9.5	9.7	195.0
s.d. ±	6.6	8.0	4.5	3.8	2.1	1.2	1.7	9.0
% change	−5.7	−19.3	−8.7	+8.5	−3.1	−13.6	−17.4	0
3% (w/w) SDS	97.5	54.0	33.0	20.0	26.7	7.5	5.2	155.0
s.d. ±	15.5	6.6	1.7	0.5	2.2	0.5	0.5	5.7
% change	−20.4	−28.0	−2.9	−37.5	−13.8	−31.8	−54.7	−20.5
1% (w/w) Cysteine hydrochloride	92.0	39.0	32.0	27.1	30.0	8.5	5.5	150.0
s.d. ±	4.0	3.1	2.1	2.5	2.5	1.9	2.0	8.0
% change	−24.9	−48.0	−5.8	−15.6	−3.1	−22.7	−52.1	−23.1

Figures refer to mean values based on five replicates.
s.d., Standard deviation.

Electron micrographs of gels, which were prepared from the proteins of 6% (w/w) commercial porcine plasma, 6% (w/w) egg albumen and a mixture of 3% (w/w) porcine plasma and 3% egg albumen all in 45% sucrose solution, are shown in Fig. 2.

On heating these proteins at 95°C for 15 min, a similar type of protein network was observed for plasma, egg albumen and the mixture of these proteins, indicating the compatibility of these proteins in gel formation. It is obvious, however, that the plasma

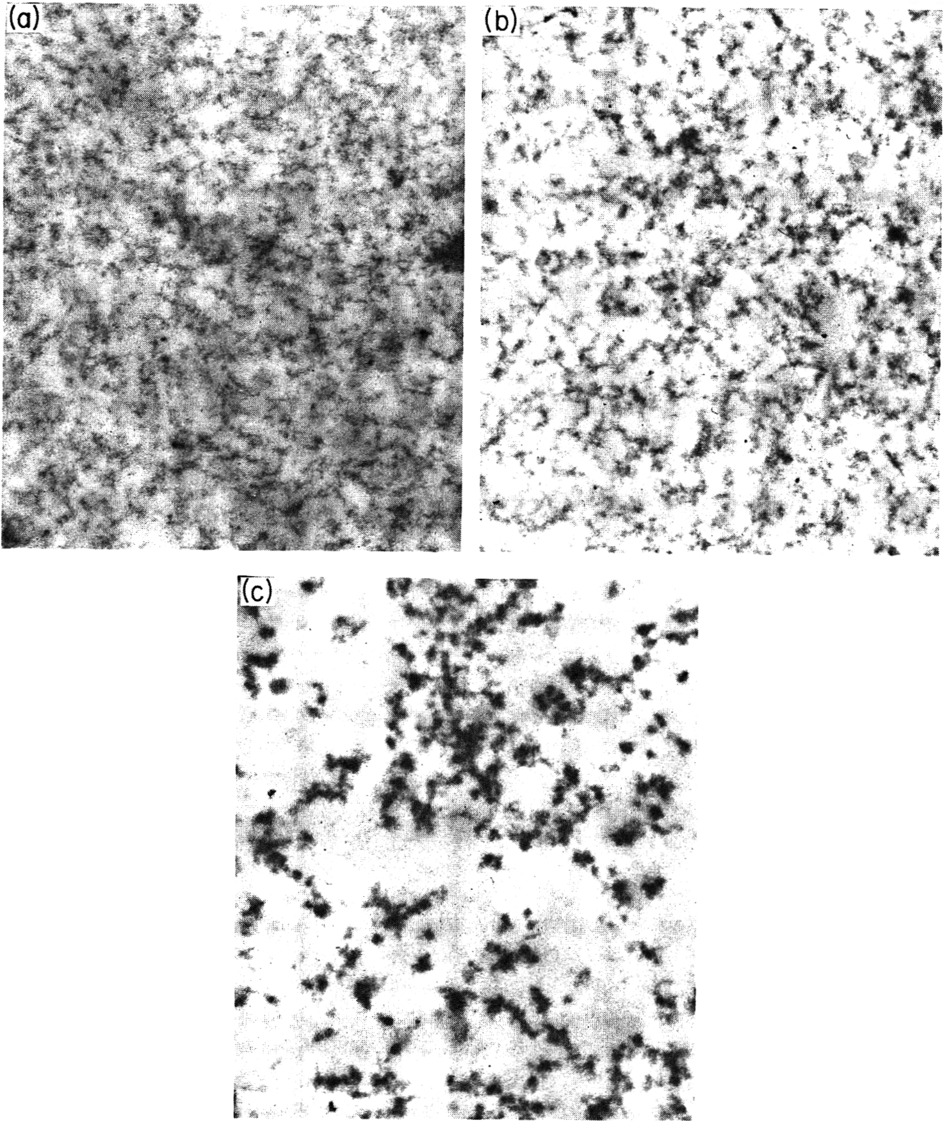

Figure 2. Electron micrographs of gels prepared by heating the proteins of (a) 6% (w/w) plasma; (b) a mixture of 3% plasma and 3% (w/w) egg albumen; or (c) 6% (w/w) egg albumen all in 45% (w/w) sucrose solution for 15 min at 85°C.

protein gel had a 'close' and dense network. In contrast, the egg albumen gel showed an 'open' structure and the mixed proteins a structure in density between the two. The density of the gel network structure, probably due to the aggregation of the protein molecules, reflects the gel strength and breaking strength of these gels: thus the plasma proteins produced a very strong gel, the mixture a strong gel and the egg albumen proteins a weak gel (Howell & Lawrie, 1984a,b).

Discussion

Based on the findings related to the gelation of all the plasma proteins, a mechanism for the heat reversible and heat setting properties of the plasma proteins can be suggested.

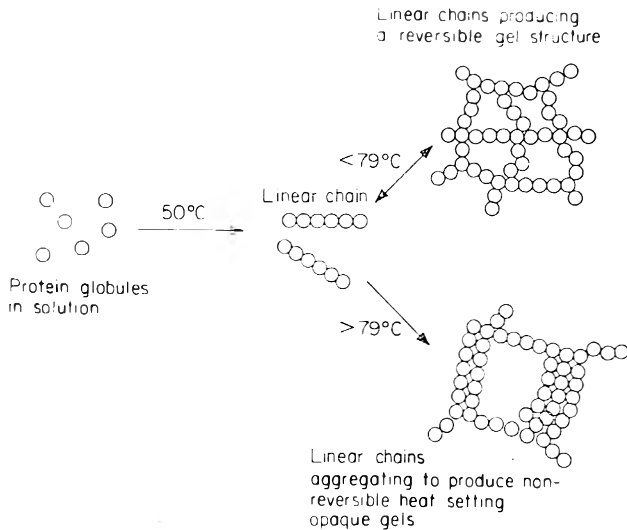

Figure 3. Possible gelling mechanism for plasma proteins.

Below 79°C the proteins of whole plasma and of plasma Fractions I, II and III produced heat reversible gels consisting probably of linear polymer chains linking up to form a three dimensional network (Fig. 3). This view is supported by Joly (1965) and Tombs (1970) who studied the conformational changes of bovine serum albumin in phosphate buffer on heating. The reversible plasma gels are thought to be stabilized by non-covalent hydrogen, hydrophobic and electrostatic linkages as these linkages are weak ($\Delta G < 20$ kcal/mol) and thermo-labile (Jones, 1964).

As the heating of plasma proteins proceeded to temperatures greater than 79°C it is likely that a more complete unfolding of the polypeptide chains occurred and aggregates were formed which bonded together laterally to produce an opaque three dimensional irreversible gel network (Figs 2 and 3). Tombs (1974) pointed out that the most probable aggregate formed by bovine serum albumin as a result of random interaction between spherical particles must be non-spherical (ellipsoid), serving as a nucleus for linear structure and for a three dimensional network. Differing views on the mechanism for the aggregation of the plasma proteins exist. Tombs (1970) suggested that protein gelation could be considered in terms of statistical theories developed by Flory (1942) for synthetic polymers. However, Bezrukov (1979) reported that statistical aggregation is unlikely to occur because protein globules have a surface mosaic structure with sites differing in charge, density and sign, degree of hydrophobicity and the presence of disulphide groups. The present work indicates that the reactions took place in an orderly sequence until heating was stopped, thus resulting in reproducible gels provided that the protein concentration and time and temperature of heating were carefully controlled. The contribution of strong covalent ($\Delta G > 50$ kcal/mol) (Whitaker, 1977), disulphide links to the structure of irreversible gels of whole plasma, serum and plasma Fractions, I, II and III was evident. In the presence of cysteine hydrochloride or β-mercaptoethanol and 4 M urea, the gel strength and breaking strength of whole plasma was considerably reduced (Tables 1–4). Urea helped to break down the hydrogen bonds, thus opening up the structure and making the disulphide bonds accessible to the reducing agent β-mercaptoethanol.

As with whole plasma, the reduction of disulphide bonds by cysteine hydrochloride

and of hydrogen bonding by urea, in gels made from Fraction III (albumin), resulted in a reduction of the gelling properties (Table 1). The structure and properties of Fraction III (albumin), which makes up about 50% (w/w) of the plasma proteins, may be used to explain the gelling behaviour of this protein.

Albumin is a single polypeptide chain which contains seventeen disulphide bonds and one free sulphydryl group. The following hypothesis is proposed. When whole plasma or Fraction III (albumin) were heated at low temperatures of 80 and 85°C for a short time (15 min) part of the albumin molecule unfolded, exposing a limited number of disulphide bonds for sulphydryl-disulphide interchange. This explanation is consistent with the fact that the resultant gel was weak. When the temperature was increased to 90 or 95°C, however, and with longer heating times (30 or 60 min) the unfolding of the albumin molecule continued, making available more disulphide groups and thus increasing the gel strength and breaking strength (Howell & Lawrie, 1984). Peters (1975) concluded that unfolding of the albumin molecule is needed to produce disulphide group reactivity. This conclusion was based on his finding that the accessibility of the seventeen disulphide bonds to reduction and oxidation varied with pH. Moreover, the free sulphydryl group is also important for gelation. This view is supported by Hospelhorn & Jensen (1954) who found that in dilute solutions of bovine plasma albumin, the sulphydryl-containing albumin aggregated more rapidly than the sulphydryl-free albumin.

As was the case with plasma Fraction III, the gel strength and breaking strength of plasma Fraction I gels were reduced in the presence of cysteine hydrochloride and 4 M urea (Tables 1 and 2). Fraction I was composed of fibrinogen, the immunoglobulins and β-globulins, all of which contain numerous disulphide groups (Putnam, 1975). In contrast, a slight increase in gel strength and breaking strength was noted for serum and Fraction II gels when treated with cysteine hydrochloride (Tables 1 and 2). The behaviour may be due to the predominance of electrostatic and hydrophobic linkages in the gel network which were exposed on the reduction of the few disulphide bonds of the α_1-globulins. It is not clear why the serum protein gels increased in gel strength in the presence of cysteine hydrochloride.

In addition to covalent disulphide bonds, hydrophobic bonds were apparently involved in the gelation of the plasma proteins. Sodium dodecyl sulphate, which prevents hydrophobic interactions, reduced the gel strength and breaking strength of whole plasma considerably and prevented the gelation of serum, and plasma Fractions I, II and III (Tables 1 and 2). Sodium dodecyl sulphate thus induced changes in the plasma proteins which prevented the formation of disulphide bonds as well as the hydrophobic interactions. According to Tanford (1980) albumin exhibits a specific site for binding sodium dodecyl sulphate and does not self-associate to block any binding site. However, information on the binding of sodium dodecyl sulphate to the other plasma proteins is lacking. It is possible that the binding of SDS to positively charged groups by electrostatic linkages or steric hindrance may have prevented hydrophobic interactions and disulphide bond formation and thus gel formation. This aspect, however, requires further investigation.

Electrophoretic characterization of the modified plasma proteins confirmed that most of the plasma proteins were sensitive to the modifying reagents (Tables 6–10). In particular the proteins of plasma were affected by β-mercaptoethanol, 4 M urea and sodium sulphite, ascorbic acid and trypsin indicating the importance of the preservation of the plasma protein entity of all the proteins for gelation.

The difference in the gelling behaviour between the proteins, whole porcine plasma

and egg albumen may be due to the limited number of available disulphide and sulphydryl groups in the latter. In particular ovalbumin, which constitutes 54% of egg albumen proteins, contains only one disulphide bond and four buried sulphydryl groups per molecule of 45 000 daltons (Osuga & Feeney, 1977).

Egg albumen also reacted differently to the chemical modifying reagents, again reflecting the difference in the gelling mechanism of egg albumen and the plasma proteins. However, as with the plasma proteins, the importance of disulphide bonds was evident from the decrease in gel strength and breaking strength in the presence of cysteine hydrochloride, β-mercaptoethanol and sodium sulphite (Tables 1–4).

On the other hand, it was surprising to observe that sodium dodecyl sulphate, sodium nitrite, ascorbic acid and trypsin increased the gel strength and breaking strength of the egg albumen gel (Tables 1–4). It is likely that these reagents promoted the unfolding of the polypeptides thus exposing (for example) the buried sulphydryl groups of ovalbumin which could then participate in the disulphide-sulphydryl interchange. Prior to heating however, ovalbumin characterized by electrophoresis did not appear to be denatured by sodium dodecyl sulphate, cysteine hydrochloride or urea. But, like the other egg albumen proteins, ovalbumin was changed by β-mercaptoethanol+urea, sodium nitrite, ascorbic acid and trypsin (Table 5). It is interesting to note that Simpson & Kauzmann (1953) found that the optical rotation of ovalbumin changed with time in the presence of urea. They attributed this reaction to complex formation between urea and ovalbumin.

In the presence of sodium dodecyl sulphate, and on heating egg conalbumin, Hegg, Martens & Lofqvist (1978) observed a delay in the precipitation of conalbumin. Similarly sodium dodecylsulphate protected ovalbumin from thermal denaturation (Hegg & Lofqvist, 1974). In this study, the effect of chemical modification on unheated egg albumen proteins, which were characterized by electrophoresis, indicated that conalbumin was the most sensitive protein (Table 5). It is likely that unprecipitated conalbumin (probably due to its modified form) and ovalbumin may be more effective for gelation and so were able to increase the gel strength of the egg albumen gel.

The increase in the gel strength and breaking strength of the egg albumen gel by ascorbic acid may have been due to a mechanism similar to that described for the action of this reagent on flour. Sullivan (1954), Sokol & Mecham (1960) and Bauernfeind & Pinkert (1970) all suggest that ascorbic acid is oxidized to dehydroascorbic acid which oxidizes sulphydryl groups to disulphides and thus influences the interchange process. Alternatively, hydrogen bonding of ascorbic acid with arginine residues (Lewin, 1974) may lead to increased gel strength.

The enhancement of the gel strength of egg albumen gels by trypsin may be due to its effect on the arginine and lysine residues, due to the partial proteolysis of proteins to low molecular weight components, or due to the interaction of the positively charged trypsin (pI 10.5) with the predominantly negatively charged egg albumen proteins at pH 8.0.

When the proteins were heated at 85°C for 15 min the type of gel network structure revealed by electron microscopy was similar to that exhibited at 95°C. However, the network of porcine plasma gel and that of the plasma-egg albumen protein mixture, exhibited similar structures and were less dense at 85° than at 95°C. At 85°C these networks consisted of fine protein strands and fewer aggregates than at 95°C. In contrast the egg albumen gel at 85°C was only slightly less dense than at 95°C. Again, the density could be related to the gel strength and breaking strength. Gels were prepared at 85°C from either 6% (w/w) porcine plasma or 3% (w/w) porcine plasma and

3% (w/w) egg albumen had very similar gel strength and breaking values (Howell & Lawrie, 1984a,b). These values were lower than those obtained at 95°C. Similarly the egg albumen gels prepared at 85°C had lower gel strength and breaking strength than those prepared at 95°C. At 85°C the egg albumen gel strength and breaking strength were considerably lower than those of either the porcine plasma gel or the porcine plasma-egg albumen gel.

Conclusion

Gelation in whole porcine plasma in plasma fractions including albumin and in egg albumin proteins predominantly involves disulphide bonds. Reduction of hydrophobic and hydrogen bonds with urea and sodium dodecyl sulphate lowered the gel strength of the plasma protein gels but increased the gel strength of the egg albumen proteins. This indicated the importance of the hydrophobic and hydrogen bonds in the former and the effect of these reagents in exposing sulphydryl groups in egg albumen which are normally inaccessible, leading to enhanced gel strength via disulphide-sulphydryl interchange reactions.

Chemical and enzymic modification can be used not only to elucidate the mechanism of gelation but might also be used successfully in the enhancement of functional properties. Thus the addition of ascorbic acid, which is both a permitted additive and a vitamin, and of the protease enzyme trypsin, might be used in product formulation to increase the gelling properties of certain globular proteins such as egg albumen.

It is evident that, although similar and compatible gels are formed by both blood plasma and egg albumen proteins, as indicated by electron microscopy, and that synergistic interactions between the two types of proteins can result in enhanced gelation, the mechanism of gelation is different for each type of protein, reflecting differences in the structure and functional groups.

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Effect of seed coat thickness and blanching on the water absorption by soybeans

R. D. KING AND S. J. ASHTON

Summary

Water absorption by whole soybeans and by soybeans with their seed coat partially abraded was compared. The rate of absorption by the whole seeds was less than that for the abraded seeds. The effect of blanching on the rate cf water absorption and absorption capacity in whole soybeans and in beans with their moisture content adjusted by storage to 33 and 65% ERH was examined. Blanching the seeds resulted in an increase in the rate of absorption but reduced the absorption capacity of the beans. The samples with the higher water activity showed the largest increase.

Introduction

Water uptake by legumes during soaking has been examined by a number of workers. Smith & Nash (1961) reported that the principal factor controlling absorption in whole beans was the seed coat and that the rate of absorption was influenced by the initial moisture content of the beans. The effect of soaking temperature has also been studied by a number of workers using soybeans (Leopold, 1980; Hsu, 1983a; Hsu, Kim & Wilson, 1983) and other legumes (Quast & Silva, 1977). The soaking solution can have an effect on the rate of water inhibition by legumes (Hsu *et al.*, 1983). For winged beans it has been shown that a short period of blanching prior to soaking accelerates the rate of water absorption (King & Puwastein, 1984).

Quantitative analysis of the water absorption process has been undertaken for wheat by Becker (1960), Chung, Fan & Shellenberger (1961) and more recently for soybeans by Hsu *et al.* (1983) and Hsu (1983a,b). Hsu (1983b) demonstrated the inadequacy of using a radial diffusion model with a constant diffusion coefficient for soybeans. He proposed the use of the radial diffusion model in which the diffusion coefficient was a function of the moisture content of the seed. The objective of this study was to examine the effects of seed coat thickness and blanching on the water absorption of soybeans.

Materials and methods

The soybeans used were of Canadian origin and were harvested in 1982. The beans were sieved and those with a diameter between 5.76 and 6.56 mm were collected.

The moisture content of two samples of these beans was adjusted by placing them in closed containers above saturated solutions of sodium chloride and magnesium

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chloride. The equilibrium relative humidity above these solutions is 33 and 75% respectively.

The beans were blanched by immersing in boiling de-ionized water for the required period of time.

Partial removal of the seed coat was achieved by placing samples of the soybeans in a domestic abrasive potato peeler which was then run at top speed for 30 min. The beans were then examined and any broken, pitted or cracked beans removed.

Measurement of sample equilibrium relative humidity (ERH)

Samples of the seeds were ground in a domestic coffee grinder to make them fine enough to pass through a 1 mm sieve. The ERH of the ground samples was determined using a Nova Sina water activity meter, model DAL 20. Samples in triplicate were left to equilibrate in a sample cell for several hours at 25.5°C prior to measurement.

Measurement of water absorption

Six replicate samples of dry soybeans (100 g per sample) were placed in 70 ml of de-ionized water at 25°C. The beans were removed from the water at various time intervals, blotted dry and weighed. This was repeated until the water absorption by the samples reached a steady value. The results were expressed as the fractional weight gain M_t/M_∞; where: M_t = weight at time (t)—weight at time (0); and M_∞ = final weight—weight at time (0).

Results and discussion

The rate of water absorption by beans was determined using whole and abraded seeds. The rate of absorption by the whole seeds was less than that for the abraded seeds (Fig. 1), particularly during the early part of the soaking period. As the soaking time increased the characteristics of the two sets became closer. The total amount of water absorbed per unit weight of dry beans was the same for both the abraded and the whole beans (Table 1). If the seeds are assumed to be spherical, then the diffusion equation for radial diffusion in a sphere might be used to predict the water absorption by the seeds. This equation can be solved analytically and the total amount of moisture entering the seed (M_t/M_∞) can be calculated as function of time (Crank, 1975):

$$M_t/M_\infty = 1-6/\pi^2 \sum_{n=1}^{\infty} \frac{1}{n^2} \exp(-Dn^2\pi^2 t/R^2)$$

where R = Average radius of the seeds, D = diffusion coefficient and t = time.

An estimate of the diffusion coefficient that gives the best fit of the data to the above equation was estimated and used to predict the water absorption process (Fig. 1). It can be seen that the radial diffusion model is an inadequate model for the description of water inhibition by soybeans. This is in accord with previous workers (Hsu, 1983b). However, for the abraded beans this model fits much closer to the data. The major difference between the whole and the abraded beans is the thickness of the hull. As the integrity of the beans must be maintained if the radial diffusion in a sphere model is to be tested, the amount of hull which can be abraded is limited. These results suggest that diffusion across the seed coat is an important factor in the hydration of soybean seeds.

Samples of the soybeans were subjected to a short period, 30 sec or 1 min, of blanching prior to soaking. This resulted in a higher rate of water uptake by the seeds, the samples blanched for 1 min having a slightly higher rate (Fig. 2). The total amount of

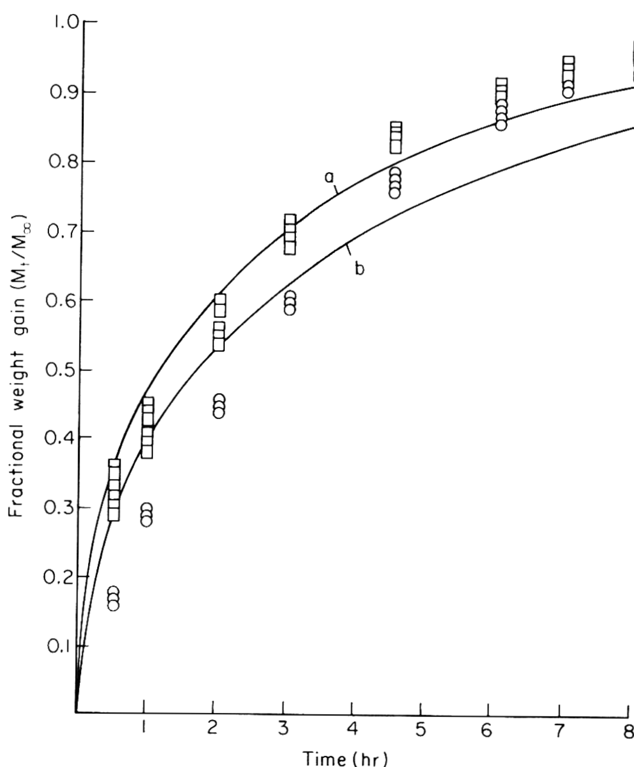

Figure 1. Fractional weight gain during soaking in water for whole (O) and abraded (□) soybeans, compared to the best fit radial diffusion model for whole (a) and abraded (b) beans. The results of six replicate experiments are plotted.

Table 1. The total amount of water absorbed (per unit weight of dry beans) by soaking soybean seeds using whole, abraded, blanched and blanched beans with an initial a_w of 0.35 and 0.65

			Blanched (time, sec)			
	Whole	Abraded	Whole		0.35 a_w	0.65 a_w
			30	60	30	30
Mean*	1.25	1.26	1.10	1.01	1.06	1.09
s.d.†	0.0052	0.02	0.05	0.06	0.03	0.05

*Mean of six trials.
†Standard deviation.

water absorbed by the beans was reduced considerably by the heating (Table 1), indicating that there is some denaturation of the protein, especially for the sample blanched for 1 min.

Samples of the soybeans were stored in atmospheres of known ERH, 33 and 75%, this resulted in the seeds having water activities of 0.35 and 0.65 respectively. The rate of water absorption by these seeds on soaking was measured using blanched and unblanched beans. In the case of the unblanched seeds the two sets of samples had very similar characteristics. After blanching the samples with the higher water activity

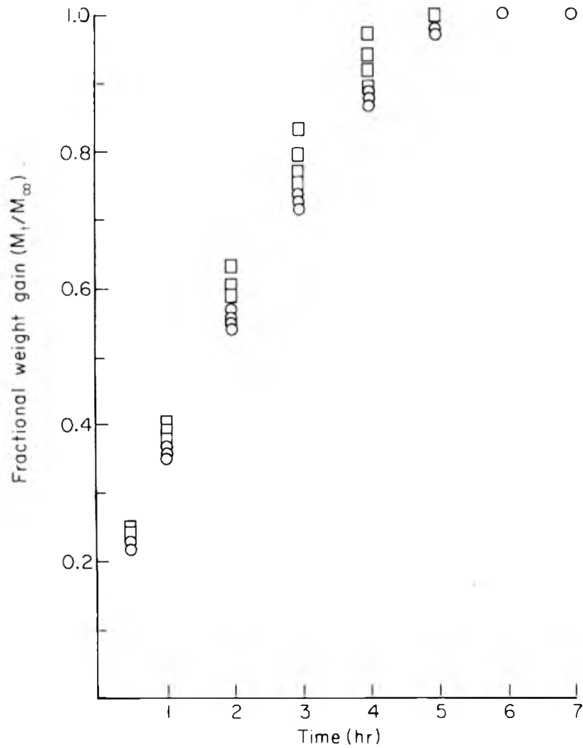

Figure 2. Fractional weight gain during soaking in water for the whole beans blanched for 30 sec (O) and 1 min (□). The results of six replicate experiments are plotted.

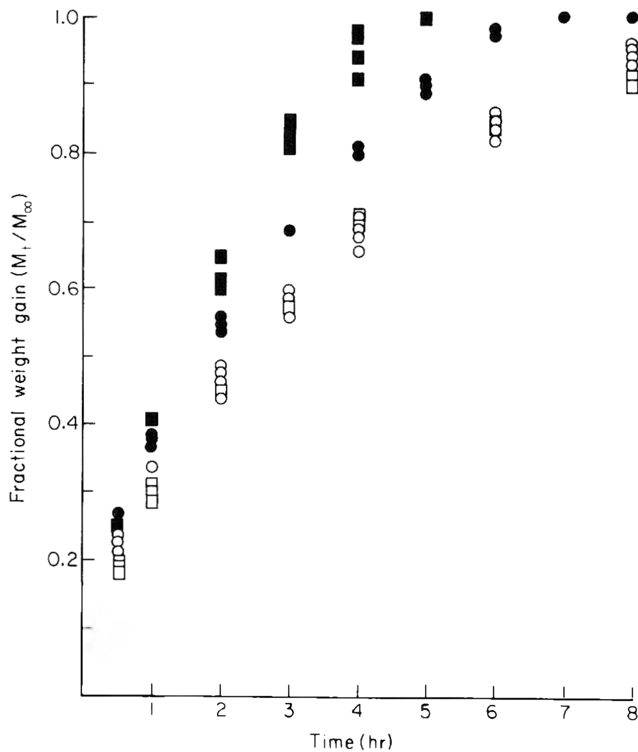

Figure 3. Fractional weight gain during soaking for soybeans with an initial aw of 0.35 (O) and 0.65 (□), and these beans blanched for 30 sec (●) and (■) respectively. The results of six replicate experiments are plotted.

absorbed water at a far greater rate (Fig. 3). The protein denaturation in these seeds can be expected to be more severe, but the effect on the total amount of water absorbed was not significant.

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Description of the Inter-laboratory Comparison Programme in the New Zealand dairy industry

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Summary

The Inter-laboratory Comparison Programme (ILCP) is a quality assurance system administered by the Dairy Division of the Ministry of Agriculture and Fisheries to monitor the performance of agency certified laboratories (ACLs). The programme works on a monthly basis during the milk producing season and is co-ordinated from the National Dairy Laboratory (NDL). Samples are despatched to Regional Dairy Laboratories (RDL) and to the ACLs 2 weeks later. The RDL results are used to calculate an official mean. The ACL results are sent to the RDL where they are scrutinized immediately; hence, a rapid feedback on the ACL performance is possible. The NDL despatches a detailed statistical evaluation of results of ACLs and RDLs. The programme has increased from seven products in 1977 to eleven in 1984. Microbiological and chemical analyses are performed. A bacterial identification scheme to monitor *E. coli* and *S. aureus* was included in 1982. A scoring system (0 poor–10 good) which includes a weighting for specific tests and which is measured against an 'official' score has been introduced into a report which is distributed to all participants. The data generated by the programme are used by both ACLs and RDLs to check their analytical performance and quality control. Data are used by Dairy Division, as a national compilation of results issued by it which serves to highlight problem analyses, season trends and analytical limits.

Introduction

New Zealand is the only country where the majority of milk produced is used to manufacture products for export. The dairy industry in New Zealand is aware of the need to manufacture a high quality dairy product as economically as possible. Product testing laboratories on site are responsible for checking quality. Government laboratories check on the reliability and quality of the testing laboratories. The Inter-laboratory Comparison Programme (ILCP), along with method standardization, regular laboratory inspection and assistance with the setting up of quality assurance systems, ensures that laboratory analyses are reliable and accurate.

New Zealand is not unique in having an Inter-laboratory Comparison Programme as part of a total Quality Assurance System. Other ILCPs are found, for example in the U.K. and in the U.S.A. for testing water and clinical samples (Skendzel & Copeland, 1975; Wilson, 1979). Where New Zealand is unique is in the running of a continuous monthly comparison programme with mandatory participation for all Agency Certified Dairy Laboratories (ACL). (Agency Certified Laboratories are dairy laboratories who have been accredited as agents for the Dairy Division to perform chemical and microbiological tests on dairy products.)

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The ILCP has three functions:

(i) To provide laboratories with an evaluation of their analytical performance. This is enhanced by the system being independent, external, and with nationwide coverage.

(ii) To provide information to the dairy industry about its laboratory performance.
 (a) conformity of analysis within the Dairy Division and industry;
 (b) data which can be used to improve analytical standards;
 (c) general statistical data allowing insight into analyses, to determine trends, variability and analytical accuracy over a number of dairy seasons.

(iii) To provide data for regulatory purposes (Standard Operating Procedures for Agency Certification):
 (a) information on analytical performance to supplement data obtained by grading comparisons. (The initital objective of ILCP was to assist in monitoring of the agency certification for laboratories);
 (b) to enable the Dairy Division to take action when problems in analysis are detected.

Development

The programme began in 1977. Samples of wholemilk, skimmed milk, casein, caseinate, cheese, butter and AMF were sent to seventy-two dairy factories. During the first season 2130 samples were distributed. In 1979/80 the number of samples sent almost

Table 1. Products and analyses for ILCP 83/84 season

	Products									
Analyses	WMP	SMP	BMP	Casein	Caseinate	WPC	Lact'm	Butter	AMF	Cheese
Moisture	√	√	√	√	√	√	√	√	√	√
Fat	√	√	√	√	√	√	√			√
Acidity	√	√	√	√						
WPNI		√								
SI	√	√	√							
Protein				√	√	√	√			
Ash					√	√	√			
Bulk density				√	√	√	√			
pH					√					√
Salt			√					√		√
Curd								√		
Peroxide									√	
Lactose				√	√	√	√			
Free fatty acids									√	
Foreign matter	√	√	√	√						
Calcium					√					
SPC	√	√	√	√		√	√	√		
Coliform	√	√	√	√		√	√	√		
Yeast moulds	√	√	√	√		√	√	√		

WMP (Wholemilk powder).
SMP (Skimmed milk powder).
BMP (Buttermilk powder).
WPC (Whey Protein Concentrate).
Lact'm (Lactalbumin).

doubled. In 1980/81 buttermilk and lactalbumin were added to the list of dairy products sent out and the number of samples distributed increased to 20 000. During the 1983/84 season both unsalted and salted butter were included.

The number of analyses performed on the samples has increased since 1977; originally only fat, moisture, acidity, solubility index and protein were included. In 1978/79 the following microbiological tests were included: (i) standard plate count; (ii) yeasts/moulds count; (iii) fungi (for certain products); and (iv) thermophilic count. The last test is no longer included. In 1980 a bacterial identification scheme was included (see Operation section). During 1983/4 over 100 000 analyses were performed. Table 1 outlines the products and analyses for the 1983/84 season. The ILCP aims to be flexible while maintaining a reasonable level of participation from laboratories and coverge of analyses. Data from all basic tests are fed into the computer, additional data are analysed manually (see Operation). The ILCP is now used as both a problem solving tool and as an information system.

Operation

The programme is planned and coordinated from the National Dairy Laboratory (NDL). The national coordinator acts through Regional Dairy Laboratories (RDL) which are Dairy Division reference laboratories. They are responsible for ensuring adequate participation by factory laboratories and the instigation of action pertinent to laboratories performance within their region. The programme includes:

(i) Routine analysis comparison;
(ii) additional analysis comparison;
(iii) reference samples; and
(iv) bacterial identification scheme.

(i) Routine analysis comparison

The programme starts with unknown samples sent to the RDLs for pre-testing. The samples are tested and results returned to the NDL, where a 'preliminary mean' (see Data Interpretation) of all tests is obtained. ACLs receive the samples 2 weeks later and are given 2 weeks to carry out the tests and return the results to their RDL (Fig. 1). The RDL checks the result against the inter RDL 'preliminary mean' and discrepant results

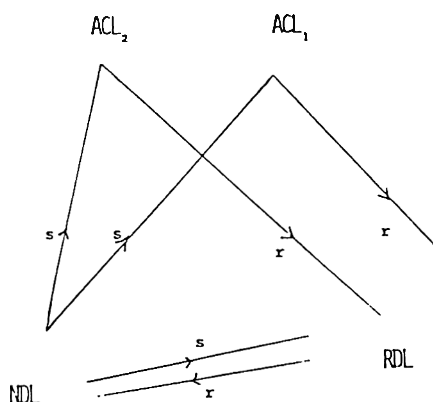

Figure 1. Schematic diagram of the elements in ILCP: s = samples; r = results.

Table 2. Additional analyses for ILCP 83/84 season

	Products			
Analyses	Wholemilk	Skimmed milk powder	Casein	Caseinate
Bulk density	\			
Vitamin A	\			
Cu	\	\	\	\
Fe	\	\	\	\
Na				\
Ash			\	
Alkali requirement			\	

are followed up as required. The ACL results are then sent to the NDL for statistical analysis (see Data interpretation section). A report of this analysis is sent to all participating laboratories.

(ii) Additional analysis

This can be divided into those performed by the RDLs bimonthly (Table 2) and those done at irregular intervals. The latter consist of:

(a) Method comparisons: determination of the most suitable or accurate method for routine use, e.g. chloride in whey protein concentrate, ash in caseinate;

(b) Problem analysis: for the 83/84 season this included alkalinity in AMF, lactose in casein and particle size in casein;

(c) New tests: e.g. instant wholemilk functional tests, calcium and phosphate in cheese (done by request of other Dairy organizations).

Additional analyses examined variability and new methods, and endeavoured to solve problems arising in the analysis of dairy production.

(iii) Reference samples

Once samples have been analysed by the RDLs further samples are despatched as known 'reference samples'. These are used in laboratory quality assurance systems. The values for the references samples are obtained as for preliminary means (see Data interpretation section).

Values given on the samples are not absolutes, but relative, and laboratories work within prescribed analytical limits. (Ministry of Agriculture and Fisheries, 1978.) The samples can be used to calibrate methods to ensure that laboratories are running within Dairy Division prescribed limits. As stability and accuracy are essential qualities of chemical reference samples, an expiry date (usually 3 months) is given so that samples will not be used continually.

(iv) Bacterial identification

The bacterial identification scheme was introduced in early 1980. This involves artificially contaminating casein and skim milk powder with a bacterial culture.

The artificially contaminated product was introduced because sufficient naturally contaminated products could not be obtained. This was due to the changes in bacterial populations in stored dairy produce and the general unwillingness of companies to supply out-of-specification products to Dairy Division. Pure cultures (on agar slopes)

were also introduced to complement the product part of the Bacterial identification scheme.

To ensure a homogeneous distribution of bacteria in the product the following methods have been tried:

(a) *Aerosol method.* This was used initially in the Bacterial identification scheme. The disadvantage of the system is that the products clump with the moisture added on spraying, and thus distribution of bacteria throughout the product is not ensured.

(b) *Freeze drying.* This is the method used at present on a regular basis. It involves culturing the bacteria to be used (*E. coli, S. aureus*). The culture is then centrifuged to separate the bacteria from the supernatant liquid. The residual bacteria are mixed with milk, frozen in dry ice/alcohol mix and then freeze dried. The resulting freeze dried product is mixed by tumbling with the powder. The main difficulties with this method are the time involved with the preparation, uncertainty of bacterial distribution and the possibility of contamination at the mixing stage.

(c) *Spray drying.* (Thompson, 1978). This method is still in the experimental stages, but appears to have the greatest potential. *Staphylococcus aureus* has been used in trials so far because the bacteria are more resistant to heat than *E. coli*. A culture of bacteria is mixed with reconstituted instant skim milk powder (40% total solids) to give a bacterial population of approximately 10^8/ml. The reconstituted powder is spray dried using a Niro Production Minor drier at varying inlet temperatures. Good recovery of the bacteria is found even at high inlet temperatures (200°F or 93°C). A study into the distribution of bacteria in dairy products using this method is being undertaken.

The ACLs are required to isolate organisms for the samples sent according to Dairy Division Standard Methods (Ministry of Agriculture and Fisheries, 1977).

The correct result for each sample is calculated from the most common RDL result. A computer printout has been developed to print out the factory result for each RDL area (Table 3). From this printout the percentage of factories getting any particular test positive or negative can be determined. Thus a check can be made on factory performance over a period of time on any particular test.

Results have shown a high correlation between the RDLs but with problems in many ACLs. Failure to identify *E. coli* by incubation on Eosin Methylene Blue Agar and Brilliant Green Bile Broth (BGBB) (44.5°C), (Eijkman) and misinterpretation of the coagulase test of the *Staphylococcus* method, were the major problems (Table 4).

A trial was undertaken by the Dairy Research Institute for the 83/84 season on contamination of cheddar cheese, with a:

(i) coliform species, and

(ii) *Staphylococcus* species.

The contaminated samples were sent to the RDLs. It is planned to continue this in the 84/85 season, with distribution including the ACLs.

ILCP Printout: data interpretation

Initially asterisks were used in the report to identify those results differing from the mean by two (or three) standard deviations. In 1978 three statistical analyses were used, giving the bias, variability and consistency of tests. The aim of these was to give an assessment of accuracy of analysis in terms of standard deviation and the degree of bias

Table 3. Computer printout of bacterial identification

				Bacterial identification result																
				Micro analysis	Eijkman		Gram stain			IMVIC				*E. coli* interpret-ation	Baird Parker Agar	Gram stain		Coagulase test		*Staph* interpret-ation
Factory	Region	Prod	Samp	ebgbb30	evrba	ebgbb44.5	cemb	egram	egr	eind	emr	evp	ecit		sbp	sgram	sgr	sslide	stube	
1	2006	s	42	1	1	1	1	r	0	1	1	0	0	*E. coli*	0					neg
2	2006	s	42	1	1	1	1	r	0	1	1	0	0	*E. coli*						neg
3	2006	s	42		1	1	1		0	1	1	0	0	*E. coli*						
4	2006	s	42	1	1		1	r	0					*E. coli*						neg
5	2006	s	42	1	1	0	1	r	0	1	1	0	0	*E. coli*						
6	2006	s	42	1	1	1	1	r	0	1	1	0	0	*E. coli*						
7	2006	s	42	1	1	1	1	r	0					*E. coli*	0					neg
8	2006	s	42	1	1	1	1			1	1	0	0	*E. coli*						
Mean	all	s	42	1	1	1	1	r	0	1	1	0	0	*E. coli*	0	r	0		0	neg

Prod = product; either casein, skimmed milk, slant or cheese.

Samp = sample number; 4 is month, 2 is sample.

e, indicates test for *E. coli*; bgbb, brilliant green bile broth agar, at 30 and 44°C; vrba, violet red bile agar; emb, eosin methylene blue agar; gram, indicates rods (r) or cocci (c); gr, positive or negative stain; ind, indole test; mr, methyl-red; vp, voges proskauer; cit, citrate; s, indicates test for *Staphylococci*.

1 = positive, 0 = negative.

Table 4. Examples: *E. coli* identification, comparison between 2 months in 2 years

	% of ACLs with incorrect eikjman (bgbb 44.5°C)		% ACLs with negative eosin methylene blue agar	
	1982/83	1983/84	1982/83	1983/84
Products	27	12	15	19
Slants	26	16	9	4

Each sample is analysed under standard conditions and positively identified for *E. coli* (reference lab).

in a particular analysis. Variability was included as an index of the reproducibility of a given laboratory for a particular analysis. Consistency is a measure of the imbalance of laboratory results on the high or low side of the RDL average (Twomey, 1980). These gave an indication of performance by each participant, but were not easy to understand by the majority of managers who lacked statistical knowledge.

In 1982 the data analysis report was changed to increase impact in factory laboratories. This new printout identified each laboratory and differentiated results into product type and analysis for all samples despatched for a month.

Terms used in the printout (Tables 5 and 6 are examples of the computer printout).

(a) Your result: the result which the laboratory obtained for the analysis sample indicated.
(b) Official result: this is the preliminary value obtained by the RDL.
(c) Your score: this is obtained from the difference between your result and the official result and expressed as a score.
(d) % Labs 5 or more: percentage of laboratories scoring greater than 5. This gives laboratories an indication of overall factory performance and where their own results lie in comparison.
(e) National result: average of all results.
(f) This month: bias score indicator.

The data are dealt with under three areas:

(1) Official result (preliminary mean);
(2) scoring system;
(3) low score/score summary.

(1) Official result

As RDL results are returned to NDL a 'preliminary mean' is calculated. To give a valid result at least three non-zero sample values are required. To obtain this 'preliminary mean', results are subject to an outlier test. This test compares the standard deviation of the RDL results with a long term standard deviation for that test, at a 95% confidence level. Results outside this limit are excluded and the new RDL standard deviation calculated. This new standard deviation is again compared with the long term standard deviation at a 90% confidence level (the rationale is that once a laboratory is rejected, the sample itself may be suspect and deserves greater scrutiny). If on this second comparison no values are excluded then the calculated mean from the remaining RDL values is used as the 'preliminary mean'. If any of these remaining RDL results are

excluded by the outlier test then all results for that analysis are rejected and no preliminary mean is given.

The preliminary means are returned to the RDLs to be used in initial assessment of ACL results. This value is also incorporated into the printout as the 'official result' which is then used to calculate the score.

(2a) Score

Results for all analyses are scored on a common scale ranging from 10 (good) to 0 (poor). The score is calculated from the Standardized Normal Deviate (SND). This is the difference between the laboratory result and official result. divided by a standard deviation. The standard deviation is a pre-set value for a particular test obtained from data over a number of seasons.

$$\text{SND} = \frac{\text{Factory}^* - \text{official result}^*}{\text{Standard deviation}}$$

For SND negative values are ignored*. Data are transformed using either square root, log or none depending on analyses, so that the distribution of results approximate normality. Score = 10−3×SND. Data are transformed to approximate a normal distribution, thus the Standard Normal Deviate lies within certain standard deviations. SND up to 1 in 67% of instances; up to 2 in 95% of instances; up to 3 in 99% of instances. Therefore:

Score = 10−3×SND.

Score = greater than 7 in 67% of instances; greater than 4 in 95% of instances; greater than 1 in 99% of instances.

Examples of scoring. Fat analysis of wholemilk powder:

Transformation	$\times^{1/2}$
Standard deviation	0.04
Factory score	28.5
Official score	28.351

Then:

$$\text{SND} = \frac{\sqrt{28.5} - \sqrt{28.351}}{0.04}$$

$$= 0.35.$$

$$\text{Score} = 10 - 3(0.35)$$

$$= 8.95 \text{ rounds } 9.$$

Scores for all chemical analyses listed in Table 1 are determined as above. Due to the problems involved in quantifying microbiological results the score is obtained in a slightly different manner.

(i) SPC (Standard Plate Count). Calculated as above except only an overall score is given for all samples.

(ii) Coliform. Coliform results are reported as the number of positive tubes out of the total counted. Because of the positive/negative nature of the results neither square root or log transformations were possible. The difficulty in scoring is to give a factory a score of 10 if its result is as close to the official as it is possible to get with the number of

tubes counted. To overcome this the difference between the factory and official result is adjusted by $\frac{1}{2}n$.

Factory—official result = x.

$$\text{If } x < \tfrac{1}{2}n, \text{ then } X = 0,$$

where n = Total number of tubes counted; and X = adjusted result difference.

If $x < 0$, then $X = x + \frac{1}{2}n$;
If $x > 0$, then $X = x - \frac{1}{2}n$.

The adjusted result difference (X) is then divided by the standard deviation to give the standardized normal deviate. The score is then calculated as detailed above.

(iii) Yeast/moulds. For yeast/moulds the count is often low. Because of the relative difference in values under 10 compared to those above, no score is given when counts are below 10. The score is calculated as for the Standard Plate Count.

Table 5. Example of chemistry results computer printout. Inter-laboratory Comparison Programme for month 6, season 83/84

	Sample	Your result	Official result	Your score	% Labs 5 or more	National result	
Product: 2A Skim milk powder	1	0.6	0.5	7	82	0.4	Fair
Analysis: C47 Solubility index	2	0.6	0.6	10	79	0.6	Good
	3	0.7	0.6	7	75	0.7	Fair
Cumulative results since start season	4	0.3	0.3	10	96	0.3	Good
	5	0.5	0.5	9	74	0.4	Good
				7	66		
No samples analysed / Overall score / % Labs 5 or more / 'this month':							
9 / 6 / 54							
Product: 2A Skim milk powder	1	6.4	6.7	8	96	6.7	Fair
Analysis: C53 WPNI	2	4.3	4.5	8	92	4.5	Fair
	3	4.3	4.7	6	81	4.5	Fair
Cumulative results since start season	4	6.3	6.6	8	92	6.4	Fair
	5	6.2	6.6	7	89	6.7	Fair
				4	69		
No samples analysed / Overall score / % Labs 5 or more / 'this month':							
9 / 6 / 53							

*Each sample in itself is satisfactory but there is evidence of overall bias.
Scoring: 0–4, poor; 5–8, fair; 9–10, good.
RDL: Auckland.
Laboratory: 999, Milkflow Dairy Company, Hamilton.

(2b) 'This month'

'This month' is given in the report as an indicator of the bias of that particular product analysis. It should be regarded as an indicator of possible problems and not as

the best measure of performance. The 'this month' score is calculated by cumulating the standard normal deviate for each individual sample.

$$\text{'This month'} = \frac{10-3\,(\Sigma\ \text{SND sample})}{\sqrt{N}}$$

where N = total number of samples per product. A good score on individual samples may still result in the 'this month' score being less than 5. This occurs when there is bias present in the individual sample result. When the 'this month' score is less than 5, a message is printed out with the results.

Table 6. Example of microbiological results computer printout. Inter-laboratory Comparison Programme for month 5, season 83/84*

		Your result	Official result	Your score	% Labs 5 or more	National result	
Coliforms	All samples						
Skim milk powder	(tubes)	0/15	0/75	10	90	0/15	Good
Butter milk powder		0/15	1/75	10	100	0/15	Good
Acid casein		4/15	26/75	10	65	5/15	Good
Lactalbumin		0/15	0/60	10	100	0/15	Good
	All products			10	87		
Standard plate count	All samples						
Skim milk powder	(count)	2369	2464	10	74	1826	Good
Butter milk powder		334	385	9	80	453	Good
Acid casein		155434	3606	0	54	5015	Poor
Lactalbumin		31	71	4	44	47	Poor
	All products†			6	72		
Yeasts and moulds	All samples						
Skim milk powder	(count)	22	16	7	65	16	Fair
Butter milk powder		2	2			2	
Acid casein		119	135	9	86	110	Good
Lactalbumin		1	1			1	
	All products			9	100		

RDL: Mount Maunganui.
*See footnote to Table 5.
†The samples are too variable but there is no evidence of overall bias.

The message: 'The samples are too variable but there is no evidence of overall bias', is printed when individual sample results have scored poorly, but 'this month' is greater than 7. This indicates that samples are scattered above and below the official result. Overall, the scoring system presents a clear and easily understood report, which enables some comparison between results reported by other participants. It has also meant that laboratories performing well are rated accordingly instead of only poor performers being given ratings.

(3) Low score/score summary

The prime printout includes a low score/score summary (Table 7) which is distributed to the RDLs. The low score report prints for all product/analysis combinations,

Table 7. Example of computer printout: Low score/score summary

	ILCP Low Score Report Month 8 83/84 Season					
	(Score under 5)	Samples				
Product: 2A Skim milk powder	Lab	1	2	3	4	5
Analysis: C47 Solubility index	0001	—	—	0	—	—
	0004	4	2	—	—	—
	0008	—	—	—	0	—
	0010	4	0	—	—	—
	0333	—	1	0	—	—
	0788	4	2	—	—	—
	1333	4	2	—	—	—
	1444	—	4	—	—	—
	1777	—	0	0	—	—
	2008	4	—	—	—	—

	ILCP Score Summary Month 8 83/84 Season						
		Samples					
Product: 2A Skim milk powder	Score	1	2	3	4	5	Total
	9	12	7	0	0	0	2
	8	0	9	0	0	0	3
	7	0	3	0	0	0	7
	6	13	0	9	0	0	2
	5	0	4	0	18	0	0
	4	5	1	0	0	0	3
	3	0	0	0	0	0	1
	2	0	3	0	0	0	2
	1	0	1	0	0	0	1
	0	0	2	3	1	0	6

those laboratories scoring less than an input parameter value (set score, 0–10), for a particular sample. The score summary prints the number of laboratories scoring a particular score per sample for all product/analysis combinations. These reports enable monitoring of poor performance laboratories and potential problem analysis.

ILCP as a quality control tool

Laboratories have to meet certain conditions before the ICLP can be used as a quality control tool:

(i) All ILCP analyses are carried out according to the procedures specified in the Dairy Divisions' analytical methods (DDM3 and DDM4).

(ii) In routine analysis, samples are treated in the same way as other samples analysed by the laboratory with no special treatment.

(iii) Analyses are carried out as soon as possible to reduce chances of variation. This is guaranteed by including a deadline for the return of results.

Results obtained under these conditions can be used in a laboratory quality control programme. ILCP is increasingly being used to provide management with information

to gauge performance, check methods and equipment and solve problems. Samples are also used in training programmes and to monitor performance. Reference samples are also used in calibration, problem solving and assessment of a method.

To maximize the impact of ILCP it is important that:

(i) There are criteria against which performance of laboratories is assessed and real problems identified.

(ii) There is a rapid feedback of results. A full statistical confirmation is returned to participating factories 2 weeks, after results are received. (We are endeavouring to decrease the time of this to only 1 week using the PRIME computer network.) Preliminary feedback on discrepant results is within a day.

(iii) There are suitable samples available and conditions which may affect the integrity of the results are known. This involves selection of products which will give a range of results, and includes those outside specification.

Conclusion

The ILCP plays an important role, in the New Zealand dairy industry, as a means of 'testing the tester' and as a quality control tool. Initially the programme was met by considerable resistance from laboratories. Today, with an understanding by laboratories of the importance and relevance of the programme and the relationship it bears to the manufacture of high quality products, there is a more positive attitude. This is prevalent in dairy factories where the laboratory staff assist with process control procedures.

As ILCP is only one part of the monitoring of laboratories, it should be used to complement an internal quality control programme. If a general appraisal reveals problems it should be relatively simple to determine the cause by use of other quality control systems.

ILCP provides data for participants, the dairy industry and related groups. Along with factory inspection and official sampling, the Dairy Division can get a complete picture of the ability of ACLs to maintain national standards. Data obtained has also enabled Dairy Division to improve and investigate existing methods. It has also highlighted problem analyses, and problem laboratories. Some analyses have shown a decrease in overall variability and in the future these may be tested less frequently. A detailed statistical analysis of the data is also being undertaken to identify major trends with time, and to reassess Dairy Division analytical limits.

Thus ILCP acts as an external quality assurance system across the total spectrum of the dairy industry in New Zealand guaranteeing quality in dairy products.

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Technical note: Water activity measurement near to 1.00

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Introduction

Lang *et al.* (1981) described the use of a proximate equilibration cell (PEC) to rapidly equilibrate a sample to an atmosphere of known a_w. Recently McCune, Lang & Steinberg (1981) developed a new method for a_w determinations with the PEC, applicable over the range 0.40–0.98. It is well known that at very high water activities (i.e. > 0.98) the true a_w value is difficult to measure (Labuza *et al.*, 1976; Prior, Casaleggio & Van Vuuren, 1977).

The aim was to adopt the PEC method of McCune *et al.* (1981) but with the idea of replacing the PEC with standard Petri dishes and using larger filter papers, pre-equilibrated to a known relative humidity, in order to determine a_w's above 0.98, by extrapolation.

Materials and methods

Materials

Whatman's No. 42 ashless circle filter papers (90 mm diameter) equilibrated to 75% RH and plastic Petri dishes with outside diameters of 90 mm were used. Weighings were performed on an analytical balance (Mettler Model Nr. H10TW) and a bacteriological incubator, set at 20±0.5°C was used for equilibration. Saturated solutions of analytical reagent salts were used to establish known a_w's: NaCl (a_w = 0.75), NaC_6H_5COOH (a_w = 0.88), KNO_3 (a_w = 0.94), and Na_2HPO_4 (a_w = 0.98). (Rockland, 1960).

Method

Circles of equilibrated filter paper were weighed (to 0.1 mg) and each was placed on the rim of three Petri dishes containing the samples, ensuring they lie flat on the adhesive paper supports (Fig. 1). Lids were put on the dishes and fastened with rubber bands. The reference a_w's was prepared by replacing the samples with saturated salt slushes of a known a_w.

After incubation (24 hr at 20°C), weigh again the re-equilibrated circles of paper and calculate the water activity of the sample from the equation:

$$a_w = 1 - 10^{(a_0 + a_1 x)},$$

where x is the relative weight gain of the paper as a percentage of the final weight ($100\,(f_w - i_w)/f_w$), and a_0 and a_1 are the regression constants for the reference salts. Alternatively, plot the relative weight gains of the references on a linear scale against

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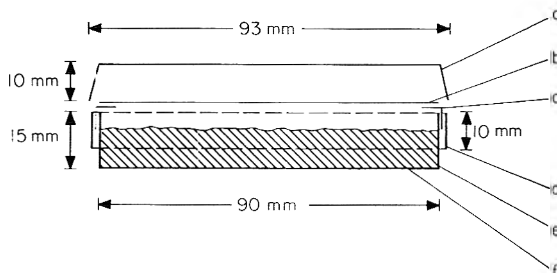

Figure 1. The systematic arrangement of an a_w plastic Petri dish: (a) lid; (b) circle of filter paper; (c) adhesive paper support; (d) elastic rubber band; (e) body of the Petri dish; (f) salt slush or sample.

their respective a_w values on an inverted log scale. Interpolate or extrapolate graphically the a_w of the sample from the relative weight gain.

The time required to weigh the filter papers on removal from the incubator (to 0.1 mg) was about 10 sec. No change in weight was noted during weighing of the initially equilibrated filter papers but in those re-equilibrated the loss in weight would lead to errors of less than 0.002 a_w.

Results and discussions

In order to test the linear relationship between the relative weight gain and log $(1-a_w)$, a calibration curve was constructed using saturated salt slushes (Rockland, 1960). Least square regression analysis resulted in a straight line (Fig. 2) with $a_0 = -0.6$ and $a_1 = -0.1$; the coefficient of determination, r^2, was 1.000.

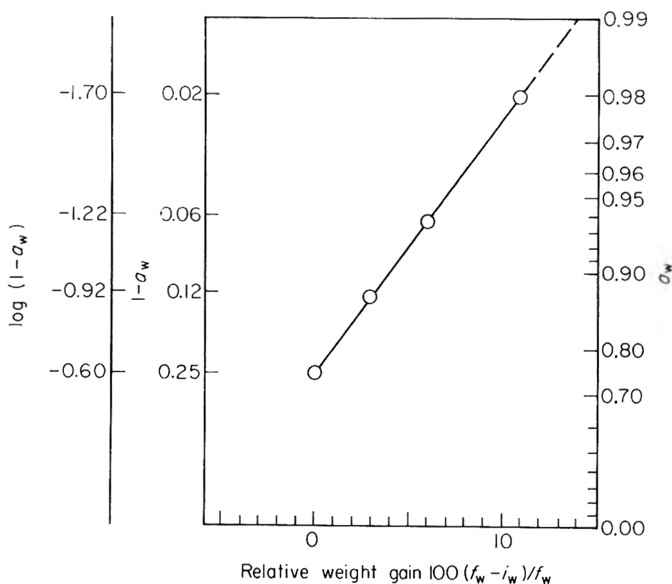

Figure 2. The calibration curve relating the water activity, on an inverted log scale, to the relative weight gain of the filter paper at 20°C. f_w, Final weight; i_w, initial weight.

Analysis of the data indicated that, for triplicate determinations, standard deviation of a_w, was 0.007 for an a_w of 0.88, 0.004 for an a_w of 0.94 and 0.003 for an a_w of 0.98.

We applied the method to calculate a_w by extrapolation to the third log cycle, from 0.990 to 0.999. The results obtained on dilute culture media (Table 1) were satisfactory because the repetitiveness (s_x) was better than 0.001 and the procedure's third decimal figure was significant.

Table 1. Water activity of some culture media

Culture media	Determination First	Second	Third	$\bar{X}$	S_X
Nutrient agar	0.999	0.998	0.998	0.998	0.0004
Nutrient broth	0.997	0.996	0.997	0.997	0.0009
Lactose broth	0.997	0.996	0.996	0.996	0.0008
McConkey broth	0.995	0.995	0.995	0.995	0.0003
Desoxicholate lactose agar	0.994	0.994	0.995	0.994	0.0006

Lenart & Flink (1983) reported a change of slope in the calibration curve above 0.95, but in our experiments the point for K_2SO_4 at an a_w = 0.976 is in the same straight line, as in fact McCune *et al.* (1981) reported. A break in the straight line might be expected above a_w of 0.99 but using NaCl solutions of a_w 0.993, 0.996 and 0.998 as references the errors produced by extrapolation of our calibration curve are less than 0.002 a_w. On the other hand, Lenart & Flink (1983) reported that sorption by the filter paper over pure water gave an a_w of approximately 0.99. With the same technique of least square regression analysis, we obtained an a_w of 0.999 for pure water.

The accuracy of the extrapolation value above a_w = 0.980 has been tested on fresh cheese samples (Marcos *et al.*, 1982) in which the a_w can be calculated from the chemical composition (Marcos *et al.*, 1981). Most of the calculated a_w's were in good agreement with the measured ones (Marcos *et al.*, 1982).

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Book Reviews

Toxic Hazards in Foods. Ed. by D. M. Conning and A. B. G. Lansdown. London: Croom Helm, 1983. Pp. vi+297. ISBN 0 7099 0253 0. £22.50.

The various chapters in this book are written by different authors in contrasting styles, mostly in the form of advanced reviews. The book is aimed at those involved in clinical nutrition as well as those concerned with food manufacture and its monitoring in industry.

Chapter 1, 'Systematic toxicity due to foodstuffs' by D. M. Conning, is presented in dictionary form, listing diseases caused by toxic substances, under organ-system headings (e.g. cardiovascular, central nervous etc.) and excludes carcinogens and immunotoxic diseases, which are dealt with in other chapters. Since some of these diseases involve more than one organ system, inevitably this approach leads to some overlap in the discussion of toxic substances between sections.

Chapter 2 is entitled 'Intolerance and allergy to foods and food additives: its relevance to toxicology' and is written by W. E. Parish. It is a very comprehensive, up-to-date account of the mechanisms involved in the development of allergy reactions. Chapter 3, 'Teratogenicity and reduced fertility resulting from factors present in food' by A. B. G. Lansdown, emphasizes the tremendous impetus to the study of teratogenicity which followed the tragic effects of thalidomide administration to pregnant women in the early 1960s. As far as food is concerned, the greatest risk of malformation of the foetus and reduced fertility comes from general or specific malnutrition, which, although not strictly a toxic hazard in food, nevertheless occasions considerable discussion. At the end of this chapter, the author concludes that although mercury and excessive alcohol intake and possibly lead and cadmium may be harmful to the foetus, most food substances or additives are without serious risk of teratogenicity.

Chapter 4, on 'Carcinogens in foods', by P. Grasso, gives a good overview of the subject, and includes a well-balanced discussion of carcinogens in foodstuffs (mostly only those eaten by a minority of the world's population), fungal carcinogens, carcinogens produced in food processing, nitrosamines, food additives and pesticides, as well as a discussion of the effect of the role of diet in the aetiology of cancer.

Chapter 5 is a useful reference chapter on 'The mutagenicity of food' by D. Anderson and I. F. H. Purchase. It deals with the methods of mutagenicity testing and includes a well-referenced list of foods and food components which have shown mutagenicity mainly in *in vitro* tests with bacteria, as well as a discussion of the relevance of mutagenicity testing to man. My only comment is that this chapter might have been more logically placed in front of Chapter 4 on carcinogenicity, rather than following it.

Chapter 6, 'The gastrointestinal tract in food toxicology' is by I. R. Rowland and R. Walker and is a much longer chapter than the others—some 67 pages with 488 references. Its coverage verges on the exhaustive, which is very useful for the research worker, but the approach renders the style of writing somewhat turgid for other readers. There is a discussion of the role of gut microflora in intestinal metabolism—a subject not normally discussed at length under the heading of toxic hazards in foods,

despite the considerable influence of gut flora on the toxicity of substances in foods. The known effects of gut flora on nutrients, pollutants and pesticides are discussed in turn. Unfortunately, this chapter lacks a concluding section, giving the appearance that a section has been left out by mistake.

The final chapter, (7) by J. R. Mansfield on 'Food allergies; clinical aspects and natural allergens' is something of an enigma. Allergy reaction has already been dealt with in considerable detail in Chapter 2. Chapter 7 describes the 'masked food allergy'—a condition not recognized by those working in the field of allergy research. Patients suffering from a 'masked allergy' to a certain food, felt better after eating it than before. Nevertheless, the patient exhibits delayed allergic response which can only be alleviated by more of the same food. This results in alternate masking and unmasking of the symptoms in a cyclical response. This interpretation of certain clinical conditions as allergy reactions was propounded in the best-selling book 'Not all in the mind' by R. Mackarness (Pan Books, 1976), to which the author of this Chapter refers. Although some evidence has now been presented to show that certain migraine headaches (one of the principle symptoms discussed by Mackarness) involve the immune response, the involvement of the immune system in many of the other clinical conditions discussed has not yet been shown in clinical trials. It is confusing that there are two chapters on allergy representing different interpretations of the condition. The approach taken in this book is unusual, but for those interested in the toxicity of specific substances in foods the index will prove indispensible. The advanced nature of most of the chapters in the book make it more suitable for the needs of research workers rather than for food manufacturers.

Ann F. Walker

Post-Harvest Pathology of Fruits and Vegetables. Ed. by Colin Dennis. London: Academic Press, 1983. Pp. xi+264. ISBN 0 12 210680 6. £25.00

Notwithstanding the large increase in plant pathological literature during the last decade, the subject of post-harvest diseases has been much neglected. This in spite of severe and economically damaging losses of produce encountered in storage, marketing and during transport from the field.

The present text edited by C. Dennis is therefore a timely and much needed addition to our libraries. Seven of the nine chapters deal with specific crops or crop groups: soft fruit (C. Dennis); onions (R. B. Maude); carrots (B. G. Lewis and B. Garrod); brassicas (J. D. Geeson); salad crops (C. Dennis); and potatoes (C. Logan).

An introductory chapter by T. R. Swinburne deals with the important subject of latency or quiescence in disease development and expression. These chapters give a proficient coverage in diverse styles of symptoms, epidemiology, biology, biochemistry and control. In contrast to the other chapters, diseases caused by bacteria are covered for a wide range of crops under the heading of 'Bacterial spoilage' by B. M. Lund using as a basis for her chapter the common features of bacterial diseases. This opportunity for synthesis was not explored by some authors who treated crops and pathogens in an encyclopaedic manner with some unavoidable repetition. The authors rightly identify that many of the problems of post-harvest disease originate in the field and that control measures must begin here. Other aspects of control dealing with storage conditions apply to a wide range of fruit and vegetables and could have been considered to advantage under a collective heading. It is a pity that the title excluded the coverage of

many important crops, for example, the diseases of flower bulbs could have been dealt with appropriately in the chapter on onions. No mention was made of mushrooms or tropical fruit and vegetables except in the chapter on bacterial spoilage.

The magnitude of the problem of post-harvest losses was not emphasized and some authors made only passing reference to it in relation to their own topic.

In the light of the current debate and crisis surrounding the funding of Agricultural Research, the editor could have provided a valuable introductory chapter spelling out the scale of the problem and the cost of post-harvest losses to various agencies. Such an introduction (or epilogue) could have identified obvious future research targets and perhaps by reference to control have opened or demolished the 'farm gate' division between 'food', and fruit and vegetable production. The book makes essential reading to all interested in the subject and hopefully will stimulate others dealing with post-harvest diseases in tropical fruit and vegetables, flowers, bulbs, seeds and corms.

G. F. Pegg

Gums and Stabilisers for the Food Industry, Vol 2. Application of Hydrocolloids.
Ed. by Glyn O. Phillips, David J. Wedlock and Peter A. Williams.
Oxford: Pergamon Press, 1984. Pp. xii+569. ISBN 0 08 029819 2. £59.50.

The book is a compilation of the Proceedings of the Second International Conference on 'Gums and Stabilisers for the Food Industry', held in Wrexham, Wales, in July 1983. This is the second volume in the series and emphasizes the applications of hydrocolloids in the food industry.

The book is divided into six chapters, namely: 'Characterization–analysis'; 'Characterization–rheology'; 'Product and end-use areas'; 'Food processing/hydrocolloid–cause and effect'; 'Legisation and toxicology'; and 'Recent developments–future trends'. Each chapter consists of several 'papers', many given by experts in their own fields of research, and this reflects the whole standard of the book. Poster presentations have also been included in a similar format.

The importance of the subject area is reflected in the interest shown by many food scientists, linking food formulations of the naturally occurring and modified polysaccharides with the quality and characteristics imparted to the final product. Many of the examples quoted and data presented are of 'real-food' systems. The book thus highlights how many facets of the food industry are using this knowledge to give benefit to the consumer.

The physico-chemical properties of these compounds vary so widely with the source, structure, and can be modified and extended substantially within minor modification (e.g. derivitization). The analysis of these compounds is thus of primary importance. Chapter 1 presents an overview of this important area covering the latest rheological and chromatographic techniques. Although adequate, I would have liked to have seen more techniques such as C^{13} NMR and GC-MS in polysaccharide analysis. Chapter 5 provides a review to the current status of the legislation and toxicology concerning exudate gums, stabilizers and modified starches. I found this particularly useful and interesting.

Although a photocopy-set book, the editors' initial contribution is evident since most of the papers follow a similar format, each also being well illustrated, making the

book very 'readable'. A weakness of the book is the subject index which is by no means exhaustive. This book, following the first volume, is essential reading to all those workers in this area of research and technology. It is expensive.

S. Z. Dziedzic

Biophysical Methods in Food Research (Critical Reports on Applied Chemistry, Vol. 5). Ed. by H. W.-S. Chan.
Oxford: Blackwell Scientific Publications, 1984. Pp. viii+204. ISBN 0 632 01212 9. £19.50.

Until recently the very complexity of food systems has largely dissuaded physically trained scientists from engaging in serious research in these areas. However, this situation is being reversed and this volume details both the theoretical basis of a number of techniques and some applications to food materials. The problem is, as the authors recognize, that most foodstuffs arc multiphasic, heterogeneous and obscure. Therefore some of the classical physicochemical spectroscope techniques are largely inappropriate.

A question on the reviewer's mind, which no doubt also resided in those of the editor and chapter authors, is to whom is the book addressed. If its intention is to alert the physically trained scientist to the possibilities of food research, then the sections on applications are rather thin. If, on the other hand, the aim is to inform the food scientist about the theory and practical use of the techniques, the subject-matter may be too physically biased. Undoubtedly, the authors have served the food scientist well by presenting an account of the techniques in a relatively concise fashion, even though some intellectual labour may be required to comprehend them fully.

The title of the chapter on thermoanalytical methods could more accurately be limited to differential thermal analyses (DTA) and differential scanning calorimetry (DSC) as only passing reference is made to thermogravimetric (TGA) and thermomechanical analysis (TMA), both of which offer some potential in the field. After a general and useful outline of the techniques, applications to proteins, polysaccharides and lipids as well as some more complex systems are described.

In contrast, the chapter on 'Optical methods as applied to biopolymer solutions', by its very title accurately indicates a severe limitation in application of optical methods except in fundamental studies of relatively dilute systems. The chapter gives, however, an excellent, if forbidding (for the average reader), account of scattering of electromagnetic radiation. Interestingly, the author is finally tempted to stretch the boundaries of optics to refer to small angle x-ray and neutron scattering. The problem is that very few food systems are dilute biopolymer systems. However, there is no doubt that, with caution, useful information can be obtained which is relevant to more concentrated and complex systems.

A further chapter is devoted to reviewing the theory and more recent developments of nuclear magnetic resonance relevant to food systems. The treatment of the theory is concise but valuable. The discussion is then extended to the problem of interpreting the mobility of water molecules in food systems, the use of cross polarization techniques in examining solid components and n.m.r. imaging of biological systems. A number of references show how valuable is the technique and give some idea of its future potential.

The section on photoacoustic spectroscopy (PAS) is perhaps a little less satisfactory in its overall presentation since the justification for its use does not appear clearly.

The final chapter on rheological methods gives an excellent review of both theory and applications. This perhaps reflects the fact that rheological techniques have been more widely used in food systems over a longer period than has been the case with the other methods. The author's background in polymer science does possibly colour his approach.

This, then, is not an easy book to read. It is, however, one that is well worth purchasing and studying if you have the need and inclination, and certainly provides some ideas of what information the techniques described can give and where they may prove valuable.

J.M.V. Blanshard

Extrusion Cooking Technology. Ed. by Ronald Jowitt.
Barking, Essex: Elsevier Applied Science, 1984. Pp xx+2 2. ISBN 0 85334 297 0. £34.00.

The text is largely comprised of a series of papers taken directly from the *Journal of Food Engineering*, **2**, (2–4), 1983. These papers are presented in the same order as they appeared in the journal and offer a random selection of topics in the extrusion cooking field. A preface has been added giving useful suggestions for authors presenting papers on extrusion cooking. These guidelines were drawn up by Subgroup 1 (Extrusion Cooking) of the European Scientific Collaboration Programme Cost 91.

The text has an index drawn up by using key words extracted from the papers. This is only partially successful and is sometimes misleading (e.g. extrusion stability, p. 7, refers to the product not the extrusion process). It is generally better to search the papers than rely on the index.

The papers themselves are varied and interesting covering recent research in the extrusion cooking field. They include studies on soya texturization and the direct expansion of cereal starches, flours and grits for the production of products ranging from pre-gelled starches and animal feeds to expanded flatbreads. The use of extrusion cooking in bioconversions for alcohol production is covered in two papers and the nutritional impact of the process is summarized in a paper by Professor Asp.

The engineering aspects of extrusion cookers receive a brief examination with comparisons of the shearing actions of single and twin screw extruders.

Many of the papers which present experimental results describe the types of experimental designs which it is necessary to use in empirical studies of multivariate systems such as extrusion cooking. These are very useful for comparisons and for newcomers to the field. The book is of interest to research workers in the field who have not already seen the *Journal of Food Engineering*. However it is not a textbook for students, and the empirical nature of many of the studies limit their general usefulness in the longer term. The book is well presented with adequate illustrations and good text layouts and many useful references.

R. C. E. Guy

Food Industries Manual, 21st edition. Ed. by M. D. Ranken.
Glasgow: Leonard Hill, 1984. Pp. xiv+530. ISBN 0 249 44166 7. £75.00.

The 21st Edition of the Food Industries Manual will undoubtedly become a standard work of reference on the technical aspects of virtually every branch of the food industry. The last edition was published more than 14 years ago, and during that time there have been many changes in processing and manufacturing techniques. The editor has skilfully combined the basic properties of food materials and the well established methods and procedures with additional up-to-date information. The editor states that all the chapters have been extensively revised and some completely rewritten; two completely new chapters have been added—one on the beverages, coffee, tea and cocoa and the other on snack foods. The total work is, therefore, a comprehensive and authoritative handbook. All the authors have considerable practical experience in their areas of expertise, and the book is published under the auspices of BFMIRA, the Leatherhead Food Research Association.

The contents of each chapter of this sturdily-bound book are arranged alphabetically, thus forming a handbook and manual for easy reference. The majority of the chapters have their own bibliography containing up-to-date key reference books, review articles and important scientific papers, which will permit the reader to follow up any topic of particular interest. The index is substantial, allowing speedy location of the contents.

The first chapter on 'Meat and meat products' by M. D. Ranken provides a thorough compilation of topics including ageing, colour, flavours, muscle structure and function, together with newer information on nitrites and nitrosamines, pre-packed meats and re-formed meat. Chapter 2 is devoted to fish and fish products. Much valuable data is provided on storage life and the nutritional composition of fish along with the fatty acid composition of fish fats. The author, M. J. Urch, discusses fish farming, freezing and the case of the battered fish finger! The section on quality grading is particularly helpful with a multitude of descriptors. K. J. Burgess provides a chapter on Dairy products including useful summaries of butter and cheese manufacture, spray-drying and UHT processing. Flour and baked goods are presented by W. E. Estow and D. J. Wallington in Chapter 4. Breadmaking, including the Chorleywood bread process is summarized, and reference is made to a large number of topics. Although this chapter fulfils the purpose of a technical dictionary, there are several cross references and repetition which might have been avoided by longer summaries of a process and greater reliance on the final index.

There is an increasing interest in fats and fatty foods, and the chapter by J. B. Russell is exceptionally well written with a brief introduction, excellent summaries of chromatography, emulsification, fatty acids, hydrogenation of fats and the production of edible oils and fats. This chapter is well referenced and contains tables giving fatty acid composition and physical data on a wide range of vegetable oils and animal and marine fats and oils. Chapter 6 contains two sections by D. J. Millin on coffee and tea together with a third section on cocoa by L. Bradford. Each section outlines the production, processing, composition and quality assessment of these beverages; the sensory descriptors are particularly useful.

In the seventh chapter on fruit juices and soft drinks, D. C. Simmonds states in a short but pertinent introduction that although the processing of fruit juices and fruit juice products has altered little over the last decade, the scale of operations and the packaging and transport methods have changed dramatically, for example in the use of

frozen concentrates and the introduction of bottles made from polyethylene teraphthalate (PET). In Chapters 8 and 9, D. Cross and K. G. Anderson list the key aspects of 'preserves' and 'pickles and sauces', respectively. The former chapter deals in admirable detail with the manufacture of jams, jellies and marmalade, and the latter chapter describes thoroughly the uses of vinegar, the preparation of chutney, mustard, piccalilli and tomato ketchup, as well as having an excellent section on quality control.

Chapter 10 on confectionery products by R. Lees reflects on the fact that most of the developments have been improvements and refinements in established materials and processes rather than revolutionary changes. The chapter refers to the manufacture of boiled sweets, caramel and toffee, and chocolate. Chapter 11 on snack foods by R. G. Booth gives a good summary of extrusion processing, the production of potato snack products as well as sections on dips, nuts, olives and raisins. Whereas the alphabetical format of the chapters is used for all other chapters, the chapter on nutrition by A. E. Bender is arranged according to traditional concepts with energy, fats, proteins, vitamins and minerals as the main sections. Some of the areas of interest to food scientists were omitted such as recommended daily amounts, nutrient fortification, the factors influencing mineral bioavailability, dietary fibre, sodium, nutrition guidelines and nutrition labelling. Unfortunately this chapter did not identify any key references. Chapter 13 on freezing and refrigeration provided a succinct guide to the subject together with additional reading. Chapter 14 on dehydration and dried products by J. M. Dalgleish includes the general principles of this method of preservation, driers, freeze-drying and the storage of dehydrated foods. The following chapter by J. A. G. Rees and J. Bettison provides an excellent manual on canning. The power-packed fifty-two pages delve into the intricacies of the subject in commendable detail. Finally, in Chapter 16, A. E. V. Lilly conveys the reader through the terms used in the handling and storage of food products.

In my opinion, it is a pity that there was little reference to the general principles of food law, labelling and the various codes of practice governing advertising and promotions. Although it would be unwise and virtually impossible to cover all the ramifications of the regulations, summaries of the basic requirements and suggestions for further reading would have been useful in this vitally important and complex area. There are also a small number of typographical errors.

Nevertheless, there is no doubt whatsoever that this manual will be a standard reference work for all those concerned with food manufacture, and for the suppliers of ingredients, packaging and machinery. Most food scientists and technologists have a core of essential reference books to hand, and this volume would be a most worthy addition to the collection.

D. P. Richardson

Food Analysis: Principles and Techniques, Vol. 1. Physical Characterization. Ed. by Dieter Gruenwedel and John R. Whitaker.
New York: Marcel Dekker, 1984. Pp. xi+338. ISBN 0 8247 7181 8. SFr. 166.

This book is the first volume of an eight-volume treatise on food analysis. It consists of chapters contributed largely by individuals with considerable experience in their respective fields of study. Though entitled 'Physical characterization', the subject

matter includes chapters on statistical evaluation of data, sensory analysis, automated chemical analysis, as well as a chapter on the structure and operation of laboratory computers. In the stricter sense, physical properties are dealt with under the headings of colour measurement, rheological techniques and mechanical properties of foods. The editors intend the treatise to be used by graduate students and scientists involved in food analysis. With the exception of Chapter 1, (statistical evaluation of experimental data), the material in Volume 1 is introduced at a level appropriate to a newcomer with a chemical or physical background and is suitable for some undergraduate teaching. Chapter 1, on the other hand, requires a feel for statistical analysis and a knowledge of appropriate terminology. The chapters are referenced individually, the total number of citations being in the region of 640. These are important since the book is not intended as a handbook of methods of analysis and the reader is expected to refer to original work. The only chapters which contain a significant number of citations after 1979 are those on rheological techniques and computers; the greatest frequency of referencing in the book as a whole is for the period 1975–1979.

An advanced coverage of the topics (detailed above) to satisfy the research worker, together with introductory principles in 328 pages is ambitious and requires a concise format. This has been achieved without loss of interest or academic value. It is encouraging to see due emphasis being given to proper experiment design and sampling under the heading of statistics. Factorial experiments, which are frequently omitted from textbooks on statistics, are also discussed. The chapter on colour measurement is an excellent summary of the principles of tristimulus colorimetry and includes comments on the characteristics of rough surfaces and translucent materials. Listings of the interrelationships between various colour scales are useful and comparison of coordinates obtained using various instruments, interesting. The chapter on rheological techniques is stimulating and deals with a wide range of approaches to the measurement of mechanical properties of foods. This chapter should be very useful to anyone embarking, for the first time, on characterization of mechanical properties of foods. The author uses mainly c.g.s. units for stress and elastic moduli but unfortunately mixes these in places with the S.I. system of units. The chapter on automated chemical analysis is clearly out of place in this volume. It is a pity that the author devotes some fifteen pages to describing and showing equipment manufactured by his company. Use of this space for greater attention to principles would have been preferable. Numerous applications are described; one omission under the subheading of food additives is the use of an auto-analyser system for the determination of sulphur dioxide.

Overall this book is a worthwhile investment and should find its way onto many library shelves. The book has a very small number of typographical errors. There is a useful index, containing some 900 entries.

B. Wedzicha

The EEC and the Food Industries. Ed. by Alan Swinbank and Jim Burns.
Reading: Department of Agricultural Economics and Management, University of Reading, 1984. Pp. viii + 178. ISBN 0 7049 0815 8. £7.50 (including p. and p.)

The last few years have seen a growing awareness that the traditional approach to analysis of food policies within the Community has suffered from serious drawbacks.

Such studies as were undertaken tended to be limited in scope or examine this sector in terms of sectoral needs or a particular perspective. The most common of the latter views being that which saw the Food Sector in terms of agricultural policy, notably in terms of the CAP. Much of the credit for the re-examination of this approach must be given to the researchers of the Department of Agricultural Economics and Management at the University of Reading. Earlier works by this group did much to highlight the importance of:

(i) analysis which recognized the particular importance and distinguishing features of this sector;

(ii) research which examined the central role of the food sector in any process of policy formulation or implementation directed at the agricultural sector.

In this context this collection of readings, derived from the second in a series of Conferences on this theme is greatly welcomed.

The papers presented reflect certain aspects of the industry itself. Harris, in the first of the papers, points out that 'the food industry is not a monolithic structure'. Many of the authors highlight the diversity and the heterogeneity of the industry. The collection of papers is characterized by the same features. Harris's paper: 'The CAP and its impact on the EEC's food industries' is a well rounded overview which describes the challenge posed and dilemmas faced when exploring the interface between policy and practice in a diverse and difficult to define area such as this. In particular he notes the definitional problems posed when the Commission 'blurs' the distinctions between sectors in recent references to 'the food industry proper' as covering first and second stage processors. Despite these problems Harris skilfully identifies the challenges posed to the food industry by:

(i) 'The Commission's . . . implicit attitude to the Communities food industries . . . that they are the mechanism whereby the CAP is implemented.'

(ii) 'The lack of any formal involvement by the food industry in CAP decision making.'

(iii) 'The special difficulties of adjustment to this detailed regulatory management by newcomers from a different administrative tradition.'

He highlights the difficulties these factors pose to the process of producing effective strategies which are effective, implementable and useful throughout the food chain in the Community not merely in its parts. At the same time administrative action here will significantly influence relationships with other parts of the world, notably developing countries seeking to develop their processing industries.

This overview provides a valuable basis for the analysis of the ensuing papers. These vary considerably in the perspective taken on their areas. Blackburn's paper on 'EEC structural policy and the food and drink industries' highlights the policy gap in this area. She indicates how those concerning the food sector are typically derived from other areas or drawn up with virtually no reference to the sector at least in terms which could be thought of as an 'identifiable structural policy'; her paper raises many thoughtful issues for both policy makers and students of policy formulation in this field.

The theme of the importance of coherence and consistency in policy formulation is returned to in Flowerdew's paper. She explores the EEC food harmonization programme in terms of additives, standards and labelling. Here the technical problems of integrating policy in a diverse and dynamic situation are handled in a thorough and comprehensive way. She explores the reasons for the painfully slow progress in this field. Particular emphasis is given to the two levels of agreement required in this field: across the Community and within member states. Despite the thoroughness of the

review there is a sense in which this paper would be stronger with greater attention to influences outside the Community on this process. At the same time this paper might be expected to arrive at rather more specific policy recommendations.

Stocker's paper 'EEC Competition and the food and drink industries' ranges widely over the forces which influence competition. This paper deliberately goes far beyond the confines of the impact of EEC competition policy to incorporate examination of national policies, monetary compensatory amounts as well as market forces such as distribution policy and retail relationships. All these are considered in terms of the broad principles guiding Commission policy; the 'unity of the market' concept and specific Articles. Stocker highlights both the specific nature of the Commission's concept and the ways in which this can be used to explain apparent inconsistencies in implementation of policy. At the same time he draws out the constraints on the freedom of action and effectiveness of policy in an environment in which other major forces, national and commercial, can be more significant in the market-place. A particularly important aspect of this paper is the recognition given to the role of retail trade policy in the food chain. The combination of retailer power and increases in vertical integration make the neglect of this sphere of activity increasingly untenable. Stocker's paper is an important step in this direction.

In the type of programme described here sectoral analysis provides the bedrock of work. The papers by Wilkinson, Rees, Graham and Locke on Dairy products, Cereals, Sugar and Sweeteners and Meat and Meat products respectively effectively supply that core of work. Each highlights the direct and profound impact of policy on key industry sectors while the authors sound warning notes on the ways in which specific actions in this field can have much more wide ranging effects than perhaps anticipated. All these papers are well written and invaluable overviews of key sectors. They will be required reading for all those with a serious interest in this field.

The paper by Gray returns to the themes raised by many of the authors especially the challenge of integrating the 'supply side' perspective of the Commission especially in terms of its Articles and the importance and needs of 'down-stream industries'. His paper illustrates a welcome recognition of the chain-like nature of the food industry with its complex network of interconnected relationships. Despite this, the specific proposals are limited. It is especially disappointing that there is a failure to realize that a request for 'clear unequivocal statements' is likely to be disappointed. The recurrent comments on the diversity and heterogeneity of the industry would appear to make such a request unrealizable.

The concluding paper by Swinbank and Burns provides an excellent overview of both the preceding papers and the food industry within the EEC. They highlight the scale of the sector while drawing attention to its neglect in terms of both academic study and consistent policy at least on an EEC wide basis. Their paper pays particular attention to four themes:

(i) the difficulty of building Food Policy on the basis of policies notably CAP designed for other sectors and purposes;
(ii) the significance of external trading relationships on internal programmes;
(iii) the complex, diverse and, occasionally, divergent nature of the internal market;
(iv) the distorted and poorly balanced nature of the organizational arrangements set up to formulate and implement policy in this area.

In this paper Swinbank and Burns maintain the quality of their earlier work while providing an invaluable overview which will help both the expert and the novice to

increase their understanding of this challenging field. This reflects the wider value of the collection. At the same time there remains a disappointing lack of sustained empirical work especially of the interconnections in the chain. A similar problem exists with the relative neglect of the distributive sector in this area. These comments aside the authors are to be complimented on a well rounded and valuable collection.

T. Cannon

Bergey's Manual of Systematic Bacteriology. Vol. 1, 9th edition. Ed. by John G. Holt and Noel R. Krieg.
Baltimore: Williams and Wilkins, 1984. Pp. xxvii+964. ISBN 0 683 04108 8. £53.00.

The long-awaited ninth edition of *Bergey's Manual* has started to appear; 'started', because it will now be published in four volumes, of which the first substantial volume (nearly 1000 pages of 28×21 cm carrying text in a small typeface) has been submitted to this journal for review. The contents of this first volume, after introductory sections on how to use the manual, and on classification, nomenclature and identification, are divided into eleven sections describing the Gram-negative bacteria of general, medical or industrial importance. The other three volumes will cover: the Gram-positive bacteria other than the actinomycetes; the archaeobacteria, cyanobacteria and remaining Gram-negative bacteria; and the actinomycetes.

One immediately obvious change is that the title has been changed from '*Bergey's Manual of Determinative Bacteriology*'. This reflects a deliberate policy change by the Trust in expanding the scope of the *Manual* to include 'more information of importance for systematic bacteriology'. Aspects such as ecology, procedures for enrichment and isolation, and the maintenance and preservation of cultures are therefore included. Eventually the 'determinative' information will be incorporated into a smaller publication to appear under the original name of the *Manual*. Obviously when this second, shorter publication appears, it is likely to be the book preferred for day-to-day use by quality assurance microbiologists and similar workers whose main problem is concerned with the identification of isolates. However, the usefulness of the more comprehensive volumes should not be overlooked: for example in the discussions of the isolation procedures, detailed recipes for media are provided in footnotes.

The philosophy behind *Bergey's Manual* can be summed up by quoting from page 3 of the book:

> 'Some microbiologists seem to have the impression that the classification presented in *Bergey's Manual* is the "official classification" to be used in microbiology. It seems important to correct that impression. There is no "official" classification . . . (This is in contrast to bacterial *nomenclature*, where each taxon has one and only one valid name, according to internationally agreed-upon rules . . .) . . . bacterial classifications are devised for microbiologists, not for the entities being classified. Bacteria show little interest in the matter of classification. For the systematist, this is sometimes a very sobering thought!'

The book is attractively presented with many photomicrographs, electron micrographs, tables and diagrams. So much information is packed in by the use of the small typeface that frequent use of the book requires good or well-corrected eyesight in combination with good illumination! This is a book which *does* repay frequent use.

It is to be hoped that this Manual is given a place not only in libraries of colleges, universities, research institutes, but also in any industrial laboratories undertaking microbiological work of a non-routine nature. Even the smaller and/or routine quality assurance laboratory is likely to benefit from the acquisition of the ninth edition of *Bergey's Manual of Determinative Bacteriology* when it appears.

W. F. Harrigan

Books received

Torry Research Station, Annual Report, 1983.
Pp. iii+41. Torry Research Station, Aberdeen.
Short reports are presented on topics such as: Making the most of our fish; Surveys; Handling and processing; Shelf life studies; Composition, safety and standards; Monitoring and control of cold stores; and Byproducts.

Long Ashton Research Station, Annual Report, 1983.
Pp. 205. ISSN 0368 7708. £4.50 (post free in U.K. from the Station, Bristol BS18 9AF).

Amongst the work reported in this issue are: Extension of the use of plant growth regulators; Measuring leaf wetness to aid disease forecasting; Modelling the growth of wheat; Improving yield of winter barley; Plant pigments as potential food colours; A computer program (SENPAK) for handling the analysis of sensory profile data; and Rapid detection of microbial spoilage in food products by bioluminescence.

AFRC Letcombe Laboratory, Annual Report, 1983.
Pp. x+130. ISBN 0 7084 0312 3. London: HMSO, £4.00.

Reports include: Cellulolysis by soil fungi; NMR studies of nitrogen compounds in plant materials.

Lipid Histochemistry. By O. Bayliss High.
Oxford: Oxford University Press, 1984. Pp. iv+68. ISBN 0 19 856405 8. £4.50 (paperback).

The sixth in a series of microscopy handbooks produced by the Royal Microscopical Society. After a very brief introduction to the structure and properties of lipids, and a discussion of the application of lipid histochemistry to clinical pathology, methods are given for tissue fixation, and staining and other procedures.

The Production and Storage of Dried Fish. FAO Fisheries Report No. 279, Suppl. Ed. by D. James.
Rome: FAO, 1983. Pp. vi+265. ISBN 92 5 101343 8.

This book constitutes the proceedings of a workshop held at the Universiti Pertanian Malaysia in 1982. The main topic areas covered are: Production of dried fish; Methods

of drying; Alternative products (e.g. fish protein concentrate); Storage losses and quality standards; Physics of drying, water activity and its effect on storage; Economics, packaging and marketing.

Storage Pests, 1982. (Research and Development Report, Reference Book 251 (82)). London: HMSO, 1983. Pp. vi+81. ISBN 0 11242638 7. £3.75.

This book describes the research carried out during 1982. Topics covered include: The significance of diapause in the control of insect populations; Monitoring and detection of infestation; Effects of mites on feeds, and on man and animals (e.g. allergic responses); Control by modified atmosphere storage; Resistance to pesticides and the genetics of resistance; Control of malathion-resistant pests; Use of residual insecticides; Treatment of structures; Use of hormones, attractants and pheromones.

Development in Food Proteins, Vol. 3. Ed. by B. J. F. Hudson.
Barking: Elsevier Applied Science. 1984. Pp. x+304. ISBN 0 85334 271 7. £30.00.

The topics covered in this volume are: Composition of meat (M. D. Ranken); Haemoproteins in meat and meat products (D. A. Ledward); Proteolysis and protein-protein interactions in cheese manufacture (P. F. Fox); Food uses of sunflower proteins (F. W. Sosulski); Bacterial proteins (M. Schlingmann, U. Faust & U. Scharf); Chromatographic methods in the investigation of food proteins (R. Macrae); Reactions of food proteins during processing and storage and their nutritional consequences (R. F. Hurrell); and Antinutritional proteins in plants (A. M. R. Gatehouse).

The Book of Edible Nuts. By F. Rosengarten.
New York: Walker & Co., 1984. Pp. xxv+384. ISBN 0 8027 0769 9. US$ 35.00.

The history, botany, ecology, cultivation, processing and use (including typical recipes) are given for almonds, brazil nuts, cashews, chestnuts, coconuts, filberts, macadamia nuts, peanuts, pecans, pistachios, sunflower seeds and Persian walnuts. Shorter entries are also provided for a further thirty other nuts, seeds etc. including cola, jojoba, pumpkin seeds, sesame seeds, soybeans and watermelon seeds.

Practical Application of Azolla for Rice Production. (Developments in Plant and Soil Sciences, Vol. 13.) Ed. by W. S. Silver and E. C. Schroder.
Dordrecht: Martinus Nijhoff/Dr. W. Junk, 1984. Pp. viii+227. ISBN 90 247 3068 6. £24.25.

Enough is Enough: The Common Agricultural Policy.
London: Consumers in the European Community Group (UK), 1984. Pp. 25. £1.00 (from CGEC, 24 Tufton Street, London SW1).

Allergy to Chemicals and Organic Substances in the Workplace. (Occupational Hygiene Monograph No. 12.)
Leeds: Science Reviews Ltd., 1984. Pp. v+79. ISBN 0 905927 51 6. £7.00.

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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	$\mu = 10^{-6}$ m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

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