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A review of microwaves for food processing

A. J. H. SALE

Summary

Ideas for the application of microwave heating to the processing of food are reviewed. A selection has been made of ideas for pasteurizing, sterilizing, defrosting, dehydrating, cooking and other applications that are described in the literature. Several are discussed to illustrate particular aspects and characteristics of microwave processing, and to try and show some reasons for the successes and failures.

Microwave heating on its own has led to few commercially successful processes; however, when combined with conventional sources of heat, microwave heating appears to have greater potential, and has led to several successful processes.

Introduction

The idea of heating by microwaves has been around for over thirty years and its potential use for heating foods has always been one of the applications that has been studied. The predominant development has been in the use of microwave ovens for cooking and reheating food in the domestic and catering situations. Equipment manufacturers' efforts to introduce microwave heating into the food processing industry have met with little success: although many ideas have been put forward, often involving the development of special equipment, the majority have been discarded in various stages. However, there are several successful microwave processes in use.

The purpose of this paper is to review a range of ideas for applying microwave heating to food processing, to illustrate particular aspects of microwave processing, and to try and show up some reasons for success and failure.

Microwave heating has characteristics that make it different from conventional heating and are relevant to reviewing the applications. These are set out in the Appendix, but the main ones are as follows. Microwaves generate heat within the food itself, no heat transfer medium is involved and, there is little reliance on heat conduction within the food itself so the temperature of the food can be raised very rapidly. Micro-

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waves do not penetrate metal, so food cannot be heated in a can. In contrast microwaves pass through plastics, paper and glass so food sealed in these materials can be heated.

For the purpose of reviewing applications and ideas the subject may be rather arbitrarily divided in four main areas: heat preservation; change of state; cooking; others. The design of equipment will not be discussed.

Heat preservation

Heat preservation includes pasteurization, sterilization and blanching. In general the hope is that the short time of microwave heating compared to conventional heating will reduce the amount of heat damage to the food, and will cut down overcooking such as may occur in canning. However, the potential gain in quality may not be achieved unless cooling is also rapid. As there is no heat transfer medium the elimination of leaching during blancing is expected.

Pasteurizing

Bakery products provide an example of pasteurizing. Some experimental work on pasteurizing bread was published by Olsen (1965) and shortly afterwards a factory pilot plant was installed in England for extending the shelf life of cakes (Evans & Taylor, 1967). The problem being tackled was that between baking and wrapping, the cakes were inevitably contaminated by mould spores carried in the atmosphere. This contamination resulted in mould growth and spoilage of the cakes in a short time. One of the ways of inactivating mould spores is by heat, but to heat a wrapped cake by hot air would take an inordinately long time because the heat conduction through a foam structure is very poor. In contrast microwaves can heat such a structure easily.

Experiments on sponge cakes showed that the heating, and therefore temperature rise was reasonably even throughout, so the pasteurizing temperature could be reached without serious overheating. A microwave tunnel oven was installed, and then practical problems arose. Cakes were baked batchwise, the tunnel operated continuously, and the factory organization was such that cakes could not be left around for a long time to cool to room temperature after baking. So the cakes were fed into the tunnel at a wide range of temperatures, and emerged also at a wide range of temperatures, because it is the temperature rise that is constant. Consequently, to ensure that all cakes were pasteurized, many were overheated. Furthermore, when it came to pasteurizing filled or iced cakes, not only was there differential heating, but melting occurred. These problems, which could have been foreseen, led to the abandonment of the process.

Sterilizing

Sterilizing in the pack is an idea that has been around for a very long time. It is often suggested that the long heat treatment that is required in canning impairs the quality of the food; the long time results from the limitation of heat transfer internally, so

that to achieve adequate heat treatment throughout, much of the food receives excessive treatment. By using microwaves for the heating, and plastic packs to let the microwaves through, very much more rapid heating is possible. So although the cooling stage may be slow the overall process time is shortened and the hope is that better quality may result. The concept has several problems. First it presupposes that the heating is even, but this is extraordinarily difficult to achieve. With uneven heating the temperature reached in the food differs from place to place. As it is necessary to ensure that the coolest part reaches a high enough temperature for a particular time, other parts of the food reach higher temperatures and are overtreated. This illustrates another characteristic of microwave heating: that there is no natural temperature limitation when heat is continually generated in the food, except, for example, when boiling is reached and the heat goes into vapourizing water. In contrast when using a heat transfer medium, the food cannot get hotter than the medium. A practical problem in microwave sterilizing is the need to maintain the integrity of the flexible plastic pack; because temperatures in excess of 100°C are required the system must be pressurized, both for heating and cooling. Research continues, for example at the U.S. Army Natick Labs; they have built special pressurized microwave equipment to study the processing parameters involved in heating and cooling flexible packs of food (Kenyon *et al.*, 1971; Ayoub *et al.*, 1974).

The examples so far have utilized heating times of the order of a few minutes, which constitutes a considerable shortening over conventional heating methods. But even these microwave heating rates are modest in comparison with those potentially possible in special circumstances. Where we have a foodstuff being pumped through a pipe the microwave zone can be very compact; with high microwave power being absorbed in a small volume the heating time can drop to less than a second.

Some years ago we studied the sterilization of milk using microwaves to achieve extraordinarily short heating times (Unilever, 1970; Assinder, 1974). Ultra high temperature processes employing temperatures of say 140°C for a few seconds yield milk that tastes fresher than the product from lower temperature longer time sterilization. We were investigating the effect of higher temperatures and shorter times on flavour and sterilization. There is no benefit in achieving a very fast heat up and a short hold at high temperature, unless the product is also cooled extremely rapidly, so we had to devise a special form of apparatus.

We had to overcome the additional problem that if very hot milk is in contact with a surface, a deposit gradually builds up. If this is in the microwave zone, this deposit burns. Therefore, the whole process of heating, holding and cooling was carried out with a free falling jet of milk not touching the walls of the equipment, which was pressurized to prevent boiling because the temperature rose to 200°C. The heating time was about 40 msec with a throughput of 25 kg/hr at 5 kW microwave power. Holding at 200°C occurred during the free fall which lasted 130 msec, and virtually instantaneous cooling was achieved by turbulent mixing of the hot milk with jets of cold sterile milk

before reaching the base of the container. Thus only cold milk was in contact with solid surfaces. The sterile milk was kept cold by recirculation through a heat exchanger and was taken off at a rate to balance the hot milk input. This extreme high temperature short time process yielded a sterile product virtually indistinguishable from the starting material. Although technically successful, the process was too complex and costly to develop.

Blanching

Blanching of vegetables is an area where considerable work has been done to establish whether microwaves can offer an improved process (Anon, 1970; Dietrich, Huxoll & Guadagni, 1970). A big problem with the conventional methods using water or steam is the loss caused by leaching of solids—nutrients and flavours—during the prolonged contact with water. The claim for microwaves is that reduced heating time and the dry process improves the yield of solids. But without cold water the cooling stage is prolonged and there is some desiccation. Whether there really is any worthwhile improvement of quality is questionable but the reason that blanching of vegetables has not become a factory process is the economic one. Microwave equipment is expensive and should be used throughout the year rather than on seasonal operations like vegetable blanching.

Change of state

Defrosting

A range of important food raw materials are deep frozen in order to preserve their quality during transport and storage. Meat and fish are prime examples, and factories have the problem of thawing or tempering large blocks of the frozen material prior to further processing. Various procedures are used, a common one being to place the blocks in tempering rooms where they may stay for several days while reaching the desired temperature. In order to accelerate the process, warm air or water have been used; unfortunately, although these steps speed up thawing rates, they also tend to degrade the product. As the surface is warm for a long time, drying by air or leaching by water may occur, and there is a bacteriological hazard.

An opportunity exists here for the use of microwaves, which have a considerable penetration depth into frozen organic material. In theory, by overcoming the heat conduction problem, operations taking 24 hr or more could be reduced to a few minutes. A major difficulty prevents the straightforward use of microwaves in this application. The problem is that water absorbs power much more readily than ice, on account of relative dielectric properties. As soon as water is formed in any part of the food, then gross differential heating starts; the water absorbs energy and rapidly heats up, while large parts of the block are still frozen, a phenomenon known as thermal runaway. In general, surface regions will thaw first, absorb an ever increasing proportion of the available power and prevent effective power from reaching central regions.

So any operation involving complete thawing is fraught with problems. On the other hand, microwave tempering is much more attractive, i.e. raising the temperature of meat or fish to about —3°C. By taking care to avoid water formation, the thermal runaway does not occur and successful tempering should be possible. Applications to meat and fish blocks (Meisel, 1972) and to shrimps (Anon, 1973) have been reported. However, difficulties arise from irregularities in the food, e.g. salt pockets and voids in meat, which give rise to uneven heating (Decareau, 1975).

There are several manufacturers of large-scale tempering equipment including ABR,* Raytheon† and L.M.I.‡ The latter have introduced refrigerated air into the microwave tunnel to try to reduce thermal runaway. The equipment can temper in 10–30 min with throughputs from half to several tons per hour. The short thawing times can lead to simpler factory management. It is also claimed that drip loss is reduced with economic benefit, but the gain is only real if the drip is normally lost and not incorporated in the later stages of processing.

Dehydration

There are two distinct transfer phenomena that control drying rates and one or the other normally dominates in a particular drying situation.

One of these limiting mechanisms is the rate at which water vapour can diffuse to the product surface in order to escape. A process whose drying speed is limited by diffusion is mass transfer limited. Any attempt to increase the drying rate by putting in more heat, is accompanied by temperature rise. So if the upper permissible temperature limit for the product is reached, then no further increase in speed can be achieved.

The other limiting mechanism is the rate at which heat can be supplied to the product interior in order to promote evaporation. No matter how porous the material is to water vapour, the drying speed will be limited by heat transfer across the product surface and subsequent conduction, i.e. the process is heat transfer limited. In conventional processing the heat transfer is increased by raising the surface temperature (e.g. using hotter air) but this is limited by the need to avoid heat damage.

Examples of process limited in these ways, might be drying of vegetables (mass transfer limited) and drying of a porous sponge (heat transfer limited). We can at once see which of these two types of process could benefit from microwave heating. In the mass transfer limited process, there will be no benefit from internal microwave heating and the most probably result will be a scorched product; heat transfer limited processes, however, can be expected to benefit from the extra rate at which microwaves can supply heat internally.

Heat transfer imposes a severe limitation to the freeze drying process. At first sight microwave heating would appear to be a natural answer to the problem as microwave

* ARB Food Machinery Co. Ltd, Bletchley, Milton Keynes.

† Raytheon Co., Waltham, Mass., U.S.A.

‡ Les Micro-Ondes Industrielles, Epone, France.

energy passes through a vacuum, and heats the food internally. Although microwave freeze drying has been studied for many years, no commercial process has evolved: this is probably due to the poor economics as well as technical problems such as the tendency to thermal runaway, and corona discharge which can occur in a very low pressure atmosphere (Grimm, 1969; Ma & Peltre, 1975).

Microwave heating is also being applied by LMI to vacuum dehydration, where the pressure is not so low as in freeze drying and the food is not frozen (Huet, 1974). Especially when the food forms a foam in the vacuum, the ability of microwaves to heat such a good thermal insulator is an advantage over the conventional vacuum drier with its heated belt or trays. This should lead to much shorter drying times.

The thermal drying of potato crisps at one time accounted for most of the installed microwave power in the food industry (Anon., 1969; Porter *et al.*, 1973). The problem that needed a solution was the excessive discolouration that occurred during frying when potatoes of high reducing sugar content were used as raw material. The occurrence of such potatoes was both seasonal and variable from year to year, the sugar level depending on weather conditions during growth. A large part of the harvest was often unsuitable for production of crisps and made raw material costs high.

When it was discovered that if the crisps were subjected to finish drying by microwaves after frying to the correct colour acceptable products resulted even if high-sugar potatoes were used, it was believed that any capital cost expended to introduce the microwave systems would be repaid in a very short time in terms of raw material cost savings.

Unfortunately many factors combined to render the process obsolete, and these are discussed as a case history by O'Meara (1973). The main factors were: the difficulty of controlling the final moisture content at a low enough level, with attendant texture problems; the organization of the industry into fewer larger units; and the installation of improved raw potato storage facilities, so that the need to use high sugar potatoes tended to disappear. In fact the microwave process had stimulated the development of improved and new processes based on conventional technology. Since the original problems have disappeared the outcome is that microwave terminal driers have become obsolete and are no longer used for crisp production.

In contrast the drying of pasta is proving successful (Maurer, Trembley & Chadwick, 1971; Anon., 1974). The process uses a combination of microwave heating with hot air for drying. Ordinarily pasta is dried at around 40°C in controlled humidity and it takes about 10 hr. These conditions are ideal for bacterial growth so that bacterial contamination is an ever present problem. The driers have to be very large to cope with the long drying time. Typically the microwave process consists of a first stage air drying to around 25% moisture, followed by drying with microwaves combined with air at about 100°C and ending with cooling in a controlled atmosphere. The overall time is about half an hour. The advantages are: great saving in floor space as the equipment is compact, negligible bacterial problems as high temperatures are used, the product is

claimed to have improved rehydratability, and the overall energy consumption is reduced. Several pasta manufacturers in the U.S.A. are using the process with equipment made by Microdry.*

Cooking

There have been many ideas for cooking with microwaves, but for many the high capital cost of microwave equipment rules them out when conventional methods are cheap. Nevertheless, some processes are viable.

The precooking of chicken parts by combined microwave and steam heating had considerable publicity (May, 1969; Smith, 1972). Plants with throughputs of 1·5 tons/hr have been described. The product advantages claimed included reduction in bone darkening and a succulent product. On the economic side, the extra cost of processing was to be more than offset by increased yield and reduced processing time. In at least one operation an important fact was lost sight of: the final breaded chicken pieces were sold on an item basis and not on a weight basis. So the money and effort put into increasing the yield was wasted. This should have been realized without doing any microwave work. However, another manufacturer has used a microwave process for cooking chicken for several years.

A process has been developed in Sweden for cooking meat patties prior to freezing, using a Scanpro† microwave tunnel (Bengtsson & Jakobsson, 1974; Nilsson, 1975). The process has superseded deep fat frying with the following advantages claimed: a shorter processing time and improved economy resulting from higher yields and lower fat usage.

Precooking can also be used to heat set meat to make it suitable for subsequent process operations or handling. An example of this type of operation has been demonstrated by ABR. Comminuted meat is extruded through a plastic tube passing through a microwave applicator, where in a short heating length the meat is heat set in about a second, and emerges as a rod and is cut into lengths. The compactness, high speed, cleanliness and efficiency make this process attractive. It opens up the possibility of producing skinless sausages without the use of temporary casings thus making the process cheaper.

Also in the meat industry microwaves have been used to precook bacon (Lactronica & Ziemba, 1972), and at the Natick Laboratories there is a study using a special oven employing two microwave frequencies, infra-red, and steam to find the optimum combination for roasting (Decareau, 1975).

In the bakery industry there is one of the most successful applications of microwave power in the food industry in U.S.A. (Schiffmann *et al.*, 1971). This is the doughnut proofing system developed by DCA Food Industries.‡ The proofer was developed to

* Microdry Corp., San Ramon, Ca., U.S.A.

† Skandinaviska Processinstrument AB, Bromma, Sweden.

‡ DCA Food Industries Inc., New York, U.S.A.

provide a rapid, compact, hygenic means of proofing yeast-raised dough with little labour. Conventional proofers tend to be large, slow, labour intensive, difficult to clean, and difficult to control. The microwave system reduces the proofing time from 30–40 min to 4 min, and does it with production cost savings. More than twenty units have been installed.

DCA have also developed a microwave doughnut frier. Microwaves are combined with deep fat frying to provide larger doughnuts from a given weight of dough with better quality than from conventional frying. It is the property of microwaves to generate heat internally that is being used here to advantage by the baker.

The use of microwave energy combined with hot air for baking bread has also been studied (Chamberlain, 1973). The process has been shown to be technically feasible and can utilize soft wheat flour. However, the very high air temperature gives rise to the problem of finding a suitable container for the dough that is transparent to microwaves, i.e. non-metallic.

Other applications

There have been numerous other ideas for exploiting microwaves for a wide variety of foods and associated operations. A few examples are listed here, without any attempt at completeness or criticism.

Pasteurization of hams (Bengtsson, Green & Del Valle, 1970)
Dehydration of potatoes and apples (Huxoll & Morgan, 1968b)
Quick cooking rice (Huxoll & Morgan, 1968a)
Cooking meringues (Baldwin, Upchurch & Cotterill, 1968)
Inactivation of alpha-amylase in flour (Aref, Noel & Miller, 1972)
Controlling insects in stored grain (Nelson, 1972)
Heating soya beans to improve the nutritional properties (Wing & Alexander, 1975)
In Japan, preparation of snack products, roasting Laver (a seawood food) and roasting nuts (Suzuki & Oshima, 1973)

Finally, the activity in the field of the use of microwaves for catering should not go without mention. *The Journal of Microwave Power*, for example, carries reviews of this aspect as well as reviews of microwave heating in industrial application (see, for example, Vol. 8, p. 123–78). Bengtsson & Ohlsson (1974) have also reviewed industrial applications.

Economics

The economic aspect must be examined when considering ideas for microwave heating. As a source of heat, microwaves are basically expensive and the capital cost is high. Therefore the maximum utilization of equipment should be aimed for, and ideally the applications should be to processes operating all the year round and operating continuously rather than batchwise. The introduction of microwave heating can have

repercussions on other parts of a process and therefore cost comparisons should be done on whole processes from raw material to final product, that is if conventional processes exist. Savings sometimes appear from places that are not obvious, but in doing comparisons, at least the following should be included.

Services (electricity, steam, water, gas)
Capital (depreciation)
Maintenance
Labour (and the degree of skill used)
Cleaning
Space
Yield of product and wastage

Rough costings should be done as soon as they can realistically be done in a project. Some examples have been given where effort was wasted by not stopping uneconomic projects. Where economics are reasonable one can obviously consider proceeding with technical development.

Conclusions

In considering whether to invest capital in microwave heating, a food processor should acquire a sufficient understanding of microwaves, of the advantages and the pitfalls, and benefit by past experience of the projects that have gone wrong (O'Meara, 1973) and those that have succeeded. He should systematically examine both the technical (Bedrosian, 1973; Assinder, 1974) and economic aspects at the appropriate stages of a project.

Microwave heating is so different from conventional heating that its use should be borne in mind when technical problems arise with products and processes. It should also provide new thinking that may sometimes result in improved traditional techniques, but also generate new ideas.

Microwave heating on its own has not led to many viable processes. Rather it is the combination of microwaves with conventional sources of heat that has generated the recent successes like pasta drying and doughnut frying. It is the combination that has been somewhat ignored in research and development. Here probably is the greatest potential.

Appendix

Microwave characteristics

Microwave heating differs radically from conventional heating. It has a number of characteristics that need to be appreciated in the assessment of potential ideas and the development of applications, whether or not it is allied with conventional heating.

(a) Microwaves generate the heat directly within the food. Heat transfer at the surface and heat conduction internally are not involved except as secondary effects that modify the temperature distribution. They show to particular advantage in heating

foams, e.g. sponges, which have a structure that makes them naturally good heat insulators, and are most difficult to heat conventionally.

(b) Microwaves can generate rapid temperature rises, very rapid in special cases; but this advantage may sometimes be offset by a limitation that only conventional processes are available for cooling.

(c) Microwaves cannot heat food inside cans or aluminium foil containers because metal acts as a barrier and reflector of microwaves. This property is used in equipment to confine and control microwaves to heat in specific ways.

(d) Microwaves readily pass through many materials which can be used to contain food, e.g. polythene, polypropylene, paper, glass.

(e) When a food is being heated by microwaves the generation of heat is continuous and so there is a continuous rise of temperature. The longer the microwaves are applied the higher the temperature and there is no automatic limit to the temperature reached, in contrast to conventional heating in which the food does not rise above the temperature of the heat transfer medium. If the food is wet, microwaves raise the temperature to 100°C when the continued generation of heat boils off free water. When the free water is gone there is nothing to hold the temperature at 100°C any more and the temperature of the nearly dry food can rise rapidly with the risk of burning.

(f) Both wet and dry food can be heated by microwaves, but wetter food is heated more strongly than drier food. This effect is more marked when wet and dry food are present together. Other differences in composition (e.g. lean, fat, salt content) can also cause differential heating.

(g) Uneven heating also happens as a result of the geometry of the food. In an ordinary microwave oven a frequent phenomenon is edge overheating (e.g. a disc of meat). A flat rectangular slice may have not only exhibit edge overheating but corners heating even more so. Thin sections may heat more than thick ones. Not only shape but also size is important. For example, if an apple is heated by microwaves the core boils before the skin gets hot, but when the sphere is large enough the surface heats more than the centre. Nevertheless, a great deal can be achieved by specially shaping the microwave applicator and also the food to get reasonably even heating. When uniform rise of temperature is achieved, reproducible final temperature requires reproducible starting temperature, or automatic control to allow for varying input.

(h) One of the most difficult situations is thawing when thermal runaway may easily happen. This is due to the fact that water absorbs microwaves more strongly than ice. So the first region to thaw tends to preferentially absorb the microwave power and runaway in temperature, and boiling may occur close to a still frozen region.

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Quantitative analysis of food products by pulsed NMR

I. Rapid determination of water in skim milk powder and cottage cheese curds

R. E. HESTER AND D. E. C. QUINE

Summary

Water contents in the range 1–5% for skim milk powder and 77–81% for cottage cheese curds have been determined quantitatively by pulsed nuclear magnetic resonance methods. The procedures for establishing optimum operating conditions for a commercial pulsed NMR process analyser are described, and temperature-dependences of the analytical results are given. For skim milk powders, integrated measurement times of 72 sec per sample gave a standard deviation of 0·2% water, while at the other end of the range the cottage cheese samples yielded 0·3% standard deviation in the water content with measurement times of 10 sec per sample. The results demonstrate the versatility of the pulsed NMR technique for analysis of food products over a very wide range of water contents.

Introduction

The water content of many food products has an important effect on their physical, chemical and biochemical properties. For many foodstuffs a maximum water content is prescribed by law. Producers of skim milk powder aim to control the water content of their products to particular levels within the range 2–5% water, the figure used depending on the intended use of the product. For cottage cheese manufacture, a legal maximum of 80% water is specified. These foods are produced commercially on a very large scale, both continuous (e.g. spray driers for milk powder) and batch processes being used. In neither case is there a rapid method for water determination in common use in the industry, although for process control and for economic reasons rapid methods clearly are recognized as being highly desirable.

A variety of physical methods have been applied to the problem of determining water in foodstuffs (Kern *et al.*, 1974; Goulden & Manning, 1970; Anderson & Berlin, 1974; MacLeod, 1973), including infra-red absorbance and reflectance methods, dielectric measurements, microwave absorption, and broad line continuous wave proton magnetic resonance (cw NMR), but none of these have been widely adopted

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for use with either of the two major food products considered here. Pulsed NMR instrumentation has been available for several years, but only recently have instruments suitable (in terms of capital cost and simplicity of operation) for process control work in the food industry been manufactured. The pulsed NMR technique now offers possible solutions to a number of hitherto intractable problems in food analysis. Compared with the oven-drying methods currently in general use in dairy laboratories, pulsed NMR offers speed, analytical simplicity, and the potential for on-line process control. The extent to which these advantages may be realized will become apparent through the results reported in this paper.

Experimental methods

All measurements reported in this work were made with the Praxis model PR-103 pulsed NMR process analyser (manufactured by the Praxis Corp., San Antonio, Texas 78228 USA). This instrument incorporates a temperature-controlled permanent magnet of 2·51 kG (0·251 T), between the pole pieces of which is fixed a 25 mm diameter sample holder. The radiofrequency pulser and receiver coil which surrounds the sample holder operates with a frequency of 10·72 MHz. This magnet/sample probe assembly is housed separately from the electronic control and readout unit, providing some flexibility in the experimental arrangement. Plates 1 and 2 show photographs of the instrument.

The operation of the instrument may be understood in general terms with the aid of the block diagram given in Fig. 1. Measurement commences automatically with the

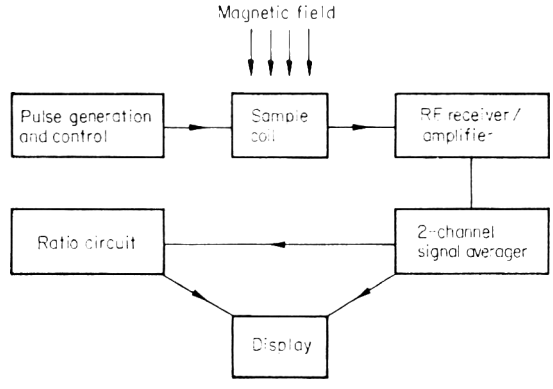

FIG. 1. Block diagram of the Praxis PR-103 spectrometer.

insertion of the sample tube into the probe assembly. Signals received after applications of short pulses of radiofrequency radiation are accumulated in a two-channel box car integrator and displayed, after automatic signal averaging, on the front panel digital voltmeter (see Plates 1 and 2). A wide variety of operating modes are available with the

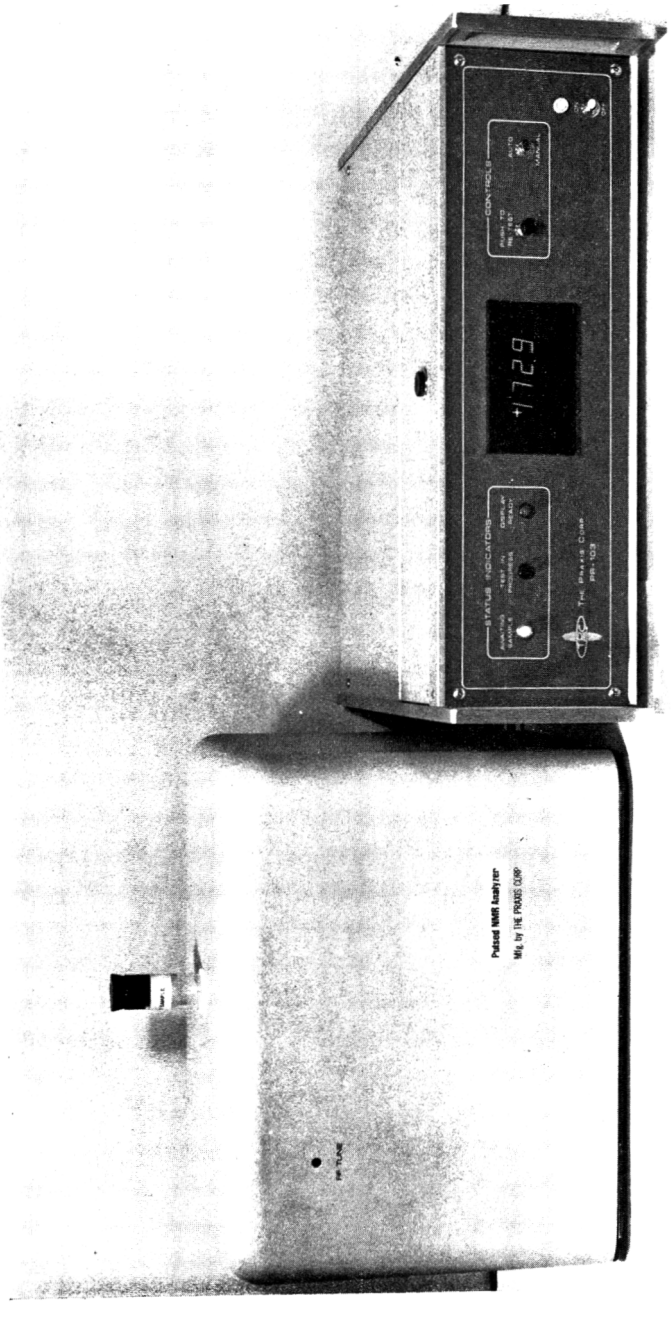

PLATE 1. The Praxis PR-103 pulsed NMR spectrometer, showing the separate sample probe and control/readout units.

(*Facing p.* 332)

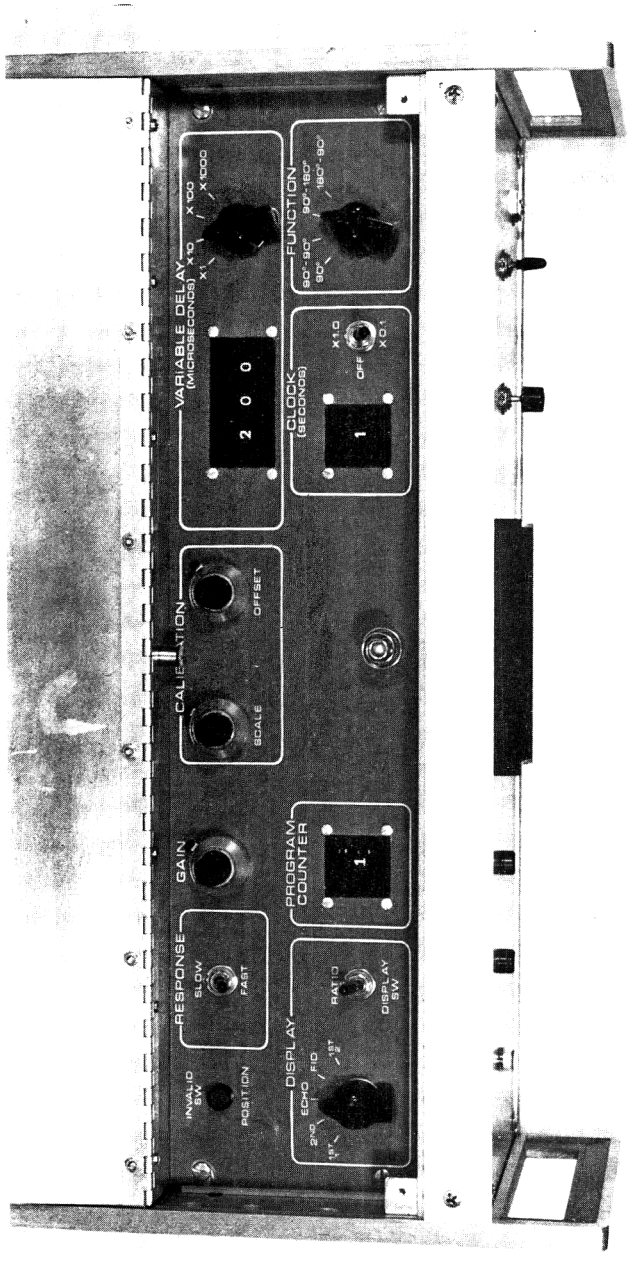

PLATE 2. An expanded view of the recessed control panel housed below the lid at the front top of the control/readout unit.

instrument, involving different combinations of 90° and 180° pulse sequences (Farrar & Becker, 1971; Slichter, 1965; Abragam, 1971), different pulse delay times, signal averaging controls, direct or ratio signal output, etc. The meter may be calibrated once the operating mode has been established, so that direct readout of the analyte sought (e.g. % water) is displayed. Typically, a complete measurement sequence, including full relaxation and return to the starting point, occupies approximately 0·6 sec, so that operating in the automatic mode around 100 consecutive measurements may be made and averaged by the instrument in a minute. This feature greatly improves the signal-to-noise ratio (SNR) achievable as compared with direct display of the measured signals.

For the work reported here a simple 90° pulse measurement was made for the skim milk powder samples. A more complex 90°–τ–180° spin-echo sequence was used for the cottage cheese curds, with the second pulse delay time, τ, set at 15 msec. For both types of sample the signal averaging facility was employed.

Standardization of the sample presentation was necessary for the milk powder, and this was achieved by pressing the powder to a standard tablet form in a conventional laboratory press. However, for the cottage cheese curds no sample standardization was needed due to the automatic instrumental correction provided by the ratio mode of operation. Samples were blended to a smooth and uniform consistency in order to reduce sampling errors, and were loaded into the 25 mm diameter sample tubes by extrusion through a simple plastic syringe. No weighing of these samples was required.

The instrument probe was set at several degrees above ambient temperature for this work, specifically at 31°C. Measurements were made directly on room-temperature samples and also on samples pre-equilibrated in a thermostat to the probe temperature.

Skim milk powder samples were subjected to an oven drying procedure to determine their water contents. This involved 5 hr at 100°C in a fan-assisted oven, the samples being cooled in a desiccator before re-weighing in dishes with lids. The method for cottage cheese curds involved drying 5 g samples in a vacuum oven at 70°C and 15 torr (i.e. $2{\cdot}0 \times 10^3$ Pa) for 1·5 hr, the curds being spread in the weighing dishes to expose a large surface area. In each case the methods were checked for reproducibility. The samples used for analysis were taken directly from tubes with which pulsed NMR measurements had been made.

Results

For measurement of water in skim milk powder, the instrument was found to have best sensitivity when operated in the free induction decay (FID) mode. Figure 2 shows results of measurements on one type of skim powder, with two different water contents obtained by part-drying. Full instrument gain was needed for these measurements due to the intrinsically low amplitude of the water signal. Averaging of the noise in the measurement was made in Auto mode with a Program Counter setting of 5,

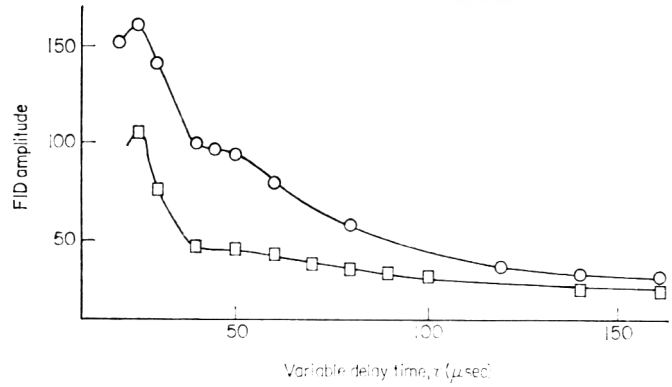

FIG. 2. Free induction decay (FID) signals from skim milk powders of differing water content. ○ Water content 78%; □ water content 80%.

corresponding to 180 sequential measurements, each of 0·4 sec duration, i.e. with a total measurement time per sample of 72 sec.

In contrast to the skim milk powder results, the instrument yielded effectively zero sensitivity in the FID mode to variations in water content in cottage cheese curd samples. For these samples, which gave very much higher signal amplitudes, requiring an instrument gain of only 1·9 (19% of full scale), both the 90°–90° and the 90°–180° functions gave reasonable sensitivity to water content. Figure 3 shows results obtained

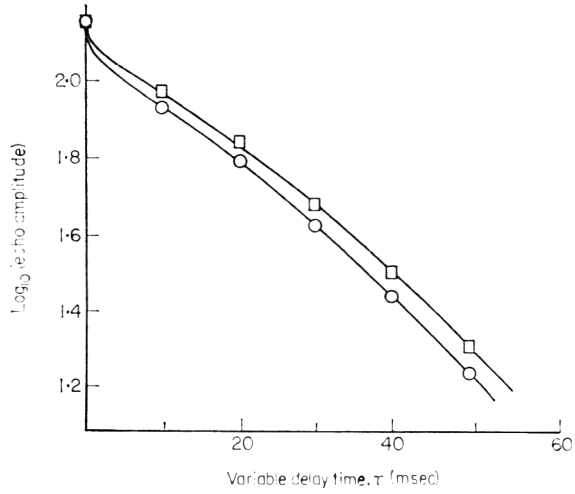

FIG. 3. Spin-echo measurements from cottage cheese curd samples of differing water content.

from cottage cheese curd samples with 78% and 80% water, using the 90°–180° 'spin-echo' function. These results were obtained in the Manual mode with no signal averaging, approx. 10 sec time being allowed for stabilization of the readings.

Direct readout of first pulse amplitudes for skim milk powder tablets of varying water contents in the range 1–5% gave the results shown in Fig. 4. Several skim powders of different origin all conformed to this calibration within a standard deviation of 0·20% water. Whey powders and full cream milk powders did not, however, fit the calibration established in Fig. 4, interference from their fat contents resulting in positive deviations

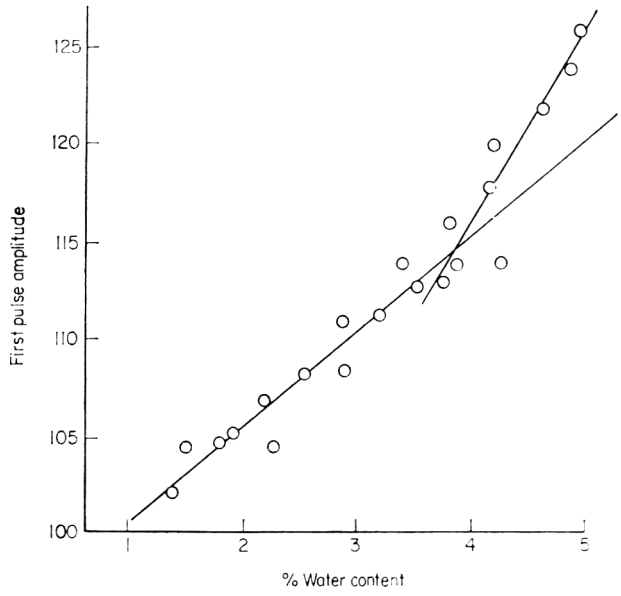

FIG. 4. Calibration of instrument first pulse amplitude v per cent water content for skim milk powder samples.

from the skim powder calibration. The results shown in Fig. 4 were obtained with a measurement time of 72 sec per sample.

From the results shown in Fig. 3, measurement of the echo amplitude with a variable delay time setting of 15 msec was selected for the analysis of water in cottage cheese curds. The ratios of these signals against the first pulse amplitudes were measured automatically to obtain a set of results which were independent of the sample weight and packing density. Results of such ratio measurements for samples prepared from a single batch of curd, with water content variation produced by whey drainage, partial air drying, or addition of small amounts of water, are shown in Fig. 5. These gave a standard deviation from the calibration line of 0·38% water. However, the reproducibility of individual measurements, including re-packing the sample, corresponded to only 0·05% water. No significance is to be attached to the absolute values of the instrument readings plotted, since these may be changed at will by adjustment of the

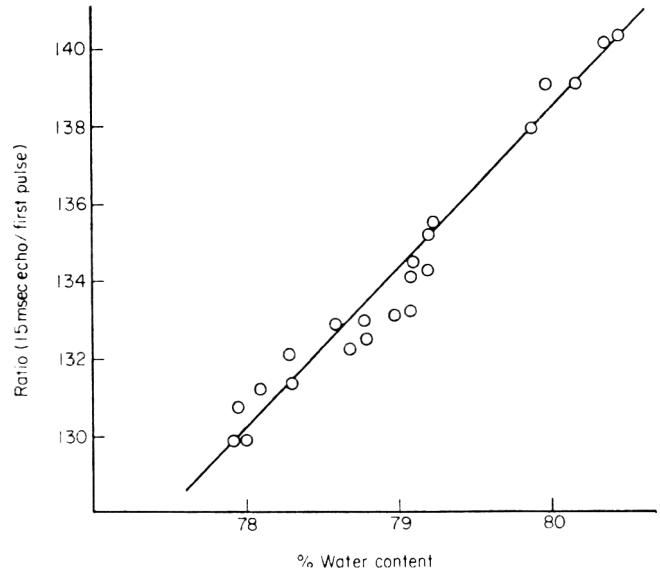

FIG. 5. Calibration of instrument readout in ratio (15 msec echo/1st pulse) mode *v* per cent water content for cottage cheese curd samples.

instrument Scale and Offset controls (Plate 2), these controls being set for a series of measurements simply to give conveniently large readout values.

The data given in Table 1 are a typical set of results obtained with cottage cheese

TABLE 1. Ratio (15 msec echo/1st pulse) readings obtained from cottage cheese curd samples taken from many different batches

Reading	% water	Reading	% water	Reading	% water
135·5	78·8	140·7	80·7	132·8	78·8
136·1	78·9	132·8	78·6	132·9	79·0
139·0	80·4	132·3	78·8	132·1	78·2
138·9	80·2	129·8	77·9	137·8	80·1
140·0	80·7	130·2	78·5	132·0	78·2

curd samples taken on a daily basis from a dairy and thus from many different batches of curd. Different Scale and Offset control settings were used here, but the data still fit a straight line with a standard deviation of 0·31% water compared with the standard vacuum oven drying results. For routine analytical use, clearly the instrument controls would be left at fixed settings in order to maintain a constant calibration line.

Discussion

The preliminary stage of a pulsed NMR analysis of any food product consists of establishing optimum operating conditions for maximizing instrumental sensitivity to the parameter sought: in this case, water content. In case of cross-interferences from other components, some trade-off of sensitivity may be required to minimize interferences. Considerable flexibility is offered by the technique, which readily yields values for characteristic relaxation times (Farrar & Becker, 1971; Slichter, 1965; Abragam, 1971) T_1, T_2 and T_2^*. For hydrogen-containing materials in the liquid state (oil, fats, water) the relaxation times normally are much longer than those for solid state materials (proteins, carbohydrates, etc.), so that these are readily distinguishable. Persyn & Rollwitz (1970) have demonstrated the principal features of the relaxation behaviour in food products, and van Putte & van den Enden (1974) have applied the method to the analysis of fats.

Skim milk powder samples with water contents in the range 1–6% were found in this work to give only weak liquid signals, necessitating the use of high instrumental gain conditions for their analysis. This led to relatively high noise levels on the received signals, and correspondingly poor measurement reproducibility. This problem was, however, overcome by the simple expedient of compressing the powder to form standard tablets, thus increasing the SNR to an acceptable level. Although a manual procedure was used here, involving a standard weight (8·8 g) of powder in a 22 mm diameter stainless steel die at 5 tons pressure, the tableting process may also be automated. Suitable reciprocating and rotary tableting presses are commercially available (e.g. models F3 and D3B from Manesty Machines Ltd, Liverpool). Preliminary experiments have resulted in the successful production of standard tablets, although in the absence of a binder these tend to be rather fragile. Manual preparation of standard tablets is a 5 min task.

The normal operating modes for the spectrometer are: (i) a 90° pulse, followed by measurement of the free induction decay signal as a function of time, τ; (ii) a 90° pulse, followed by a second 90° pulse after a variable time interval, τ, with measurement of the signal amplitude after the second pulse; and (iii) a 90° pulse followed by a 180° pulse after a variable time interval, τ, with measurement of the signal amplitude at time 2τ. One channel (hard wired) of instrument always monitors the signal amplitude following the first pulse (strictly, after the 'dead-time' interval of 11 μsec, during which the receiver is blanked off). A second channel monitors signals at any other time interval following the pulse or sequence of pulses. By ratioing the signals in the two channels the instrument can automatically compensate for variations in sample size, bulk density, etc.

For skim milk powder samples the magnitude of the free induction decay (FID) signal was found to depend strongly on the water content (see Fig. 2). However, the relative amplitudes of the FID signals at different time intervals following the first pulse were found to show little dependence on water content. Thus an analysis for water using the FID ratio mode would give very poor sensitivity. The 90–τ–90 mode similarly

was found to be insensitive to water content, and the spin echo measurement following a 90–τ–180 sequence was too weak for useful measurement. Accordingly, direct measurement of the total signal amplitude following the first pulse is seen to provide the best method for performing this analysis. As indicated earlier, standardization of the sample presentation is necessary in this mode. The change in slope in the calibration line at *c.* 4% water (see Fig. 4) may be due to a change in the nature of binding of the water molecules in the powder matrix.

At the much higher levels of water which are present in cottage cheese curds, a totally different set of nuclear spin relaxation characteristics were found. The 90–τ–180 mode was found to be most sensitive to the water content. Ratio measurements of the spin-echo amplitude for a delay time $\tau = 15$ msec against the first pulse signal amplitude were made, thus eliminating any need for sample standardization. These signal amplitudes were much larger than those obtained from the milk powder samples, and the better SNR values resulting obviated the need for signal averaging. The excellent repeatability of the pulsed NMR result for a single sample suggests strongly that the major sources of error for the cottage cheese curds analyses are in the sample inhomogeneity (sampling error) and oven drying procedures (S.D. of repeatability found to be 0·20% water). Serious instability in measurements obtained with whole curds was associated with the draining of whey from the samples, and this was greatly reduced by homogenization.

The analytical results reported in the previous section all were obtained from samples pre-equilibrated to the temperature of the sample probe. For both types of sample the first pulse signal amplitude increased by approximately 4% in 40 min on raising the temperature by 10°. Clearly, this temperature pre-equilibration could be eliminated at the cost of some increase in the variance of a set of measurements.

Finally, it must be emphasized that the useful results demonstrated in this work may be invalidated by the addition of a few per cent liquid fat to the products analysed. Thus, for whey powder, full cream milk powder, or for cream-dressed cottage cheese, different procedures must be established to compensate for the cross-interferences between their water and liquid fat contents.

Conclusions

(1) The Praxis PR-103 process analyser offers a rapid method of determining water in foodstuffs over a wide range of composition.

(2) Compared with oven drying methods, the pulsed NMR method gives a standard deviation of 0·2% water in skim milk powder and 0·3% water in cottage cheese curds.

(3) The pulsed NMR instrument offers several different modes of measurement, and the optimum mode must be determined empirically for each foodstuff.

(4) Pulsed NMR measurements may be temperature-sensitive. Best results are obtained by using samples which have been pre-equilibrated to the instrument probe temperature.

Acknowledgment

We should like to thank Foss Electric (U.K.) Ltd for loan of the Praxis PR-103 instrument, the English M.M.B. and Northern Dairies for providing samples, and the Science Research Council for a research studentship (for D.E.C.Q.).

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The control of enzymic browning in fruit juices by cinnamic acids

JOHN R. L. WALKER

Summary

The use of cinnamic, *p*-coumaric and ferulic acids to control the browning of apple juice has been investigated. Concentrations of cinnamic acid greater than 0·5 mM effectively prevented browning for over 7 hr. A simple method for estimating the minimum concentration of inhibitor required for a particular variety or blend of juice is outlined.

Introduction

The enzymic browning of fruits and fruit juices is brought about by the interaction of *o*-diphenol oxidase* (*o*-DPO) with its substrates which are naturally present in the fruit and this oxidation is usually controlled by the addition of ascorbic acid during juice preparation. An alternative approach is to use *o*-DPO inhibitors and a number of workers have shown that the *o*-DPOs from apples (Walker, 1969), potatoes (Macrae & Duggleby, 1968), pears (Rivas & Whitaker, 1973) and sweet cherries (Pifferi, Baldassari & Cultura, 1974) were inhibited by low levels of *p*-coumaric and ferulic acids. More recently Walker (1975) investigated the effect of a range of substituted cinnamic acids upon particulate and soluble *o*-DPO preparations from apples and found cinnamic and *p*-coumaric acids to be potent inhibitors of this enzyme. In the light of these observations it was considered that these substituted cinnamic acids might prove useful for the control of enzymic browning during the preparation of apple, or other fruit juices, and the results of laboratory tests are reported here.

Experimental and results

Earlier workers (Walker, 1969, 1975a; Macrae & Duggleby, 1968; Pifferi *et al.*, 1974) had shown that the K_i value and type of inhibition of apple *o*-DPO depended upon both the phenolic substrate and the substitution pattern of the cinnamic acid. Moreover different varieties of apple differ widely in the levels of phenolic compounds (*o*-DPO substrates) present (Walker, 1962; van Buren, 1970) and since the inhibition of apple

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* *o*-Diphenol: O_2-oxidoreductase, E.C. No. 1, 10, 3, 1.

o-DPO by cinnamic acids is predominantly competitive in nature (Walker, 1975a) the amount of inhibitor required to prevent browning will depend upon the variety of fruit being processed. A simple test, based on the procedure of Walker & Reddish (1964), was devised to estimate the amount of inhibitor required for the prevention of browning.

A suitable quantity (100–200 ml) of opalescent apple juice was prepared by mincing the fruit and filtering the resultant juice through fine gauze. Small amounts (50–100 mg/100 ml) of ascorbic acid were added, if necessary, to provide *temporary* control of browning. A series of 25 × 150 mm test tubes was set up; each tube contained different quantities of the inhibitor under test in 1 ml water plus 9 ml of apple juice. A reference tube contained 1 ml 1% (w/v) sodium dithionite in place of the inhibitor solution.

TABLE 1. Effect of cinnamic acids upon the browning of apple juice; test tubes containing 9 ml juice plus 1 ml inhibitor were aerated at 30°C until browning of control tube fully developed (20–30 min); absorbance recorded at 450 nm (blue filter) against blank tube treated with Na dithionite

Final concn of inhibitor (mM)	Degree of browning			
	Granny Smith		Sturmer Pippin	
	Colour developed	Absorbance	Colour developed	Absorbance
Nil (control)	Brown	0·48	Dark brown	0·69
Cinnamic acid				
0·1	Light brown	0·21	Dark brown	0·52
0·25	None	0·05	Light brown	0·41
0·5	None	0·05	None	0·06
0·75	None	0·02	None	0·04
1·0	None	0·02	None	0·04
p-Coumaric acid (4-Hydroxycinnamic)				
0·1	Light brown	0·26	Dark brown	0·57
0·25	Light brown	0·19	Brown	0·37
0·5	None	0·07	None	0·04
0·75	None	0·06	None	0·03
1·0	None	0·05	None	0·02
Ferulic acid (4-Hydroxy-3-methoxycinnamic)				
0·1	Light brown	0·25	Dark brown	0·66
0·25	Light brown	0·21	Dark brown	0·50
0·5	Pale brown	0·17	Light brown	0·28
0·75	Pale brown	0·11	Pale brown	0·10
1·0	None	0·03	None	0·03

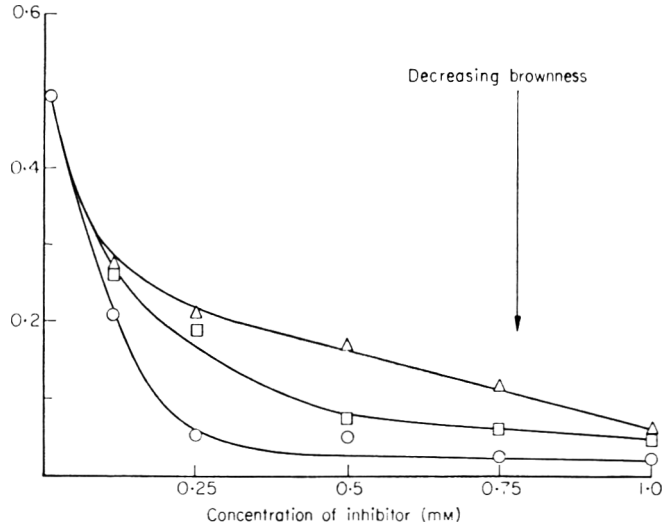


FIG. 1. Effect of concentration of inhibitor upon the browning of juice from Granny Smith apples, cinnamic acid (○), *p*-coumaric acid (□) and ferulic acid (△).

The tubes were then incubated at 30° and each tube was strongly aerated by a stream of fine bubbles from a capillary tube. After 20–30 min, by which time the control tube (no inhibitor) had turned dark brown, the colour of each tube was recorded. If quantitative measurements were required the samples were clarified by centrifuging at 15 000 × ***g*** for 20 min and their absorbance recorded in a colorimeter fitted with a blue filter (450 nm) and using the dithionite-treated sample as a zero-blank.

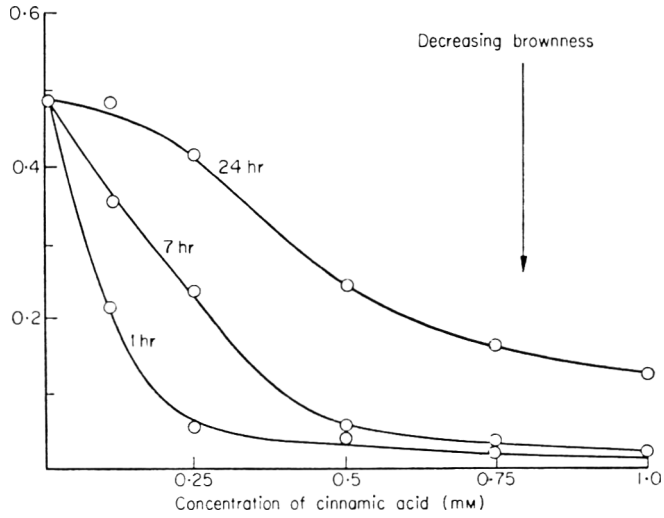
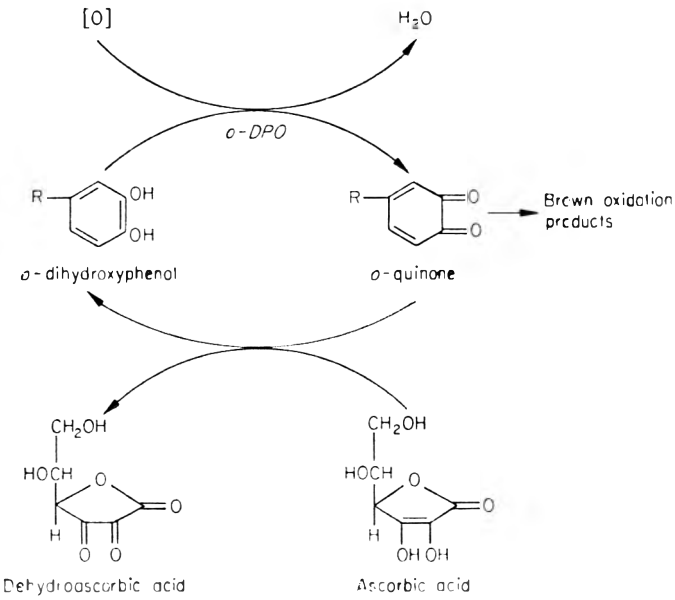


FIG. 2. Effect of holding time on the control of browning of juice from Granny Smith apples.

The effects of different cinnamic acids upon the browning of juice from Granny Smith and Sturmer apples are compared in Table 1 and Fig. 1 where it will be seen that cinnamic and *p*-coumaric acids were more effective than ferulic acid. A study of the long-term stability of the inhibitory effects of cinnamic acid is shown in Fig. 2, and similar results were given by *p*-coumaric acid. This shows that low levels of these compounds are able to provide relatively long lasting control of enzymic browning especially when compared with control by ascorbic acid.

Discussion

Addition of ascorbic acid is the common method of control of enzymic browning during the pre-pasteurization stages of the manufacture of 'natural' or 'opalescent' apple juice and similar products. This compound functions by reducing the primary quinone oxidation products of the fruits' natural phenolic substrates and is consequently consumed during the process as shown below.



Other methods of control of enzyme-catalysed browning have been proposed and these have been reviewed by Mathew & Parpia (1971) and Walker (1975b) but the proposal of Finkle & Nelson (1963) is of special interest here. These authors treated apple juice with *o*-methyltransferase plus S-adenosylmethionine and showed that this enzyme system converted the natural *o*-DPO substrates, caffeic and chlorogenic acids, to ferulic and feruloylquinic acids respectively. This treatment prevented browning

but it was not realized at that time that the ferulic acid could also act as an inhibitor of *o*-DPO. Unfortunately this procedure is too expensive for routine use.

The experiments reported in this paper are based on earlier fundamental studies of the properties of *o*-DPOs from apples and other plant tissues and suggest that additions of cinnamic acid, or its more soluble sodium salt, may offer an inexpensive, long-term, control of browning. The minimum quantity of inhibitor required will depend upon the level of naturally-occurring phenolic substrates present and this has been shown to vary widely for different varieties of apples (Walker, 1962) and other fruits (van Buren, 1970).

Cinnamic, *p*-coumaric and ferulic acids, or their esters, have all been reported to occur naturally in many edible fruits or plants and their essential oils so it would seem most unlikely that there would be any health hazard associated with the use of these compounds to control enzymic browning.

Acknowledgment

The author is grateful to the N.Z. Apple and Pear Marketing Board for a supply of fruit, to the University Grants Committee for equipment and to Mrs Margaret Stevens for technical assistance.

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Loss of thiamin from potatoes

T. E. OGUNTONA AND A. E. BENDER

Introduction

Potatoes provide 10% of the calculated average intake of thiamin in the British diet (National Food Survey, 1974) so processing losses are a matter of some importance. Much attention has been paid to losses of vitamin C through poor methods of preparing potatoes, especially soaking in water for long periods, but little attention has been paid to the corresponding losses of thiamin.

These losses may be considerable due to the modern development of selling and consequently storing peeled and chipped potatoes preserved from discolouration with sulphur dioxide. Sulphite splits thiamin into inactive thiazole and a pyrimidine sulphonic acid. Although it is normal practice to immerse the peeled or chipped potatoes into sulphite for only a few minutes, they may subsequently be stored for periods up to several days before cooking and consumption. Mapson & Wager (1961) reported 25% loss after sulphite treatment and three days' storage, and 35–40% overall loss on subsequent cooking.

In some institutions it is common practice to peel potatoes the day before they are used and to leave them soaking in water overnight or for 24 hr. The effects of soaking in water, in sulphite and subsequent cooking on the thiamin content of potatoes were examined in the laboratory and values compared with those found in a number of commercial samples of fried, chipped potatoes.

Materials and Methods

Potatoes

King Edward potatoes, presumably harvested the previous November–December, were used during the month of March. They were peeled mechanically and washed in tap water and in distilled water.

For the preparation of chipped potatoes pieces were cut approximately $12 \times 12 \times 50$ mm. Chips from different tubers were combined to provide mixed samples for treatment and analysis to obviate differences in the thiamin content of different tubers.

Sulphite

A solution of sodium metabisulphite, 0·5% w/v was used to inhibit polyphenol

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oxidase; and to maintain constant conditions of treatment the cut potatoes were soaked overnight (16 hr) at 5°C. This is far more drastic treatment than the commercial process of a few minutes immersion but provides constant conditions.

Frying

After sulphite treatment samples were fried in commercial cooking oil (Medyna 'Golden', mixed vegetable oils) for 5 min at 150°C using sufficient oil just to cover the chips.

Thiamin estimation

The A.O.A.C. method was used (A.O.A.C., 1965) with modifications because of the large amount of fluorescent material in the potatoes and the high fat content of the fried material. The sample was crushed and dispersed in 250 ml 0·1 N HCl and digested for 30 min at 96–100°C on a steam bath. The pH was then adjusted to 4·2 with NaOH solution. Thiamin was liberated from combination by overnight digestion with takadiastase (200 mg) and papain at 40°C. The digest was filtered, purified on a Decalso column and the thiamin eluted and oxidized with alkaline potassium ferricyanide solution in the dark.

The solution was assayed in a P. E. (Colman) fluorimeter using thiamin hydrochloride standards.

Results

Penetration of sulphite

Tubers were cut into halves, and one half soaked in metabisulphite solution while the other half served as control. Slices were cut at 2 mm intervals to examine the depth at which destruction of thiamin occurred.

Table 1 shows losses by leaching into water without preservative. The outermost 2 mm layer lost 40% of the thiamin but even the fifth layer, i.e. 10 mm from the

TABLE 1. Loss of thiamin from half potato tubers soaked 16 hr in water or metabisulphite solution (thiamin content in 2 mm slices, μg/100 g)

	Fresh	Soaked in water	Loss %	Fresh	Soaked in sulphite	Loss %
1st layer (surface)	79·0	45·1	43	77·9	15·6	80
2nd layer	76·9	54·6	29	77·2	22·5	71
3rd layer	76·7	53·7	30	77·2	23·2	70
4th layer	76·9	57·7	25	77·1	44·7	58
5th layer	77·0	61·0	21	77·3	42·5	55

surface, lost 20%. When the potato halves were soaked in metabisulphite these losses increased to 80% in the outermost layer and were as high as 55% in the fifth layer. At various depths losses of thiamin were approximately twice as great in metabisulphite solution as in water.

Effect of subsequent frying

Half tubers were fried after soaking either in water or in metabisulphite solution (Table 2).

TABLE 2. Loss of thiamin from half potatoes soaked in water or metabisulphite solution and subsequently fried (thiamin content in 2 mm slices, μg/100 g)

	Soaked in water			Soaked in sulphite		
	Raw	Fried	Loss %	Raw	Fried	Loss %
1st layer	39·2	35·2	11·0	20·6	16·5	19·9
2nd layer	42·5	38·2	10·1	30·3	23·6	22·1
3rd layer	41·7	37·3	10·6	32·0	25·2	21·2

The results indicate that sulphite soaking doubles the loss caused by frying from 10% loss in tubers soaked in water to 20% loss in samples soaked in sulphite. These losses are due to frying and are additional to the leaching and destruction shown in Table 1.

Chipped potatoes (12 × 12 × 50 mm) were soaked in water or sulphite and fried with the results shown in Table 3.

Sulphite doubles the loss of thiamin from 40% to 80% but there is only a small additional loss in the subsequent frying (from 40 to 45% for the water soaked samples and from 75 to 80% for the sulphite-treated samples).

Table 3 also shows the thiamin content of raw and fried chips from the Queen Elizabeth College refectory (King Edward potatoes). Raw chips had been peeled mechanically, washed, cut into chips by hand and soaked in water for 20 hr. The fried chips were kept hot for 2 hr before analysis (average time of consumption). They were, on average, slightly smaller in size than the laboratory preparation.

The thiamin content of both raw and fried chips was similar to that of the laboratory samples soaked in water. The latter has suffered a 40% loss from the original raw material by soaking. The loss of the refectory sample on frying and keeping hot was 15%. The laboratory samples soaked in sulphite before cooking were much lower in thiamin than the refectory samples where no sulphite was used.

Five commercial batches of chips purchased from five shops are also shown in Table 3.

TABLE 3. Thiamin content of raw and fried chipped potatoes, μg/100 g

	No. of samples	Range	Mean	Loss %
Laboratory sample				
Raw chips	9	60·1–76·6	62·6	
(a) Soaked in water 16 hr	10	36·5–40·1	37·3	40·4
subsequently fried	10	30·2–40·6	34·4	45·0
(b) Soaked in sulphite 16 hr	7	10·9–19·3	14·8	76·4
subsequently fried	9	10·1–16·7	12·0	80·8
Refectory sample				
Raw, after soaking in water 20 hr	10	35·0–39·4	36·4	
Subsequently fried and kept hot	10	23·8–40·6	30·6	
Commercial samples				
I	5	30·1–30·8	30·5	
II	5	9·4–31·2	23·5	
III	5	27·2–35·2	30·0	
IV	5	5·1–21·2	16·2	
V	5	15·9–18·5	16·8	

In each case five samples were analysed. Even between only five samples two batches revealed a fourfold range in thiamin content. Mean values ranged from 30 μg/100 g (the same as found in the refectory sample and the laboratory water-soaked sample) to as low as 16 μg/100 g (the same as found in the laboratory sulphited samples).

Discussion

The commercial method of preserving peeled potatoes is to immerse them in sulphite solution and drain before subsequent storage, which would leave much less sulphite in the product than in our samples which were soaked overnight in the solution. This was demonstrated by finding 200 ppm of sulphite in our raw samples and 100 ppm after frying compared with only traces, insufficient to quantify, in commercial samples of fried chips. However, the thiamin content of the commercial samples at 23 μg/100 g was intermediate between that found in the laboratory for chips that had been soaked in water and those soaked in metabisulphite. Anderson, Esselen & Fellers (1954) immersed their samples in sulphite in the usual way but then stored them for as long as eight days, when there was a 50% loss of thiamin. A further factor beyond the time of soaking and storage is the amount of sulphite absorbed. Furlong (1961) showed that sulphite was more readily taken up the more damaged the surface of the chip, which might account for the wide range of values found between some of the commercial samples. Mapson & Wager (1961) immersed their samples for 2 min and drained for

2 min. They showed that it was possible to store potatoes for seven days at 5°C and fourteen days at 1°C; losses of thiamin reached 24% after three days' storage at 5°C. Subsequent boiling caused a greater loss from preserved than from fresh potatoes; boiling resulted in 15% loss in the absence of sulphite and twice this with sulphite; frying caused 10% loss without sulphite but this rose to as much as 35–45% after sulphiting. Storage hot cause a loss totalling 75%. These authors also showed losses of 9–17% by leaching. Balfoort & Stevelink (1966) using higher concentrations of sulphite than commercially used reported 10–50% losses on cooking and showed that cooking losses were increased by previous sulphite-treatment.

Sulphite is sometimes incorporated into various forms of dehydrated potato both to preserve the colour and protect the vitamin C (U.S. Patents 3 800 047 and 3 027 264). In such a powdered, granulated or flaked form it is likely that a considerable part of the thiamin would be destroyed but neither of these patents makes any mention of this loss. However in Patent 3 343 970 the loss is compensated by restoring thiamin and ensuring that it is added at a stage removed as far as possible from the addition of sulphite.

Thiamin intake

Most determinations of nutrient intake are calculated from food composition tables. If this were done for potato chips there could be a fourfold overestimate of intake. The standard U.K. Tables (McCance & Widdowson, 1960) give a value of 110 μg thiamin/100 g raw potatoes and 100 μg/100 g chips. We found values of 77–79 μg in raw potatoes (Wright (1935) found 30 μg) but only 5–35 μg (mean $23{\cdot}4 \pm 3{\cdot}08$ μg)/100 g commercial chips.

It is likely that the values in the Food Tables may have been determined before it became common practice to preserve the potatoes with sulphite and there is no indication of a soaking period.

Soaking half tubers in water for 16 hr resulted in losses of 43% in the outer 2 mm and even at a depth of 10 mm there was a loss of 20%. Lowe (1969) reported 20–40% loss of thiamin on soaking overnight and 50–70% total loss on subsequent cooking. It is well recognized that vitamin C can be lost in this way so that the generally deplored practice of peeling potatoes and sometimes chipping them the day before they are to be cooked and consumed results in a serious loss of nutrients (Eddy & Stock, 1972). We have encountered a practice in school meal preparation (A. E. Bender, A. Getreuer & M. Harris, unpublished) of peeling and storing potatoes on Monday morning for use at Wednesday's lunch, while for the rest of the week peeled potatoes were soaked for over 24 hr. While it is well recognized that food composition tables can give only a rough approximation of the nutrient content of foods cooked with different degrees of severity, little attention appears to be paid to the destruction of nutrients that commonly takes place in catering establishments. True intakes of labile nutrients under such conditions will be only a fraction of calculated values.

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Some chemical changes in fish silage

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Summary

During ensilage of cod in the presence of 3% (w/w) formic acid, the enzymes mainly responsible for the liquefaction were those of the gut, skin and other parts of the fish other than flesh; those of the last were only marginally implicated. During the storage of the silage the formation of non-protein nitrogen (free amino groups, volatile bases, and polypeptides) was measured. Silages were produced using whole cod and herring. Tryptophan was found to be partially lost: during ensilage and storage, the values for available lysine remained constant.

Introduction

Fish silage has been popular for animal feeding in some Scandinavian countries and was first introduced in Finland in 1920 by A. I. Virtanen. The production of silage on an industrial scale started in Denmark in 1948 and three years later its production was of the order of fifteen thousand tonnes per annum (Petersen, 1951). The production of fish silage on an industrial scale is limited because its high water content makes it uneconomic to transport the material for long distances. However, in areas close to the sea, silage could be used instead of more expensive animal feeds. Although fish silages produced with formic acid are more expensive than those produced with inorganic acids (Lisac, 1961), the process of liquefaction can be carried out within a range of pH from 4 to 4·5 (Olsson, 1942). This is due to the antiseptic properties of formic acid. However, when silages are produced using inorganic acids, the pH of the final product should be around 2 to avoid bacterial growth (Edin, 1940); and therefore it is necessary to neutralize the product before it is used for feeding purposes.

The production of fish silage using formic acid involves grinding the fish and adding formic acid in such a concentration that the final product will have a pH around 4 (Tatterson & Windsor, 1974).

Although some work has been done concerning production of fish silage, there is little known about the chemical changes taking place during the liquefaction and storage of the product. The object of the present work was to study the degree of

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involvement of enzymes from the different parts of the fish in the process and to investigate some of the chemical changes taking place duing the production and storage of the product.

Materials and methods

Production of silage

For the first set of experiments (those in which changes in non-protein nitrogen were determined) ten cod (*Gadus morhua*) measuring from 70 to 80 cm were dissected very carefully to avoid contamination with gut. The fish was divided into four parts: (i) gut (liver included); (ii) skin (not including fins but including the skin from the head); (iii) flesh (fillets); and (iv) head (with bones, fins and flesh from the backbone and ribs).

Ten different silages were prepared using from 200 to 300 g of sample in each batch. In each case the silage was produced by mincing the raw material sufficiently to pass through a 4 mm mesh and adding 3% (w/w) of 98% formic acid. The ten silages were as follows: gut, skin, flesh, head, skin and gut, skin and head, skin and flesh, gut and flesh, gut and head, flesh and head. The two-component silages were made by thoroughly mixing together equal parts by weight of the respective components. The samples were stored in plastic containers under aerobic conditions at 30°C. In each case the pH was initially 3·9.

An experiment was also carried out in which enzymes were inactivated. In this experiment the four main components of the fish were minced and the formic acid added as described above. Samples (50 g) were transferred to closed plastic containers and heated for 1 hr in a water bath at 100°C. The samples, after blanching, were cooled and the containers opened. The silages were then stored in plastic containers under aerobic conditions at 30°C. Evaluations of total nitrogen and moisture were made in each sample after it had been heated.

In the experiments in which tryptophan and available lysine were measured thirty whole herring (*Clupea harengus*) each about 25 cm in length and fifteen cod of about 45 cm in length were used. The silages were prepared by mincing the whole fish and adding formic acid as described above. All the silages were stored in covered plastic buckets under aerobic conditions at 5°, 15° and 30°C. The pH of the silages made from herring and cod respectively were 3·7 and 3·9.

The cod and herring were stored in ice for about one day and seven days respectively before the silages were made.

All the measurements were made in duplicate with the exception of total nitrogen which was made in triplicate.

Analytical determinations

Moisture. Ten grams of sample were dried for 12 hr in a vacuum oven at 70°C and moisture determined as a percentage by the difference in weight of the sample before and after drying.

Total nitrogen. The determinations of total nitrogen were carried out in the individual components (flesh, skin, head, gut) with corrections for the formic acid added. The total nitrogen for the silages formed by two components was calculated by adding the total nitrogen of each one of the samples and dividing by two.

Total nitrogen was determined on 1·5 g by the micro-kjeldahl method (Pearson, 1970).

Non-protein nitrogen. Determinations of non-protein nitrogen, nitrogen in polypeptide links and free amino groups were obtained separately for each component of the fish before the addition of the acid. For silages formed by two components the values were obtained by adding together the non-protein nitrogen figures of each component and dividing by two. Zero time values for polypeptides and free amino groups in two-component mixtures were calculated in the same way.

Non-protein nitrogen was determined as follows: to 5 g of sample 10 ml of 20% (w/v) trichloroacetic acid was added and after homogenization the sample was filtered. The nitrogen content was determined in the filtrate by the micro-kjeldahl method (Pearson, 1970).

Polypeptides. In this work the term polypeptides included all molecules with peptide links in soluble form (remaining in solution after the addition of 20% trichloroacetic acid). Dipeptides were not measured because the formation of colour will only occur with peptides having more than one linkage (Joslyn, 1970).

For the evaluation of polypeptides a sample was taken from the above filtrate (from the non-protein nitrogen determination) and the polypeptides determined by the biuret reaction (Snow, 1950).

Free amino groups. A sample was taken from the filtrate as used for the non-protein nitrogen determination, and the free amino groups determined by the ninhydrin reaction (Bailey, 1962).

Volatile bases. The determination was carried out by the method described by Ronold & Jakobsen (1947). This technique measures trimethylamine oxide, trimethylamine and gives a combined value for (ammonia, mono- and dimethylamine).

Tryptophan. The method described by Miller (1967) was employed.

Available lysine. This determination was carried out by the Silcock method (Ostrowski, Jones & Cadenhead, 1970).

Note: all the chemicals used were of analytical grade.

Results

Total nitrogen and moisture

The levels of total nitrogen (in respect of the single-component silages) were as follows: skin (3·95%); flesh (2·95%); head (2·44%); gut (2·47%). Moisture contents were as follows: skin (76·1%); flesh (79·0%); head (78·8%); gut (72·6%).

In the inactivation experiment where samples were heated in sealed containers, no effects on the values for total nitrogen and moisture were found.

Total non-protein nitrogen

Figures 1 and 2 show the rate of formation of non-protein nitrogen at 30°C for the different parts of the fish and for different combinations of these. The rate of proteolysis in skin and gut was high during the first 24 hr and relatively little change was detected after that. Three days after the addition of formic acid, over 50% of the total nitrogen

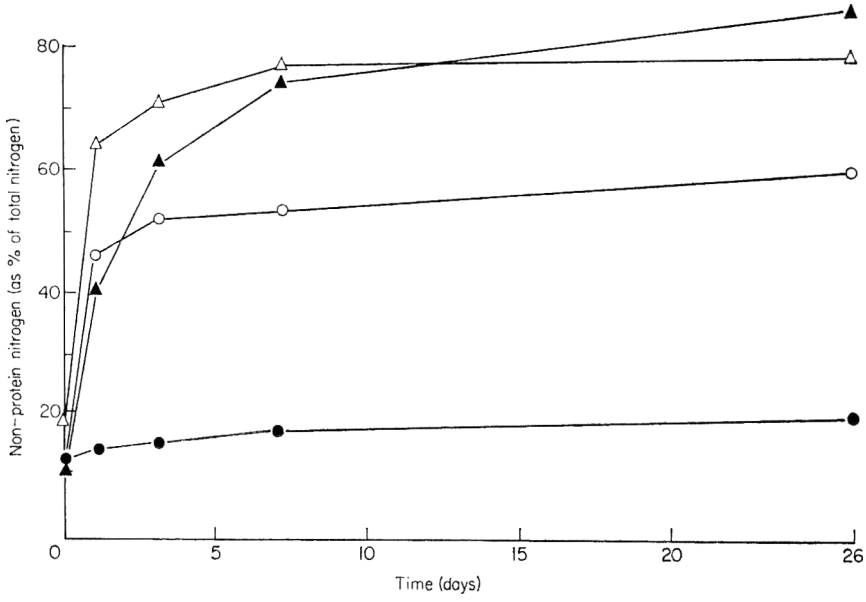

FIG. 1. Formation of non-protein nitrogen in silages produced from different parts of cod at pH 3·9 and 30°C. △ Silage from gut; ○ silage from skin; ▲ silage from head; ● silage from flesh.

was in non-protein form in all the one-component silages with the exception of that made from flesh which showed very little proteolytic activity at pH 3·9. The head showed less proteolytic activity than the skin and gut in the first 24 hr, but similar non-protein nitrogen contents had been reached by the twenty-sixth day in the silages made from the head and the gut. In all the samples in which gut was one of the two components, the amounts of total non-protein nitrogen reached by the twenty-sixth day were higher than those when silages were produced from each component individually. The non-protein nitrogen attained a value of 83·5% of the total nitrogen in gut and flesh whereas corresponding values for gut and flesh individually were 77·5% and 20·5% respectively. A mean value of only 49% would be expected if the components were in a ratio 1:1.

On the other hand, the levels of total non-protein reached by the twenty-sixth day in respect of the mixtures of flesh and skin (42% of total N) and head and skin (71% of total N), were clearly additive, since the corresponding values for the separate components were 20·5%, 59·5% and 85% of the total nitrogen for flesh, skin and head, respectively. There was no synergistic effect.

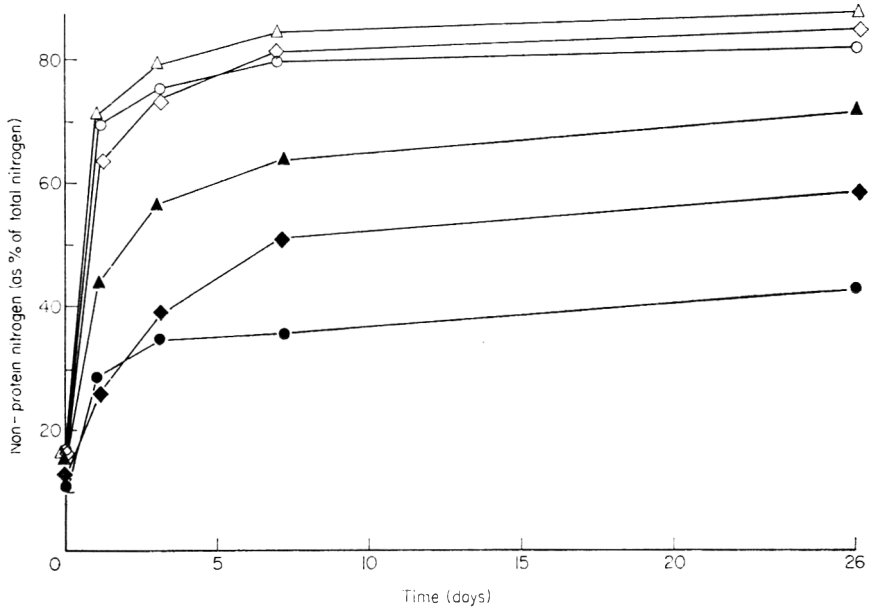

FIG. 2. Formation of non-protein nitrogen in silages produced from combinations of different parts of cod at pH 3·9 and 30°C. △ Silage from gut and head; ◇ silage from flesh and gut; ○ silage from skin and gut; ▲ silage from skin and head; ◆ silage from flesh and head; ● silage from flesh and skin.

The effect of adding head to flesh was similar but less than of gut (e.g. the non-protein nitrogen was slightly higher for the latter).

Polypeptides

In the silages formed by a single component, flesh showed the lowest formation of polypeptides: this fraction accounts for 7·8% of the total nitrogen by the twenty-sixth day. The highest value obtained was for skin (39·5%). The most rapid changes in all the samples were found to occur in the first five days of ensilage, as shown in Table 1. All the samples in which gut was a component showed a decrease in the polypeptides between the seventh and the twenty-sixth day. The same effect was registered, but to a lesser degree, in samples containing head.

TABLE 1. Polypeptide nitrogen in cod silage as % of total nitrogen

Raw material ensiled	Duration (days) of ensilage at 30°C and pH 3·9				
	0	1	3	7	26
Skin	3·8	32·1	41·1	40·2	39·5
Flesh	1·4	2·6	3·2	4·8	7·8
Gut	7·9	26·4	28·6	28·1	25·0
Head	4·4	22·2	32·8	35·8	31·6
Flesh and skin	*	16·9	23·1	21·8	25·3
Flesh and gut	*	35·1	34·5	26·9	30·6
Flesh and head	*	13·0	24·2	25·2	29·4
Skin and gut	*	37·0	34·3	29·7	27·4
Skin and head	*	28·8	38·5	39·4	41·6
Gut and head	*	36·5	35·8	31·1	32·3

* Not determined.

Free amino groups

Although by the twenty-sixth day the concentration of free amino groups was low in ensilaged skin and (especially) flesh (6·3% and 1·9% respectively), values of over 20% of the total nitrogen were found in ensilaged gut and head. A rapid increase of free amino groups during the first day of ensilage was shown in all the samples in which gut was a component. In all combinations containing head the formation of free amino groups slowed down after the seventh day of storage. Table 2 shows the changes observed.

TABLE 2. Free amino nitrogen in cod silage as % of total nitrogen

Raw material ensiled	Duration (days) of ensilage at 30°C and pH 3·9				
	0	1	3	7	26
Skin	1·2	1·9	3·9	6·8	6·3
Flesh	1·3	1·4	1·9	2·4	1·9
Gut	5·3	15·5	20·3	25·2	23·5
Head	1·8	5·6	12·1	18·1	21·2
Flesh and skin	*	2·1	2·8	3·8	3·8
Flesh and gut	*	12·5	20·1	25·1	23·8
Flesh and head	*	3·5	5·6	10·1	10·9
Skin and head	*	11·0	18·5	24·7	22·6
Skin and head	*	3·1	6·1	10·0	11·7
Gut and head	*	13·0	20·7	26·3	27·6

* Not determined.

Inactivation

Figure 3 shows the effect of heating the silages in boiling water for 1 hr. The same level of non-protein nitrogen was found in all the samples for the first two weeks of ensilage. A slight reactivation was noticed in silages from skin and head after that period.

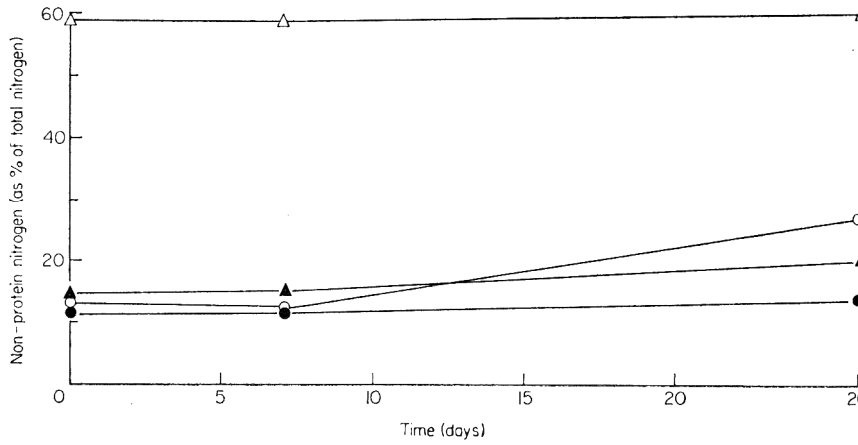

FIG. 3. Formation of non-protein nitrogen in cod silages treated for 1 hr in boiling water at pH 3·9 and stored at 30°C. △ Silage from gut; ○ silage from skin; ▲ silage from head; ● silage from flesh.

Trimethylamine oxide and trimethylamine

The levels of trimethylamine and trimethylamine oxide were the same for all the samples during the twenty-six days of the experiment. Trimethylamine was found to be in very low concentrations in all the samples (with a range of 0–11 mg of N/100 g of sample). The values of trimethylamine oxide in the individual components were approximately as follows (mg N/100 g of sample): skin (19), flesh (60), gut (6), head (29). The values obtained for the samples with two components corresponds to the arithmetic average of their components.

Ammonia, mono- and dimethylamine (AMD)

By the twenty-sixth day of ensilage the concentration of AMD had reached a value of approximately 100 mg of N/100 g of sample in both gut and head. For skin and flesh the values obtained were approximately 50 mg of N/100 g of sample. Although there was low proteolytic activity in the flesh, the concentration of AMD was relatively high after the first week. In the silages with more than one component all the samples containing gut showed the highest concentration of AMD. Table 3 shows those results.

TABLE 3. Ammonia, mono- and dimethylamine in cod silage stored at 30°C and pH 3·9 (mg N/100 g of sample)

Raw material ensiled	Duration of ensilage (days)				
	0	1	3	7	26
Skin	8	17	16	29	50
Flesh	3	8	5	5	56
Gut	35	40	66	66	99
Head	14	9	40	54	97
Flesh and skin	*	3	3	8	21
Flesh and gut	*	43	60	82	123
Flesh and head	*	18	13	39	62
Skin and gut	*	38	58	85	112
Skin and head	*	13	14	34	85
Gut and head	*	49	47	79	101

Tryptophan

During the storage of the silages from herring and cod, some loss of tryptophan was detected. Figures 4 and 5, respectively, show those changes. The rates at which tryptophan decreased at 5°C and 15°C in cod silage were very similar and after fifty-four days 20% of the total tryptophan was lost. Those losses were greater than in silage from herring stored at the same temperatures. Cod silage stored at 30°C showed a loss of 25% of tryptophan after the same time. The silage from herring at 5°C showed no loss after forty-two days, whereas these stored at 15°C and 30°C lost 10% and 33% respectively in this same period. Twenty-five per cent of the total tryptophan in herring was lost in the first seventeen days in the silage stored at 30°C.

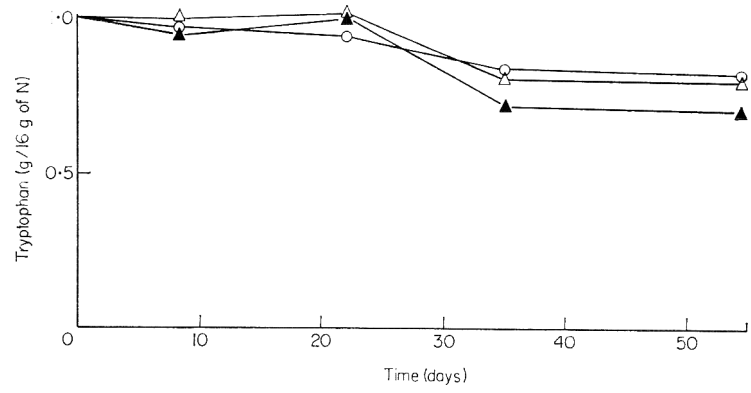

FIG. 4. Loss of tryptophan in cod silage at pH 3·9. ○ Silage stored at 5°C; △ silage stored at 15°C; ▲ silage stored at 30°C.

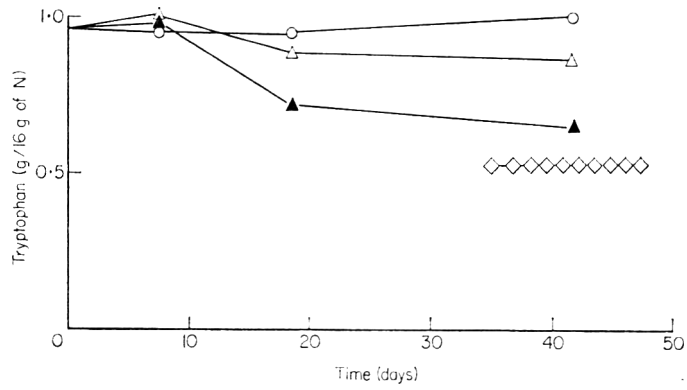

FIG. 5. Loss of tryptophan in herring silage at pH 3·7. ○ Silage stored at 5°C; △ silage stored at 15°C; ▲ silage stored at 30°C; ◇ level of tryptophan in a three-year-old herring silage (stored at room temperature and pH 4·0).

NOTE: the zero time for Figs 4 and 5 corresponds to the raw fish.

Some determinations carried out in a three-year-old silage made from herring (stored at room temperature and with a pH of 4) showed a level of tryptophan of 0·51 g/16 g of N. This corresponds approximately to 53% of its original value.

Available lysine

Silages from cod and herring stored at 5°C, 15°C and 30°C, showed the same values for available lysine as those of the raw fish (9·6 and 9·8 g/16 g N respectively).

Discussion

During the liquefaction of the different parts of cod (flesh, skin, gut, head) in the presence of 3% formic acid, flesh enzymes were found to be only marginally implicated.

Proteins in skin, flesh and head were hydrolysed by gut enzymes resulting in an increase in non-protein nitrogen.

The level of non-protein nitrogen in samples of skin and head, and skin and flesh showed values similar to these obtained from the arithmetic average of each one of their components, therefore it can be stated that skin proteolytic activity is limiting or that skin proteolytic enzymes do not act on flesh proteins. The formation of non-protein nitrogen in silages produced from head or where it was a component indicates a strong proteolytic activity in this part of the fish. The formation of non-protein nitrogen occurred mainly in the first day or so after ensilage. After twenty-six days of storage, high values of non-protein nitrogen were registered in samples of gut containing two

components. Lower levels were registered when the silages were produced individually showing some resistance of gut itself to self digestion. Although the formation of ammonia, mono- and dimethylamine was relatively low compared with the total nitrogen and therefore of little importance in the quality of the product, the values obtained for gut or head were twice as large as those obtained for skin or flesh.

During the liquefaction and storage of the product in the presence of formic acid, the formation of trimethylamine (which can indicate bacterial growth) was not detected. Ancillary microbiological analysis* carried out in silages from cod and herring offal showed that only a small number of bacteria survived the treatment (a range of 40–480 total colony count/ml).

The inactivation experiment showed that the breakdown of proteins stops after heating, and because the samples already contained acid it appears that hydrolysis in the various ensilages was purely enzymic.

In relation to the individual components, the formation of free amino groups was higher in gut and head than skin and flesh and higher amounts of free amino groups were detected in all the silages in which gut was one of the components indicating a more extensive breakdown of the proteins.

Although herring silage was slightly more acid than cod silage (pH 3·7 compared with 3·9) the losses of tryptophan at 5°C, and 15°C were higher in the latter. However, at 30°C, herring showed a greater destruction.

In relation to cod silage stored at 5°, 15° and 30°C by the thirty-fifth day the values for tryptophan ranged from 0·75 at 30°C to 0·85 at 5°C (in grams of tryptophan/16 g of N) which are near the lower limits reported by Becker *et al.* (1955) for the feeding of young swine on a diet containing 15·3% protein. Griminger, Scott & Forbes (1956) reported a limit of 0·7 g of tryptophan/16 g N in a diet of 20% protein for young fowls and for laying hens. Thus these data would imply that additional tryptophan might be necessary in diets containing 15·3% or less of protein for young swine and in diets containing 20% or less protein for young fowls and laying hens if the silage were to be used to supply the non-cereal protein. On the other hand, cod silage stored at 5°C, 15°C and 30°C could be used without being supplemented to supply the non-cereal protein for laying hens, and feeding of young swine and fowls if it is used before the first twenty days of storage.

In the case of the herring silages there is no need to supplement those stored at 5°C and even 15°C after forty days' storage (0·95, 0·9 g tryptophan/16 g N). However after twenty days in the sample stored at 30°C supplementation would be desirable if the silages were used at or below the levels of proteins described above.

Since the values for available lysine in the raw material and silages were the same, it is clear no exception can be taken to the product in this respect.

* The analyses were as follows: total colony count/ml; coliform orga/ml; presumptive coli-aerogenes; clostridia/ml; E. coli I MPN/100 ml; yeast+fungi/ml; actinomyces; lactic acid bacteria/ml.

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The construction of grading schemes based on freshness assessment of fish

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Summary

Standard acceptance sampling schemes can be modified for use in grading, where grades are defined by one or two boundaries and a percentage permitted to fall below the lower boundary. Schemes are constructed to grade fish by freshness, using sampling by attributes or by variables, where the method of assessment is Raw Odour. When another method of testing, such as the Torry Fish Freshness Meter, is used, it is not sufficient in the attributes scheme to convert to new grade boundaries; the corresponding percentages falling below the grade boundaries for the new test must be determined. The procedures for deriving a scheme are not restricted to grading of fish, but may be applied to any similar quality control situation.

Introduction

One of the most widely used methods of quality inspection is 'Acceptance Sampling' (e.g. Grant, 1964). A product is presented to an inspector in batches, a sample is taken from a batch by a predetermined plan and a decision on the destiny of the batch is made on the basis of the results obtained from the sample. In most industrial processes a batch is either accepted or rejected but the method can also be used for grading, where a batch is placed in one of several previously defined grades.

One of the United Kingdom's obligations on entry to the European Economic Community was the introduction of schemes for grading of wet fish, by size and by freshness, on landing at port markets. The Regulation (EEC, 1973) laid down no statistical requirements such as sampling procedure or an acceptable degree of mis-grading, and these and other aspects were left to individual member states to determine. This paper describes some ways in which such a scheme for grading by freshness might be constructed, using a sensory method, the Torry Raw Odour scale (RO) of Shewan *et al.* (1953) or an instrumental method, the Torry Fish Freshness Meter (TFM) (Jason & Richards, 1975). Both methods had been found to be suitable for the grading of fish under commercial conditions (Connell *et al.*, 1976).

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The following definitions for grades based on the RO scale are proposed.

(1) There would be three freshness grades, E, A and B, the lower grade boundaries being at scores of $8\frac{1}{2}$, 7 and $4\frac{1}{2}$ respectively.

(2) A batch would be of a particular grade if not more than 15% of the fish in it had RO scores below the lower boundary for that grade. Consequently, a batch with more than 15% of its fish scoring less than $4\frac{1}{2}$ would not have a grade assigned to it.

Grades have in general an upper and a lower boundary, but where batches are reasonably homogeneous it may be assumed that a decision is required at only one boundary.

For cod (*Gadus morhua*) and similar gadoid species the grades correspond to distinct phases in the spoilage of the fish. At a score of 7, changes due to biochemical reactions have taken place, but bacterial spoilage has not yet become really noticeable. The fish have lost the original fresh odours and have a neutral, bland odour. An extra grade has been recognized, corresponding to scores of $8\frac{1}{2}$ or higher, to distinguish extremely good material. A score of $4\frac{1}{2}$ corresponds to the stage at which fish becomes 'putrid' as distinct from 'stale', and beyond this stage fish is generally considered to be unfit for human consumption. The percentage of fish allowed below a grade boundary must be chosen to be acceptable to both producer and buyer. With such a material as wet white fish, the buyer might be willing to allow up to 15% of a batch to be below a grade boundary before refusing to accept the batch as that grade, and the producer might agree that with a greater percentage of lower grade fish a batch could not be expected to be accepted in a higher grade.

Grading by Raw Odour

Sampling by attributes

In a scheme of sampling by attributes each sampled item (e.g. each fish) is judged on whether it passes a certain requirement. For example, at the A/B boundary, a fish would be considered to be of high grade (A) if its RO score were 7 or more, and of low grade (B) if less than 7. The grade assigned to the batch would depend on the number of low grade fish in the sample.

The performance of a sampling scheme is shown by its operating characteristic (O–C) curve, in which the probability $P(\theta)$ of accepting a batch is plotted against the proportion defective θ, or proportion of items in the batch which fall below the boundary.

In an ideal scheme, all batches with proportion defective not more than a given value would always be accepted and all batches with more than the same proportion defective would always be rejected. The O–C would be

$$P(\theta) = \begin{cases} 1 \text{ if } \theta \leqslant \theta_0 \\ 0 \text{ if } \theta > \theta_0 \end{cases} \text{ for some } \theta_0.$$

This is impossible to achieve with less than 100% inspection of a batch, but the aim in practice is a scheme using a reasonable sample size, which will accept with a high probability batches where $\theta \leqslant \theta_0$ and will accept with a low probability batches where $\theta > \theta_0$.

For the scheme to be constructed here, it is desirable that the degree of misgrading (due to sampling) should not only be low but should equally affect the producer and the buyer, so that the 'indifference quality' or proportion defective for which probability of accepting a batch is 0·5 (Hamaker & Van Strik, 1955) should be θ_0, the maximum allowed proportion defective in an acceptable batch. In the scheme to be derived, $\theta_0 = 0{\cdot}15$.

A previous attempt to apply quality inspection, based on freshness assessment, was a scheme introduced by the Hull Fishing Vessel Owners' Association (HFVOA) in 1958 and modified with the advice and assistance of staff from Torry Research Station's

TABLE 1. HFVOA quality control scheme

Sample size	No. of defectives	Action
4	0	Accept
	1,2	Continue to sample of twelve
	3,4	Reject
12	1,2	Accept
	3–10	Reject

Humber Laboratory (Burgess, Spencer & Baines, 1962). Its purpose was not to grade but 'to withdraw from the market before auction fish that, although not condemned by the health inspectors as "unfit, unsound or unwholesome", might nevertheless reach this stage by the time it had gone through the normal distribution chain and had been displayed by the retailer'. A double sampling plan was used, an initial sample of four and a further eight when necessary, and batches were accepted or rejected on the basis of the number of 'defectives', or number of fish with a RO score less than 4, as shown in Table 1.

The O–C curve is shown in Fig. 1, and its derivation will be found in the Appendix. It will be seen that the indifference quality of 0·25 is too high for the requirements of the scheme to be constructed, so the sampling scheme used by HFVOA could not be used.

The usual method of choosing a sampling plan is by reference to sampling inspection tables, some of the most important of which have been described and compared by Hill (1962). However, these were mainly produced for use in industry, where regimes

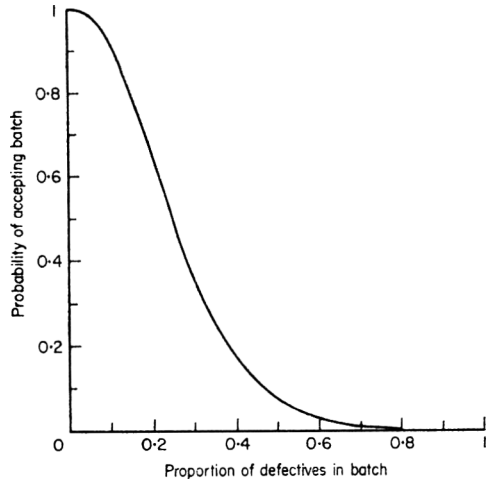

FIG. 1. HFVOA scheme—operating characteristic.

in which proportion defective can reach 0·15 before a batch is unacceptable are seldom considered.

If a single sampling plan is desired—where a sample of n items is taken, and the batch rejected if there are more than c defectives in the sample—n and c are determined so that the O–C curve goes through two suitable points (Wetherill, 1969).

When double sampling schemes are to be used there are more variables and it is much more difficult to derive a scheme by algebraic means, even with crude approximation. One is often reduced to examining a number of schemes and choosing the one whose O–C is as close as possible to that which is desired. It was felt that a situation where a batch could be accepted as upper grade, if a first sample of only four fish was taken and none of them were below the boundary, favoured the producer unduly, so the first sample was increased to six. Double sampling schemes using a first sample of six and a further six if necessary were examined, and of these the scheme shown in Table 2 was chosen. Its O–C curve is shown in Fig. 2, where proportion below the

TABLE 2. Attributes scheme using Raw Odour

Sample size	No. of lower grade	Action
6	0	Upgrade
	1	Continue to sample of twelve
	2–6	Downgrade
12	1	Upgrade
	2–7	Downgrade

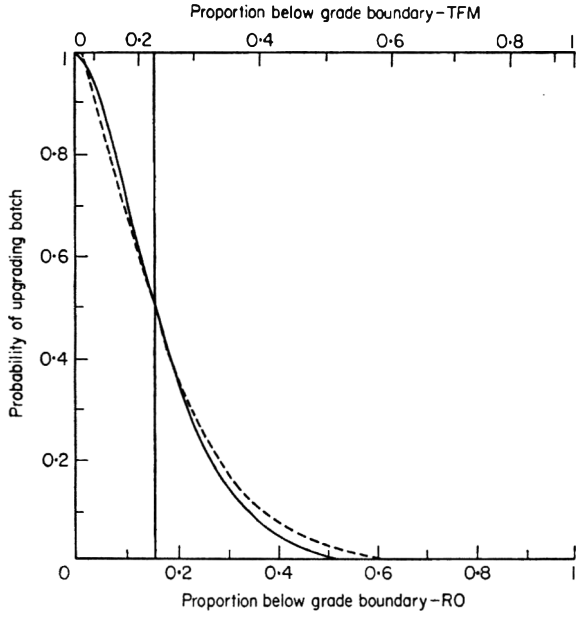

FIG. 2. Attributes schemes—operating characteristics. ——— Scheme using RO; – – – – scheme using TFM.

boundary is shown on the bottom scale. The derivation of the O–C is given in the Appendix.

It may be seen that the scheme achieves the desired aim of an indifference quality of 0·15, indicated by a vertical line on the figure. The areas above the curve to the left of 0·15, and below the curve to the right of 0·15, represent misgrading due to sampling error.

However, it may be assumed that the sampling scheme, operated by experienced, trained graders, need not be practised on batches where a grade can be easily assigned on superficial inspection. It may be further assumed that such batches can be graded without error. For any quality the theoretical probability of misgrading will never be higher than 0·5 which will occur if all batches have 0·15 lower grade. Away from this critical quality the probability of misgrading falls quickly.

Sampling by variables

In previous experiments on freshness of fish (e.g. Connell *et al.*, 1976), sampling was by variables. In this method the required number of fish are sampled from a batch, each scored separately and the sample mean calculated. The decision on which grade is to be assigned to the batch is made on the basis of the sample mean.

A grading scheme using sampling by variables may be constructed so that for any given batch, its chance of being assigned to any grade is almost or exactly the same

whether sampling is by attributes or variables. It is necessary to know the distribution of freshness within a batch or to make certain assumptions. The standard deviation within a batch may be known from earlier investigations or may have to be estimated from the current data. It is assumed here that the scores from a batch are normally distributed with a known standard deviation σ.

The O–C for the scheme using sampling by attributes gave $P(\theta)$, the probability of accepting as upper grade a batch which had a proportion θ of its fish with scores below the lower boundary for that grade.

In a normal distribution with mean μ and standard deviation σ, an expected proportion θ of the population will lie below $\mu - z_\theta\sigma$, where z_θ is the value of the standard normal variate z such that $P(z \leqslant -z_\theta) = P(z \geqslant z_\theta) = \theta$. Hence, a normal distribution with standard deviation σ and probability θ of lying below a value L has a mean of $L + z_\theta\sigma$.

In the case of sampling by attributes, a batch was to be put in the upper or lower grade depending on whether the proportion below a grade boundary was more or less than a certain value θ. In the case of sampling by variables, the criterion will be whether the batch mean is more or less than some decision level. If these criteria are to be equivalent, decision levels must be chosen so that a batch whose mean is at a decision level will have a proportion θ below the grade boundary L. Thus the decision level will be at $L + z_\theta\sigma$.

The sample number is now calculated, using a suitable point on the O–C curve for the attributes scheme, where a batch containing a proportion q below the grade boundary has probability r of being placed in the upper grade. A suitable point (see Fig. 2) has $q = 0{\cdot}3$, $r = 0{\cdot}15$.

The mean of such a batch will be $L + z_q\sigma$, and the difference between decision level and batch mean is

$$(L + z_\theta\sigma) - (L + z_q\sigma) = (z_\theta - z_q)\sigma.$$

Sample means, of sample size n, will have a normal distribution with mean equal to the batch mean and standard deviation $\sigma/\sqrt{n}$. The distribution must be such that the sample mean has probability r of being above the decision level, which must therefore be at $L + z_r\sigma/\sqrt{n}$.

Thus

$$z_r \frac{\sigma}{\sqrt{n}} = (z_\theta - z_q)\sigma$$

and

$$n = \left(\frac{z_r}{z_\theta - z_q}\right)^2.$$

In the present case, where $\theta = 0{\cdot}15$, $q = 0{\cdot}3$ and $r = 0{\cdot}15$, $n = \left(\frac{1{\cdot}04}{1{\cdot}04 - 0{\cdot}52}\right)^2 = 4.$

The O–C curve can be constructed from the relationship $z_r = (z_\theta - z_q)\sqrt{n}$, where θ and n are fixed for the scheme and r is calculated for each value of q.

Using the results obtained by Connell *et al.* (1976) where $\sigma = 0{\cdot}78$, the decision levels lie at scores of 9·3, 7·8 and 5·3. The standard deviations were obtained from samples which had been shared between two judges, so the desired performance of the present scheme would be achieved by two graders assessing two fish each. Because the RO scale has an upper limit of 10, the assumed normal distribution of scores within a batch cannot hold for very fresh batches. If normality is assumed, a batch with mean 9·3, and consequently 15% of its scores below 8·5, would also have 15% above 10·1, which is impossible. It may be assumed that such very fresh batches will be landed from inshore vessels making only short trips and are more homogeneous than those from middle and distant water vessels of the types considered by Connell *et al.* The effect on misgrading, due to the inadequacy of the model at this point, would be negligible.

The probability of misgrading will depend on the range of freshnesses present. For instance, if a uniform distribution of batch means from 4·0 to 10·0 is assumed, approximately 15% of batches will be misgraded in the long run.

Grading by Torry Fish Freshness Meter

Sampling by variables

If a different method of freshness assessment, such as the Torry Fish Freshness Meter (TFM), is used, the grading criterion must be such that a batch which is of a particular grade by the original method shall still be of that same grade. A sampling procedure has then to be established so that a batch will have the same chance as before of having a particular grade assigned.

The information required is the relationship between scores by the two methods and the standard deviation within a batch for each method. In the case of RO and TFM, a linear functional relationship between batch means was derived by Connell *et al.* (1976).

Let the relationship be $X_{\mathrm{TFM}} = a + bX_{\mathrm{RO}}$, the within-batch standard deviations be σ_{RO} and σ_{TFM} and the grade boundaries under consideration be L_{RO} and L_{TFM}.

If the RO scores within a batch with mean μ_{RO} and standard deviation σ_{RO} are converted to equivalent TFM scores using the functional relationship, the resulting distribution will have mean $a + b\mu_{\mathrm{RO}}$ and standard deviation $b\sigma_{\mathrm{RO}}$, and this distribution may be compared with that of the actual TFM scores, as in Fig. 3. The ratio $D = b\sigma_{\mathrm{RO}}/\sigma_{\mathrm{TFM}}$ is a measure of the relative precision of the two methods of testing, being the ratio between their standard deviations when converted to the same scale. Using the data of Connell *et al.*, $D = 0{\cdot}74$, showing that TFM is less precise than RO.

Given two distributions with different standard deviations, two situations might be considered:

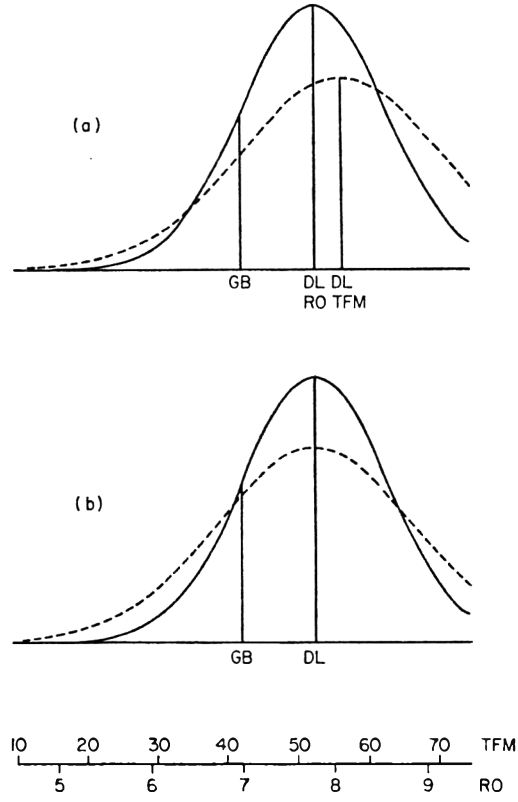

FIG. 3. Distribution of RO and TFM scores in a batch, showing grade boundary (GB) and decision levels (DL). Case (a): equal proportions below grade boundary; Case (b): means are equivalent. ——— RO; - - - - TFM.

(a) the proportion θ below a given value is the same in both distributions;

(b) the means are equal.

Case (a) corresponds to the requirement that not more than a given proportion θ (in the present case 15%) shall lie below the grade boundary for a batch to be acceptable in the upper grade, and the same proportion shall apply for RO or TFM.

When testing by RO, batches with means greater than the decision level $L_{\mathrm{RO}}+z_\theta\sigma_{\mathrm{RO}}$ will be acceptable. The grade boundary in the TFM scale is at $L_{\mathrm{TFM}}=a+bL_{\mathrm{RO}}$ and the decision level $L_{\mathrm{TFM}}+z_\theta\sigma_{\mathrm{TFM}}=a+bL_{\mathrm{RO}}+z_\theta\sigma_{\mathrm{TFM}}$.

The TFM equivalent of the RO decision level is

$$a+b(L_{\mathrm{RO}}+z_\theta\sigma_{\mathrm{RO}})=a+bL_{\mathrm{RO}}+bz_\theta\sigma_{\mathrm{RO}}.$$

This will not be equal to the actual TFM decision level unless $\sigma_{\mathrm{TFM}}=b\sigma_{\mathrm{RO}}$, i.e. the standard deviations are equivalent.

Batches with TFM means between these different decision levels will be acceptable

by one method and rejectable by another. Case (a) therefore results in an inconsistency in the defining of a grade for a batch.

Case (b) corresponds to the requirement that a batch with a mean greater than the RO decision level or the equivalent by another method of assessment shall be acceptable in the upper grade.

The grade boundary for TFM is again $a+bL_{RO}$ but the decision level is now $a+b(L_{RO}+z_\theta\sigma_{RO})$ and is equal to the TFM equivalent of the decision level in the RO scale.

The difference between the decision level and grade boundary is

$$a+b(L_{RO}+z_\theta\sigma_{RO})-(a+bL_{RO})=bz_\theta\sigma_{RO}.$$

If ψ is the proportion of TFM readings below the grade boundary in a batch whose mean is at the decision level it also follows that the decision level is at $L_{TFM}+z_\psi\sigma_{TFM}$, and the difference between decision level and grade boundary is $z_\psi\sigma_{TFM}$.

Thus ψ may be obtained from the equation

$$z_\psi\sigma_{TFM}=bz_\theta\sigma_{RO}$$

and hence

$$z_\psi=\frac{b\sigma_{RO}}{\sigma_{TFM}}z_\theta=Dz_\theta.$$

Using the results of Connell *et al.* (1976), where $a=-50{\cdot}6$, $b=13{\cdot}3$, $\sigma_{RO}=0{\cdot}78$, $\sigma_{TFM}=14{\cdot}0$, it follows that $D=0{\cdot}74$ and hence if $\theta=0{\cdot}15$ then $\psi=0{\cdot}22$. Table 3 shows the relationship between the proportions falling below the grade boundary when a batch is tested by both methods. Thus, in order to have a consistent rule for grading,

TABLE 3. Percentage below grade boundary when testing a batch by different methods

RO	TFM
1	4
5	11
10	17
15	22
20	27
25	31
30	35
35	39
40	43
45	46
50	50

TABLE 4. Decision levels for sampling by variables using different methods

	RO		TFM	
Sample sizes	4		8	
Grades	Lower grade boundary	Decision level	Lower grade boundary	Decision level
E	8·5	9·3	62	73
A	7·0	7·8	42	53
B	4·5	5·3	9	20

it is necessary to recognize that a batch with 15% of its RO scores below a grade boundary will have 22% of its TFM readings below the equivalent TFM grade boundary.

The grade boundaries and decision levels for TFM have been calculated using the functional relationship $X_{TFM} = a + bX_{RO}$ and are shown in Table 4.

Batch means have to be estimated with the same relative precision and the required sample sizes can be calculated in the same manner as for RO, or, more simply, directly from the RO sample sizes by the relationship

$$n_{TFM} = \frac{n_{RO}}{D^2}$$

which takes account of the different precisions of the tests. Using the values above, with $D = 0{\cdot}74$ and $n_{RO} = 4$, it follows that n_{TFM} (rounded up to the next integer) is 8.

Sampling by attributes

It has been shown above that a batch with 15% of its fish having RO scores below a grade boundary will contain 22% fish giving scores below the corresponding TFM boundary. Thus if a TFM scheme using sampling by attributes is to be comparable to

TABLE 5. Attributes scheme using Torry Fish Freshness Meter

Sample size	No. of lower grade	Action
6	0	Upgrade
	1,2	Continue to sample of twelve
	3–6	Downgrade
12	1,2	Upgrade
	3–8	Downgrade

the RO attributes scheme, a batch would require to be upgraded if not more than 22% of its fish give readings below the grade boundary.

The scheme shown in Table 5 has, as required, an indifference quality of 0·22, and as may be seen in Fig. 3, its O–C agrees closely with that for the RO attributes scheme when the differing distributions are taken into account. The O–C is derived in the Appendix.

Although the graders using the RO attributes scheme are assumed to have sufficient experience in sensory assessment to be able to grade some batches on a superficial inspection, it is assumed that TFM operators do not and would not need such experience, and the scheme would be applied to all batches to be graded.

Discussion and conclusions

It is possible to construct a freshness grading scheme for wet fish, using Raw Odour as a method of assessment. If batches are reasonably homogeneous a decision will be required at only one boundary between grades, and the situation is equivalent to one in which batches are either accepted or rejected.

Where a scheme using sampling by attributes has been developed, it is possible to construct a scheme using sampling by variables with an operating characteristic nearly identical. The latter method is more informative, and the data obtained can be used to monitor the distribution of freshness in a batch and, for example, can detect abnormal

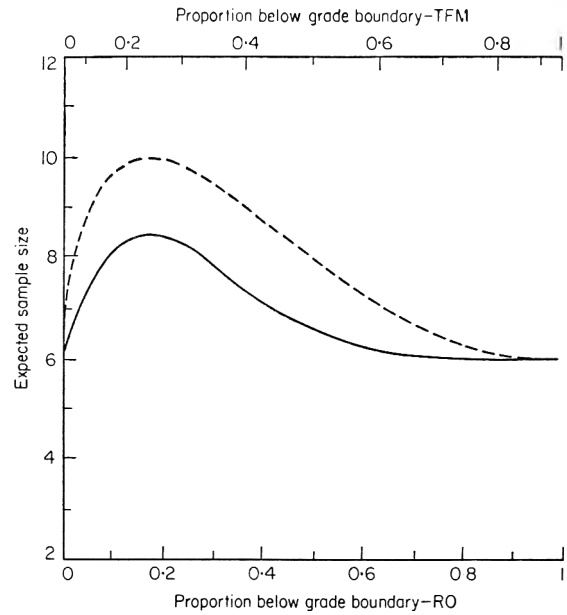

FIG. 4. Expected sample size. ——— RO; – – – – TFM

mixing. Although more computation is required, sampling by variables generally needs fewer samples. Expected sample sizes for the attributes scheme, which vary with proportion below the boundary, are shown in Fig. 4. The values are highest near the indifference quality, and tend towards six in the more extreme cases when a batch can be graded on the basis of the first sample.

When another method of testing, such as the Torry Fish Freshness Meter, is considered, it is not sufficient to convert to new grade boundaries; the corresponding percentages in a batch falling below the grade boundaries for the new test must also be determined. It is then possible to choose an attributes sampling plan, or determine sample numbers and decision levels for a variables scheme, to give similar operating characteristics for the two methods.

Although the derivation of grading schemes has been illustrated by freshness assessment of fish, the approach may equally be applied to size grading, or to any similar quality control situation.

Appendix

Derivation of operating characteristics

Let θ be the proportion defective (or below a grade boundary) in a batch, and let $\phi = 1 - \theta$.

In a single sample of size n taken from a large population (or batch) with proportion defective θ, the probability that there are exactly c defective items is the Binomial probability

$$Pr(c \mid n;\theta) = \binom{n}{c} \theta^c \phi^{n-c}$$

Let $P(\theta)$ be the probability that a batch of proportion defective θ is accepted.

(1) *HFVOA scheme* (Table 1)

$$\begin{aligned} P(\theta) &= Pr(0 \mid 4;\theta) + Pr(1 \mid 4;\theta)\ [Pr(0 \mid 8;\theta) + Pr(1 \mid 8:\theta)] \\ &\quad + Pr(2 \mid 4;\theta)\ Pr(0 \mid 8;\theta) \\ &= \phi^4 \quad + 4\theta\phi^3\ [\phi^8 + 8\theta\phi^7] \\ &\quad + 6\theta^2\phi^2\phi^8 \\ &= \phi^4 + 4\theta\phi^{11} + 38\theta^2\phi^{10} \end{aligned}$$

(2) *RO attributes scheme* (Table 2)

$$\begin{aligned} P(\theta) &= Pr(0 \mid 6;\theta) + Pr(1 \mid 6;\theta)\ Pr(0 \mid 6;\theta) \\ &= \phi^6 \quad + 6\theta\phi^5\phi^6 \\ &= \phi^6 + 6\theta\phi^{11} \end{aligned}$$

(3) *TFM attributes scheme* (Table 5)

$$\begin{aligned} P(\theta) &= Pr(0\,|\,6;\theta) + Pr(1\,|\,6;\theta)\ [Pr(0\,|\,6;\theta)Pr(1\,|\,6;\theta)] \\ &\quad + Pr(2\,|\,6;\theta)\ Pr(0\,|\,6;\theta) \\ &= \phi^6 \quad + 6\theta\phi^5\ [\phi^6 + 6\theta\phi^5] \\ &\quad + 15\theta^2\phi^4\phi^6 \\ &= \phi^6 + 6\theta\phi^{11} + 51\theta^2\phi^{10} \end{aligned}$$

Expected sample size

In both the Raw Odour and Torry Fish Freshness Meter schemes using sampling by attributes, the sample size can be either six or twelve. The expected sample size is the weighted average of these two values, where the weight attached to each is the probability of that sample size.

Let $P(m)$ be the probability that the required sample size is m. Then the expected sample size, $E(n)$, is:

$$E(n) = 6P(6) + 12P(12)$$

Since the only two possible sample sizes are six and twelve.

$$P(6) = 1 - P(12),$$

so

$$E(n) = 6[1 - P(12)] + 12P(12) = 6 + 6P(12).$$

RO attributes scheme

$$\begin{aligned} P(12) &= Pr(1\,|\,6;\theta) \\ &= 6\theta\phi^5 \end{aligned}$$

Thus

$$E(n) = 6 + 36\theta\phi^5.$$

TFM attributes scheme

$$\begin{aligned} P(12) &= Pr(1\,|\,6;\theta) + Pr(2\,|\,6;\theta) \\ &= 6\theta\phi^5 + 15\theta^2\phi^4 \end{aligned}$$

Thus

$$E(n) = 6 + 36\theta\phi^5 + 90\theta^2\phi^4$$

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The use of bromelain in the hydrolysis of mackerel and the investigation of fermented fish aroma

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Summary

A fish hydrolysate was produced from homogenized mackerel using bromelain to increase the rate and extent of proteolysis. Measurement of the extent of hydrolysis and conversion of insoluble to soluble nitrogen compounds after 1, 2, 3, 5 and 14 days of incubation at 38°C showed that a hydrolysate having some of the characteristics of an oriental fish sauce could be formed from mackerel within this period with a net protein conversion rate of over 75%, but it was necessary to include a 24 hr incubation period prior to the addition of salt.

The technique was used for the investigation of the causative agents of aroma production in the mackerel homogenate. Assuming that the proteolysis was still carried out by the protease, the mackerel homogenate was subjected to heat treatment, or mixed with antibiotics, or TCA prior to addition of bromelain. These treatments caused the loss of some of the constituents (associated with oriental fish sauce aroma) which suggests that with mackerel, micro-organisms play a significant role in aroma development. The method could be used for the investigation of sauces prepared from oriental fish; as the causative agents of aroma production might well be different. The bacteria, involved with mackerel could easily produce harmful products which were not investigated.

Introduction

Large quantities of fish caught throughout the world are wasted because of poor consumer acceptability, or the occurrence of a seasonal glut. Methods of preserving the fish or of converting it into a more palatable form would be of great value. One method would be to convert the insoluble protein to a soluble form which would give a good

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yield of protein from the fish bulk and might be more easily preserved. This is done in many South-East Asian countries by fermenting certain types of fish to produce a sauce, which is accepted as part of the daily diet of over 250 million people (Saisithi, 1966).

A typical fermentation involves the placing of layers of small tropical fish (e.g. *Sardinella* sp. and *Stolephoreus* sp.) in contact with layers of salt in large concrete tanks (Saisithi *et al.*, 1966; Van Veen, 1965b). Proteolysis occurs and approximately 50% of the protein is converted to the soluble form (Saisithi, 1966), as the fish disintegrates over a period of about a year to yield a clear brown liquid, which is decanted off. Some lipolysis also occurs (Spinelli, 1971; Lerke, Farber & Adams, 1967).

It would be advantageous with the fish sauce, (a) if the fermentation period could be shortened to reduce the capital costs and increase the throughput, (b) if a maximum conversion of insoluble to soluble protein could be achieved, (c) if the flavour and aroma components could be established and their production accurately controlled, (d) if the high salt concentration could be reduced to allow for a higher individual consumption of fish sauce (providing that undesirable spoilage does not occur) and (e) if fish, normally discarded, could be incorporated.

The main criteria for consumer acceptability of these sauces are flavour and aroma. Although the product varies according to locality (Van Veen, 1965), fish sauces generally have a predominantly salty taste with amino acids and nucleotides contributing to the flavour (Saisithi, 1966; Spinelli, 1971). The aroma is complex but can be divided into three main contributing aromas. These are (a) a fishy aroma due mainly to trimethylamine, (b) a cheesy aroma believed to be due to low molecular weight fatty acids (Saisithi, 1966; Howard & Dougan, 1975) and (c) a meaty aroma which is complex and varies with the origin of the sauce. The brown colour is attributed to the Maillard reaction (Saisithi, 1966).

A variety of methods of increasing the fermentation rate of the traditional sauce have been investigated. Raising the temperature reduced the fermentation rate to a small extent (Amano, 1962). Santos (1968) added papain and obtained a fairly good product although little information is given concerning its quality. A preparation of enzymes from *Bacillus subtilis* has been used to liquefy fish over 3–5 hr at 50°C, but the quality is again not reported (Amano, 1962). 'Pronase' has been used with some success to produce a fish sauce after four months but was found to be expensive (Sulit & Tiongson, 1967). Fresh pineapple juice has been reported to have been added to eviscerated fish to increase proteolysis in the Western part of former Cochin China (Chevy, 1931–32).

The object of the present work is to investigate the possibility of using bromelain (a by-product of the pineapple industry) to increase the rate of proteolysis of fish to produce an oriental fish sauce. The causative agents of aroma production are also investigated since the slow autolysis stage is replaced by a rapid hydrolysis with added enzyme and the fish can be subjected to a variety of pre-treatments which would normally make the preparation of a natural fish sauce impossible.

Materials and methods

Preparation of fish sauces

Mackerel, with head and tail removed, were homogenized in a Sunbeam blender for 5 min at 5°C. The mixture was stored at −20°C. Samples were removed from the freezer as required.

Samples (300 g) were mixed in a 1-litre conical flask with water, cysteine solution and enzyme, as shown in Tables 1, 2 (a) and (b). The pH was adjusted as required. The flasks were plugged with cotton wool and incubated in water baths at the temperature indicated. In some cases, salt was added to the flasks after an interval of time.

TABLE 1. Duplicate samples prepared for fish sauce fermentation

	A	B	C	D	E
Mackerel (g)	300	300	300	300	300
Salt (% w/w)	20	20	10	20	20
Initial time (hr)*	0	0	7	7	20
Enzyme (% w/v)	0	0·2	0·2	0·0	0·2
Cysteine (M)	0·006	0·006	0·006	0·006	0·006
Temp (°C)	38	38	38	38	38
Water (cm^3)	45	45	45	45	45
pH	6·45	6·2	6·2	6·2	6·2
Final pH	7·05	7·20	7·00	7·15	6·20

* Initial time is the time that elapsed between the start of the incubation and the addition of salt.

Degree of hydrolysis (Formol titration)

One-gram samples were removed at intervals from the incubation flasks, mixed with 40 cm^3 of H_2O, and titrated to pH 7·0 with 0·1 N NaOH, and then 10 cm^3 of formalin solution (48% v/v) was added.

The titration was continued to pH 8·5 with 0·1 N NaOH.

Examination of fish sauces

Total soluble solids. One gram of fish hydrolysate was added to 10 cm^3 water, and allowed to stand for 10 min. After filtering through Whatman No. 90 filter paper and washing the residue twice with water, the filtrate and the washings were evaporated to dryness in a tared dish at 110°C and re-weighed.

pH was measured using an EIL Model No. 5417 pH meter.

Nitrogen. The method was a modified version of the A.O.A.C. method. 1 cm^3 of fish hydrolysate was placed in a Kjeldahl flask together with two copper catalyst tablets and 5 ml conc. H_2SO_4. Heating was continued for 45 min, after the solution had cleared.

TABLE 2

(a) Samples prepared to determine the effect of pasteurization temperature on the formation of the cheesy aroma using the rapid proteolytic method

	F	G	H	I	J	K
Mackerel (g)	300	300	300	300	300	300
Salt (%)	0	20	20	20	0	0
Initial period (hr)*	0	24	48	48	0	0
Enzyme (%)	0·2	0·2	0	0·2	0·2	0·2
Cysteine (M)	0·006	0·006	0·006	0·006	0·006	0·006
Water (cm^3)	45	45	45	45	45	45
Temp (°C)	38	38	45	38	38	38
Pretreatment temp.†	—	—	55	63	63	100
Pretreatment time (min)‡	—	—	30	30	30	30
Final pH	5·65	5·85	5·7	5·8	7·6	7·2

* Time elapsed between the commencement of the fermentation and the addition of salt.
† Temperature of heat treatment, prior to the addition of bacteria free enzyme solution.
‡ Time of pasteurization.

(b) Samples prepared to evaluate the effect of TCA and antibiotics on the development of the cheesy aroma

Mackerel (g)	300	300	300	300
Treatment	1% TCA§	0·2% antibiotic¶	—	—
Salt (%)	20	0	0	20
Initial period (hr)	24	0	0	0
Enzyme (%)	0·2	0	0·2	0
Cysteine	0·006	0·006	0·006	0·006
Water (cm^3)	45	45	45	45
Temp. (°C)	38	38	38	38

§ Fish homogenized with 1% TCA, filtered, washed with water neutralized to pH 6·4 with NaOH, then treated with the enzyme, cysteine, etc.
¶ Mixture (1:1) of obenine and penbritin.

After diluting to 50 ml the ammonia was distilled and the remainder of the method was as published (A.O.A.C., 1970).

Free volatile fatty acids. 10 cm^3 filtered fish sauce was acidified with 2 N H_2SO_4 to a congo red end point, the solution was steam distilled in a Markham still. The distillate (150 cm^3) was neutralized with 0·1 N NaOH and 0·5 cm^3 was added in excess. The solution was evaporated at 38°C to dryness. It was dissolved in the requisite concentration of 2 N dichloracetic acid in acetone and made up to 1 cm^3. The final acid

concentrations in acetone were determined by GLC using a Perkin Elmer F11, at 140°C with a N_2 gas flow of 30 ml/min and a column of 5% FFAP on chromasorb G AW-DCMS (180–100 mesh, 2 m × $\frac{1}{8}$ in O.D.).

Trimethylamine was determined by the Conway Micro diffusion method (Santos, 1968; Murray & Gibson, 1972).

Stability of bromelain in salt solution

The activity of bromelain after various time intervals was determined by incubating 1% enzyme solution in 0%, 10% and 20% NaCl solutions at 38°C and in 0% salt at 45°C.

The assay was carried out by adding 2 cm^3 of the incubated enzyme in salt solution to 0·2 cm^3 of 0·3 M cysteine and 5 cm^3 of 0·5% casein solution. 0·1 cm^3 of 1 M citrate buffer (pH 6·0) was added, and the mixture was incubated at 38°C for 10 min. 4 cm^3 of solution was transferred to 5 cm^3 5% TCA, filtered and the absorbance of the filtrate at 280 nm was measured in a 1 cm cell.

Results and discussion

Stability of the enzyme in NaCl solutions

Twenty per cent salt concentration caused a loss of 65% of bromelain enzyme activity within 30 hr at 38°C. Even in 10% salt, enzyme activity was reduced by 45%. The higher the temperature the greater was the loss of activity, therefore 38°C with both the 10% and 20% salt concentrations was selected for production of fish sauces.

Hydrolysis of the fish

The degree of hydrolysis, followed by measuring the carboxyl groups released with the formol titration, in the first series of sauces (Table 1) are presented in Fig. 1.

Samples C, D and E allowed an initial incubation period without salt. This will encourage the growth of bacteria which might well give a different mechanism to that found in oriental fish sauce manufacture, and could produce harmful compounds.

The extent of the hydrolysis was much greater in those flasks containing added enzyme (E, B, C) than the natural fermentation (A, D) within the time period selected. Hydrolysis of samples approached the maximum value in five to ten days. With sample E, this was in the order of 13·5% hydrolysis of the initial product (assuming the MW of an average amino acid to be 90). The proteolysates produced were compared with some commercial samples of fish sauce from Thailand and Malaysia. Most of the experimental samples had a total solids content similar to the commercial samples.

Sample E, yielded a good product with a typical fish sauce aroma, although the 'meaty' aroma present in the Thai sauce was not so pronounced. This sample had a higher soluble nitrogen content than the commercial samples as measured by the

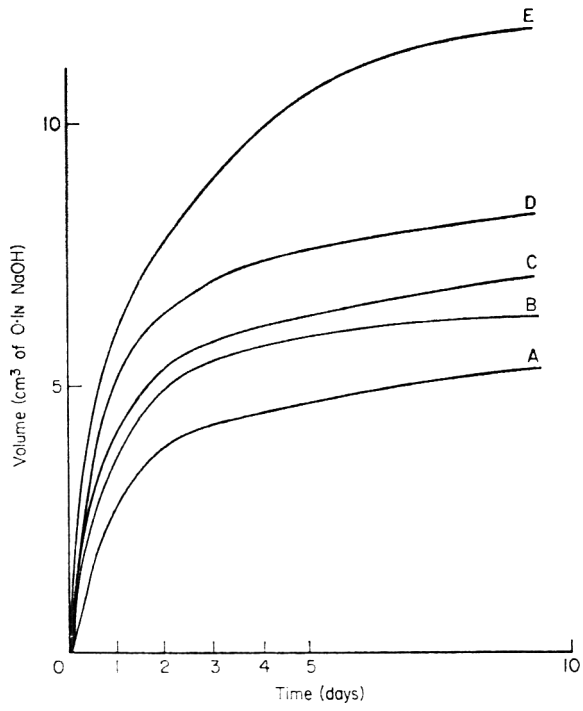

FIG. 1. Degree of hydrolysis (expressed as volume of 0·1 N used in the formal titration) *v.* time for a series. A, No enzyme, 20% salt; B, 0·2% enzyme, 20% salt; C, 0·2% enzyme, 10% salt, 7 hr initial period; D, 0·2% enzyme, 20% salt, 7 hr initial period; E, 0·2% enzyme, 20% salt, 20 hr initial period.

modified Kjeldahl method. It has been reported that bacterial action does not occur with the commercial preparations (Hamm & Clague, 1950).

Samples A–D had only oily aromas that did not resemble the commercial samples.

Within fourteen days only 41·7% of the insoluble protein nitrogen in A, had been hydrolysed by bromelain. Delayed addition of salt aided proteolysis. With a 7-hr delay before addition of salt, protein conversion was increased to 76·5% and a 20-hr delay increased the conversion to 89·7% (Table 3).

Repulsive odours, which occurred slowly during the incubation period, became too strong with initial incubation periods much longer than 24 hr and the aroma of the resulting fish sauce showed no resemblance to commercial fish sauce.

The length of the initial incubation period had a pronounced effect on the aroma. The samples, after a 7-hr incubation prior to the addition of the salt, followed by a full incubation period, yielded sauces which after 24 hr had a slight typical fish sauce aroma which gradually disappeared with continued fermentation.

The 20-hr incubation produced a fish sauce with fishy and cheesy aromas of typical commercial fish sauces. This aroma was investigated further.

TABLE 3. Examination of the products of hydrolysis

Sample	Vol. obtained (cm^3)	Total solid (%)	Formal nitrogen % Amino N	Total N/ml* (%)	Conversion of nitrogen† (%)
A	30	32·4	1·4	2·0	20·7
B	80	22·2	0·20	1·5	41·7
C	110	31·7	0·80	3·0	76·5
D	55	31·3	0·64	1·2	23·0
E	120	34·7	1·43	2·20	89·7
Thai	—	36·0	1·22	1·7	—
Malay	—	28·5	0·67	1·3	—

* By modified Kjeldahl method.

† Per cent protein in original fish = 17·9% (N × 6·24).

Liberated amino acids could act as aroma precursors and be involved as flavour components (Co & Anderson, 1970). The amino acid profiles of the samples are presented in Table 4. The colour of sample E was very similar to that of the Thai and Malay sauces.

TABLE 4. Amino acid profiles of samples compared to commercial samples of Thai and Malay fish sauces (in mg/cm^3)

	A	B	C	D	E	Thai	Malaysian
Asp	1·11	2·78	0·88	0·52	1·26	2·76	1·10
Thr	0·98	0·48	0·46	0·32	0·26	1·61	0·70
Ser	0·84	0·00	0·26	0·20	—	0·74	0·16
Glu	1·84m	3·7	2·20	1·44	3·42	5·82	1·78
Pro	0·44	0·32	0·20	0·12	0·32	6·88	0·26
Gly	0·58	0·72	1·80	0·28	0·92	1·10	0·44
Ala	1·74	0·48	1·5	1·08	3·96	2·84	1·52
Cys	0·20	1·04	0·52	0·28	2·38	0·24	0·42
Val	1·14	2·14	0·64	0·64	2·20	1·82	1·0
Ileu	1·10	2·0	0·62	0·60	1·94	1·82	0·98
Met	0·72	1·18	0·60	0·26	0·96	0·74	0·48
Leu	2·30	2·94	1·82	1·28	2·80	2·02	1·64
Tyr	0·18	0·94	0·50	0·42	1·40	0·14	0·32
Phe	0·80	0·34	0·78	1·38	2·46	1·16	0·66
Lys	0·80	5·1	0·20	—	4·0	0·72	0·40
His	2·92	5·2	1·48	1·44	3·42	3·06	1·66
Arg	1·2	—	0·40	0·18	—	—	—

The use of the rapid hydrolysis method in investigating the causative agent in the aroma

Because the proteolytic stage could be controlled, the agents that are potentially responsible for aroma formation could be investigated. Fish enzymes, (Saisithi, 1966) microorganisms (Saisithi *et al.*, 1966) and fat oxidation (Howard & Dougan, 1975) have all been implicated as potential causation agents in the development of the aroma of oriental fish sauces. When the causative agent has been established, then the metabolic route can be investigated.

The method was used to investigate the causative agent of the aroma of the sauce obtained from mackerel, which resembled oriental fish sauce with respect to the cheesy and ammonical components (sample E). The meaty aroma was not sufficiently strong to merit investigation.

The cheesy note was related to the concentrations of low molecular weight fatty acids, as indicated by Howard & Dougan (1975).

A series of fish samples were subjected to a number of heat treatment (pasteurization) processes as given in Table 2 (a). In each case, except for the rapid hydrolysis method control sample G, the typical cheesy aroma did not develop, possibly due to the difference in the time of incubation before adding salt. The rate of hydrolysis was followed in samples H and I which were incubated at a higher temperature and should have had an increased rate of proteolysis. The rate and extent of the hydrolysis were similar to that of B in Fig. 1.

The low molecular weight fatty acid concentrations of the sauces resulting from samples F and G were analysed and are given in Table 5. The suggested range for a large number of Thai sauces (J. Dougan, personal communication) would be in the order indicated. Samples J and K gave very low values for neutralization of the distillate, and so must be far removed from a typical fish sauce.

As only sample G (control) yielded a satisfactory aroma, the trimethylamine value for this was measured and shown to be 98. The Thai sauce gave a value of 48.

TABLE 5. Concentrations of the volatile organic acids present in some fish sauce samples (mg/cm^3)

Acid	F	G	I	J	Thai*
Acetic	1·10	2·30	1·20	0·99	2·0
Propionic	0·57	0·110	0·055	0·033	0·2
i-Butyric	1·06	0·078	0·31	0·107	0·02
n-Butyric	0·16	0·156	0·043	0·470	0·2–0·4
i-Valeric	0·063	0·074	—	0·021	0·02
n-Valeric	—	0·036	—	0·798	—

* Values taken as representative of a typical commercial Thai fish sauce (Howard & Dougan, 1975).

It would appear that pasteurization which destroys the majority of enzyme activity and microorganisms results in no development of the cheesy aroma during fermentation.

Another batch of samples were fermented (Table 2 (b)) but in one case the fish was treated with 1% trichloracetic acid which would destroy both bacteria and enzymes. Also a batch was incubated with a fairly broad spectrum mixture of antibiotics. In the latter case, it was unnecessary to add salt as the fish was effectively preserved. In neither of these batches was a satisfactory aroma developed, by subjective testing, this is confirmed by the analysis (Table 5).

From this it would appear that bacteria play an important role in the development of the cheesy aroma of the sauce obtained from mackerel. The contribution by the fish enzymes could be destroyed by heat or by chemical treatment, and then the substrate could be inoculated with microorganisms present in fish or additions of bacterial enzyme extracts could show the importance of microorganisms compared to the fish enzymes. The role of lipids in aroma development could also be assessed using this method. However, this would be of greater value if the tropical species and micro-organisms actually present in these fish fermentations were investigated.

It is quite possible that a different mechanism is involved with the natural tropical fish sauce manufacture, since the present investigation with mackerel involved the use of North Sea fish which have a relatively high bacterial count. The fish were also macerated, thus introducing surface bacteria, and a salt free incubation period was necessary to produce the cheesey note. Dougan & Howard showed that the fatty acids were produced after three months in the traditional process. Whereas the present 'rapid' method undoubtedly involved bacteria and took fourteen days. The prolonged period in the traditional method could produce a completely different situation.

The bacteria involved could also produce harmful compounds, so the method involving the salt free period cannot be recommended as satisfactory for producing a fish sauce, without further investigation.

Conclusions

A high degree of proteolysis of mackerel was obtained at 38°C with the addition of 0·2% bromelain. However, the addition of salt as a preservative (as in tropical fish sauce manufacture) decreased the extent of conversion of soluble to soluble protein from 89·7% to 41·7% in fourteen days, without a salt free period.

A 24-hr incubation period prior to the addition of salt allowed the development of the constituents of the cheesy aroma of an oriental fish sauce. Investigation, using the rapid hydrolysis method demonstrated that microbial action is important in the development of the aroma with mackerel. The importance of the proteolytic stage in aroma formation could be the provision of amino acids as substrates for the trans-formation or syntheis of flavour compounds by bacteria. However, toxins and other

contaminants could be produced by microbial action, and these would have to be investigated. The rapid hydrolysis method would facilitate the investigation of the development of typical aroma in tropical fish sauce.

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The connective tissues of fish

VIII. Comparative studies on hake, cod and catfish collagens

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Summary

Some properties of the collagens of myocommata and skin from three species of fish are described. The musculature of hake separates readily into flakes because of intrinsic weakness in the connective tissue. The corresponding tissue of catfish is unusually strong and holds the musculature together under almost all conditions, while that of cod has properties intermediate between the other two. Skin is stronger than myocommata, thickness for thickness, but the order of strength between species is still hake < cod < catfish.

The acid-soluble collagen of hake in citrate buffer has the highest thermal denaturation temperature of the three species (19·4° compared with 13·4° for catfish) and the highest content of imino acids. It also has the highest proportion of stable *intra*-molecular crosslinks.

Introduction

Most comparative studies of fish collagens have used skin or swim bladder as the raw material, and have considered differences in the amino acid composition (e.g. Piez & Gross, 1960) and the thermal stability (e.g. Takahashi & Yokoyama, 1954) between species. The collagen found in myocommata, the thin sheets of connective tissue between the myotomes, has not been investigated until recently, neither has that from the fine processes which come from the face of each myocomma, extending between and around the muscle cells and probably connecting with the next myocomma (Love, 1970). Studies of the physical properties of such finely divided material appear at first sight to be difficult to carry out, but in fact it is possible to learn a great deal from simple observations, as follows. If the fish is filleted, any mechanical failure of the fine collagenous processes results in slits or holes appearing in the cut surface of the muscle, a phenomenon known as gaping. The extent of gaping can be assessed visually, and by using an arbitrary numbered scale, Love & Robertson (1968) were able to relate the properties of the fine processes to the biological condition of the fish. In addition, Love, Lavéty & Steel (1969) made comparisons between different species and,

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considering the nature of the measurement, obtained some surprisingly reproducible results. Among a number of marine fish they found that the haddock (*Gadus aeglefinus* L.) gaped more severely than other species, while catfish (*Anarhichas lupus* L.) and skate (*Raja batis* L.) did not gape at all, even when the muscle was roughly handled.

Later studies (Love, Lavéty & Garcia, 1972) showed that the isolated myocommata of catfish were in fact mechanically stronger than those of, for example, cod (*Gadus morhua* L.) but that those of haddock were *not* weaker, the excessive gaping in that species being attributable to a low post-mortem pH (Love & Haq, 1970) which severely, though reversibly (Love *et al.*, 1972), weakens the myocommata. It was assumed in the reasoning that the myocommata were of the same material as the fine collagenous processes.

However, it is well known among fishermen that the musculature of hake (*Merluccius merluccius* L.) also tends to fall apart on filleting, and as its weakness appears in this case to be a characteristic of the connective tissue rather than a pH effect, it seemed a good subject for study in the present work.

Our purpose, then, is to compare in more detail the connective tissues of hake, which gape, with those of catfish, which do not, and also to examine those of cod which appear to have properties lying somewhere in between.

Materials and methods

Raw material

The catfish were caught east of Faroe (62° 01′ N, 06° 12′ W) on 22 September 1972 and the hake and cod were caught in the Minch (Ru Stoer) (58° 15′ N, 5° 30′ W) on 26 September 1972. All fish were gutted and chilled as quickly as possible after death and packed in drained melting ice for one day before being frozen in a vertical plate freezer (about 3 hr). They were placed in a store at —30°, first on the boat and then at the laboratory, until needed. The measurements of mechanical strength of the connective tissue were thus made on frozen-thawed material. It was not usually possible to make them on the fresh tissue, since hake and catfish are difficult to obtain on grounds near Aberdeen and the use of unfrozen but stale fish was considered to be less satisfactory. However, local fish were obtained on one occasion and used in the salt-solubility experiment. Myocommata were dissected as described by Love *et al.* (1972).

Preparation of acid-soluble collagen from skin and myocommata

Samples of myocommata or skin weighing 5–9 g were finely chopped with scissors and mechanically stirred for 24 hr with 150 ml 0·5 M sodium acetate. The insoluble material was stirred with a further quantity of sodium acetate after being separated by centrifugation (20 000 ***g*** for 30 min). It was then centrifuged again and washed with 50 ml cold deionized water, centrifuged as before and extracted with 250 ml 0·5 M acetic acid for 24 hr to get most of the collagen into solution. All subsequent steps were

performed essentially according to the procedure of Lewis & Piez (1964). At the end of the preparative procedures, all of which had been carried out at about 3°, the precipitated collagen was freeze-dried and stored at about 3° over calcium chloride until needed. It should be emphasized that treatment with acid in this way destroys some of the intermolecular cross links, so that the collagen is not in the same state as in the original tissue. However, the acid treatment is essential to obtain a solution.

Other techniques

The water content of the musculature, an indication of the biological condition of the fish (Love, 1960) was calculated from the weight lost by about 6 g of mixed tissue (myotomes + myocommata) from the anterior end of the fish after seven days at 100° in an open vessel. The pH of the musculature and the breaking stress of strips of skin or myocomma were measured as described by Love *et al.* (1972). Thickness was calculated from the weight of a given area of tissue, taking the specific gravity of cod connective tissue as 1·09. This value was found to be valid also for hake, but that of catfish was 1·07.

The nitrogen content of the acetic acid soluble collagen was measured by Kjeldahl's method, and the amino acid composition by a Technicon auto-analyser after hydrolysis in 6 N HCl under pressure at 125° for 1 hr. Hydroxyproline was also determined separately by the method of Leach (1960).

The thermal denaturation temperature was measured on solutions of collagen (about 0·7 mg/ml) in sodium citrate buffer ($I = 0{\cdot}089$, $pH = 3{\cdot}5$). The solutions were each kept for 60 min in a bath at a constant temperature between 9° and 23°, inclusive, at single degree increments, the variation in any temperature being $\pm 0{\cdot}03°$. After the incubation period the viscosity of the solution was measured at the incubation temperature in a constant-temperature jacketed Ostwald viscometer (Technico type BS/U, size B, Gallenkamp & Co., London).

Sedimentation analysis of collagen denatured at 30° for 2 hr was carried out at 25° with a Spinco model E analytical ultracentrifuge at 59 780 rev/min with an An-D analytical rotor. Photographs were taken using a phase plate angle of 55°. The relative proportions of the α- and β-components were calculated by cutting out and weighing enlarged paper tracings of the peaks in the Schlieren diagrams, correcting for the effect of radial dilution (Schachman, 1959).

Tissue solubility was measured on skin and myocommata which had been finely chopped with scissors and then homogenized for 1 min in an Ultra-Turrax homogenizer in fifty times its weight of 0·5 M NaCl, all operations being carried out at 0° to 4°. The mixture was stirred mechanically overnight, centrifuged until clear and, after decanting, rehomogenized with further NaCl solution and stirred a further night. After centrifuging and again decanting the insoluble material was rehomogenized and immediately centrifuged. The three supernatants were combined and the procedure repeated on the insoluble material using sodium citrate buffer (pH 3·5) as the solvent.

The proportions of collagen soluble in salt and dilute acid and the insoluble fraction were calculated from their hydroxyproline contents, measured by the procedure of Leach (1960).

Results

Breaking stress of tissue

Some properties of the fish and their connective tissues are shown in Table 1.

TABLE 1. Characteristics of the fish used and properties of their connective tissues

	Body length (cm)	pH of muscle (post-mortem)	Water content of muscle	Thickness of myocommata (mm)	Breaking stress of myocommata (N/cm^2)	Thickness of skin (mm)	Breaking stress of skin (N/cm^2)
Hake	87	6·42	79·4	0·12	34·7 ± 12·0	0·36	159·0 ± 13·9
Hake	89	6·43	80·3	0·10	42·3 ± 15·7	0·38	224·1 ± 46·6
Hake	82	6·38	77·7	0·11	14·2 ± 0·9	—	—
Cod	94	6·51	80·3	0·17	65·0 ± 24·4	0·39	402·1 ± 65·3
Cod	86	6·43	81·6	0·20	109·6 ± 21·3	0·45	274·3 ± 21·7
Catfish	52	6·28	80·1	0·11	162·3 ± 69·1	1·05	456·1 ± 64·6
Catfish	47	6·32	79·1	0·09	153·8 ± 36·0	1·11	464·1 ± 151·1
Catfish	44	6·39	80·5	—	—	1·05	429·2 ± 50·5

Values of breaking stress shown are each the mean of ten determinations, with standard deviations.

The relatively low pH values and water contents of the muscle indicate well-fed fish, as we should expect since they were caught in the month of September. The myocommata of the three species seem to be roughly comparable in thickness. The skins of hake and cod are also comparable, but that of catfish is much thicker—perhaps for protection, since this is an aggressive species.

Turning now to the breaking stress, we see that the relative strengths of the myocommata of the three species mirror their tendency to gape, that of hake being weakest and that of catfish strongest.

The same relationship between the species is seen in the stresses needed to break the skin. They were, however, much greater than the stresses which would break the same thickness of myocommata. The difference between the strengths of skin and of myocommata has already been reported in cod by Mohr (1971), and the difference between the myocommata of cod and of catfish by Love *et al.* (1972).

Solubility of collagen

The solubility of the two tissues from the three species is shown in Table 2. For this experiment only, two specimens of each species of fish were caught in the North Sea

near Aberdeen on 11 October 1975. The mean water contents of the muscle were: hake: 80·1%; cod: 80·1%; catfish: 79·6%. Body lengths averaged 76, 63·8 and 64·5 respectively.

TABLE 2. Solubility of collagen from myocommata and skin from hake, cod and catfish

	Hake	Cod	Catfish
Myocommata			
Salt soluble	5·14	4·16	8·26
Citrate soluble	54·94	76·31	42·00
Insoluble	39·92	19·53	49·74
Skin			
Salt soluble	5·18	1·49	2·29
Citrate soluble	60·03	85·88	62·60
Insoluble	34·80	12·63	35·11

Results are expressed as percentage of the total collagen.

Since the native collagen of the myocommata of the catfish is the least soluble among the three species it presumably possesses the greatest proportion of *inter*-molecular cross links. The observation is therefore in accord with the mechanical superiority of catfish myocommata, but otherwise the results of Table 2 are not informative in the general context of this paper. Cod has less insoluble material than hake, in spite of cod collagen being the stronger of the two, so one can surmise that if its strength stems from *inter*-molecular bonding, the bonds are labile at pH 3·5.

Properties of acetic acid soluble collagen

Amino acid composition. Harrington & von Hippel (1961) pointed out that fish collagens exhibit an appreciably wider range of amino acid composition than the mammalian species, in keeping with the greater evolutionary time scale which they span. In the present analyses (Table 3), the acid soluble collagen of catfish myocommata seems to show the biggest divergence from those of the other two species, with much higher contents of histidine and hydroxylysine.

Glycine makes up close to one-third of fish collagens, despite variations in other amino acids (Eastoe & Leach, 1958; Piez & Gross, 1960), and this pattern is also to be seen in the fish collagens of Table 3, the glycine of catfish skin collagen being rather higher than the others.

The proportions of alanine given in the table are quite close to the generalization of Eastoe & Leach (1958) of one alanine in every nine residues, though the figure for catfish is rather lower.

At least part of the tyrosine was considered by Eastoe & Leach (1958) to be an

TABLE 3. Amino acid composition of fish acetic acid-soluble collagens (residues/1000 residues)

	Myocommata collagen			Skin collagen		
	Hake	Cod	Catfish	Hake	Cod	Catfish
Ala	118·0	106·0	92·7	129·0	105·6	99·1
Gly	323·1	313·6	311·8	328·3	332·2	367·0
Val	24·4	26·1	25·0	24·2	25·2	21·8
Leu	28·0	32·3	27·6	21·7	29·7	23·6
Isoleu	15·6	18·6	14·5	12·5	17·1	11·2
Pro	91·5	87·6	79·2	99·7	89·6	87·3
Phe	15·1	14·5	14·4	13·9	13·8	15·1
Tyr	4·3	6·0	3·6	2·2	5·0	3·1
Ser	48·3	62·9	64·2	49·0	60·8	66·9
Thr	30·2	25·8	25·4	28·3	26·1	24·8
Met	20·6	20·4	19·2	19·2	20·6	16·5
Arg	61·3	59·1	56·5	64·6	62·5	62·1
His	12·7	16·3	40·4	11·5	11·8	9·2
Lys	38·4	36·9	48·8	34·0	33·1	31·5
Asp	39·1	42·3	37·6	37·1	41·5	39·4
Glu	81·7	82·2	72·2	75·6	77·4	74·2
Hypro	41·2	40·7	39·5	43·8	40·7	41·9
Hylys	6·7	8·2	27·6	6·3	7·6	6·0
Recovery by weight (%)	88·7	88·1	94·9	98·6	98·6	91·8
Total N (g/100 g)	17·2	17·8	18·0	17·9	18·0	18·1

impurity in the collagen preparation, because the concentration decreased when the collagen was converted to gelatin. Mohr (1971) found the low values of 3·8 and 3·9 residues of tyrosine per thousand in purified acetic acid soluble collagen from cod myocommata and skin, respectively. The present values, ranging from 2·2 to 6·0 residues, seem therefore to indicate a reasonable state of purity.

Hydroxylysine comprises just three to twelve residues per 1000 (Harrington & von Hippel, 1961), but since it cannot be removed by purification procedures it is thought to be an essential part of the collagen molecule. The values for hydroxylysine in Table 3 fall within this range, apart from the high value in catfish myocomma collagen already noted.

The levels of hydroxyproline, which stabilizes the collagen molecule, are shown in the results of column chromatography in Table 3 and, as a check, by colorimetric measurement in Table 4. In both cases there is more in hake than in catfish, though the differences are not great and the position of cod is not always the same in the series of three species. Our value (Table 4) for the hydroxyproline content of cod skin collagen, 7·61%, is close to the values obtained by Doty & Nishihara (1958), 7·0%, by Mohr (1971), 7·27%, and by Young & Lorimer (1960), who found 8·0% hydroxyproline.

TABLE 4. Hydroxyproline in acetic acid-soluble fish collagens (wt %) as determined by Leach's method

	Myocommata collagen	Skin collagen
Hake	7·63±0·57	8·36±0·40
Cod	7·64±0·02	7·61±0·34
Catfish	7·00±0·18	7·20±0·14

Values are the means of triplicate determinations, with standard deviations.

On the other hand, the value obtained by chromatography for this tissue, 40·7 residues per 1000, is lower than that obtained by Piez (1965), fifty-six residues, and by Mohr (1971), sixty residues.

Proline, the other imino acid which stabilizes the collagen, shows clear differences between the species, increasing in concentration in both kinds of acetic acid soluble collagen in the order catfish–cod–hake.

Eastoe & Leach (1958), Piez & Gross (1960) and Pikkarainen & Kulonen (1969) noted that an increase in the proportion of hydroxyproline between species was matched by a decrease in serine, which is confirmed in the present results, hake having the least serine of the three and catfish the most. However, there does not seem to be a matching decrease in threonine corresponding with that reported by these authors.

Pikkarainen & Kulonen (1969) also noted that the sum of serine, threonine, imino acids and alanine, which were all related by the genetic code, were constant in vertebrate collagens. In the present work it can be seen that the proportion of alanine matches that of the imino acids, being highest in hake and lowest in catfish.

The total nitrogen content of cod skin collagen was reported by Doty & Nishihara (1958), Young & Lorimer (1960) and Mohr (1971) to be 18·3%, close to the value in Table 3. Piez (1965) reported a value of 18·6%.

Thermal denaturation temperature. Collagen solutions are viscous, but when they are heated beyond a certain temperature the viscosity suddenly decreases as the molecules are denatured and uncoil. The thermal denaturation temperature is taken as that at which the viscosity has fallen to 50% of its initial value after incubation for an hour.

TABLE 5. Temperature (°C) at which the viscosity of collagen in citrate buffer solution falls to half its initial value

	Myocommata collagen	Skin collagen
Hake	19·5	19·4
Cod	15·3	15·0
Catfish	—	13·4

In the present experiments, five solutions were incubated at any one temperature and the mean viscosity was plotted. The results, read from the graphs obtained, are summarized in Table 5.

There was insufficient material left from the catfish myocommata to do these determinations, but the difference between the denaturation temperatures of catfish skin and that of cod is almost certainly real, being of the same order as that found by Gustavson (1956, Table 25, p. 224), who studied the shrinkage temperatures of the intact tissue.

Doty & Nishihara (1958) showed that the denaturation temperature of different collagens was proportional to the hydroxyproline content, and it was shown by Piez & Gross (1960) that both the proline and the hydroxyproline were involved in establishing the denaturation temperature. They interpreted the observation as support for the hypothesis that the molecular structure of collagen was stabilized by the restriction on changes in the secondary structure of the polypeptide chain imposed by the pyrrolidine rings of these two imino acids.

The present results (Tables 3–5) show that the collagen with the highest proportions of proline and hydroxyproline (hake) has the highest denaturation temperature, while that of catfish with the lowest proportions has the lowest. This not only confirms the earlier work, but suggests that the rather small interspecies differences in imino acid content are real.

In agreement with the present results, Mohr (1971), using the same solvent as we did, found a difference between the denaturation temperatures of cod collagen from myocommata and that from skin but the actual temperatures he found, 13·2° and 12·3°, respectively, were slightly lower than the present ones.

Sedimentation analysis. When heated above its denaturation temperature, the collagen helix disintegrates and the individual chains ('α-chains') of amino acids are freed. However, if stable *intra*-molecular cross links are present, some of these chains remain linked to one another as dimers (β-components) or trimers (γ-components) after denaturation.

In the ultracentrifuge, the most slowly-sedimenting component in solutions of denatured collagen has been shown to consist of the separated α-chains, which are preceded by the heavier β-components, and, most rapidly, γ-components if any are present (various authors, reviewed by Piez, 1967). Thus an examination of the Schlieren diagram will give an indication of the amount of cross-linking between the individual chains in the collagen molecule.

In the present work, 15–18 mg dried collagen were dissolved in 3 ml sodium formate buffer ($I = 0{\cdot}079$, $pH = 3{\cdot}8$) with stirring for 24 hr at 3°. This was cleared by centrifuging and then held at 30° for 2 hr to denature, the concentration as determined by Kjeldahl analysis being 0·3–0·5% in different runs. Ultracentrifugation was then carried out at about 25°; it is necessary to keep the temperature up, because the reaction is reversible to some extent (von Hippel & Harrington, 1959).

TABLE 6. Relative proportions of α- and β-components (%) in a formate buffer solution of denatured acid-soluble collagen prepared from myocommata and skin of three species of fish

	Myocommata collagen		Skin collagen	
	α	β	α	β
Hake	70·4	29·6	62·2	37·8
Cod	73·2	26·8	71·4	28·6
Catfish	100	0	100	0

In the Schlieren diagrams there was no sign of γ-component (trimer), but two clear peaks marked the presence of α- and β-components. The proportions of each, calculated as the areas under each peak expressed as a percentage of the total (see experimental section) are shown in Table 6.

The present results indicate that hake tissue has the biggest proportion of thermally stable *intra*-molecular cross-links, while catfish has none, confirming that such links do not relate to the mechanical strength of the collagen. Piez (1972) states that even in the intact animal their purpose is not clear—they would not contribute significantly to the stability of the collagen molecule or of the fibril. He concludes that they may be intermediates in the formation of *inter*-molecular cross-links.

Discussion

The results shown in Table 1 confirm that the physical strength of isolated myocommata at constant pH reflects the tendency of the species to gape. This has in the past been assumed, but does not necessarily follow. Gaping probably results from the rupture of the fine collagenous processes which surround the muscle cells ('endomysial connective tissue') and which merge with the myocommata at the myotome–myocomma interface (Love *et al.*, 1969), not from the rupture of the myocommata themselves. It was assumed that the pericellular collagen possessed the same properties as that of the myocommata, and the present results lend support to this idea. It is interesting to note that the breaking stresses of the skins of each species, while much greater than those of the corresponding myocommata, still follow the tendencies of the different fish to gape. Although Mohr (1971) has shown that chemical and physical differences exist between the acetic acid soluble collagens of myocommata, skin and swim bladder within one species (cod), it is clear that each species tends to have a characteristic collagen. The way in which collagens of variable strengths relate to the way of life of the different species is still unknown, although as already stated the catfish, with the strongest collagen, is also possibly the most aggressive.

It has been well established in other species that the intact connective tissue shrinks and the corresponding collagen in solution denatures at progressively higher temperatures as the proline and hydroxyproline increase in proportion to the other amino acids (Gustavson, 1953, 1956, p. 220; Piez & Gross, 1960). The way in which these two substances stabilize the triple helix has been discussed by von Hippel & Harrington (1959) and Josse & Harrington (1964). In the present study, it is interesting to note (Tables 3–5) that the mechanically weakest collagen (that of hake) is the most thermally stable, and vice versa.

At least one other member of the hake family is very prone to gape. The phenomenon has been described qualitatively in the Cape hake (*Merluccius capensis*) by Burt *et al.* (1968), who point out that in spite of the relatively high temperature of the surface waters off South Africa where the fish is caught, it is really a cold-water species, living and feeding in the bottom waters of the Benguela current. It would be interesting to know whether the collagen is as thermally stable as that of its Northern counterpart, *Merluccius merluccius*.

The results of the sedimentation analysis in formate buffer solution (Table 6) show that the number of stable *intra*-molecular cross-links joining adjacent α-chains in the collagen molecules differ according to species. The figures for α and β components have not been described previously for hake or catfish, but in cod we may compare our results with published values. Piez (1967) showed that the proportions of α, β and γ components in denatured soluble collagen of cod skin were 40 : 50 : 10 respectively. Mohr (1971), working in this laboratory, could not detect any γ component, as in the present work, but found 46% and 54%, respectively, of α and β components in cod skin and 54 and 46%, respectively, in cod myocommata. Thus the particular specimens of cod investigated in the present study appear to have contained a collagen less cross-linked with thermally stable intramolecular bonds than were the collagens investigated by the other workers.

To sum up, the mechanical strengths of the myocommata and skin reflect the tendency of different species to gape, but studies on the acetic acid soluble collagen do not extend our knowledge since hake connective tissue, mechanically the weakest, contains the most thermally stable collagens and also the collagen with the greatest degree of stable *intra*-molecular cross-linking. Differences in the number of thermally—and probably acid—labile *inter*-molecular bonds may therefore perhaps govern the interspecies differences in the mechanical properties of the connective tissues.

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Eating quality of hot deboned beef

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Summary

One side from each of six beef carcasses was treated in a standard commercial fashion (control) whilst meat from the other sides was removed and packed either as joints or muscles within 3 hr after stunning (hot deboned). The eating quality of the meat was assessed by laboratory and consumer taste-panels and the texture determined instrumentally. When the meat was held at 10°C until the development of rigor mortis, the ultimate eating quality was equal to that of the controls. Ageing enhanced the tenderness of hot deboned meat.

Introduction

The traditional handling of beef carcasses for the UK retail trade involves chilling in the abattoir over 24 hr, wholesale distribution in quarters and a holding period in retail premises which may vary from one to many days. Meat is, therefore, never cut before the completion of rigor mortis. Holding before cutting improves quality because of the tenderizing effect of conditioning; it is also expensive because of the cost of refrigeration and because of the inevitable loss of weight due to evaporation. Evaporation is most rapid while the carcass is still hot and hence rapid chilling immediately after slaughter is advocated to reduce weight loss. Rapid chilling also minimizes bacterial growth which reduces the risk of bone taint internally and increases shelf life by reduction of surface spoilage organisms.

Against these desirable consequences, a fall in the temperature of meat below 15°C pre-rigor induces contraction of the muscle which can cause a severe increase in the eventual toughness of the meat after cooking (Locker & Hagyard, 1963). This process of cold-shortening will occur in any part of the beef carcass which is cooled, in rough terms, below 10°C within 10 hr after slaughter (Bendall, 1972), hence an efficient cooling system applied immediately after dressing will cold-shorten the surface layers of any carcass to a depth depending on the circumstances.

Adequate control of temperature throughout the meat is, therefore, essential if abattoir throughput is to be maximized without the risk of cold-shortening but this is difficult to achieve in the case of cooling the whole side of beef. Removal of the meat from the skeleton pre-rigor would allow much greater control of cooling since cuts

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could be packaged in small units (Follett, Norma & Ratcliff, 1974; Schmidt & Keman, 1974). Such a procedure has the added benefits of preventing evaporative weight loss, avoiding the unnecessary chilling of bones and excess fat and more efficient use of refrigerated space. Colour and drip loss are also improved in meat which is cooled more rapidly.

Although cold-shortening would be avoided in such a controlled hot deboning operation, the overall effects on eating quality are not predictable and this effect has been studied in the present work.

Experimental

Materials

Five steers (aged eighteen to twenty-four months) and one Jersey cow (aged four to five years) were slaughtered and the carcasses dressed and split conventionally. One side (control) of each carcass was held at ambient temperature for 5 hr and then transferred to a chillroom at 1°C. Boneless joints or muscles were removed from the other side of each carcass as soon as possible and no later than 3 hr after stunning. The muscles were packed individually in plastic bags (which had a very low permeability to oxygen and were impermeable to water vapour) which were then heat shrunk on to the meat by placing the pack in water at about 88°C for 2 or 3 sec. The whole procedure was completed in less than 3 hr after stunning. The packs were stored at 10°C for 24 hr. Subsequently they were stored at 1°C for a further six to ten days; in conditioning experiments the meat was stored for a further one or five days at 1°C.

Determination of eating quality

The eating quality was assessed by taste panelists who tasted the control and hot deboned meats in pairs. Nine tasters judged for tenderness on a scale: extremely tough (scored —7), very tough (—5), moderately tough (—3), slightly tough (—1), slightly tender (+1), moderately tender (+3), very tender (+5) and extremely tender (+7); juiciness on a scale: dry (0), slightly juicy (1), moderately juicy (2), very juicy (3) and extremely juicy (4); flavour on a scale: not acceptable (0), slightly good (1), moderately good (2), very good (3) and extremely good (4); overall acceptability on a scale: extremely unacceptable (scored —7) to extremely acceptable (+7), similar to that for tenderness.

A consumer trial was carried out in which employees of the Institute and their families were given similar grilling or roasting joints from control and hot deboned meats on consecutive days, to be eaten as part of their evening meal. Tasters were asked to judge for tenderness on a scale: very tough (scored —5), moderately tough (—3), slightly tough (—1), slightly tender (+1), moderately tender (+3), and very tender (+5); juiciness on a scale: dry (1), slightly juicy (2), moderately juicy (3) and very juicy (4); flavour on a scale: not satisfactory (1), poor beef flavour (2), good

beef flavour (3), excellent (4); overall acceptability on a scale: not acceptable (1), just acceptable (2), moderately good (3), very good (4), extremely good (5). In all, 238 tasters from 108 households assessed the meats; approximately half of the tasters received the meat from the control side on the first tasting.

Texture determinations

After conditioning for at least ten days each of eighteen muscles was trimmed free of fat and any large connective tissue sheets were removed with a scalpel. The remainder of the meat was cut into pieces weighing 15 to 20 g and heated in water either at 60°C for 20 min, 75°C for 1 hr or 90°C for 3 hr. These conditions were chosen to simulate grilling, roasting or stewing conditions respectively, since the temperatures and times are those attained at the centre of meat when grilled or roasted to medium donness or when stewed. The texture was determined using a compressive test (Rhodes *et al.*, 1972) and the total work done during the test was recorded as the toughness value.

Results

The overall eating quality of cuts of beef

Steaks and joints were taken from a carcass of one Friesian steer and the meat used for laboratory and consumer taste-panels. Taste panel assessments of the roast chuck joint were not affected by the hot deboning procedure (Table 1). Taste panelists judged the flavour as good although the meat was regarded as tough and dry, a combination which undoubtedly produced the low scores for acceptability.

Consumers judged the grilled and roast beef (Table 2) as moderately acceptable, of good flavour and moderately juicy. As expected, the tenderness varied widely between the different cuts and, in the control meats, the fillet steaks were consistently judged to be the most tender whilst the fore-rib was one of the toughest of the roasting joints. An important effect of hot deboning was to cause a marked toughening of the fillet, in which the average score was reduced by three units (Table 2). As only thirteen

TABLE 1. Eating quality of roast beef assessed by taste panelists

Treatment	Taste panel scores (mean; S.D. in parentheses)			
	Tenderness	Juiciness	Flavour	Overall acceptability
Hot-deboned	−2·33 (2·24)	1·00 (1·16)	3·00 (2·83)	0·78 (2·92)
Control	−1·67 (2·65)	1·11 (1·17)	2·78 (2·56)	0·33 (3·16)

Chuck joints from control and hot-deboned sides of one carcass were roasted under standard conditions and were tasted in pairs by nine panelists.

For each attribute, the hot-deboned meat did not differ significantly from the control by paired '*t*' test.

TABLE 2. Eating quality of cuts of meat assessed by consumers

Joint	Number of tasters	Treatment	Average scores			
			Tenderness	Juiciness	Flavour	Overall acceptability
Fillet	13	Hot deboned	0·69	2·69	2·85	3·15
		control	3·62**	3·08	2·69	3·54
Sirloin	29	Hot deboned	1·89	2·86	3·07	3·76
		control	1·83	2·79	2·89	3·34
Rump	31	Hot deboned	0·73	2·97	2·90	3·16
		control	−0·29	2·68	2·87	2·90
Thick flank	30	Hot deboned	3·00	2·73	2·70	3·17
		control	3·00	2·20	3·03	3·63*
Topside	37	Hot deboned	1·60	2·60	2·87	3·46
		control	2·41	2·30	3·05	3·65
Silverside	57	Hot deboned	1·00	2·26	2·72	3·32
		control	1·04	2·35	2·90	3·33
Chuck	16	Hot deboned	−0·24	2·73	3·02	2·93
		control	0·00	2·85	2·67*	3·02
Fore-rib	25	Hot deboned	1·48	2·32	2·52	2·80
		control	−0·36*	2·88*	2·64	3·04

238 tasters from 108 households assessed the meat from one carcass. The fillet, sirloin and rump were cut as steaks for grilling; the others as joints for roasting. Scales are given in the experimental section.
* $P<0·05$; ** $P<0·01$; other differences were not significant by paired 't' test.

consumers received the fillet, the results were grouped into individual households, when it was found that five out of the seven households noted a toughening; there is no doubt therefore that the toughening was the result of hot deboning and was not due to any differences in the handling or cooking which the two samples may have received. Hot deboning produced a slight increase in the tenderness of the fore-rib roast but had no effect on the tenderness of the other meats and little or no effect on the other qualities. When the judgment from all the meats were pooled the overall effect of hot deboning was not significant.

Effect of hot deboning on the texture of individual muscles

Muscles from four Hereford × Friesian steers were taken from a wide range of cuts: in all, eighteen muscles were chosen which represent 54% of the muscle mass of the beef side. The toughness values, determined by a shear test, of each of the muscles are shown in Table 3. Comparing the two treatments for each muscle individually, hot deboning produced a significant toughening in the *M. supraspinatus*, but had no effect on the toughness of the other muscles. When all the muscles were pooled (seventy-two

comparisons), hot deboning increased the toughness values by 10%. It was also noticeable that the variability in toughness values between the animals was increased in the hot deboned meats; the coefficients of variation were on average, 20 and 13% for the hot deboned and control muscles respectively. Cooking losses were unaffected by the hot deboning procedure.

TABLE 3. Texture of hot deboned muscles

Cooking conditions	Muscle	Location	Toughness values	
			Hot deboned	Control
'Grilling'	Gluteus medius	Rump	11·3 (1·5)	11·9 (1·0)
	Psoas major	Fillet	12·2 (0·7)	12·1 (0·8)
'Roasting'	Longissimus dorsi	Sirloin	16·3 (5·0)	14·6 (2·2)
	Rectus femoris	Thick flank	22·4 (1·7)	20·5 (2·7)
	Biceps femoris	Silverside	20·7 (2·7)	20·6 (1·9)
	Semitendinosus	Silverside	26·3 (1·9)	24·2 (2·7)
	Semimembranosus	Topside	24·3 (5·7)	23·8 (2·4)
	Pectoralis profundus	Brisket	24·7 (4·6)	23·8 (3·6)
	Serratus ventralis	Brisket/chuck	22·6 (4·2)	16·4 (0·9)
	Infraspinatus	Chuck	15·4 (2·4)	14·6 (2·0)
	Supraspinatus	Chuck	22·9 (2·7)**	18·8 (2·3)
	Triceps Branchii	Shoulder	19·1 (2·1)	19·0 (0·8)
	Latissimus dorsi	Fore-ribs	22·4 (3·8)	20·7 (1·1)
'Stewing'	Complexus	Chuck	12·8 (3·0)	12·8 (1·8)
	Gastrocnemius	Hind leg	15·2 (4·5)	13·3 (3·0)
	Biceps brachii	Clod	17·3 (8·6)	13·8 (2·3)
	Rectus abdominis	Flank	20·3 (8·1)	16·3 (4·3)
	Extensor carpi radialis	Shin	20·6 (6·1)	18·4 (4·3)
	All muscles (means)		19·3 ***	17·6

Muscles from four carcasses were aged for at least ten days at 1°C. The muscles are grouped according to their suitability for cooking, details of which are given in the experimental section. Toughness values (J. 10^{-2}) are the mean, S.D. in parentheses, of the four animals.

Within the roasting group shown in Table 3, the muscles are arranged conventionally, i.e. muscle from the first quality joints (sirloin, thick flank, silverside and topside) appear above those of the second quality joints (brisket, chuck, shoulder and fore-rib); the ranges of toughness values (15 to 25) within each class were identical and the means were not profoundly different. Furthermore, in the 'roasting' class the *infraspinatus*, *serratus ventralis* and *triceps brachii* muscles showed such low values (< 20) that it must be concluded that, after removal of gross connective tissue, these muscles should be used as steaks for grilling or frying rather than roasting.

TABLE 4. Effect of conditioning on the texture of control and hot deboned beef

Muscle	Initial		Conditioned	
	Hot deboned	Control	Hot deboned	Control
Psoas major	13·2	13·0	11·5	10·7
Gluteus medius	23·4	20·3	16·0 **	18·3
Longissimus dorsi	24·4 *	28·1	12·9	12·4
Semimembranosus	23·8	26·9	17·3	16·2
Biceps femoris	25·8	29·5	13·9	17·4
Semitendinosus	31·1 **	26·9	24·2	21·5
All muscles (means)	23·6	24·1	16·0	16·1

Muscles from one carcass were cooled to 10°C during the first 24 hr after slaughter and were subsequently stored at 1°C for one day (initial) or five days (conditioned). Toughness values are the mean of ten determinations of the work done (J. 10^{-2}) during as tandard shear test.

* $P<0·05$; ** $P<0·01$; other comparisons do not differ significantly by 't' test.

Effect of storage on the texture of hot deboned beef

Texture was determined instrumentally on six hindquarter muscles taken from the carcass of an old cow. The toughness values (Table 4) were lowest in the muscles from the fillet and rump and highest in those of the silverside. The order of increasing toughness of the conditioned muscles was similar to that previously found in the consumer trial (Table 2). Storage for five days at 1°C reduced the toughness values of all the muscles and the overall reduction was the same in both control and hot deboned meats. When the muscles were taken individually hot deboning had a small but inconsistent effect on the toughness values.

Discussion

When individual muscles or joints of beef were excised soon after slaughter and held at 10°C for 24 hr prior to chilling, the eating quality was, in general equal to that from meat cut 24 hr after slaughter. Only in one cut (the fillet) was the hot deboned meat found to be substantially tougher by the consumer panel. Detailed examination by a shear test showed that hot deboning increased the toughness values by 10%, a level which is negligible compared to the variability found between animals, different cuts of meat or due to the extent of ageing. Such small increases were also found by Billault *et al.* (1973) and Kastner & Russell (1975). Schmidt & Keman (1974) found no differences in the eating quality of hindquarter muscles. The increase in toughness is due to shortening of the muscles when cut from their attachments pre-rigor. In carcass form, hanging from the Achilles tendon stretches many muscles, the *M. psoas major*

particularly, which then shorten passively when cut from their attachments. When held at 10°C a further small amount of shortening occurs later when the muscles enter rigor. The extent of toughening, and the accompanied increased variation in toughness, are small and would not affect consumer acceptance.

In commercial practice, the rate of cooling depends upon the size, composition and packaging of the meat as well as the operating temperature and the delay before chilling. Kastner, Hendrickson & Morrison (1973), using hindquarter muscles or muscle groups showed that hot deboned meat had to be held for 8 hr prior to chilling in order to maintain tenderness.

The eating quality of hot deboned beef was improved by storage under chill conditions, the improvement in tenderness was similar to that in the control meats. Many studies on traditional beef have shown that there is little improvement in tenderness by storage for more than ten days at chill temperatures. An equivalent extent of tenderizing can also be obtained by storage at 15°C for three days, although this procedure increases significantly the level of bacteria. In hot deboned meat bacterial counts were satisfactorily controlled by storage at 16°C for two days followed by freezing at —14°C (Schmidt & Gilbert, 1970).

Hot deboning, either in the form of muscle seaming or as conventional jointing appears therefore to be without any great effect on the eating quality of beef. Muscle seaming would be the more advantageous since more effective use could then be made of the carcass by classifying muscles (or muscle groups) according to their method of cooking or their use in formulation and processing. In fact, the shear values of several muscles from the forequarter suggested that at least three muscles could be upgraded for use as grilling or frying steaks.

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The effect of lupin protein isolation procedures on the emulsifying and water binding capacity of a meat-protein system

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Summary

A study on the emulsifying capacity and water binding capacity of lupin protein (isolates and concentrates) using various isolation procedures was carried out. These procedures included protein extraction in single or double step in alkaline or acid conditions by the use of a wet or dry milling technique. Different ion conditions were also used. For comparison, soya bean and lupin protein were extracted through a co-precipitation procedure.

Protein extraction by wet milling and then precipitation and drying at isoelectric pH promoted water binding capacity, while dry milling and alkaline extraction enhanced the emulsifying capacity.

Protein co-precipitation and ion environmental conditions are other factors influencing the emulsifying capacity and water binding capacity. The proper choice of these parameters will determine the optimum conditions for emulsifying and water binding capacity of a protein isolate or concentrate.

Introduction

Increased concern has been shown in the last decade for improving protein quality and increasing protein content of various existing foods. This situation unfolded the need for developing a source of proteins with specific functional behaviour in certain food systems. Consequently, proteins have been isolated from various origins, mainly from soybean (Circle & Whitney, 1959; De Paolis, 1972), cottonseed (Berardi, Martinez & Fernandez, 1969; Lawhson & Catter, 1971) and peanut (Bhatia, Parpia & Baliga, 1966; Eapen, Kalbag & Subrahmanyan, 1966). Protein isolates from other food protein sources such as sunflower (Gheyasuddin, Catter & Mattil, 1970), rapeseed (Sosulski & Bakal, 1969), lupin (Manrique, Cole & Edwards, 1974a, b), are receiving increasing attention.

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For incorporation into food, protein products should possess certain functional properties, the requirements of which vary for different types of foods. It seems likely, therefore, that the functional and physical properties rather than the nutritional value of a new protein isolate or concentrate will determine its range of application (Hermansson, 1972).

In sausage manufacture the relevant functional properties are the emulsifying capacity (EC), emulsion stability (ES) and water binding capacity (WBC), (Hansen, 1960; Swift, Lockett & Freyer, 1961; Helmer & Saffle, 1963; Trautman, 1964; Pearson *et al.*, 1965; Thomas, Baumgartner & Hyde, 1974a, b).

Hansen (1960) describes the first step in the manufacture of sausages which is preparing a finely comminuted batter. The two main components of this batter are the muscle protein slurry and the subdivided fat particles. Sausage batter is therefore commonly referred to as an emulsion of fat globules in a continuous aqueous protein phase.

In making sausage products, the processor depends upon the binding properties of meat proteins, myosin and actomyosin, contained in red skeletal meat (Rock, Sipose & Meyer, 1966). Because these meats are expensive, they are often used sparingly, resulting in marginal formulations. In addition to this, mishandling of the meat, natural variations in the meats and processing can weaken the emulsion structure greatly and may lead to emulsion breakdown. Non-meat protein additives are often used as insurance ingredients to keep such failure to minimum (Rakosky, 1970).

Soybean protein isolates and concentrates, sodium caseinates, non-fat dried milk, milk co-precipitates and cereal flours have been used in sausage manufacture (Saffle, 1968; Thomas *et al.*, 1974b). The advantages claimed for the use of non-meat proteins in sausages are an improvement of emulsifying properties, water binding, consistency, colour and appearance, protein enrichment and as inexpensive extenders (Schut, 1969; Rakosky, 1970). Their functional properties have been reported by Pearson *et al.* (1965), Inklaar & Fortiun (1969) and Thomas *et al.* (1974a).

Lupin protein isolates have been developed by using several protein isolation procedures (Manrique *et al.*, 1974b). Different aspects appeared to change in accordance with the selected protein isolation route. These include protein yield, protein electrophoretic behaviour, amino acid profile and molecular weight distribution (Manrique, 1976). This circumstance led to the present investigation into the influence of protein isolation procedures on the pertinent functional properties involved in sausage manufacture.

Conventional non-meat protein additives are added at a rate ranging from 1 to 3% of the total sausage products (Meester, 1969). Considering sausages, e.g. luncheon type sausage contains 10–12% protein, these protein additives in actual fact may contribute approximately 20% of the total sausage proteins. Also the greatest effect on the functional properties seems to be at the 20% replacement level of the meat with protein additives (Thomas *et al.*, 1974a). Evaluation of the EC and WBC was done on

the lupin proteins alone and on a mixture of eighty parts meat protein and twenty parts lupin protein.

Materials and methods

Raw material

Certified seed and meal of sweet *Lupinus angustifolius*, variety Uniwhite, were obtained from the Grain Pool Ltd, Perth, Western Australia. The whole raw seed was used to obtain the protein isolates through a wet milling technique. The lupin meal produced from decoated seed is a commercially available product produced by a dry milling process which was used to extract the protein.

Protein isolation procedure

Methods of protein isolation are similar to those reported earlier (Manrique *et al.*, 1974) and a flow diagram (Fig. 1) and a summary of sample identification (Table 1) are presented.

The milling of whole seeds was carried out by two distinct methods. In the wet milling whole seeds were soaked in water at 65°C for 2 hr and 50°C for 1·5 hr respectively. The wet seeds were wet milled in a colloid mill to yield the protein slurry. The dry milling process was carried out by using lupin meal commercially. A slurry was formed and in both cases protein extraction was carried out in alkaline (pH 8) or acid (pH 2) conditions, in single or doube protein extraction steps. In the case of double extraction an acid extraction was followed by an alkaline extraction or vice versa. Another protein extraction was carried out in a salt-water system (0·5 M NaCl; 0·5 M Na_2SO_4)at pH 8. Protein extraction was carried by a dry milling

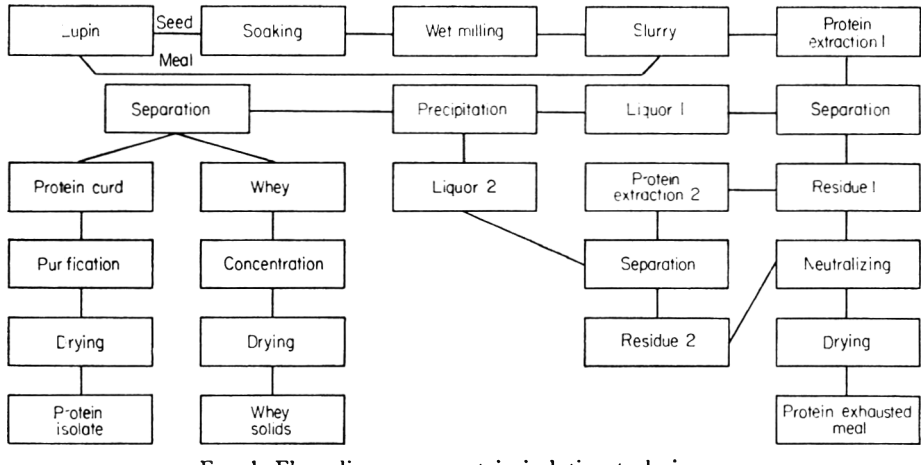

FIG. 1. Flow diagram—protein isolation techniques.

TABLE 1. Identification and extraction methods employed for various samples and their protein contents

Extraction method	Isoelectric proteins				Proteinates			
	Wet milling		Dry milling		Wet milling		Dry milling	
	Sample no.	Protein %	Sample no.	Protein %	Sample no.	Protein %	Sample no.	Protein %
Acid (single step)	1	45·72	2	81·26	3	43·84	4	85·58
Alkaline (single step)	5	53·16	6	92·56	7	53·68	8	94·88
	9*	62·13	10	70·22	11*	59·40	12	56·08
	13†	67·92	14	76·04	15†	68·69	16	75·67
			17	71·91			18	79·97
Acid/alkaline (double step)	19	48·14	20	72·76	21	45·25	22	71·32
Alkaline/acid (double step)	23	48·87	24	81·93	25	48·14	26	82·94
Salt extraction: 0·5 M Na_2SO_4			27	56·88			28	57·42
Salt extraction: 0·5 M NaCl			29	59·90			30	59·26

* Lupin + soya bean in equal quantities.
† Soya bean.

process, an alkaline protein extraction and an isoelectric protein precipitation and sample 6 (water system) was used for comparative purpose since it was obtained in the same manner without the salt addition.

Protein precipitation was carried out upon the soluble protein obtained from the various protein extraction steps. When a double protein extraction was involved, the soluble protein from each extraction was mixed and then the protein precipitation was carried out in all cases at pH (4·65–5·0). A protein curd was then separated from the whey by centrifugation and prior to the drying, was divided into two parts. One was pH adjusted (pH 7·0) to yield the proteinate, while the other half was dried to form the isoelectric isolate.

Co-precipitation

Whole soybeans obtained from Quirindi Co-operative, N.S.W., Australia, were used to produce two samples for comparative purposes. One of them consisted of a 50% mix of soybean and lupin seed, while the other was 100% soybean. Protein from both lupin and soybean was made soluble through a wet milling and an alkaline extraction and was co-precipitated isoelectrically, since both legumes have a very similar protein solubility profile (Manrique, 1976).

Drying methods

The protein curd obtained after centrifugation was deep frozen (−20°C) on trays prior to drying. Most of the samples were freeze dried in a cyclic type tray freeze drier made by Budge Ellis Co-operative Ltd, N.S.W., Australia. A protein curd obtained by an alkaline dry milled single step protein extraction procedure was separated into fractions and dried under different conditions to yield several protein isolates obtained from the same protein curd. Samples 10 and 12: drum drier made by Blaw-Knox Co., Buflovak Equipment, New York, USA. Samples 14 and 16: cabinet drier of the cross-flow type 23 m^3/min. 75°C. Sample 17: A/S Niro Atomizer Spray Drier, Denmark. Sample 13: high frequency wave drier, Phillip No. 2006/02, 6 kW, 27·12 MHz.

Protein slurry

Slurries containing 2% protein were prepared by soaking each material in 3% sodium chloride solutions for 1 hr at 5°C and then dispersed in a Waring Blender* for 1·5 min, using first speed.

Meat

Longissimus dorsi muscle from sheep obtained from abattoirs and local butchers was used. Samples were stored at 5°C in a plastic bag. The muscle contained 20·2–20·6% protein. The meat (50 g) was cut into small pieces and then homogenized for 1·5 min with 450 g of 3% sodium chloride solution in the Waring Blender, using low speed. The meat homogenate therefore contained 2% protein. In each of the trials the meat homogenates were divided into two halves, the first half was used with its original pH of 5·68 and the other half was adjusted to pH 6·0 using 5% concentration of sodium carbonate.

Oil. Safflower oil obtained from Pacific Safflower (Aust.) Pty Ltd, Sydney, was used.

Functional Properties

The emulsifying capacity and water binding capacity tests were conducted as reported earlier by Thomas *et al.* (1974a).

Chemical analysis

Protein contents of lupin and soya proteins were determined using an automated Kjeldahl procedure as reported by Crooke & Simpson (1971). Protein contents of meat were determined using the A.O.A.C. method (1965). pH measurements were carried out on all slurries using a pH meter.

Results

The EC of the proteins alone and of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0 of all the samples are reported in Table 2. The data for the WBC is reported in Table 4.

* Model MR-9CR-Science Essentials Company, 2100 Howell Ave, Anahein, California 92806. U.S.A.

Protein isolation procedure

The results of protein isolation procedure on EC and WBC are reported in Table 3.

TABLE 2. The EC of the proteins alone and of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0

Sample nos.	Proteins		A mixture of twenty parts lupin protein and eighty parts meat protein			
	pH	EG (ml)	pH	EC (ml)	pH	EC (ml)
Meat only			5·68	27·30	6·00	32·50
1	4·70	0·0	4·94	0·0	5·22	27·5
2	5·13	0·0	5·53	8·0	5·63	32·0
3	8·04	0·0	6·66	30·7	7·26	34·0
4	5·95	0·0	5·80	22·3	5·93	34·5
5	4·92	0·0	5·35	12·0	5·50	31·5
6	4·98	0·0	5·26	9·0	5·64	35·5
7	6·83	0·0	6·21	27·0	6·23	33·0
8	5·62	0·0	5·68	19·5	5·88	28·5
9	4·80	0·0	5·40	24·9	5·44	37·0
10	5·05	0·0	5·22	8·0	5·61	36·0
11	6·56	0·0	6·13	26·6	6·19	30·0
12	6·13	0·0	6·00	15·5	6·03	30·0
13	4·74	0·0	5·06	1·0	5·22	29·0
14	4·84	0·0	4·97	0·0	5·21	29·0
15	5·84	0·0	5·76	23·0	5·88	33·0
16	6·06	0·0	5·91	13·0	6·01	29·0
17	4·84	0·0	5·22	6·0	5·26	27·5
18	5·64	0·0	5·66	12·5	5·84	31·0
19	4·65	0·0	5·05	0·0	5·30	31·5
20	4·40	0·0	5·22	5·5	5·32	30·0
21	7·55	0·0	6·76	28·5	6·87	31·5
22	5·88	0·0	5·80	26·0	5·90	34·0
23	5·03	0·0	5·35	7·5	5·40	31·5
24	4·50	0·0	5·20	22·5	5·24	34·0
25	6·30	0·0	6·14	22·5	6·21	28·0
26	5·62	0·0	5·64	23·1	5·74	31·5
27	4·93	0·0	5·12	4·5	5·26	34·5
28	6·33	0·0	6·00	22·2	6·11	33·0
29	4·64	0·0	4·96	0·0	5·20	34·0
30	6·18	0·0	5·93	16·7	6·03	29·0

TABLE 3. Effect of protein isolation procedure on EC (ml) and WBC (g) of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0

Protein isolation procedure	Sample no.	Wet milling					Dry milling			
		pH 5·68		pH 6·0			pH 5·68		pH 6·0	
		EC (ml)	WBC (g)	EC (ml)	WBC (g)	Sample no.	EC (ml)	WBC (g)	EC (ml)	WBC (g)
Isoelectric protein										
Acid (single step)	1	0·0	7·26	27·5	17·44	2	8·0	6·50	32·0	16·80
Alkaline (single step)	5	12	6·96	31·5	18·34	6	9	6·10	35·5	19·78
Acid/alkaline (double step)	19	0·0	9·24	31·5	23·28	20	5·5	6·00	30·0	12·64
Alkaline/acid (double step)	23	7·5	7·34	31·5	20·82	24	22·5	7·84	34·0	17·10
Proteinate										
Acid (single step)	3	30·7	11·32	34·0	22·38	4	22·3	8·36	34·5	20·24
Alkaline (single step)	5	27·0	11·12	33·0	22·70	8	19·5	7·16	28·5	20·66
Acid/alkaline (double step)	21	28·5	9·86	31·5	23·46	22	26·0	8·98	34·0	20·10
Alkaline/acid (double step)	25	22·5	8·98	28·0	22·74	26	23·1	7·88	31·5	19·12

Protein extraction system

The results of the EC and WBC of proteins under different ionic conditions are reported in Table 5.

Co-precipitation

The results of the EC and WBC of soybean, lupin and equal mix of soybean and lupin are presented in Table 6.

Drying

The influence of the drying methods on EC and WBC are given in Table 7.

Discussion

The EC value of the meat of pH 6·0 (Table 2) is higher than the meat of lower pH 5·68. This was expected, because as the pH of the meat homogenate was increased more salt soluble protein became available. Swift & Sulzbacher (1963) and Hegarty, Bratzler & Pearson (1963) reported that salt soluble protein is the major emulsifier in a sausage-type emulsion and is greatly influenced by pH.

None of the protein samples (Table 2) formed an emulsion when used alone. However, different results were obtained when used in a mixture with eighty parts meat protein and twenty parts lupin protein. With low pH meat all the proteins produced EC lower than the low pH meat (100% meat). Some of the samples coming from the isoelectric precipitation procedure did not form an emulsion at all, as the proteins decreased the

TABLE 4. The WBC of the proteins alone and of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0

Sample nos.	Proteins		A mixture of twenty parts lupin protein and eighty parts meat protein			
	pH	WBC (g)	pH	WBC (g)	pH	WBC (g)
Meat only			5·68	8·00	6·00	24·16
1	4·70	15·52	4·94	7·26	5·22	17·44
2	5·13	6·46	5·53	6·50	5·63	16·80
3	8·04	13·44	6·66	11·32	7·26	22·38
4	5·95	5·06	5·80	8·36	5·98	20·24
5	4·92	11·37	5·35	6·96	5·50	18·34
6	4·98	2·24	5·26	6·10	5·64	19·78
7	6·83	10·25	6·21	11·12	6·28	22·70
8	5·62	3·04	5·68	7·16	5·88	20·66
9	4·80	4·30	5·40	7·68	5·44	22·22
10	5·05	7·16	5·22	8·54	5·61	23·24
11	6·56	6·31	6·13	11·42	6·19	23·48
12	6·13	11·14	6·00	9·46	6·03	29·22
13	4·74	2·11	5·06	7·32	5·22	23·90
14	4·84	5·17	5·97	7·52	5·21	27·02
15	5·84	4·43	5·76	11·11	5·88	25·30
16	6·06	6·44	5·91	7·10	6·01	27·38
17	4·84	7·24	5·22	7·02	5·26	25·50
18	5·64	5·31	5·66	8·02	5·84	30·64
19	4·65	15·02	5·05	9·24	5·30	23·28
20	4·40	6·70	5·22	6·00	5·32	12·64
21	7·55	11·68	6·76	9·86	6·87	23·46
22	5·88	15·44	5·80	8·98	5·90	20·10
23	5·03	13·56	5·35	7·34	5·40	20·82
24	4·50	6·01	5·20	7·84	5·24	17·10
25	6·30	14·44	6·14	8·98	6·21	22·74
26	5·62	4·36	5·64	7·88	5·74	19·12
27	4·93	10·52	5·12	9·46	5·26	30·32
28	6·33	9·13	6·00	10·68	6·11	24·94
29	4·64	12·27	4·96	7·84	5·20	24·72
30	6·18	11·02	5·93	10·84	6·03	27·74

TABLE 5. Effect of ionic condition of the protein extraction on EC (ml) and WBC (g) of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0

Extraction system	Isoelectric protein				Proteinate			
	Sample no.	Dry milling			Sample no.	Dry milling		
		pH 5·68		pH 6·0		pH 5·68		pH 6·0
		EC (ml)	WBC (g)	EC (ml)	WBC (g)	EC (ml)	WBC (g)	EC (ml)	WBC (g)
Water	6	9·0	6·10	35·5	19·78	8	19·5	7·16	28·5	20·66
0·5 M Na_2SO_4	27	4·5	9·46	34·5	30·32	28	22·2	10·68	33·0	24·94
0·5 M NaCl	29	0·0	7·84	34·0	24·72	30	16·7	10·84	29·0	27·74

TABLE 6. Effect of protein curd co-precipitation of proteins on EC (ml) and WBC (g), of a mixture of twenty parts protein and eighty parts meat protein of pH 5·68 and 6·0

	Sample no.	Protein WBC (g)	A mixture of twenty parts protein sample and eighty parts meat protein		A mixture of twenty parts protein sample and eighty parts meat protein	
			pH 5·68		pH 6·0	
			EC (ml)	WBC (g)	EC (ml)	WBC (g)
Isoelectric protein						
Lupin	5	11·37	12·0	6·96	31·5	18·34
Lupin + soya bean	9	4·30	24·9	7·68	37·0	22·22
Soya bean	13	2·11	1·0	7·32	29·0	23·90
Proteinate						
Lupin	7	10·25	27·0	11·12	33·0	22·70
Lupin + soya bean	11	6·31	26·0	11·42	30·0	23·48
Soya bean	15	4·43	23·0	11·11	33·0	25·30

TABLE 7. Effect of protein curd drying procedure on EC (ml) and WBC (g) of a mixture of twenty parts lupin protein and eighty parts meat protein of pH 5·68 and 6·0

Sample no.	Drying procedure	Mixture of twenty parts lupin protein and eighty parts meat protein		Proteins WBC (g)	Mixture of twenty parts lupin protein and eighty parts meat protein	
		pH 5·68 EC (ml)	pH 6·0 EC (ml)		pH 5·68 WBC (g)	pH 6·0 WBC (g)
10 I	Drum dryer	8·0	36·0	7·16	8·54	23·24
12 P		15·5	30·0	11·14	9·46	29·22
14 I	Cabinet dryer	0·0	29·0	5·17	7·52	27·02
16 P		13·0	29·0	6·44	7·10	27·38
17 I	Spray dryer	6·0	27·5	7·24	7·02	25·50
18 P	High frequency wave dryer	12·5	31·0	5·31	8·02	30·64
6 I	Freeze dryer	9·0	35·5	2·24	6·10	19·78
8 P		19·5	28·5	3·04	7·16	20·66

I, Isoelectric protein.
P, Proteinates.

pH of the meat bringing it close to the isoelectric point, and hence, a decrease in salt soluble protein (Swift & Sulzbacher, 1963).

The WBC value of high pH meat (pH 6·0) is much higher than the low pH meat (Table 4). This pH dependent characteristic, which was also typical in the EC values, is in agreement with the results reported by Bouton, Harris & Shorthose (1971), Hamm (1960) and Thomas *et al.* (1974a, b). The WBC of the proteins alone (Table 4) varied greatly and thirteen of the lupin protein samples produced higher WBC than the meat of low pH.

The use of a mixture of twenty parts lupin protein and eighty parts meat protein resulted into two distinct WBC values. The high pH meat produced WBC values much higher than the low pH meat and samples 12, 14–18, 24, 27 and 30 produced WBC values higher than the meat of high pH.

Protein isolation procedure

EC. There was an increase in EC values when a proteinate sample was used (Table 3). This seems to indicate that the role of the pH of the protein additive is as important as that of the pH of the meat homogenate. The EC values at pH 6·0 are high and for samples 3, 4, 6, 7 and 22 are still higher than the meat values.

Dry milled, isoelectric samples, 2, 20 and 24, yielded higher EC than wet milled samples, 1, 19 and 23, for equivalent protein extraction techniques using low pH meat. This tendency was not seen for the corresponding proteinate samples, 3, 4, 21, 22, 25 and 26 at pH 5·68.

To observe the contribution of the protein isolation technique on the EC values, it is necessary to situate in the most unfavourable condition isoelectric protein and low pH meat. Looking at samples 1, 5, 19 and 23 (Table 3) it is seen that an alkaline extraction yields higher values of EC than acid extraction. This pattern is also followed for the two-step protein extraction when the first step is in an alkaline condition. The same behaviour is followed with the dry milled samples 2, 6, 20 and 24, but the overall EC values are higher. This indicates that dry milling favours better EC. As dry milling is a more rigorous process than wet milling, it is likely that the protein would be more denatured. This may mean that, as the protein becomes more dissociated, there are more ionic groups to interact with the fat globules to give origin to a better emulsion. This trend is well shown with the isoelectric samples, where the pH is in the isoelectric area and the meat is of low pH.

WBC. Proteinates gave higher WBC values than isoelectric proteins for the same protein isolation techniques as one would expect from higher pH (Table 3).

Dry milled samples give lower WBC values than wet milling. This difference is smaller for samples within the same pH condition, as proteinates, but this gap becomes bigger as we compare samples from a low pH meat and isoelectric type protein to one of the high pH meat and proteinate form. This is the case, for example, with sample 6

(6·10 g) as compared to samples 7 (22·70 g), in which the WBC value has been more than tripled for the same protein extraction procedure. WBC is therefore favoured by wet sampling and acid extraction where the protein has longer polypeptide chains, thus permitting greater quantities of water to be bound in the emulsion (Manrique, 1976).

Protein extraction system

EC. Proteinate yielded higher EC values (Table 5) when a low pH muscle was used. The EC was further increased by the use of a high pH meat. Sample 6, 27 and 29 in this condition gave higher EC than the meat. The water system (sample 6) gave the highest EC values followed by 0·5 M Na_2SO_4 (sample 27) and 0·5 M NaCl (sample 29). It seems that a proteinate sample with a high pH meat gives an optimum condition for EC. If a low pH meat is available, it is better to use a proteinate and the Na_2SO_4 extracting medium would be the most suitable.

WBC. The pattern that follows WBC values is more consistent with the ionic system used. 0·5 M Na_2SO_4 gives the highest WBC followed by 0·5 M NaCl and water system.

Samples 27 and 29, 28 and 30, gave higher WBC values than the meat control when a mixture of twenty parts lupin protein and eighty parts meat protein of pH 6·0 was used. This seems to indicate that protein extracted in the presence of sodium ions and alkaline pH forms protein products which have high WBC values. If the effect of ion condition is added to a wet milling process, higher WBC values would be expected.

EC. EC was the highest (Table 6) for all the samples, using a mixture of twenty parts lupin protein and eighty parts meat protein of high pH (6·0). The isoelectric protein No. 9 gave the highest EC (37 ml). To observe the influence of co-precipitation on EC, the conditions of low pH muscle (pH 5·68) and isoelectric protein are chosen. Soybean has EC value (1 ml) which is very small as compared to lupin (12 ml). By co-precipitation, sample 9 gave an EC value of 24·9. This synergistic action may be very promising since cheaper protein sources can be blended to enhance or maintain EC.

WBC. In the case of WBC of the protein samples, lupin presents higher values than soybean and the co-precipitated samples give intermediate WBC. When a meat replacement is made, WBC is improved substantially by a high pH muscle. The synergistic effect observed very markedly for EC in the co-precipitated sample is not seen to such extent with the WBC. In this case the WBC values are the result of a geometric summation rather than a synergistic effect. This conclusion proves to be correct since EC and WBC are physical properties which increase in opposite condition as far as molecular weight distribution is concerned. Low molecular weight protein components will promote higher EC whereas high molecular weight component will promote WBC (Manrique & Edwards, 1976).

Curd drying

EC. The drying methods are listed (Table 7) according to the extent of heat treatment given to the protein curd, i.e. the lowest for freeze drying and the greatest for the drum drying. Drum and freeze drying, the two extreme situations, appear to give the greatest EC when used with a muscle of pH 6·0. The proteinate increases EC for low pH meat (5·68), while for high pH meat (6·0) there is a tendency to lower or maintain EC values.

WBC. WBC values are increased by meat replacement at pH 6·0. In this condition the high frequency drying yields a sample with the highest WBC, followed closely by the drum drying and cabinet drying. The high frequency dryer seems to help in the WBC values, but it may not be an industrially feasible way to produce isolates. It appears that the high frequency dryer develops low temperature in the product during a short time and this helps to preserve the protein. The freeze dryer does not help in obtaining good WBC values. The cabinet dryer operated at a temperature of 75°C gives reasonable WBC figures which are especially consistent for the high pH meat.

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Technical note: Rapid method for the estimation of protein in maize

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Introduction

Maize is of economic importance in many countries. Its quality is mainly assessed from the protein content, which is normally determined by the fairly laborious Kjeldahl procedure. There is need, especially considering the current stage of development of the Central American countries, for a rapid method of determination which can be used by semi-skilled personnel.

Numerous workers have recommended methods which are shorter and less laborious than the Kjeldahl procedure (Cole, 1969; Pearson, 1973a). One possibility is to apply volumetric or colorimetric methods directly to the Kjeldahl digest thus avoiding the distillation step (Hashmi, Ali & Umar, 1962; Mann, 1963). The biuret reaction, involving the formation of a coloured complex when peptides are treated with copper sulphate in alkaline solution, has been applied to cereals (Williams, 1961). Ronalds (1974) has employed the Kofranyi method which essentially recovers the ammonia liberated when the sample is distilled from alkaline solution.

The simpler more rapid methods for estimating protein in cereals apply colorimetric methods to a directly prepared extract. Most frequently used have been the dye binding methods, which measure the capacity of the polar groups of the protein to bind with selected dyes of opposite charge, thus causing a reduction in the colour of the solution (Udy, 1956, 1957). The formation of a turbidity when sulphosalicylic acid is added to a protein solution has been employed in biochemical work for many years. Proteins flocculate in the presence of sulphosalicylic acid and this has been used particularly for the detection of albumin in urine (White & Delory, 1952) and for the estimation of plasma (Mawson, 1942). The reaction was applied for the rapid estimation of protein in wheat flour by Feinstein & Hart (1959). For the rapid estimation of protein in maize this method was selected for trial as it satisfied the desired criteria, i.e. it is rapid, simple to perform (no heat or distillation is necessary), no corrosive reagents are employed and the absorbance can be measured on a photoelectric colorimeter. The instrument used was the EEL colorimeter (one of the commonest of the simpler types used in the UK) replacing the Klett-Summerson colorimeter used by Feinstein & Hart (1959).

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Experimental

In order to apply the Feinstein & Hart (1959) procedure to maize it was first necessary to consider the main factors that might affect the results, i.e. the weight and particle size of the sample used and the extraction and reaction times. Good reproducibility was attained with samples which had been milled through screens of up to (but not more than) 1 mm. It seemed advisable to use only samples containing less than 0·50 g protein as with higher weights it was difficult to keep the precipitate in suspension. The extraction and reaction times recommended by Feinstein & Hart (1959) were in general found to be applicable to maize. It was found necessary to take the readings 20 sec after addition of the sulphosalicylic acid reagent as the absorbance tended to fall rapidly particularly with the solutions containing the higher weights of protein (0·4–0·5 g). The following procedure was finally adopted.

The maize samples were prepared in a Christy and Norris Laboratory Mill (Size 8 with 0·8 mm screen). For each estimation not more than 5 g of ground sample was weighed, to an accuracy of ± 0·01 g, into a 250 ml glass-stoppered conical flask and 100 ml of 0·05 M sodium hydroxide solution was added. The flask was shaken for 15 min using a Griffin and George Microid Flask Shaker and the suspension was centrifuged at 3000 rpm for 10–15 min. Then 5 ml of the separated liquid was pipetted into a stoppered cylinder and 3% m/V sulphosalicylic acid was added to fill to the 50 ml mark. After stoppering, the cylinder was immediately inverted three times to ensure uniform distribution of the colloid formed and the absorbance measured 20 sec after addition of the reagent in an EEL Portable Colorimeter fitted with a No. 625 filter. Checks on reproducibility, etc. were made using a Perkin Elmer Spectrophotometer Model 6/20 (10 mm cell at 540 nm). The instruments were adjusted to zero using the sulphosalicylic acid solution.

The calibration graph was prepared using the same procedure from maize samples of known protein content. After determining the protein content of the maize by the macro Kjeldahl method (Pearson, 1973b), samples corresponding to the following protein (N × 6·25) weight: 0·05, 0·1, 0·2, 0·3, 0·4 and 0·5 g were employed for constructing the graph.

Results and discussion

The calibration graph was prepared from samples of maize of protein content ranging from 8·05–8·82% (N × 6·25). The regression equation for the samples is:

$$y = 6{\cdot}13x + 0{\cdot}31 \quad (r = 0{\cdot}91)$$

where x = protein in sample (g. N × 6·25)

and y = absorbance reading (EEL Colorimeter).

The correlation was only slightly inferior to the results obtained when the absorbances

of the same colloidal suspensions were measured with a Perkin Elmer spectrophotometer. Not surprisingly, the correlation was improved ($r = 0{\cdot}98$) when only results from individual maize samples were used. This suggests that the method would be ideally suited for the routine control of maize derived from the same source. It is in any case advisable for each laboratory to construct its own calibration graph to allow particularly for differences in sample preparation and colorimetric equipment.

From the above regression equation the protein and nitrogen contents can be calculated directly:

$$\% \text{ Protein } (N \times 6{\cdot}25) = \frac{y - 0{\cdot}31}{0{\cdot}0613W}$$

$$\% \text{ N} = \frac{y - 0{\cdot}31}{0{\cdot}383W}$$

where W = weight of sample used (g).

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Technical note: Aseptic technique for obtaining sterile beef tissue

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The changes which take place in meats during refrigerated storage may be caused by external bacterial sources as well as by naturally occurring enzymatic and chemical reactions. To obtain an understanding of these changes and of their relative importance in meat spoilage, it is necessary to differentiate between the bacterial-induced changes and those due to muscle autolysis. The success of such a study depends upon the method used for obtaining sterile tissue. The size of the animal from which the muscle tissue is to be taken determines to a great extent the procedure and precautions to be used. The larger the animal the more difficult it is to obtain sterile tissue and the more expensive the study becomes.

TABLE 1. Techniques for obtaining sterile muscle tissue

	Method	Animal	Author
1	Surgical room technique	Rabbit and lamb	Zender *et al.* (1958); Radouco-Thomas *et al.* (1959)
2	Alcohol flame	Rabbit	Sharp (1963)
3	Alcoholic dye-dip	Rabbit	Sharp (1963)
4	Chlortetra-cycline solution	Chicken	Van den Berg *et al.* (1964)
5	Gnotobiotic	Small animals	Ockerman *et al.* (1964)
6	Surgical isolator	Beef	Ockerman & Cahill (1967)

Methods of obtaining sterile muscle tissue have been reviewed by Ockerman *et al.* (1969) and are classified in Table 1. Sharp (1963) reported that the alcoholic-dye-dip gave sterile tissue approximately 50% of the time and he recommended the use of an alcoholic-flame procedure which gave about 70% success. This is an extremely useful method when a large number of sterile samples are required from the same muscle. The chlortetracycline procedure used by Van den Berg, Lentz & Khan (1963) and by Khan & Van den Berg (1964) can only be used in autolytic studies while the gnotobiotic method of Ockerman *et al.* (1964) is a very expensive procedure. Of the procedures listed above the surgical isolator procedure outlined by Ockerman (1966) and Ockerman & Cahill (1967) is perhaps the most suitable for larger animals, such as the bovine and

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porcine species. Hasegawa *et al.* (1970) used essentially this procedure to obtain porcine muscle, but without the surgical isolator. The procedure is time consuming and the success of the method depends entirely on having complete control over the slaughtering operation.

This note reports on an alternative and relatively inexpensive method for obtaining sterile muscle tissue from a beef carcass. The method was developed with the co-operation of a local abbatoir.

Materials and methods

Knives, calipers, grinder and grinder plates were sterilized for 15 min at 121°C prior to use. Sterile disposable gloves were worn by the operators during the entire sampling procedure.

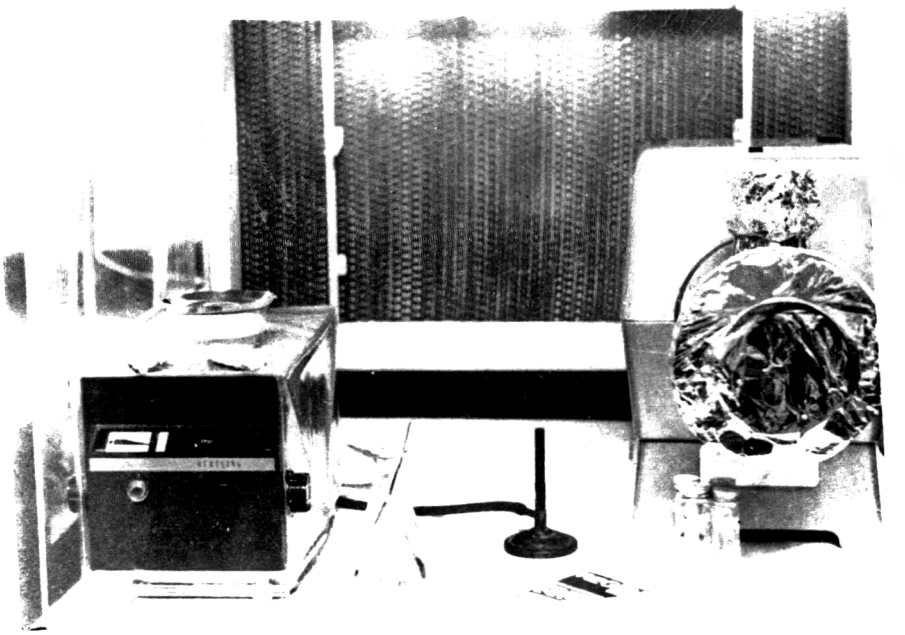
Slaughtering and evisceration of animals were carried out in the usual manner on an automated line in a local abbatoir. The total time from stunning to entry into chill room was approximately 45 min. Carcasses were held overnight at 2° to 5°C and then boned out at 10°C. The boner wore sterile gloves and used a sterile knife to remove the strip loin or lumbar region of the *longissimus dorsi* muscle (about 5–6 kg) from the carcass which was then placed in a sterile covered container and transported to the laboratory.

In this study, the samples were prepared using aseptic technique in a laminar air flow unit. Plate 1 shows the design of the laminar flow assembly. Air was filter-sterilized and blown over the table and grinder towards the operator, by an air sterilizing unit supplied by Microfilm Ltd, Fleet, Hants., England. The air flow was directed by a Perspex hood. As an added precaution the table and hood were washed with a hypochlorite solution prior to use.

The strip-loin with skin-side down, was placed on sterile aluminium foil and an incision made longitudinally along the exposed surface with a sterile knife. A second sterile knife was used to strip off a 2–3 cm thick slice longitudinally along the strip loin while a second operator held the slice with a sterile calipers. This was performed on both sides of the incision. Slices about 2 cm thick were then removed aseptically from the exposed interior and transferred to a sterile container. It is possible to obtain 2 kg sterile tissue from a 5 kg striploin using this procedure. The excised slices were ground through a sterile grinder having a 4 mm grinding plate and collected in a sterile container. Samples were then prepared for each sampling period by transferring approximately 5 g minced tissue to sterile 50 ml screw-cap bottles with caps loosely screwed on so as to maintain aerobic conditions. Using one bottle at each sampling period the danger of contamination during the storage period was minimized. All samples were stored at 7°C until required.

A number of spoilage type microorganisms which were isolated from spoiled beef, were purified and reinoculated into sterile mince. The reinoculated mince was then distributed in 5 g amounts into sterile 50 ml screw-cap bottles as for the sterile mince

Sterile beef tissue



(*Facing p.* 428)

TABLE 2. Typical example for microbial numbers and pH values for sterile control and reinoculated samples

Day	Control		*Enterobacter* aerogenes		Non-pigmented pseudomonas	
	S.P.C.	pH	S.P.C.	pH	S.P.C.	pH
0	None	5·64	$1·0 \times 10^{2}$	5·70	$7·0 \times 10^{4}$	5·79
4	None	5·68	$2·0 \times 10^{4}$	5·69	$3·0 \times 10^{8}$	6·56
6	None	5·72	$4·0 \times 10^{6}$	5·65	$1·4 \times 10^{10}$	7·42
8	None	5·62	$1·0 \times 10^{8}$	5·60	$2·1 \times 10^{10}$	7·66
11	None	5·72	$1·0 \times 10^{10}$	6·64	$3·0 \times 10^{10}$	7·96
13	None	5·66	$2·0 \times 10^{10}$	6·50	$1·0 \times 10^{10}$	7·98
15	None	5·75	$3·0 \times 10^{10}$	6·86	$1·0 \times 10^{10}$	7·90
18	None	5·63	$1·0 \times 10^{10}$	6·83	$2·5 \times 10^{10}$	7·94
20	None	5·70	$1·0 \times 10^{10}$	6·94	$1·9 \times 10^{10}$	7·85
25	None	5·70	$1·0 \times 10^{10}$	7·0	$1·6 \times 10^{10}$	7·90

and stored at 7°C. Every other day one each of the control and inoculated samples were analysed for total counts using the standard plate count at 20°C for 72 hr, during a storage period of twenty-seven days. This procedure has been used on six occasions up to this time. On each occasion approximately 200 × 5 g samples have been prepared, half of these being used as sterile controls. Only two bottles out of a total of 600 controls have been found to contain any bacteria. It is critical to use aseptic precautions at all stages particularly, during the transfer into the sterile 50 ml bottles.

Table 2 summarizes and is a typical example of the microbial numbers and pH values obtained for the sterile control plus two species of bacteria which had been purified and reinoculated.

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Book Reviews

Refined Carbohydrate Foods and Disease. Some Implications of Dietary Fibre. Ed. by D. P. BURKITT and H. C. TROWELL.
London: Academic Press. xiii + 356 pp. £7.80.

Taking the Rough with the Smooth. Dietary Fibre and Your Health—a New Medical Breakthrough. By A. STANWAY.
London: Souvenir Press. 255 pp. £3.50.

The food industry is frequently, almost continuously, under attack. Most detractors are ill informed and the attacks have little or no basis, but the 'fibre story' is supported by highly reputable medical practitioners, and 'fairly and squarely' [*sic*] lays the blame at the door of the food manufacturer. The list of diseases involved is frighteningly comprehensive—diverticular disease, appendicitis, tumours of the large bowel, ulcerative colitis, varicose veins, hiatus hernia, gallstones, diabetes and duodenal ulcers.

Although the attack is aimed at the milling, baking and sugar industries the backwash affects the entire food industry which makes use of white flour or sugar, and food scientists and technologists will be well advised to find out all about it.

The 'fibre story' must be one of the fastest growing scientific stories ever; it was only in 1969 that Dr Burkitt first put forward his criticism of low fibre diets and 1972 that Mr Painter reported successful treatment of patients with bran. Certainly 'roughage' has long been recommended as a preventative of constipation but it is somewhat startling to be reminded by Dr Trowell that the 1962 edition of a standard physiology textbook stated that 'bran is a disadvantage'.

The observation is that many diseases are common only in industrialized communities —and there only in recent years—where the diet is relatively low in 'fibre' and the theory is that lack of fibre is their main cause. Dr Trowell calculated that 200 years ago 'we' ate 500 g whole wheat/head/day providing 2·5–10 g of fibre, 100 years ago fibre intake was 1·3 g, today it is 0·2 g. At the same time the consumption of sugar and fats has risen vastly.

The low-fibre diet gives rise to small bulk of slow travelling intestinal contents and difficulties in defaecation cause increased pressure in the colon. This is the basic cause of the related diseases listed above, which are linked in the same communities and in the same individuals. Sieved, low fibre diets were used for the management of diverticulitis for fifty years without any benefit through the wrongly held belief that undigested particles caused harm. Painter on the contrary treated patients with fibre and reported in 1972 marked benefit in 90% of them.

The culprits are refined carbohydrates, meaning milled cereals (fibre removed) and sugar (as distinct from fruits).

Dr Heaton says that white bread is eaten more quickly than wholemeal, is less satisfying so more is eaten, needs less chewing which stimulates less saliva and less gastric juice. The latter is less well buffered and the pH lower; high sugar diets give rise to higher osmotic pressure. This is the basis of the explanation of some of the 'saccharine diseases'.

There has been, and still can be, much criticism of matters of detail. Do the dates of refining fit all that closely with the massive increase in diverticular disease? Can one really state that slow bowel movements lead to *over efficient* absorption of nutrients? Can there be such a product as 'unprocessed bran'? When the surgeon successfully treats his patients with bran does that really prove that lack of bran was the cause of their disorder?

The book edited by Burkitt & Trowell, with six other authors, presents a reasoned argument. Certainly over the past few years the protagonists have been criticized on many points of fact but Dr Trowell stated in a letter in the *British Medical Journal* (12 July 1975) that after discussions with the milling industry 'we have learned much' and his chapter describing what is meant by 'dietary fibre' demonstrates this.

On the contrary the book by Stanway is a typical piece of journalism—badly presented, sensational, ill informed and Comic Cuts to those who are not seriously involved. If his book were your only source of information you would reject the fibre theory out of hand. He reads as though he has collected his information solely by reading Burkitt & Trowell's book (which was published a little earlier) but has not understood it very well. In fact it looks as if he does not understand his own writing since there are numerous contradictions in the text. After telling us how much our health improved during the war because of 85% extraction bread he then tells us that brown bread of any kind is no good, we must insist on the whole grain. 'Don't add sugar to your breakfast cereal, add dates instead' (but dates are 74% sucrose). He equates all factory-made foods with synthetic foods. He says 5 lb overweight is just as important as 50 lb then goes on to show that health risk rises in proportion to the amount overweight. He damns all packeted and canned foods then says they are a great standby. His figures for fibre intake over the centuries differ from those of Trowell. He recommends 'gentle grinding' of wheat rather than 'harsh smashing' by rollers because 'we simply don't know what happens to the intrinsic properties of the grains when we treat them roughly'. If this is how medically qualified journalists write what can we expect from the daily press?

The story is summed up in Sir Richard Doll's preface (to Burkitt & Trowell's book): 'once every ten years or so a new idea . . . captures the imagination and . . . seems to provide the key . . .';'. . . whether it (deficiency of dietary fibre) will be as seminal an idea as that of vitamin deficiency or as sterile as that of stress we shall probably not know for another ten years.' (He himself has little doubt.)

Meanwhile every food scientist needs to become well informed on the subject and Burkitt & Trowell's book is a painless and interesting way of doing so.

A. E. Bender

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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

SI UNITS

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

NON SI UNITS

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

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

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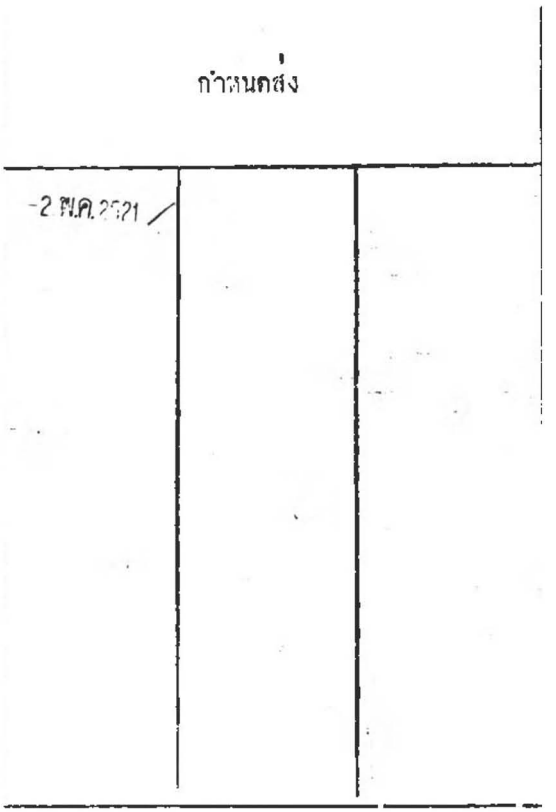
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