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Scope. Food technology is the application of scientific and technological knowledge to the production, storage, distribution, marketing and use of food and beverages from raw materials. The Journal covers a wide range of topics relevant to food, from raw material composition to consumer acceptance, from physical properties to food engineering practices, and from quality assurance and safety to storage, distribution, marketing and use. It covers basic food science and all relevant technological applications leading to the product on the table. The Journal provides a broadly based international forum for publication in English of original research and new applications in the form of research papers, concise but critical topical reviews, technical notes, and letters on material published in previous issues. All but letters are subject to multiple peer review. The Journal also publishes reviews of relevant books.

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Editorial

The *Journal of Food Technology* has now completed its twenty-first year with this issue—it has reached maturity. As I indicated in the August issue, all areas of activity change with time, generating different needs as they develop. The field of food technology has changed greatly over that period, from being relatively 'low-tech', but with a science base, to being 'high-tech' and science-led. The Journal, which serves research workers in this field, has been subjected to an extensive review of its role and the way in which it is fulfilled, and a number of changes have already been made. One change, already announced in August, is to make explicit its role as a broadly based vehicle for the international exchange of novel information relevant to the interests of the Institute—food science and technology.

The Journal has always included some food science as well as technology, although the emphasis has always been on the latter. This was entirely appropriate in its early days, when many developments in technology were led by a large measure of pragmatism, and, with notable exceptions, understanding of the underlying science followed. The situation has changed, and food science is now an established family of taught disciplines with a body of knowledge that is sufficiently mature to lead most developments in today's food industry. Food technology is now so predominantly science-based in its application that it is both meaningless and inappropriate to try to separate it from the science.

The present title is therefore inaccurate—it reflects neither the current role nor content of the Journal. More importantly, it deters some workers in the more basic aspects of food science from using the Journal as an appropriate place in which to publish their research. A specific and frequently mentioned reason is the absence of 'Food Science' from the title. Thus, there are strong reasons for a change in title to one that is more appropriate.

There are, of course, counter arguments. The Journal is well known and established, and a change would break with tradition and cause problems for abstracting and data retrieval services. Established practice and tradition should not be lightly discarded: but the world is dynamic and is constantly evolving; artifacts become redundant if they do not change with changing needs, and the Journal is no exception.

Beginning with the first issue of Volume **22,** in February 1987, the Journal will be known as *The International Journal of Food Science and Technology*. It will have a subtitle *Formerly The Journal of Food Technology*, which will be used for the first few years. The new title also reflects the fully international content and circulation of the current Journal.

I also wish to express the grateful thanks of the editorial team to all those colleagues who have helped us by refereeing papers. The publication of research is totally dependent on the system of independent peer review to ensure that high standards are maintained. This has a cost—a lot of time and effort is required to review someone else's work thoroughly and constructively. It is also unacknowledged because referees work anonymously, for obvious reasons. However, following a few other Journals, we now publicly thank our referees. In this issue we publish the names of those who have helped us in this way over the last few years.

One of our Deputy Editors, Jeremy Selman, has now resigned. I thank him for all the work he did whilst with us, and welcome the new Deputy Editor, Paul Nesvadba to the team.

Finally, although many of our readers may not celebrate Christmas, I would still like to wish you all a happy and peaceful Christmas season.

Derek G. Land
Editor

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Review: Application of science and technology to poultry meat processing

J. M. JONES

Introduction

The poultry processing industry has progressed dramatically, passing from the essentially 'cottage industry' of three or four decades ago to today's highly automated food production business. Its extent may be gauged from the fact that in 1985 the United Kingdom output of poultry meat was 875000 tonnes (Anon., 1986a). In addition to whole raw and cooked carcasses, there are some 100 types of poultry products available on the British market, ranging from portions through sausages and hams, to speciality products such as 'Chicken Kiev'. In 1985, within the U.K., some 50000 tonnes of poultry meat were further processed into products other than simple roasted or cooked birds and portions (Anon., 1986a).

While the ability of the poultry breeding companies to develop the types of birds required for particular markets has contributed greatly to the poultry processing industry's success, much can be attributed to the dramatic advances that have been made, notably in Europe, in the design of equipment used to transform poultry from the live bird to the finished carcass or product.

The purpose of this paper is to review the science and technology that have played a part in recent developments in poultry meat preparation.

Processing

The process of going from the live bird to the final product is a multi-stage operation, the principal steps of which are shown in Fig. 1.

The majority of the stages up to and including packaging are semi- or fully automated (Schipper, 1981), while grading and process control within the plant may be carried out using computerized weighing systems (Veerkamp, 1983, 1984).

Chilling and carcass quality

Of the various processing stages, the chilling operation has attracted a great deal of attention because of its possible effect on carcass quality. The purpose of chilling is to reduce rapidly the temperature of the eviscerated carcass from approximately 30°C to below 10°C, and hence to prevent the multiplication of micro-organisms of public health significance as well as delaying the proliferation of organisms that cause 'spoilage' of the carcass. Generally the chilling method will be one of two types, immersion (water) and cold air. Water chilling is frequently used in the U.S.A. to cool chickens destined for the 'fresh' or 'chill' market, while in Europe immersion chilled poultry is generally frozen, with 'fresh' poultry being air chilled.

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In the early 1970s concern was expressed within the EEC that, with hundreds or thousands of poultry carcasses passing through immersion chillers, there would be bacterial cross-contamination of carcasses. However, an extensive investigation, involving a number of research laboratories, showed that there was no hazard if the temperature and volume of water used in the chiller were carefully controlled (Anon., 1976). This study led to the acceptance of the 'counter current' immersion chiller where the eviscerated carcasses enter the system at one end to meet increasingly cool water, which enters the system at the point where the carcasses are discharged. According to Veerkamp (1985), counter current immersion chilling should be regarded as a first-stage chilling operation for poultry.

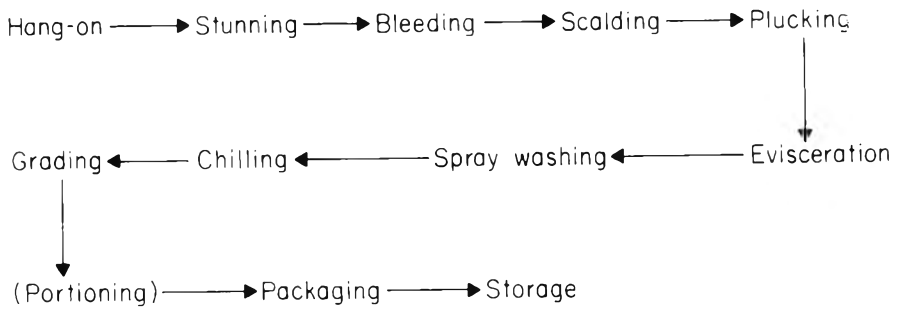

Figure 1. Stages in poultry processing.

In addition to supposed problems with bacterial cross-contamination, water chilling has been frowned upon because of the fact that during the process the carcass takes up water, some of which will be lost later as 'drip', thus leading to consumer complaints. Because of this, EEC Regulations limit the amount of water picked up during water chilling to approximately 5%. Despite this limitation, there has been an increasing move away from immersion to air chilling procedures in parts of Europe.

While the air chilling process itself gives fewer opportunities for cross-contamination, the fact that most air chilled poultry is 'soft scalded' at 50–52°C means that the avoidance of cross-contamination at the early stages of processing is extremely important (Mead, 1980). However, there may be disadvantages to air chilling in that a weight loss of some 1–2% is possible during chilling, and conventionally air chilled carcasses may show signs of changes in flavour due to oxidation or autolysis after only 3 months in the frozen state (Grey *et al.*, 1982). In order to overcome these disadvantages, recent research in The Netherlands has been directed towards the use of evaporative air chilling as the first-stage cooling process in poultry plants. In this process, water is sprayed onto carcasses as they pass through an air chiller and is then removed by evaporation to give a rate of cooling that is only 30% less than that of counter current immersion chilling (Veerkamp, 1985). In addition, the evaporative air

chilling method allows the cooling of carcasses that have been scalded at 58 to 60°C without the usual discoloration problems noted during air chilling of chickens scalded at this relatively high temperature (Veerkamp, 1981). A suggested variation on the evaporative method is the wetting of chicken carcasses with an electrically charged spray of water (Dew, 1982), although this method does not yet seem to be used commercially.

Chilling and meat tenderness

Frequently the cooked breast meat of chickens from batches processed under apparently identical conditions shows a wide variation in texture (Grey *et al.*, 1982). In some cases this variation may be due to the speed of the processing operation. For instance, under commercial conditions, water chilled chickens may be packaged and placed in a blast freezer within 1 hr of slaughter when the carcasses are in varying degrees of *rigor mortis*. Although Grey *et al.* (1982) were able to reduce the texture range of immersion chilled chickens to that of air chilled carcasses by storing the former in ice for 1.5 hr prior to freezing, the toughness problem was not eliminated entirely by this procedure, nor by the application of electrical stimulation in the early stages of processing (Jensen, Jul & Zinck, 1978).

One means of increasing the tenderness of chicken breast meat, and at the same time reducing the bird to bird variation in tenderness, is to inject sodium chloride solution (4% w/v) into the muscle prior to chilling the carcasses (Jones *et al.*, 1980), and recently in the U.S.A. there have been a number of studies aimed at achieving the same effect by chilling the chickens in brine rather than in water.

When broiler chickens were assessed for tenderness after overnight chilling in tanks of 5% brine, they were found to be significantly more tender than chickens chilled in ice-water, with the brine having a greater tenderizing effect on 'tougher' than on 'more tender' meat (Janky *et al.*, 1978; Janky, Koburger & Oblinger, 1982). A 16-hr immersion chilling time would be unacceptable to the commercial chicken processor. However, subsequent studies showed that if chickens were cooled in brine under commercial time and temperature conditions, and with agitation of the cooling medium, the resulting meat was significantly more tender than water chilled chickens. This is possibly because the combination of chiller agitation and a gradual decrease in the chilling medium temperature resulted in a more efficient brine penetration than was achieved merely by soaking the carcasses in brine (Dukes & Janky, 1984, 1985).

In the light of current trends to limit the dietary intake of sodium, the observation of Dukes & Janky (1985) that cooked meat from brine chilled chicken contained sodium levels three to four times higher than those normally found in breast meat could be considered a disadvantage of the brine chilling process. Consequently, the possibility of using KCl rather than NaCl in the cooling medium was investigated by Sams, Dukes & Janky (1986) who found that the potassium salt was equally effective in increasing breast muscle tenderness and apparently did not produce adverse flavour effects.

Despite the seeming advantages of using sodium or potassium salts to improve meat quality, the process does suffer from the disadvantage that carcasses chilled in salt solutions take up significantly more water during chilling than those chilled in water alone (Sams *et al.*, 1986). Thus, if processors were permitted to use brine chilling, they might have difficulties in complying with regulations on the water content of the chicken carcass and might also be required to label their product to make the consumer aware of the fact that chickens had been brine chilled.

Influence of portioning and deboning on meat texture

As stated earlier, chicken and turkey meat is available in forms other than the intact carcass. The preparation of these 'value added' products involves either portioning the carcass, or removal of the meat by manual or mechanical deboning methods. While the evolution of this facet of the poultry industry has led to a number of technological developments that have increased efficiency, some basic problems persist to affect the eating quality of the meat.

Portioning

A number of 'fast food' outlets specify the number of portions to be obtained from a chicken carcass, and there are at least eight cutting techniques used commercially (Hudspeth *et al.*, 1973). Portioning may be performed manually or by machine.

The time *post-mortem (p.m.)* at which the bird is portioned and the type of cut made on the carcass have been shown to influence the tenderness of the resulting portions. Klose *et al.* (1972) showed that meat from chickens cut into eight pieces within 25 min of slaughter was 30% tougher than portions from birds treated in the conventional manner, i.e. cooled for at least 6 hr. Some of the cuts made on warm or *pre-rigor* carcasses influenced toughness more than others: those having the greatest effect were a transverse cut of the breast, removal of the wing at its junction with the breast, and flattening of the portioned breast (Klose, Sayre & Pool, 1971; Lyon, Lyon & Hudspeth, 1973).

Subsequent to these studies, the commercial practice of holding carcasses for 4–6 hr before portioning was adopted.

Deboning

If meat is to be removed from the poultry carcass to produce breast fillets or rolls, there would seem to be a case for the processor to remove meat as soon as possible after slaughter, thus saving time, water and space. Consequently, there has been an increased interest in the question of hot deboning of poultry meat.

The finding of Dawson & Janky (1985), that a *p.m.* deboning time between 5 and 15 min did not affect the difference in tenderness of chicken breast fillets, was supported by the observation of Stewart *et al.* (1984b) that while the instrumentally measured tenderness of meat stripped from an uneviscerated carcass at 0 min *p.m.* was numerically the greatest, it did not differ significantly from that of muscles excised at intervals up to 60 min *p.m.* The latter authors reported that tenderness was significantly greater for meat excised at 120 and 240 min *p.m.* when it was approximately equal to the value for meat held on the bone for 24 hr before excision.

In contrast to the above findings obtained using uneviscerated chickens, another investigation indicated that if the birds were eviscerated and chilled commercially, the time of holding before deboning would need to be extended. Lyon, Hamm & Thomson (1985) examining chickens immediately after chilling (less than 1 hr *p.m.*) found that the meat was significantly less tender than that from birds held for 1 or more hr after chilling. Indeed, the data indicated that, in the case of chickens, deboning should be delayed for at least 6 hr after chilling to assure a large percentage of birds having tender meat. The situation has not been investigated so extensively with turkeys.

In many cases the deboned chicken, or more particularly turkey, meat may be transformed into 'rolls' or 'roasts'. In their simplest forms the meat might be fabricated

into products without further treatment. It is not surprising, therefore, that an early study showed that hot deboned turkey meat made into rolls, without any mechanical or chemical treatment, gave products that were tougher than those made from conventionally chilled breast meat (Nixon & Miller, 1967). However, the meat may be cubed or ground and agitated in the presence of sodium chloride or polyphosphate in order to enhance binding of the meat pieces, and there have been a number of recent investigations into how these processes might affect the tenderness of hot deboned poultry meat.

Wardlaw, McCaskill & Acton (1973), comparing loaves formulated from chicken breast meat that was ground *pre-rigor* (hot deboned) or *post-rigor* (chilled) and blended with 1.5% NaCl, found that the loaves from *pre-rigor* meat were tougher, and concluded that there appeared to be no significant industrial advantage in the use of such meat in poultry loaf production. On the other hand, the *rigor* state of breast meat destined for use in turkey rolls was found to be of no significance, providing that the meat cubes were mixed with NaCl for an adequate period prior to transformation into rolls (Kardouche & Stadelman, 1978).

A subsequent study, however, suggested that the boning method and salt addition significantly affected the tenderness of both chicken and turkey rolls (Furumoto & Stadelman, 1980). Surprisingly, in the absence of added NaCl, rolls prepared from hot deboned chicken meat were more tender than those from cold boned meat. The reason for the apparent anomaly is not immediately obvious, but may have been a consequence of the rapid chilling of the chickens used for cold deboned meat. When the chicken rolls contained salt, there was no significant difference between hot and cold deboned meat. In contrast to chicken, the turkey rolls examined by Furumoto and Stadelman were tougher in the case of hot deboned meat, both in the presence and absence of sodium chloride.

While it is not clear whether the differences in tenderness noted in the above studies would be readily detected by the consumer, it is obvious that the use of hot deboned poultry meat in certain products warrants further investigation.

Investigations into the cause of toughness

The fact that severing or excising muscle shortly after slaughter of the bird causes toughness of poultry muscle has led to a great deal of research over the past 25 years. However, despite this effort, the precise mechanism by which portioning or hot deboning increases toughness has not been fully elucidated.

After the death of the bird, the concentration of the principal cellular energy source of the muscle, adenosine triphosphate (ATP), is maintained firstly by the breakdown of creatine phosphate and then by the degradation of the storage carbohydrate glycogen. Glycogen breakdown (glycolysis) produces lactic acid, and thus ultimately increases the acidity of the *post-mortem* muscle from an initial value in the breast muscle of the live bird of approximately pH 7.0 to an ultimate value in the region of 5.6–5.8. Initially, the rate of glycolysis is slow as the ATP level in the muscle is maintained by creatine phosphate breakdown, but with the disappearance of the latter, the rate of glycolysis increases and the ATP concentration falls. When the ATP level has reached less than 30% of its initial value, the muscle passes into *rigor*. In the case of hot deboned chicken breast muscle the onset of *rigor* commenced within 30–60 min *p.m.* (Kijowski, Niewiarowicz & Kujawska-Biernat, 1982).

Many of the investigations on the tenderness of poultry meat have attempted to establish the relationship between *post-mortem* biochemical changes and the onset of

rigor mortis. De Fremery & Pool (1963) concluded that an increased rate of *post-mortem* glycolysis resulted in tougher poultry meat, and that an increased rate of lactic acid formation was a decisive factor. This view seemed to be supported by the work of Peterson (1977) who found that if polyphosphate was injected into chicken breast muscle some time prior to hot deboning, the normal toughening effect was eliminated. Since the ultimate pH value of the injected muscle was higher (6.15 compared with 5.81 for untreated meat), Peterson concluded that the observed tenderization effect was due to a slowing down of the rate pH decline in the injected muscle.

However, despite the fact that hot deboned poultry meat is generally less tender than that prepared conventionally after chilling, the pH decline of hot excised or severed muscle is more gradual than that of intact muscle left on the carcass, although the ultimate pH values of the muscles are similar (Stewart *et al.*, 1984a).

Stewart *et al.* (1984a) found no differences in the rate of temperature decline between excised and intact muscle and thus concluded that the observed difference in the rate of pH decline was not due to differences in muscle temperature decline. Rather, they postulated that the slower pH decline resulted from a temporary decrease in the rate of *post-mortem* glycolysis brought about by the cutting of the muscle. On severing the fibres, the muscle could contract unimpeded, reducing the need for high levels of ATP and thus reducing, for a short period at least, the need for glycolysis to maintain the ATP concentration. This in turn would limit the production of lactic acid in the muscle.

Lyon, Hamm & Thomson (1985) found that the pH value of muscle excised immediately after commercial chilling (approximately 1 hr *p.m.*) was significantly higher than the values in muscles held in ice at 2°C after chilling, and that the most rapid pH decline occurred within 1 hr of chilling, falling from pH 6.22 to 5.64 after 1 hr and then to 5.51 after 4 hr. However, Stewart *et al.* (1984b) found a muscle pH value of 5.94 after chicken carcasses had been held in slush-ice for 4 hr and concluded that the *rigor* processes were incomplete at this time.

The study of Stewart *et al.* (1984b) indicated that the pre-excision holding temperature influenced the rate of pH decline since, in contrast to the results obtained at 4°C, the *post-mortem* glycolysis and *rigor* rates had levelled out at 2–4 hr *p.m.* in the case of carcasses held at room temperature prior to muscle excision. Under the latter conditions, the pH value of the excised muscle was highly and negatively correlated with the *post-mortem* time of excision. Stewart *et al.* (1984b) concluded that pH decline and muscle tenderness were parallel functions of the *post-mortem* glycolysis and *rigor* processes, but were not necessarily related in a cause–effect relationship.

Recent research has emphasized the requirement for a delay prior to the cutting of poultry meat, which should be noted by those processors intending to install the 'on-line' automatic portioning and deboning equipment now appearing on the market. However, even if the toughness problems associated with the portioning and deboning of *pre-rigor* carcasses are overcome, the concept of 'hot' cutting may not be accepted for a number of reasons. For instance, chickens deboned immediately after plucking produced a reduced total meat yield when compared with conventionally processed and chilled chickens (Benoff *et al.*, 1984), probably because the hot deboned carcasses had not been subjected to the normal washing and chilling operations and, thus, had not taken up moisture.

More important than the yield consideration is the fact that the omission of the evisceration, washing and chilling stages would probably make a hot cutting method unacceptable in those areas such as the EEC and U.S.A. where there are strict regulations regarding the *post-mortem* inspection and chilling of slaughtered poultry.

Mechanically recovered meat

The increasing popularity of portions and further processed poultry products left processors with a surplus of necks, wings and 'frames' (body skeleton) for which it was necessary to find uses in order to avoid wastage of valuable protein and to maintain profits. Twenty to 30 years ago this led to the poultry processing industry adopting the technique of mechanical deboning, which had already been in operation with the fish processing industry for about 10 years. The procedure readily gained acceptance, particularly in the U.S.A. where 90 million kg of mechanically deboned poultry meat was being produced annually in 1979 (Anon., 1979). The product is used in a variety of forms, such as frankfurters, sausages, patties, luncheon meat and dry soup mixes.

There are basically two types of deboning machine in use in the poultry industry. In the older, auger-type machine, the raw material may be used intact but is more likely to be broken into smaller pieces before being forced against screens, slots or filter plates in the deboner. The edible mechanically recovered meat (MRM) passes through the openings, while the bulk of the bone residue does not.

In contrast to the above continuous two-stage machines, a single stage batch deboner, which does not require the preliminary grinding of the bones, has been developed in Europe. In this machine the meat is separated from the bones by the application of high pressure and is filtered through grids. The action of this 'pressure'-type machine causes the meat temperature to rise by only 3 to 5°C, compared with the 7 to 10°C rise caused by the mechanical action of some auger-type deboners (Mast *et al.*, 1982; Newman, 1983).

Composition of poultry MRM and effect on product quality

(a) Gross structure. The physical processes involved in mechanically deboning poultry meat mean that there is considerable disruption of the muscle structure with the MRM emerging as a paste-like material. Mast *et al.* (1982) reported that MRM from a 'pressure'-type deboner contained larger intact muscle fibres than material produce by 'auger'-type machines, although even among these there was a variation in the structure of the several batches of MRM examined.

The paste-like consistency of poultry MRM has generally limited its use to chopped emulsion products, and in the U.S.A. almost all poultry frankfurters are made from 100% MRM (Marsden, 1982), although the consumer acceptibility of the frankfurter may vary according to the source of MRM. For instance, frankfurters prepared with MRM from chicken backs, from which fat was removed prior to deboning, were rated more highly than those prepared from neck MRM (Baker & Kline, 1984). On the other hand, if the fat was not removed, the MRM from chicken backs was rated less well than neck MRM (MacNeil, Mast & Leach, 1978).

Maurer (1979) considered that potential uses for poultry MRM might be increased by texturizing the product in some way, such as extrusion, pan-frying or the addition of soya protein, although he subsequently found that incorporation of 30–40% heat treated turkey MRM into turkey summer sausages resulted in a product that was less juicy and visually less acceptable than sausages made with 'raw' turkey MRM or ground beef (Maurer, 1986). On the other hand, a turkey roast exhibiting good binding and textural properties was obtained when meat chunks, prepared by heat setting salted turkey MRM, were mixed with either 'raw' turkey MRM or cubed thigh meat (Lampila, Froning & Acton, 1985). Megard, Kitabatake & Cheftel (1985) concluded that by mixing chicken MRM with binding agents such as wheat flour or egg white it was possible

to convert the mixture into a texturized material resembling meat loaf by high temperature extrusion cooking in a twin-screw machine.

Clearly the use of texturized MRM in products warrants further research.

(b) Bone content. Concern has been expressed over a possible health hazard caused by the inclusion of bone fragments in MRM. While the material has been shown to contain some bone particles, these were considered to be so small as to be undetectable in the mouth on eating products containing MRM and would not constitute a health risk to consumers (Anon., 1979).

On examining bone fragments isolated from mechanically and hand deboned turkey meat by digestion with papain, Froning (1979) found the particles from MRM to be substantially smaller. A subsequent investigation showed that bone particle sizes in MRM from chicken and fowl (spent layers) varied considerably, but those from fowl were larger, having an average width of 299 μm and length of 557 μm compared with 240 and 374 μm, respectively, for chicken (Froning *et al.*, 1981). This latter study showed that bone particles actually aggregated when papain was used to isolate fragments, thus giving rise to erroneous estimates of particle size. Froning *et al.* (1981) found that the short-term digestion of MRM in alcoholic potassium hydroxide gave accurate estimations of particle size and concluded that the rapidity of this method made it more practical for use in the processing industry than the enzyme method.

From their examination of MRM derived from various sources ranging from young turkeys and chickens to year-old spent laying fowl, Grunden & MacNeil (1973) concluded that the pre-slaughter age of the bird was an important factor in determining the bone content of poultry MRM. The higher levels found in spent layer MRM (Table 1) exceeded the 1% level set by USDA and probably reflected an increased degree of bone calcification in these older birds. However, Mast *et al.* (1982) reported bone levels of 1–2% in broiler chicken MRM obtained from some deboning machines and stressed that proper adjustment of the machine was necessary in order to avoid high bone contents in the product.

(c) Proximate analysis. Froning (1981) reported a wide variation in the proximate analysis (protein, fat, moisture) of MRM prepared from raw chicken, fowl and turkey, with protein levels ranging between 9.3 and 15.5% and fat from 12.7 to 27.2%. The variability might have arisen from a number of factors, such as the age of the bird, with fat content of the carcass increasing with age, or it might be related to the amount of meat and skin left on the bones or 'frames' to be mechanically deboned.

Table 1. Bone content of MRM from various sources*

Source	Bird age (weeks)	Sex	Percentage bone solids†
Broiler backs	7–8	Male	0.79
Spent layer (Fowl) carcasses	> 52	Female	1.44
Turkey racks	24	Male	0.32
Turkey racks	52	Female	0.55

*data of Grunden & MacNeil (1973).

†estimated using atomic absorption spectrophotometry.

On using broiler chicken backs and necks from a common source in trials using four types of deboning machines, Mast *et al.* (1982) reported a much lower quantity of fat in one trial than the other two. There was also considerable variation between the MRM produced by the different machines, although the compositions of the various meats did not differ significantly ($P > 0.05$). This suggested that the type of deboning machine did not materially influence the MRM composition, thus supporting the view of Orr & Wogar (1979) that the source of chicken backs and necks used for producing MRM had a greater influence on its fat and moisture content than the deboning machine used. Since the trials of Orr & Wogar (1979) and Mast *et al.* (1982) were carried out over a period of time using machines located in food processing plants, the possibility of machine settings being altered between trials cannot be ruled out. As well as affecting yields, deboning machine settings may markedly influence the fat content of MRM (Froning, 1981).

Since the protein and fat content of the raw material may be used to calculate the meat content of products, a wide day-to-day variation in MRM composition could be of practical consequence to a food processor who would need to be aware of the possible sources of variation.

(d) Stability of MRM. Poultry MRM may be held for 5–6 days at 3 or 4°C before rancidity, due to auto-oxidative reactions in the product, is detected (Dimick, MacNeil & Grunden, 1972; Moerck & Ball, 1974), with chicken MRM being more stable than turkey MRM because of its higher tocopherol content (Dawson & Gartner, 1983).

The relative instability of poultry MRM may be related to its composition. In addition to introducing oxygen into the product, the process of mechanical deboning releases haem pigments from the bone marrow, and these find their way into the MRM (Froning & Johnson, 1973). This may be particularly acute in the case of the young chickens (42–49 days old) currently used by much of the broiler industry, since the bones of these birds will contain more red marrow than those of older birds such as laying fowl.

While triglycerides constitute some 93% of the total lipid content of chicken MRM, they seem to be relatively unimportant in promoting auto-oxidation (Moerck & Ball, 1974). Rather, the determining factor is the 1–2% of phospholipids, 62–65% of which may be unsaturated and which, unlike the triglycerides, contain fatty acids with three to six double bonds (Moerck & Ball, 1974; Dawson & Gartner, 1983). Generally, the higher the proportion of unsaturated fatty acids present, the more susceptible the lipid system will be to oxidation. It is generally accepted that lipid oxidation is catalysed by the iron present in muscle, although whether haem or non-haem iron plays the dominant role in the process is open to conjecture in the case of certain red meats such as beef and pork (Love, 1983). Although approximately 50% of the total iron in poultry MRM was present as haem and approximately 50% as non-haem iron, Lee *et al.* (1975) concluded that the haemproteins were the predominant catalysts of lipid oxidation in poultry MRM, and that the rate of oxidation increased as the molar ratio of polyunsaturated fatty acids to haem proteins increased from 350:1 to approximately 500:1. Thus it is highly likely that the problem of rancidity will be more acute in those cases where skin is included with the meat to be deboned, since the deboning operation will express fat from the skin into the meat.

The onset of oxidative rancidity during refrigerated storage may be substantially delayed by the incorporation of antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene or rosemary oleoresin, into turkey sausage containing MRM (Barbut, Josephson & Maurer, 1985).

As regards the frozen storage of raw turkey MRM, storage time rather than

temperature between −13°C and +32°C was the most important factor affecting the eating quality of products subsequently prepared from the MRM (Johnson, Cunningham & Bowers, 1974). These results, together with those of Froning *et al.* (1974), showed that in the case of turkey, the MRM should not be held in the frozen state for more than 6–10 weeks.

Shelf life of poultry and poultry products

Probably the fastest growing sector of the poultry meat industry is that of the chilled product. Naturally, such a product has a relatively short shelf life when held at normal chill temperatures (−1 to 4°C), and much research effort had gone into developing means of extending shelf life and also into trying to establish reliable methods of predicting the shelf life of chilled poultry.

The most notable indication of quality deterioration with time in the chilled poultry carcass is the transition from a 'fresh' product having virtually no smell, to meat having a distinct 'off' odour and a variable amount of slime on its surface. In the case of the bird conventionally packaged in polythene, the development of 'off' odours equates with the number of bacteria present on the bird [1×10^8 organisms/cm^2 (Shrimpton & Barnes, 1960)]. Consequently, a number of studies have been directed towards relating 'off' odour to the volatiles produced by spoilage bacteria. Among these volatile substances are sulphides, esters, alcohols and ketones (Freeman *et al.*, 1976; Pittard *et al.*, 1982). Some of these, such as hydrogen sulphide, dimethyl sulphide and methanol, were considered to be associated with the typical 'off' odour of chicken breast meat undergoing spoilage at refrigerated temperatures, but it was not established that the compounds were produced in large quantities prior to spoilage. Thus this avenue of investigation appears to be of limited value.

Although odour is the normal quality criterion by which shelf life is judged, it is not ideal since it does not take into account biochemical changes occurring in the meat, possibly altering the flavour before an 'off' odour is detected. For instance, nucleotides undergo major catabolic changes with ATP passing to inosinic acid (IMP), which is used commercially as a flavour enhancer, and thence to hypoxanthine, which is said to impart a bitter flavour to meat. It has been claimed that the level of hypoxanthine in the tissue might be used as an index of freshness in beef (Pizzocaro, 1978) and thus several investigations have been carried out into its usefulness as a quality index in poultry. Khan, Davidek & Lentz (1967) had noted that hypoxanthine could be used as an index of chicken muscle quality, but felt that erroneous results could be obtained in the case of ice chilled birds where nitrogenous material might be lost by leaching. Recent work with turkey meat (Jones *et al.*, 1982) showed that while hypoxanthine levels increased during chill storage, a taste panel did not report a steadily increasing 'bitter' flavour as the hypoxanthine levels reached those known to influence the acceptability of red meats (22–27 mg/100 g muscle). Furthermore, the work of Jones *et al.* (1982) indicated that the hypoxanthine concentration was influenced by the pH value of the stored muscle.

Currently it seems that much more work is required before acceptable biochemical means of estimating poultry meat shelf life are available. Therefore, attention is turning to the use of time–temperature monitoring of the product, since it is well known that temperature is the major factor influencing the shelf life of chilled poultry (Pooni & Mead, 1984). Mead (1985) recently described a battery operated temperature function integrator, available in the U.K., which may be used to monitor thermal abuse along the processing and distribution chains. The instrument integrates the effects of time and

temperature to predict the remaining shelf life, presuming that the relationship between temperature and spoilage rate is known. According to Mead (1985), the predictive equation is given by:

$$R = (0.1T+1)^2$$

where R is the relative spoilage rate (relative to the rate at a reference temperature, usually 0°C) and T is the storage temperature in degrees C.

A knowledge of the initial bacterial load of the product is necessary if shelf life is to be predicted accurately.

As reported by Wells & Singh (1985), there are a number of other time–temperature indicators, some of which are abuse indicators, and others of which are full history time–temperature devices. Although these seem to have been used principally with frozen meat, further development may result in their being used with chilled poultry.

In addition to the organisms that cause spoilage, food poisoning bacteria such as salmonellas may contaminate high numbers of poultry carcasses. Many attempts have been made to reduce the bacterial load on carcasses by using dips or sprays of chemicals such as phosphates, organic acids or glutaraldehyde, as well as by super-chlorination (Mead, 1980). Such treatments are frequently of limited value because they either produce adverse quality effects or may not be approved for food use by regulatory agencies, even if the efficacy of a certain treatment has been established. For instance, chlorination of processing water has been shown to reduce the total bacterial load, but is not legally permitted in some European countries (Mulder, 1982). For these reasons, other techniques for reducing bacterial load and extending the shelf life of poultry carcasses and portions have been studied.

Effect of reduced temperature

The efficacy of using low temperature for prolonging the shelf life of poultry carcasses has been well documented. For instance, holding chickens at 2°C resulted in a 2 day extension of shelf life when compared with storage at 5°C (Bailey *et al.*, 1979). However, the effect of using even lower storage temperatures was strikingly demonstrated by Barnes *et al.* (1978) who examined turkey carcasses held in oxygen permeable polythene bags at temperatures between 5°C and −2°C. At 5°C the average time that elapsed before the detection of 'off' odour was 7.2 days; at 0°, 22.6 days, but at −2° the time which elapsed before the detection of 'off' odour was 38 days.

Thus, holding carcasses at −2°C might enable a poultry processor to ensure a more even flow of product at certain times of the year. However, the processor must exercise careful temperature control since, although the meat is not frozen at this temperature, it is close to its freezing point of between −3 and −4°C (Barnes, Impey & Griffiths, 1979; Shrimpton, 1965). It is not clear whether poultry held at −2°C could be sold as 'chilled' within parts of Europe, since within the EEC, for instance, the recommended temperature range for chilled poultry produce is from 4°C to −1°C.

Modification of atmosphere surrounding the meat

(a) Packaging material and vacuum packaging. The simplest means of modifying the atmosphere around the carcass is to make use of the fact that *post-mortem* respiration of the poultry meat will absorb oxygen and release carbon dioxide. Thus, with a carcass held in an oxygen impermeable film or package, there will be a decrease in the oxygen

content and an increase in the carbon dioxide level in the space between the bag and the carcass.

In an early experiment, Shrimpton & Barnes (1960) compared chickens wrapped in polythene bags with others in vinylidene chloride-vinyl chloride co-polymer bags that were evacuated and heat shrunk. After 12 days at 1°C, the carbon dioxide content in the polythene bags was 2.1% (0.2% at 0 days) and the carcasses had developed a definite 'off' odour. In the case of the evacuated packs 'off' odours were not detected until after 16 days of storage, by which time the carbon dioxide in the packs had reached a level of 10%.

Later work with ducks and chickens (Barnes, Impey & Griffiths, 1979; Igbinedion *et al.*, 1981) showed that wrapping in heat shrunk oxygen-impermeable film would extend shelf life by more than 50%, when compared with storage in polythene bags, and the authors assumed that this benefit was due to the build up of CO_2 in the packs.

Studies using vacuum packaged turkey drumsticks and breast muscle fillets revealed that levels of CO_2 as low as 3% could inhibit the growth of spoilage bacteria and that the development of 'off' odour in the drumsticks was delayed from 14 to 20 days and from 16 to 25 days in the case of breast fillets (Jones *et al.*, 1982; Mead *et al.*, 1983). However, a trained taste panel found marked flavour changes to precede the detection of definite 'off' odours, thus supporting the earlier contention that odour detection alone might not be the best criterion for judging the shelf life of poultry meat. Further possible drawbacks to the use of vacuum packs for the retail sale of poultry are the leakage of fluid from the muscle during storage, leaving unsightly 'drip' in the packs; a bluish tinge to the meat resulting from the reduction of the muscle myoglobin, and lastly, a slight odour perceived when the pack is opened. However, the odour disappears and colour is restored shortly after the meat has been exposed to air (Mead *et al.*, 1983; Humphreys, 1985).

(b) Gas flushing. The results obtained by Shrimpton & Barnes (1960) using evacuated packs for the storage of chickens were not too surprising in view of earlier observations. Coyne (1933) and Haines (1933) found that CO_2 at levels of 10–20% inhibited the growth of a number of spoilage organisms if the storage temperature was kept below 4°C. Although use was soon made of the findings of Coyne and Haines to develop systems for the shipment of red meat on long voyages, the method of transporting poultry meat in a constant level of CO_2 at chill temperature (controlled atmosphere, CA) does not appear to have been widely used in recent years.

Ogilvy & Ayres (1951) carried out the first extensive study on the use of CO_2 to prolong the shelf life of chicken portions when they examined the effects of both controlled or modified atmosphere (MA), where portions were packed in CO_2 but no attempt was made to control the gas composition during storage. Both systems gave an extension of shelf life, although the effect was greater in the case of CA. These authors also found that within the range of 0–25% CO_2 the ratio of keeping time of chicken in the presence of CO_2 to that in air was a linear function of CO_2 concentration. They considered the 25% level of CO_2 to be the maximum to be used because of the rapid discoloration of the meat which occurred during storage at higher levels. These observations seem to be at variance with those of other workers (Hotchkiss, Baker & Qureshi, 1985) who found that the colour of chickens stored in a MA containing 80% CO_2 at 2°C was maintained for at least 28 days. The study of Hotchkiss *et al.* (1985) indicated that the odour of raw chicken quarters stored in 80% CO_2 was acceptable for up to 35 days, in contrast to the observations of Wabeck, Parmetee & Stadelman (1968) who found that

carcasses held at 1°C in a CA of air containing 10 or 20% CO_2 developed 'sweet' or 'fruity' 'off' odours 6 days before control carcasses held in air. A similar sweetish, non-characteristic spoilage odour was also noted when chickens were stored in containers in solid CO_2 (Thomson & Risse, 1971) and it was felt that this CO_2-related 'off' odour could be a problem in storage facilities or tightly closed boxes.

A common feature of studies on chickens held in modified atmospheres has been the rapid decline in CO_2 levels in the packs. It may go from 100 to 40% in 24 hr and down to 20% after 2 days of storage (Sander & Soo, 1978; Gray, Elliot & Tomlins, 1984), and it is generally assumed that this disappearance results from the high solubility of CO_2 in the liquid film on the surface of the meat.

In contrast to the considerable amount of work carried out on chicken, there is little information relating to the gas packaging of turkey meat. Mead *et al.* (1983), using skinless turkey breast fillets, showed that packs containing 30% CO_2 in nitrogen held at 1°C were acceptable for up to 3 weeks of storage, although the meat quickly lost its 'bloom'. In an attempt to improve meat colour, some fillets were stored in packs containing 10 or 20% oxygen in addition to 20 and 30% carbon dioxide. However, there was a rapid development of unpleasant flavours in the cooked meat, apparently related to the CO_2 concentration in the pack. In addition, the raw meat developed a salmon pink colour, which sometimes persisted into the cooked meat. The authors concluded that the inclusion of O_2 in gas packs of skinless turkey breast fillet would be of little commercial value and that further research was required in this area. This view is clearly reinforced by a recent report that at least one company in the U.K. is marketing bulk packs of chilled turkey portions held in barrier bags under an atmosphere of carbon dioxide/nitrogen/oxygen (Anon., 1986b). Some of these products have skin attached and so may behave differently from the breast fillets used by Mead *et al.* (1983).

Irradiation of poultry meat

An alternative means of prolonging shelf life, as well as reducing the levels of pathogenic bacteria on poultry meat, is to subject the carcasses to ionizing radiation, a process used for the pasteurization and sterilization of foodstuffs for 40 years. In this paper only pasteurization will be considered.

Although importation or sale of irradiated foods is not currently allowed in the U.K., the irradiation of selected foods is allowed in a number of countries. For instance, in The Netherlands the maximum dose approved for refrigerated poultry carcasses is 3 kGy while in Israel and South Africa poultry carcasses may be treated with up to 7 kGy: the Gray (Gy) is the absorption of 1 joule of energy/kg irradiated material and is equivalent to 100/rads.

Two forms of radiation pasteurization may be used to prolong shelf life.

Radurization is designed to kill or inactivate the spoilage organisms, and for poultry this is generally carried out at dose levels of about 2.5 kGy. The irradiated product needs to be stored under refrigerated conditions.

Radicidation is designed to kill, or render harmless, non-spore forming bacteria of public health importance and is generally carried out at doses of 2.5–5.0 kGy for chilled (fresh) poultry and up to 7.5 kGy for frozen poultry where the reduced availability of water results in an increased bacterial resistance to radiation. As with radurization, the 'fresh' irradiated product is stored in the refrigerated state.

Of the various methods of radiation described by Mead & Roberts (1986), dosage by gamma-radiation would seem to be the one of choice for poultry carcasses because the high penetration would allow it to affect bacteria in the carcass cavity.

(a) Extension of shelf life and elimination of pathogenic bacteria

Doses of irradiation as low as 1 kGy may eliminate spoilage bacteria and thus increase the shelf life of poultry meat, although the extension of shelf life attained will also be influenced by the storage temperature. For instance, Mulder (1982) reported that in the case of poultry carcasses irradiated at 2.8 kGy and held at 1.6°C the extension of shelf life was 21 days, but at 4.4°C it was 14 days. The limiting factor to the shelf life of poultry does not seem to be microbial spoilage but a decline in the eating quality of the irradiated meat. Recently Basker *et al.* (1986) found that the eating quality of breast meat from chickens irradiated at 3.8 kGy and held at 1°C was maintained for 3 weeks and declined after a further week. Apparently, microbial spoilage did not become a problem until after 35 days of storage in the case of irradiated chickens, compared with a shelf life for chilled non-irradiated carcasses of about 4 days.

As well as being a tool for extending shelf life, irradiation is currently of interest to the food microbiologist as a possible means of removing pathogenic bacteria, particularly *Salmonella*, commonly found on processed poultry carcasses. In the U.S.A., an expert panel concluded that irradiation of poultry at 7 kGy would eliminate *Salmonella*, while smaller doses of approximately 2 kGy could adequately reduce, or even eliminate, *Salmonella* in chickens raised and processed under good sanitary conditions (Froning, 1978). However, Mulder (1982) reported that while irradiation with 2.5 kGy caused a ten-fold decrease in the total number of *Salmonella*-positive chicken carcasses, as well as reducing the number of organisms per carcass, this dose did not guarantee a *Salmonella*-'free' carcass. Greater elimination of *Salmonella* was achieved if the irradiated carcasses were held at −18°C for 3 months.

While microbial susceptibility to irradiation seemed to be greater when chicken carcasses were treated at 5°C than at −18°C, there was no statistical difference between the two temperatures (Mulder, 1982).

(b) Effect of irradiation on product quality

Irradiation pasteurization may be considered to be a 'cold' process since it causes only a slight rise in product temperature, and thus changes in nutritional quality, such as reduction in vitamin content, are generally minimal. However, the sensory quality of poultry may decline as a result of irradiation as may be judged by the fact that chicken or turkey meat irradiated at 1.25–8.0 kGy and held under chill conditions developed a pinkish or red colour which was maintained throughout the storage period (Coleby, Ingram & Shepherd, 1960; Mead & Roberts, 1986). It is not clear whether the slight colour change produced by irradiation would prejudice consumers used to white skinned or fleshed poultry.

Changes in product quality are not confined to the colour of the meat, since early studies showed that raw chicken meat can develop an 'irradiation' odour at dosages as low as 1 kGy, with the odour increasing with increasing dose. While sensory panels were said to be able to rank carcasses in order of increasing radiation dose on the basis of their odour (Coleby *et al.*, 1960; Hanson, Brushway & Lineweaver, 1964), other studies gave somewhat different results. Mercuri, Kotula & Sanders (1967) did not find an 'irradia-

tion' odour with chicken irradiated at 1 or 3 kGy, but reported the typical 'wet hair' or 'feathers' odour with a dosage of 5 kGy. The odour resulting from irradiation seems to be only transitory, lasting up to 4 days of chilled storage after treatment at 5 kGy (Mercuri *et al.*, 1967; Froning, 1978). Froning (1978) reported that, when the treatment was reduced to 2.5 kGy, the 'irradiation' odour was detected on the day of treatment, but not after 4 days of storage. The foregoing refers to experimental data and it is not clear whether the consumer would detect the 'irradiation' odour in poultry that had passed along the normal distribution chain.

In addition to the production of 'irradiation odour', flavour changes in cooked meat may occur as a result of irradiation. Coleby *et al.* (1960) found that the flavour of cooked meat from chickens dosed with between 1.25 and 5 kGy could be ranked in order of increasing dose if cooking was carried out by steaming. This was not the case with roasted meat. In fact, an experienced taste panel generally failed to select roasted carcasses that had received up to 8 kGy in the raw state. The masking of 'irradiation flavour' by roasting has been confirmed by Mead & Roberts (1986).

(c) Feasibility and acceptance of food irradiation

Irradiation technology has advanced to the stage where the process is ready for application by the food industry, as evidenced by the fact that gamma-radiation plants are already operating in a number of European countries (Franken, 1981; Webb & Henderson, 1986). If food irradiation were to be legalized in the U.K., presumably more plants would be developed at a start-up cost of about £1 million each. The current literature gives little indication of the cost of irradiation to the poultry processor, although Froning (1978) suggested that application to the packed and boxed chickens would be in the region of 2 cents/kg, while Franken (1981) pointed out that the price would be dependent upon the applied dose, and that for a dose of 3 kGy the cost would be in the range of 0.16–0.22 Dutch guilders per kg chicken.

A major public relations exercise might be needed to get consumers to accept irradiation, despite the fact that the process may improve the microbiological safety of poultry as well as extending its shelf life. According to Mulder (1982), the main reason for irradiation not becoming widely used for poultry is the consumers' resistance to any process in which atomic energy is involved, even though some authorities believe that the irradiation process as applied to food does not induce radioactivity (Anon., 1986c). Indeed, some of the radiolytic products formed in meat may also be found in non-irradiated products (Brynjolfsson, 1985).

A recent report from the Advisory Committee on the Safety of Irradiated and Novel Foods (ACINF, 1986) stated that ionizing radiation presented no toxicological hazard and introduced no special nutritional or microbiological problem if properly applied up to a dosage of 10 kGy. Webb & Henderson (1986) felt that the ACINF report did not cover possible health effects in depth, and also pointed out that no test is currently available to identify food that has been irradiated. This lack of a definitive test was suggested as a reason for not yet legalizing food irradiation in the U.K., while the pilot survey carried out by Webb & Henderson also showed that food producers felt that irradiation should not be used to compensate for poor manufacturing practice.

Consumer reaction to irradiated food may, however, vary with the consumer group, as shown in a recent survey carried out in the U.S.A. by Bruhn, Schutz & Sommer (1986). Here, conventional consumers were more likely to accept irradiated foods than

'alternative' (ecologically sensitive) consumers, and their attitude towards food irradiation could be positively influenced by an educational programme. In general the authors found that consumers showed a higher level of concern about the use of food preservatives than for food irradiation, but willingness to buy irradiated food was based on the safety of the process rather than the advantage of the food product.

Conclusion

The increase in the efficiency of production and the continuous development for further processed and 'value added' poultry products would not have been possible without the application of science and technology. However, some of the innovations have been concerned solely with maximizing the efficiency of production and have not taken into account their possible effects on meat quality, such as tenderness. In fact, the area of meat tenderness is one in which a more comprehensive understanding of the *post-mortem* biochemical reactions accompanying physical changes that occur in poultry muscle is needed before current deboning methods can be fully utilized.

Further areas for investigation include the elucidation of the sensory changes occurring during the storage of poultry and the establishment of definitive methods for predicting the shelf life of poultry products.

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Effect of polysaccharide stabilizers on the rate of growth of ice

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Summary

Measurements of the linear rate of advance of ice into undercooled aqueous solutions and gels have been made. The solutes consisted of sucrose with smaller quantities of other substances, mostly polysaccharides. The linear crystallization velocity in sucrose solutions is mainly governed by the rate of diffusion of sucrose molecules, and the addition of soluble polysaccharides causes only a small extra retardation. However, the growth of ice into undercooled sucrose solutions is drastically retarded and altered in morphology by the presence of a gel network. This effect is discussed qualitatively in terms of the interaction of the ice front with the gel fibres, which reduces the equilibrium freezing point and may lead to rupture of the fibres.

Introduction

It is widely believed that hydrophilic gums ('stabilizers') interfere with ice crystallization in ice-cream. No direct evidence of an effect of stabilizers on ice crystal size has been published, but the use of stabilizers does result in a product that is perceived as being 'smoother'. Since the coarseness of a population of crystals depends (for a given rate of heat removal) on the relative rates of nucleation and growth (Fennema, Powrie & Marth, 1973) it might be inferred that stabilizers either enhance the nucleation of ice, or reduce the rate of growth. As stabilizers affect nucleation of ice to an insignificant extent in frozen confectionery products (Muhr, Blanshard & Sheard, 1986), the second possibility is examined in this paper.

The three important potential rate determining steps for crystal growth are heat transfer, mass transfer and interface reaction kinetics. It was envisaged that stabilizers would have an insignificant effect on heat transfer (Callow, 1952) but might influence either or both of the other steps. To clarify the theoretical framework, consider the growth of an ice crystal in an undercooled unstirred solution, originally having a solute concentration of c_b and temperature T_b (and retaining these values sufficiently far from the interface at all times). Exclusion of the solute from the growing interface will result in an enhanced solute concentration at the interface (c_i). The temperature of a stationary interface, of radius of curvature R, should have the equilibrium value T_M, where

$$T_M = T_E(c_i) - 2\sigma/R\Delta S_V \qquad (1)$$

where $T_E(c_i)$ is the equilibrium freezing point for a plane ice interface with a solution of concentration c_i, σ is the ice/solution interfacial energy, and ΔS_V is the entropy change

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when the unit volume of ice is formed from an infinite reservoir of the solution. However, for a finite rate of molecular deposition (corresponding to a linear crystal growth velocity V) the interface temperature T_i will differ from T_M by an amount ΔT_i related to V by a growth law

$$V = f(\Delta T_i, c_i). \tag{2}$$

For the sake of clarity, the relationship between the various temperatures is given in Fig. 1.

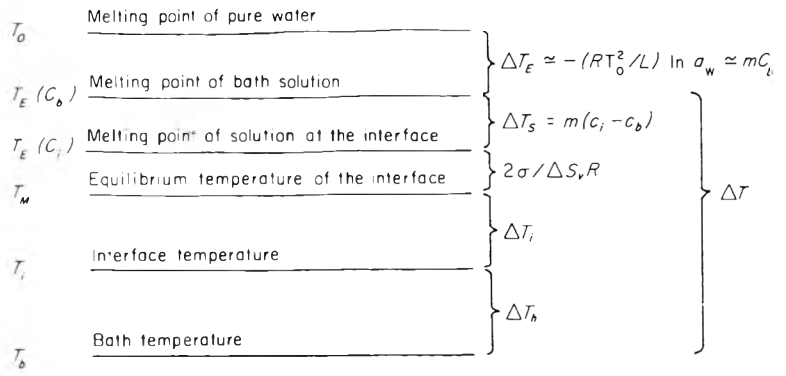

Figure 1. The relationship between temperatures for crystal growth into an undercooled solution.

It is apparent that, for steady-state growth, c_i and T_i must attain such values that the macroscopic freezing rate (V) determined by mass transfer (driving force ΔT_s) equals that determined by heat transfer (driving force ΔT_h), or by the molecular deposition rate (driving force ΔT_i). From Fig. 1,

$$\Delta T_s + \Delta T_i + \Delta T_h = \Delta T - 2\sigma/\Delta S_v R. \tag{3}$$

If one of these processes (say that corresponding to ΔT_s) is very much slower than the others then it follows that (neglecting capillarity),

$$\Delta T_s \simeq \Delta T$$

and a considerable simplification is achieved, since the rather inaccessible values of c_i and T_i are removed from the problem. Use is made of this simplification in a theoretical treatment in the discussion section.

In the experimental work the linear growth rate V was measured as a function of c_b and T_b. It proved necessary to use two techniques of measurement, namely the 'tube' and 'flask' techniques.

In the first technique, the undercooled solution was contained in a U-tube and, after seeding ice at one end, the rate of growth of ice along the tube was measured. The 'tube experiment' was inapplicable to gels, since the ice spread rapidly between the gel surface and the tube wall, thus concealing the progress of any ice growing inside the gel. For this reason a second technique was devised in which an ice seed was introduced into the centre of a round flask filled with the undercooled gel (or solution). The 'flask experiment', however, proved unsatisfactory for sucrose solutions in the absence of stabilizers, since convective currents arose which were observed to seriously disturb the ice-crystal growth. The two techniques were thus complementary.

Materials and methods

The source and specification of the stabilizers used have already been reported (Muhr, *et al.* 1986).

1. *The tube experiment for ice-crystal growth rates*

Background. The tube experiment has been used to measure linear crystallization velocities by many workers (Hartmann, 1914; Walton & Judd, 1914; Brann, 1918; Freundlich & Oppenheimer, 1925; Tammann & Büchner, 1935; Kost, 1953; Lusena, 1955; Pruppacher, 1967; Glicksman, Schaefer & Ayers, 1976). What is measured, as presently understood, is the rate of advance of the front of a dendritic invasion of the contents of the tube. Such a dendritic invasion is (for a pure liquid) essentially an isenthalpic process, i.e. the time taken for the invasion of a particular volume element is short compared to the time required for a significant transfer of heat from the volume element to the bath (Glicksman *et al.*, 1976). Under these conditions, the undercooling of the tube contents determines the rate of advance of the invasion front (V), and also the fraction of water transformed to ice. (Immediately after the invasion, the temperature of the ice/water is close to the melting point.) Heat transfer to the surrounding cold bath should only influence the subsequent 'occupation' of the invaded territory. Similar observations apply to the dendritic invasion of an under-cooled solution when mass transfer is the rate-limiting step. The tube serves merely as an experimental convenience and to provide graduation marks for spatial reference. Unfortunately, the tube may also complicate the situation by providing a solid substrate, and the dendrites may be encouraged to grow as a close packed parallel array and thus their thermal or concentration fields may overlap (Glicksman, *et al.*, 1976). The isolated needles assumed in the theory of dendrite growth are more nearly attained by free radial growth of dendrites from a point source. Hobbs (1974) provides a useful figure in which literature values of the 'linear crystallization velocity' (i.e. tube experiment results), and of the free growth velocity of ice in pure water, are compared as a function of undercooling. The former are generally larger, presumably the advantageous properties of the tube wall outweigh any retarding effect from the mutual thermal interference of the dendrites.

The tube experiment should be carefully distinguished from the capillary method for determining the interface reaction kinetics for crystal growth (Jackson, Uhlmann & Hunt, 1967).

Method. The apparatus (Muhr, *et al.*, 1986) consisted of a range of U-tubes (see Table 1) which contained the trial solutions, and a well-insulated Perspex freezing tank containing industrial methylated spirit (IMS). Cooling was achieved by a Neslab Cryocool (model CC-60II) heat exchanger. The IMS was vigorously stirred and the temperature was maintained to within ± 0.05°C of the desired value using a Neslab Exatrol heater. The U-tubes were filled to a uniform height with the trial solutions using a syringe, and then immersed in the freezing tank.

Reliable results required considerable attention to experimental detail in a fashion that has not always been evident in the previous literature. For example:

(a) The temperature of the filled U-tube should be equilibrated to that of the bath temperature. The procedure can conveniently be standardized by establishing the time constant of the cooling curve, and thereafter assuring that samples are immersed for

a minimum of six time constants prior to taking measurements of the velocity of crystallization.

(b) U-tubes of approximate dimensions should be employed. There is some apparent conflict in the literature as to whether or not V depends on the tube dimensions. To clarify the situation in the present study, a range of tubes of differing dimensions and materials was employed in a preliminary experiment. One copper tube was used; here the crystallization velocity was calculated from the time between abrupt increases in temperature, detected by thermocouples inserted into the copper tube every 10 cm (and sealed with epoxy resin). As a representative solution for these experiments 0.15 g/cm^3 sucrose solution, in every 100 cm^3 of which has been dissolved 0.3 g Manucol DM, was used. The results are given in Table 1.

Table 1. The effect of tube size and material on V (for 0.15 g/cm^3 sucrose, 0.3% Manucol DM, T_f = −6.00°C)

Tube material	Inside diameter (cm)	Outside diameter (cm)	Time constant of cooling (min)	V (cm/sec)	Standard deviation (±)
Glass	0.195	0.370	0.08	0.113	0.008
Glass	0.240	0.420	0.09	0.126	0.002
Glass standard	0.500	0.815	0.34	0.126	0.003
Glass	0.695	0.995	0.54	0.126	0.002
Glass	0.885	1.215	0.80	0.125	0.003
Glass	1.670	2.050	2.24	0.125	0.002
Perspex standard	0.370	0.650	—	0.131	0.004
Perspex	0.935	1.600	2.94	0.126	0.002
Copper	0.440	0.625	0.190	0.134	0.006

It is apparent from Table 1 that the only significant change in V with tube size occurred for the smallest bore glass tube (V exceptionally low); while the only significant effect of tube material on V occurred for the copper tube (V a little high). The fact that V was not much larger in the copper tube probably reflects the greater importance of the rate of mass transfer rather than the rate of heat dissipation. Two tube types were selected for subsequent experiments, and these are denoted 'standard' in Table 1. Several standard glass tubes were made up for the first experiments but subsequently the standard Perspex tubes were used.

(c) Concentrations within the tube should be equilibrated prior to an experiment, and crystal type should be consistent in any series of experiments. Where a second determination of V was made for a tube soon after thawing, a higher value usually resulted. This was interpreted as being the result of incomplete water/residual syrup remixing after ice separation. More than one set of results for V from each filling of the tube was usually taken and, to avoid the incomplete remixing problem, several hours were allowed to elapse before a rerun. The standard deviation of the results was in general no worse than 10% of the mean (and usually rather better). Occasionally, however, the ice front exhibited two alternative modes of growth; 'rotational' in which the rays spiralled around the tube, and 'spear' in which the ice rays were directed parallel to the tube axis. Spear growth was the more common form, and also corresponded to the most meaningful values of V. Similar observations of morphological

variability have been reported by other workers (Brann, 1918; Kost, 1953; Glicksman *et al.*, 1976).

Three series of experiments were undertaken using the tube technique.

(i) Solutions were prepared by dissolving, with the aid of a magnetic stirrer, a range of stabilizers (and quantities thereof) into 100 cm^3 samples of 0.15 g/cm^3 sucrose solution. The value of V was then determined for each sample at T_b = 6.0°C, corresponding to ΔT = 5°C. The effect of stabilizers was put in context by determinations of V as a function of ΔT and sucrose concentration in the absence of stabilizer, and also in the presence of Manucol DH.

(ii) V was determined for a series of solutions (all having Manucol DM and sucrose in the mass ratio 2:100) that modelled the rising concentration of the residual syrup as ice separates out from a 'typical' frozen confectionery product. These solutions were prepared by first dissolving the alginate in an appropriate amount of water using a magnetic stirrer, and then dissolving the sugar into this solution in a volumetric flask, making a final volume adjustment with distilled water after air bubbles had been removed under low pressure (a slow process!). V was measured at a constant value of ΔT = 5°C.

Measurements of the viscosity of the trial solutions were obtained at a temperature of 4±0.5°C using a Deer Rheometer with a cone-and-plate geometry.

(iii) V was determined for a series of solutions made by dissolving additives (using a Silverson vortex mixer) in 0.75 g/cm^3 (58.6% w/w) sucrose solution. T_b was kept at −16.2°C for the ice growth experiments corresponding again to ΔT = 5°C for the stabilizer 'additives' (glucose and citric acid).

2. *The flask experiment for ice crystal growth rates*

Method. A wide range of techniques for studying the free growth of dendritic crystals has been reported in the literature. The apparatus used here (Fig. 2) was a simplified form of the apparatus of Glicksman *et al.* (1976), employing the same method of initiation of crystallization in the centre of a spherical flask by means of a nozzle. Round bottom Quickfit flasks were used, and the nozzles were made from Pasteur pipettes. The nozzle tip was arranged (by eye) to be as close as possible to the flask centre. Having almost filled the flask with the sample (prior to gelation), a layer of

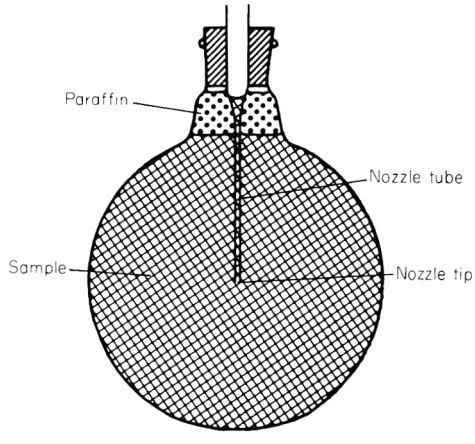

Figure 2. Diagram of the flask and adjuncts employed in studying the ice-crystal morphology and kinetics in three dimensions.

paraffin was added (to discourage ice nucleation at the sample surface) and then the nozzle was inserted.

As with the tube experiment, the cooling time constant of the flasks was determined. A minimum of six time constants was allowed to elapse before crystallization was initiated.

The time of emergence of the ice from the nozzle, and the time at which the ice reached the side of the flask was recorded, visually and photographically, and notes were made of the morphological appearance of the ice.

Four series of experiments were undertaken.

(i) *The effect of firmness of alginate gels.* In the first series of experiments the effect of 'firmness' (i.e. calcium content) of alginate cells on ice growth was studied. A stock solution of 0.40 g/cm^3 sucrose (35% w/w) was prepared, and into 1 litre of this was dissolved 10 g of Manucol DM (using a Silverson high speed vortex mixer). Gels were made by mixing 1 g of a blend of $CaHPO_4$ and Na_2HPO_4 into 150 cm^3 measures of the alginate and sucrose solution, and mixing with 50 cm^3 of the stock sucrose solution immediately after dissolving 2.0 g glucono-δ-lactone in the latter. The gels were aged for 24 hr before initiating ice crystallization. The bath temperature (T_b) was −6.5°C for the fullest range of calcium content, although ice growth in gels containing 0.00, 0.01 and 0.025 g of $CaHPO_4$ per 100 cm^3 were also studied at −4.5, −7.5 and −8.5°C, and a few additional experiments were performed at −4.7°C. At −9.5°C, spontaneous nucleation occurred preventing growth-rate studies. The freezing point of 0.404 g cm^3 sucrose solution was close to −3.5°C, although it should be noted that the presence of the phosphate salts (and to a lesser extent the glucono-δ-lactone) will depress the freezing point somewhat.

(ii) *The effect of gel age.* In the second series of experiments, the effect of the age of alginate gels on ice growth within them was studied. The gels were prepared as in (i) using 0.01 g $CaHPO_4$ per 100 cm^3.

(iii) The effect of stabilizers. In a third series of experiments, the effect of a variety of stabilizers on the growth of ice in 0.404 g/cm^3 sucrose solution was studied. The solutions were prepared by dissolving 1.125 g of stabilizer (using the Silverson high speed vortex mixer) into 150 cm^3 of 0.404 g/cm^3 sucrose solution. To allow a comparison with the gel results (above), 0.75 g Na_2HPO_4 and 1.5 g glucono-δ-lactone were also dissolved in each measure of 150 cm^3 sucrose solution. In the case of celacol (methyl celluose), a more complete dissolution was obtained by first dissolving the stabilizer in water and then adding the sucrose. In the case of xanthan, half the quantity was used; even at this concentration, xanthan gave exceedingly 'thick' solutions. For these stabilizer–sugar–water solutions, ice growth was studied at T_b = −5.0°C and at −6.5°C.

(iv) *Results for other gels.* Finally, some exploratory experiments were made on agar, gelatin and silica gels. The agar gels were made by adding 0.5 or 1.0 g of agar to 100 cm^3 of 0.404 g/cm^3 sucrose solution contained in a conical flask and stirred by a magnetic flea, using just sufficient heat to dissolve the agar. Gelatin gels (1.0 or 2.0 g in 100 cm^3) were prepared by the same method, although a lower temperature sufficed. Silica gels were prepared from mixtures of an appropriate ratio of sodium metasilicate–and–sucrose solution (0.0411 g/cm^3 SiO_2, 0.0129 g/cm^3 Na_2O, 0.404 g/cm^3 sucrose) and hydrochloric acid–and–sucrose solution (0.0365 g/cm^3 HCl, 0.404 g/cm^3

sucrose). It was found that a ratio of 20:5.5 (silicate: acid by volume) gave the most rapid setting time (3 min). The gel concentration was in some cases reduced by adding, in addition to the silicate and acid solutions, a volume of 0.404 g/cm^3 sucrose solution.

Results

(i) *The effect of stabilizers on ice growth in 0.15 g/cm^3 sucrose solution (tube experiment)*

The results of the determination of the dependence of the linear ice crystallization velocity on sucrose concentration (0.10, 0.15 and 0.20 g/cm^3), bath temperature and presence or absence of Manucol DH are shown in Fig. 3.

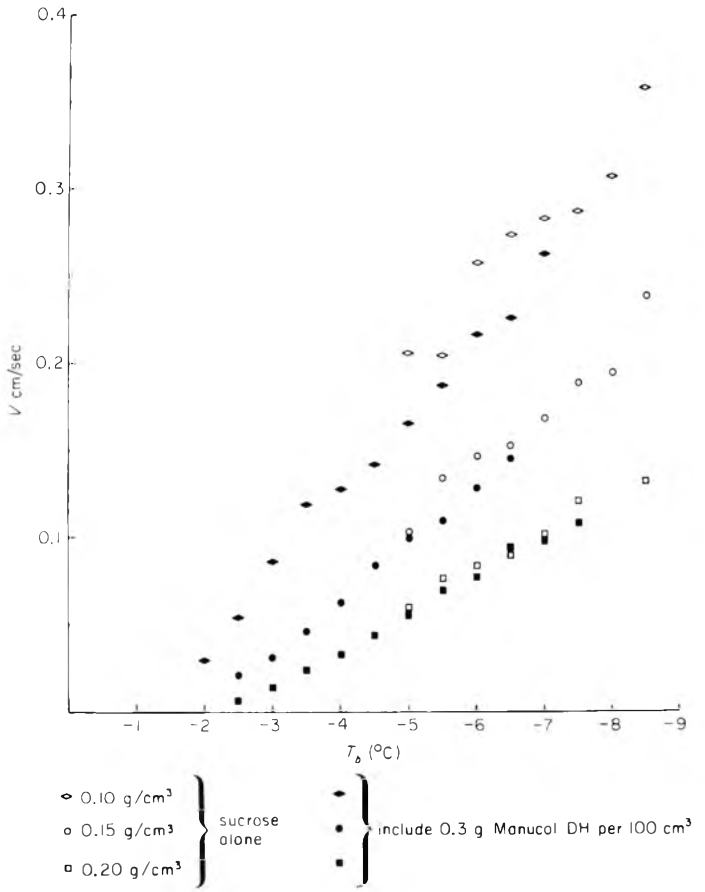

Figure 3. The dependence of the linear crystallization velocity (V) on bath temperature (T_b) with sucrose concentration as a parameter (using standard glass tubes).

It is apparent that the reproducibility of the technique is not good, nevertheless clear trends can be detected as T_b or the sucrose concentration is altered. The presence of Manucol DH results in a consistent (albeit small) reduction in V.

The effect of different concentrations of methyl cellulose on V for 0.15 g/cm^3 sucrose, ΔT = 5°C, is displayed in Fig. 4. Methyl cellulose reduces V by a small but significant amount, somewhat in excess of the reduction caused by the same weight of

extra sucrose (shown by the solid line in Fig. 4). The retarding effect appears to be greatest for the highest molecular weight (Celacol M2500), although variation with molecular weight is scarcely significant (the error bar in Fig. 4 represents twice the typical standard deviation).

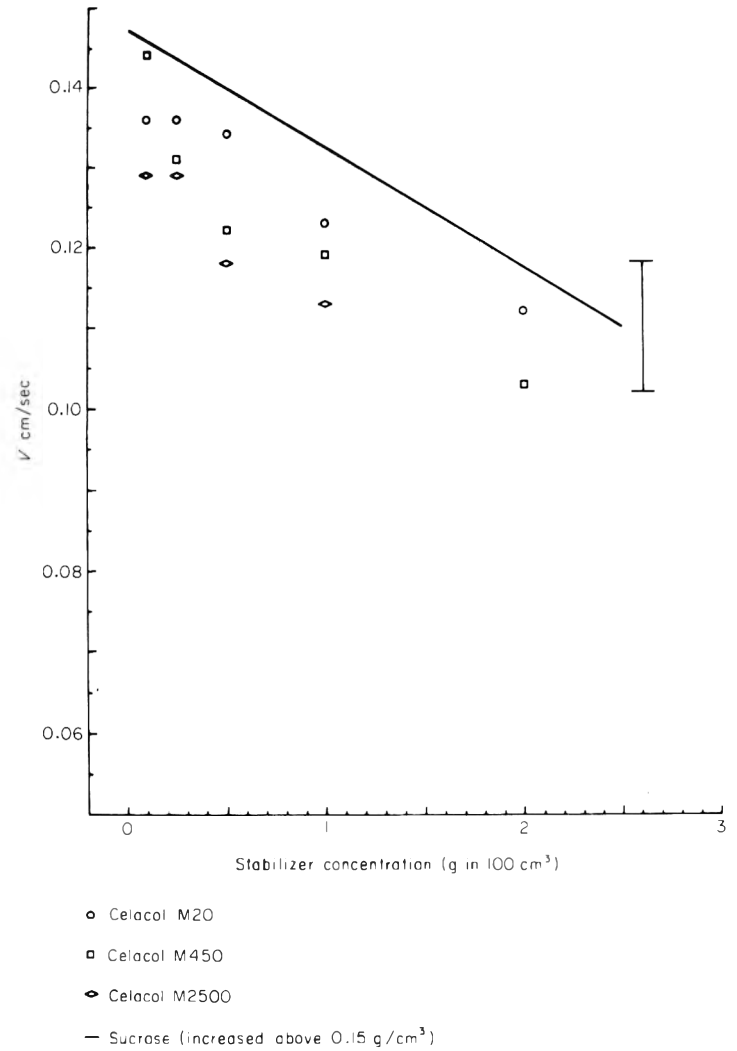

Figure 4. The effect of methyl cellulose on the linear ice crystallization velocity (V) in sucrose solution (0.15 g/cm^3) using standard glass tubes.

The effect of different concentrations of sodium alginate on V for 0.15 g/cm^{-3} sucrose, ΔT = 5°C, is displayed in Fig. 5. For alginate concentrations up to 1%, V is reduced by a similar amount as with the methyl celluloses, although there is no significant difference for the different molecular weight alginates. For 2% alginate solutions, a far more substantial reduction in V appears to occur. However, the scatter in the results also becomes much larger (the longer error bar again represents two standard deviations for V in solutions with 2 g alginate per 100 cm^3). The greater scatter was associated with a changing morphology of the ice growth (from 'spear' to

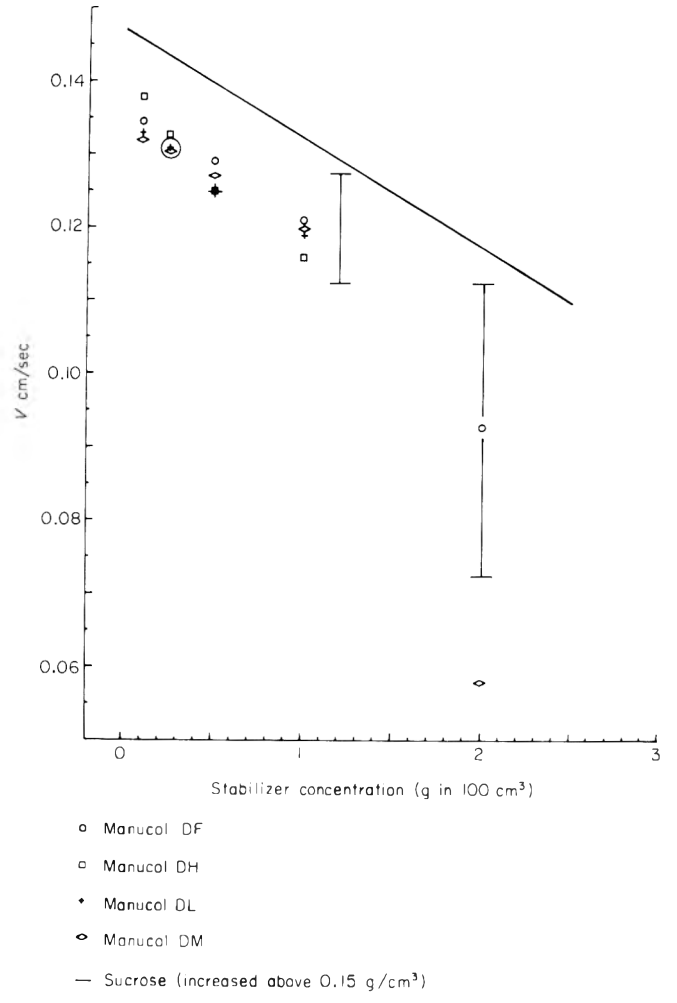

Figure 5. The effect of alginates on the linear ice crystallization velocity (V) in sucrose solution (0.15 g/cm^3) using standard glass tubes.

'rotational'). The substantial apparent reduction in V observed, therefore, is chiefly the result of some poorly understood morphological effect, and no true comparison of crystallization kinetics is possible. A similar instability in the morphology of ice growth was sometimes observed for the 2% methyl cellulose solution, but it was possible to select data applying almost only to 'spear' growth, with the consequence that the standard deviation and reduction in V were in line with the results for lower stabilizer concentrations.

(ii) *The effect of the quantity of water in the sucrose–alginate–water system (tube experiment)*

The results are shown in Fig. 6. This plot has the same shape as the results for $\Delta T = 10$°C of Lusena (1955). Three values of V, interpolated from the data of Tamman & Büchner (1935) are also given in Fig. 6; the agreement with the present results is reasonably good. It is apparent from Fig. 6 that the Manucol DM exerts only a small effect on V at lower sucrose concentrations (solids 0.6 g/cm^3), but there is a somewhat

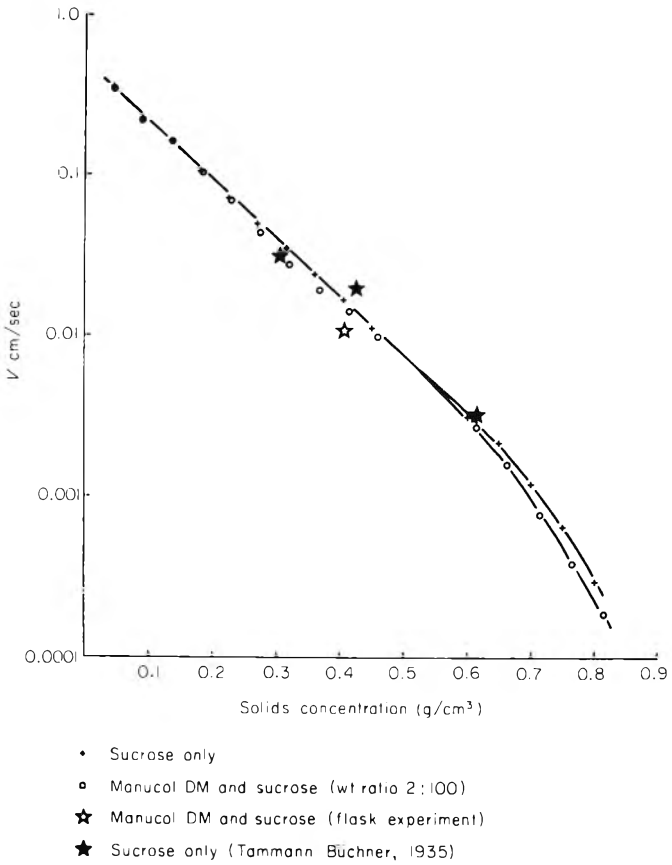

Figure 6. The dependence of the linear ice crystallization velocity (V) on sucrose concentration, with and without Manucol DM (ΔT = 5°C, standard Perspex tubes).

greater effect at higher concentrations. These results may be contrasted with the large effect of Manucol DM on the viscosity shown in Fig. 7.

(iii) *The effect of additives on ice growth in 0.75 g/cm³ sucrose solution (tube experiment)*

The results are given in Table 2. The standard deviations have been entered only for systems for which many replicate experiments were performed. From Table 3 it is again apparent that stabilizers (at a concentration that is typical of their commercial use) cause a modest reduction in V, although the retardation of growth is somewhat greater than that caused by the same mass of micromolecular solutes (glucose, citric acid).

(iv) *The effect of firmness of alginate cells on ice crystal growth and morphology (flask experiment)*

The results are given in Tables 3 and 4, Figs 8–15. The term 'MR Snowflake' refers to the characteristic double snowflake form described by Macklin & Ryan (1966); an example is given in Fig. 13. MR snowflakes are thus a special case of straight dendrites. The other morphological descriptions are also represented in the plates. The value of V = 0.0104 cm/sec for a solution of 0.75 g Manucol DM in 100 cm³ of 0.404 g/cm³ sucrose solution at T_b = −8.5°C (from Fig. 8) can be compared with the value of

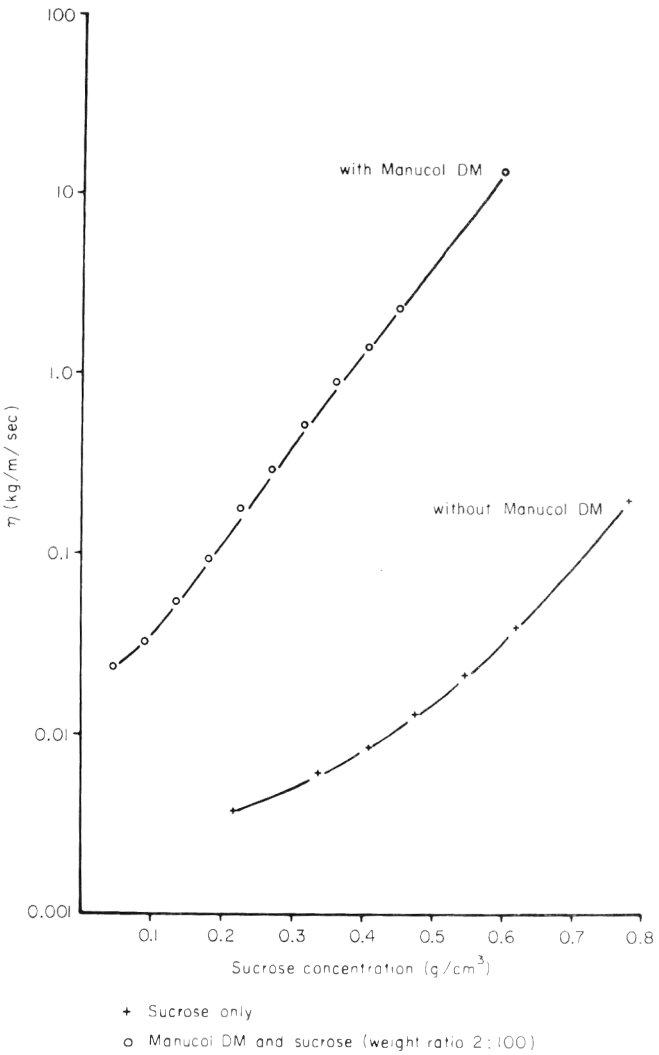

Figure 7. The viscosity (η) of sucrose solutions, with and without Manucol DM, as a function of concentration at 4°C.

V = 0.0141 cm/sec for a solution of 0.0081 g/cm^3 Manucol DM, 0.405 g/cm^3 sucrose at T_b = −8.5°C obtained using the tube technique (see Fig. 7). Bearing in mind the presence of phosphates and glucono-δ-lactone in the former system, this comparison can be regarded as indicating a fair agreement between results obtained by the different techniques.

It is apparent from Fig. 8 and Table 3 that a significant effect on ice crystal morphology and mean linear growth rate (V) first occurs for 0.01 g $CaHPO_4$ per 100 cm^3 (equivalent to about 20 ppm Ca^{2+}). This is a very low concentration of calcium (for example, ice lollies are usually made with about 800 ppm Ca^{2+}). According to Dow (1974), enhancement of the viscosity of 1% Manucol DM solutions due to Ca^{2+} content could be detected by conventional cone-and-plate rheometry (shear rate 5190/min) only for concentrations greater than 300 ppm. It is thus manifest that ice growth is very sensitive to gel structure (at least for alginate gels).

Table 2. The effect of additives on V (for 58.6% w/w sucrose solution, $T_b = -16.2°C$, using standard perspex tubes)

Additive	Quantity added to 100 cm³ (g)	$V \times 10^4$ (cm/sec)	Standard deviation
—	—	6.5	0.3
Courlose F75	1.5	5.1	—
Manucol DM	0.1	6.0	—
Manucol DM	0.2	5.9	—
Manucol DM	1.5	3.9	0.2
Xanthan	1.5	4.4	—
Sodium pectate	1.5	5.7	—
Glucose	1.5	5.6	—
Citric acid	1.5	5.3	—

Table 3. The effect of calcium content on ice growth in 0.75% of Manucol DM gels (35% w/w sucrose, $T_b = -6.5°C$, aged 24 hr)

System	$CaHPO_4$ (g/100 cm³)	$V \times 10^3$ (cm/sec)	Morphology
No alginate	0	6.0	Straight dendrites, tended to float up
Alginate solution	0	4.1	Straight dendrites
Alginate solution	0.005	4.0	Straight dendrites
Extremely weak gel (can be poured)	0.01	1.8	Curved dendrites
Very weak gel	0.015	1.1	Cloud with discernible curved dendrites
Weak gel	0.02	1.2	Cloud with discernible curved dendrites
Weak gel	0.025	1.1	Cloud with discernible curved dendrites
Gel (can't be poured)	0.05	1.0	Cloud with large lobes
Firm gel	0.10	0.4	Cloud with large lobes

For very firm gels it was found that a liquid film covered the gel surface, and allowed an easy path for ice growth up the outer surface of the nozzle tube and down the flask walls (Fig. 12). This problem prevented the study of ice growth in gels of a $CaHPO_4$ content in excess of 0.2 g per 100 cm³.

Adding the freezing point depressions, which would occur if each solute was dissolved separately in the total amount of water, gives a reasonable estimate for the total freezing-point depression.

The 0.5 g of Na_2HPO_4 and 1.0 g glucono-δ-lactone dissolved in every 100 cm³ of 0.404 g/cm³ sucrose solution is thus anticipated to depress the freezing point by ~ 0.6°C from −3.5 to −4.1°C. However, when a fine thermocouple junction (with a reference junction immersed in an ice/water slush) was inserted into the dendritic ice in a flask containing an alginate solution ($T_b = -7.5°C$), the potential difference registered corresponded to a temperature of −4.3°C. For two alginate gels (0.01 g $CaHPO_4$ per 100 cm³ and 0.025 g $CaHPO_4$ per 100 cm³, bath temperature −7.5°C) the apparent temperature was −4.5°C. The fact that these temperatures lie so far below

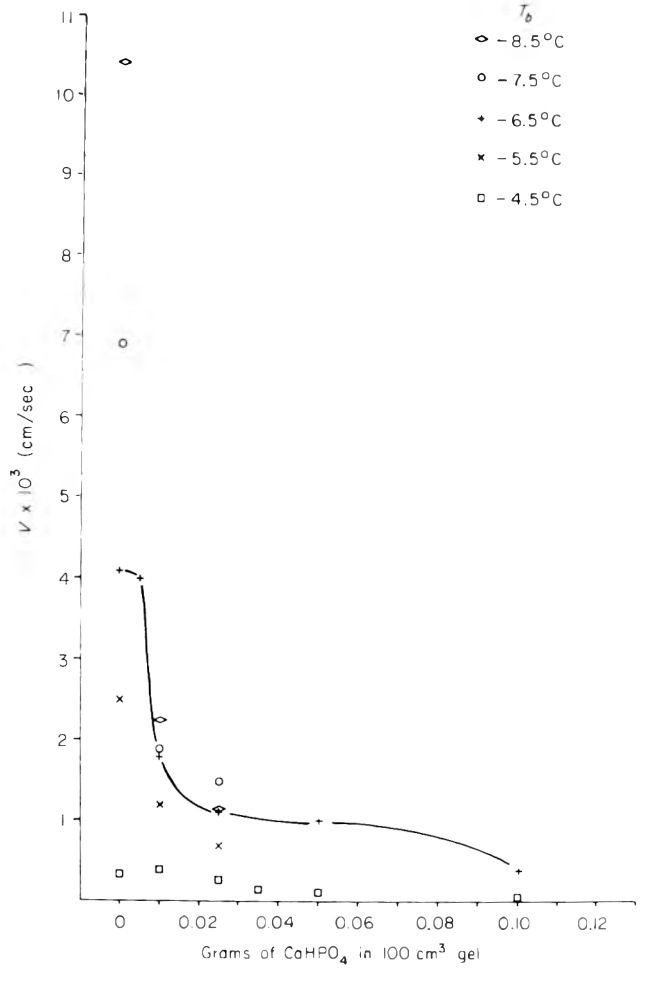

Figure 8. The effect of calcium content on ice-growth rate in 0.75% Manucol DM gels (0.404 g/cm^3 sucrose, age 24 hr) with bath temperatures as a parameter.

−4.1°C is surprising, but its significance is not clear. For systems which cannot be stirred, in which the ice growth rate is extremely slow, it is difficult to measure a well-defined freezing point (a similar difficulty was faced in concentrated sucrose solutions by Young & Jones, 1949).

Table 4. The effect of calcium content on ice growth in 0.75% Manucol DM gels (35% w/w sucrose, T_b = −4.7°C, aged 24 hr)

System	$CaHPO_4$ (g/100 cm^3)	$V \times 10^3$ (cm/sec)	Morphology
Alginate solution	0	0.58	MR snowflake
Firm gel	0.1	0.12	Several fan-shaped sheets of ice
Very firm gel	0.2	0.02	Distinctly non-planar fan-shaped sheets of ice

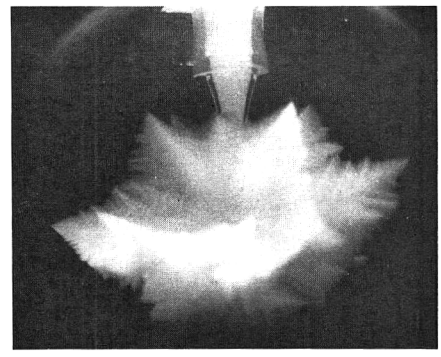

Figure 9. Floating dentrites; 35% w/w sucrose+0.5 g Na_2HPO_4+1.0 g glucono-δ-lactone per 100 cm^3, T_b = −6.5°C, 100 cm^3 flask, 6 min after emerging.

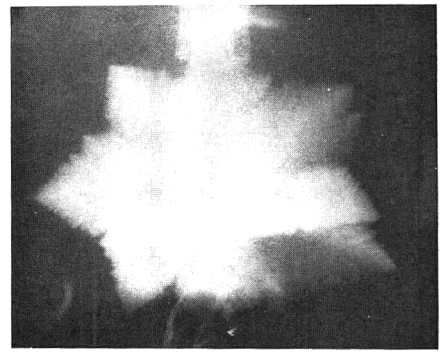

Figure 10. Straight dentrites; 35% w/w sucrose+0.75 g Manucol DM+0.005 g $CaHPO_4$ per 100 cm^3, T_b = −6.5°C, 100 cm^3 flask, 10 min after emerging.

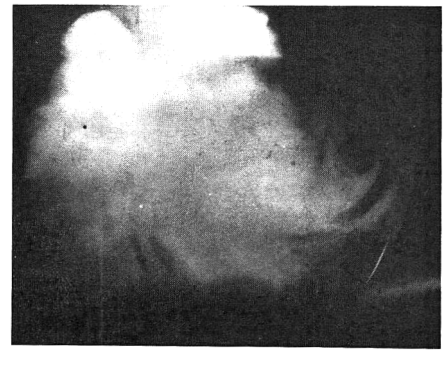

Figure 11. Curved dentrites; 35% w/w sucrose+0.75 g Manucol DM+0.01 g $CaHPO_4$ per 100 cm^3, T_b = −6.5°C, 100 cm^3 flask, 25 min after emerging.

Figure 12. Cloud; 35% w/w sucrose+0.75 g Manucol DM+0.1 g $CaHPO_4$ per 100 cm^3, T_b = −6.5°C, 100 cm^3 flask, 50 min after emerging.

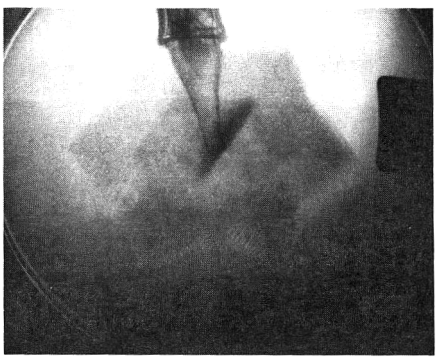

Figure 13. Double snowflake (so-called MR snowflake (Macklin & Ryan, 1966)). 35 % w/w sucrose +0.75 g Manucol DM+0.0 g $CaHPO_4$ per 100 cm^3, T_b = −4.7°C, 100 cm^3 flask, 49 min after emerging.

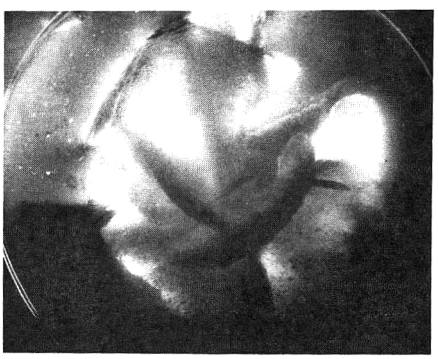

Figure 14. Fan-shaped sheets of ice; 35% w/w sucrose +0.75 g Manucol DM +0.2 g $CaHPO_4$ per 100 cm^3, T_b = −4.7°C, 100 cm^3 flask, 30 hr after emerging.

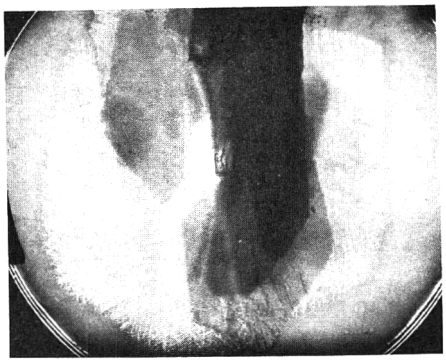

Figure 15. Straight dentrites; 35% w/w sucrose + 0.75 g Manucol DM + 0.085 g $CaHPO_4$ per 100 cm^3, $T_b = -4.5°C$, 100 cm^3 flask, 5 hr after emerging.

In view of the curved dendrites observed at greater undercooling, the straight dendrites observed for a weak gel close to the equilibrium temperature (Fig. 15) are most surprising. Moreover, it is also apparent that such dendrites grow at about the same rate as those in the alginate solution (Fig. 8), in contrast to the substantial retardation observed at greater undercooling. However, altered morphology and a substantial reduction in ice growth rate were observed for firmer gels at −4.7°C (Table 4, Fig. 14).

For gels with 0.2 g $CaHPO_4$ per 100 cm^3, two simple experiments showed that the gel structure had been irreversibly ruptured by the growth of the ice sheets. Firstly, refreezing (again at $T_b = -4.7°C$) a thawed sample after 24 hr resulted in a very rapid growth of ice (7×10^{-4} cm/sec, compared with 0.2×10^{-4} cm/sec originally, from Table 5) with almost exactly the same position and shape as the original growth. Secondly, for another thawed sample, the nozzle was removed and replaced by a Pasteur pipette. On squeezing the teat, air was injected into (previously scarcely visible) pockets in the gel, which then strikingly resembled the original ice in appearance, even showing the fine structural features (lines etc.) on the surface of the curved sheets.

(v) *The effect of gel age (flask experiment)*

The results are given in Table 5. It can be deduced that at 4 hr the gelation process

Table 5. The effect of age on ice growth in 0.75% Manucol DM gels (35% w/w sucrose, $T_b = -6.5°C$, 0.01 g $CaHPO_4$ per 100 cm^3)

Age (hr)	$V \times 10^3$ cm/sec	Morphology
4	3.4	Straight dendrites
24	1.8	Curved dendrites
96	1.1	Curved dendrites
120	1.1	Curved dendrites

was far from complete, and that most of the gel structure developed in the first 24 hr, although subsequently some further development occurred.

(vi) *The effect of different stabilizers (flask experiment)*

The results are given in Tables 6 and 7. It was difficult to discern the fine structures of ice in locust bean gum, guar gum and xanthan gum solutions due to their turbidity. It is apparent that all stabilizers retard ice growth to some extent, but that at $T_b = -6.5°C$ only xanthan gum has a substantial effect on ice crystal morphology (Table 6). In this, xanthan gum in solution resembles a weak alginate gel, which fits in with the rheological evidence of weak chain-aggregation in xanthan solutions (Pettitt, 1979). The effect of the various stabilizers appears to depend on the undercooling, since at $T_b = -5.0°C$ (Table 7) guar gum had the most substantial influence on ice growth. This is also borne out when Tables 6 and 7 are compared with Table 2 (where the undercooling is greater, as well as the concentration of stabilizer and sucrose, and the tube technique was used). However, since most of the values of V were obtained from only one or two determinations, the degree of confidence attached to this evidence of a complicated variation of the influence of stabilizers on ice-crystal growth with ΔT and/or sucrose concentration is not very great.

For solutions without stabilizers (and thus of low viscosity) a vertical column of liquid of a different refractive index was generally observed directly below the nozzle. On some occasions small particles of ice could be observed to sink slowly in this column.

Table 6. The effect of a variety of stabilizers on ice growth (35% w/w sucrose, $T_b = -6.5°C$, 0.75 g stabilizer per 100 cm^3)

Stabilizer	$V \times 10^4$ cm/sec	Morphology
None	6.0	Imperfect MR snowflake, tended to float up
Courlose F75	4.3	Imperfect MR snowflake
Manucol DM	4.1	Imperfect MR snowflake
Guar gum	2.7	Straight dendrites in all directions
Locust bean gum	3.0	Straight dendrites in all directions
Xanthan gum (only 0.375 g per 100 cm^3)	1.5	A tight mass of curved dendrites

Table 7. The effect of a variety of stabilizers on ice growth (35% w/w sucrose, $T_b = -5.0°C$, 0.75 g stabilizer per 100 cm^3)

Stabilizer	$V \times 10^4$ cm/sec	Morphology
None	12.0	Straight dendrites, visible convection currents
Celacol M2500	9.4	Straight dendrites, in one experiment forming a MR snowflake
Courlose F75	9.4	Straight dendrites, in one experiment forming a MR snowflake
Manucol DM	9.0	Straight dendrites, in one experiment forming a MR snowflake
Guar gum	4.8	Many straight dendrites in all directions
Locust bean gum	7.5	Straight dendrites, tending to lie in one plane
Xanthan gum (only 0.375 g per 100 cm^3)	7.1	Many dendrites in all directions

and rise again near the wall of the flask. Convection and movement of the ice, due to buoyancy, made the measurements of V for solutions without stabilizers rather unreliable controls.

Table 8. The morphology and kinetics of ice-crystal growth in aged agar, gelatin and silica gels (35% w/w sucrose, $T_h = -6.5°C$)

System	Age (hr)	$V \times 10^3$ (cm/sec)	Morphology
0.5 g agar in 100 cm³ (firm gel)	24	3.1	Cloud with very small, well-defined lobes
1.0 g agar in 100 cm³ (firm gel)	3	2.5	Cloud with very small, well-defined lobes
1.0 g agar in 100 cm³ (firm gel)	24	2.3	Cloud with very small, well-defined lobes
1.0 g agar and 1 g Courlose F75 in 100 cm³ (firm gel)	24	2.0	Cloud with somewhat larger lobes
1 g gelatin in 100 cm³ (very weak gel)	3	1.1	Angular cloud
1 g gelatin in 100 cm³ (very weak gel)	24	0.7	Angular cloud
2 g gelatin in 100 cm³ (weak gel)	24	0.1	Cloud with spiky edges
Volume ratio of silicate : acid : 35% w/w sucrose			
20 : 5.5 : 25.5 (Not gelled)	3	3.4	Imperfect MR snowflake
20 : 5.5 : 25.5 (Not gelled)	72	3.4	Imperfect MR snowflake
20 : 5.5 : 0 (Gel)	24	0.5	Stacks of sheets, curved at edges
20 : 5.5 : 0 (Gel)	3	0.5	Stacks of sheets
20 : 5.5 : 10 (Weak gel)	3	0.8	Stacks of sheets
20 : 4.0 : 0 (Not gelled)	3	2.1	Straight dendrites

Results for other gels

These are given in Table 8. It should be noted that the silica gels contained significant concentrations of ionic solutes in addition to the 36% w/w sucrose, so that the results for them are not strictly comparable to other gels. It is apparent that, in spite of being the softest gel, gelatin caused the greatest retardation in ice crystal growth. For all three types however, there was a very significant interaction between gel structure and ice growth.

Summary of the results for ice crystal growth

To sum up, the existence of a gel network grossly affects ice growth even if the rigidity of the gel is not very great. The effect seems to be less pronounced when the undercooling is very small. Stabilizers that do not form a network retard ice-crystal growth (at the concentrations typical of their commercial use, the retardation of growth is by a factor of between one-third and two-thirds, and is thus rather greater than would be produced by the same weight of a micromolecular solute) but do not greatly influence the dendritic morphology. The magnitude of the effect exerted by stabilizers may depend on the undercooling in a manner specific to each type of stabilizer, but more experiments would be required to clarify this issue.

Discussion

The observation that ice growth is severely retarded in gels provides strong evidence that if stabilizers (at least gelatin, agar and alginate) do not diffuse sufficiently rapidly away from the ice interface, then they exert an influence on the ice. Furthermore, the fact that all the stabilizers (in solution) affect ice growth in a similar way (and to only a small extent) points away from specific effects on interface kinetics (which might arise from adsorption caused by the presence of appropriate functional groups—Ohara & Reid, 1973; Raymond & deVries, 1977) towards an effect on transport kinetics (which would depend primarily on molecular size).

We therefore need to consider what mechanisms may account for the retardation and/or modification of ice crystal growth in gels on solutions of stabilizers.

(i) *Ice growth in gels*

Since the existence of a gel network (of low concentration) does not greatly affect the diffusion of micromolecules such as sucrose (Muhr & Blanshard, 1982), we need only consider the direct interaction between the network and the ice. Freezing of the water in a network can only occur if the water migrates out of the gel to the ice front ('cryogenic suction') or if the ice penetrates the gel pores, perhaps by rupturing them. However, the growth of ice by cryogenic suction requires a curvature of the ice interface. The curvature must be such that the corresponding Laplace pressure difference (Aguirre-Puente & Azouni, 1973) is sufficient to supply the water against the hydraulic resistance of the network and the resistance of the network to shrinkage. It is difficult to quantify the latter effect. Moran (1926) found that during the freezing of gelatin gels (12–40%) in air, ice did not penetrate the gel but instead drew water out until the gel phase reached a constant composition (54% gelatin). However, equilibrium took several days to attain.

The more dilute gels studied here suffered rupture during freezing, and it is apparent that the ice penetrated the gel. For this case it is also difficult to quantify the effect of the gel on the kinetics of ice growth. However, a crude model (based on classical capillary theory), which may identify the relevant parameters, will now be considered.

Treating the gel as a mechanical network of fibres (of radius R) and the ice as a 'fluid' with the surface free energy σ (against the solution entrained in the gel) we may visualize the ice/gel system as in Fig. 16. The model is the same as that used by Everett for porous media (Everett & Haynes, 1965).

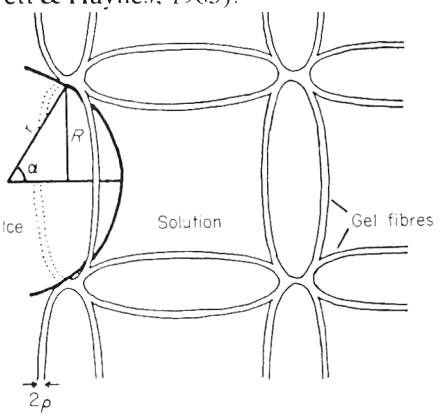

Figure 16. Impingement of an ice interface on an idealized gel network.

Consider an ice front of radius of curvature r which is attempting to penetrate a mesh in the gel of radius R. Assuming, for simplicity, that the contact angle of ice on the fibre is 180°, it can be readily shown that each element of length dl of the fibre ring is subject to a force 2σ dl normal to the surface of the ice (this may be calculated either from the surface tension or by considering the difference in pressure across the ice/fibre interface and across the fibre/water interface).

The fibre ring is thus subjected to a force ($\mathcal{F}$), of magnitude $2\pi R.2\sigma.\cos\alpha$ along the axis of propagation, whence

$$\mathcal{F} = 4\pi\sigma(r^2-R^2)^{1/2}R/r. \tag{4}$$

The radial components of force will result in a tension ($\mathcal{T}$) in the fibre, which may be calculated from balancing the forces exerted on halves of the ring:

$$\mathcal{T} = 2\sigma R^2/r. \tag{5}$$

Equation 5 is essentially the result of Kuhn (1956). In equations 4 and 5 the effect of the enhanced pressure ($2\sigma/r$) in the ice phase has been neglected. However, this pressure will only provide a normal force on each element dl of magnitude $(2\sigma/r).\rho$ dl which may be neglected in comparison to the above 'flotation' term (since for a reasonable model $\rho \ll R \leq r$).

When a plane ice front advances towards a network such as depicted in Fig. 16 it will thus initially repel each ring, until compression of the network is sufficient to produce a counter force of $4\pi\sigma R$ per ring. Further encroachment of the ice front (imposed by continued extraction of latent heat) is then only possible if the interface curves and begins to penetrate the rings. According to equation 5, the tension $\mathcal{T}$ rises. If exceeds the tensile limit ($\mathcal{T}^*$) of the fibres, they will be broken and the ice can proceed to the next obstacle in the network. The curvature at which this happens is, from equation 5, $r^* = 2\sigma R^2/\mathcal{T}^*$ whence the freezing-point depression, according to equation 1, is

$$\Delta T = \mathcal{T}^*/R^2\Delta S_V. \tag{6a}$$

If $\mathcal{T}^* > 2\sigma R$ the ice front will penetrate the ring without rupture and the freezing-point depression will be

$$T = 2/RS_V \tag{6b}$$

Equation 6b is essentially the result of Kanig (1960).

There are major problems in applying the Everett model to gels. The value of σ is not known precisely, but probably lies between 15 and 35 mJ/m² Hobbs, 1974, p. 441). Much less is known about the contact angle of ice against the gel fibre, but it is likely to be less than 180°. The order of magnitude of R (the mesh size of the gel) can be obtained from diffusion or permeability experiments; some results from the literature are given in Table 9. A method of estimating $\mathcal{T}^*$ for covalent bonds has been given by de Boer (1936) who obtained a value of 5.6×10^{-9}N for the C—C bond.

Assuming (as above) that $\theta = 180°$, and putting $\sigma = 25$ mJ/m² and $R = 100$ nm the maximum tension in the polymer ring will be $2\sigma R = 5\times10^{-9}$N, which is comparable to $\mathcal{T}^*$ for non-associated carbon-chain polymers. Thus for networks with $R \geq 100$ nm we would anticipate major damage to the gel on freezing and only a small freezing-point depression.

The rupture of gel structures by crystal growth and freezing-point depressions caused by the network has been observed for a variety of systems (e.g. Kanig & Karge, 1966; Henisch, 1970).

Callow (1925) investigated ice growth in gelatin gels, and noted an enormous decrease in the linear crystal growth rate (at −3.0°C) when the gelatin concentration was increased above 1%. He found that the gel structure was irreversibly destroyed by the separation of ice. He also mentioned that ice-crystal growth was not so drastically retarded in gels of agar (or starch) as in gelatin gels. His results are thus broadly in line with those presented here.

It appears (according to equation 6a) that $\mathcal{T}^*/R^2$ is larger for gelatin gels than for agar or alginate gels of comparable rigidity. Unfortunately, estimates of R from the literature are widely scattered, and it is not immediately evident from Table 9 that R is smaller for gelatin gels than for agar gels (of comparable rigidity). Similar uncertainty surrounds estimation of $\mathcal{T}^*$ (since the molecules may associate). Thus, further experiments are required before a semi-quantitative check of the model can be made. The obvious differences in ice morphology for the different gels (see Table 8) cannot yet be interpreted, but the technique may eventually provide a useful probe for gel structure.

The lower influence on ice growth that alginate networks appear to exert when the undercooling is very low (Figs 8 and 15) may perhaps be explained by a yielding of the network if enough time (for ice growth) is allowed.

(ii) *Ice growth in stabilizer solutions*

Theory. As explained above, only the influence of stabilizers on transport kinetics need be considered. If convection were the dominant mechanism of transport we would expect that stabilizers would drastically retard ice-crystal growth (because they greatly enhance viscosity). This was not found for the experimental conditions used in this work, but has been reported for other conditions (Shipe, Roberts & Blanton, 1963).

Superficially, it might seem that macromolecules (which diffuse slowly) would also retard ice-crystal growth if diffusion (of water) were the dominant rate-determining transport mechanism. To show that this is fallacious, a criterion for the effectiveness of a solute at retarding the growth rate of an ice crystal (for a given undercooling) will now be developed. The treatment also provides a criterion for judging the relative significance of heat flow and solute diffusion as rate-limiting factors.

Table 9. Structural parameters for aqueous gels inferred from permeability data

Gel and reference	Gel volume fraction ϕ	Permeability τ ($cm^2 \times 10^{-14}$)	Fibre radius (Signer & Egli's model A)† ρ (nm)	Pore radius (White's model)‡	RMS spherical space radius (combination of the models of Ogston§, and Signer & Egli†) (nm)
Agar*	0.012	440	3.3	59	26
	0.050	20	1.8	13	6.5
Gelatin*	0.056	200	6.2	40	20
Gelatin†	0.009	61	1.0	22	10
	0.071	1.4	0.65	3.5	1.7
Silica gel†	0.012	920	4.9	86	40
	0.049	17	1.7	12	6.0
Polyacrylamide‡	0.045	0.38	0.22	1.8	0.85
	0.091	0.21	0.30	1.4	0.70
	0.326	0.02	—	0.5	—

*Pallman & Deuel (1945), †Signer & Egli (1950), ‡White (1960), §Ogston (1958).

Consider an ice-crystal growing into an unstirred solution of concentration c_b and temperature T_b places remote from the interface (Fig. 1). The undercooling is $\Delta T = T_L(c_b) - T_b$. We assume that the rate of growth is controlled by diffusion (of either heat or solute) from the interface (since there is no heat sink in the ice phase, no heat can be transported through it). The conditions for conservation of solute at the interface is

$$c_i V_n = -D \left(\frac{\partial c}{\partial n}\right)_i, \tag{7}$$

where n indicates the direction normal to the interface and i refers to the interface. Similarly, the condition for conservation of heat at the interface is

$$LV_n = -k \left(\frac{\partial T}{\partial n_i}\right), \tag{8}$$

where L is the latent heat of fusion per unit volume of ice and k is the thermal conductivity. Equations 7 and 8 are necessarily true for non-steady state conditions and for a moving interface.

Equation 7 can be rewritten as

$$V_n = -\frac{1}{c_i} D(c_i - c_b)\left(\frac{\partial \theta}{\partial n_i}\right), \tag{9}$$

where $\theta = (c - c_b)/(c_i - c_b)$ is a dimensionless concentration obeying Fick's Second Law with the boundary conditions

$\theta_i = 1, \theta_b = 0.$

Similarly, (8) can be rewritten as

$$V_n = -\frac{1}{L} k \cdot \Delta T_b \left(\frac{\partial \theta}{\partial n_i}\right), \tag{10}$$

where $\theta = (T - T_b)/\Delta T_b$ is a dimensionless undercooling also obeying Fick's second Law and $\theta_i = 1, \theta_b = 0$.

As a first approximation for a small compact crystal we may take θ at any instant to have the value which satisfies $\nabla^2\theta = 0$ throughout the solution. That this should be so follows if we consider a small increase in volume (δV) in time δt of a crystal enclosed by an arbitrarily close stationary surface S. Thus for heat conservation

$$\delta t \int_S \text{outward flux} = L\delta V - c_p T \delta V \tag{11}$$

where c_p is the specific heat of the solution. The second term on the righthand side of equation (11) is an overestimate for the 'consumption' of undercooling within S arising from motion of the interface. Provided that

$$L \gg c_p \Delta T \tag{12a}$$

this term can be neglected so that the interface may be considered to be stationary and $\partial\theta/\partial t \simeq 0$. For diffusion controlled crystal growth, the equivalent criterion is

$$c_i \gg (c_i - c_b) \tag{12b}$$

If the appropriate criterion is met, it follows that $(\partial\theta/\partial n)_i$ in equation (9) or (10) depends (to a first approximation) only on the geometry and is independent of D or k. It follows that just two independent factors contribute to a sluggish crystal growth rate; a low rate of diffusion (D or k) and a large amount of diffusant expelled per unit of volume of crystal formed (c_i or L). If we assume that the freezing curve is linear:

$$T_L(c) = T_L(0) - mc$$

then (see Fig. 1)

$$\Delta T_s = m(c_i - c_b)$$

so that equation (9) becomes

$$V_n = \frac{-D}{\Delta T_s + \Delta T_E} \Delta T_s \left(\frac{\partial\theta}{\partial n}\right)_i, \tag{13a}$$

but equation (12b) can similarly be rewritten as

$$\Delta T_E \gg \Delta T_s$$

whence

$$V_n \simeq \frac{-D}{\Delta T_E} \Delta T_s \left(\frac{\partial\theta}{\partial n}\right)_i. \tag{13b}$$

For diffusion controlled growth (i.e. $\Delta T_s = \Delta T$) equation (13b) may not be grossly in error even when equation (12b) is not valid, since V_n should then be greater than anticipated from equation (13a), and should tend to infinity as $c_b \to 0$ (i.e. $\Delta T_E \to 0$).

Assuming either diffusion or heat transport is the dominant rate effect ($\Delta T_s = \Delta T$ or $\Delta T_h = \Delta T$, respectively) comparison of equation (13b) with equation (10) shows that the growth rate is controlled by solution diffusion if

$$D/\Delta T_E \ll k/L, \tag{14}$$

and conversely. Strictly, the domain of validity of equation (14) is restricted by criterion 12a or b, but we might expect it to be a conservative restriction.

For a fixed compact morphology the crystal growth rate is predicted to be proportional to $\Delta T.D/\Delta T_E$ or $\Delta T.k/L$ as is appropriate. The constant of proportionality has dimensions of 1/length, and from the solution of $\nabla^2\theta = 0$, $\theta = 1$ on the surface of a sphere we may guess that

$$V \propto \frac{D}{R\Delta T_E} \Delta T \tag{15a}$$

or

$$V \propto \frac{k}{RL} \Delta T, \tag{15b}$$

as is appropriate, where R is the radius of curvature of the interface.

Application of the theory to the experimental results. We may now apply the criteria to aqueous sucrose solutions. First of all, $L/c_p \sim 80°C$ for water, so that equation (12a) is satisfied for all moderate undercoolings. Equation (12b) will be satisfied for concentrated solutions and moderate undercoolings ($\Delta T_E \gg \Delta T$).

Criterion (14) may be applied using the estimated data given in Table 10. It appears that diffusion of sucrose away from the interface will control the rate of ice-crystal growth for solutions having a concentration in excess of a few weight percent. The linear dependence of V on ΔT over the narrow range shown in Fig. 3 lends some support to equation (15). A more exacting test is given in Fig. 17 where a log–log plot of V against $D/\Delta T_E$ (from Table 10) is given, which strikingly confirms the dependence of V on $D/\Delta T_E$ over most of the concentration range. The growth rate falls below the straight line plot for concentrations less than 0.1 g/cm^3 perhaps because heat diffusion is becoming a significant retarding mechanism (although it must be admitted that equation (12b) is certainly invalid if we assume it is diffusion controlled since $\Delta T > \Delta T_E$ for these low concentrations).

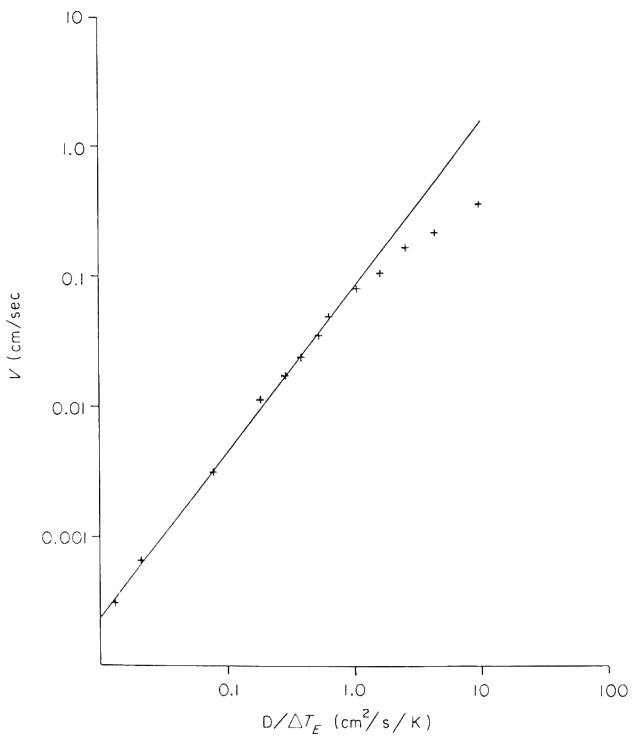

Figure 17. The dependence of the velocity of linear ice-crystal growth (V) on $D/\Delta T_E$ for sucrose solutions of varying concentration where $\Delta T_E = 6°C$.

However, the slope of the linear portion of Fig. 17 is not unity. Instead V (for $\Delta T = 6°C$) is given by

$$V \simeq 0.08\,(D/\Delta T_E)^{1.3} \tag{16}$$

where V is in cm/sec and D in cm^2/sec.

Table 10. Transport data for sucrose solutions at the freezing point

c_b (%w/w)	ΔT_E (°C)	$k \times 10^3$ (J/sec/cm/deg)	$D \times 10^6$ (cm²/sec)	L (J/cm³)	$\frac{k}{L} \times 10^6$ (cm²/sec/deg)	$D/\Delta T_E \times 10^6$ (cm²/sec/deg)
0	0.0	5.60	2.75	307	18.2	*
5	0.3	5.44	2.50	306	17.8	8.33
10	0.6	5.27	2.24	304	17.3	3.73
15	1.0	5.11	1.99	303	16.9	1.99
20	1.5	4.95	1.74	301	16.5	1.33
30	2.7	4.61	1.22	296	15.6	0.45
40	4.5	4.27	0.85	288	14.8	0.19
50	7.3	3.92	0.51	277	14.2	0.07
60	12.3	3.55	0.20	256	13.9	0.016
70	19.3	3.13	0.04	227	13.8	0.002

ΔT_E—From Pancoast and Junk (1980).

k—Interpolated from the International Critical Tables.

D—Extrapolated from the data of Henrion (1964) for D at 25°C by using the Arrhenius Law with activation energies from Gladden & Dole (1953).

L—From Hobbs (1974, p. 361). The contribution to L from the heat of dilution has been neglected.

The disagreement with the form of equation (13) remains when ΔT_E is considerably larger than ΔT so it would not seem to be a consequence of failure to satisfy equation (12b). Instead the disagreement may arise because the assumption that the morphology of the ice crystal is independent of temperature and concentration is not justified. Indeed, such an effect plays a crucial role in recent theories of dendritic growth (Langer, 1980), which should eventually lead to a fuller understanding. However, it appears that the magnitude of $D/\Delta T_E$ will be a useful guide to the effectiveness of a solute in retarding ice-crystal growth (for a given value of ΔT, the lower is $D/\Delta T_E$ the lower will be V).

We may now consider whether or not stabilizers could significantly retard ice-crystal growth. Unfortunately, not very much data is available as to typical values of D and ΔT_E. However, Laurent *et al.* (1976) gave a value of $D = 5 \times 10^{-7}$ cm²/sec for a dilute dextran solution (molecular weight ~ 150,000). Using

$$\Delta T_E \simeq -104 \ln a_w,$$

where a_w is the activity of water in the solution, and using the value of 5.1×10^{-4} mol/cm³/g for the second viral coefficient for dextran solutions (Basedow & Ebert, 1979), we may estimate that $\Delta T_E \simeq 3 \times 10^{-3}$°C for a 0.05 g/cm³ dextran solution. Thus, for a 5% dextran solution $D/\Delta T_E \simeq 170 \times 10^{-6}$ cm²/sec/deg compared to 8.3×10^{-6} cm²/sec/deg for a 5% sucrose solution. It thus appears that the rate of diffusion of dextran is not sluggish enough to offset its very small effect on the freezing point, so that dextran should be less effective than sucrose at retarding ice-crystal growth. However, the effectiveness of dextran at retarding ice-crystal growth may well increase rapidly as the concentration is increased, because of the highly non-linear dependence of $\ln a_w$ on polymer concentration.

The superficial expectation that macromolecules should be very effective at retarding ice-crystal growth (because they diffuse sluggishly) is thus in conflict with both theory and experiment. However in the experiments, stabilizers did cause a somewhat

greater retarding effect than an equal weight of sucrose. This small but definite effect may arise because the systems are ternary rather than binary solutions.

If the presence of sucrose renders stabilizers more effective at bringing about a freezing-point decrement, or reduces the rate of diffusion of stabilizer molecules, then the theory may be adequate to explain the results. There is reason to believe that both these effects may occur. The diffusion of macromolecules will be retarded by the enhanced 'microviscosity' due to the presence of sucrose molecules, according to the Stokes–Einstein equation. Also, the freezing-point curve for polymeric solutes is generally highly non-linear, $\partial \Delta T_F / \partial c$ being almost zero at low c but becoming quite appreciable at higher values (Farrant, 1969). An examination of theoretical models for $\ln a_w$ indicates that this steepening effect of the freezing-point curve does not depend so much on the polymer concentration as on the total solute concentration, so that the addition of a small concentration of polymer may reduce the freezing point of a concentrated sucrose solution by much more than it would reduce the freezing point of water (Muhr, 1983).

The elimination of convection currents (on addition of stabilizers) may make a contribution to the retardation of ice-growth velocity. Such convection currents were observed in experiments on ice growth in undercooled sucrose solutions contained in flasks.

Glicksman & Huang (1982) showed that natural convection can become the dominant mechanism for the growth of dendrites from the pure melt if the undercooling is less than a critical amount. Comparison of Figs 6 and 7 shows that convection cannot be the dominant transport mechanism for our system, but it could still make a significant contribution in the absence of stabilizers.

Conclusions

Stabilizers in solution (at concentrations typical of their commercial use in frozen confectionery) reduce the growth rate of ice in sucrose solutions by a factor of one-third to two-thirds. They do not greatly affect the morphology of the ice. This effect is larger than anticipated from considerations of their mobility and effect on the freezing point for a binary solution, but may be a consequence of the presence of sucrose as an additional solute. It is not possible to judge whether this reduction in ice-growth rate is sufficient to explain the belief that stabilizers interfere with ice-crystal growth in frozen confectionery, since quantitative evidence for the belief is lacking.

A gross effect on both ice morphology and growth rate is observed for stabilizers which form a gel network. This effect can qualitatively be explained in terms of mechanical interference of the growth of ice. The gel fibres are expected to cause the ice-interface to develop a radius of curvature comparable in size to the mesh size of the network and hence to depress the freezing point. The gel fibres will be subject to a tension, which may result in rupture, especially for coarse networks. Again, the significance of this result for frozen confectionery is uncertain, especially as the effect seems to be smaller at very small undercoolings (such as would prevail in ice cream). However, the phenomenon should provide further insights into gels, as the ice crystal growth rate and morphology is highly sensitive to network structure.

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Infant food preparations from cowpea, coconut and soybean

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Summary

Composition and sensory properties of 'milk' extracted from cowpea (*Vigna unguiculata*), coconut (*Cocus nucifera*) and soybean (*Glycine max*) were compared with Frisolac (a commercial infant formula), cow's milk and human milk. Cowpea milk and soybean milk were higher in nutrients than cow or human milk and Frisolac except for calcium and energy. The flavour of the products were rated acceptable. There were no differences in the flavours but the odours and colours of the milks were significantly different ($P < 0.05$).

Introduction

Although soybean has become a very important food crop because of its high quality protein, oil, vitamins and minerals (Nicholas, Sinclair & Jellife, 1961; Oyenuga, 1978), cowpea (*Vigna unguiculata*) is the most widely consumed food legume in the diet of Nigerians. The seeds are valuable as a source of protein, and especially as a lysine supplement to the lysine deficient cereals in the diet of Nigerians (Oyenuga, 1978; Onayemi & Potter, 1976). Coconut (*Cocus nucifera*) is also a valuable source of nutrients (Oyenuga, 1978) and has a good quality protein (Loo, 1968).

Attempts have been made to process these food items into forms in which their nutrients were more readily available and optimally utilized. Among these is the extraction of the juice in coconut and solubilization of the seeds to prepare milk substitutes. Hot water extraction (Wilkens, Mattick & Hand, 1967), acid grinding (Kon *et al.*, 1970) and alkali soaking (Badenhop & Hackler (1970) have all been used for soymilk extraction. Nelson, Steinberg & Wei (1976), attempted to establish a standard method for the production of legume milks by soaking, blanching and adding sugar, flavour, sucrose, vanillin and starter distillate. Ebiringa (1983) attempted to produce cowpea-coconut-milk using the alkali soaking and wet grinding method of Nelson *et al.*, (1976) with cowpea, corn and coconut. Coconut milk was a by-product of the coconut oil industries (Roxas, 1963, Robledana-Luzuriage, 1956) but the temperatures employed led to a decrease in the nutritive value of coconut protein (Mitchell, Hamilton & Beadles, 1945). The protein extracts from coconut are almost fibre-free and high in quality (Gunetileke & Laurentius, 1974).

It was the purpose of this study to extract 'milk' from cowpea, coconut and soybean with the primary objective of establishing their potential value for human nutrition especially for infants, children and those adults who are unable to digest the raw fruit and seeds.

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Materials and methods

Materials

The soybean seeds (*Glycine max*) were obtained from the International Institute of Tropical Agriculture, Ibadan while the cowpea (*Vigna unguiculata*) and coconut (*Cocus nucifera*) were purchased from a local market in Ibadan.

Sample preparation for chemical analysis

The seeds of soybeans and cowpeas were winnowed to remove particles, defective seeds and stones before being pounded in a mortar. Samples were reduced to fine powders in a Moulinex blender (*Model 241*). The coconut was dehusked and broken into pieces before blending and the mash produced was dried (Gallenkamp moisture oven model OV-440, 60°C, 24 hr). The sample powders were stored in air-tight containers before analysis.

Extraction of milk

The methods used were modifications of the method of Nelson *et al.*, (1976). Soybean milk (SBM) and cowpea milk (CPM) were extracted by soaking in tap water (18 hr), blanching (80°C, 5 min), dehulling and blending to smooth pastes, diluting with water (SBM, 1:3; CPM, 1:2), sieving through muslin cloth, boiling the milk (filtrate) and finally cooling to room temperature. The coconut fruits were broken and the flesh was blended to a very smooth consistency. The mash was diluted 1:2 with water and subsequently treated as for CPM and SBM to obtain the coconut milk (CCM).

Chemical analysis

Sample preparation. The SBM and CPM were dried in a moisture oven (AOAC, 1980) and the residues ground to a fine powder. The CCM sample was prepared using the AOAC (1980) method for preparing samples from a colloidal suspension. The residue, after centrifugating and decanting, was dired to constant weight (moisture oven, 60°C) and then ground to a powder.

Moisture content. The raw milks were evaporated in a vacuum rotary evaporator (40°C) and dried to constant weight (AOAC, 1980). The moisture content was obtained by difference.

Proximate Analyses were: crude protein ($N \times 6.25$) and fat (AOAC, 1980); available carbohydrate (a combination of methods Southgate, 1976; Dubois *et al.*, 1956); minerals (atomic absorption Perkin Elmer model 305B), after dry-ashing and solution of the ash in distilled deionized water acidified with nitric acid), caloric values (ballistic bomb calorimeter, Gallenkamp model CB 370).

Sensory evaluation

The CPM and SBM were sweetened with sucrose (25 g per litre of milk) because of their bland taste. The multiple comparison test (Larmond, 1977) with eight assessors used soymilk (R) as reference compared with SBM (414), CCM (411) and CPM (404) for flavour preference and differences in odour or colour. The correct identification of the reference SBM (R) as being equal to SBM (414) by seven out of eight of the panel demonstrated their sensory ability.

Results

Table 1 shows the mean of duplicate determinations of the nutrient composition of the raw samples, the milks and the residues. Soybean had the highest solids (91.2 g%), ash (5.2 g%), protein (43.9%) and energy value (534 kcal/100 g), while cowpea and coconut had the highest available carbohydrate (57.1 g%) and lipid (36.0 g%), respectively.

Table 1. Percent proximate composition of soybean, cowpea and coconut: their milks and residues

	Soybeans			Cowpea			Coconut		
Component	Whole	Milk	Residue	Whole	Milk	Residue	Whole	Milk	Residue
Mositure (g)	8.8	91.7	72.8	10.4	92.0	63.6	40.2	92.5	60.0
Total solids (g)	91.2	8.3	27.2	89.6	8.0	36.4	59.8	7.5	40.0
Crude protein (g)	43.9	3.7	11.5	21.6	2.8	12.4	6.5	1.4	5.7
Crude fat (g)	20.5	1.9	5.0	2.7	0.4	1.2	36.0	4.1	19.5
Available carbohydrate (g)	15.0	2.1	5.8	57.1	4.3	17.5	12.2	1.7	7.8
Ash	5.2	0.6	1.8	3.8	0.5	1.7	1.8	0.3	1.6
Crude fibre (g)	6.6		3.1	4.4		3.6	3.3		5.4
Calcium (mg)	206.4	22.0	58.8	97.0	10.7	38.4	16.2	3.7	17.6
Iron (mg)	8.4	1.1	2.5	5.8	0.8	2.4	1.3	0.2	0.7
Energy (kcal)	534	59	136	426	44	179	510	67	281

Of the milks, SBM, CPM and CCM were significantly different from CM, HM and FM (chi-squared $P < 0.05$). SBM, CPM and CCM were also significantly different from each other ($P < 0.05$).

Table 2 represents the percentage of the RDA (Recommended Dietary Allowances, U.S. 1980) that can be met by 100 ml (of 7–8% solid matter) of milks. The

Table 2. Percent RDA met by 100 ml of HM, FM, SDM, CPM and CCM for infants and children

Nutrient	Energy (kcal)	Protein (g)	Calcium (mg)	Iron (mg)
RDA for				
< 1 year	820	14	550	5–10
1–3 years	1360	16	450	5–10
Infants (< 1 year)	%	%	%	%
HM*	8.2	9.3	6.0	0.8–1.6
FM†	8.2	10.0	8.2	4–8
SBM	7.2	26.4	4.0	11–12
CPM	5.4	20.0	1.9	8–16
CCM	8.2	10.0	0.7	2–4
Children (1–3 years)				
HM	4.9	8.1	7.3	0.8–1.6
FM	4.9	8.8	10.0	4–8
SBM	4.3	23.1	4.9	11–12
CPM	3.2	17.5	2.4	8–16
CCM	4.9	8.8	0.8	2–4

*McCance and Widdowson (1978).
†Frisolac Composition (1983).

SMB and CPM can supply higher percentage of the RDA of protein and iron for infants and children (1–3 year) than HM and FM. The CCM is as rich as HM and FM in energy and protein for both age groups, and can meet more of RDA for iron than HM (v/v), but less than FM.

Sensory evaluation

There were no significant differences ($P < 0.05$) in the flavours of both the CCM and CPM, but there was a significant difference in their colours and odours ($P < 0.05$). No beany flavours were observed in the SBM and CPM. They had natural bland tastes while CCM had a natural sweet taste. The sweetening of the SBM and CPM was largely responsible for the similarity in flavour of the samples. The SBM had a cream colour, the CCM had a whitish milky colour and the CPM had an off-white colour. The CPM was rated the least acceptable in odour and colour. However, all the samples were acceptable to the taste panel.

Efficiency of the extraction techniques

Evaluation of the efficiency of nutrient extraction has not been measured previously (Nelson *et al.*, 1976, Thio Gaon, 1978, and Ebiringa, 1983). Table 3 shows the percent recovery (PR) of the nutrients present in the original samples.

Table 3. Percent nutrient recovery in the 'milk' samples

	Nutrient recovery (%)		
Nutrient	SBM	CPM	CCM
Energy	53	42	37
Protein	42	69	85
Fat	44	60	32
Ash	67	55	68

Discussion

The nutrient composition of the raw samples was reflected in the milks, although the energy value of CCM was higher than that of the raw 'coconut flesh', probably because of the high fat content. The nutrient content of SBM was slightly higher than that of Nelson *et al.* (1976) but lower than that of Thio Goan (1978), probably due to differences in the extraction methods. No data was available with which to compare CCM. The presence of large amounts of nutrients in the residues suggests the need for further improvement in the extraction procedure. As a child might consume up to 1000 ml of milk per day, the milks could supply ten times the percentage of the RDA shown in Table 2, if consumed as much per day. The lack of crude fibre and high level of available carbohydrate in the milks could make them more tolerable sources of nutrients than the raw samples.

Since the level of crude protein is not satisfactory evidence of protein quality further studies are needed on the amino acid score, digestibility and bioavailability of the protein in the 'milks'. Data on the composition of the available carbohydrates is also necessary because of the claimed association between the raffinose family of the oligosaccharides and the incidence of diarrhoea and flatulence in infants (Kon *et al.*,

1973, Steggarda & Dimmick, 1966). However, this study has demonstrated that soybean, cowpea and coconut are potential sources of nutrients, especially for infants and children.

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Release of metals from soya products by the action of some gastric and intestinal enzymes

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Summary

Measurements have been made of the amount of calcium, magnesium, zinc, iron, copper, manganese and nickel released into solution from three types of soya product prior to, and following the use of, *in vitro* enzymatic degradation procedures. The amount of solubilized metal has been shown to depend upon the enzymes used, the metal under consideration, the pH and the type of soya being studied. Such experiments may help to improve understanding of the behaviour of metals released from soya by the action of enzymes in the gastro-intestinal tract.

Introduction

In order to maintain an adequate standard of health it is necessary to ingest and absorb sufficient quantities of nutrients to enable the body's metabolic processes to function efficiently. Ingested food is degraded in the gastro-intestinal tract to produce a mixture of metal ions and complexes, amino acids, peptides, fats, fibre and many other substances. However, relatively little is known about the way in which these substances react together *in vivo* and it is therefore difficult to predict which foods or mixtures of foods can provide a given nutrient in its most bioavailable form.

The bioavailability of essential metals from different soya products has been the subject of discussion for some time (Welch & Van Campen, 1975; O'Dell, 1979; Erdman, 1981; Young & Janghorbani, 1981; Bodwell, 1983). Whilst soya products are a potentially rich source of some metals, the extent to which they exist in a bioavailable form in the gastro-intestinal tract is not known. The literature on this subject is somewhat confused, partly by the failure of some workers to adequately characterize the different types of soya product used in their experiments (Rackis & Anderson, 1977; Waggle & Kolar, 1979).

An important stage in obtaining a better understanding of the bioavailability of a metal is to determine how much is released from a given food in soluble form under the conditions found in the gastro-intestinal tract. There are many problems associated with *in vivo* studies and consequently a number of *in vitro* techniques have been developed to investigate the release of metals from foods (Narasinga Rao & Prabhavathi, 1978; Furuya, Sakamoto & Takahashi, 1979; Schricker, Miller & van Campen, 1982; Crews, Burrell & McWeeny, 1983, 1985).

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One such technique (Crews *et al.*, 1983, 1985) has been used in this work to investigate the release of calcium, magnesium, zinc, iron, copper, nickel and manganese from three different types of soya product.

Materials and methods

Materials

The hydrochloric, nitric and sulphuric acids, hydrogen peroxide, sodium chloride (Aristar grade reagents), 1000 μg/g Zn, Fe, Cu, Mn and Ni and 10000 μg/g Ca and Mg solutions (Spectrosol grade) used in this work were obtained from BDH Ltd. Sodium bicarbonate was obtained from Sigma Chemical Company Ltd. The *in vitro* digestion of the soya products was carried out using Porcine enzymes (Sigma). Gastric enzymolyses were carried out using a solution of 10 mg/cm^3 Pepsin (cat. no. P7000) in acidified saline (150 mmol/dm^3 NaCl, 20 mmol/dm^3 HCl). Intestinal enzymolyses were carried out using a solution containing 1.5 mg/cm^3 Pancreatin (cat. no. P1750), 5 mg/cm^3 Amylase (cat. no. A6880) and 0.75 g/dm^3 bile salts (cat. no. B8756) in saline (150 mmol/dm^3 NaCl).

Sample homogenization was carried out using a Colworth Stomacher Laboratory Blender 400 with samples being contained in the appropriate stomacher bags. All volumetric glassware and polypropylene bottles (1 dm^3) were acid cleaned prior to use and all analytical solutions were made up to volume with double distilled deionized water.

Three different types of soya product were used in this work. A full-fat soya flour, sample A, was donated by British Soya Products Ltd., and a concentrated soya protein, sample B, and a textured soya protein (mince variety), sample C, were donated by Lucas Ingredients Ltd. Further details of these products are given in Table 1.

Table 1. Some typical composition data (percentage dry matter basis), supplied by the manufacturers of the three soya products studied

	A Trusoy (full-fat flour)	B Newpro (concentrated protein)	C Solus (textured protein)
Protein	44.1	65 (Min)	42.5–52
Carbohydrate	25	17	29.5
Fat	21	2.5	1.5
Fibre	2.0	3.5 (Max)	3.0
Ash	4.9	4.5 (Max)	6
Moisture	Packed at approx. 7	6 (Max)	8

Wet digestion of all samples was carried out using a Tecator Auto Digestion System which could deal with up to twenty samples simultaneously. Analytical determinations of metal concentrations were carried out using a Beckman Spectraspan IIIA DC plasma emission spectrometer.

Trace element analysis

Details of the wet digestion procedure used in this work have been reported previously (Crews *et al.*, 1983). In brief, however, the total amount of Ca, Mg, Zn, Fe, Cu, Mn and Ni in each of the three types of soya produce was determined in the following manner. The organic matrix of duplicate aliquots of each soya (1 ± 0.1 g) was decomposed using hot concentrated sulphuric and nitric acids followed by hydrogen peroxide (100 cm^3/dm^3) using a Tecator Wet Digestion System. Recovery determinations were also carried out by addition of a solution containing a known amount of each analyte to replicate aliquots of each soya. The aqueous solution produced by the wet digestion procedure was made up to 100 cm^3 with distilled deionized water and Zn, Fe, Cu, Mn and Ni levels were then measured by Plasma Emission Spectrometry. This method was also used to determine the Ca and Mg levels, although a further hundred-fold dilution of the analyte was necessary. The wet digestion procedure was also used to determine the metal levels in aliquots (20 cm^3) of the saline and enzymolysis supernatants.

Saline extractions

Extractions of the soya products were carried out using saline solution (150 $mmol/dm^3$) at either pH 2.5 or pH 7.0. Duplicate aliquots of each sample (25 ± 0.1 g) were placed in Stomacher bags and pummelled with saline (100 cm^3) for 30 sec. The resulting mixture was transferred to polypropylene bottles and incubated with continual shaking for 4 hr at 37°C. After incubation the pH was checked and adjusted as necessary to pH 2.5 or pH 7.0. The mixtures were then centrifuged and the supernatants analysed as described under Trace Element Analysis. Saline blanks were also taken through the procedure.

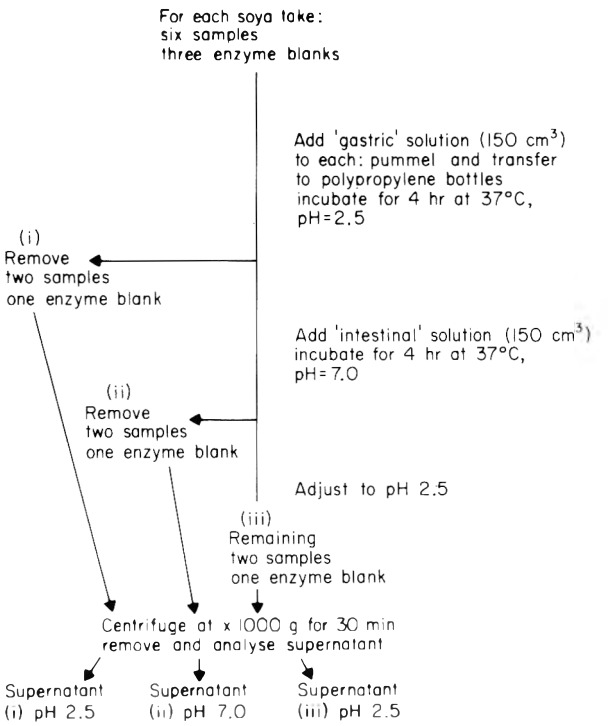

Figure 1. Schematic of enzymolysis procedures.

Enzymatic degradations

Identical aliquots of each sample were prepared for use in the enzymolysis experiments. An aliquot (25±0.1 g) for each sample was placed in a Stomacher bag and pummelled for 30 sec after the addition of gastric solution (150 cm^3). The resulting mixture was then transferred into a polypropylene bottle and the enzymolysis was carried out as shown schematically in Fig. 1. After centrifugation, the enzymolysis supernatant was removed and the metal levels were determined as described under Trace Element Analysis. An aliquot of the solid residue was taken, dried, estimated gravimetrically and the amount of soya solubilized by the enzymolysis was calculated. Enzyme blanks were also prepared as control samples. The post enzymolysis adjustment to pH 2.5 (Fig. 1; supernatant (iii)) was carried out to provide further information about the metal species present.

Table 2. Total element levels in three types of soya and percentage solubility before and after treatment with digestive enzymes at acid and neutral pH.

		% Soluble				
Sample	Total (mg/kg)	Saline* (pH 2.5)	Gastric†	Gastric intestinal‡	Saline* (pH 7.0)	Gastric intestinal§
Zinc						
A	46.6	80	91	105	36	63
B	31.7	60	69	74	66	3
C	55.1	76	90	101	28	46
Iron						
A	80	10	42	89	19	31
B	132	20	61	78	65	32
C	108	5	50	100	22	42
Copper						
A	13.8	38	65	89	70	92
B	15.6	15	51	76	64	79
C	16.4	24	61	88	68	88
Manganese						
A	30.5	83	102	111	6	9
B	19.8	76	90	96	67	6
C	36.3	85	106	113	10	9
Nickel						
A	6.30	112	112	125	123	114
B	3.64	70	67	78	95	81
C	8.86	86	83	91	95	95
Magnesium						
A	2420	89	104	101	55	63
B	1140	91	94	100	85	49
C	2984	92	92	96	55	64
Calcium						
A	2750	56	53	56	13	−3
B	3090	37	46	44	37	−10
C	2930	58	61	66	20	−1

*25 g soya: 100 cm^3 saline; †25 g soya: 150 cm^3 'gastric' solution (pH 2.5);
‡as † followed by addition of 150 cm^3 'intestinal' solution (pH 7.0) and acidification to pH 2.5 after enzymolysis; § as ‡ but no acidification after enzymolysis

Results and discussion

Metal content of soya produce

The amount of each metal found in the different soya products is shown in Table 2. Duplicate sample analyses gave a mean relative standard deviation of 5%.

Physical reactions of the soya produce with enzyme solutions

Quite different physical reactions were observed for the three different types of soya when the enzymolysis solution was first added. Sample A immediately formed a thin slurry, whereas sample B formed a thick paste that was relatively difficult to handle. Sample C, however, absorbed a quantity of solvent but retained its physical integrity. The amount of each sample solubilized by the enzyme treatment was found to vary according to the nature of the sample and the stage of digestion as shown in Table 3.

Table 3. Percent (w/w) of soya solubilized by the enzymolysis procedure

Sample	Gastric enzymolysis (pH 2.5)	Gastric + intestinal enzymolysis (pH 7.0)
A	47	78
B	23	52
C	48	85

The relatively small amount of sample B released into solution by the digestive enzymes may have been due to the inhibition of enzyme transport through the thick slurry that was produced when the aqueous enzyme solution was added to the soya.

Solubilization of metals by enzymes

The *in vitro* procedure described here permits measurement of the amount of metal solubilized by saline or enzyme solutions. It cannot accurately mimic complex *in vivo* systems to give precise information as to the amount of metal that is absorbable by the gastro-intestinal tract. However, the soluble metals found in the *in vitro* supernatants are at least potentially available for absorption. The final bioavailability will depend upon a number of other factors including the chemical form of the solubilized metal, nutritional status and the influence of other elements in the diet.

The percentage of metal released into solution from each soya after treatment with either saline or enzyme solutions is shown in Table 2. These results have been corrected for the amount of metal contributed by saline or enzyme solutions, which for Zn, Fe, Cu, Mn and Ni was < 0.1 mg/kg for the saline blank and ≤ 1.2 mg/kg for the enzyme blank. For Mg and Ca, saline and enzyme blanks were ≤ 7.0 mg kg^{-1} with the exception of the 'gastric' and 'intestinal' blanks for Ca which contributed 55–70 mg Ca/kg. Duplicate sample analyses gave a mean relative standard deviation of 4.4% (range 0.23%).

Two factors which may effect the solubility of all the elements studied should be noted. Firstly, the soya products were used without prior cooking, a factor that has been shown to influence the release of metals from soya (Lease, 1967; Weaver *et al.*, 1984). Secondly, the sample to extractant ratios for the saline, 'gastric' and 'gastric' plus

'intestinal' treatments (1:4, 1:6 and 1:12, respectively) are different. Consequently, any change in metal solubility may arise not only because of the action of 'intestinal' enzymes for example (where the ratio is greatest), but also because there is more liquid present and changes in equilibrium between solid and liquid phases may take place. This may be of proportionately greater importance if only slightly soluble species are present. Further work is needed to investigate the effects of equilibrium changes and an approach incorporating a dialysis technique (such as that reported by Schricker *et al.*, 1982) might be useful.

The saline treatments do, however, provide some indication as to how effective are the enzyme treatments. Some trends apply to all the soya products for several of the elements studied. At acid pH the amounts of soluble Zn, Mn, Ni, Mg and Ca released by 'gastric' enzymolysis are much the same as released by saline treatment alone. Enzymolysis releases only a small additional amount of soluble element. Similarly, at pH 7.0, saline extraction releases much the same amounts of soluble Cu, Ni and Mg (sample B behaves differently for Mg and this will be discussed below), as does the enzyme treatment.

Further interactions between analytes and the soya matrices studied, as affected by pH, enzymes and other parameters, are discussed below.

Zinc. At pH 7.0 most Zn is released by saline treatment from sample B, the concentrated soya protein. Treatment with the 'gastric' plus 'intestinal' enzymes, increases the amount of soluble Zn from samples A and C but in contrast drastically reduces that from B. The Fe, Mn, Mg and Ca from the protein concentrate behave in a similar manner at neutral pH. The enzyme treatment either releases or provides a ligand(s) that significantly reduces the solubility of these elements from sample B at neutral pH. Although sample B was the least solubilized by enzyme treatment (Table 3) this may not wholly account for the reduced solubility since it appears that enzymolysis is not necessary to release substantial quantities of these particular elements. Lease (1967), found that enzymic digests (pH 6.8) of soya flour solubilized more Zn than did similar treatment of soya protein isolate. A 'carrier' molecule, (postulated to be a large molecule containing a sugar moiety and amino acids as integral constituents), was found to be present from soya flour which was able to render Zn soluble and dialysable at pH 6.8 from insoluble phytate complexes. Furthermore, animal studies with chicks fed either defatted soya flour or soya protein isolate have found that only chicks fed the latter product required supplemental Zn (Rackis & Anderson, 1977).

It may be that the soya concentrate (B) in this present study is behaving in a similar fashion to Lease's protein isolate, having no 'carrier' to maintain Zn (and possibly other elements) in a soluble form when enzymes are present at neutral pH.

Iron. Enzyme treatment increases the amount of acid soluble Fe released from all three soya products. Approximately one-third of the Fe from each matrix was released at neutral pH following complete enzymolysis. Work with soya products has produced conflicting reports on Fe availability. For example, Weaver *et al.* (1984), investigating the bioavailability of Fe to rats from processed soya bean fractions, found no significant differences between Fe retention from any of the soya fractions. However, Schricker *et al.* (1982), using an *in vitro* technique, found that soya isolates as a group had lower relative Fe availability than the group of soya flours evaluated. Of the soya products studied here, sample A, the full-fat flour, released the least amount of soluble Fe after enzyme treatment. It is difficult to speculate without further studies whether the

phytate content (typically 1.15–2.17%, Rackis & Anderson, 1977) of soya, the soya protein itself or some other parameter (for example, the formation of insoluble ferric hydroxide) is responsible for the relatively low percentage solubility of Fe at pH 7.0 reported here.

Copper. Surprisingly much less Cu is soluble in saline at pH 2.5 than at pH 7, (Fe behaves similarly). Enzyme treatment increases the amount of soluble Cu appreciably at acid pH. Almost identical amounts are soluble at both acid and neutral pH after complete enzymolysis.

Manganese. The bulk of Mn in the food was released into solution under acidic conditions but very little was found in solution at pH 7.0 with the exception of sample B after saline treatment. Manganese carbonate is not very soluble at neutral pH and may explain the lack of solubility here at this pH following enzyme treatment, since sodium bicarbonate solution is used to neutralize the 'gastric' digests prior to the 'intestinal' stage. This ligand would also be found *in vivo* and it is reported that, in the normal adult human, only about 3% of orally ingested Mn from food is absorbed. After subsequent biliary excretion a net amount $\leq$ 1% is retained (Scheuhammer & Cherian, 1983).

Nickel. The levels of Ni, in the final diluted acid digests, were relatively low and the resulting increased measurement errors produced a situation in which apparently more than 100% of the metal was released from the soya. Even so, the results in Table 2 show that most of the Ni from all three soya products is soluble both with and without enzyme treatment and at both pH 2.5 and pH 7.0. The high solubility of Ni is of interest since Flyvholm, Nielsen & Andersen (1984), in a review of eighty-six foodstuffs, found that soya beans and soya products had very high Ni contents, (mean levels of 5.2 and 5.1 μg/g, respectively; only cocoa, mean 9.8 μg/g was higher), relative to other foodstuffs. This, combined with the apparent ease of solubilization of Ni from the products studied here, suggests that foodstuffs containing soya may make a significant contribution to Ni uptake.

Magnesium and calcium. At high pH the amount of soluble Mg was less than that found under acidic conditions (with the exception of sample B as mentioned earlier). Some Mg may be precipitated as the phytate at neutral pH and this will be discussed in connection with Ca below.

The insoluble food components and/or enzyme residues remaining after enzymolysis at pH 7.0 appeared capable of abstracting Ca from solution to the extent that correction for Ca in the enzyme blank gave rise to the apparent negative values shown in Table 2. Some of the insoluble Ca at neutral pH may be present as the carbonate (solubility of calcium carbonate in cold and hot water is approximately 1 mg/100 cm^3; Weast, 1984). The presence of phytate in the soya products may also have decreased the solubility of Ca. In the absence of protein, precipitation of calcium phytate will occur at pH 4.2 and higher (for magnesium phytate precipitation occurs at pH 5.2 and higher, de Rham & Jost, 1979). However, in the presence of protein from soya, the phytate in solution binds directly to the protein at pHs below the isoelectric point (for soya proteins this point lies around pH 5.5). When bound to protein, phytate cannot be precipitated as the Ca (or Mg) salt (de Rham & Jost, 1979). Phytate-protein binding can be disrupted at high pH (usually at pH $>$ 10) or by high ionic strength solutions (e.g. 1.5 M NaCl or saturated ammonium sulphate). Prattley, Stanley & van de Voort, (1982),

found that soluble protein–Ca–phytate complexes which were formed at pH > 6, were dissociated above pH 10 at high ionic strength. For the soya proteins studied here, it is likely that both industrial processing and *in vitro* enzyme treatment have caused denaturation of the proteins which will alter the binding sites available for Ca^{2+} and release phytate to form the insoluble Ca salt at neutral pH (Kroll, 1984).

Wien & Schwarts (1985), found that Ca solubility from full-fat soya flour could be decreased at pH 6.9, *in vitro* by the presence of bile salts. Further work is needed to determine whether the bile salts used in the *in vitro* system reported here influence trace element solubility.

Conclusions

The present work has shown that both the action of enzymic digestion and the pH of the digest solution can play important roles in determining the amount of metal released from the food. Some elements were solubilized by the action of saline solution alone. Calcium and manganese were found to be present in relatively low concentrations in the intestinal digest of all three soya products at pH 7. In addition, a relatively small amount of zinc was solubilized by the intestinal enzymolysis of the concentrated soya protein at this pH.

The results presented in this report permit some assessment of the potential *in vivo* solubility of metals from the three soya products studied. This solubilized portion may provide an estimate of the upper limit that is potentially bioavailable. However, these soya products have been treated in isolation. They are likely to be ingested *in vivo* as part of a mixed food matrix and this, as well as other factors discussed above, will greatly influence the final bioavailability of metals from these products. Additional research is necessary to study metal speciation in the digest solutions to investigate the factors that influence the bioavailability of metals from soya *in vivo*.

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Rice bran as a source of dietary fibre in bread

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Summary

Samples of wheat bran, parboiled rice bran and fat extracted rice bran were analysed for chemical composition, particle size distribution and farinogram dough characteristics. Fat extraction increased the fibre and protein content of rice bran. Wheat bran had the highest fibre content, water holding capacity and particle size. The farinogram properties indicated that there was no significant change when parboiled or fat extracted rice bran was added to wholemeal dough. However, wheat bran markedly changed the farinogram properties of wholemeal flour dough. Bread was prepared using wholemeal flour and the wheat and rice brans. Wheat bran or fat extracted rice bran decreased the loaf volume, acceptability, texture, flavour and appearance but parboiled rice bran only reduced the loaf volume by 6.9%.

Introduction

Rice bran is light brown, slightly oily, unstable meal produced as a by-product of the rice milling process. It contains approximately 17% fat and 6% crude fibre (Houston, 1972). The instability is a result of the high levels of unsaturated fatty acids and the presence of lipase (James & Sloan, 1984); parboiling or removal of the lipid with solvent stabilizes the bran (Houston, 1972).

James & Sloan (1984) investigated the functional properties of rice bran and wheat bran and found that defatted, extruded rice bran had higher stability and water holding, fat holding, and foaming capacities than non-defatted rice bran or wheat bran. These properties may affect the quality of bread made with added bran.

The development of high fibre breads has increased recently because of implications of dietary fibre deficiency in many diseases (Nelson, 1985; Meers, 1981). Wheat and soy bran and powdered cellulose have been used in bread making (Dubois, 1978), and a recent study has shown that up to 5% of acid or alkali treated rice bran could be added to wheat flour to produce acceptable bread (Fukui, 1982). The present study has shown that parboiled or fat-extracted rice bran may also be used to produce an acceptable high-fibre bread.

Materials and methods

Commercial parboiled rice bran (Ricegrowers Co-operative Mills Ltd., Leeton, NSW) was obtained by dehulling paddy rice cooked in steam at 120°C for 20 min. Fat-extracted rice bran was obtained from the same source by extracting the lipids with hexane. The bran was air-dried at 55°C with intermittent agitation; no residual solvent could be detected by taste. The wheat bran, wheat flour, yeast and bread improver were obtained from Allied Mills, Lidcombe, NSW, Australia.

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Moisture, fat, crude protein, fibre, water absorptivity and bulk density were determined using the methods described by the AACC (1983). Particle size distribution was carried out on duplicate 50 g samples using a Foss Electric Sieve Shaker with sieve sizes of 1, 0.6, 0.25, 0.15, 0.125, and 0.075 mm.

Farinograms were produced using the Brabender farinograph model 820500, and dough development time, tolerance index, stability, resistance and dough elastically were calculated from duplicate curves (AACC 1983).

The bread making formulation was: wholemeal flour (85), bran (15), saturated fat (2.0), gluten (10), sucrose (1.0), milk powder (4.0), yeast (3.2), bread improver (1.0), water (73), salt (2.5) and calcium propionate (0.2) (parts by weight). The control loaves (wholemeal) contained 100 parts wholemeal flour and no bran.

The mixing, moulding, proofing and baking were carried out according to standard methods (James & Sloan, 1984). Coded samples of the loaves were rated by twenty-four experienced assessors on a nine point hedonic scale for flavour, texture, appearance and overall acceptibility. The results were analysed by analysis of variance and compared using Duncan's Multiple Range Test (Snedecor & Cochran, 1967).

Results and discussion

Lipid extraction markedly increased the proportion of apparent crude protein and fibre contents of rice bran (Table 1) and probably caused the large difference in the visual and physical characteristics of each material. The crude protein, fibre and ash content of the lipid extracted rice bran was higher than expected. However, there may have been a difference in the concentration of other constituents of the bran. The parboiled rice bran was a light brown colour powder with slight cohesive properties. The lipid extracted rice bran was lighter in colour, and a lack of cohesiveness led to 'dustiness' and difficulty in handling the powder, probably because it has a greater proportion of particles less than 0.25 mm in diameter (46.7%) compared to wheat (2.6%) or parboiled rice (7.5%) bran. However, the high fibre content of extracted rice bran did not lead to a higher water absorptivity than the two other brans (Table 1), unlike that of James & Sloan (1984) where partially defatted rice bran had a higher water absorptivity than parboiled bran. This discrepancy may be explained by the particle size, as water absorptivity decreases with decreasing particle size (Lorenz & Lee, 1977). The farinograph data indicated that adding bran to wholemeal flour did not significantly increase the water absorption (Table 2) but there was a significant difference in the water absorptivity of the individual brans (Table 1). The farinograph values were significantly increased by the addition of wheat bran, but not by the addition of parboiled and lipid extracted rice bran (Table 2). The problems associated with the addition of wheat bran have been well documented (Pomeranz *et al.*, 1977: Roiter *et al.*, 1981; Shogren *et al.*, 1981).

Table 1. Composition and water absorptivity of brans*

Sample	Crude protein (g/100 g)	Lipid (g/100 g)	Ash (g/100 g)	Fibre (g/100 g)	Water absorptivity (g/100 g)
Wheat bran	14.2a	2.6a	5.7a	43.2a	338.2a
Parboiled rice bran	12.3b	16.4b	5.3a	7.3b	764.9b
Fat-extracted rice bran	17.7c	0.2c	10.8b	13.2c	133.9c

*Means of three replications; means followed by a different letter differ significantly ($P < 0.01$).

Table 2. Dough characteristics obtained from farinograms*

	Addition to wholemeal flour			
	No addition	Wheat bran	Parboiled rice bran	Fat-extracted rice bran
%Water absorption	66.4a	68.1a	67.5a	67.2a
Development time (min)	6.0a	9.2b	6.5a	7.5c
Stability (min)	5.5a	35b	4c	5a
Resistance (min)	11.5a	44.2b	11.3a	12.5a
Tolerance index (Brabender units)	60a	20b	60a	80c
Time to breakdown (min)	7.5a	29.5b	8.4c	9.0d
Dough elasticity	90a	120b	90a	90a

*Mean of duplicates; means within a row followed by a different letter differ significantly ($P < 0.01$).

The loaves with added wheat bran had a significantly lower loaf volume and sensory evaluation scores (Table 3). Loaf volumes were much greater with the added rice brans compared to wheat bran but the loaf volume and sensory evaluation scores were lower for the loaves with added fat extracted rice bran compared to parboiled rice bran. The loaves with added parboiled rice bran were 91.3% of the wholemeal loaf volume and there was no significant difference in flavour, texture and appearance (Table 3). However, the added parboiled rice bran loaves had a lower acceptability to this panel than wholemeal loaves, and there may be factors other than those tested that contribute to acceptability.

Pomeranz *et al.* (1977) have suggested that added fibre reduces the loaf volume because of a dilution of the gluten content and changes in crumb structure which impair carbon dioxide retention. The wheat bran particles were larger than the rice brans and this may explain the higher loaf volumes obtained with added rice brans (Roiter *et al.*, 1981).

Overall it appears that parboiled rice bran may be a possibility for increasing the fibre content of bread. A larger survey, involving a greater number of assessors may give more accurate results.

Table 3. Loaf volume moisture and sensory evaluation of loaves containing different brans*

	Addition to wholemeal flour			
	No addition	Wheat bran	Parboiled rice bran	Fat-extracted rice bran
Loaf volume (cm^3)	2490a	1690b	2275c	1920d
Moisture	41.7a	45.5b	42.5a	42.8a
Flavour	7.6a	6.3b	7.1a	5.9b
Texture	7.5a	5.3b	7.0a	5.3b
Appearance	7.8a	5.3b	7.3a	6.6c
Acceptability	7.7a	5.9b	6.5c	5.9b

*Means of duplicate or means of the scores from twenty-four panelists; means in rows, followed by different letters are significantly different ($P < 0.01$).

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Multiple mechanisms of bean hardening

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Summary

Several aspects of the hard-to-cook phenomenon in black beans were investigated. Bean hardening had commenced by the fourth month of storage at 30°C and 85% RH. The heat treatment used in this study caused initial softening but actually potentiated the defect once the beans were subjected to high temperature and high humidity. Both hydration and soaked hardness analyses indicated that the small differences in water absorption between hard and soft beans could not account for the large differences in cooked hardness. Increased hardness of raw defective beans was not related to cooked hardness but to elevated moisture levels. A multiple mechanism for bean hardening is presented which includes phytate loss as a minimal contributor to cooked hardness during initial storage and phenol metabolism as a major contributor on extended storage. The phenol metabolism is thought to be a lignification-like mechanism.

Introduction

Storage of legumes under adverse conditions of high temperature and high humidity renders them susceptible to a hardening phenomenon characterized by extended cooking times for cotyledon softening. Not only does this present an energy problem but nutritionally the cooked product is inferior and acceptance by the consumer is low (Aguilera & Stanley, 1985; Stanley & Aguilera, 1985). A more complete understanding of the underlying mechanism(s) causing this defect is necessary to prevent its development.

The objectives of this research were to monitor several chemical and physical indices in black beans (*Phaseolus vulgaris*) under various storage conditions and to establish their role(s) in the hard-to-cook defect. These included water absorption, phytate levels, phytase activity, phenols, moisture, and structural and hardness changes in the raw, soaked and cooked product.

Materials and methods

Materials

Black beans (*Phaseolus vulgaris*, var. orfeo) were provided by the Catholic University of Chile, SA. The beans were field dried in the traditional manner to a final moisture content of 11.7% (dwb). A portion of these were subsequently roasted in a granular bed heat exchanger (Aguilera *et al.*, 1982) at 150°C for 2 min. This heat treatment reduced the moisture content to 9.4% and resulted in an equilibrated internal temperature of 80°C. There was a delay of approximately 2 months between harvest

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and commencement of the storage studies as a result of sample preparation and shipping.

Methods

Storage conditions. Regular dried and heat treated beans were studied under two storage conditions: high temperature, high humidity (30°C, 85% RH) and low temperature, low humidity (15°C, 35% RH). They were contained in jute bags in lots of approximately 10 kg and placed in appropriate environmental chambers (Conviron Controlled Environments, Winnipeg, Manitoba). For ease of discussion the different treatments and storage conditions will be referred to as follows: RDLL-regular dried beans, low temperature, low humidity; RDHH-regular dried beans, high temperature, high humidity; HTLL-heat treated beans, low temperature, low humidity; HTHH-heat treated beans, high temperature, high humidity.

Soaking and cooking procedures. Bean samples were soaked for 18 hr in five volumes of distilled water at 25°C. The protocol for cooking beans consisted of soaking as described above, replacing the same volume of distilled water and cooking at 100°C on a hotplate for 2 hr while maintaining the volume of water. Cooktime commenced when the water started boiling. The cooked bean samples were drained and allowed to cool for 1 hr to facilitate accurate mass measurements for hardness.

Hardness measurements. The hardness of raw and soaked beans were measured using an Instron Universal Testing Machine (Model 1122, Instron Canada, Burlington, Ontario) employing the wedge system of Sefa-Dedeh, Stanley & Voisey (1978). Maximum force required to cut through the seed coat and cotyledons was measured for 20 individual beans and the average force reported as kg/bean. The load cell used and instrumental settings were those reported by Sefa-Dedeh *et al.* (1978).

Cooked bean hardness was determined using a 10 cm^2 Ottawa Texture Measurement System (OTMS) test cell with an eight bar wire extrusion grid (Canners Machinery, Simcoe, Ontario) as described by Sefa-Dedeh, Stanley & Voisey (1979). The maximum force to compress and extrude a 30 g sample was measured. Determinations were performed in triplicate and the results reported as kg/30 g cooked sample.

Phytate. The anion exchange method of Harland & Oberleas (1977) was used to recover phytate and the colourimetric procedure of Latta & Eskin (1980) used for phytate determination. The initial extraction was made using 2.4% HCl according to Latta & Eskin (1980). Phytate was expressed as mg/g of beans, on a dry matter basis. Four replications were performed per treatment.

Phytase activity. Crude phytase extracts were obtained and phytase activity monitored according to the method of Lolas & Markakis (1977). The rate of increase in inorganic phosphorous was determined by the method of Chen, Toribara & Warner (1956). Protein content was measured using the Biuret reaction (Gornell, Bardawill & David, 1949). Specific activity of phytase was expressed as μg P_i/mg protein/30 min. Activity values were corrected using a control containing enzyme which had been boiled for 10 min. Three assays were performed per treatment. The enzyme extracts were frozen and stored at −40°C until needed.

Phenols. The previously frozen enzyme extract was assayed for total phenol content. A 1.0 ml aliquot of crude enzyme fraction was extracted with 2.0 ml of methanol for 10 min on a wrist shaker and centrifuged for 10 min in a clinical centrifuge set at maximum speed. A 1.0 ml portion of this supernatant was analysed for phenols using the Folin-Ciocalteu reagent (Schanderl, 1970). Results were expressed as mg tannic acid /g dry bean. It must be emphasized that these results were obtained from the enzyme extract and not on an extract of whole beans. Extracts were available for months 1,2,4,8 and 10.

Moisture. Moisture content of the beans was determined in duplicate according to the AACC air oven method (44 15A).

Water absorption. Water absorption characteristics of the beans were followed on triplicate 10 g samples that had been soaked in 50 ml of distilled water for 1, 3, 6, 12 and 24 hr at 25°C. After soaking for the appropriate time the water was drained and surface water removed by blotting. Gain in weight was taken as the amount of water absorbed and expressed as a percentage of the dry weight. The hardness of these samples was also measured.

Microscopy. Beans were immersed in liquid nitrogen and freeze fractured. Samples were fixed in 2% glutaraldehyde in 0.01 M HEPES buffer, pH 6.0 for 6 h. They were then rinsed five times in buffer over a period of 2 hr to remove glutaraldehyde and dehydrated in an ethanol series (10, 20, 30, 40, 50, 60, 70, 80, 90 and 3 × 100%).

Samples for scanning electron microscopy were critical point dried, mounted on aluminium stubs and coated with ~ 30 nm of gol/palladium. These were viewed and photographed on an ETEC scanning electron microscope at an accelerating voltage of 10 kV.

Samples for light microscopy were embedded in Epon 812 after dehydration. Blue-green sections (0.5–1.5 μm) were cut with a diamond knife on a Sorvall Porter Blum ultramicrotome (Ivan Sorval Inc., Norwalk, Conneticut) and examined on a Zeiss photomicroscope (Model 62727, Zeiss Optical Instruments, Toronto, Ontario). Photomicrographs were taken on Kodak Ektachrome film (ASA 400). Specific stains employed are described in the discussion.

Results and discussion

Water absorption

Bean hydration patterns are illustrated in Fig. 1. They were characterized by an initial period of rapid water uptake followed by a progressively decreasing rate until saturation had been attained.

Beans stored under high temperature and high humidity conditions (HH) exhibited higher initial rates of water absorption when compared to the low temperature, low humidity (LL) samples. This increased rate has been attributed to the higher moisture content (Crean & Haisman, 1963). Two explanations for this rate difference are possible. Sefa-Dedeh *et al.* (1979) identified the seed coat as the primary barrier to water uptake during the initial stages of absorption. Essentially, beans with an elevated moisture content are 'primed' for further water absorption during the soaking process and do not show the longer lag times of low moisture legumes. This effect is best demonstrated by the transition in water uptake from month 0 to 1 in the HH beans,

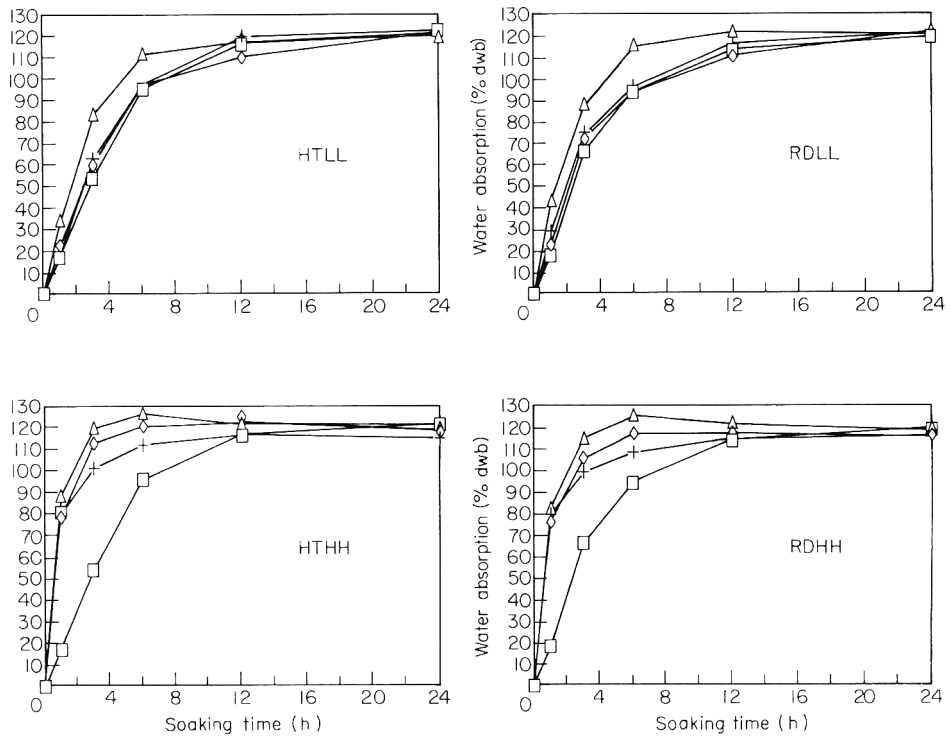

Figure 1. Water absorption during hydration of samples stored for. □ = 0 months. + = 2 months. ◇ = 6 months. △ = 10 months. (average standard deviation was 2.55%).

consistent with the equilibration of the beans in their new storage environment and resultant increase in moisture (Fig. 2).

Swelling of seeds during water absorption has been attributed to the increase in volume of absorbed atmospheric gases when displaced by water (Parrish & Leopold, 1977). These authors suggested that as gas expansion proceeds, a 'pressurized bubble' forms which resists further uptake of water until solubilization increases and the gas can eventually escape. This back pressure is a function of the amount of absorbed gases and/or the degree of solubilization of the gases prior to wetting. In the case of the HH storage, either less atmospheric gas is present or the higher moisture content precludes development of back pressure until further hydration occurs.

This back pressure-desorption process helps explain the slower absorption rate of the LL beans seen in Fig. 1. With greater quantities of absorbed gases and lower moisture contents back pressure develops much more rapidly and retards the rate of water uptake.

Another characteristic of HH storage was that the rate of absorption increased as storage time advanced. This trend was evident in both the regular dried (RD) and heat treated (HT) beans. LL beans showed a fairly consistent rate of absorption over time except for the samples stored for 10 months where the noticeable increase in rate may have been caused by the slight increase in the initial moisture content of the beans (Fig. 2).

RDHH beans had significantly lower absorption values than the other samples (119.8 *versus* 123.2%, $P < 0.05$). These data must be interpreted with care as this

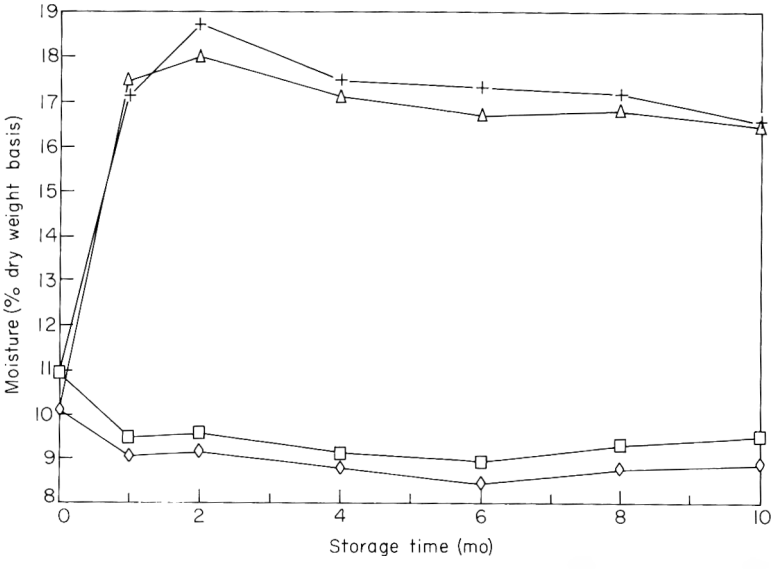

Figure. 2. Moisture content during storage, □ = RDLL, + = RDHH, ◇ = HTLL, △ = HTHH.

difference may be explained by the loss in soluble solids during soaking. Both HH storage treatments exhibited an apparent decrease in absorption (by 24 hr) as storage time increased and this was greater in the RD samples. Jones & Boulter (1983) found a negative relationship between 'imbibition value' and solute leakage when examining black beans stored under HH and LL conditions.

The roasting process may serve to counteract the effect of the HH storage, causing changes to either the testa or cotyledon components, increasing their resistance to solubilization and subsequent leaching during soaking. Molina *et al.* (1976) reported that heat treatments of increasing duration caused progressively greater water absorption. It is of interest to question whether this resistance to leaching is related to the development of hardness.

Water absorption is actually a balance between the rate at which water is absorbed and the rate at which soluble solids are lost. Towards the end of the soaking time, when water absorption is slow, the loss of solids must be greater in the HH beans to show the loss in absorption. In contrast, the rate of soluble solids lost must be minimal in LL beans since no loss in absorption was observed.

Solute leakage has been related to membrane degradation and/or abnormalities (Ching & Schoolcraft, 1968; Hallam, Roberts & Osborne, 1973; Varriano-Marston & Jackson, 1981). The breakdown of intercellular components, for example, protein, may contribute to these lost solids (Stanley & Aguilera, 1985; A. Hohlberg and D.W. Stanley, unpublished data). Although there is a significant difference between treatments it is doubtful whether a 4% change would be significant with respect to cooked hardness, especially if the loss of soluble solids is taken into account. Solute leakage rather than hardshell is indicated as the latter phenomenon is characterized by severe depression of water absorption (Antunes & Sgarbiera, 1979; Hsu, Kim & Wilson, 1983). Such a depression was not evident in this study. Thus hardness changes which occurred during storage cannot be attributed to hardshell, and fall into the 'hard-to-cook' category.

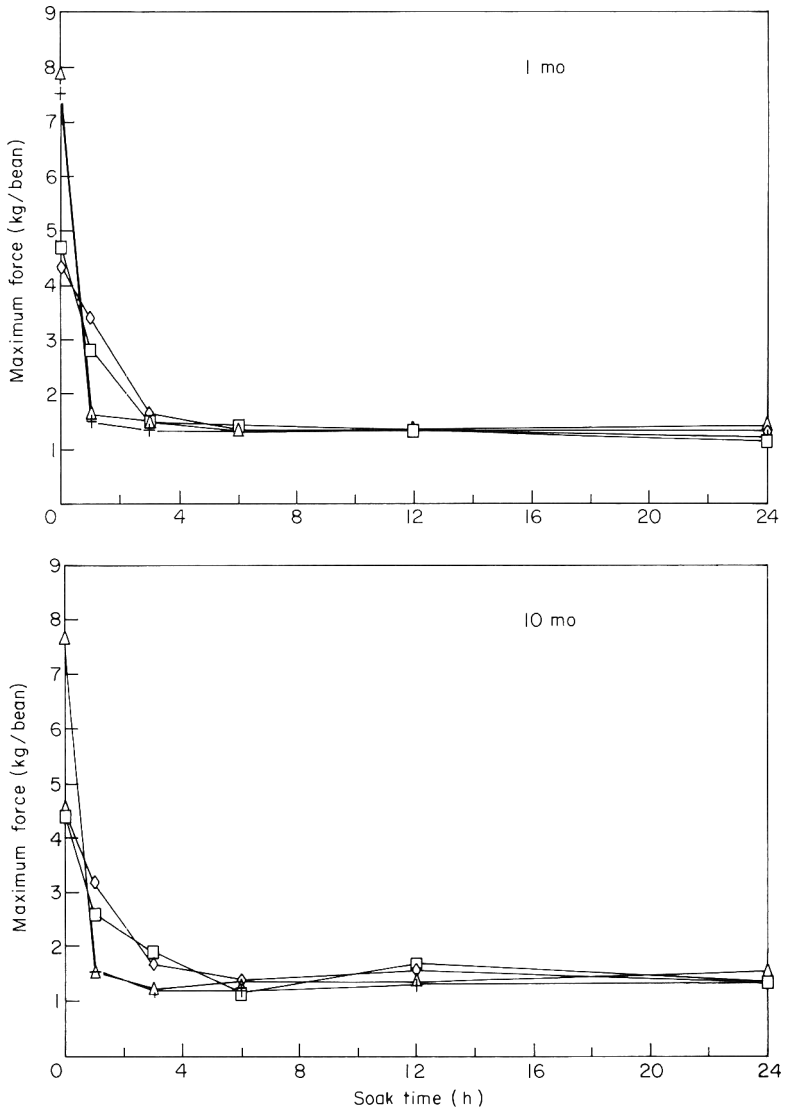

Figure 3. Wedge force of soaked beans during storage, □ = RDLL, + = RDHH, ◇ = HTLL, △ = HTHH.

Effect of soaking time on hardness

Texture of the beans was measured after 0, 1, 3, 6, 12, 18 and 24 hr soaking. In general, as soak time increased the force required to cut the bean decreased until saturation had occurred (Fig. 3) and thereafter remained constant. Beans stored under HH conditions showed a faster rate of softening than the LL beans. Once saturation is reached the softness of the seeds is the same, regardless of storage condition or heat treatment.

The data suggest a logarithmic relationship between soaked softness and water absorption once equilibration to the environments has been reached (i.e., after 1 mo). Correlation coefficients for log force against water absorption for each treatment were highly significant ($P < 0.001$). The rate of softening was dependent on storage

conditions, with HH storage yielding the fastest rates, while previous heat treatment had little effect. This difference in softening rate is related primarily to the increased hardness of the raw product under HH conditions (Fig. 4). When the beans have imbibed approximately 100% water, on a dry weight basis, the force values become constant. This occurs after about 3 hr for the HH beans and 6 hr for the LL beans (Fig. 3).

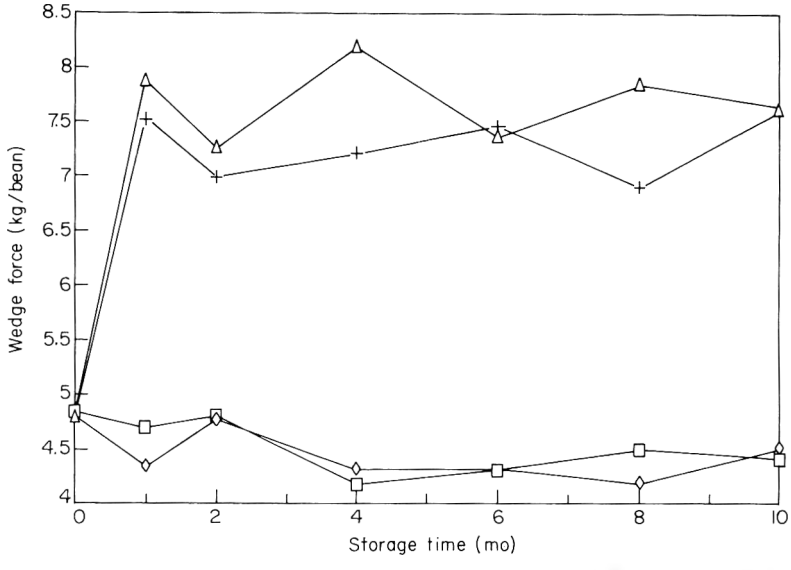

Figure 4. Wedge force of raw beans during storage, □ = RDLL, + = RDHH, ◇ = HTLL, △ = HTHH.

Typical force-time deformation curves for the raw and soaked beans are illustrated in Fig. 5. The shape of the curves for raw seeds indicated an initial rapid increase in force followed by a relaxation period and a second resistance peak of diminished magnitude. This pattern may be interpreted as the first peak being the force required to cut through

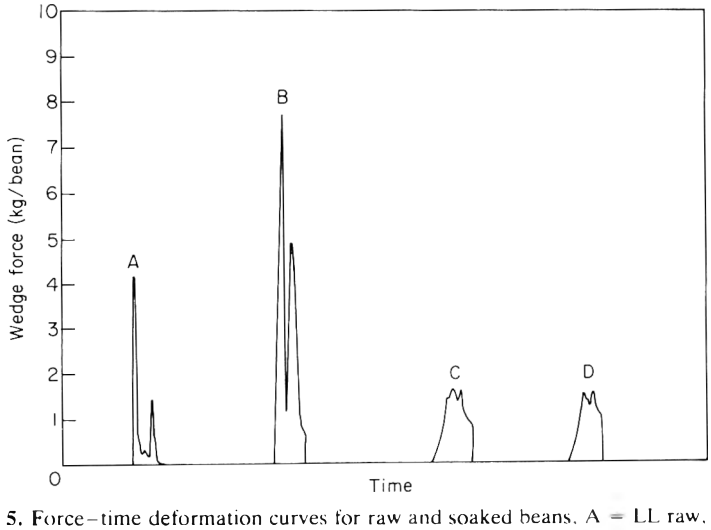

Figure 5. Force–time deformation curves for raw and soaked beans. A = LL raw, B = HH raw, C = LL 24 hr soak, D = HH 24 hr soak.

the seed coat and first cotyledon while the sudden drop occurs as a result of the space created by the inside curvature of the two cotyledons that offers little resistance. The second peak is thought to reflect the renewed force necessary to cut through the second cotyledon. This peak is generally smaller than the first as there is not the added resistance of the seed coat. The abrupt decrease following the second peak is caused by the ultimate splitting of the bean.

Characteristics of these curves may be used to interpret the increased force required to cut through seeds stored under HH conditions. The magnitude of peak 2 reflects the contribution of the cotyledon only while the ratio of peak 1 to peak 2 is related to the contribution of the seed coat. Under HH environments there is a significant increase in both peaks (peak 1–4.49) *versus* 7.59; peak 2–2.45 *versus* 4.82, $P < 0.05$), suggesting that the effect of accelerated storage on the raw seed hardness is not related to the seed coat but to the cotyledons themselves. The significantly higher ratio of the two peaks in LL beans (1.99 *versus* 1.67, $P < 0.05$) indicates that the seed coat makes a greater contribution to texture than in the HH beans. This effect is probably related to the reduced moisture content and increased brittleness of the seed coat under LL conditions.

Differences in force-time deformation curves are no longer evident once water has been absorbed (Fig. 5). After 24 hr soaking the contribution of the seed coat is negligible and the force required to cut the cotyledons is similar. Nor is the decrease in resistance between peaks as pronounced since the cotyledons have swelled upon imbibing water and the space between them has been reduced.

It has been shown that there is a striking difference in the hardness of the raw beans after storage under HH conditions. This increase correlates well with the elevated moisture content ($r = 0.972$, $P < 0.0001$). It is logical to assume that the increase in hardness of the raw bean is related to the intracellular components, of which starch is the primary constituent, comprising approximately 40% of the cotyledons on a dry weight basis (Powrie, Adams and Pflug, 1960). It is believed that the initial 8–10% of water absorbed by starch is 'bound water' and that further hydration upon equilibration in a saturated atmosphere causes expansion, the degree of which is dependent upon the source of the starch (Leach, 1965). Jones & Boulter (1983) found that the isolated starch from beans in accelerated storage had somewhat greater swelling power than controls stored at 4°C. It is probable that the LL beans essentially contain bound water only and solvation is minimal. The wedge may simply slide by dry starch granules and protein bodies, pushing them aside as it passes through the cotyledon. In the HH beans, with increased moisture and expansion of the starch, as well as a more fully hydrated cytoplasmic matrix, the wedge would not be allowed to slip by as easily, meeting with increased resistance. This 'stickier' nature of the intracellular components would provide drag on the wedge. Further hydration upon soaking, initially very rapid for both HH and LL beans, causes softening and eliminates the differences.

Figure 6 depicts SEM micrographs of raw beans from both storage conditions after the wedge texture test. These samples were not fixed but immediately coated and viewed. The starch granules were not fractured in either case. The increased force, therefore, cannot be due to the wedge being forced to cleave the granules. It was observed, however, that the cotyledon cells of HH beans were often devoid of granules, with only a depression remaining. This could be a result of the starch granules being dragged from the surrounding matrix by the wedge due to their more adhesive properties at the higher moisture contents. When this occurs, the matrix is compressed as a result of this dragging resistance. Where starch granules remained in the cell they appeared closer together, possibly from partial swelling. In cells from LL samples,

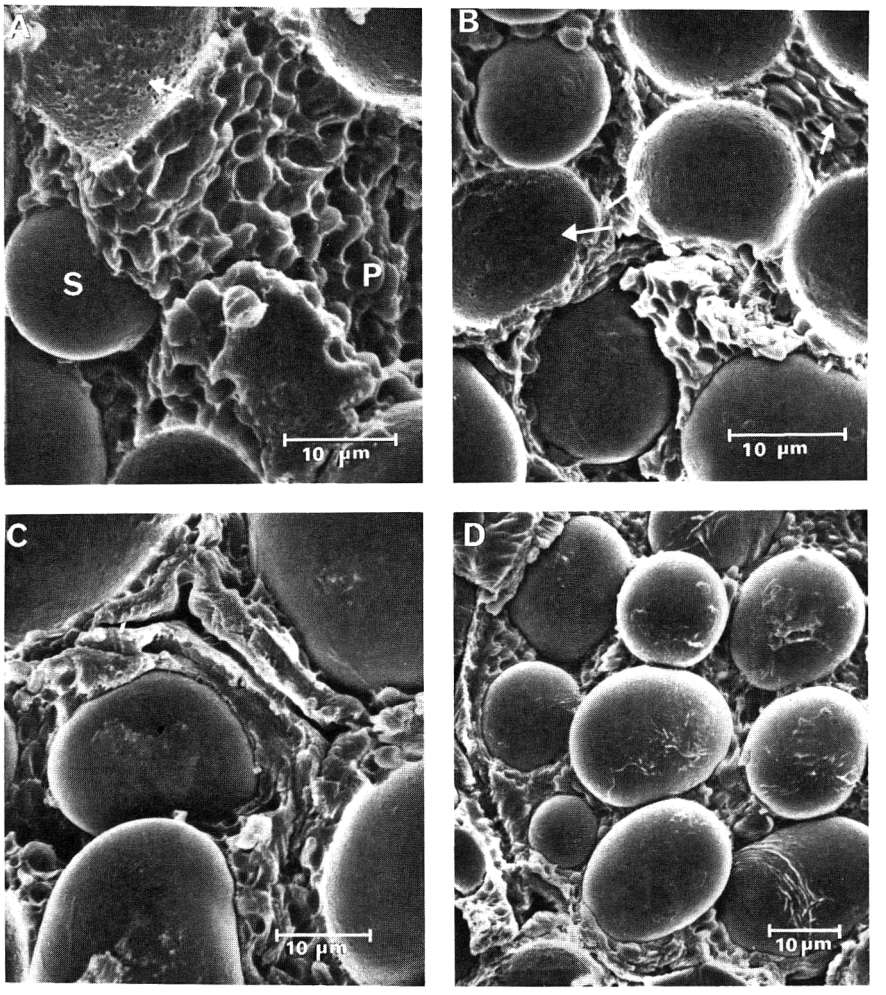

Figure 6. Scanning electron micrographs of raw beans subjected to the wedge texture test. (A) LL cotyledon showing loss of a few starch granules (open arrow) but no evidence of stress to the surrounding matrix. S = starch granule, P = protein body. (B) HH cotyledon with greater loss of starch granules (open arrows) and compression of the cytoplasmic matrix in these areas (solid arrow). (C) LL cotyledon with minimal wrinkling on the starch granule surface. (D) HH cotyledon showing extensive wrinkling on granule surface.

where starch granules were not in place, there was no evidence of compression of the cytoplasmic matrix. The starch granules of the HH beans had an extensively wrinkled surface; this was not nearly as pronounced in the LL beans. This may be a membrane-like material (Silva & Luh, 1978) which was affected by the elevated temperature and humidity.

The immediate increase in hardness of raw beans upon exposure to the HH environment and the consistent elevated levels under these conditions seems to preclude the cell wall from being involved in this increase. If the cell wall were a major contributor to raw hardness, force values would be expected to increase consistently over time, similar to that seen for the cooked hardness, not remain constant upon a change in the environmental conditions.

Cooked hardness.

Beans kept at elevated temperature and humidity developed the hard-to-cook defect by the fourth month of storage while those stored at low temperature and humidity changed little (Fig. 7). Table 1 gives a statistical analysis of the force values.

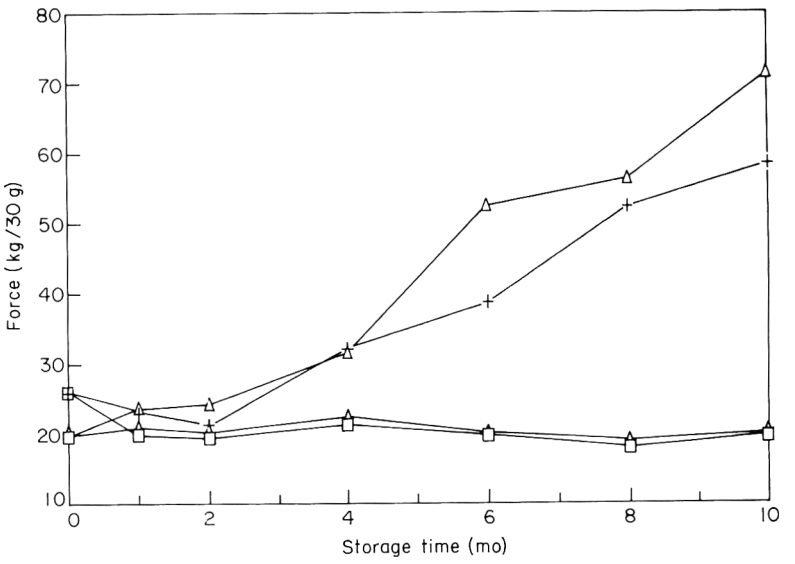

Figure 7. Cooked texture of black beans during storage. □ = RDLL, + = RDHH, ◇ = HTLL, △ = HTHH.

At month 0, prior to storage, the cooked force values were low, but those for the HT samples were significantly lower than for the RD samples ($P < 0.05$). The RD samples actually grew softer during the first 2 months of storage after which time storage at elevated temperature and humidity led to the defect. HTHH samples grew progressively harder during storage and were significantly harder than the RDHH beans from the sixth month onward.

These data have several implications. The heat treatment prior to storage reduced cooked hardness when compared to the regular dried samples at month 0. However, under higher temperature and humidity conditions this pre-treatment seemed to

Table 1. Effect of treatment and storage on mean force to extrude cooked beans (kg/30 g)

	Month					
Treatment	0	2	4	6	8	10
RDLL	$^{a}26.17^{a}$	$^{bc}19.43^{a}$	$^{b}21.27^{a}$	$^{bc}19.73^{a}$	$^{c}18.00^{a}$	$^{bc}19.77^{a}$
RDHH	$^{a}25.40^{a}$	$^{a}21.23^{a}$	$^{b}32.10^{b}$	$^{c}38.77^{b}$	$^{d}52.40^{b}$	$^{e}58.43^{b}$
HTLL	$^{a}20.10^{b}$	$^{a}20.10^{a}$	$^{b}21.70^{a}$	$^{a}20.07^{a}$	$^{a}18.97^{a}$	$^{a}20.17^{a}$
HTHH	$^{a}20.10^{b}$	$^{ab}24.20^{b}$	$^{b}31.50^{b}$	$^{c}52.60^{c}$	$^{c}56.4^{c}$	$^{d}71.27^{c}$

NB Left-most superscript refers to rows. Right-most superscript refers to columns.

Means with a different superscript are significantly different ($P < 0.05$).

accentuate the development of the defect following 4 months of storage. Aguilera & Steinsapir (1985) found that a high temperature-short time treatment caused a reduction in cooked hardness, as measured by puncture force. In that study, however, the hardness remained lower than in the control during storage. This difference is probably a result of a more intensive heat treatment (exposure to 250°C for 3 min, final bean temperature 105°C) than the present samples received. It seems that although the lesser heat treatment caused initial softening, it eventually led to the development of increased hardness. A similar response to that of Aguilera & Steinsapir (1985) was reported by Molina *et al.* (1976), although in this case the beans were either retorted or heated under steam, both processes allowing more heat penetration due to the higher temperature, time and/or water content.

The differences in our data may relate either to enzymatic activation or to an initial injury of the enzymes responsible for the hardening process, which subsequently recover and initiate the phenomenon, especially at the higher temperatures and moisture levels. Although it will be seen that the enzyme we studied did not behave in this manner, the possibility of other enzymes participating in the defect cannot be ruled out.

In summary, there is evidence that the application of a heat treatment can reduce the toughness of cooked beans if they are stored under suitable conditions. However, if the storage conditions are not favourable, a heat treatment, if sub-lethal to the components participating in the hardening mechanism, can actually accentuate the defect as storage progresses.

Microscopy

Microscopic examination of cooked beans revealed reduced cell separation in defective beans. Figure 8 shows the difference in cotyledon cell separation between RDHH and RDLL beans stored for 4 months. These sections were stained with Congo red for fluorescence microscopy (Fulcher & Wong, 1980), a technique specific for beta-glucans in the cell wall that provides a striking highlight of the cell boundary. Discrete starch granules are quite noticeable in hard beans whereas in the soft beans they appear as dark bodies, often fused together. This, in conjunction with reduced cell expansion of hard beans, suggests that a secondary result of restricted cell separation could be the lack of starch gelatinization during cooking, which would contribute to the defect.

Scanning electron micrographs of regular and hard beans are shown in Fig. 9. Beans from elevated storage conditions exhibit dense packing of the cotyledon cells with minimal separation. Middle lamella remnants are also observed, resulting from structural breakdown of the middle lamella during cooking (Sefa-Dedeh *et al.*, 1979). Although it was difficult to discern any differences in the amount of middle lamella remnants between hard and soft beans it was noted that cell walls of defective beans had actually broken open or pulled off where adjacent cells had been situated before fracturing. This indicates that the adhesion between cells was greater in hard beans.

Phytate

Initial statistical analysis of the phytate data indicated that each treatment/storage combination should be treated separately. Regressions were performed and the slopes obtained for each replicate, estimating the rate of phytate degradation and appropriate analysis of variance was performed on the slopes. The data indicated that there was a significant difference in the rate of phytate hydrolysis over time. Preplanned multiple

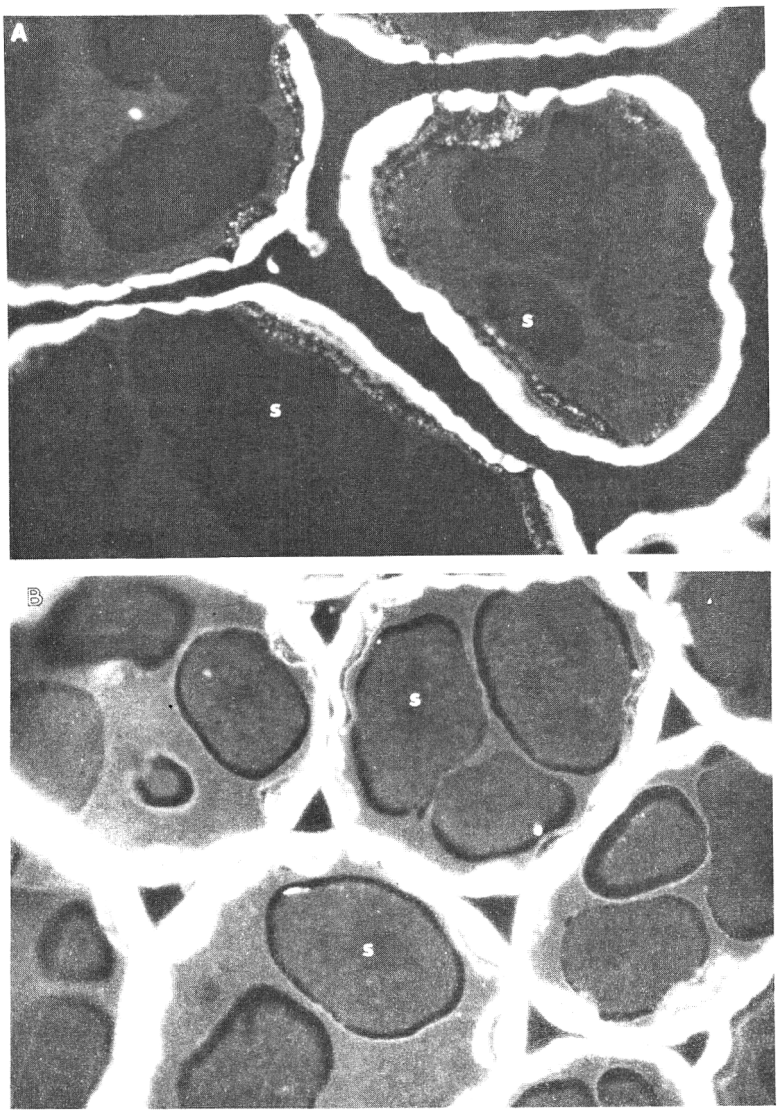

Figure 8. Light micrographs of cooked bean. (A) RRDL sample showing separated cells, S = starch granule. (B) RDHH sample indicating lack of cell separation.

comparisons using Duncan's procedure revealed that the rates fell into three categories: LL's, RDHH and HTHH ($P < 0.05$).

Phytate degradation was similar for beans stored under LL conditions and these rates were significantly lower than those for the HH storage ($P < 0.05$). Heat treatment retarded phytate loss when compared to beans that had been field dried, but this was significant only for beans stored under high temperature and humidity. A general trend, therefore, was seen between higher rates of phytate loss and the development of hardness in the cooked beans. The correlation coefficient between these two parameters was significant ($r = -0.716$, $P < 0.01$).

Although the phytate data have been presented in Fig. 10 as a comparison of slopes it should be noted that intercepts for the heat treated samples were higher than those of

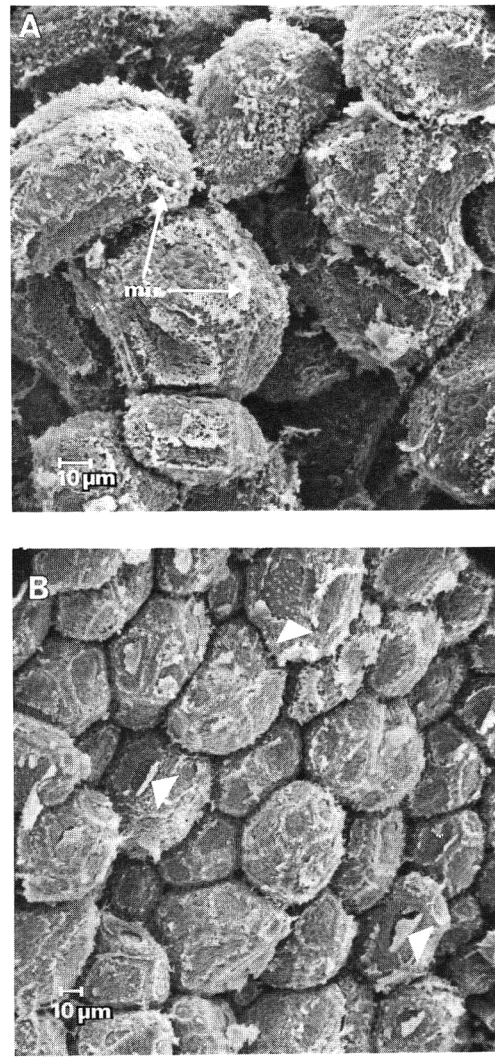

Figure 9. Scanning electron micrographs of cooked bean. (A) RDDL sample showing loose association of cells, mlr = middle lamella remnants. (B) RDHH sample showing tightly packed cells and areas (arrowheads) where cell wall has been broken open or pulled off.

the RD beans at time 0. The RD samples also exhibited a slight increase in phytate over the first 2 months of storage before showing any decrease. These aberrations are possibly a result both of initial damage to phytase by the application of dry heat and of phytate synthesis. Parallel changes are seen in the cooked hardness during the first few months of storage. The overall rate of phytate loss was used because the phytate levels for the raw samples prior to any treatment (at true time 0, i.e., harvest) were not known.

Mattson was the first to suggest that legume cookability was dependent upon phytate (Mattson, 1946; Mattson *et al.*, 1950). Since his pioneering work several studies have examined the relationship between the hard-to-cook defect and phytate content. The majority of this research has supported Mattson's work and has covered several

species and varieties of legumes and cereals, i.e., Peers (1953) wheat; Rosenbaum & Baker (1966) peas; Kumar *et al.* (1978) varied; Longe (1983) cowpea; Shehata, Abu Bakr & El-Shimi (1983) faba beans; and Moscoso, Bourne & Hood (1984) kidney beans. Other researchers have reported low correlations between these two parameters (Crean & Haisman, 1963; Henderson and Ankrah, 1985).

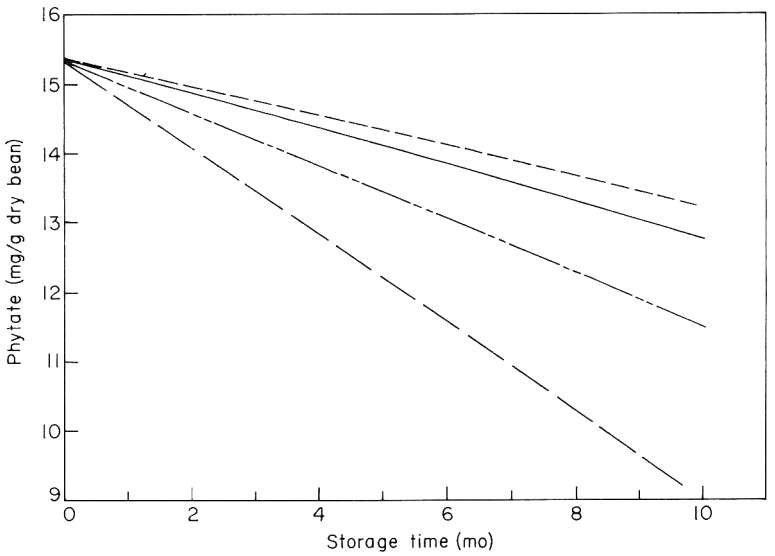

Figure 10. Phytate loss during storage, ----, RDLL; ——, RDHH; ——, HTLL; ———, HTHH.

The low but significant correlation coefficient found in the present study implies that phytate is a contributor but perhaps not the sole operating mechanism in the hardening defect. This is emphasized by the rate of phytate loss in the HT samples stored under HH conditions. It is lower than in the RD beans, yet the cooked hardness increases as time progresses.

Phytase

Although the phytase data fluctuated, a general quadratic trend was indicated (Fig. 11). This fluctuation may be the result of an on/off mechanism acting on the enzyme which subdues the trend at these low levels of activity. In general, beans in HH storage exhibited increasing activity over time. LL storage suppressed activity initially after which activity increased to the original levels. The specific activity of the heat treated samples was reduced in comparison to the RD beans and this was evident for both storage conditions.

As expected, the activity profiles paralleled the loss of phytate. Though the specific activities are quite low, those for month 0 storage are similar to values reported for other raw legumes, e.g. Henderson & Ankrah (1985) found a specific activity of 0.24 μg P_i/mg protein/30 min for *Vicia faba*.

The substantial initial decrease in activities for beans stored at lower temperature and humidity appears to be due to suppression of the enzyme. Lower activities of the heat treated samples may indicate partial denaturation or disruption of the enzyme, though it remains active and eventually recovers. Although no data are available on

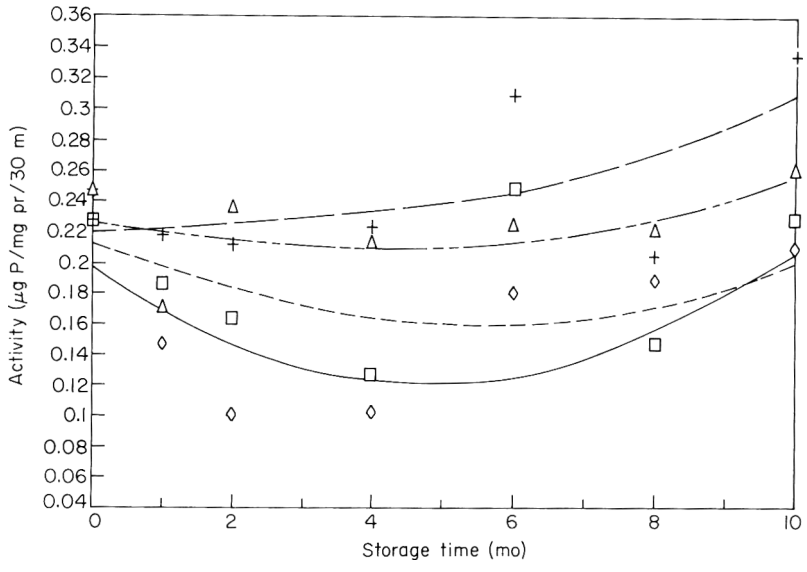

Figure 11. Phytase activity during storage, □, – – – –, RDLL; +, ——, RDHH; ◇, ——, HTLL; △, ———, HTHH.

phytase regeneration, this phenomenon is well documented for other enzymes such as peroxidase.

Phytase is a thermostable enzyme (Peers, 1953; Chang & Schwimmer, 1977; Reddy, Sather & Salunke, 1982), the degree of stability against thermal inactivation being dependent upon species and on whether the study is done *in vitro* or *in vivo*. The phytase activities suggest that the dry heat applied to whole dry beans was not sufficient to cause inactivation, although it did lower activities compared to regular dried beans.

An important consideration when examining enzymes is the environment in which they operate. Beans have a low moisture content which means that small changes in moisture can cause large changes in water activity (a_w). Beans in the HH environment have a moisture content of between 17 and 18% compared to 8–9% for LL samples. This difference has some important implications when biological reactions are considered. Data on black beans indicate a_w s of about 0.77 and 0.40 at moisture contents of 18 and 9% (dwb), respectively, at 25°C (Aguilera & Balliviar, 1986). Under HH conditions the solvent capacity and mobility of water would be only slightly decreased while in LL conditions severe reduction in these attributes will occur. In HH storage enzymatic activity and oxidative reactions could proceed unimpeded and would also be enhanced by elevated temperatures.

Extractable phenols

The extracts used for phytase activity analyses were observed to change colour during storage. Extracts from low temperature, low humidity beans retained their dark blue/purple hue while high temperature, high humidity bean extracts were observed to lose colour, turning progressively lighter during storage. By 10 months the extracts of HH beans were a light blue to whitish grey.

The limitations of the phenol method used should be mentioned. Values of tannic acid are not absolute but depend on the conditions used and the nature of the starting material. Water was used as the initial extracting medium and this is known to be less efficient than methanol or acidified methanol in extracting the soluble phenols (Ma &

Bliss, 1978). The colorimetric test was performed on a sample of the enzyme extract and subsequent extraction with 100% methanol may not have been complete since protein–phenol interactions can interfere with the extraction. Because all samples were treated identically and the protein content of the extracts were similar (36.6±3.8 mg/ml), the relative values of total phenols were considered valid to use as a preliminary probe into possible mechanisms.

LL beans lost significantly more phenols to the extracting medium than the HH beans ($P < 0.01$) (Fig. 12). The phenol losses were similar for all treatments up to the fourth month, after which the HH extracts had much less extractable phenol compared to their LL counterparts.

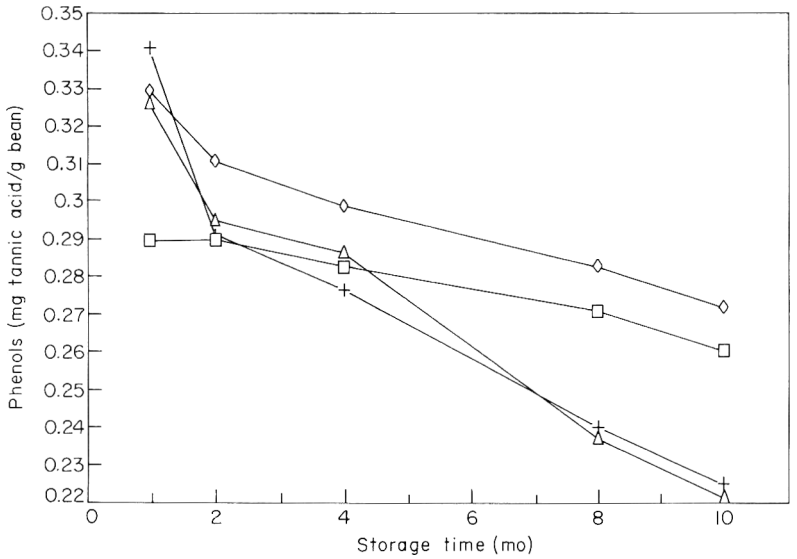

Figure 12. Extractable phenols from bean enzyme extract. □ = RDLL. + = RDHH. ◇ = HTLL. △ = HTHH.

The levels of extractable phenols were compared to cooked hardness, which remained low over the higher range of phenol contents (Fig. 13) but an abrupt change occurred below about 0.25 mg tannic acid/g dry bean where the hard-to-cook defect became evident. Statistical analysis indicated that phenol content could account for only 58% of the variation in cooked hardness over the entire storage period, although this was significant ($P < 0.001$). It was evident though that this parameter was important only in the latter stage of storage, i.e., beyond 4 months.

An obvious question which arises over the difference in extractable phenols is why there is a difference between storage and how these relate to cooked hardness. Similar changes have been documented by other researchers. Deshpende & Cheryan (1985) found that assayable tannins of milled bean flour decreased during storage and that high relative humidities accentuated this decrease. Kadam *et al.* (1982) showed that polyphenol content decreased as maturity of winged bean increased, with a concomitant increase in cooking time over similar maturity stages. Changes in extractable phenolic substances exhibited during post-harvest storage have been attributed variously to: polymerization to produce insoluble high molecular weight polymers (Kadam *et al.*, 1982); attachment to carbohydrate matrix (Salunkhe *et al.*, 1982); or as a result of binding to protein (Bresani *et al.*, 1983).

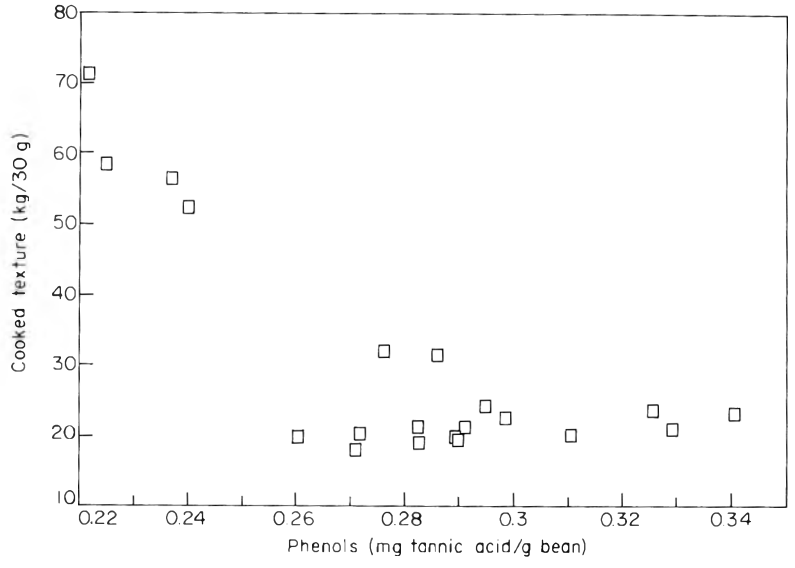

Figure 13. Extractable phenols *versus* hardness after cooking.

It is suggested here that the change in extractable phenols is, in part, due to their increased polymerization during storage and that a possible lignification-like mechanism is functioning to restrict cell separation on cooking. Lignin is a product of the oxidation of polyphenolic compounds. The process of lignification can occur either enzymatically (mediated by cell wall bound peroxidase) or, at least partially, non-enzymatically (Blouin, Zarins & Cherry, 1982). That lignification may be occurring is not a new concept (Molina *et al.*, 1976), but it has not been conclusively proven. From the present data, it would appear that the decrease in extractable phenols does not affect cooked hardness until fairly low levels are seen.

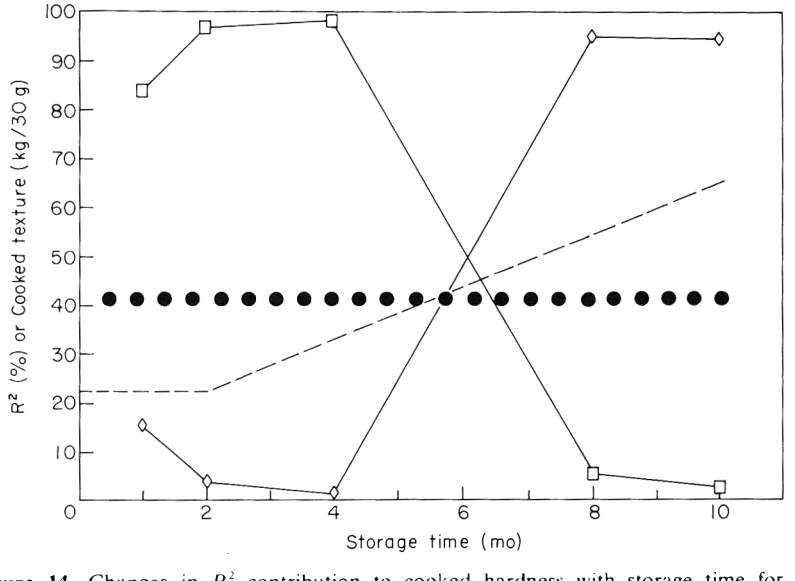

Figure 14. Changes in R^2 contribution to cooked hardness with storage time for two independent variables, □——□ = phytate, ◇——◇ = extractable phenols, ---- = typical bean hardening curve, ●●● = maximum subjective acceptability level.

Two parameters, phytate and extractable phenols have been examined in this study at the bean hardening process. Each when analysed separately has been shown to relate to cooked hardness, however the R^2 values, though significant, were low. To gain more insight into the role of each and their interrelationships, a multiple regression analysis was performed to relate both factors to the cooked hardness at different storage times (Fig. 14).

When both variables are included 97 to 100% of the total variation is accounted for at each storage period. During the initial months of storage any changes are predominantly due to phytate while in the latter months extractable phenols become the major contributor. Figure 14 indicates that the transition occurs between 4 months and 8 months. This is around the time that the hardening defect is accelerated (Fig. 7). Aguilera & Ballivan (1986) state that a relative hardness of 1.8 is the maximum for subjective acceptance. This translates to about 41 kg/30 g, which occurs within the transition region.

It would appear that phytate levels only cause minor differences in hardness of cooked black beans, possibly not even to the point of consumer differentiation. This would explain why, even though phytate levels continue to decrease in LL beans beyond 10 months, only minor changes in hardness of cooked beans were noted. With time, as phenols become polymerized or bound, in what is believed to be associated with a lignification-like mechanism, a major change in hardness after cooking results, completely overriding the minor effects of phytate.

If this is the case, the aspects of a multiple mechanism in bean hardening must be accepted. Future research, presently under way, necessarily includes further evidence of lignification in the cell walls of hard beans and the fate of polyphenols under different storage conditions. An investigation of the differences in hydration of defective and control beans during cooking is also being conducted.

Acknowledgments

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Improving pectin technology: extraction using pulsating hydrodynamic action

C. KRATCHANOV,* K. MAREV, N. KIRCHEV AND A. BRATANOFF

Summary

A model of an extraction process of pectin from vegetable raw material is proposed, and factors determining the extraction efficiency are discussed. Based on the model, a new method of pectin extraction has been developed using pulsating hydrodynamic action on fruit mass during turbulent recirculation. An appropriate flow pattern and diagrams of typical cyclic changes in local time-mean velocity and pressure of the fluid are presented. These show steep local gradients which are thought to cause flexing and disruption of cell walls thereby accelerating pectin removal. Laboratory and industrial tests of the new method and equipment resulted in an increased yield of pectin (30–60%), faster extraction (2–5 times) and increased extract concentration (over 0.5%) compared to already established processes.

Introduction

Industrial extraction of pectin from fruit pomace using aqueous solutions of mineral acids is carried out mainly by two types of processes:

(a) Periodic multiple extraction in separate apparatus (Kertesz, 1951);

(b) Continuous counter-current extraction with screw or belt conveying of raw materials (Kertesz, 1951; Doesburg, 1965; Nowlin, 1974).

Slow stirring of the mixture is a special feature common to both methods. This results in slow diffusion both of solvent molecules into the fruit pomace and of pectic molecules outwards. Consequently, the duration of processing is long (6–24 hr), the degree of extraction is low (approx. 75% of the laboratory extracted pectic substances), the pectin content of the aqueous extract is below 0.3% and energy consumption is considerable. These disadvantages of the existing extraction methods necessitate theoretical and experimental studies aimed at improving the extraction process.

Materials and methods

Materials

All reagents used in the analysis were of AR grade. Table 1 shows the characteristics of the apple pomace samples used in the laboratory and industrial experiments.

Analytical methods

The polyuronic acid content and the degree of esterification of the polyuronides of apple pomace, the purity and the degree of esterification of pectic preparations were all

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Table 1. Characteristics of apple pomace and apple pectin

	Apple pomace		Apple pectin		
	Polyuronic acid content (%)	Extractable pectin, (%) (standard)	Degree of esterification, (%)	Purity (%)	Gel strength
1. Pomace for laboratory experiments	12.3	6.75	68.9	92.4	180
2. Pomace for industrial experiments	16.6	10.80	71.4	56.4	220

determined by standard methods described in a preceding publication (Kratchanov *et al.*, 1981). Gel strength was determined by Tarr and Baker's method (Bender, 1949).

The photomicrographs (Fig. 2a, b, c) of apple pomace under extraction processes were taken under magnification × 100. Technical measurements and calculations of the operational mode of the EP-16 extractor were carried out in accordance with Reynolds (1974). The measurement of static pressure was taken by probing at specific places of the extractor—at the sucker, in front of the propellor and into the diffuser after the guide blades.

Laboratory process experiments

150 l of 0.5%, 1.0% or 2.0% nitric acid at 73 or 83°C were poured into a 300 l EP type laboratory extractor (produced in our laboratory). 7.5 kg apple pomace were added upon starting the propeller (for experiments with 20:1 hydromodule). Extraction temperature was maintained at 70 or 80°C for a total period of 45 min, during continuous turbulent stirring (at 8 m/sec peripheral velocity of the propeller). Samples of the mixture were taken at 10, 15, 20, 25, 30 and 45 min. Upon filtration, the pectin concentration of the samples was determined by coagulation with ethanol. The pectic preparations thus isolated were then analysed (degree of esterification, purity, gel strength).

Industrial experiments

Comparison experiments were conducted under industrial conditions by the new technology elaborated by the authors (Marev *et al.*, 1983) and by the old technology used previously (Solms & Deuel, 1962) using the same kind of apple pomace.

Model of the extraction process

Hindered diffusion during the extraction of pectin is mainly due to the mode of binding of the pectic molecules in the vegetable tissue. A diagrammatic model of the instantaneous state of extracted mass is shown in Fig. 1. The difference in the solution concentrations inside and outside the fruit mass was the main driving force according to Fick's law of diffusion. However, in the case of the extraction process of pectin the following specific features were considered:

(a) The fibrillar pectic molecules are complex polymers of a relatively high average length-to-diameter ratio ($l/d > 1000$). Even under more favourable conditions these cannot be instantly transferred from the vegetable tissue to the liquid phase. Therefore,

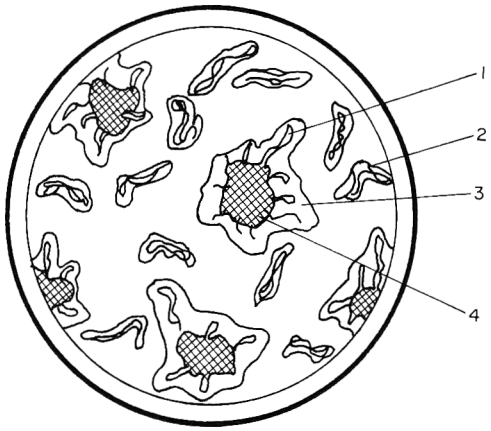

Figure 1. Model of extraction process: (1) pectic molecules passing through the surface of the fruit particle; (2) pectic molecules passed into solution; (3) gelatinous coating around the fruit particle; (4) fruit particles.

a special action (physical or mechanical) and time are required to achieve this transfer.

(b) In the vegetable tissue the pectic macromolecules are tightly attached to the other polymer components of the cell (polysaccharides, lignin, proteins) secondary valent bonds and covalent (ether, ester) bonds. These must be broken to release free, mobile pectin by using a dilute aqueous solution of mineral acids in the extraction.

(c) The swelling of fruit particles during the extraction process (mainly due to increased hydration of hydrophilic colloids in the cell) causes additional difficulties as a result of the narrowing of passages for diffusion of macromolecules. The effect of this factor should be reduced by distintegration of raw materials.

(d) The pectic molecules partly extracted from the fruit particles, together with the water molecules solvating the fruit particles, form a gelatinous coating. The coating thickness would be increased both by the low molecular carbohydrates available in the solution (especially with insufficiently washed raw materials) and by the presence of mineral acids. This coating hinders further free mass-transfer. The photomicrographs

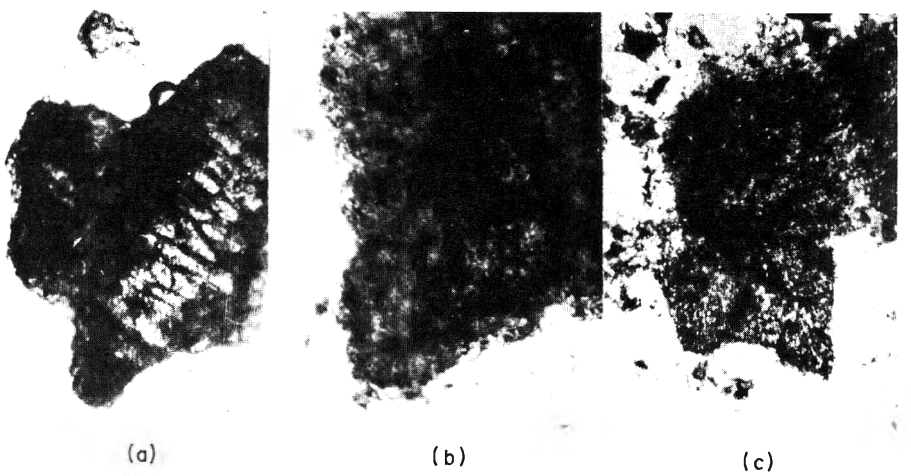

Figure 2. Photomicrographs of apple pomace particles during extraction using dilute nitric acid: (a) after 5 min, (b) after 25 min, (c) after 45 min.

of apple pomace under extraction process using dilute nitric acid (Fig. 2a–c) serves as some experimental evidence to support this assumption. Apparently, with the progress of the extraction process the gelatinous coating of a separate particle gradually changes, passing through a maximum during the first half of the process (Fig. 2b).

These specific features of the pectin extraction require multiple and prolonged extraction with a relatively high hydromodule (pomace:solvent = 1:40–1:50), and application of a hydrolysis catalyst (mineral acids such as hydrochloric, nitric and sulphurous acids).

New method and equipment for pectin extraction

The model of extraction of pectin shown in Fig. 1, and the special features of the process required substantial change to develop new technological solutions to the problems. Initially an appropriate mode of action was neded to treat the mass in order to loosen the bonds between the pectic molecules and the other components, to ensure their accelerated and sufficient removal from the extracting particles, and to accelerate the entire diffusion process of extraction with minimum energy consumption and processing time.

Innovative experiments led to a new method of periodic extraction of pectin under conditions of an intensive hydrodynamic field. The fruit mass was mixed with the extracting agents using a propeller homogenizer. The latter was operated periodically by a pre-set program and provided a fountain-like turbulent mixing at relatively high velocities ranging from 3 to 10 m/sec, while the whole mass was repeatedly transferred from zones of low pressure to zones of high pressure. Thus during a fixed time, the working mixture of raw materials and solvent was subjected to an intensive mass-transfer with a specific impulse action. A recent version of production equipment (Fig. 3), consisted of a vertical cylindrical heat-insulated vessel closed at the top with a lid and an access hatch, and equipped with a double conical bottom. The vessel was supplied with an internal cylindrical drainage wall made of perforated sheet steel completed with radial ribs, and the homogenizing device was located in the centre of the vessel. An operating propeller wheel with spaced turned paddles and cowling was mounted on the lower end of the vertical shaft which was enclosed in a special bearing fastened by curved ribs for directing the flow to a prolonged diffuser. The latter was connected at the lower end to a suction inlet fixed by bolts to the conical bottom. The lid had hot and cold water inlets, as well as with vents and charging holes. The downwards sprayer of the injector installation for vapour, gas, mineral acids and other reagents needed for the process, was suspended from the top. Under the upper lid, a shower device for washing the extractor was inserted. The driving gear of the homogenizer pump, controlled semi-automatically by programme setting to a two-speed mode, was mounted on the lid. In this version the extractor had the following operating data:

—net working volume	16 m^3
—height of working volume	3.5 m
—internal diameter of cylindrical body	2.54 m
—diameter of working wheel	0.4 m
—rotational speed of working wheel	720/360 rpm
—rate of circulation	0.360/0.180 m^3/sec
—mean flow velocity in the diffuser	3.40 m/sec
—power of two-speed motor	12/9 kW

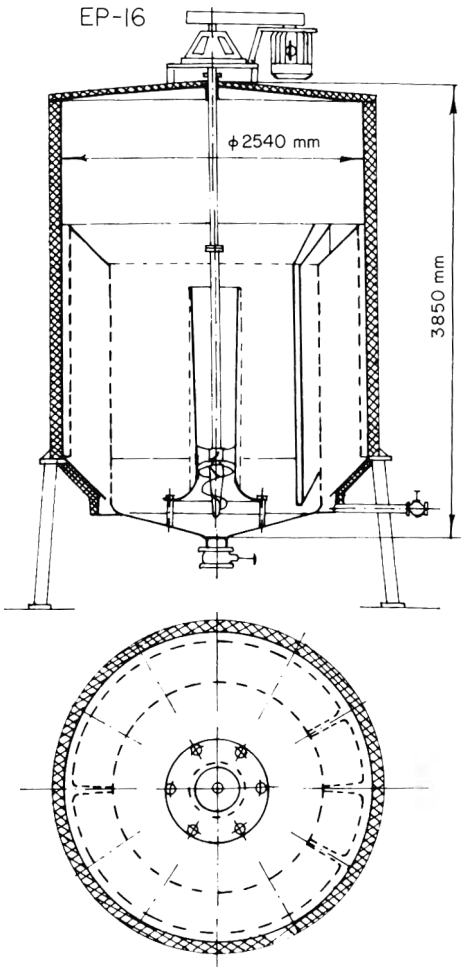

Figure 3. Construction of the extractor.

The extractor was fitted with a control and measuring unit for level, volume, temperature and pH.

The mode of operation and the performance of the equipment were as follows: the solvent and raw materials were simultaneously fed to the extractor while the homogenizing device ran at low rotational velocity. Under these gentle operating conditions a continuous mixing and homogenization of the working mixture was achieved. After filling up the working volume and adjusting the temperature and the acidity, using the injector installation, with feed-back to the control instruments of the extractor, the programme action of the homogenizer was activated. At a high rotational speed the working wheel applied its complete pumping power to the fluidized mass causing intensive motion within the entire working volume. The mass was sucked in from the bottom of the extractor and forced upwards through the guide ribs and the diffuser to the surface. The powerful flow of turbulent mass ran over the surface and sank down, and was flooded continuously by subsequent quantities of ejected mass. Upon reaching the bottom, the mass was sucked in again and thus underwent continuous circulation. A complex fountain and turbulent motion in vertical and radial direction in all zones of the

working volume was thus achieved. The high velocities, the relatively high rate of circulation, and particularly the impulse hydrodynamic action of the propeller on the fruit mass in the passage channel, ensured rapid and complete diffusion of the pectic substances. Alternation of the two speeds used the driving energy more economically.

The driving gear was either stopped manually or automatically by a programme. The extract was separated by gravity rapidly and completely due to the special drainage system. The hydrodynamic field created throughout the entire working volume of the extractor is indicated in Fig. 4a. The particles of mass followed the lines of the field in a circular motion. The approximate form of the trajectory of moving particles in the field of the extractor is outlined in Fig. 4a. The motion continuously varied in direction and magnitude of its velocity.

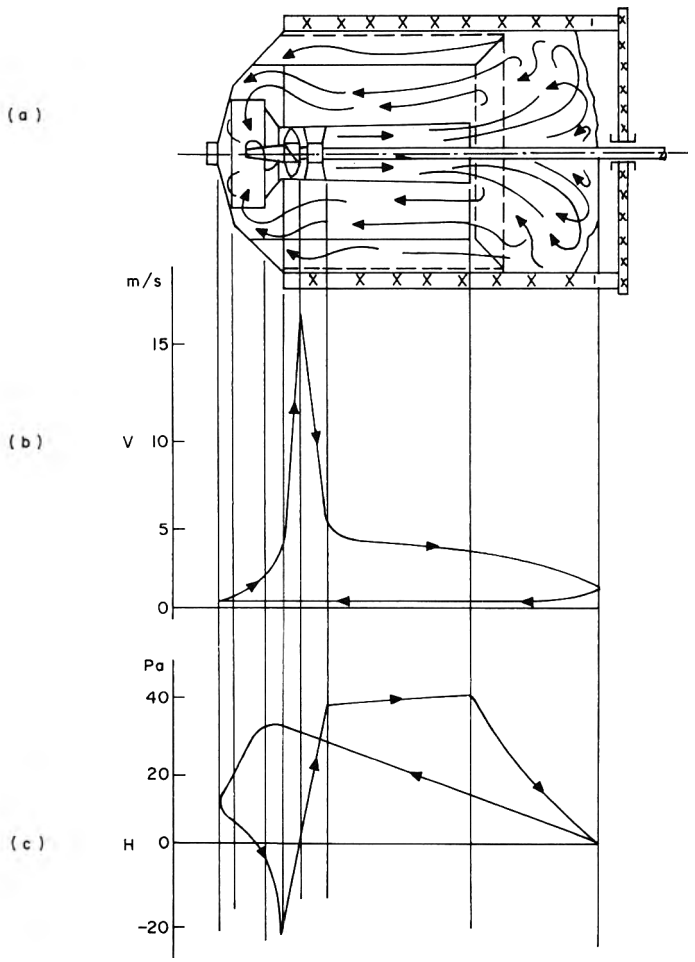

Figure 4. (a) Scheme of the hydrodynamic field. (b) Velocity of fluid. (c) Pressure of fluid.

The character of motion of the fluid flow was evaluated by the Reynolds number (Re):

$$\mathrm{Re} = \frac{c \cdot D}{\nu},$$

where: c = flow mean velocity; D = flow diameter and ν = the kinetic viscosity coefficient. The latter varied with the nature of the fluid and the temperature. For example, in water of 90°C, $\nu = 0.3\times10^{-6}$ m²/sec. In this instance $\nu = 4.2\times10^{-6}$ m²/sec was adopted. In the case of ascending flow through the diffuser:

$$\mathrm{Re} = \frac{3.2\times0.4\times10^{6}}{4.2} = 305000 \gg 2320 = \mathrm{Re}_{crit}.$$

In the case of descending flows:

$$\mathrm{Re} = \frac{0.08\,(2.54-0.4)\times10^{6}}{4.2} = 40762 > 2320 = \mathrm{Re}_{crit}.$$

In both cases turbulence ensured good rates of mass transfer.

As the particles of vegetable mass crossed different zones of the extractor, they were subjected to the action of variable flow accelerations and various hydropressures. Typical variation in the velocity of a particle on the trajectory for one cycle is estimated in Fig. 4b. This velocity determined the hydrodynamic pressure of the flow. Fig. 4c shows the variation in the hydrostatic pressure of the flow for the same cycle. Data were theoretically established from the laws of hydraulics, and experimentally confirmed by measurements of specific sections of the flow. The variation of these two factors in the course of a single cycle is of a particular interest. Based on operating data for an EP-16 extractor, the mean period of a cycle was estimated as:

$$T = \frac{V}{Q} = \frac{16}{0.360} = 44 \text{ sec}$$

where: V = volume of the extractor, m³; Q = rate of circulation, m³/sec.
The cyclic plots in Fig. 4b and c were transformed to a corresponding time-ordinate in

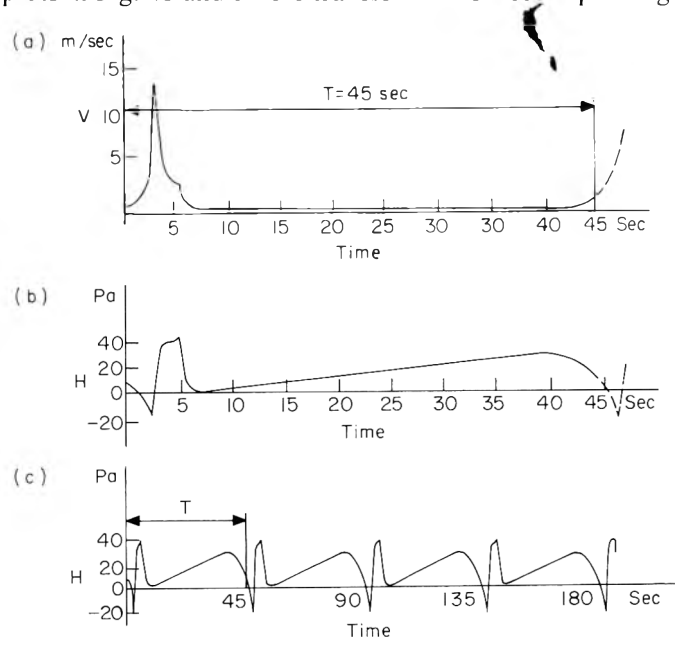

Figure 5. (a) Variation of the velocity of fluid for one cycle. (b) Variation of the pressure of fluid for one cycle. (c) Cyclic variation of the pressure of fluid during extraction.

Fig. 5a and b, where the position of the particles in the lower part of the extractor was adopted as the origin of the coordinates.

The character of the diagrams revealed a succession of brief dynamic treatment and subsequent more gentle and prolonged action. In the active part of the period the particles of mass received abrupt aceleration (Fig. 5a), when taken up by the propeller paddles, and then a deceleration when dashed against the flow-directing paddles of the diffuser. During the same interval, an abrupt change in pressure occurred from vacuum to maximum overpressure (Fig. 5b). This impulse action of pressure caused distortions in the cells, cracking and even breaking them, thus loosening the links between the pectic molecules and the tissue, and removing the former from the latter. The multiple repetition of these convulsions is shown in Fig. 5c. The changing acceleration and friction eliminated continuously the gelatinous coating formed around the particles (Fig. 2). The pectic molecules released as a result of this action were rapidly dispersed and homogenized in the turbulent flow. The physical nature of such treatment achieved the necessary requirements enumerated by the model, for the extraction of pectic substances.

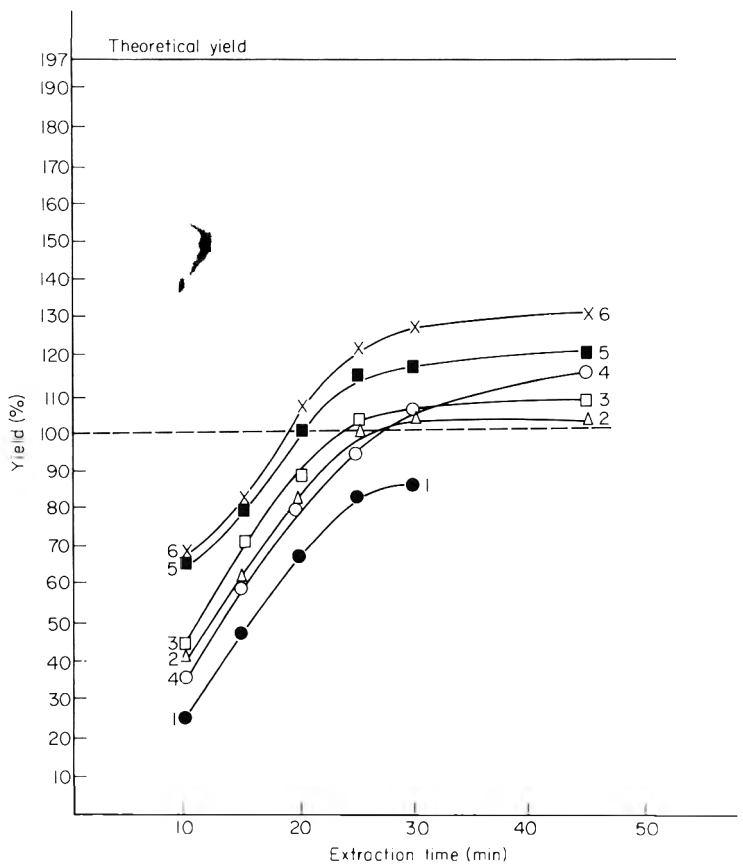

Figure 6. Effect of extraction parameters on pectin yield (1) 0.5%; (2) 1.0%; (3) 2.0% nitric acid at 70°C; (4) 0.5%; (5) 1.0%; (6) 2.0% nitric acid at 80°C.

Results and discussion

The newly developed method and equipment required were tested under laboratory and industrial conditions. The first series of pilot extractor experiments were conducted in order to study the effect of temperature on the process. The data in Fig. 6 represent pectin yield at 70°C (curves 1, 2, 3) and at 80°C (curves 4, 5, 6), the usual temperature range for pectin extraction. The tests were carried out using three acid concentrations (0.5, 1.0 and 2.0% nitric acid). An increase in temperature and acidity caused the expected acceleration of the extraction process. The data presented indicate that the yield of pectin increased to a maximum after about 30 min. Note that the limit of the acid-extractable pectin under the adopted conditions was considerably lower than the 'theoretical' yield estimated by the polyuronide content, but at the same time rather higher than the yield achieved by the conventional method marked as 100%. The data about the gel strength obtained from the series of pectic preparations (Fig. 7) show that the increase in the acidity at 70°C had no negative effect upon the gel strength in the whole time-interval studied, whereas the prolonged treatment of the mass at 80°C and at a high acidity (2% nitric acid) had a definite unfavourable effect.

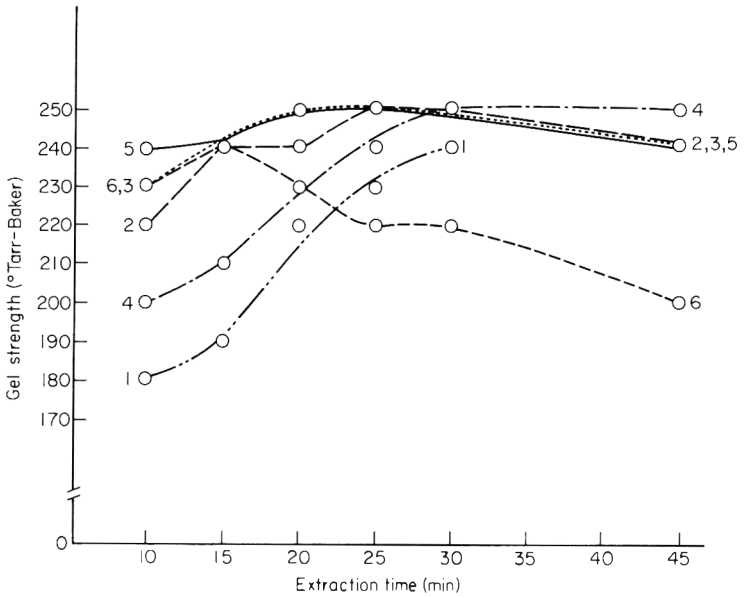

Figure 7. Influence of acidity, extraction time and extract temperature on gel strength of pectin (1) 0.5%; (2) 1.0%; (3) 2.0% nitric acid at 70°C; (4) 0.5%; (5) 1.0%; (6) 2.0% nitric acid at 80°C.

The next series of experiments were designed to study the effect of rotational speed of the propeller homogenizing device upon the extraction rates. Figure 8 presents part of the data obtained which shows that an increase in the speed caused only a minor increase in rate of pectin diffusion. Speed had a lesser effect compared to the medium, the temperature and the acidity. Therefore, the working mode of the propeller homogenizing device was appropriately chosen. Further experiments with an extractor having a capacity of 16 m^3 (Fig. 3) were carried out under industrial conditions. The results (Table 2) were in agreement with the results obtained under laboratory conditions.

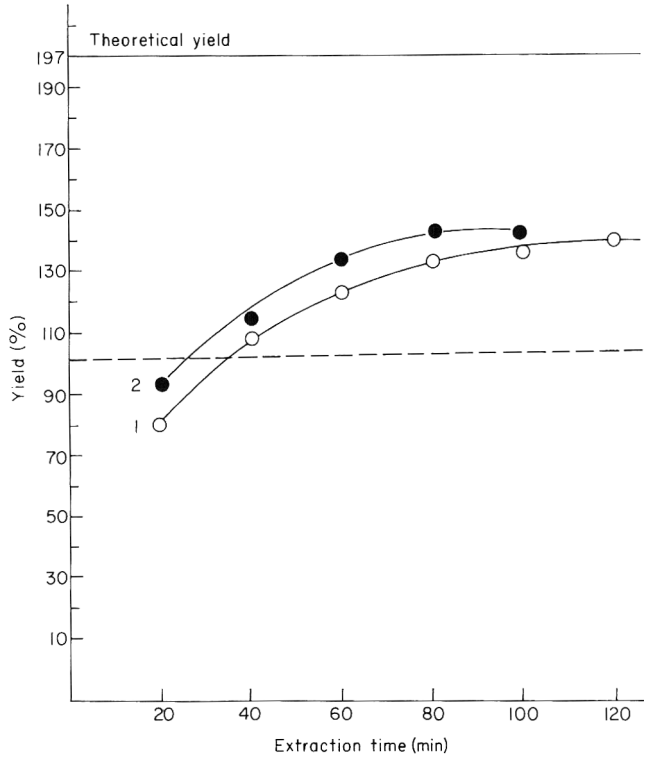

Figure 8. Effect of rotational speed of the propeller upon the extraction rate (1) 1570 rpm; (2) 2000 rpm.

Another peculiarity of the new method was the reduced energy consumption. The data in Table 2 show that increasing the hydromodule ratio increased pectin yield, but also increased energy consumption.

Table 2. Data from industrial experiments

Run	Experimental conditions	Pectin concentration in extract (%)	% of pectin yield in respect to standard	Energy consumption (%)
Blank	Triple extraction in conventional extractor (40:1 hydromodule)	0.21	75	100
1	Double turbulent extraction (20:1 hydromodule)	0.60	112	50
2	Double turbulent extraction (28:1 hydromodule)	0.43	121	62

Conclusions

These studies showed that the new method of pectin extraction, which was developed on the basis of pulsating turbulent homogenization of raw material, represents a rational technological solution characterized by the following advantages when compared to the existing methods and installations for pectin extraction.

1. The periodic running of the process provides optimum conditions for extraction in a turbulent state of mass-exchange. This corresponds to the specific features of the pectic raw materials, giving a high yield of pectin.

2. Other merits of the method were the versatility of the technological conditions, the easy and simplified control of the process, and the high reliability in servicing the equipment.

3. Energy consumption of this extraction process was substantially lower than conventional methods. Equipment was designed to provide fountain-like turbulent mixing of the vegetable tissue and the extracting agent, using a propeller homogenizer centered in a cylindrical vessel with drainage walls. Trials using this equipment gave an increased yield of pectin (30–60%), the duration of the process was reduced 2–5 times and the concentration of pectin in the extract was also increased (over 0.5%) compared to those of established processes.

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Mussel protein recovery—batch dissolved air flotation studies

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Summary

Batch dissolved air flotation is employed as the solid/liquid separation technique in the recovery of flocculated mussel protein solutions. Maximum flocculated suspended solids recoveries are obtained in all cases at an air/solids ratio of 0.02. Results show that the performances of the flocculating agents used, sulphuric acid, ligninsulphonic acid, Calgon and chitosan, are pH and dosage dependent with chitosan, having a protein recovery of 80%, being the superior material.

Introduction

The increasing demand for protein has led to intensive searches for cheap sources of protein. Because of the fecundity, rate of growth and wide distribution of cultures of mussels, they may provide an important alternative source of protein. On-going research is taking place into the development of a continuous process for the production of a protein concentrate from fresh sea mussels. Initial processing involves the size reduction of the whole mussels followed by the separation of flesh from shell by hydraulic classification in a grit tank. The liquid effluent contains the protein in the proportions of 40% w/w insoluble and 60% w/w soluble protein.

Suspensions of the proteins having micron and sub-micron particle sizes may be more effectively removed if they are caused to aggregate. This may be achieved by 'coagulation' and 'flocculation'. In engineering convention, coagulation is seen as the destabilizing process brought about by the addition of some reagent to a dispersion of particles in a continuous liquid phase in which it is possible for the particles to adhere and form flocs, whilst flocculation is the hydrodynamic process in which these collisions are brought about. In this paper the term 'flocculation' will be used to describe any phenomena that cause aggregation of particles.

Flotation is a very effective method of solid–liquid separation and has distinct advantages for the removal of low density particles which have a tendency to float. Various flotation processes are available, but the current study is concerned with dissolved air flotation. In this process a liquid stream is saturated with gas, primarily air, at elevated pressures. Reducing the pressure to atmospheric liberates small bubbles that attach to the solid particles or flocs, lowering the apparent density below that of water, allowing the bubble/solid agglomerate to float to the surface.

The paper reports research work on the assessment of various materials to precipitate and flocculate mussel proteins and the subsequent solid–liquid separation in a batch dissolved air flotation vessel.

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Materials

1. Ligninsulphonic acid (LSA)

Ligninsulphonic acid is a by-product from the sulphite wood pulping industry and is commercially available in a variety of forms and quality. The spent sulphite liquors contains wood lignins that have been made soluble by the introduction of sulphonate groups in the lignin. Ligninsulphonic acid is not a standard chemical, but a rather complex mixture of these sulphonated lignins plus minor wood extract impurities. Sulphonated lignins are large molecules (molecular weights up to 15000) which have approximately 50% of major repeating units (polymerized coniferyl alcohol) sulphonated, primarily on the aliphatic carbon attached to the aromatic ring.

In solution the sulphonate group is essentially completely ionized resulting in a negative charge on the molecule. It maintains this charge even at pH 2.0. It is this anion that confers its reactivity and potential as a protein precipitant and flocculant. It has been used successfully on numerous effluents from industrial waste waters. A few examples are: Tonseth (1968) Jørgensen (1971) and Hopwood (1980).

2. Calgon

Calgon is the registered trademark of Albright and Wilson's glassy sodium phosphates or, as one of several synonyms, sodium hexametaphosphate. It is a condensed phosphate, which are those containing more than one P atom and having P–O–P bonds. Calgon, when in solution, will undergo ionization as illustrated in Fig. 1.

With acidification the phosphate group continues to carry a negative charge at low pH values. Therefore, precipitation of soluble protein with Calgon in acidic aqueous medium will be a reaction involving the negatively charged PO_3^{2-} and PO_3^- groups and positively charged amino groups on the protein molecules.

The literature contains numerous examples of the application of condensed phosphates to the treatment of effluents from fish and meat processing plants at pH levels varying from 2.0 to 8.0, (Spinelli & Koury, 1970; Johnson & Peniston, 1971; Finley, Gauger & Fallers, 1973; Finley & Hautala, 1976; Graham & Yacob, 1975 and Cooper & Denmead, 1979).

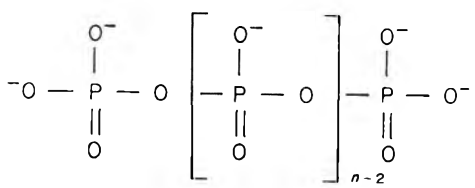

Figure 1. Ionized Calgon.

3. Chitosan

Chitosan is the product after the alkaline N-deacetylation of the naturally occurring polymer chitin. The principal repeating units are given in Fig. 2.

Chitosan is difficult to get into solution being insoluble in water and in organic solvents. It will, however, dissolve in dilute solutions of acetic acid. In solution the positive hydrogen ion from the acid will associate with the amino group in chitosan producing a net positive charge on the polymer giving it potential precipitation and flocculating properties.

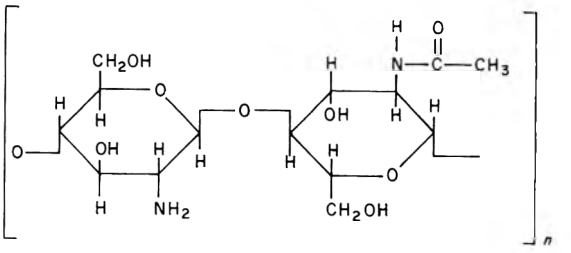

Figure 2. Principal repeating units of chitosin.

Chitosan has not experienced the wide application in waste water treatment unlike the other two materials. Adsorption processes to remove metal ions are reported (Muzzarelli & Rocchetti, 1974; Averbach, 1982; Sakaguchi & Nakajima, 1982) but little work on industrial proteinaceous wastes. An exception is Bough (1976) who reported suspended solids recoveries of up to 77% on effluents from meat and poultry processing plants.

The above materials were made up into the solutions:

(i) 5% w/w ligninsulphonic acid;

(ii) 5% w/w Calgon;

(iii) 1.66% w/w chitosan (in 5% v/v glacial acetic acid).

Experimental

The batch dissolved air flotation apparatus, which consists of a flotation vessel and liquid saturator, is shown in Fig. 3. The flotation vessel was constructed from Perspex, with a capacity of 2.25 litre, and a diameter of 0.14 m. A sample point was provided at the base for clarified effluent sampling. A lip was provided at the top of the cessel to enable the concentrate to be 'scraped off'.

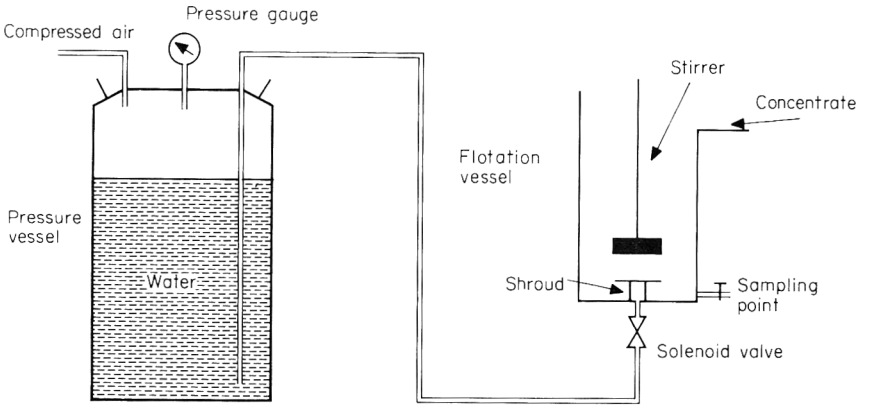

Figure 3. The batch dissolved air flotation system.

The stainless steel pressure vessel, have a capacity of 11.4 litres was two-thirds filled with distilled water. Air was introduced at an absolute pressure of 5 bar. Unless agitation was employed, poor saturation was achieved. The vessel was, therefore, inverted and violently shaken several times, a 'crude' but effective method of achieving consistent saturation levels, at the systems temperature and pressure, of 98%.

Discharge of the water from the pressure vessel was achieved by activating a time controlled solenoid valve. The constant elevated air pressure in the saturator forces the water at a controllable rate through the solenoid valve and into the base of the flotation vessel. Just before the water entered the flotation vessel it flowed through two 8 mm diameter eccentric orifice plates situated 10 mm apart. The purpose of these orifice plates was to reduce the pressure to atmospheric pressure at low velocities. A shroud section was also provided to further reduce the outlet velocity (Fig. 3).

In the flotation tests, proteinaceous samples contained in the flotation vessel are subjected to chemical treatment to promote flocculation. The floc is then tested for its ability to be flotated by the use of the dissolved air mechanism. The ratio of the volume of air-saturated water introduced to the volume of proteinaceous sample is referred to as the recycle ratio.

Test procedure:

(a) a bulk proteinaceous solution is obtained and well mixed;
(b) the bulk solution is sampled to assess its protein, suspended and total solids contents;
(c) 1.5 litre sample is added to the flotation vessel;
(d) rapid stirring (200 rpm) is applied for 1 min;
(e) the desired pH of the sample is obtained by the addition of acid or alkali;
(f) a known dosage of flocculant is added and pH readjusted if required;
(g) rapid stirring is maintained for 1 min;
(h) the solution is sampled to assess its suspended and total solids contents;
(i) slow stirring (20 rpm) is applied for 15 min;
(j) a controlled volume of air-saturated water is admitted to the flotation vessel;
(k) flotation is allowed for 15 min;
(l) clarified liquid and concentrate samples are obtained and assayed for protein, suspended and total solid contents.

A series of these tests were conducted at a fixed flocculent dosage but varying pH for each flocculant in order to obtain the optimum pH for maximum protein recovery. A second series of tests was undertaken at constant optimum pH but varying flocculant dosages. The protein contents of the solids and solutions were assessed by Kjeldahl and Biuret tests, respectively. The soluble protein of the untreated solution was assessed as the protein content of the liquid remaining after the untreated solution had been centrifuged for 10 min at 1.70×10^3 **g**. Insoluble protein was assessed on the solids obtained (the suspended solids) after the above centrifugation. Standard evaporation procedures were used for solids contents of concentrates and solutions. The results obtained on the clarified liquid (step 1) were adjusted to the volume of the untreated sample (step c).

Results and discussion

The initial processing, by grinding and hydraulic classification, of the mussels produced typical bulk solutions of the order:

(i) total solids contents of 4 g/litre;
(ii) total suspended solids contents of 1.3 g/litre;
(iii) protein contents of 2.5 g/litre;

Thus approximately 70% of the mussel tissue was in a soluble form and protein analyses revealed that about 60% of the protein present was in a soluble form. All the initial tests to assess the various flocculants were conducted at 50% recycle ratio.

Initially the bulk solution was tested for the effectiveness of acidification alone in recovering protein. A 10% v/v sulphuric acid solution was used and the results are shown in Fig. 4. An optimum pH of 4.5 was obtained giving protein, total and suspended solids recoveries of 54.1, 27.1 and 98.6%, respectively. The quality of the floc at a pH of 4.5 was very fine and fragile and the effluent obtained after flotation was slightly turbid. At pH values either side of 4.5 the flocculation fell dramatically with no noticeable flocculation at pH values of 2 and 7.

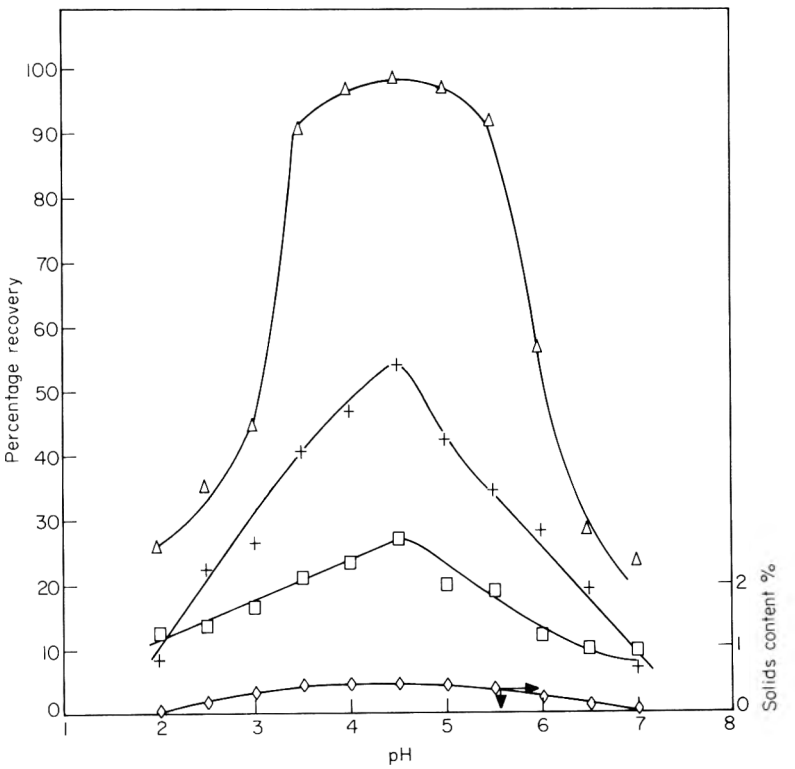

Figure 4. The effect of pH, using acidification alone, on the concentrate (◇), total solids (□), suspended solids (△) and protein (+) recoveries.

A poor maximum concentrate solids content of 0.45% was obtained at pH 4.5 because poor quality floc, which did not drain well, was being produced by acidic flocculation.

The mechanism of protein precipitation in operation is isoelectric precipitation. The net charge on the protein macromolecule is zero resulting in precipitation, but, because of the fragility and small size of the flocs, only 54.1% of the protein was recovered using the dissolved air technique. The small size of the floc reduces the possibility of bubble capture within the surface irregularities of the floc particles. Adhesion between the bubbles and particles must also have been poor.

The various chemicals were individually tested to improve protein recovery. With ligninsulphonic acid protein recovery improved to 62.8% at pH 2.5 (Fig. 5) and at a dosage of 330 mg/l (Fig. 6). Recoveries of total and suspended solids of 31 and 100% were obtained. Flocculation occurred immediately on the addition of ligninsulphonic acid and the floc produced at pH 2.5 was large and voluminous, forming a concentrate

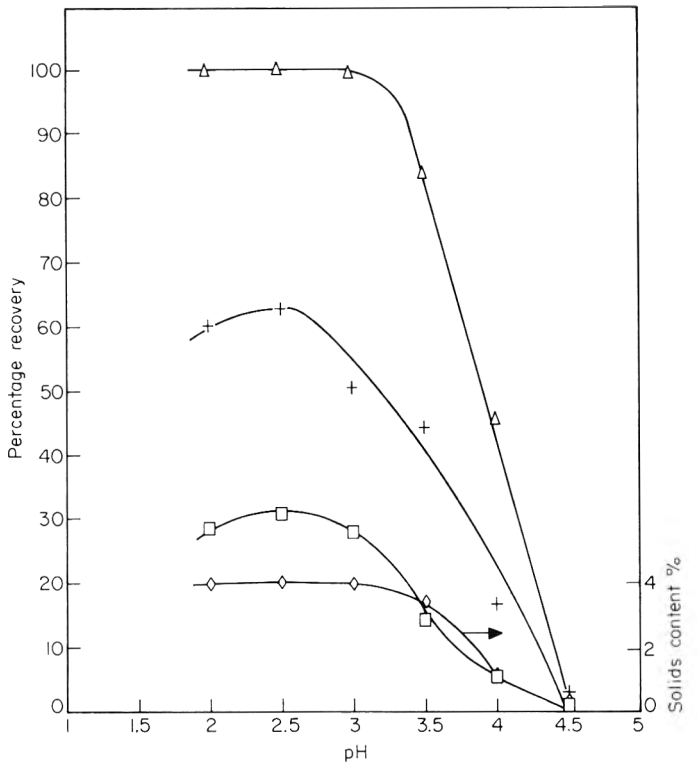

Figure 5. The effect of pH, using ligninsulphonic acid as flocculant on the concentrate (◇); total solids (□), suspended solids (△), and protein (+) recoveries.

which was compact with good self-draining properties. It had a maximum solids content of 4.2% w/w. At pH 2.5 with acidification only, a very poor concentrate of 0.2% solids was obtained with a protein recovery of 22.4%. The effluent obtained after flotation was clear. As the pH was increased above 2.5 the visual quality of floc deteriorated, and at the isoelectric pH little or no flocculation occurred; the protein recovery decreased to 3.0% at pH 4.5. The corresponding value for acidification alone was 54%. The presence of the negatively charged ligninsulphonic acid molecules solubilized the proteins by either producing organic micellar or solvation effects at the protein isoelectric point. Also at pH 4.5 the negatively charged ligninsulphonic acid took up positively charged amino groups on the proteins producing a net negative charge on the lignin–protein complex. An increase in acidity below pH 4.5 would be required to produce more positively ionized amino groups and lower isoelectric point of the lignin–proteins. Figure 5 shows this as maximum insolubility and floc formation, as protein recovery occurred at pH 2.5. This value can be taken as the isoelectric point of the lignin–protein complex, although 32.7% of the protein still remained in solution.

Calgon shows a similar trend to ligninsulphonic acid. The optimum pH occurred at 2.5 (Fig. 7) with maximum protein, total and suspended solid recoveries of 62.7, 30 and 100%. The optimum chemical dosage was again approximately 330 mg/litre. Visually the floc was not as good as that produced by ligninsulphonic acid and a poorer concentrate, 2.5%, at optimum conditions was obtained, although the effluent after flotation was clear. The mechanism of flocculation with Calgon is similar to that in the ligninsulphonic acid system, i.e. reaction between the positive amino groups and the

negatively charged Clagon along with a desolvating effect. However, comparison of Figs 5 and 7 indicates that desolvating effect was more active in the Calgon system. At a pH of 4.5, 41% protein recovery was obtained with Calgon compared with only 3.0% with ligninsulphonic acid. Flocculation is enhanced by a bridging mechanisms, which is a concept proposed in situations where charge neutralization effects can not be explained. In this mechanism, dispersed high molecular weight polymers (e.g. Calgon) are adsorbed onto the solid's surface then either compressed at the surface, leading to peptization, or collision of particles covered, to form bridges. In the latter case large flocs are formed having structured rigidity. A notable feature of this series of tests was the fact that at the higher Calgon dosages protein recovery was lowered; the quality of the floc deteriorated and there was a consequential drop in float solids concentration (Fig. 8). At these higher concentrations, the effluent after flotation was hazy. This could possibly be explained by the phenomenon of peptization where excess flocculant 'insulates' the coaguli formed and prevents bridging flocculation. Cooper, Russell & Adam (1982), using Calgon to precipitate fibrogen and haemoglobin, also found increasing precipitant dosages increased solubility of organic carbon. However, they reported that equivalent recoveries could be obtained by a subsequent lowering of pH.

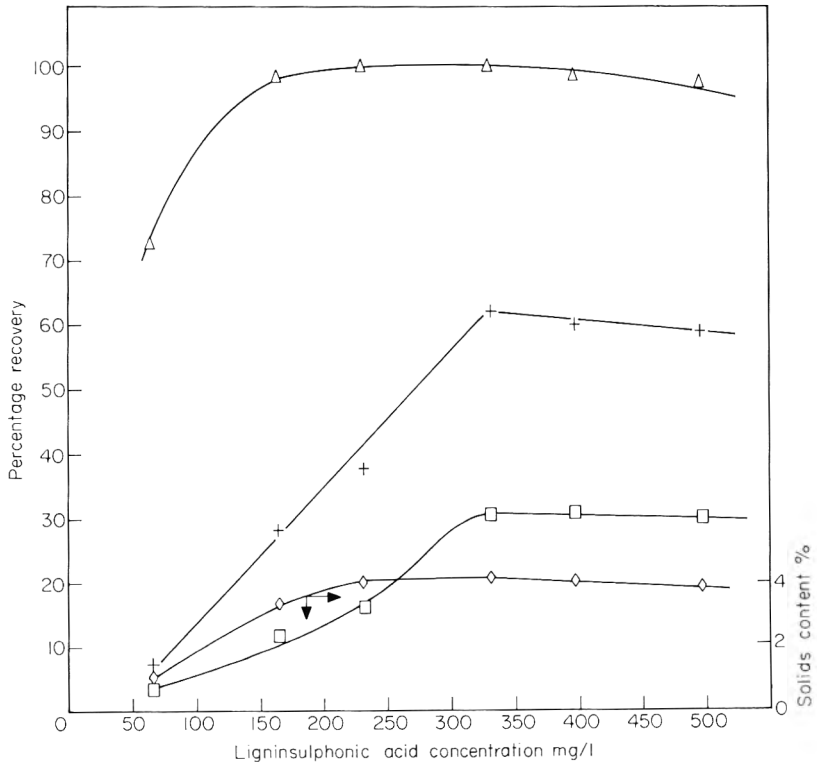

Figure 6. The effects of ligninsulphonic acid dosage, at pH 2.5, on the concentrate (◇), total solids (□), suspended solids (△), and protein (+) recoveries.

Chitosan presents a very different situation to those previously discussed (Fig. 9). A maximum protein recovery of 80% was obtained at pH 6.4 and total and suspended solids maximum recoveries were 60 and 100%, respectively; an improvement on all the other chemicals. A large voluminous floc was formed immediately upon chitosan

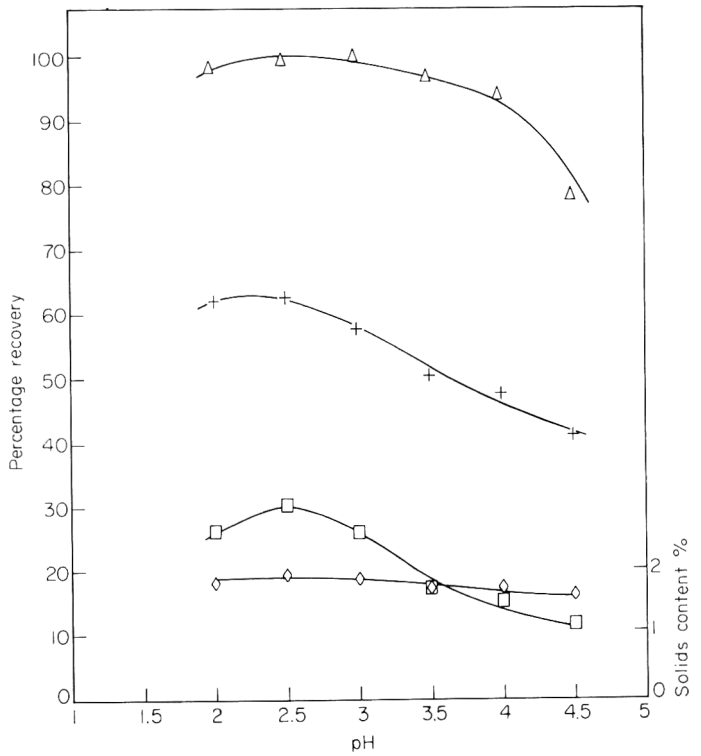

Figure 7. The effect of pH, using Calgon as flocculant, in the concentrate (◇); total solids (□), suspended solids (△) and protein (+) recoveries.

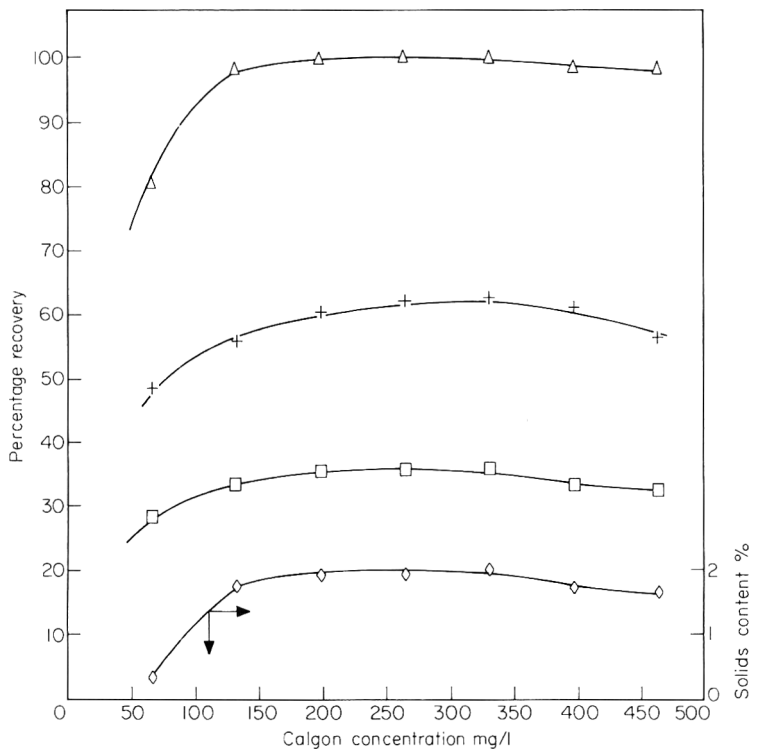

Figure 8. The effect of Calgon concentration, at pH 2.5, on the concentrate (◇), total solids (□), suspended solids (△) and protein (+) recoveries.

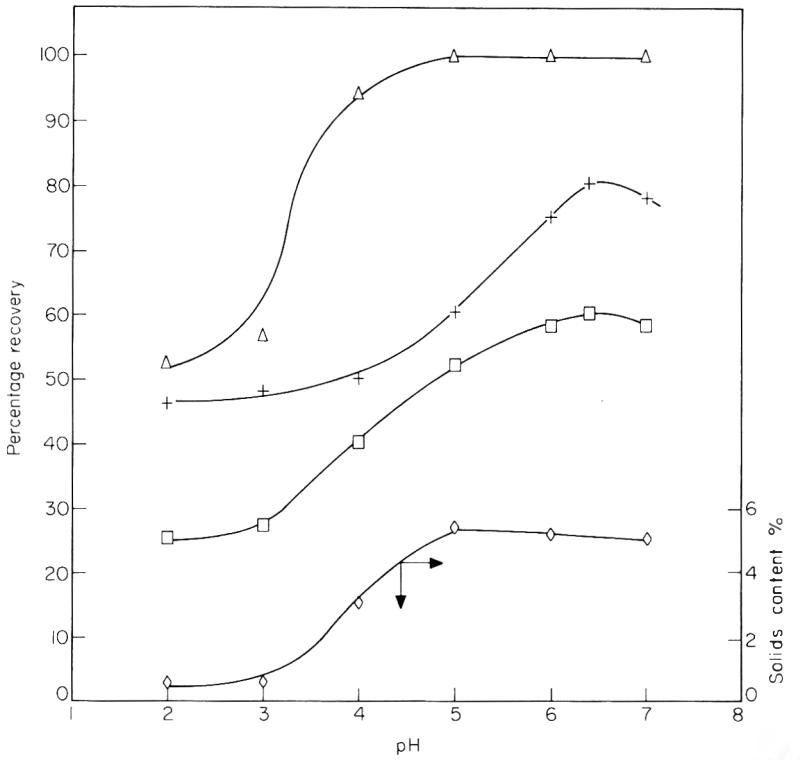

Figure 9. The effect of pH, using chitosan as flocculant, on the concentrate (◇), total solids (□), suspended solids (△) and protein (+) recoveries.

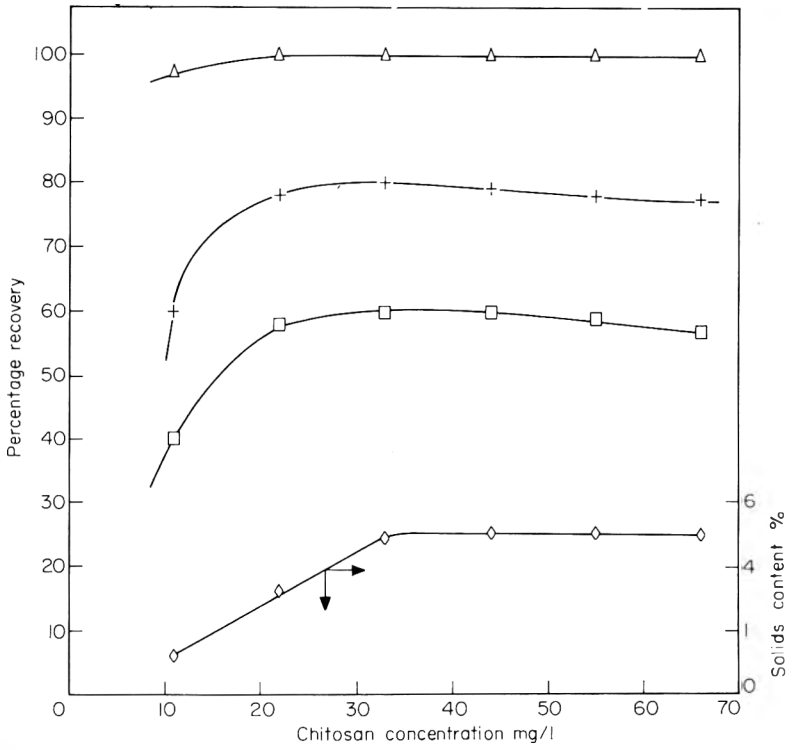

Figure 10. The effect of chitosan concentration, at pH 6.4, on the concentrate (◇), total solids (□), suspended solids (△) and protein (+) recoveries.

addition, and after flotation, a clear liquid and a compact concentrate having a solids content of 5% were obtained. Protein recoveries remained relatively constant at 45% at and below the isoelectric point of the mussel proteins, rising significantly as the mussel proteins become more negatively charged enabling them to react with the positively charged NH_3^+ group in chitosan. The latter comes from the repeating glucosamine unit, which may be regarded as a chelating ligand, which possess the important property of acting as bridging groups. It is suspected that this chelation is responsible for the precipitation of soluble protein by chitosan. As reasonable levels of flocculation and protein recoveries were obtained over the whole acidic pH range and because of the size of the chitosan molecules, bridging flocculation may also be taking place. No flocculation occurred in the alkaline region. Maximum protein recovery (Fig. 10) is obtained at a chitosan dose (30 mg/litre), ten times lower than either ligninsulphonic acid or Calgon, both of which give inferior protein recoveries and clearly establishes chitosan as the superior protein recovery agent (Table 1).

Table 1. Summary of batch flotation tests

	Optimum pH	Optimum dosage (mg/litre)	Total solid recovery (%)	Suspended solids recovery (%)	Protein recovery (%)	Concentrate (%) solids
Acid	4.5		25.2	100	53.5	0.45
LSA	2.5	330	31.1	100	62.8	4.17
Calgon	2.5	330	35.8	100	62.7	1.59
Chitosan	6.4	33	60.0	100	80.0	5.03

In addition to establishing the optimum conditions for flocculation, the technique of dissolved air flotation, which is a solid/liquid separation technique, requires sufficient air to flotate the solids. There are three basic methods of bubble-particle attachment, (Vrabilk, 1959):

(i) bubble capture within the surface irregularities of floc particles;

(ii) formation of flocs around bubbles;

(iii) adhesion of bubbles to particles.

The first two mechanisms require destabilization between particles and the third requires a destabilization between bubbles and particles.

The quantity of air introduced into the flotator depends upon the saturator pressure, the degree of saturation and the recycle ratio. The amount of air required for flotation can be related to the total suspended solids concentration in the flotator after flocculation by the air/solids ratio, A_s:

$$A_s = \frac{19.5\,P\,R\,f}{C},$$

where A_s = air/solids ratio, mg/mg;

P = absolute pressure in saturator, atmospheres;

R = recycle ratio;

f = fraction of saturation of air in water;

C = suspended solids concentration

The suspended solids was the total quantity of flocculated solids under conditions of optimum pH and flocculant dosage. A saturator pressure of 5 bar and recycle ratios of

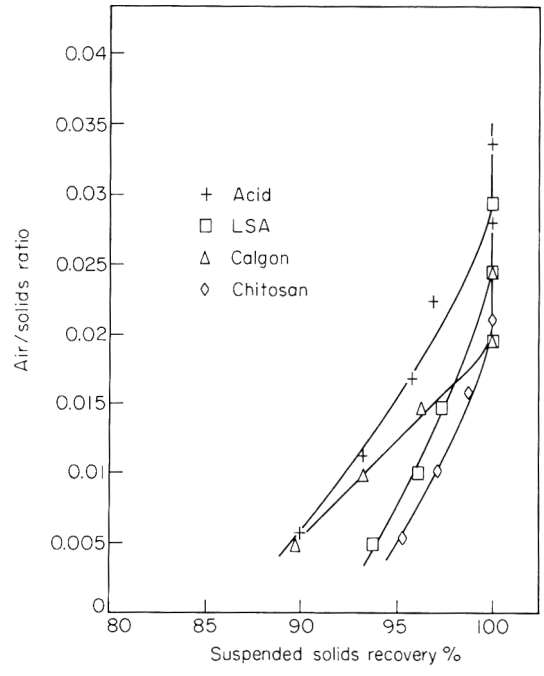

Figure 11. The variation of suspended solids recovery, using various flocculants, with air/solids ratio.

10, 20, 30, 40 and 50% were used. The relationships between air/solids ratio and percentage suspended solids removal and float concentration are given in Figs 11 and 12, respectively. With all the flocculants tested an air/solids ratio of 0.02 would give

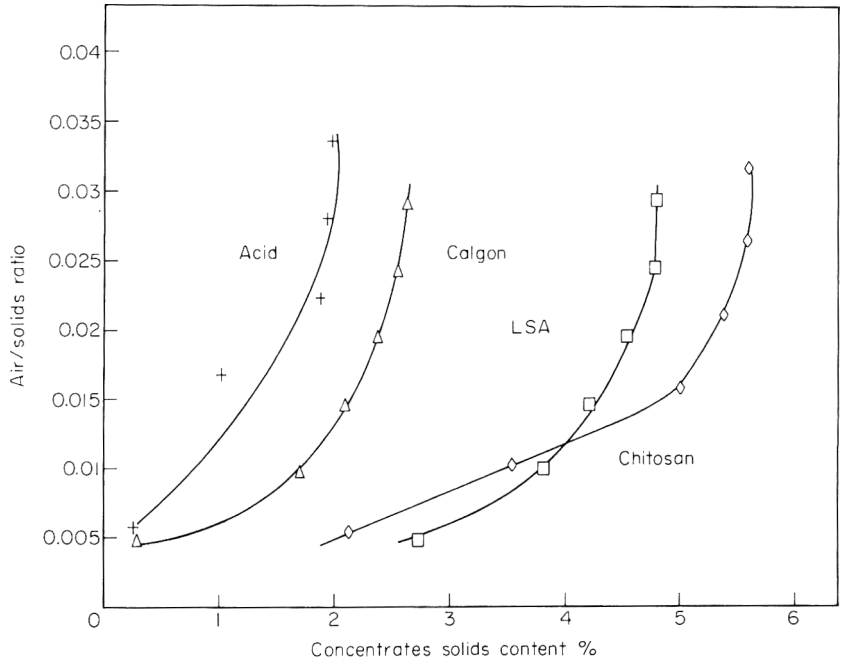

Figure 12. The variation of concentrates solids content, using various flocculants, with air/solids ratio.

100% removal of the suspended solids. As can be seen from Figs 11 and 12, chitosan again has a superior performance at corresponding A_s levels.

It was noticeable, in this study, that the quality of the floc seemed to influence the concentrates solids content directly. Visual observations made on floc quality assessed, as voluminosity, revealed that the best floc was produced by chitosan with the other materials decreasing in the following order:

Chitosan > ligninsulphonic acid > calgon > sulphuric acid.

This order is reflected in the sludge concentrations and also in their suitability for solids removal. It would seem, therefore, that the bigger or the better quality floc produced, the better the self draining properties of the concentrate obtained by flotation.

Conclusion

The batch studies, using dissolved air flotation as a means of protein recovery, have shown that the technique is feasible. The results show the importance of pH in the flocculation process and on the prevailing recovery mechanism. Of the materials tested, chitosan is shown to have a much superior performance in requiring comparatively smaller dosages but giving higher protein recoveries. Similar air/solid ratios, for optimum performance, are required for all the chemicals tested but the solids content of the concentrates formed vary with the individual chemical.

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Using classical psychophysics to determine ideal flavour intensity

R. L. McBRIDE* AND D. A. BOOTH†

Summary

In Experiment 1 a classical psychophysical method, the method of constant stimuli, is adapted to determine the most preferred flavour intensity of an orange drink. Twenty-five assessors were each presented with five concentrations of the drink, and were required to respond either 'too strong' or 'not strong enough', the notional concept of ideal-intensity serving as the standard in the comparison. All assessors exhibited an ideal-point within the stimulus range presented, and the overall estimate of optimum concentration was close to that of previous work. In Experiment 2, the method of constant stimuli was applied in orthodox manner, with the optimum concentration (as determined from Experiment 1) physically presented as the standard in a series of paired comparisons. Assessors were required to identify the stronger drink within each pair. The estimates of discriminability in Experiments 1 (the 'hedonic' just noticeable difference) and 2 (the conventional just noticeable difference) were found to be almost identical.

Introduction

The methods of classical psychophysics are primarily concerned with the determination of sensory differences. For example, the *method of constant stimuli* is traditionally applied as follows.

Assessors are presented with a pair of stimuli and are required to nominate the more intense with respect to a particular characteristic (e.g. for two sugar solutions the question might be 'which is sweeter?'). One member of the stimulus pair is the preselected (but undeclared) standard, the other is variable—either slightly more or slightly less intense. After many judgements (e.g. 100) of each of several pairs, the proportions of 'stronger' responses are transformed into normal deviates (z-scores, see Guilford, 1954, pp. 123–128) and plotted against log stimulus level (Rubin, 1976; Thurstone, 1928); a curve is commonly fitted by regression or probit analysis. Normal-probability paper, which positions the raw proportions on the y-axis such that their spacing corresponds to z-scores, may alternatively be used for graphical presentation. The stimulus level that evokes a 50% 'stronger' response is labelled the point of subjective equality (PSE), and the difference between the stimulus levels that evoke, respectively, 75% and 50% 'stronger' responses is known as the (ascending) just noticeable difference, or JND. The Weber ratio is given by JND/PSE. This method has been used previously on several taste stimuli (e.g. Lundgren *et al.*, 1976, 1978; McBride, 1983).

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Although traditionally confined to intensity judgement, the above methodology might, in some cases, be appropriate for hedonic assessment. Many taste stimuli exhibit single-peaked hedonic functions: that is, their pleasantness increases with stimulus intensity up to an ideal-point (breakpoint), then decreases with further increase in stimulus intensity (e.g. sugars; see Moskowitz, 1971). Assuming the assessor has a clear notion of what is ideal intensity, then he or she should, when presented with a number of different stimulus levels, be able to respond either 'too strong' or 'not strong enough'. In other words, the task would be analogous to that in the method of constant stimuli described above, except that the individual's *notional* ideal would serve as the pre-selected standard.

Single-peaked hedonic functions are ubiquitous among food and drink stimuli, and are not restricted to the taste modality. For example, an aroma may be too strong or not strong enough, a sample too hot or not hot enough, a texture too soft or not soft enough, and so on. Furthermore, there is now much evidence that the ideal-point for a given stimulus is indeed a clearly held concept (Booth, Thompson & Shahedian, 1983 Booth *et al.*, 1986; Frijters & Rasmussen-Conrad, 1982; Griffiths, Clifton & Booth, 1985; Mattes & Lawless, 1985; McBride, 1982, 1985; Moskowitz, 1972). Accordingly, this study explores the feasibility of using the method of constant stimuli to determine the ideal flavour intensity for a *group* of assessors.

When used in this role, the terms PSE and JND take on different meaning. The PSE, the stimulus level at which responses are split fifty/fifty, will now correspond to the ideal-point; and the JND will relate to hedonic discrimination rather than to pure intensity discrimination.

Experiment 1

Materials and methods

The stimulus material was an artificially flavoured orange drink mix ('Tang', Cottees General Foods) dissolved in distilled water. A previous investigation using this material (McBride, 1985) had suggested 15.0% w/v as the mean optimum concentration. Therefore, five stimulus levels were chosen to fall symmetrically about this value: 9.0, 12.0, 15.0, 18.0, and 21.0% w/v (0.6, 0.8, 1.0, 1.2, and 1.4×15.0%, respectively). Stimuli (80 ml) were served at 10°C in clear glass tumblers.

Twenty-five employees of the CSIRO Food Research Laboratory, most of whom were experienced in sensory testing, served as assessors. They attended four replicate sessions on consecutive days, providing one hundred judgements per stimulus. At each session the five stimulus concentrations were presented simultaneously. Assessors were instructed to drink a portion of each in the (individually randomized) order specified on the response sheet, and, after tasting each, to state whether the flavour was 'too strong' or 'not strong enough' (forced-choice). Rinsing with distilled water was mandatory before and between stimuli.

Results

Assessors reported the task to be straightforward, signifying that the notional ideal was able to function as a *de facto* standard. Figure 1 is a normal-probability plot of the percentage of 'too strong' responses (circles) against log stimulus concentration (see Guilford, 1954). The 21% concentration evoked a perfect (100%) 'too strong' response and therefore could not be included in the analysis; nevertheless, the remaining four

points, when transformed to z-scores, are well described ($r = 0.99$) by the semi-log regression

$$z = 13.32 \log C - 15.15 \quad (1)$$

where C is stimulus concentration.

From equation (1), the ideal stimulus concentration, which corresponds to the 50% ($z = 0$) 'too strong' response on the ordinate, is found to be 13.72% (illustrated graphically by the dotted line in Fig. 1). Similarly, by inserting the appropriate z value (0.675) in the regression equation, the concentration corresponding to the 75% 'stronger' response is found to be 15.42% (also obtainable graphically from Fig. 1). The difference between these two estimates, 1.70%, is the hedonic JND, and the hedonic Weber ratio = 1.70/13.72 = 0.12. It now remains to compare these hedonic parameters with those obtained in the equivalent intensity discrimination task.

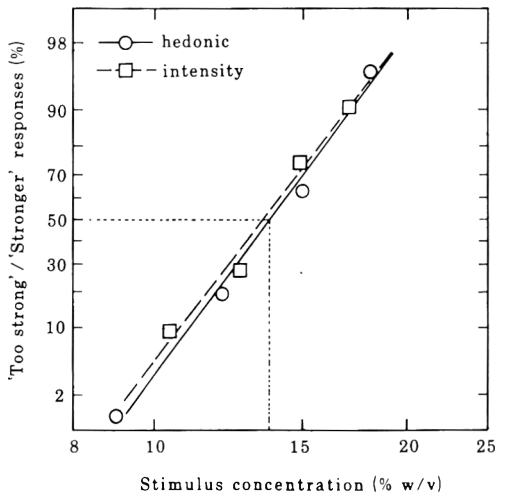

Figure 1. A normal-probability graph of the hedonic ('too strong') responses in Experiment 1 (circles), and the intensity ('stronger') responses in Experiment 2 (squares), plotted against (log) stimulus concentration. The best-fitting semilog regressions for the hedonic (solid curve) and intensity (broken curve) data are almost coincident. The ideal-concentration corresponds to the 50% 'too strong' response on the ordinate (dotted line).

Experiment 2

Materials and methods

In Experiment 2, conducted 6 weeks after Experiment 1, the method of constant stimuli was applied in an orthodox manner. Twenty-five assessors participated, twenty-one of whom had taken part in Experiment 1; the remainder were drawn from the same pool. At each of four replicate sessions over consecutive days, they were presented with four *pairs* of stimuli, one member of which was always the (undeclared) standard. They were required to taste each pair in the (randomized) order specified, and to identify the stronger stimulus with each. The concentration of the standard was 13.72%, the level determined as ideal in Experiment 1, with the four comparison stimuli distributed symmetrically about this value in ratios of 0.76, 0.92, 1.08, and 1.24 (the stimulus range was truncated to preclude the perfect discrimination that had occurred in Experiment 1). Stimuli were presented in opaque red tumblers to mask any possible differences in

colour density within stimulus pairs; in other respects the experimental conditions remained as in Experiment 1.

Results

Figure 1 gives the percentage of 'stronger' responses (squares) plotted against log stimulus concentration. The four data points are well described ($r = 0.99$) by the semi-log regression

$$z = 13.14 \log C - 14.83 \quad (2)$$

From equation (2), the PSE ($z = 0$) is found to be 13.45%. The concentration that corresponds to the 75% 'stronger' response is 15.14%, giving a JND of 1.69%, and so the Weber ratio = 0.12. These discriminantly estimates are almost identical to those in Experiment 1, and similar to other previously determined values for taste stimuli (Lundgren *et al.*, 1976, 1978; McBride, 1983; Schutz & Pilgrim, 1957).

Discussion

As demonstrated by Experiment 1, the method of constant stimuli is viable even when the standard in the pairwise comparison is purely notional. Assessors found the task straightforward, and all clearly exhibited an ideal-point: of the 100 judgements for the 9% stimulus, 99 were 'not strong enough'; conversely, all 100 judgements of the 21% stimulus were 'too strong'.

Convergence of different psychophysical methods

The present estimate of optimum concentration, 13.72%, is close to (less than 1 JND from) 15.0%, the estimate suggested by an earlier rating study on the same stimulus material (McBride, 1985). (In the rating study, assessors were required to evaluate various concentrations on a graphic rating scale, labelled 'Not nearly strong enough' at one end, 'Much too strong' at the other, with 'Just right' in the centre.) Furthermore, the responses in the present study appear to be normally distributed about the ideal-point; this accords with data from rating studies that show no change in slope or intercept at the ideal-point (Booth *et al.*, 1983). This result is of some importance to psychophysics, for it represents a further instance (cf. McBride, 1983) of agreement between direct (rating) and indirect (classical) psychophysical methods, traditionally held to be disparate.

Hedonic-intensity link

The estimates of discriminability from the ideal-point discrimination task (Experiment 1) and from the pure intensity discrimination taks (Experiment 2) were found to be almost identical. The simplex interpretation of this result is that, on a panel basis, the notional ideal in Experiment 1 functioned just like the perceived intensity of the physically presented standard in Experiment 2; assessors judged the same perceptual attribute in both conditions, but made a preference judgement in one case and an intensity judgement in the other. This result is also of some significance to psychophysics, as it again raises the possibility of a simple link between the hedonic and intensity attributes of a stimulus (Henion, 1971; McBride, 1985).

Further questions

The present method may, like the rating approach (McBride, 1982, 1985), be susceptible to range bias: presentation of a different range of stimuli might result in a different estimate of ideal. The present study is unlikely to be significantly range-biased, because previous work suggested appropriate concentrations. In general, however, pilot work would be needed to select the stimulus levels. Other ways of avoiding range bias include the use of monadic-sequential stimulus presentation (McBride, 1982), an individually applied 'staircase' approach (Booth *et al.*, 1983), or the use of a separate-groups design (Poulton, 1979).

Several questions remain. The present method provides a group estimate of ideal, but to what extent do the ideal-point, and discriminability about the ideal-point, vary within and between individuals? Recent data (Conner, Haddon & Booth, 1986) suggest that individuals' ideal points are normally distributed about log stimulus concentration. The confounding of intra- and inter-assessor variabilities in the present study precludes any such analysis, but it seems as though in this case variability within assessors was just as great as variability between: only seven of the twenty-five assessors in Experiment 1 responded in exactly the same way at all four sessions; only eight responded in the same way at the last three sessions. It also remains to be determined whether the present methodology is so readily adapted to other types of stimulus, and to what extent it depends upon the degree of familiarity with the stimulus material.

Notwithstanding these issues, the present study has demonstrated yet another application for classical psychophysics in sensory and consumer research. In particular, the method may have potential in product development. The task is easy to understand, requires only a simple forced-choice response, and estimates not only the optimum level of a stimulus component but also the tolerable deviation from that level.

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Book Reviews

Rice: Chemistry and Technology, 2nd edn. Ed by Bienvenido O. Juliano. St. Paul, Minn.: American Association of Cereal Chemists, 1985. Pp. xvi+774. ISBN 0 913250 41 4. US $87.00.

The present monograph updates that of 1972, with emphasis on recent developments, and is wider in scope, with more detail of Asian practice, particularly in the chapters on parboiling, milling, and rice products, though, with this a stated intention, it is a pity the universal term 'paddy' is not used instead of the American term 'rough rice'.

Such monographs are intended primarily as source books so the approach is detailed and prior knowledge of the basic sciences to first degree level is a prerequisite for the reader. This book covers all aspects of post-harvest rice, starting from the grain and its gross composition, its polysaccharides, proteins and lipids, and biochemistry—the Editor elected to write these parts and, as the world authority on rice chemistry, he has done a splendid job.

Parboiling (60 pp.) receives a complete and thorough rewrite by Bhattacharya from Mysore, giving the subject a more useful perspective by the inclusion of Indian technologies and costings. The overview of milling by van Ruiten covers all scales and techniques, from village upwards. The section on the comparison of milling systems (10 pp.) is far too short, as is this whole chapter (40 pp.). The reviewer would have liked the chapter to have also included the excellent material provided by Wimberly in *Rice Post-Harvest Technology* edited by Araullo, de Padua & Graham for IDRC (1976)—this was more practically oriented.

Drying is covered by Calderwood with Kunze as co-author. This chapter does not deal adequately with small-scale dryers designed for use in villages and small mills in developing countries, such as the IRRI designs, and the reader is referred to other publications. The book would have benefited from a section of, say, five pages, on this important subject.

Cogburn contributes a short chapter (only 23 pp.) on Storage—barely longer than in 1972. This could have been enlarged to include greater detail on entomology of storage pests, rodent types and their habits, and much more detail on modified atmosphere storage.

Juliano himself writes a comprehensive account of rice bran (42 pp.)—the best account the reviewer has read. It is amusing to note that the subject of solvent extraction milling, given 13 pages in 1972 and which has impressed a generation of students by its neat technology, is now given 8 lines as a discontinued process.

The reviewer has found this new edition up-to-date and very comprehensive. There are few books on rice with the IDRC manual mentioned above being an important and practical supplement to the standard monograph, of which the present book is the sole example devoted only to Chemistry and Technology.

The book is well-produced, clearly printed with good illustrations. The index, so far as can be judged from a short period of use, is accurate and covers the contents well (the 1972 edition was not free from errors). The binding does not seem strong enough for a large book intended for constant use. The reviewer's 1972 edition has been a bench and mill companion throughout the world, yet is still in fair condition. It is doubtful if the

second edition will stand up to constant use for such a long period: the quality of the binding is not up to the generally excellent quality of the contents. Nonetheless, an essential book and good value for the price.

D.A.V. Dendy

Interactions of Food Components. Ed by Gordon G. Birch and M.G. Lindley. London: Elsevier Applied Science, 1986. Pp. xi+343. ISBN 0 85334 398 5. £42.00.

This book is the proceedings of a Symposium held at the National College of Food Technology, Reading, in April 1985. As such the editors, publishers and authors must be congratulated on producing such a well-written text so quickly. Almost all food science and food technology is concerned with understanding, and controlling, the nature of the interactions between food components and thus the Symposium could, to some extent, have been very diverse and lack cohesion. This is not the case as the 16 papers presented are grouped in such a way as to make a logical sequence and appeal to scientists and technologists, manufacturers, nutritionists and psychophysicists working with food although the balance of the papers will obviously appeal to some readers more than others. For example there is only one paper (chapter 2) concerned with the interaction of water with food components although this area has of course been of major research interest for many years. Incidentally this short chapter is an excellent introduction to the topic and should be compulsory reading for all interested in the rather nebulous concepts of 'water activity' and 'bound' water in foods.

Chapters 3, 4 and 5 are all concerned with the possible interaction between minerals, especially iron, and carbohydrates or proteins. It is good to see rational chemical approaches being made to metal binding in foods and the attempts to interpret these results in nutritional terms. However one must interpret the results of such studies with care and ensure that the nutritional conclusions are based on a thorough understanding of both the chemistry and physiology of the system. Thus, although iron undoubtedly binds to many proteins and prevents its passage through an everted rat gut (chapter 4), *in vivo* such proteins will be digested and the low molecular weight iron complexes so produced may actually aid absorption; it is known that high protein diets aid iron absorption.

Chapters 6, 7 and 8 discuss the interactions between selected additives (antioxidants, nitrate and sulphur dioxide) and food components. All three chapters are concise up-to-date accounts of the role of these preservatives in foods. Chapter 9 is concerned with the interactions of food components affecting microbial growth and rightly stresses that much microbiological research has been devoted to determining 'minima' for growth of most microbes under laboratory conditions which may be irrelevant to food commodities and lead to unnecessarily harsh legislation. As the authors state 'increasingly there is a need for data bases, perhaps commodity based, to enable the shelf-life and microbiological safety to be estimated with respect to the microbes of concern. Food microbiology has failed to organize its resources to begin providing that information'.

Chapters 10, 11 and 12 are all concerned with interactions of importance in the development and perception of flavour and aroma in foods and specifically deals with phenol-protein interactions and their possible significance for astringency, physicochemical interactions involved in aroma transport processes from solution and interactions of non-volatile and volatile substances in foods. These chapters would be of

interest to workers in the appropriate fields but are of less general interest than the earlier chapters.

Chapter 13 is concerned with the functional properties of pectins in various food systems and may have benefited by a less specific treatment as the role and function of the pectin in many of the systems could be duplicated by other ingredients.

The last three chapters are concerned with interactions in specific foods and systems, namely milk components in food systems, of confectionery products and those between protein and lipid in bread dough. These three chapters, which take up about 25% of the text, are obviously of interest to workers in the relevant areas and are excellent, relatively concise reviews, which should be widely appreciated.

The book itself is well-produced, remarkably free of typographical errors and the legibility of the typeface and clarity of the illustrations make for very easy reading and, although not extensive, the index is adequate.

In conclusion, this book is a worthwhile addition to the ever-expanding literature concerned with food science and will find a place in many libraries but, one suspects, few studies or offices.

D.A. Ledward

Rheology of Wheat Products. Ed by Hamed Faridi. St. Paul, Minn.: American Association of Cereal Chemists, 1985. Pp. vi+273. ISBN 0 913250 42 2. US $41.00.

This book is based on contributions to the symposium of *Rheology of Wheat Products* as part of the annual meeting of the American Association of Cereal Chemists (24 September, 1985, Orlando. Florida).

Consumer acceptance of many wheat based products is dependent not only on how such materials respond to deformation but also to their rheological 'states' at various stages of processing. Currently there is increasing interest in the rheology of such products and, therefore, the appropriate assessment of rheological properties is of vital importance in controlling both their development and formulation.

This volume is intended to present 'an objective state-of-the-art discussion of various aspects of wheat rheology'. However, it in fact contains papers that take us from the more empirical testing traditionally used in the baking industry through to the more fundamental types of rheological measurements, which are finding increased application in the food industry in general.

The papers provide a very good introduction to the theory and application of wheat flour rheology, an interesting insight into the trend toward non-destructive mechanical testing and a useful source of up-to-date reference material. The inclusion of papers from some of the large industrial laboratories has ensured a good balance between the theoretical considerations and the more practical or applied aspects of cereal rheology.

In general the book is clearly written; however, it has been reproduced directly from the submitted typewritten copy and as a consequence has many of the problems associated with such material (Variable typeface, single margin justification, double margin justification etc). This is most obvious in the poor clarity of some of the illustrations.

While I would not consider, as the editor claims, that the book is 'state-of-the-art', it is a useful book and would be most helpful to anyone entering the field of cereal rheology.

A.E. Bell

Functional Properties of Food Macromolecules. Ed by J.R. Mitchell and D.A. Ledward. London: Elsevier Applied Science, 1985. Pp. xii+433. ISBN 0 85334 373 X. £52.00.

This is a book that comprehensively covers the major mechanisms of the interaction of food macromolecules. These components find significant usage in various sectors of the food industry, in particular, being used to control the rheological properties of many modern convenience food products.

Each chapter provides essentially a good review of the subject area. (The viscosity of polysaccharides and proteins; gelation of polysaccharides, gelatin and globular proteins, water and fat holding, foaming, emulsification, and texturization of proteins.) In these areas, recent developments are considered and their implications relating to our understanding of the functional properties of food macromolecules are examined. The references at the end of each chapter are both useful and generally well researched.

The various contributions from industrial, institutional and academic authors have ensured wide coverage both of the range of molecules considered and the underlying fundamental phenomena. In particular, attention has been given to the properties of aqueous protein–polysaccharide mixtures, a situation approaching the behaviour of multicomponent systems such as most foods.

The general layout design and appearance of the volume is attractive. There is an excellent subject index but no author-index; however, a list of contributors is given. Both the printed text and the figures are very clear, on the other hand some of the photographs have lost a certain amount of detail by having been scaled down. This is particularly noticeable in some of the electron micrographs.

Aimed primarily at food scientists and technologists, it is a book which is clearly written and, with some prior background in rheology, is very readable. It is a good companion to your copy of *Ferry* if you are interested in the functional behaviour of interacting food macromolecules.

A.E. Bell

Microbial Toxins and Diarrhoeal Disease. Ed by David Evered and Julie Whelan. (CIBA Foundation Symposium No 112). London: Pitman, 1985, Pp. ix+286. ISBN 0 272 79786 3. £27.95.

This is a detailed and specialized book containing the contributions and *verbatim* discussions of a Ciba Foundation Symposium held in 1984. I doubt if many readers will stay long with it unless they have an active interest in gut physiology and toxin action. A good knowledge of the subject is assumed by the author of the opening chapter, and this is the pattern throughout the book. However, the topics and their complexity are fascinating, so perseverance is rewarded. The book is well produced and has a detailed index. The text is clear, typographical errors few, and illustrations adequate (although labelling with some illustrations is too small). The cover bears a charming photograph of healthy, although apparently poor children—a firm reminder that the goal for those that research this field is the control of diseases that kill many children in developing countries. There are chapters on: introduction to diarrhoeal disease and involvement of microbial toxins; regulation of water and ion movement in the intestine; the mucosal barrier to attachment of bacteria and toxins to cell surfaces; cholera and other toxins which activate adenylate cyclase; heat-stable enterotoxins that activate guanylate

cyclase; possible mode of action of heat-stable 'b' enterotoxin of *Escherichia coli;* intracellular mechanisms for regulating secretion; role in watery diarrhoea of serotonin and other neurohumoral substances produced by *Entamoeba histolytica;* pharmacology of electrolyte transport and possible drug treatment for diarrhoea; salmonellosis; shigellosis; staphylococcal delta toxin as an enterotoxin; toxins that act locally in the intestine and that are produced by *Clostridium difficile* and other clostridia; nature and roles of future vaccines in the control of toxin-induced diarrhoeal diseases; and finally the Chairman's summing up.

One is struck by the complexity of the field, by the difficulty of isolating causal factors, and by the problems of relating findings *in vitro* to the situation *in vivo*. As the Chairman writes 'It is difficult to sum up the remarkable range and depth of things that have been reviewed from the many different perspectives in the meeting'. The reader will be rewarded by finding that microbes produce substances that act by mimicking human endocrine mediators; that components of mucus (and so, one wonders, components of food) may protect the host by competing with host cell surfaces for contact with gut pathogens and their products; and so on. This valuable collection of modern concepts and detailed information relating to gut physiology and diarrhoea should be in all university libraries and on the shelves of specialists working on the subject.

R.W.A. Park

Errata

Please note the following corrections of errors that occurred in the August issue of the journal.

On page 419, the title of the paper *should* read:

Review: Lactic acid: considerations in favour of its acceptance as a meat decontaminant.

On page 426, the entry in Table 4 concerning the Visser and Bijker (1985) reference 'Calf tongues' under the heading 'Aerobic colony (3d.30°C)' *should* read:

Initial count: 5.6, Reduction: 2.9.

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REFEREES

No reputable journal can operate without a willing, able and dedicated team of referees. The Editors wish to express their grateful thanks to the following for their help in this onerous but essential task. It makes a substantial contribution to science. We humbly apologize to anyone inadvertently omitted.

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JOURNAL OF FOOD TECHNOLOGY: INSTRUCTIONS TO AUTHORS

Original contributions relevant to the science and technology of food and beverages are accepted on the strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. Topics of only narrow local interest will not be accepted unless they have wider potential or consequences. Papers accepted become the copyright of the Journal, and may not be published elsewhere in whole or in part without the Editor's permission in writing. No page charges are levied.

Contributions are: *Papers*. These are reports of substantial research (typically <6000 words equivalent, including tables, figures, references), and comprise:

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Technical Notes (<2000 words). These describe smaller investigations and applications, useful techniques and apparatus. They have no summary, a brief Introduction, Materials and Methods, Results and Discussion, Acknowledgements and Reference. They can provide accelerated publication if needed.

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Arrangement and presentation. As the purpose of publishing work is to communicate it to others, it is necessary to design the arrangement and presentation to do this in the most efficient way. This requirement is often quite different from the sequence in which the work was done. The logical sequence is to describe a problem or need, analyse it in operational or testable terms, obtain the data, and then use them to clarify, solve or satisfy the problem or need. This is an excellent basis for both design and reporting of investigations, although in practice the path may be devious. This should not cloud the report, which should aim to clarify the situation for the reader rather than demonstrate how difficult and complex the work was. It is rarely necessary to present all data. It is much more difficult and time consuming to produce clear and concise reports than rambling and woolly ones, but the end result is of far greater value to the reader, both in the shorter time taken to read it and in greater understanding. Furthermore, it reaches more people, for many will not waste time on unnecessarily extensive reports.

Authors should very carefully consider the preparation of papers to ensure that they communicate efficiently. We are much more likely to accept those that are carefully designed and laid out, have few or no errors, are concise, and conform to the style and instructions. They will also be published with much less delay than those that require much scientific and editorial correction. Authors who are not fluent in written English should seek help from a fluent person before the final version is typed.

The Editor reserves the right to make literary corrections and to make suggestions to improve brevity.

Typescripts (three complete copies) must be single-sided, double-spaced and with wide margins. The title page should carry an informative title that reflects the content, a running title (<46 characters+spaces), the names of the authors, and the place(s) where the work was carried out. The full postal address of the indicated corresponding author must be given as a footnote. Up to six keywords or very brief phrases must be given to aid data retrieval and indexing.

Standard Usage, Abbreviations and Units. The Concise Oxford English Dictionary is the reference for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it is spelt out unless it is 100 or greater.

Abbreviations should be used sparingly, only when long or unwieldly names occur frequently, and never in the title; they should be given at the first mention of the name.

Metric SI units should generally be used except where they conflict with current practice or are confusing. For example, 1.5 l rather than 1.5×10^{-3} m^3 or 3 mm rather than 3×10^{-3} m.

Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units.

Common species names should be followed by the Latin binomial (underlined) at the first mention. For subsequent use the generic name should be contracted to a single letter if it is unambiguous.

Figures should be drawn boldly in black Indian ink on separate sheets of white paper. They should be drawn so that on 50% reduction they will fit within the type area, and that lines, figures and symbols will be still clearly legible and not overcrowded. Photographs must be unmounted, glossy prints that have not been retouched. Colour prints will incur charges to the author. All figures must be given Arabic numbers, e.g. Fig. 3, in the text and on the back of the figure, where the title of the paper and the authors' names must also be marked. Self-explanatory legends of all figures should be typed separately under the heading 'Legends to Figures'.

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Chen, J., Reineccius, G. A. & Labuza, T. P. (1986). Prediction and measurement of volatile retention during extrusion processing. *Journal of Food Technology*, **21**, 365–383.

Stone, H. & Sidel, J. L. (1985). *Sensory Evaluation Practices*. Pp. 56–59. Orlando: Academic.

Dubois, P. (1983). Volatile phenols in wines. In: *Flavour of Distilled Beverages* (edited by J. R. Piggott). Pp. 110–119. Chichester: Ellis Horwood.

Unpublished work must only be cited where necessary, and only in the text. Copies of references in press in other Journals must be supplied with submitted typescripts. It is essential that all citations and references are carefully checked before submission, as mistakes or omissions will cause delays.

Proofs will be sent to the corresponding author.

Offprints. Fifty will be supplied free. Further copies must be ordered when the proofs are returned.

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