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Man's food supply in ancient times: some aspects indicated by archaeological remains*

MICHAEL F. LUCK AND SAMUEL A. GOLDBLITH

Summary

The authors show how fresh interpretation of archaeological evidence can lead to new information on the developments of foods and their preservation.

Introduction

A study of the prehistory of the New World possesses many more questions than answers with only certain strands of human activity traceable through the maze of time. On the universal themes of man's existence – environmental adaptation, subsistence and settlement and technology – only subsistence has been the most difficult to chronicle with any degree of confidence. However, in order to make statements on methods of food preservation it is important to look at the ways in which dietary remains have been preserved from prehistoric times.

As an unnatural state, food preservation has haunted man from the beginning of time. It has created first an art, then much later, a technological process based on science, now a recognized field of study, and an existence of billions of people employed as non-agriculturalists. However, food preservation as discovered from archaeological remains has also produced surprising configurations and theories as to the basic principles surrounding food preservation. Analysis of the physical factors leading to the food preservation process seems to demonstrate emerging patterns of sameness whether intentional or otherwise.

A thorough search of any historical literature in archaeology will reveal that few individuals in the field have been concerned with the analysis or study of preservation. Generally archaeologists have tended to accept the state of

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preservation, good or bad, virtually as providential and of little or no significance. Actually an understanding of the processes which were responsible for it can be vital both for the successful conservation (laboratory preservation) and as circumstantial evidence' (Biek, 1963). One can locate volumes of data describing in the minutest detail the artifacts discovered and elaborate statements about the implications of material found; but no mention is made of the vehicle which allowed for its preservation, especially where conditions are not so obvious.

For example, in a contemporary edition of *American Antiquity*, an article concerning the diet of middle Woodland Villages appeared. The article was co-authored by one of the best known palaeo-ethnobotanists in the United States, Richard Yarnell (Yarnell, Munson & Parallee, 1971). Yarnell expended great effort trying to give quantitative analysis to dietary remains with only passing mention of the possible differential preservability of the artifacts. In fact, no mention was made as to why there was preservation at all.

However, man, like every member of the animal kingdom, has exploited the environment in order to survive. The assumption that environmental exploitation is one of the most vital aspects of man's behaviour has made it important to study man's dietary habits in order to gain a more thorough knowledge of his culture.

If through excavation a reasonably accurate statement can be made about a particular people's diet and methods of preservation, then a more reliable statement can be made about the activities of that group and the future of food production.

Dietary remains can provide reliable implication as to the relative development of its agriculture, i.e. did they have domestic animals. Dietary remains along with dating can produce a configuration showing the effect and direction of cultural drift as well as establish inter-cultural trading relationships. Even more basic than the aforementioned points, is man's relationship to his environment; what part of his natural surroundings did he find desirable and able to exploit.

In studying dietary remains, not only can the influences of nature upon man be seen, but also man's influence on nature. Man's development of greater yielding cereal grain is an excellent example. Man, it can be assumed, was the most likely factor in the development of six-row barley from two-row barley. (Halbaek, 1970). These are but a few of the many inferences that archaeologists can make when dietary preservation is found.

There is a paradoxical relationship between dietary materials and preservation. In order for a foodstuff to potentially be a part of an individual's diet, it must possess properties that allow it to quickly and easily change physical states. To this end, if man consumes a particular substance, then so can nature in its normal functioning; thereby rendering it archaeologically indistinguishable. Regarding this difficulty, nature has on occasion preserved for us portions of early man's diets in seemingly 'unnatural' surroundings or conditions that will not support life. If life cannot exist in particular

conditions, then it is very unlikely that decomposition can occur either. Nature has also cooperated with archaeologists by providing certain parts of edible food differentially consumable and some parts not consumable at all; reference here is made to the structural components in the composition of plants and animals in the form of bone for animals and xylem for plants. In addition, nature has provided investigators with non-food functioning parts of plants with properties that render them most defiant to agencies of decay, e.g. the outer shell of pollen and spores which are not used as food, but indicate that a particular plant species was in existence. All of these things decompose at a slower rate than valuable foodstuffs under 'normal' conditions and are more likely to be in evidence in archaeological sites.

In studying dietary preservation it is important to know the agencies of decay under normal conditions. In this regard, preservation depends upon minimizing three types of changes: enzymatic, chemical, and microbiological. The first agent of decomposition is *comminution,* the reducing of matter to smaller dimensions. Comminution may have been the cause of death of the item. Man may harvest a plant like greens – this is one form of comminution – ingest it where it is further broken into smaller pieces, void it, and then have soil fauna do the same process until it has been rendered indistinguishable. In this process the chemical composition has not been changed. The second agent of decomposition is *chemical change,* i.e. digestion. In digestion soluble nutrients are dissolved out of the dead tissue and absorbed by the feeding organism. The digestive juices contain chemicals and enzymes which render the material more soluble. Protein, for instance, will be hydrolysed to absorbable amino acids. Naturally, the digestion may be either internal as in the case of man and other animals, or external as with organisms such as fungi and bacteria which live on the dead tissue. The third process of decay is the *loss of soluble substances.* This is always liable to occur when material is lying on the ground. This is the leaching out of broken down products from the dead tissue that has been caused by other organisms. A fungus has decayed a piece of wood, however it is still distinguishable as a piece of wood; but then rain comes and washes the dissolved material away, making it no longer distinguishable (Dimbleby, 1967). Many organisms and especially bacteria are very responsive to their chemical surroundings. In fact, some bacteria are unable to flourish in acid conditions, however fungi can thrive. This means that in acid soil micro-organism decomposition is mainly fungal.

Having discussed decomposition, one can establish that preservation is an unnatural state, and that under normal conditions an item would very soon be chemically changed and physically unrecognizable. The time that it takes to completely decay an item beyond recognition is relatively short when considered on a geological time scale. The time may vary from a few days to many years, but it still remains a short time. Indeed, most consumable material takes a very short time to decay, usually hours or days. This means that in order for an item to appear in an archaeological site that can be called old, two steps in preservation must occur. The first is that the item must survive the immediate

short term period of normal decomposition which most items do not. A preserved object that has survived either changed states so that it was resistant to decaying agents, or it was placed in an atmosphere that was not conducive to decay. Carbonization is an example of the former and freezing is an example of the latter. After an item has survived the normal short period in which most decay occurs, it must survive the time passage from antiquity to excavation. This can better be described in terms of things that do not happen to the item, i.e. carbonized material can burn or be crushed, frozen material can thaw.

Dietary remains can be found in two states, either as artifacts or as chemical remains. The former is the easiest to recognize and the latter is by far the most common.

Artifact remains can be placed into two categories and then subdivided. The first two general divisions are 'changed' and 'unchanged' artifact remains. This division is based largely on the manner in which a remain survived the first step of the two necessary for preserved recognition. Unchanged can be defined as no observable state change from when a foodstuff was a possible dietary constituent. The definition can include coprolites and digestive tract remains of preserved humans as in the case of the *Bog People* (Glob, 1971). A 'changed' dietary artifact is one in which some agent has altered the original state which caused it to survive and become recognizable from antiquity until excavation. An example of this is carbonization.

Having discussed the conditions necessary for life, aerobic conditions, moisture, temperature and proper chemical surroundings (Dimbleby, 1967), the converse of that statement is also true; if one of these conditions does not exist, then life will not exist, hence preservation will occur. To this must be added that the state cannot fluctuate in the passage of time or life will begin and hence decomposition will occur. Sometimes more than one of these conditions exists at the same time. All of these conditions have to be considered as not normally occurring circumstances.

The first conditions of archaeological significance to preservation of dietary artifacts is aridity. Aridity has produced the so-called mummified grain from Egyptian tombs. This is the nearest we can get to the original state of the food from antiquity. In this state of complete lack of moisture, grain remains phenomenally preserved even with the tiny hairs on the kernels. Basically, dehydration reduces the water activity below the level at which microorganisms will grow and chemical/biochemical changes occur. In all probability, the viability of archaeological materials in a preserved state is due to the initial removal of moisture and the maintenance of that level by low humidity storage. This type of storage keeps enzymatic activity and biochemical changes to a minimum, inasmuch as water is a necessary component of both types of changes. In this regard, dehydration may be the oldest type of preservation. For this reason, aridity is the primary factor in the preservation of most coprolites and dry caves are the primary place they are found.

Another condition which has lent itself well to archaeological preservation has been the peat bogs. The opposite effect of the arid Egyptian tombs has

created very remarkable preservation. The peat bog presents a state in which moisture has sealed the artifacts from contact with air and therefore halted decomposition (Halbaek, 1970). Another possible reason for bog preservation may be a polyphenol effect of humic acid (Biek, 1963). Regardless of the reason for the magic powers of peat bogs, there is certainly something in the mixture which contains both anti-microbial as well as preservative compounds, e.g. formaldehyde.

Some of the best dietary remains from peat bogs have come from the visceral contents of peat burials. At this point, a comment about visceral remains and coprolites would be appropriate. When remains are found of plants or animals that would possibly be sources of food in antiquity, it is easily assumed that they were used for human consumption. One does not have to look very far to find examples where this does not always correspond. Many South Pacific inhabitants raise large numbers of pigs and no doubt there are numerous swine remains left behind. However, swine does not constitute a major constituent of their diet because pigs are considered money and only those who by their standards can throw away money can afford to eat pigs. Archaeologists are sometimes guilty of making inferences about diet based on finds that are not associated directly with diet. For instance, if there is no preservation associated with burial and no other association found, is it reliable to infer that this was a staple product of their diet, although it may be only a product strictly used in burial rites. This is the major advantage of coprolites and visceral remains analysis. There can be no question in studying coprolites and visceral remains of what is actually consumed by people of that culture. However, a drawback is that preservation may represent a staple product of the culture or an unusual dietary combination. The truth about such questions is the secret antiquity holds and man can no more than infer, with his inferences no better than the material he has to work with. Therefore, visceral remains and coprolites are one of the best tools for making proper inferences.

Visceral remains have not been the only ones recovered from bogs. Bogs have proven to be food preservatives of other types of dietary evidence. Small fruits have been found preserved in bogs with some of the meat still connected to the stone.

'Nevertheless, there is a strange power in bog water which prevents decay. Bodies have been found which must have lain in bogs for more than a thousand years, but which, though admittedly somewhat shrunken and brown, are in other respects unchanged' (Oldsag Committeen, 1837).

Strangely enough, skin, hair and brains are best preserved whereas bones will completely decalcify and disappear if peat is permeated with water. Brains generally are preserved for a much different reason than one would suspect. In the brain tissue chloresterin can be found which is a chemical insoluble in water. It is basically a matter of the soil acids associated with the bog environs. Strong soil acid destroys bone tissue.

A relatively recent find at Damendorf in Schleswig provided a body with skin and internal organs so well preserved as to defy explanation (Glob, 1971).

Whatever the cause it is sure that the tanning process of bog water is an area worthy of further exploration and study. However, we do know with some degree of assuredness that the process concerns acid in the water and the total exclusion of air.

Other examples of moisture causal preservatives unrelated to bogs occur. The tombs of Noin Ula in Russia are an example of how water flooding a recently filled grave, thereby preserved the material inside (Halbaek, 1970).

Temperature is another indispensable factor necessary for the existence of life. It is much rarer than the two already mentioned, but the Pazyryk nomad tombs are examples of objects being frozen from burial to excavation presumably because of perma-frost (Halbaek, 1970).

G. W. Dimbleby in *Plants and Archaeology* considered aridity, aerobic conditions and temperature as 'normal environmental factors which influence preservation'. He stated that chemical surroundings are 'special circumstances which can favour the preservation of organic remains' (Dimbleby, 1967). It is difficult to understand his reasoning for separating this factor because a state of complete aridity, anaerobic bogs, or perma-frost graves are not functional areas for the furtherance of any type of life. The effect is the same as a chemical environment that is not conducive to life. There are two major areas where the chemical environment differs from the first three mentioned. They are: (1) the first three are state of absences (absences of air, moisture and temperature) while the latter is a state of presences of a preservative, and (2) the first three are much easier to detect (peat bogs are known well before excavation) but polyphenol effects may not be found until excavation is in progress.

The first adverse chemical environment is the presence of copper. Relatively small concentrations of copper can prevent the activity of microorganisms in conditions that are otherwise favourable for their growth. Because of copper's toxic effect, dietary remains have been found in association with copper artifacts or possibly in old copper mines (Biek, 1963).

Another adverse environment for microorganisms is salt. Salt mines have yielded evidence of perishable material as well as dietary material (Biek, 1963).

Another group of chemical preservatives are polyphenols. This group is probably a major contributor for much of the present preservation that is found of very perishable material (this phenomenon has only recently created much interest as to its functioning because of peat bogs). Polyphenols are organic toxins made of vegetable materials, usually of oak, bark or gallnuts. Sometimes the word tannins is used to denote the same products. Early man in many locations knew the effects of tannins and used them to preserve leather. This caused hides to resist decay more easily. Since tannins are contained in many different vegetable substances, their total effect is most difficult to ascertain. About the only thing that is known for sure is that polyphenols do exist and have a profound effect on preservation. However, at this time which polyphenols work and the amounts necessary to preserve are mostly speculation. Another contributing factor to the confusion about the effect of polyphenols is that polyphenols do not have to be found in association with the

artifact in order to cause preservation. In other words, an artifact could have been in contact with a polyphenol prior to burial and absorbed enough of the toxin to preserve it and thus lie preserved with no association with the surrounding chemical state, except that small amount that is in direct contact relationship to the object.

Man's early attempts at preservation were primitive but essential. It is clear from archaeological reports that physical factors in our knowledge of man's food supply in ancient times leads to the following theories: First, preservation was a process which resulted when foodstuffs were saturated or immersed in specially treated moisture fields or placed in fields which were totally devoid of treated moisture. Secondly, preservation was an *essential* prerequisite for early man to master in his transition from hunter-gatherer to settled agriculturist.

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Potential of gamma irradiation of fruits: A review*

JAMES H. MOY

Introduction

The perishable nature of most fruits is due to insect infestation, physiological changes, post-harvest fungal breakdown and other pathological diseases. Besides canning or freezing, storing the fruits at refrigerated temperature and coupled with controlled atmosphere have been the techniques of extending their marketable life.

The demands for refrigerated storage of fruits grown in the tropical and subtropical regions are much higher than in the temperate zone because of higher ambient temperature and higher relative humidity. These tropical fruits are invariably harvested at a higher temperature before storage. Available facilities for cold storage are usually less than adequate, partly because of the need to accommodate other commodities, partly because of economic factors.

With a few exceptions, tropical fruits are prone to fruit fly, and some to seed-weevil infestations. Shipment for export market must conform with established quarantine procedures for various fruits in different countries.

The existence of these problems strongly suggests the need for a good preservation technique for fresh fruits.

Requirements of a satisfactory preservation technique

There is probably no such thing as a 'perfect' preservation technique for fresh fruits. The use of low temperature and controlled atmosphere is logical because physiological changes in a fruit are slowed down. But it is ineffective or inadequate to control insect larvae and their eggs, fungal and bacterial diseases. There are also different results and varied reactions in the use of controlled atmosphere on the fruit quality such as that of apple.

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A very recent concept of fresh commodity preservation is the use of sub-atmosphere, better known as 'hypobaric' storage. It seems to be a sensible approach because fruits stored in a partial vacuum will have a lower respiration rate, and bacteria and fungi probably would not grow as well. There might even be adverse effects on the insects and their eggs, which remains to be investigated.

A good preservation technique for fresh fruits should have the following characteristics: (1) it should have a thorough, controlling effect on insect larvae and eggs on the fruit, (2) it should not affect the qualities and nutrients of the fruit, (3) it should be very efficient, and (4) it should have a synergistic preservation effect on the fruit if and when combined with other preservation techniques.

Ionizing radiation, more commonly in the form of gamma-radiation as a post-harvest, pre-shipment treatment meets the above mentioned requirements more than any other known technique including 'hypobaric' storage.

Usefulness of low dose gamma radiation treatment

The most logical and sensible use of low dose gamma radiation for treating fresh fruits is for (1) disinfestation and (2) shelf life extension. The third use, pasteurization, which is mostly for fungal disease control, is technically feasible but sometimes offset by some undesirable effect on the fruit quality, casting doubt on the economic feasibility of the treatment.

The two currently accepted quarantine procedures used for treating Hawaii grown papaya and other fruits and plant materials for export markets are as follows:

(1) *Vapour heat treatment.* Raising the fruit tempeature to 43.3°C by means of heated air saturated with water vapour (Jackson, 1971). The time needed to complete treatment of papaya is 8–12 hr.

(2) *Fumigation.* Ethylene dibromide (EDB) is volatized in approved chambers equipped with a heating device and fans to circulate the gas in the chamber every 3 min throughout the exposure period. The time required to treat papaya is 2 hr followed by 1 hr of aeration. Methyl bromide (MB) may be used according to prescribed dosage, exposure and pressure as listed for each commodity in the USDA Plant Quarantine Treatment Manual.

Since the vapour heat treatment takes 8–12 hr to complete under saturated humidity from prevailing room temperature to 43.3°C, the fruits tend to ripen faster after treatment and sometimes become softer.

With EDB fumigation, there was the problem of small amounts of chemical residue left on the skin of the fruit and the layer immediately below the skin. Should the Food and Drug Administration (FDA) reduce the tolerance of 10 ppm inorganic bromide residue in papayas treated with EDB, the papaya industry would be forced to seek alternate treatments (Jackson, 1971).

Whether the dosage of EDB used (8.4 ml/m^3 space) always does a complete job of disinfestation is sometimes questioned.

Methyl bromide is known to cause injuries, internal discoloration, darkening and decay lesions on certain varieties of avocados and other fruits and vegetables.

Gamma radiation has definite advantages over chemical fumigation or vapour heat treatment of papaya because: (1) it is much more efficient and can be a continuous process, (2) it assures complete disinfestation, (3) it leaves no residue on the fruit while ethylene dibromide does (the gamma irradiated fruit is very much like having been X-rayed), (4) fumigation and vapour heat treatment tend to accelerate ripening while gamma irradiation will delay ripening and (5) in the long run, less pesticide could be applied to the preharvested fruit crop if irradiation were to be used for quarantine treatment, resulting in a cleaner environment.

The Hawaii grown mangoes have another infestation problem which neither of the two accepted quarantine procedures can solve. Besides the three species of fruit flies (Mediterranean, Oriental and Melon), a mango seed-weevil sometimes gets into the seed while the fruit is forming and growing. Microwave might kill the seed-weevil inside but the chances of partially cooking the fruit are high. Therefore, gamma radiation is the only technique that can disinfest the mango of all the fruit flies and the seed-weevil at the same time.

A brief review of some studies on treating various fruits with gamma radiation

Research on irradiation of fruits for several useful purposes was carried out in the 1960s to the early 1970s in various parts of the world such as Brazil, India, Thailand, Taiwan, the Phillipines, Venezuela and the United States (California, Florida, Hawaii, Puerto Rico). The purposes include: (1) disinfestation, (2) shelf life extension and (3) decay control.

Results of irradiation studies varied due to differences in: (1) fruit composition and variety; (2) physiological response to radiation treatment; (3) maturity of fruit harvested before irradiation; (4) post-treatment storage condition, especially temperature; (5) whether combined with other treatment such as hot water dip; (6) degree of infestation or infection before irradiation. Therefore, in spite of a sizable amount of literature and reports on irradiated fruit, one cannot always generalize the efficacy of a given species of fruit being irradiated. However, a certain trend has emerged when one examines and compares findings from various sources:

(A) Temperate zone fruits. Examples are strawberries, peaches and nectarines. The purpose of or the technical effect obtained from gamma irradiation is usually decay control, i.e. inhibition of grey mould in strawberries and brown rot control in peaches and nectarines.

The minimum effective absorbed dose required is in the range of 150–

200 krad, sometimes as high as 300 krad (Dennison & Ahmed, 1971; Maxie & Sommer, 1968; Maxie, Sommer & Mitchell, 1971). The sensory qualities of the fruits are retained, with a slight loss (10–15%) of nutrient qualities. The biggest problem, however, is softening of the fruits, often resulting in transit injuries and decreased marketability.

(B) Subtropical fruits. This group is best represented by the citrus family: oranges, lemons, tangerines and grapefruits. The technical effect desired is also decay control such as inhibiting moulds and stem-end rots in these fruits.

The minimum effective absorbed dose required is in the range of 100–150 krad (Dennison & Ahmed, 1971). While sensory and nutrient qualities of these fruits are retained at the treatment dose under laboratory conditions and simulated commercial shipments, there were varying degrees of peel injury and softening at 50–150 krad.

Banana is variously classified as a subtropical and a tropical fruit. Varieties that have been studied included: Dwarf Cavendish, Giant Cavendish, Fill Basket, Red, French Plantain (Sreenivasan, Thomas & Dharkar, 1971), Brazilian (Moy *et al.*, 1971), Gros Michel (Maxie & Sommer, 1968), *Masa sapientum*, Linn (Kao, 1971). The technical effect obtained was delayed ripening, varying from negligible to 7 days. The additional effects within the dose used (20–50 krad) would be the right range for fruit fly disinfestation (minimum should be 26 krad). Studies in Hawaii showed sensory and nutrient qualities of the Brazilian variety retained at doses up to 100 krad.

(C) Tropical fruits. This group is best represented by papayas and mangoes. The fruit fly problem in the tropics and subtropics of both of these fruits and the additional seed-weevil problem of the Hawaiian mangoes (var. Haden) make disinfestation the primary purpose of irradiation (Loaharanu, 1971; Moy *et al.*, 1971; Moy *et al.*, 1973). Shelf life extension of both fruits by irradiation has also been studied extensively.

On the Solo Variety of Hawaii-grown papayas, the USDA Hawaii Fruit Fly Investigation Laboratory has determined the minimum dose for fruit fly control to be 26 krad (earlier designated as 21 and 25 krad). Extensive laboratory studies on the papaya's sensory and nutrient qualities showed both retained at 100 and 150 krad respectively. Large scale shipping studies have further demonstrated the technical efficacy of gamma irradiation of papayas which showed no changes in sensory and nutrient qualities after 4000 km of ocean or air freight plus further inland transit (Dollar, Hanaoka & McClish, 1971). Combining hot water treatment (47.8°C for 20 min) with 75 krad resulted in shelf life extension of 3–3.5 days compared to control fruits stored at the same temperature (Moy *et al.*, 1971).

Using the puree of papayas irradiated at the Hawaii Development Irradiator (HDI) as 15% (wet wt) of the diet, the 2-year animal feeding tests conducted in 1969–71 by Industrial Bio-Test Laboratory have shown negative results, indicating safety and wholesomeness of irradiated papaya at disinfestation dose level.

For disinfestation of Hawaii grown mangoes (var. Haden) a minimum

absorbed dose of 33 krad was required. Shelf life extension of 8 days on the Okrong variety was obtained at 40 krad (Loaharanu, 1971); of more than 5 days on the Carabao variety at 60 krad (Pablo, Manalo & Cardeno, 1971); and of 6–8 days on the Alphonso variety at 25 krad (Sreenivasan *et al.*, 1971), and of 7 days on five varieties (Sensation, Eldon, Keitt, Kent and Rubi) at 75 krad (Cuevas-Ruiz, Graham & Luse, 1971). Control of anthracnose was obtained on the Zill variety at 105–210 krad (Dennison & Ahmed, 1971).

Pineapple of the commercial Smooth Cayenne variety grown in Hawaii is not subject to quarantine treatment though it can be infested by all three species of fruit flies under laboratory conditions. Besides its relatively harder shell, the reasons that pineapples escape infestation are partly because of the 'preferred host' situation in the field and partly because acidity of the fruit adversely affects the development of the eggs into adult flies.

Entomologists in Hawaii do not discount the possibility of pineapple being infested, especially in newer varieties. Quarantine treatment would then be required for movement of pineapple from Hawaii to the U.S. continent or other export markets. Studies at the Hawaii Research Irradiator (HRI) on two varieties of pineapples (Smooth Cayenne and Hybrid Variety A) have shown both sensory and nutrient qualities retained at 200 krad, though the fruit became more sensitive to chill and shell bronzing when treated above 100 krad.

Table 1 summarizes the effects of gamma-radiation treatment on a number of selected fruits, corresponding to those discussed above.

The most promising candidate for irradiation – papaya

In terms of tolerance and responses to achieving an intended technical effect, proven efficacy through planned shipping studies, proven safety through protocoled animal feeding tests and the effectiveness of two accepted quarantine treatment procedures *v.* gamma irradiation, it leaves little doubt that papaya is the most promising candidate to be gamma irradiated.

Texture of the papaya (var. Solo) stays firmer longer when treated in the range of 25–100 krad with 75 krad giving the best effect. Ripening is normal but delayed for 3–4 days when compared to controls stored at ambient temperature. The irradiated papaya has a clean appearance with a bright yellow-golden colour.

Since papayas harvested from the field should be refrigerated as soon as possible to delay ripening, irradiation for disinfestation can be carried out on a continuous basis when the fruits have been chilled. This is in contrast to the other two treatments where the fruits have to be at room temperature when treatment begins. The actual total delay in ripening to increase marketability is all the more significant when all the steps of cooling, packaging and treatment are considered.

The second most promising candidate for irradiation treatment for export

Table 1. Effects of gamma irradiation on various fruits*

Fruit (variety)	Technical effect (purpose)	Minimum absorbed dose (krad)	Observed tolerance dose (krad)	Sensory quality	Nutrient quality	Transit effect	Other effect	Alternative treatment	Benefit/ cost
Papaya (Solo)	Disinfestation	26	100	Retained at 100 krad	Retained at 150 krad	None	None	Fumigation or vapour heat	High
	Shelf life extension	75	100						
Mango (Haden)	Disinfestation	33	100	Retained at 150 krad	Retained at 200 krad	None	None	Fumigation (not for seed weevil)	High
(Alphonso; Okrong; Carabao; Rubi, etc.)	Shelf life extension	25 40 60 75							
(Zill)	Decay control	105–210							
Pineapple (Smooth Cayenne)	Disinfestation	26	100	Retained at 200 krad	Retained at 200 krad	None	Sensitive to chill	Fumigation	Potentially high if needed
Banana (8 var.)	Disinfestation and shelf life extension	26	100	Retained at 100 krad	Retained at 100 krad	None	Surface scald	Fumigation or refrigeration	Moderate
		20–50							
Citrus (orange, lemon, tangerine, grapefruit)	Decay control (stem-end rot and moulds)	100–150	150	Retained at 100 krad	Retained at 80 krad	None	Peel injury and softening	Hot water (5 min at 52.8°C)	Low
Peach and nectarine	Brown-rot control	150	100	Softening at 100 krad and above	Slight loss at 150 krad	Some	Red colour intensified at 200 krad	Hot water (7 min at 48.9°C)	Low
Strawberry (Shasta, Lassen, Fresno)	Inhibition of grey mould	200–300	200	Retained at 200 krad	10–15% less at 200–300 krad	Some	Softening at 300 krad	Refrigeration	Low

* References cited in the text.

markets would be mango, for purposes of disinfestation of fruit flies and shelf life extension. The seed-weevil problem is not as serious as it appears to be. The incidence is extremely low in Hawaii, according to knowledgeable entomologists. The probability of the seed-weevil infesting other fruits has not been methodically studied and defined, and warrants careful studies. Nonetheless, gamma irradiation will additionally take care of the seed-weevil.

The papaya petition

The culmination of 8 years' work on irradiated papaya was the preparation of a petition for FDA clearance. The petition was supported by results of the animal feeding tests and research conducted cooperatively between the Hawaii Research Irradiator (HRI) at the University of Hawaii and the Hawaii Development Irradiator (HDI) of the Department of Agriculture, State of Hawaii, also partly with USDA-Hawaii Fruit Fly Laboratory and University of California, Davis.

The official petitioners are the U.S. Energy Research & Development Administration (ERDA, formerly the Atomic Energy Commission, which sponsored the irradiation research) and the U.S. Department of Agriculture, which has a keen interest in the potential of the irradiation treatment as a quarantine procedure. The purpose of the proposed treatment is to apply minimum absorbed dose of 26 krad to prevent development of eggs or larvae to the next stage, thus preventing emergence of viable adult fruit flies of the species *Dacus* and *Ceratitis*. Spelled out also in the petition are proposed irradiation procedure, proposed safety measures, packaging, storage, and wholesomeness of the irradiated papaya.

As of 15 May 1976, the papaya petition has not been formally filed with the U.S. Food and Drug Administration (FDA). All the data and supporting documents have been provided to USAEC by the cooperating laboratories.

The entire project of gamma irradiated papayas and other tropical fruits and vegetable has been a good learning process for all concerned. This includes the FDA because protocols for shipping studies and feeding studies had to be developed in Hawaii and Washington and approved by FDA. The questions of food additive, safety, benefits, the logic of the Miller amendment are not always as clearcut as mathematical equations, especially when atomic energy is involved, and its applications in the past 31 years have been so varied. The question of classifying irradiation as a food additive *v.* a process received a good deal of attention at the FAO/IAEA-sponsored symposium on 'Radiation Preservation of Food' held in November 1972 in Bombay. The concensus there was that it should be classified as a process, much like thermal processing.

The prospects of gamma irradiation of fruits

Irradiated food products cleared for human consumption on the international scene to date consisted of thirty clearances covering fifteen different food

items approved in ten countries. In terms of specific applications, fourteen of the clearances are for shelf life extension through sprout inhibition (potatoes and onions); six for insect disinfestation (wheat, dried fruits, dry food concentrates and cocoa beans); two for shelf life extension through delayed maturation (mushrooms and asparagus) and the remaining eight for controlling microbial spoilage. It can be seen that gamma-radiation has so far found more applications for disinfestation and shelf life extension than in other areas.

While no clearance has been approved for radiation disinfestation of fresh fruits and vegetables, such treatments are very much needed for tropical fruits and vegetables to permit movement to various marketing areas throughout the world and to have a longer marketable life.

The future of irradiated foods as it might share part of the food market with other processed foods will depend on the following factors: (1) economic feasibility; (2) demonstrated technical effects; (3) proven safety for human consumption; (4) people's attitude.

All things considered, papaya has the best potential of being favourable in all these factors. As far back as 1966, the cost of irradiating papaya at 75 krad was estimated to be 1.5¢ per pound treated as compared with 1.0¢ per pound fumigated. The difference of 0.5¢, or even higher, can be easily absorbed in the retail price of papaya.

The technical effects and safety aspects of irradiated papaya have been amply demonstrated, tested and proven as no other fruits have been, involving efforts and man-hours supported by the U.S. Atomic Energy Commission and the State of Hawaii, conservatively estimated at $1.6 million.

Acceptance of an irradiated food, especially when treated at a low dose such as papaya, is a question of education of the consuming public, plus sanction by government regulatory agencies. According to a survey conducted in two U.S. West Coast cities in 1966–67, three out of four persons said they would buy irradiated food on the market if the treatment had been approved by the U.S. government (Moy *et al.*, 1971).

Concluded simply, gamma irradiation of fruits definitely has good potential, especially for disinfestation of tropical fruits such as papaya. It is strongly hoped that the papaya petition will soon be officially filed with FDA by ERDA and USDA. It will represent an advancement in Food Science and Technology and related fields as well as demonstrate our faith and leadership in the technology of Radiation Preservation of Foods to the rest of the world. If the U.S. can put men on the moon and feed them bread made of irradiated flour, then it can also declare irradiated papaya marketable and safe for human consumption.

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Aqueous extraction of black leaf tea

I. Leaf insolubility*

V. D. LONG

Summary

Total solids contents are reported for tea extracts prepared at 80°C from mixtures of (1) black tea and water, (2) black tea and tea extracts (up to 11 mass percent of total solids) and (3) residues from a first extraction and water, shaken for 4 hr in sealed bottles and centrifuged at 1000*g* for 30 min, and the fraction of leaf dissolving inferred by materials balances. At the highest mixing ratio of solid to extracting medium employed (1 : 10), leaf solubility is essentially independent of extract concentration. Solubility in water alone lies between two limits: 35% at the highest mixing ratio and 47% at infinite dilution (dry leaf basis), indicating the presence of highly soluble and sparingly soluble fractions. Interpretation of the results in terms of three components: solubles, insolubles and water is discussed and a simple adjunct to a triangular composition diagram proposed to allow for the variable amounts of sparingly soluble matter remaining undissolved.

Introduction

An intermediate stage in the manufacture of instant teas is the production of concentrated tea solutions which may be made by extracting black tea leaf with hot water in multistage, countercurrent, solid–liquid extraction systems comprising a train of contactors and separators, operating either continuously or batchwise. Performance analysis and process development of such systems and related extraction processes is helped by expressing extraction duty in terms of equivalent numbers of theoretical or equilibrium stages, based on a knowledge of phase equilibria between liquid extracts and solid residues. The present work describes a technique for establishing such equilibria and reports the total solids contents of extracts obtained from various mixtures of tea

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solids and water at 80°C. From these measurements it is possible to infer the degree of extraction of the residue by materials balance and to describe phase equilibrium for the tea–water system in terms of three components: water, soluble tea solids and insoluble tea solids. Obviously, in view of the complexity and wide variety of tea solids, well documented elsewhere (Roberts, 1962; Stahl, 1962; Pruidze, Gogisvanidze & Bokuchava, 1968; Bokuchava & Skoboleva, 1969; Millin, Crispin & Swaine, 1969), any description of this system by merely three components is a gross over-simplification which is justified only as a useful working guide for understanding, designing and improving processes with particular emphasis on concentration and yield of total soluble solids. Quality of extract is beyond the scope of this paper.

Experimental

The tea leaf used in these experiments was prepared from a commercial blend of large-leaf orthodox teas (80% Ceylon; 20% African) by screening out the small fraction (less than 4%) of leaf passing a 40 mesh B.S. sieve. The moisture content of leaf as received was 6.4% by weight and the water soluble solids content measured by Soxhlet extraction at 100°C for 50 hr was 58% by weight on a dry tea basis. Extracting media were water and tea extracts at three levels of concentration, around 3.8, 7.8 and 11.1% by weight total solids, prepared from separate portions of the above leaf by open extraction with water, first extract and second extract respectively at liquid-to-leaf mass ratios of 10:1, dissolving 31 ± 2% of the leaf (as received) allowing for extract retained by the residue on filtration.

Equilibrium extractions were carried out at 80°C with water-to-leaf mass ratios about 10, 20, 50, 100 and 200:1 and with extract-to-leaf mass ratios of approximately 10 and 100:1. The technique used was to weigh the predetermined quantity of leaf into a dry, leak proof, screw capped, polypropylene centrifuge bottle 250 ml capacity on an analytical balance. About 100 ml extracting liquid (water or tea solution) at 90°C were added from a measuring cylinder, the bottle top firmly secured and the added mass found by reweighing the bottle; the temperature of extracting liquid being chosen to give a mixture near to the extraction temperature when using a cold bottle.

The sealed bottle was next placed with a duplicate similarly prepared in a wire cage which oscillated transversely at 150 cycles/min in a water bath at 80 ± 0.5°C. After 4 hr in the water bath duplicate pairs of bottles were removed, check-weighed to ensure no loss had occurred, and centrifuged for 30 min at 2000 r.p.m. (nominally 1000*g*) in a specially adapted centrifuge operating at 80 ± 1°C. One of each pair was opened immediately and decanted hot while the other was cooled for 10 min under a running tap before opening and decanting cold. In both cases the supernatant extract was decanted through a conical strainer made from 80 mesh copper gauze to eliminate any stray floating leaf fragments and stored in glass stoppered conical flasks to await total solids determinations.

Decantation at two different temperatures was adopted as an approximate correction for losses of water vapour at the elevated temperature. Preliminary experiments decanting tea solutions of known initial concentration had shown that at high temperatures the concentration rises slightly due to loss of water vapour while at low temperatures the concentration falls slightly due to losses of tea solids by precipitation of tea 'cream' which on microscopic examination was found to comprise very small, highly solvated, spherical particles having an average diameter 1 μm, showing Brownian motion and settling under gravity with a velocity 10 mm/day. The changes in concentration at the two temperatures were almost identical in magnitude and opposite in sign, so that the average gave a very good estimate of the concentration before opening the bottle. In this way there was a built in check on the reliability of the result which must in any case lie between the two extremes. With tea solutions alone the difference between these extremes was less than 1% of the initial extract concentration and the average concentration within 0.1% of the initial concentration. In the presence of leaf, which required more careful decantation and gave increased collecting surface for precipitate, the difference between the two methods of decantation approximately doubled.

After decanting extract from the first extraction of a sample of leaf, the extraction bottle was immediately resealed and in most cases a further aqueous extraction carried out next day on the residual leaf plus entrained and absorbed extract. This second extraction and subsequent treatment was carried out in the same way as the first except that no leaf addition was required and in all cases water only was used as the extracting medium.

The total solids content of decanted extracts was determined gravimetrically in duplicate by weighing 10 ml aliquots into shallow aluminium dishes, evaporating to dryness overnight in a current of air in a vacuum oven at 80°C and subsequently keeping the dried residue at the same temperature for 1 hr under a full vacuum maintained by rotary pump. The moisture content of leaf was similarly determined by vacuum drying at 80°C for each batch of extractions performed.

As an independent measurement of total solubles at 80°C, 'Soxhlet-type' extractions were also made. The thimble and extraction vessel were immersed in a water bath at 80 ± 0.5°C and fed with water preheated by passing through coils in the same bath. From 5 to 10 g of leaf was accurately weighed into a previously extracted and dried filter thimble and extracted for periods up to 35 hr. After the predetermined extraction period the thimble and contents were dried to constant weight and the weight of residue found.

Results

First extraction of leaf

Values of concentration of tea solids in extracts obtained by first extraction of leaf with water are shown as a function of the mixing ratio (calculated on an

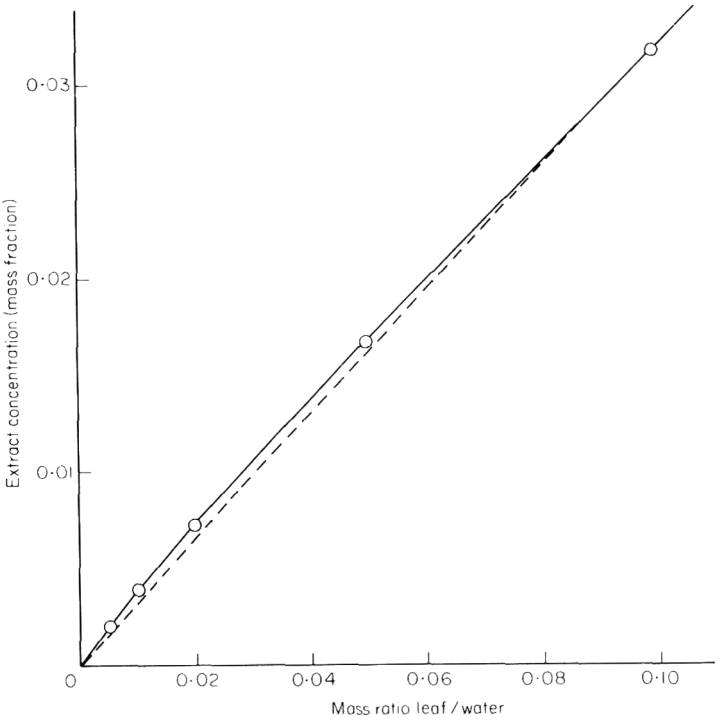

Figure 1. Extract concentration as a function of mixing ratio for extraction of black leaf with water.

'as received' basis) in Fig. 1. Each point is the mean of at least four extractions equally divided between hot and cold decantations. The broken line relates to a solubility model to be discussed later.

Concentrations obtained by extracting leaf with tea extract in place of water are shown as a function of initial extract concentration in Fig. 2. The results fall on two approximately parallel straight lines, one for each mixing ratio. The full lines, which have intercepts given by Fig. 1 and minimize variance in final concentration, have equations:

$$c_2 = 0.0316 + 0.941c_1 \quad (10:1 \text{ extractions}) \tag{1}$$

$$c_2 = 0.0039 + 0.963c_1 \quad (100:1 \text{ extractions}) \tag{2}$$

where c_1, c_2 are the fractional mass concentrations of initial and final extracts respectively. The broken lines are for later reference.

The residue in equilibrium with extract comprises insoluble solids, undissolved but potentially soluble solids and absorbed extract. Assuming all water in the residue is present as extract at the bulk concentration, it is shown in the Appendix that the mass fraction of the initial tea solids remaining insoluble and undissolved $(i+u)$ may be found by combining tea solids and water mass balances to give:

$$i + u = [1 - c_2 - w - (c_2 - c_1)/R]/[(1 - c_2)(1 - w)] \tag{3}$$

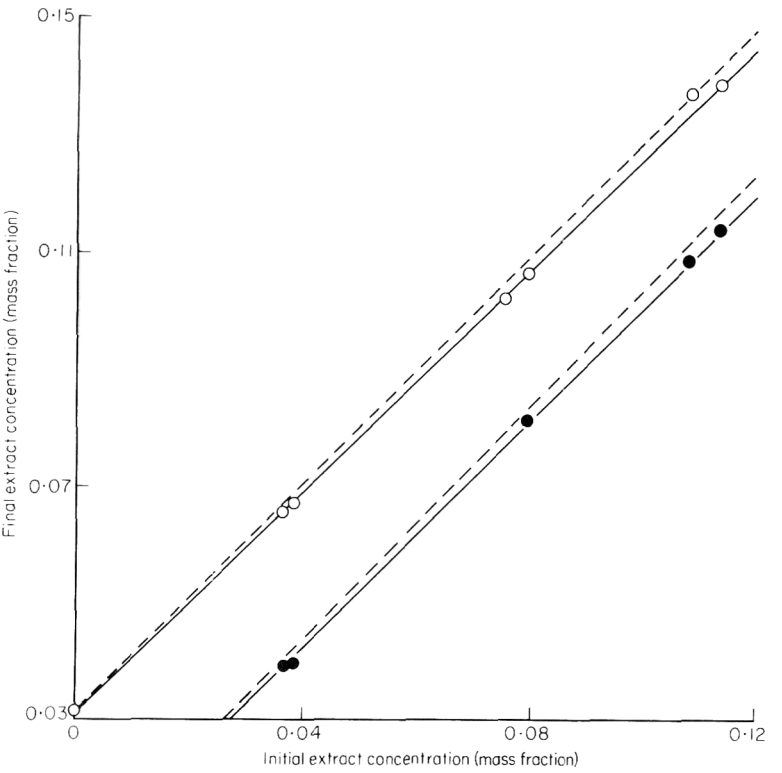

Figure 2. Final extract concentration as a function of initial extract concentration for extraction of black leaf with tea extracts; leaf/extract mass ratios: ○, 0.10; ●, 0.01.

where R is the mass mixing ratio of as received leaf to initial extract and w is the mass fraction water in the leaf before extraction.

Values of fraction insoluble and undissolved calculated by equation (3) from the experimental points of Fig. 1 and the upper curve of Fig. 2 are shown as a function of leaf-to-extract ratio in Fig. 3. For economy the results have been plotted with others relating to residue extractions detailed later. The results for water extraction of leaf lie on a well-defined curve shown as a broken line which extrapolates at zero leaf/extract ratio or infinite dilution to the true insolubles content of leaf and should correspond to the result of Soxhlet extraction. At higher mixing ratios potentially soluble material remains undissolved and at the highest ratios the fraction dissolving has become almost constant. This behaviour conforms to the well-known division of tea solids into insoluble, sparingly soluble and highly soluble fractions; the proportions of each class at 80°C being about 53:12:35 in these experiments according to the broken line in Fig. 3.

Extraction of residues

The starting materials for these extractions were water and the mixture of spent leaf plus attached extract remaining after decantation of first extracts.

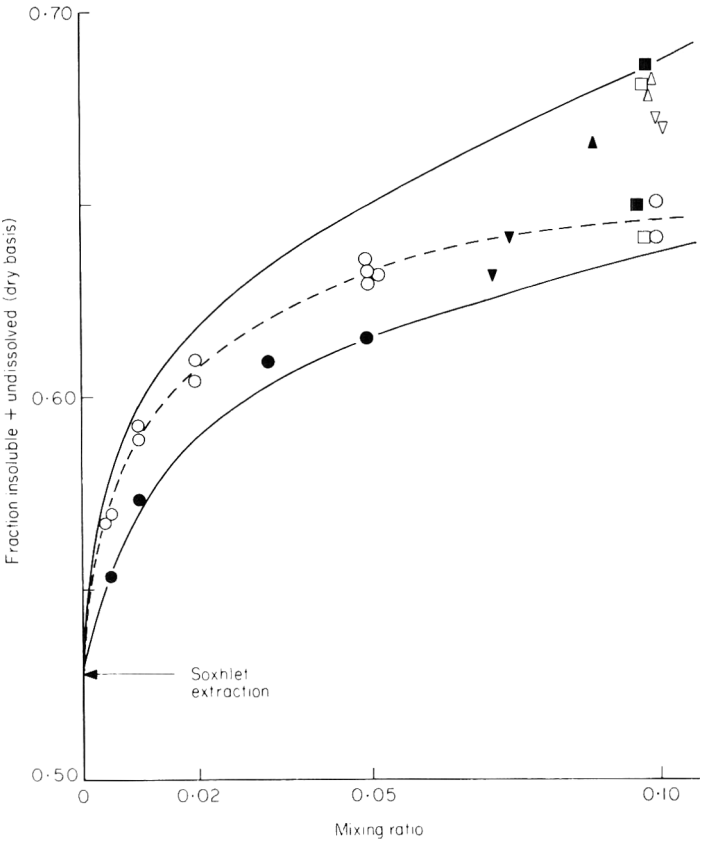

Figure 3. Insoluble and undissolved tea solids in extraction residues. Total solids content of extracting medium used in first extraction, first extractions: □, 0.11; △, 0.08; ▽, 0.04; ○, 0; second extractions: ■, 0.11; ▲, 0.08; ▼, 0.04; ●, 0.

The dry solids content of the residue to be extracted was estimated, as a mass fraction of the dry solids of the leaf used in the first extractions, from the insoluble and undissolved fraction above plus the soluble solids in attached extract. The mixing ratio was defined as the ratio of total dry solids to total water present as added water and residual extract. Because of the variable nature of the starting material strict duplicates did not exist and the results of extract concentrations obtained by hot and cold decantations were not averaged, thereby adding slightly to the overall scatter.

The array of results obtained was fairly bewildering, but analysis of variance confirmed the fairly obvious suggestion that important variables affecting the final concentration were the mixing ratio (R') used in the extraction and the fractional dry solids content of the residue to be extracted (r). Further statistical analysis showed that two additional variables; the mixing ratio (R) used in the first extraction from which the residue was taken and the product $r.R$, which is essentially the ratio of dry solids in the residue extracted to the weight

of extract used in its preparation, were needed to get good estimates of extract concentration. The correlation found:

$$c_2 = 0.494R' - 0.287R + 0.0010r + 0.213r.R - 0.0017 \quad (4)$$

gave estimates of extract concentration which are compared with measured concentrations in Fig. 4.

By applying tea solids and water mass balances detailed in the Appendix, the mass fraction of solids in the leaf fed to the first extraction which remains insoluble and undissolved after the second extraction is found to be:

$$i + u = r[1 - c_2/(R'(1 - c_2))]. \quad (5)$$

Values of insolubles and undissolved solids calculated for averaged pairs of extractions are plotted against mixing ratio in Fig. 3. The mixing ratio employed here is slightly different from that used in connection with first extractions, but is close enough to show the broad similarity of result on a common diagram. All results in Fig. 3 lie in a band bounded by two curves, lines of maximum and minimum solubility, which converge to the insolubles content of leaf at infinite dilution.

Estimating insolubles in leaf

Two methods of estimating the insolubles of leaf were used: first, 'Soxhlet-type' extraction, and second, extrapolation of the results of single batch extractions to infinite dilution. The mass of residue from 'Soxhlet' extraction decreased continuously with time of extraction and was thought to be essentially constant after 35 hr. However, despite taking great care, the results were

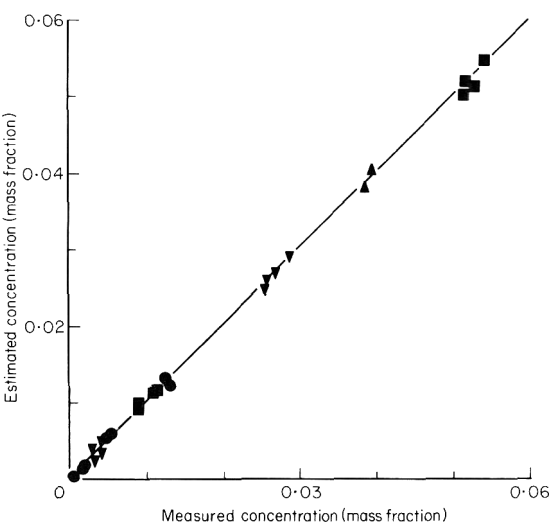

Figure 4. Precision of formula for estimating extract concentrations from second extractions (key as for second extractions in Fig. 3).

disappointingly variable, the mean of eight determinations being 52.7% of dry leaf with a standard error of 1.1%.

In view of the failure of 'Soxhlet' extraction to give precise results recourse was made to extrapolation of the results of batch extraction. One possibility was to extrapolate the previously found values of insoluble and undissolved content for water extractions to zero mixing ratio. However this was rejected because there is no certain basis for such extrapolation. By contrast, extrapolation of extract concentration to zero mixing ratio can be done with a high degree of confidence, so it was decided to extrapolate concentration measurements from extractions carried out with mixing ratios 0.02, 0.01 and 0.005 to zero concentration as shown in Fig. 5. To illustrate the precision of the method the results of hot and cold decantation are shown separately. Substitution of the limiting slope at $c = 0$ for $(c_2 - c_1)/R$ in equation (3) together with $c_2 = 0$ gives the limiting value of insolubles. In order to get the best estimate of limiting slope, cubic equations were fitted to both curves passing zero and minimizing the sum of squares of deviations in concentrations. These were found to be:

(a) *hot decantation*

$$c = 148R^3 - 6.48R^2 + 0.438R \tag{6}$$

(b) *cold decantation*

$$c = 166R^3 - 7.66R^2 + 0.441R. \tag{7}$$

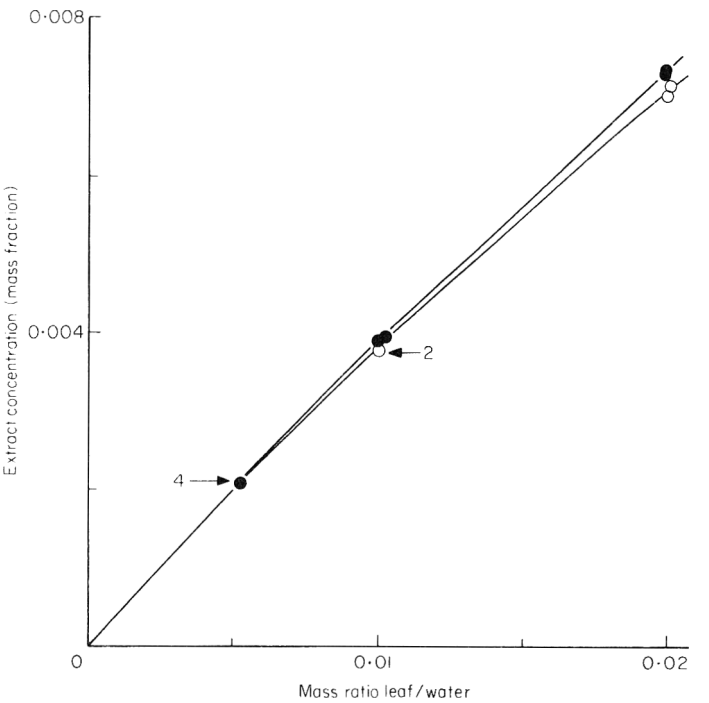

Figure 5. The approach to zero concentration in extractions of leaf with water. Mode of decantation: ●, hot; ○, cold; 2, 4, numbers of coincident points.

Hence the corresponding values of limiting slope are 0.438 and 0.441 for hot and cold decantations respectively. Substituting the average value for slope and the average moisture content of leaf extracted (0.068 for the points presented) in equation (3) gives an estimate of 52.8% for the insolubles content of leaf for extraction at 80°C. The close agreement with the average 'Soxhlet' result is probably fortuitous in view of the inaccuracies of the latter, but the result appears to be the best estimate of insolubles content of the leaf used in this work.

Discussion

The sealed bottle technique of extraction was developed to reduce and quantify losses of water compared with conventional methods involving open transfer to separation equipment such as a vacuum filter or basket centrifuge. Gudnason (1969) also sought to reduce evaporation loss and developed a special brewing apparatus allowing *in situ* filtration. This equipment was found to give reproducible extraction suitable for many investigations requiring relatively large volumes of extract but was not suited to equilibrium extractions because of continuous slow seepage of extract through the filter plate. Moreover it was a cumbersome item to replicate and the solvent and solid could not be metered with the precision of the bottle method.

It is interesting to see how far the results obtained conform to the simplest three component hypothesis of solution in which dry leaf is held to comprise constant amounts of infinitely soluble solute and inert insolubles only. This model is frequently used by chemical engineers in leaching calculations and forms the basis of a calculation of coffee extraction given by Charm (1963). The most sensitive test of applicability lies in the results for insoluble and undissolved solids shown in Fig. 3. This clearly shows that the apparent insolubles content (i.e. the matter remaining undissolved) is not constant as required by the model but depends on the mixing ratio used in extraction. However it will be noticed that at the highest mixing ratio there was little change with initial concentrations in the range 4–11 mass percent. Thus at high concentrations, where the extracting solutions are presumably already saturated with respect to the more sparingly soluble components, the model is followed quite closely. Now first extractions of leaf with water give a curve which suggests that saturation with respect to the less soluble components is approximately achieved at the highest mixing ratio. This then raises the question as to why the undissolved matter is higher in the first extractions made with extract. The answer undoubtedly lies in the fact that the extracting solution itself contained insolubles and/or matter which became insoluble during extraction. Estimates of the amounts of such material are possible from extractions made at 100 : 1 ratios in which the amount of extract remained unchanged while the amount of leaf was reduced by a factor of ten. Such correction shows that in all cases there is no significant departure from an insoluble and undissolved content of leaf of 65

mass percent. Thus the simple model seems to fit all extractions giving final concentrations greater than 3 mass percent, but at lower concentrations residue compositions based on this treatment are liable to appreciable error.

Despite its shortcomings, the above model does give good estimates of extract concentration over the complete range investigated. In the Appendix it is shown to lead to the following expression for extract concentration:

$$c_2 = [c_1 + R\,(1 - i)\,(1 - w)]\,/[1 + R\,(1 - i + i.w)]. \tag{8}$$

Taking $c_1 = 0$, $i = 0.65$ and $w = 0.06$ gives a relation applicable to first extractions with water, shown as a broken line in Fig. 1. Considering the incorrectness of the model, the prediction is remarkably close to experiment and is quite accurate enough for many engineering purposes. Rearranging equation (8) gives:

$$c_2 = c_2' + c_1/[1 + R(1 - i + i.w)] \tag{9}$$

where c_2' is the fractional mass concentration produced by extraction with water at the same mixing ratio. Equation (9) is clearly linear in c_1 with a slope not far removed from unity as found in extractions of leaf with extract. Taking the former values for i and w, and for c_2' experimental values from Fig. 1, gives relations shown as broken lines in Fig. 2. These deviate from the experimental lines by amounts which are proportional to the initial extract concentration and are approximately the same for both mixing ratios even though the latter differ by an order of magnitude. This deviation is considered to be due to insolubles deposited from initial extract and amounts on average to 2.8% of the

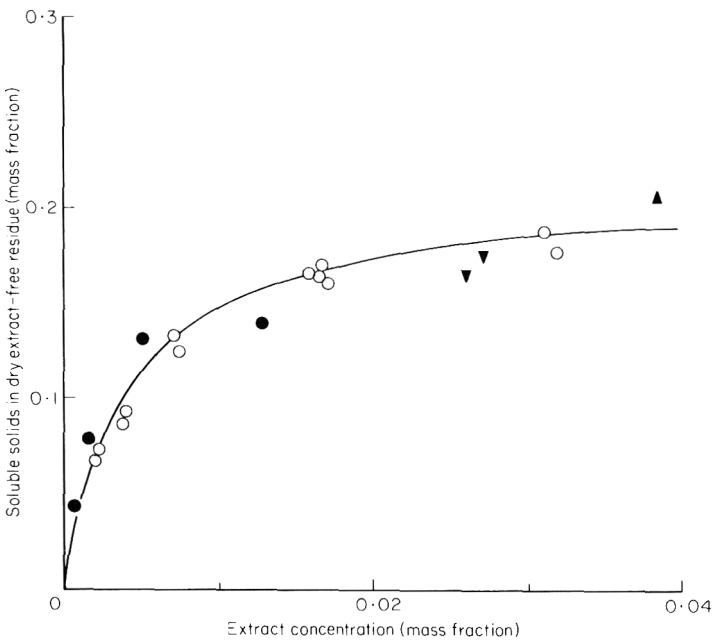

Figure 6. Undissolved soluble solids content of dry, extract-free residues in equilibrium with extract as a function of extract concentration (key as in Fig. 3).

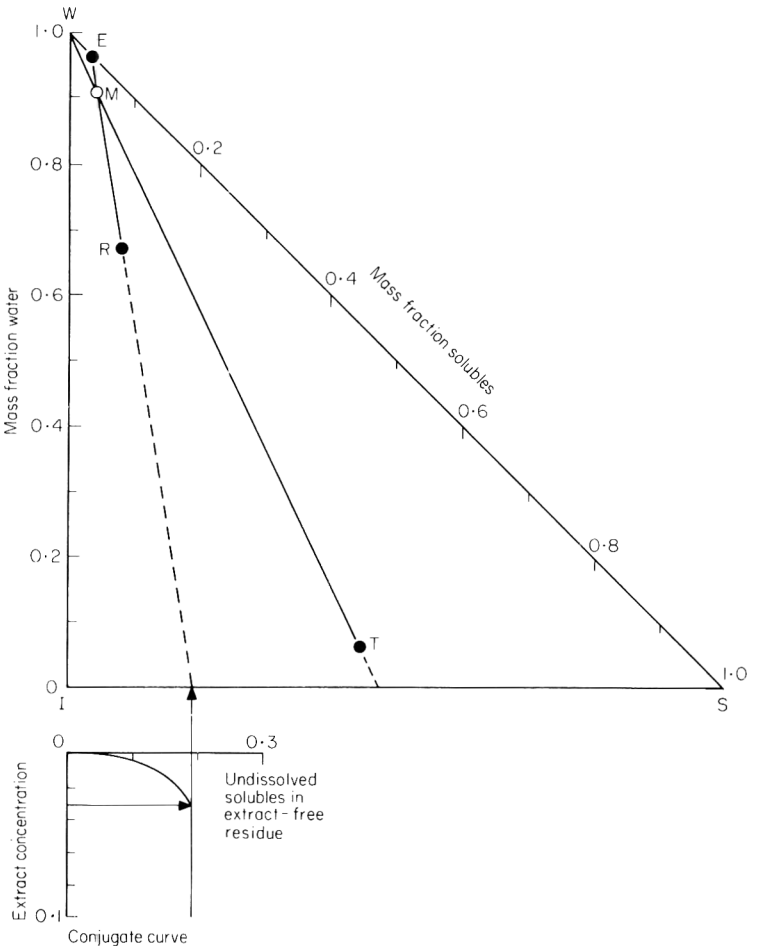

Figure 7. Triangular representation of the tea–water system to allow for variable dissolution of sparingly soluble solids. I, insolubles; S, solubles; W, water; T, original black leaf; M, mix point; E, extract; R, residue; TM/WM = water/leaf mass ratio (10 : 1); RM/EM = extract/residue mass ratio (9 : 2).

total solids of that extract. Viewed another way, if the 100 : 1 extractions are taken as a control for the 10 : 1 extractions then the latter comply with the predictions of the simple model.

To construct a more realistic model for regions of low extract concentration (say below 4 mass percent), the soluble solids content of dry, extract free residue (i.e. $u/(i+u)$) is plotted as a function of final extract concentration in Fig. 6. The results lie about a single curve which may be used as a conjugate diagram to a triangular phase diagram for predicting extract-residue equilibria as in Fig. 7. To illustrate the simplest application of this diagram the geometric construction is shown for estimating extract and residue composition from a 10 : 1 mixture of water and black tea with a residue-to-leaf ratio of 2 : 1. Application to stagewise extraction will be obvious by comparison with the method used by Charm (1963).

It will be seen that the method above is based on a model which describes the system in terms of three components: insolubles, solubles and water, where the insolubles represent the limit of exhaustive extraction (e.g. Soxhlet) and allowance is made for solubles remaining undissolved under other conditions. In particular the yield of soluble tea solids in stable solution will be a minimum when extract concentrations exceed 4% by mass and a maximum at infinite dilution. This has interesting implications for countercurrent extraction, because such processes have extracts proceeding from regions of low concentration to regions of high concentration. Thus it is to be expected that some tea solids will dissolve only to be precipitated at the high concentration end of the extractor. Now if extraction is limited to those solids soluble at the highest concentration and this concentration exceeds 4%, the equilibrium yield will correspond to the lower solubility limit (35% of dry leaf at 80°C in these experiments) and the precipitated solids will be recycled internally so that the overall behaviour of the process in the steady state will be as though they did not exist. On the other hand, if the precipitate is finely divided and settles slowly, like tea 'cream', it is quite likely that most of it will pass through the separator in suspension in the extract. Under these conditions equilibrium solubility at the high concentration end will not be the sole criterion of extractability and yield may approach the upper solubility limit (47% of dry leaf at 80°C) in extreme cases. In addition, under non-equilibrium conditions supersaturation of sparingly soluble matter is possible.

Conclusions

(1) The water soluble solids contained by black tea have a wide range of solubilities so the fraction of leaf dissolving in water or previously prepared aqueous extract depends upon mixing ratio and the solubles already present in the extracting agent.

(2) In these experiments the effect of previous history of leaf and extracting medium on the extent of dissolution correlates with the extract concentration finally attained.

(3) At final concentrations above 3–4 mass percent the fraction of leaf dissolving at 80°C is approximately constant at 35% of dry leaf.

(4) With decreasing final concentrations below 3 mass percent progessively more leaf dissolves reaching a maximum of 47% dry leaf at infinite dilution.

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Appendix

Some applications of materials balances

Nomenclature

c_1 fractional mass concentration of initial extract used as solvent.
c_2 fractional mass concentration of final extract after extraction.
e_2 mass of final extract per unit mass dry leaf extracted.
i mass fraction insolubles in dry leaf.
R mass mixing ratio as-received leaf to extract.
R' mass mixing ratio total dry solids to total water.
r mass of tea solids in residue per unit mass of dry leaf originally extracted.
u mass of undissolved soluble solids remaining in the residue from unit mass dry leaf.
w mass fraction water in as-received leaf.

A1. Derivation of equation (3)

Basis: unit mass dry leaf extracted.

(i) *Water balance*

Water in final extract = Water in leaf before extraction + Water in initial extract

$$e_2(1-c_2) = \frac{w}{1-w} + \frac{1-c_1}{R(1-w)}$$

(ii) *Tea solids balance*

Insoluble and undissolved in residue = Tea solids in leaf before extraction + Tea solids in initial extract − Tea solids in final extract

$$i+u = 1 + \frac{c_1}{R(1-w)} - e_2 c_2$$

Substituting for e_2 from i and simplifying:

$$i + u = [1 - c_2 - w - (c_2 - c_1)/R]/[(1 - c_2)(1 - w)].$$

A2. Derivation of equation (5)

Basis: unit mass dry leaf extracted in the first extraction.

(i) *Water balance*

Water in final extract = Total water input

$$e_2(1 - c_2) = r/R'$$

(ii) *Tea solids balance*

Insoluble and undissolved in residue after second extraction = Solids in residue before extraction − Tea solids as extract

$$i + u = r - e_2 c_2$$

Substituting for e_2 from i gives:

$$i + u = r[1 - c_2/(R'(1 - c_2))]$$

A3. Extract concentration by a simple three-component hypothesis

Assume that dry leaf contains a constant amount of infinitely soluble solute and that the remainder is insoluble and inert.

Basis: unit mass dry leaf extracted with initial extract mass concentration c_1 to give final extract mass concentration c_2.

(i) *Water balance* – as in A1.

(ii) *Soluble tea solids balance*

Solubles in final extract = Solubles in leaf extracted + Solubles in initial extract

$$e_2 c_2 = 1 - i + c_1/[R(1 - w)].$$

Substituting for e_2 from i and rearranging gives:

$$c_2 = [c_1 + R(1 - i)(1 - w)]/[1 + R(1 - i + i.w)].$$

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Functional properties and amino acid content of a protein isolate from mung bean flour*

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Summary

A protein isolate was prepared from mung bean flour by extraction with 0.001 N NaOH, precipitation at pH 4.5, neutralization of the dispersed precipitate to pH 6.8–7.0, and subsequent freeze drying. The isolate's amino acid composition was determined and found to be similar to that of mung bean flour except for cystine which was destroyed during isolate preparation. The following properties of the protein isolate were investigated: nitrogen solubility, buffer capacity, foamability, gelation. Except for buffer capacity, the isolate demonstrated good functional abilities in simple systems under laboratory conditions.

Introduction

Mung bean, *Vigna radiata* (L.) Wilczek is consumed in the Philippines and in many Asian countries as a vegetable in the form of cooked beans or as mung bean sprouts. Mung bean contains 20–25% protein and sufficient quantities of all amino acids except methionine, cystine, and tryptophan. The non-protein amino acid, S-methylcystine, is also present in mung bean but has not been linked to any harmful effects (Evans & Bandemer, 1967). Hemagglutinins are absent (Honovar, Shih & Liener, 1962). Mung bean was found non-toxic as a feed for animals (Adan, 1935; Vijandre, 1954; Bunce *et al.*, 1970). Good growth response was recorded when mung bean formulations were given to children in Thailand and the Philippines (Bhumiratana & Nondasuta, 1969;

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Alcaraz-Bayan *et al.*, 1972). Although trypsin inhibitors have been reported to be present in mung bean (Liener, 1965), they are rendered inactive by heat application.

In the Philippines, only about 4% of the ingested protein is furnished by beans of any type (Intengan, 1969). Mung bean is also widely used in the manufacture of noodles. During recovery of the starch from mung bean, the protein is a by product thrown away with the wash water. The utilization of the mung bean protein is possible if it possesses desirable nutritional and functional properties. Thus mung bean protein might find better acceptance as a food additive in the form of a protein isolate.

In addition to its nutritional value, such an isolate may find use in texturization, incorporation into baked goods or in other products wherein it could serve a functional role. Meyer (1966) pointed out that full utilization of protein products may depend on their functional characteristics. This study was undertaken to investigate the amino acid content and functional properties of a protein isolate extracted from mung bean. Such a process could be employed in Southeast Asia to salvage the protein waste produced from the manufacture of the noodle derived from mung bean starch.

Materials and methods

Flour preparation

Dried seeds of *Vigna radiata* (L.) Wilczek, variety MG50-10A, were supplied by the International Rice Research Institute, Los Banos, Laguna, Philippines. The grinding of the seeds and separation of the flour was done by Liberty Flour Mills, Inc., Mandaluyong, Rizal, Philippines, using a laboratory Buhler mill. Flour was used as the starting material because it contained most of the protein, was relatively soluble, required less equipment, and produced few by-products.

Isolate preparation

Mung bean flour was mixed with 0.001 N NaOH at a ratio of 20:1 (water: flour, v/w) and allowed to stand for 1 hr with moderate mixing at 15 min intervals. Insoluble compounds were removed by centrifugation at 700 *g* for 30 min. An IEC UV centrifuge (Boston, Mass.) was employed for centrifugation of samples during the study. To the liquid supernatant, 0.5 N HCl was added to effect precipitation. Precipitation was considered complete when the solution reached pH 4.4–4.5. A Corning-Eel expand portable pH meter, Model 610 (Evans Electroselenium Ltd, Holstead, Essex, England) was used for all pH determinations and adjustments. The precipitated protein was separated by centrifugation at 700 *g* for 30 min and washed three times with an acid solution of pH 4.5. The precipitate was dispersed in distilled water and the pH of the recovered protein isolate was adjusted to pH 6.8–7.0 with 0.5 N NaOH and subsequently freeze dried using a Virtis 10-145 MR BA Freeze-Mobile.

Analytical methods

Moisture, ash, crude fibre, and fat analyses were determined on the flour using standard methods given in the Official Methods of Analysis of the A.O.A.C. (1965). Nitrogen determination (protein estimated as nitrogen × 6.25) on flour, extracts, and isolates were done by micro-Kjeldahl (Chapmann & Praft, 1961). Amino acid content was obtained on a Beckman Model 120C Amino Acid Analyzer following the method outlined in Beckman/Spinco Instructional Manual and Handbook (1966). Briefly, this involved hydrolysis with 6 N HCl for 23 hr at 110°C in a sealed tube followed by elution of the hydrolysate with a sequence of sodium citrate buffers, varying from 0.2 M, pH 3.25 to 0.35 M, pH 5.25. Total analysis time per sample was 3.5 hr. The tryptophan content was not analysed.

Nitrogen solubility

Solubility tests were made at pH 2, 3, 4, 4.5, 5, 6, 7, 8 and 10. Using the method outlined by Mattil (1971), 2 g of isolates were dispersed in distilled water. Dispersions were stirred continuously with a magnetic stirrer for 30 min and the pH was adjusted using 0.5 N NaOH or 0.5 N HCl. A final volume of 40 ml gave a solvent:isolate ratio of 20:1 (v/w). The supernatant extract was centrifuged at 1300*g* for 30 min, filtered through Whatman No. 1 filter paper and analysed for total nitrogen by the micro-Kjeldahl method. The results were expressed as percent protein isolate soluble at the given pH.

Buffer capacity (BC)

Protein was dispersed in 100 ml distilled water to form a 0.5% protein concentration (w/v). The initial pH was determined and the dispersion titrated to pH 3.0 with 0.1 N HCl or to pH 10.0 with 0.1 N NaOH. Buffer capacity (BC) was calculated for 0.5 pH change by the equation of Morr, Swenson & Richter (1973);

$$BC = \frac{\text{mg titrant}}{\text{wt of protein (g)} \times \Delta \text{pH}}$$

Foamability

Sufficient isolate was added to distilled water to give 8.0% protein (w/v). Solutions were adjusted to pH 7.0 with 0.1 N dilute NaOH and mixed gently. Vigorous whipping for 5 and 10 min followed, using three electric mixers: a three-speed Hamilton Beach 79-1, a three-speed General Electric M57, and a ten-speed General Electric M68.

Volumes were recorded before and after whipping and the percentage volume increase calculated according to Lawhon, Cater & Mattil (1972) and

Lin & Humbert (1974):

$$\% \text{ vol. increase} = \frac{\text{vol. after whipping} - \text{vol. before whipping}}{\text{vol. before whipping}} \times 100$$

Weights were also taken before and after whipping and the specific volume calculated according to Baldwin & Sinthavalai (1974) and Hansen & Black (1972):

$$\text{Specific volume} = \frac{\text{vol. after whipping (ml)}}{\text{wt after whipping (g)}}$$

Foam samples were inverted and allowed to drip through a 2000 μm wire screen into preweighed beakers as reported by Watts (1937) and Baldwin & Sinthavalai (1974). The weight of liquid released from foam after 5, 10, 20, 30, 60, and 120 min was determined and calculated as percentage drip based on the original weight of the foam. Percentage drip was inversely related to foam stability.

$$\% \text{ drip} = \frac{\text{wt of liquid released from foam}}{\text{original wt of foam}} \times 100$$

Gelation

Following the method of Circle, Meyer & Whitney (1964), sufficient isolate was dispersed in distilled water to make 100 ml total volume and protein concentrations of 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 14% (w/v). The mixtures were stirred and the pH adjusted to pH 7.0 with 0.5 N NaOH. To assure complete mixing the mixture was placed in a blender (Atomix, England) at high speed for 2 min. Centrifugation for 15 min at 1400 *g* followed to eliminate any air present. The samples were distributed into test tubes in 10 ml aliquots and evaluated for gel formation by the least concentration endpoint (LCE) method of Trautman (Briskey, 1970). A series of protein concentrations were heated in a Memmert W350R water bath at 80°C for 10 min. Preliminary testing showed that an additional 5 min heating was required to equalize the sample temperature with that of the water bath. After heating, the samples were cooled at 0°C in an ice bath and the strength of the coagulum evaluated by inverting the tube. The lowest protein concentration which formed a stable gel (remained in inverted test tube) was considered the gelation endpoint.

Results and discussion

Proximate analyses of seed fractions

The proportion that each seed fraction contributed to the whole mung bean was determined by weighing each fraction. It was found that the bran and

Table 1. Proximate analyses of seed fractions as compared to whole mung bean (dry weight basis)*

Analysis	Seed fractions			Whole mung bean		
	Flour (%)	Pollard (%)	Bran (%)	C† (%)	SSS‡ (%)	FAO§ (%)
Proportion of bean	84.6	13.2	2.2	100.0	100.0	100.0
Ash	3.3	4.0	4.1	3.4	3.0	3.7
Crude fibre	1.2	19.6	30.2	4.3	3.5	4.7
Crude fat	0.8	0.6	0.1	0.8	3.0	1.2
Protein	28.0	18.0	11.0	26.3	25.6	24.6
Carbohydrates¶	66.7	57.8	54.6	65.2	64.9	65.8

* Mean of six samples.
† Calculated from seed fractions data.
‡ Singh *et al.* (1968).
§ FAO/US. Dept. of Health, Education and Welfare, 1972. Food Composition for use in East Asia.
¶ Calculated by difference.

pollard* constituted only 2.2 and 13.2% respectively. Flour made up 84.6% of the weight of the whole seed.

Results from the proximate analyses (Table 1) confirmed the high protein, low fat content of mung bean. Theoretical values were obtained from analyses of the three portions. The calculated values compared favourably with analyses of ground whole mung bean by other researchers.

Location and relative distribution were indicated by the proximate analyses of the flour, pollard, and bran (Table 1). The flour was found to be the major contributor to the good nutritive value of mung bean. The 28.0% protein found in the flour is greater than the 26.3% protein found in the whole seeds. Its high protein and low crude fibre contents accounted for its use as the protein isolate source. Flour may also be used as the starting material for starch extraction and noodle production because of its high carbohydrates content of 66.7%. Due to the presence of the embryo, the pollard yielded a relatively high 18% protein. However, the nutritive contribution of this fraction was low because of its high content of crude fibre, approximately 60% of that found in the whole bean. Crude fibre content of the bran was even higher, but the bran comprised such a small proportion of the whole bean that its effect on food value was negligible.

These observations and results were similar to those reported by Singh, Singh & Sikka (1968). Proximate analyses values given by them for mung bean cotyledon were essentially the same as those obtained from the mung bean flour. Similar results were observed with the seed coat and bran fractions. Due to difference in the milling procedure it was not possible to compare data from

* Pollard is the fraction that contains fine particles of the seed coat or hull (bran), endosperm and germ.

the third fraction. Singh *et al.* (1968) studied the embryo as a separate seed fraction while the embryo formed only a part of the pollard fraction in these studies.

Amino acids

Amino acid composition of mung bean flour and its protein isolate were found to be similar (Table 2). The mung bean and its isolate is a good source of lysine but it is deficient in cystine and methionine. Evans & Bandemer (1967) obtained the same results with mung bean seeds. Most amino acids were present in greater amounts in the isolate but these variations were small and the general amino acid pattern remained unchanged. These data agreed with those obtained by Samson, Cater & Mattil (1971) with coconut protein isolate. Meyer (1966) stated that any differences in essential amino acid content of commercial soybean isolates arose from varietal differences and the nature of the nitrogenous substances. Lawhon & Cater (1971) cited different protein species rather than protein modification as the explanation for amino acid variations in glandless cotton seed extracts.

Table 2. Amino acid composition of mung bean flour and its protein isolate (g/16 g N)

Amino acid	Mung bean flour (mean)	Mung bean isolate (mean)
Alanine	4.37	5.07
Arginine	6.81	7.01
Aspartic acid	11.85	10.17
Cystine	0.37	–
Glutamic acid	18.05	19.50
Glycine	3.94	3.60
Histidine	2.59	2.48
Isoleucine	4.79	5.47
Leucine	7.90	9.33
Lysine	6.69	6.82
Methionine	1.22	1.29
Phenylalanine	5.50	6.79
Proline	4.03	4.06
Serine	4.86	2.47
Threonine	2.82	1.77
Tyrosine	2.93	2.99
Valine	5.95	6.72
Ammonia	3.70	4.09
% Protein (% N x 6.25)	27.99	92.83

Although it was assumed that denaturation did not occur, cystine was not recovered from the isolate in sufficient quantity for measurement. The cystine content of the flour samples was recorded as 0.37 g/16 g N. Even this small

amount disappeared during isolate preparation. Roxas, Intengan & Juliano (1976) obtained 0.7% cystine in dehulled mung bean by performic acid oxidation. Circle & Johnson (1958) attributed the destruction of sulfur-containing amino acids to partial hydrolysis by alkali. Cystine is unstable above pH 10. This has been observed in some rice glutelin extracts by Tecson *et al.* (1971). Wolf (1970) reported decreased amounts of essential amino acids in soybean isolate due to fractionation of protein during isolate preparation.

Nitrogen solubility

The percentage soluble nitrogen in the mung bean protein isolate was determined at seven pH values (Fig. 1). Greatest solubility occurred at pH values far removed from the isoelectric point of the protein isolate. Thus, tests run at pH 2, 8 and 10 yielded the highest percent soluble protein with 84.12, 98.67, and 98.84% respectively. The protein isolate was nearly 100% soluble in alkaline solutions near the pH of protein extraction, pH 10.5. In general, pH Lowest solubility was recorded at pH 4–5. This was expected as the isolate proteins had been precipitated at their isoelectric point of 4.5.

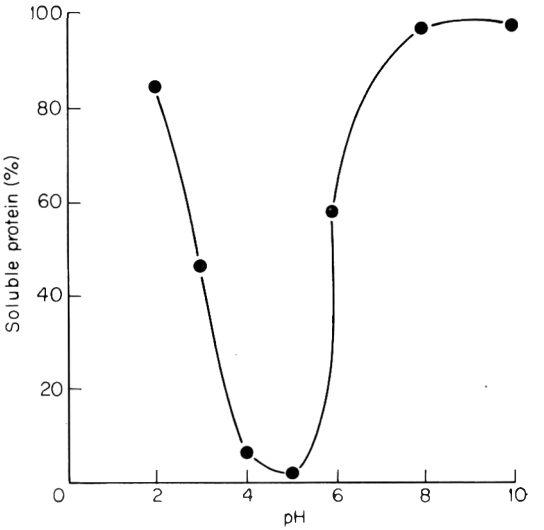

Figure 1. Effect of pH on solubility of mung bean protein isolate at room temperature (27.5°C).

Although the shape of the solubility curve proved consistent, a wide range of values was recorded among those isolates produced on different days. Variation in legume protein isolate solubility has been noted earlier (Nash, Eldridge & Wolf, 1967; Wolf, 1972; Rhee, Cater & Mattil, 1973). The main explanation centred on the nature of the globulins, the major protein fractions. In soybeans a portion of the 7S and 11S globulins was shown to exist as aggregate. These aggregates appeared to grow with each step of isolate preparation and affected

a corresponding decrease in protein solubility. The ratio and extent of polymerization was highly variable among individual samples.

Nash *et al.* (1967) noted decreased solubility in soy protein isolates with increased age. However, there was little variation in the age of the mung bean protein isolates, all samples falling in the 3–5 months age group. Observed solubility variations seemed unrelated to age of the isolate.

The protein solubility of mung bean protein isolate compared favourably with solubility profiles of soybean and coconut protein isolates. Nash *et al* (1967) recorded 33.3–81% soluble protein from soy proteinate at the same pH. The solubility curve for coconut protein isolate obtained by Samson *et al.* (1971) followed the same general shape although the percentage of soluble protein was slightly lower than that of the mung bean protein isolate.

Buffer capacity

Aside from the high buffering capacity at the titration extremes of pH 3 and 10, the best buffering action was in the pH range 5.5–7.5 (Fig. 2). The lowest values for buffer capacity were recorded at pH 4.0 and 8.0. Morr *et al.* (1973) attributed buffering action to the ionization equilibria involving protein carboxyl (pKa 1.8–2.5), citrates (pKa 3.08, 4.74 and 5.40), and phosphates (pKa 2.12, 7.21 and 12.67) and reported increased BC with mineral content.

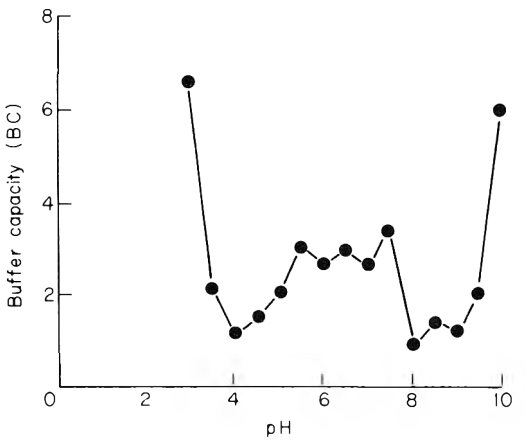

Figure 2. Buffer capacity of mung bean protein isolate at room temperature (27.5°C).

Foamability

Whipping of the mung bean protein isolate was carried out at room temperature, pH 7.0, and 8% protein concentration (w/v). The only time variables were mixer and whipping time.

It was found that stronger mixers yielded greater percentage volume increases and slightly more stable foams within a given whipping time. Specific volume

Table 3. Whipping quality of mung bean protein isolate*

Mixer	Whipping time (min)	Volume increase (%)	Specific volume (ml/g)	Drip 20 min (%)	Drip 30 min (%)	Drip 60 min (%)	Drip 120 min (%)
Hamilton Beach† 79-1, 3-speed	10	424	5.7	29.09	48.93	74.91	85.55
General Electric† M57, 3-speed	10	439	6.7	27.83	51.85	76.28	85.23
General Electric† M68, 10-speed	10	727	9.3	23.97	49.22	71.76	79.36
General Electric† M68, 10-speed	5	650	8.5	28.54	48.86	70.01	79.17
General Electric§ M68, 10-speed	5	625	8.9	5.75	19.17	44.13	54.14

* Condition of whipping: Dispersion of 8% protein in distilled water at pH 7.0 at room temperature.

† Mean of three trials.

‡ Mean of nine trials.

§ Fresh egg white.

was also higher for the ten-speed mixer as compared with the three-speed mixers. Longer whipping times for the weaker mixer did not correct these differences.

Using the ten-speed mixer, 10 min whipping time gave higher specific volume and larger percent volume increase than 5 min whipping time but foam stability was not significantly affected. Whipping times longer than 10 min were not attempted as it seemed unlikely such a product would find general acceptance.

While mung bean protein isolate showed good foamability it did not equal that of egg white. Egg white was more stable, a brighter white, and more brittle than the mung bean isolate. Mung bean foam was a light beige colour and more closely resembled whipped cream than egg white (Table 3).

Gelation

In the absence of more specialized instruments, the simple gelation test described under Materials and methods was carried out to verify the gel-forming ability of mung bean protein isolates (Table 4).

Protein concentration of 10, 12 and 14% consistently gelled upon application of heat. Gels of 10 and 11% protein were softer than gels of 12–14% protein which were very stiff. Samples of 8 and 9% protein were very viscous. In fact, a coagulum was formed but it was not strong enough to prevent gel disruption and slippage when the test tube was inverted.

Circle *et al.* (1964) reported maximum viscosity of sodium soy proteinate under the following conditions: 8% protein at 100°C for 30 min, 10% protein at 100°C for 45 min and 12% protein at 100°C for 60 min. If the time factor

Table 4. Effect of protein concentration on gelation of mung bean protein isolate*

Protein (%)	No. of samples	Gelation†	
2.0	2	–	Liquid
4.0	2	–	Liquid
5.0	3	–	Liquid
6.0	3	–	Liquid
7.0	3	–	Liquid
8.0	9	–	Viscous
9.0	6	–	Very viscous
10.0	6	+	Gel (LCE)‡
12.0	3	+	Firm gel
14.0	3	+	Firm gel

* Conditions of gelation: heating protein solution of pH 7.0 at 80°C for 10 min and cooling to 0°C.

† (–) no gelation, (+) gelation.

‡ LCE: least concentration endpoint, lowest protein concentration at which gel remained in the inverted tube.

was held constant at 30 min, higher protein concentrations required higher incubation temperature to attain maximum viscosity. Thus, the viscosity recorded for 8% protein heated at 80°C for 30 min was equal to that for 10% protein heated at 100°C for 30 min or 12% protein heated at 110°C for 30 min. By comparison, gel formation of a 10% mung bean protein solution heated at 80°C for 10 min appeared to indicate exceptional gelling ability. However, it must be remembered that due to the nature of the test employed, the maximum gelation of mung bean protein may not have been determined. It was very possible that if the time, temperature, pH and protein concentration were more thoroughly investigated, optimal gelling conditions would emerge that were different from those experimental conditions used.

Conclusions

The same amino acid pattern was observed in both mung bean flour and its protein isolate. Individual samples exhibited small variations but it was generally assumed that denaturation did not occur during protein isolate preparation. Cystine, however, was destroyed, perhaps by alkali hydrolysis during extraction. This decreased the nutritional value but did not affect the functional value of the isolate.

Nitrogen solubility at pH values less than 3 or more than 6 yielded 50% more soluble protein. Low solubility of only 2–5% between pH 4–5 limited the isolate usefulness in foods at these pH levels.

Buffer capacity of the mung bean protein isolate was not great and any buffering influence it exerted when incorporated into other foods would be minimal.

Although not equal to whipped egg white, the isolate demonstrated good whipping ability. A creamy, relatively stable foam of pale beige colour was formed from a solution of 8% protein.

Gelation of mung bean protein isolate was found to depend on the protein concentration. Increased gel rigidity occurred with increased protein concentration. The least concentration endpoint (LCE) was established at 10% protein when heated at 80°C for 10 min and cooled at 0°C. Firmer gels were formed with more concentrated solutions.

Practical testing in complicated food systems must be done to firmly establish the role of mung bean protein isolate as a nutritional and functional component in foods. However, process recovery of the waste protein from the noodle manufacture is also of primary concern and must be studied. A future publication will describe this process.

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Protein level in developing seeds of *Vicia faba* L. and their quality in relation to pod position on the stems

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Summary

Samples of two *Vicia faba* cultivars, differing in maturity and seed size were collected at weekly intervals from flowering to maturity. Dry matter and protein contents increased from 14 days after flowering until 56 days seed contained about 40% moisture, then reached a plateau with slight increase until maturity, while moisture content decreased steadily.

Data on pod number, number of seeds in a pod, dry matter, shell and hull contents, crude protein, imbibition and germination capacities and baking time at different stem heights, showed that most of these parameters decreased from bottom to top. There were varietal differences concerning the relationship between agronomic characters, protein content and seed quality and pod position.

Chemical constitutents of seeds and cotyledons showed that carbohydrate comprises about 50–55% and protein 25–30% of the sample, whereas hulls contain about 53% crude fibre. Seed imbibition capacity and baking time were determined.

Introduction

In Egypt, horse beans (*Vicia faba* L.) also called field beans or broad beans are the major legume seed crop grown for human consumption. Green pods are eaten fresh and cooked as vegetable, but mature seeds are baked (Foul Medamis).

Field beans have attracted attention as a possible home grown protein source. The biochemical transformations during seed maturity are highly complex. Draper (1976) found a rapid increase in protein, lignocellulosic material and total dry matter in maturing seeds and pods during a period extending from anthesis to a date 8–10 weeks later. Over the subsequent

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10 weeks there was a much smaller increase in total protein. Silva, Vierra & Sediyana (1976) reported that seeds of Rico-23 variety matured 40–50 days after fertilization at 30–40% moisture content. Cerning, Saposnike & Guibbot (1975) reported a study on sixteen horse bean samples from various geographical origins (Europe, North Africa and Asia) grown in different areas of France. They found that samples had a carbohydrate content of 51–66%, starch from 30 to 42.3% and the carbohydrate content inversely related to protein content, while fat content was low.

In Bulgaria, Kolarova (1976) studied the technological qualities of *Phaseolus vulgaris* seed in relation to the position of pods on the plant. The study showed that seeds of pods developed low on the plant have a smaller proportion of testa than those grown higher on the plant. These testa are thinner and the seeds are cooked easier than those of pods developed high on the plant.

The present investigation aimed to follow protein levels in developing seed and to assess the relationship between pod position on the plant and seed characteristics.

Materials and methods

Seeds of field beans (*Vicia faba* L.) were sown in Kafr El-Sheikh college of Agriculture farm in 1976. The cultivars chosen were: Giza I, a local variety, moderately early with a medium small seeds and a cultivar not licensed in Egypt but referred to commonly as I.C., which is late with long pods, and large seeds. When pods of the central portion of the plants were approximately 2–3 cm long (7 days after flowering), and before there were detectable seeds, with the assumption that since the pods were the same size they were approximately the same age; several hundred pods were tagged. Normally no more than one or two pods per node were tagged, for twenty plants on the interior row for twenty rows plot for each cultivar. At weekly intervals, forty tagged pods were harvested, half of them were hand separated into seeds and shells. Each sample was weighed and dried to a constant weight at 70°C for dry weight, moisture content and shelling percentage determinations. Dried samples were milled for protein analysis.

At maturity, pods were taken from three portions (bottom, middle and upper) of twenty plants to determine agronomic characters; (number of pods, number of seeds per pod, weight of pods and seeds), chemical composition (crude protein, fat, ash, crude fibre, moisture content and total carbohydrate was obtained by difference) and quality (imbibition and germination capacities and baking time). The chemical constituents were determined according to the A.O.A.C. (1960) and protein values were calculated by multiplying the total nitrogen by the factor 6.25. A pooled sample of seeds was separated into hulls and cotyledons for chemical analysis. The values of protein, fat and carbohydrate were multiplied by the factors 4, 9 and 4 respectively to obtain calorific values. Water uptake was determined by steeping 50 g seeds or

cotyledons or hulls for 24 hr at room temperature. The seeds were then drained, spread on a paper towel to remove excess water and weighed. The percentage gain in weight was calculated. For baking time, the steeped seeds or cotyledons or hulls were mixed with five times their weight of water in a tightly covered pan and boiled at 100°C for a period which was adequate for softening and cooking of the beans. The germination test was recorded after 11 days according to the seed book of the U.S.D.A. (1961).

Results and discussion

Dry matter and protein contents

Dry matter accumulation in Figs 1 and 2 outlines the growth pattern of individual seed and pod. About 90% of the dry matter was synthesized during the 50 day period (15–63). The synthesis of seed dry matter slowed after 63 days when the moisture reached around 40%. At this stage the seed and pod reached the maximum size as the mature seeds and pods were smaller and more wrinkled than those of 63 days due to loss of moisture. The moisture and shell percentages fell steadily to a level of 38–42% at 63 days and very rapidly to 11 and 19% after 70 days in chronological order.

Crude protein content of the seed, and pod, (Fig. 3) increased and approached a maximum at 84 days. Seed protein increased 8 and 9%, while pod protein by 6.5 and 7% for I.C. and Giza I respectively.

In general at the early stages of seed bean maturation the differences in the accumulation of dry matter content between the local cultivar (Giza I) and the

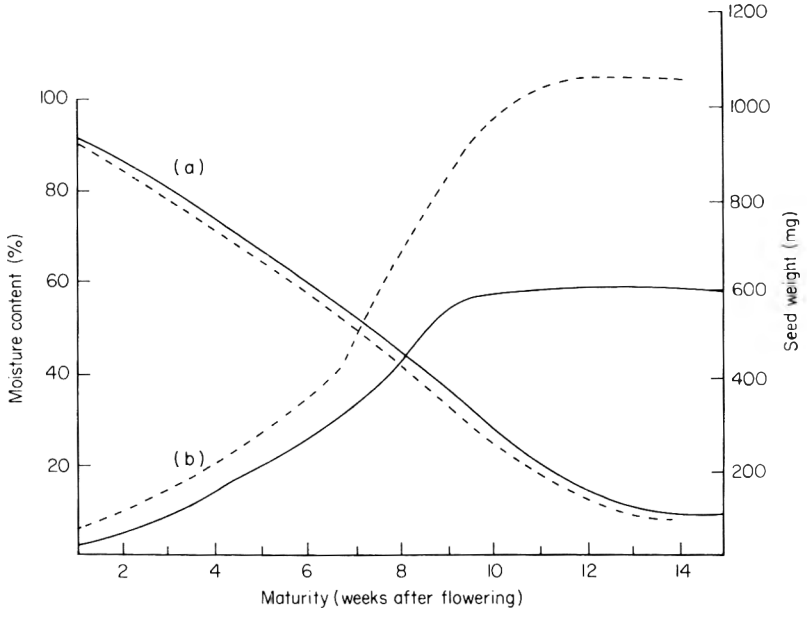

Figure 1. Moisture content (a) and seed dry weight (b) of developing seeds of Giza I (solid lines) and I.C. (dashed lines) *Vicia faba* cultivars.

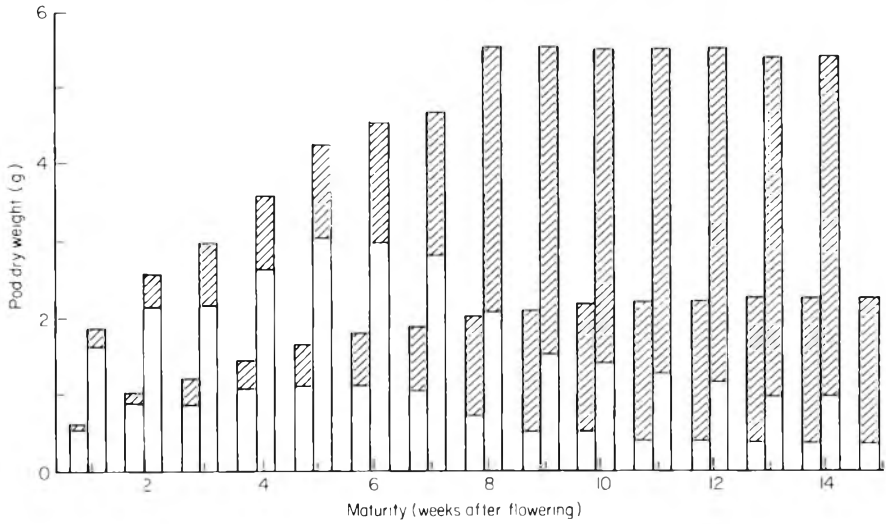

Figure 2. Proportion of pod dry weight as shells (hatched) and seeds (open). The columns at each stage of development represent Giza I (left) and I.C. (right).

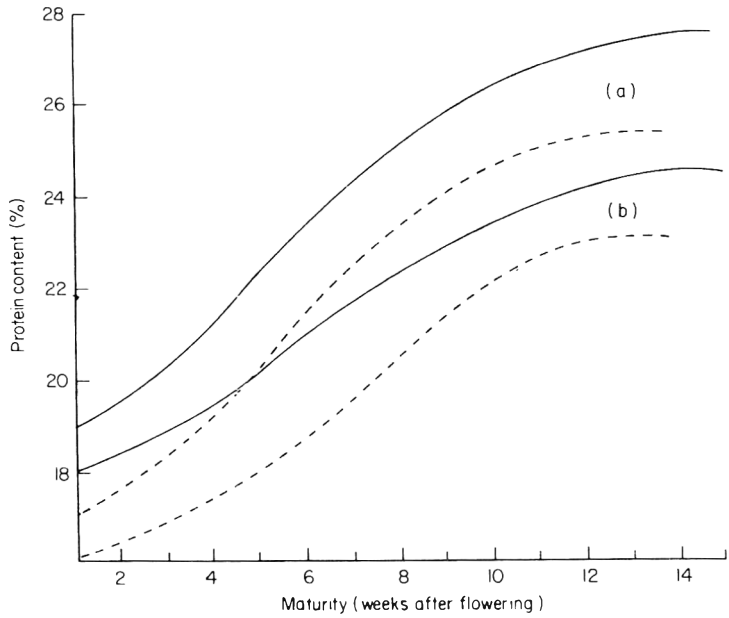

Figure 3. Protein content of seeds (a) and developing pods (b) of Giza I (solid lines) and I.C. (dashed lines) *Vicia faba* cultivars.

introduced cultivar (I.C.) were small, but the gap started to increase after 56 days, then remained virtually constant until maturity. These results are supported by the finding of Briarty, Coult & Boulter (1970). They found that the deposition of reserves in the cotyledons was complete by 85–90 days after flowering, following which water was lost until the seed became hard and ripe at 110 days after flowering.

Table 1. Agronomic characters and quality in relation to pods position on the plant

Characters	Giza I			I.C.		
	Bottom	Middle	Top	Bottom	Middle	Top
Pod						
Number of pods/plant	7.1	9.5	5.9	5.2	7.0	4.0
Pod dry weight (g)	2.0	2.3	1.5	5.3	5.5	4.8
Shell (%)	18.4	18.3	20.1	19.0	19.1	21.5
Protein (%)	24.6	24.5	24.0	23.0	23.0	22.5
Seed						
Number of seeds/pod	2.6	3.0	2.3	4.0	4.1	3.6
Seed dry weight (g)	0.6	0.6	0.4	1.0	1.1	0.8
Hull (%)	13.0	13.0	14.0	12.5	12.5	13.0
Protein (%)	27.8	27.7	27.0	25.5	25.4	25.0
Germination (%)	100	100	95.0	100	100	97.0
Imbibition (%)	96.9	97.0	94.8	109.5	110	105
Baking time (hr)	3.5	3.5	4.0	3.0	3.0	3.5

Seed characters in relation to pod position on the plant

Data in Table 1 show that fewer pods were produced from nodes at the upper portion than the lower parts of the stems, and that they contained fewer seeds which were of small size. There was little variation in the number and weight of pods/plant, seed number/pod, and shell contents between the bottom and middle portion. It is also clear that the introduced cultivar out-yielded the local variety due to the higher number and weight of seeds per pod. On the other hand, the results show little variation for seed position on protein and moisture contents. Similar findings have been reported by Graman & Kumel (1972) and Pederson (1975).

The results (Table 1) show some effect for seed position on germination and imbibition capacities, where the upper seeds were less viable and took up less water than those from the middle or bottom portions. It is also shown that the seeds from the upper portion need more time for baking than those from lower parts since their seed coat (hulls) are thicker. These results agree with that reported by Kolarova (1976).

Chemical constituents and quality

The chemical composition of seeds, cotyledons and hulls are presented in Table 2. It shows that 50.2–54.6% of seeds and cotyledon samples may be accounted for by carbohydrate, followed by protein which ranged from 25 to 29.6%, while the hulls have 5.4–6.7% and about 53% crude fibre. So hulls provide a useful source of protein for animal feeding. A similar finding has been reported by Cerning *et al.* (1975).

Table 2. Chemical constituents and quality of hulls, cotyledons and whole seeds of two *Vicia faba* cultivars

Constituents and quality	Giza I			I.C.		
	Hulls	Cotyledons	Whole seeds	Hulls	Cotyledons	Whole seeds
Moisture (%)	11.5	11.8	11.6	11.1	11.3	11.1
Protein (%)	6.7	29.6	27.5	5.4	27.1	25.0
Ash (%)	2.5	3.3	3.5	2.4	3.1	3.2
Lipid (%)	0.4	1.6	1.2	0.5	1.8	1.5
Crude fibre (%)	53.4	2.4	6.0	52.4	2.1	5.5
Carbohydrate (%)	25.5	51.3	50.2	28.2	54.6	53.7
Calories/100 g	132.4	338.0	321.6	139.3	343.0	328.3
Imbibition (%)	213.4	130.3	96.5	254.0	152.6	109.0
Baking time (hr)	3.5	0.5	3.5	3.0	0.5	3.0

It can also be seen that cotyledons were quicker to imbibe water than whole seeds, indicating the importance of the role played by the seed coat which agrees with the findings of Hollingsworth (1972).

The data given in Table 2 show that cotyledons were cooked more rapidly than their hulls and that the latter controlled the cookability of the seed beans. This agrees with Rockland & Metzler (1967), who reported that tenderization rates of seed coat and cotyledons were different and independent of each other.

Conclusions

The present study shows that the characteristics of seeds from different portions of genetically pure cultivars are significantly different. Therefore, for the experiments in which seed characteristics are measured, the samples should be taken from a defined portion of the plants to minimize the sampling error due to pod position on the plant. Also, it proves that dehulled and whole seed beans provide a useful source of low cost protein for human consumption and that hulls are useful for animal feed.

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The fractionation of porcine plasma by potential food industrial techniques

E. B. DONNELLY* AND R. A. M. DELANEY*

Summary

Chemical precipitation with polyethyleneglycol (PEG) and the technique of gel filtration (GF) were applied to the laboratory fractionation of commercial plasma protein. Both these techniques possess potential for application to large scale protein fractionation in the food industry.

Total plasma protein precipitation varied with molecular weight of PEG and the temperature and pH of the PEG/plasma mixture. High molecular weight proteins were selectively precipitated at low concentrations of PEG 6000.

Gel filtration of porcine plasma yielded three fractions. The main constituents of the first fraction were α globulins, fraction (2) contained γ globulin plus α and β globulins and fraction (3) consisted mainly of albumin with α and β globulins also present. A comparison of the amino acid composition of each of the three fractions with that of FAO whole egg reference protein (FAO/WHO, 1965) indicated that the limiting amino acids of fractions (1) and (2) were isoleucine and tryptophan respectively, while those of fraction (3) were methionine and cyst(e)ine. The values for essential amino acid index of all three fractions were higher than that of whole plasma.

Introduction

For many years blood plasma proteins have been fractionated into components suitable for clinical applications by methods of chemical precipitation (Putnam, 1960) and organic solvent fractionation (Cohn, *et al.*, 1946). Techniques used by the pharmaceutical industry generally produce a high quality product in small quantities. Recently however, continuous fractionation of serum proteins by organic solvent precipitation has been developed in the pharmaceutical industry and electrophoresis has been scaled up in a system capable of handling 1000 litres of protein solution per week (Watt & Smith, 1971).

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An acceptable food industrial technique should be capable of handling large volumes of raw material, it should be simple and easy to operate and the end product should meet the required bacteriological and toxicological standards demanded for that product. Chemical precipitation using polyethyleneglycol (PEG) 6000 offers potential for large scale protein fractionation work since it has been found that PEG 6000/plasma solutions are easy to handle, cause no protein denaturation at room temperature (Polson *et al.*, 1964) and the PEG can be recovered by techniques such as gel filtration (Zeppenzaeur & Brishammar, 1965). In the present series of experiments the effect of PEG molecular weight 400, 1500, 6000 and 20 000 on the precipitation of total plasma protein from PEG/plasma mixtures at pH values of 4, 6 and 8 and temperatures of 4 and 22°C were examined. Those conditions most suitable for the precipitation of total plasma protein formed the basis for experiments on the selective precipitation of plasma protein with PEG.

Gel filtration is at present used in the food industry in the fractionation of whey and skim milk (Delaney, Donnelly & Kearney, 1973). Plasma protein fractionation requires the use of Sephadex G200 (Pharmacia, Sweden) which is unsuitable for use in traditional gel filtration systems because of its high water regain value of 20 ± 2.0 g H_2O/g dry Sephadex. Recently however a stacked sectional column has been developed (Pharmacia in 1971) which gives high resolution of human serum at a flow rate of 13.2 litres/hr. In this study the laboratory fractionation of commercial porcine plasma by gel filtration was developed. The main constituents of the three fractions obtained were identified by polyacrylamide gel and cellulose acetate electrophoresis. The nutritive value of each of the fractions was assessed chemically by amino acid analysis.

Materials and methods

Material

Porcine plasma was supplied by Mitchelstown Co-operative Society Limited, Co. Cork. Collection and separation was as described by Delaney (1975).

Protein precipitation with polyethyleneglycol

The method used was a modification of that employed by Richter, Morr & Reineccius (1974) for the fractionation of whey proteins using polyethyleneglycol. PEG 400, 1500, 6000 (BDH, Surrey) and PEG 20 000 (Guhr, London) were added to separate aliquots of plasma to a concentration of 20% (w/v) PEG : plasma and the pH of the mixtures was adjusted to values of 4, 6 and 8. Duplicate aliquots at each pH were held for 24 hr at 4 and 22°C. The samples were subsequently centrifuged for 30 min at 12 000 *g* at room temperature. The difference in the total nitrogen content of the supernatants and that of the original plasma was used as a measure of protein precipitation. The effect of varying the concentration of PEG 6000 from 2.5, 5–50% (w/v) PEG : plasma on

the precipitation of total plasma protein at pH 4 and 6 and 22°C was measured in a similar manner.

Selective plasma protein precipitation with PEG was examined by adding PEG 6000 to aliquots of plasma in the concentration range 4, 8, 12 ... 52% (w/v) PEG : plasma. The pH was adjusted to 4 and the mixtures held for 24 hr at 22°C. Total nitrogen estimation and electrophoretic studies were conducted on the supernatants obtained after centrifugation of the mixtures at 12000*g* for 30 min.

Protein

Protein was obtained by micro-Kjeldahl estimation using a factor of N × 6.25 (Horwitz, 1970).

Viscosity measurements

PEG 6000 and 20000 were added to 100 ml samples of plasma to a concentration of 20% (w/v) and the pH of the mixture adjusted to 4, 6 and 8. After standing for 24 hr at room temperature, the mixtures were filtered through Whatman No. 1 filter paper. The rate of filtration of the plasma/PEG mixture was used as an index of viscosity. In a similar manner the viscosities of plasma solutions containing 10, 20 ... 50% (w/v) PEG 6000 : plasma were measured.

Electrophoresis

Polyacrylamide gel (PEG) electrophoresis was conducted in a vertical slab electrophoresis unit (E. C. Apps, Philadelphia, U.S.A.). The gel used was 5% cyanogum to which 0.13% NN-methylene bisacrylamide has been added. A continuous buffer system employing tris buffer pH 9.2 was used to separate the proteins. Cellulose acetate (CA) electrophoresis was conducted according to the method of Plummer (1971). Samples of the fractions obtained by gel filtration of porcine plasma were concentrated prior to electrophoresis by dialysis for 24 hr against a 50% solution of PEG 1500.

Amino acid analysis

After acid hydrolysis in 6 N HCl for 22 hr duplicate samples were analysed by the procedure of ion exchange chromatography (Spackman, Stein & Moore, 1958) using a Technicon TSMI automatic analyser. Tryptophan was determined after alkaline hydrolysis by the method of Slump & Schreuder (1967).

Results and discussion

The variation in the levels of total protein precipitated by PEG 400, 1500, 6000 and 20000 at pH's of 4, 6 and 8 at 4 and 22°C is shown in Fig. 1. As the

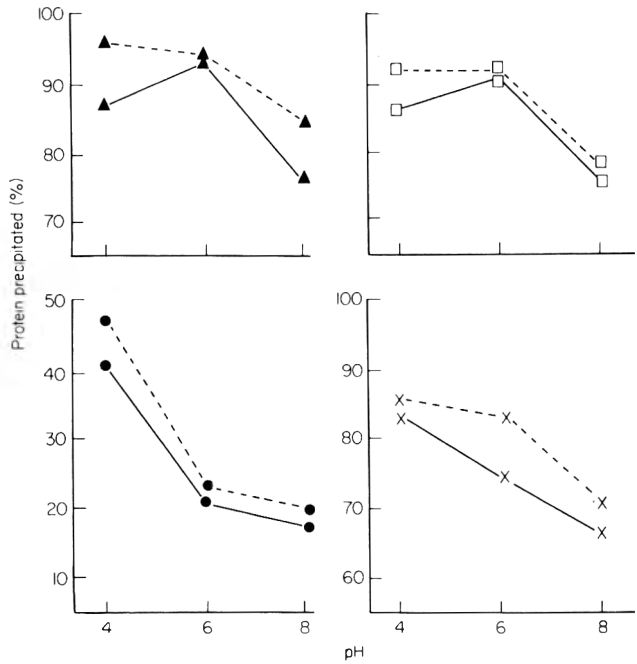

Figure 1. Effect of PEG 400 (●), 1500 (x), 6000 (▲) and 20 000 (□) on precipitation of total plasma protein at 4 (——) and 22°C (– – – –).

pH decreased from 8–4 the amount of total protein precipitated by the lower molecular weight polymers PEG 400 and 1500 increased. The decrease in protein solubility with decrease in pH can be predicted from an equation developed by Juckes (1971) which describes the variation in protein solubility in the presence of polyethyleneglycol. The equation is:

$$\text{Log } S = \text{K} - Bw$$

$$B = \frac{\bar{v}}{2.303}\left(\frac{R_s + R_r}{R_r}\right)^3$$

where S is the solubility, K is a constant, w is the polymer concentration, $\bar{v}$ is the partial specific volume, R_s is the cross sectional radius of the polymer and R_r the radius of the molecule excluded. Juckes (1971) found that a reduction in the pH of the PEG/protein mixture was attended by an increase in the value of β and a large decrease in K, resulting overall in a decrease in protein solubility with a decrease in pH. This pH effect was not evident with the polymers PEG 6000 acting at 4°C or PEG 20 000, where there was a decrease in solubility as pH increased from 4 to 6.

Total plasma protein precipitation at 22°C was equal to or greater than that at 4°C. In the precipitation of serum albumin by PEG an increase in temperature was found to lower the value of β and gave little effect on K giving an overall effect of increasing solubility with increasing temperature (Juckes,

1971). In the case of the plasma proteins an increase in temperature may lower the value of K and thus produce a decrease in solubility with a rise in temperature.

PEG 20 000 and 6000 precipitated more total protein than PEG 1500 which in turn caused a higher level of protein precipitation than PEG 400. Polson *et al.* (1964) also found that the ability of PEG to precipitate protein was an inverse function of the molecular weight of the PEG species employed. Higher concentrations of low molecular weight polymers were required to effect the same amount of precipitation as low concentrations of high molecular weight polymers. The present experimental findings support the theory of protein precipitation by PEG as an exclusion mechanism of protein from the PEG matrix. Introduction of PEG into a protein solution will lower the amount of solvent available to the protein, causing protein precipitation to occur. Both the higher molecular weight polymers PEG 6000 and 20 000 caused almost total precipitation of plasma protein under the experimental conditions. The effect of increasing the concentration of PEG 6000 from 2.5, 5–50% at pH 4 and 6 and 22°C is shown in Fig. 2. The amount of total protein precipitated remained steady at 96% after a concentration of 20% (w/v) PEG : plasma had been reached. The value of 96% would therefore appear to be the maximum level of protein precipitation attainable under the existing chemical conditions.

The viscosity indices of solutions containing PEG 20 000 were higher than those of PEG 6000 as seen from Fig. 3. Sedimentation and protein recovery from plasma solutions containing PEG 20 000 was difficult due to the high viscosity of the solutions. In Fig. 4 the increase in viscosity of plasma solutions containing increasing concentrations (10–50%) of PEG 6000 at pH 4 and 22°C

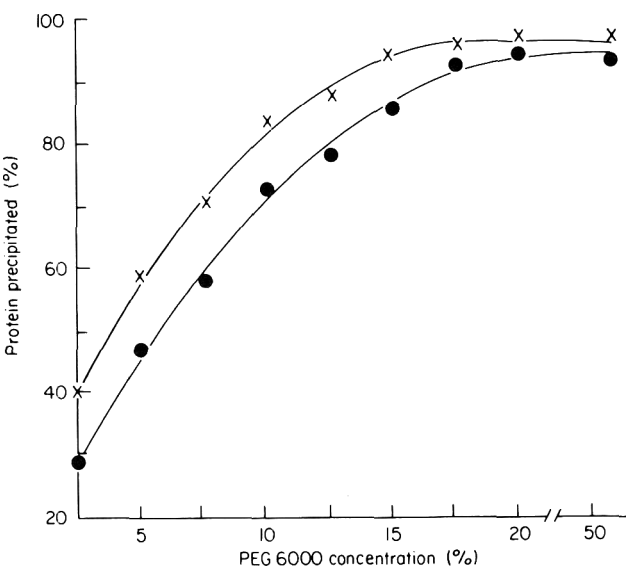

Figure 2. Effect of concentration of PEG 6000 on precipitation of total plasma protein at pH 4 (x) and pH 6 (●).

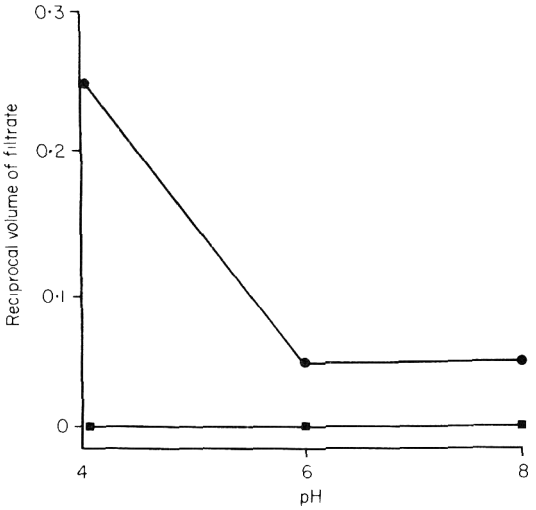

Figure 3. Effect of PEG 6000 (■) and 20 000 (●) on filtration rate of PEG/plasma mixture.

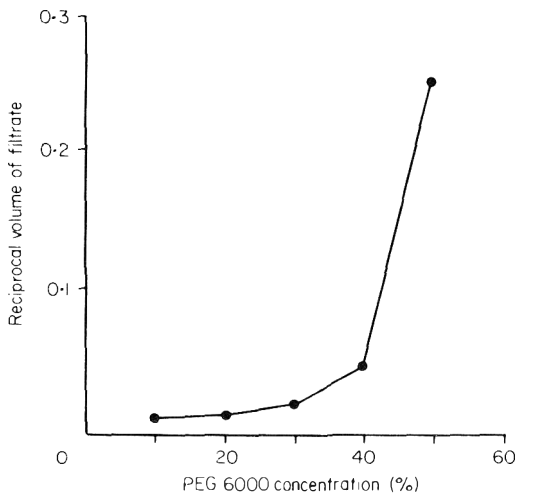

Figure 4. Effect of concentration of PEG 6000 on filtration rate of PEG/plasma mixture.

is shown. Solutions containing 20% (w/v) PEG 6000 : plasma were sufficiently viscous for easy handling.

Optimum conditions for plasma protein precipitation, i.e. using PEG 6000 at pH 4 and 22°C were chosen to investigate the precipitation of individual plasma proteins by adding increasing amounts (4, 8 ... 20%) PEG to plasma. Only those supernatants obtained after sedimentation of the precipitate formed on addition of 4 and 7% (w/v) PEG to plasma showed up clearly on electrophoresis. The protein contents of the remaining supernatants were less than 4%. Unsuccessful attempts were made to concentrate the protein by removing PEG in prolonged dialysis. At a concentration of 4% (w/v) PEG : plasma some globulins

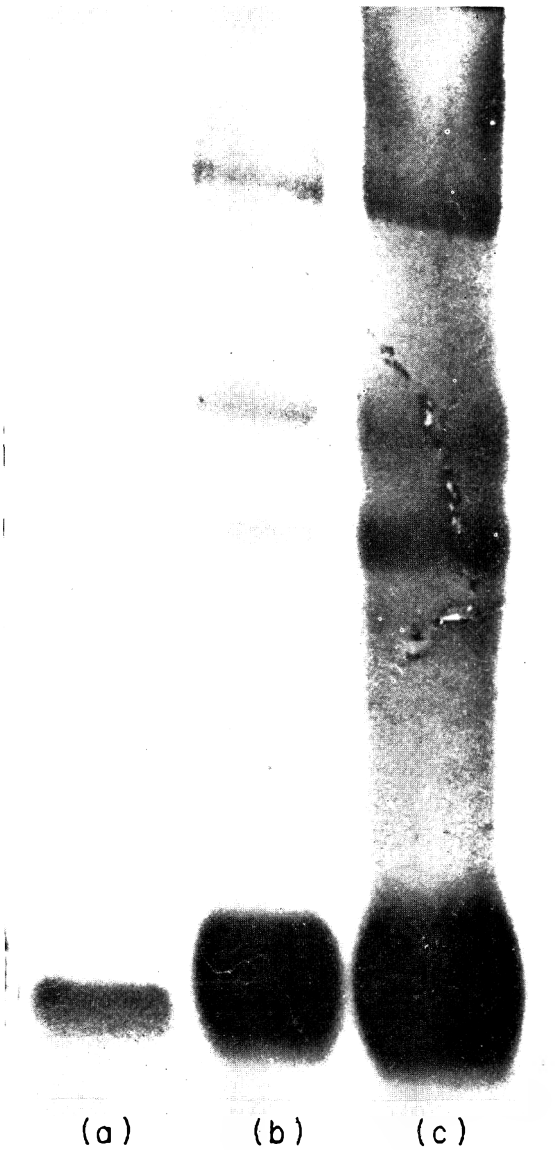

Plate 1. Polyacrylamide gel electrophoretic pattern of (a) porcine plasma, (b) supernatant formed on addition of 4% PEG, and (c) 8% PEG to plasma.

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Plate 2. Polyacrylamide gel electrophoretic pattern of fractions obtained by gel filtration of porcine plasma; (a) first fraction, (b) γ globulin standard, (c) second fraction. (d) α globulin standard, (e) third fraction, (f) albumin standard, (g) porcine plamsa

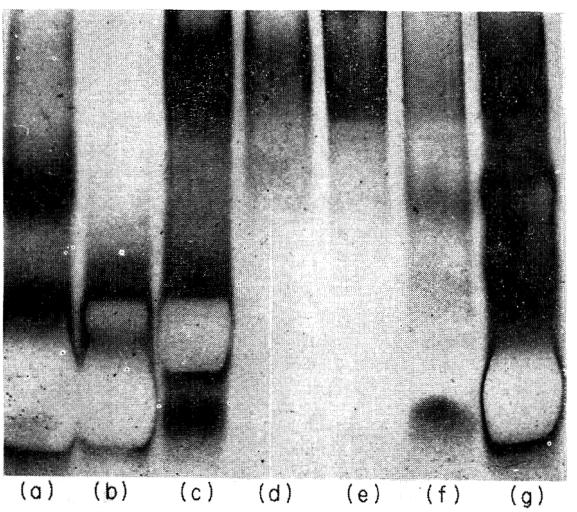

Plate 3. Polyacrylamide gel electrophoresis pattern of fractions obtained by gel filtration of a solution of albumin and globulin standards; (a) third fraction, (b) albumin standard, (c) second fraction, (d) γ globulin standard, (e) first fraction, (f) α globulin standard, (g) porcine plasma.

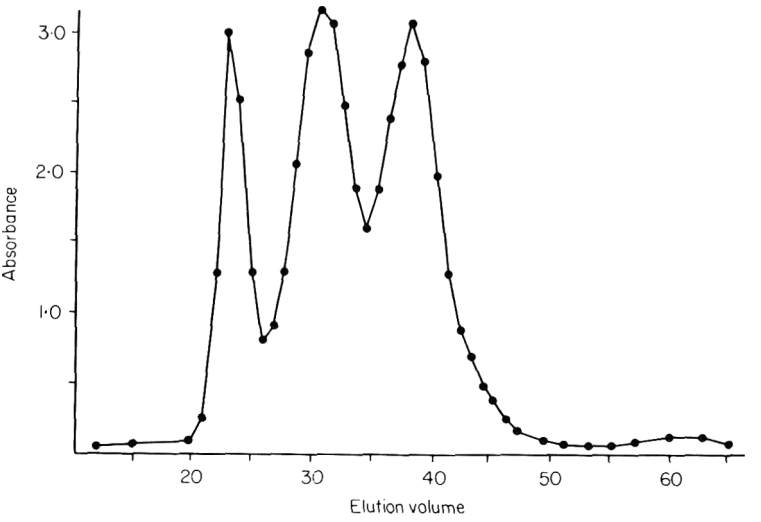

Figure 5. Gel filtration diagram of porcine plasma. Sample size 5 ml of plasma diluted 2:1 in buffer; tris buffer pH 8; column dimensions 2.2 × 70 cm; rate of development 10 ml/hr.

and a small amount of albumin were precipitated while with 8% (w/v) PEG: plasma the supernatant was almost pure albumin (Plate 1). Chesebro & Svehag (1968) investigating the precipitation of human serum proteins found that although in general higher molecular weight proteins were precipitated first, precipitation did not proceed strictly according to molecular weight. Hydrogen ion concentration has been found to be the most critical factor affecting selectivity of precipitation by PEG (Polson *et al.*, 1964). Removal of PEG from PEG/protein mixtures has been successfully achieved by salt precipitation followed by ion exchange chromatography (Polson & Ruiz-Bravo, 1972) or by gel filtration (Gambal, 1971).

Gel filtration of porcine plasma yielded three fractions the KAV values of which were 0.08, 0.42 and 0.75 respectively (Fig. 5). Identification of the main constituents of the three fractions was achieved by electrophoresis of specimens of the bulk samples of each fraction with standards. The results of polyacrylamide gel electrophoresis are shown in Plate 2. The densitometric scans of the cellulose acetate electrophoretic patterns of fractions 1, 2 and 3 and standards are shown in Figs 6 and 7. The main constituents of the first fraction were α globulins, fraction (2) contained γ globulin, albumin and other globulins while fraction (3) contained albumin with a substantial amount of α and γ globulins also present. The gel filtration diagram of a mixture of standards containing 60% albumin, 20% α globulin and 20% γ globulin is shown in Fig. 8. The KAV values for fractions 1, 2 and 3 were the same as those obtained by the fractionation of porcine plasma. The PAG electrophoretic pattern of specimens of bulk samples of the fractions shown in Plate 3 identified the main constituents of fraction (1) as α globulins, fraction (2) contained γ globulin, α globulins and albumin while fraction (3) consisted of albumin

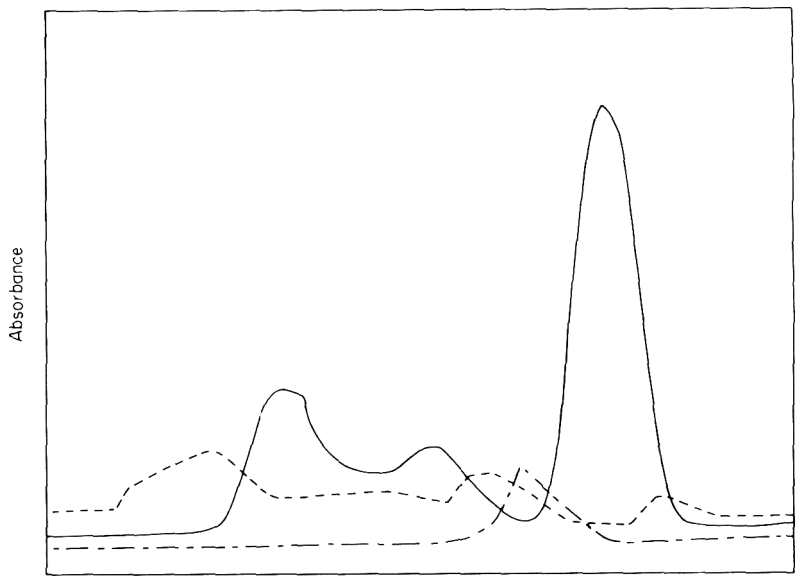

Figure 6. Densitometric scan of the electrophoretic pattern of the first (—·—) second (– – – –) and third (——) fraction obtained by gel filtration of porcine plasma.

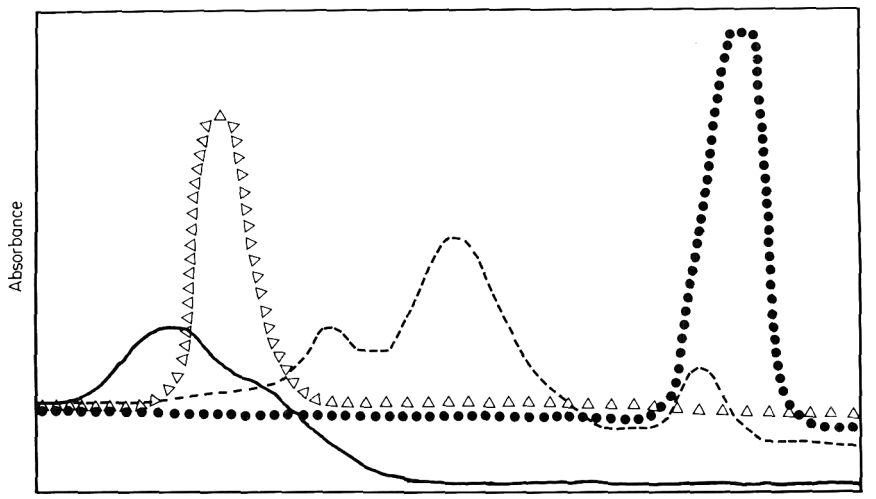

Figure 7. Densitometric scan of electrophoretic pattern of the standards albumin (●), γ globulin (——), fibrinogen (△) and α globulin (– – – –).

with some α globulins also present. The major protein constituents of porcine plasma fractions were similar to those found in the fractions obtained by gel filtration of human serum proteins (Flodin & Killander, 1962). The human serum fractions were shown by immunoelectrophoresis to contain a number of minor protein components. Large scale gel filtration of human serum has been achieved using a stacked sectional column (KS 370, Pharmacia, Sweden). It would therefore appear that plasma could be industrially fractionated in a similar manner. Plasma contains *c.* 1–3% fibrinogen, molecular weight 340 000

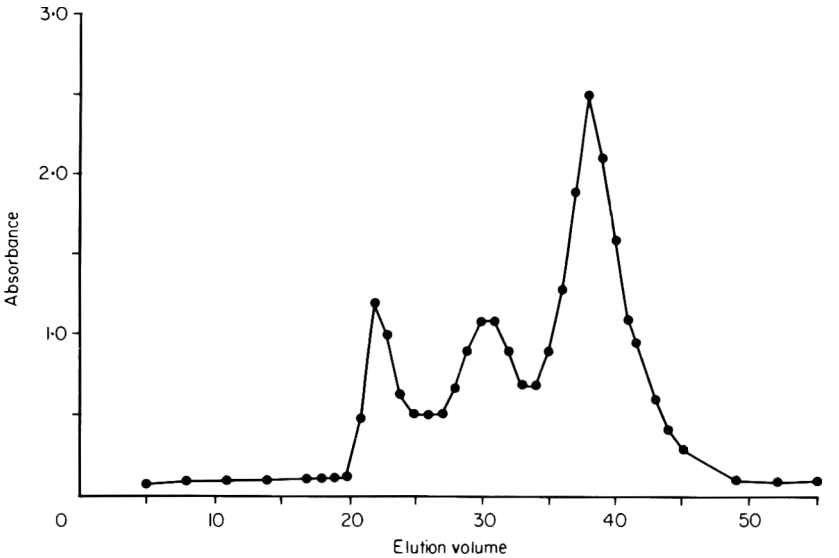

Figure 8. Gel filtration diagram of a mixture of standards containing 60% albumin, 20% γ globulin and 20% α globulin. Sample size 5 ml of standard solution diluted 2 : 1 in buffer; tris buffer pH 8; column dimensions 2.2 × 70 cm; rate of development 10 ml/hr.

daltons which is absent from serum. This high molecular weight constituent would probably appear in the first fraction although its presence was not detected in the laboratory separation of plasma.

The detailed amino acid profiles of the plasma fractions obtained by gel filtration of porcine plasma are given in Table 1. Data on the amino acid composition of porcine plasma (Delaney, 1975) are also tabulated. A comparison of the essential amino acids of fraction (1) with whole egg protein revealed that fraction (1) contained excess amounts of threonine, lysine, tryptophan and cyst(e)ine. The values for valine, isoleucine, phenylalanine, lysine, tryptophan, methionine and cyst(e)ine were higher in fraction (1) than in porcine plasma concentrate. The chemical score (CS) for fraction (1) was 58 with isoleucine as the limiting amino acid. The essential amino acid index (EAAI) for this fraction was 87.8 as compared to a value of 70.1 for plasma concentrate. In fraction (2) the values for leucine, tryosine, phenylalanine lysine and cyst(e)ine were higher than in egg protein. The CS for fraction (2) was 31 with tryptophan as the limiting amino acid. The EAAI was 73.2. Fraction (3) contained greater amounts of threonine, valine, tyrosine, lysine and tryptophan than egg protein Methionine and cyst(e)ine were the limiting amino acids in fraction (3) which had a CS of 36. The EAAI value was 79.6. Since the essential amino acids which were present in excess amounts varied with the three fractions, each fraction could act as a supplement in different foods. The EAAI values of all three fractions were higher than those of whole plasma. Delaney (1975) found a close correlation between EAAI values and the biological value of porcine plasma concentrate determined from rat feeding experiments. In the same

Table 1. Amino acid composition of porcine plasma fractions

Amino acid	Fraction (1)	Fraction (2)	Fraction (3)	Porcine plasma concentrate*	Whole egg FAO reference protein
Asp	8.2	9.5	8.2	10.1	
Thr	5.5	4.3	6.7	8.5	5.1
Ser	5.5	3.6	6.7	6.5	
Glu	11.7	13.6	10.8	14.0	
Pro	5.5	4.8	7.1	5.9	
Gly	4.3	2.6	4.2	3.3	
Ala	11.5	5.5	4.3	5.1	
Val	6.2	6.0	8.3	5.8	7.3
Ile	3.8	3.5	3.2	3.2	6.6
Leu	7.3	10.1	8.0	9.2	8.8
Tyr	4.1	5.4	6.1	4.7	4.2
Rhe	5.1	6.2	4.9	4.5	4.8
Lys	9.5	11.0	8.3	9.4	6.4
His	3.1	4.0	2.6	3.2	
Arg	6.2	6.4	6.7	5.1	
Trp	2.2	0.5	1.8	1.3	1.6
Met	2.1	0.5	1.3	1.1	3.1
Cys	3.1	4.9	0.7	2.1	2.4
Total amino acids	105.0	102.6	100.2	103.0	
Chemical score	58.0	31.0	36.0	49.0	
EAAI	87.8	73.2	79.6	70.1	

* Delaney (1975)

study the nutritional value of porcine plasma was found to be similar to that of casein. On this basis it would appear that the nutritive value of the prepared plasma fractions was comparable to that of casein, which is generally accepted as a nutritionally adequate industrial food protein.

Conclusions

PEG 6000, which is a suitable chemical precipitant for industrial use, can selectively precipitate plasma proteins under chosen chemical conditions. Plasma fractions can also be prepared by gel filtration, a separation technique already used in the food industry. The fractions prepared by gel filtration of plasma have a nutritive value similar to that of whole porcine plasma. Large scale fractionation of blood plasma therefore appears industrially feasible.

Acknowledgments

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Evaluation of some factors useful for the mathematical prediction of moisture gain by packaged dried beef

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Summary

Several factors were evaluated which may be useful for the mathematical prediction of moisture gain by air dried beef packeaged in flexible films. The absence of significant moisture gradients inside a package of dried beef pieces during storage with external water transfer was experimentally verified. This condition of equilibrium greatly facilitates the mathematical analysis of moisture gain during storage. It is also shown that the temperature at which the beef was dried may considerably affect the prediction of the kinetics of moisture gain. This is due to the influence of drying temperature on the equilibrium moisture content of air dried beef. Finally, it is shown the type of results which should be expected when different isotherm equations, including a single straight line, are used to describe the water sorption behaviour of beef.

Introduction

The prediction of storage life of dehydrated foods packaged in flexible films is of obvious importance in the area of food preservation. Traditionally, storage tests were done by storing the packaged dried foods at different external conditions (i.e. relative humidity and temperature) and testing periodically for chemical deterioration and/or for organoleptic acceptability. In the last years, however, there has been an increased interest in the development of mathematical models for optimization of flexible film packaging of dehydrated foods and promising results were obtained in this way. The development of these models is largely due to the work of Karel and coworkers (Karel, 1967; Mizrahi, Labuza & Karel, 1970; Simon, Labuza & Karel, 1971; Labuza, Mizrahi & Karel, 1972; Quast, Karel & Rand, 1972; Quast & Karel, 1972) who were able to develop the prediction of storage stability as a function of package properties for dried foods deteriorating through different moisture sensitive

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reactions. These models were based on the combination of kinetic data for the deteriorative reactions, the water sorption propoerties of the food, and the permeability characteristics (to water and/or oxygen transport) of the package.

Precooked dried beef mainly deteriorates through a mechanism of non-enzymatic browning and lipid oxidation (Sharp & Rolfe, 1958), reactions whose rate depend on the moisture content (Labuza, Tannenbaum & Karel, 1970). For this reason, the prediction of moisture gain by dried beef packaged in a flexible film would be necessary in order to predict the storage life of the product. The mathematical prediction of moisture gain by a packaged dried food is based on the rate of water vapour transport through the flexible film and the sorption characteristics of the food (sorption isotherm). This paper is concerned with the evaluation of some factors which may be useful for the mathematical prediction of moisture gain by air dried beef. These factors are, (a) the assumption of no significant moisture gradients inside the package, (b) the effect of the previous drying temperature on the shape of the water sorption isotherm of beef, and (c) the use of different isotherm equations also including the single straight line for the sake of simplicity.

Results and discussion

The rate of transport of water vapour through a flexible film is given by, (Labuza, Mizrahi & Karel, 1972)

$$\frac{dw}{d\theta} = \frac{P.A}{e}(p_e - p) \tag{1}$$

where w = weight of water transferred across the film (g), θ = time (sec), P = permeability of the film (g water. cm/cm^2. sec. cm Hg), e = film thickness (cm), A = area of the film (cm^2), p_e = vapour pressure of water outside of film (cm Hg), p = vapour pressure of water on other side of film (cm Hg).

In order to solve for water gain it is usually assumed (Mizrahi *et al.*, 1970) that the water which enters the package rapidly equilibrates with the food, that is to say that the major resistance to water transport is in the film. The internal vapour pressure p, is therefore solely determined by the water sorption isotherm of the food. This assumption is now experimentally verified for packaged dried beef pieces in the following manner. Dried pre-cooked beef samples (2.2% initial moisture content) of about 1.0 × 1.0 × 0.6 cm in size were placed in a metal container that had a membrane sealed to its top. This membrane, (Ficel K, 3L, Schcolnik, cellophane coated polyethylene) may be considered as a moderate barrier to water vapour transfer. In this experiment the moisture could enter the beef only from the top. The beef samples were arranged as closely as possible in four layers with a total thickness of about 2.5 cm, and placed in a glass desiccator containing a saturated salt solution which provided a constant relative humidity of 75%. The desiccator was placed in a constant temperature oven at 37°C. Periodically, samples of beef were

Table 1. Moisture content in specified layers of packaged (Ficel K-3L film) dried beef pieces during the course of storage at 37°C and 75% realtive humidity

Layer	Moisture content (% dry basis)	
	Storage time – 13 days	40 days
First (uppermost)	5.9	9.7
Second	5.6	9.4
Third	5.8	9.6
Fourth (lowest)	5.7	9.6

Initial moisture content = 2.2% dry basis (fat-free).
Equilibrium moisture content (75% external r.h.) = 17% dry basis (fat-free).

withdrawn from the first (uppermost), second, third, and fourth (lowest) layers and analysed for moisture content. The results are shown in Table 1. It can be seen that the moisture content of the different layers is almost the same. Thus, it appears that the assumption of equilibrium is close to reality. These results are in agreement with those reported by Mizrahi *et al.* (1970) who found that in a 2 cm thick layer of freeze dried cabbage powder packaged in Scotchpak 48 film, the major resistance to water transport was in the film. We may advance that for common packaging conditions (i.e. low film permeabilities), the situation of internal equilibrium in packaged dried beef is relatively easy to attain and could be assumed with little risk for the mathematical prediction of moisture gain.

Effect of the drying temperature on the kinetics of moisture gain

In a recent paper, Iglesias & Chirife (1976) showed that the drying temperature noticeably affects the sorption isotherm of air dried beef. It was observed that the higher the drying temperature (between 30 and 70°C), the lower was the sorption capacity of beef. The adsorption isotherms at 30°C of pre-cooked beef previously dried at 30, 55 and 70°C, were very well described by using a multilayer adsorption equation, originally developed by Halsey (1948). Halsey's equation as used by Iglesias & Chirife (1976) is:

$$p/p_0 = \exp(-a''/X^r) \tag{2}$$

where p/p_0 = water activity, a_W; a'' and r are parameters and X = moisture content (% dry basis; fat-free).

The resulting equations for the different beef isotherms were:

$$p/p_0 = \exp(-18.50/X^{1.448}), \text{ for beef dried at } 30°C \tag{3}$$

$$p/p_0 = \exp(-15.08/X^{1.392}), \text{ for beef dried at } 55°C \tag{4}$$

$$p/p_0 = \exp(-10.70/X^{1.301}), \text{ for beef dried at } 70°C \tag{5}$$

The mean relative error of those isotherm equations in the range of water activity 0.10–0.80, was shown to be, 2.6, 1.8 and 3.7% respectively. Having verified that the major resistance to water transport is likely to be in the film, equation (2) may be now used to calculate the internal vapour pressure, p, in equation (1). By the substitution of the isotherm general equation (equation 2) into equation (1), and if the moisture content, X, is substituted for the water gain, w, in equation (1), it follows that:

$$\frac{dX m_s}{d\theta} = \frac{PA}{e} [p_0 \exp(-a''/X_e^r) - p_0 \exp(-a''/X^r)] \quad (6)$$

then,

$$\frac{dX}{\exp(-a''/X_e^r) - \exp(-a''/X^r)} = \frac{PA}{e m_s} p_0 \, d\theta \quad (7)$$

where $PA/e\,m_s = P_f$ = packaging factor (g water/sec. g solid. cm Hg), X = moisture content (% dry basis; fat-free), m_s = weight of solids (fat-free), p_0 = saturation vapour pressure of water at the temperature of the experiment, X_e = moisture content of beef as as predicted by the isotherm equation if exposed to the external packaged relative humidity (% dry basis; fat-free)

Equation (7) cannot be integrated and it was numerically evaluated by using a computer, for the different isotherm equations indicated by equations (3) to (5). The following situation, likely to represent packaging and storage conditions of air dried beef was considered:

External relative humidity = 50% and 70%
Storage temperature = 30°C
m_s = 88 g
A = 600 cm^2
$P = 1.82 \times 10^{-12}$ g water.cm/cm^2.sec.cm Hg
$e = 2.5 \times 10^{-3}$ cm

The results are shown in Fig. 1 representing the predicted moisture gain by packaged dried beef samples (initial moisture content 4%) previously dried at the temperatures of 30, 55 and 70°C respectively. It can be seen that as the drying temperature appreciably changes the shape of the water sorption isotherm of beef (Iglesias & Chirife, 1976), it also significantly affects the kinetics of moisture gain. It is also observed that the beef sample dried at high temperature would gain water more slowly than the one dried at low temperature.

Linearization of the sorption isotherm equation and use of other isotherm equations

In considering the use of different isotherm equations to predict the moisture gain by dried beef, the moisture content range of interest must be first established. A moisture content of 4% is taken as the initial moisture value of beef because this is a likely value for the air dried product, and because it is

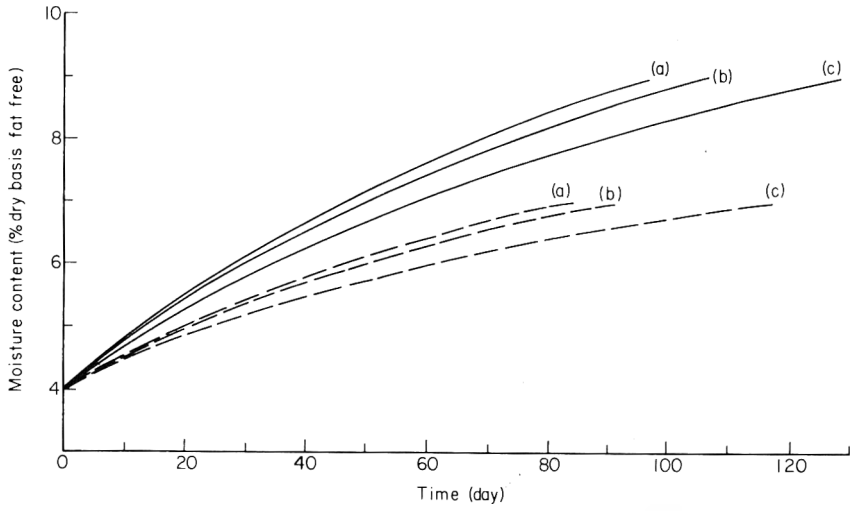

Figure 1. Effect of the previous drying temperature on the predicted moisture gain for beef dried at 30 (a), 55 (b) and 70°C (c) and stored at 30°C (arbitrary packaging factor) at relative humidities of 50 (– – –) and 70% (——). $P_f = 5 \times 10^{-9}$ g water/sec x g solid (fat-free) x cm Hg.

known that very low moisture contents may favour lipid oxidation (Martínez & Labuza, 1968). It is difficult to specify the maximum permissible moisture content in dried beef. This is due to the fact that many of the deteriorative reactions which cause changes in flavour, texture and colour of dried beef proceed in a manner that does not allow the definition of a critical moisture content. As a maximum moisture condition a water activity of about 0.70 gives reasonable protection against microbial spoilage (Mossel & Ingram, 1955). In practice, however, the moisture content of dehydrated beef must be below equilibrium at 50% relative humidity in order to reduce the rate of deteriotation caused by non-enzymic browning (Sharp & Rolfe, 1958). With this in mind, and considering the sorption isotherm of beef, dried at 55°C, (Iglesias & Chirife, 1976) at a storage temperature of 30°C, the following alternative moisture ranges will be considered, (a) 4–9% (dry basis, fat-free) and (b) 4–13% (dry basis, fat-free).

(*a*) *Moisture content range* 4–9%

If the sorption isotherm of the food is approached by a single straight line:

$$X = b a_W + C$$

where b is the slope and C the intercept, equation (1) after substitution is integrated to give:

$$\ln \frac{X_e - X_0}{X_e - X} = \frac{P A p_0}{e m_s b} \cdot \theta \qquad (9)$$

where X_0 = initial moisture content, X = moisture content at time θ.

Consequently, the moisture content-water activity data in beef (in the range here considered) were correlated by using a single straight line. A least squares analysis was used to obtain the slope of the correlating straight line which was substituted into equation (9). The corresponding curve of moisture content versus time was calculated using the same arbitrary packaging factor, as in the example of Fig. 1; external relative humidity was in this case 60%.

The B.E.T. equation (Labuza, 1968) was also considered to correlate equilibrium moisture contents in beef. The B.E.T. equation may be written (Cregg & Sing, 1967):

$$\frac{dX}{X_m} = \frac{1}{p_0} \cdot \frac{dp}{(1-p/p_0)^2} + \frac{1}{p_0} \cdot \frac{(C-1)\,dp}{[1+(C-1)p/p_0]^2}$$

where X_m = monolayer moisture content and C is constant.

The rate of water transfer through the flexible film is given by:

$$\frac{dX}{d\theta} = \frac{PA}{e\,m_s} \cdot (p_e - p)$$

substituting dX:

$$\left(\frac{1}{p_0} \frac{dp}{(1-p/p_0)^2} + \frac{1}{p_0} \cdot \frac{(C-1)\,dp}{[1+(C-1)p/p_0]^2} \right) \frac{X_m}{p_e - p} = \frac{PA}{e\,m_s} \cdot d\theta \qquad (10)$$

X_m and C were first evaluated using the usual B.E.T. procedure (Labuza, 1968) and then equation (10) was numerically evaluated by using a computer. The packaging factor was the same as in the case of the straight line isotherm analysis.

In order to compare the moisture gain curves calculated by using the different isotherm equations, the computer was also fed with the experimental data of water sorption in beef, previously reported (Iglesias & Chirife, 1976), instead of a curve fitting equation. The moisture gain curve was subsequently calculated in a stepwise procedure. The results are shown in Fig. 2, in which there are also plotted the curves obtained through the use of equation (7) based on Halsey's isotherm. It can be seen that the curves calculated with the aid of B.E.T. and Halsey's equations agree very well with that calculated with the experimental isotherm data. However, the one generated by using the straight line simplification shows considerable difference.

(*b*) *Moisture content range* 4–13%

Equilibrium moisture contents in this range were correlated by using B.E.T. and Halsey's equations and the kinetics of moisture gain subsequently calculated. The packaging factor was the same as in case (a), but a external relative humidity of 70% was now considered. The results are shown in Fig. 3. It can be seen that up to about 11% moisture content the curve calculated with the aid of B.E.T. equation is somewhat closer to the 'experimental' curve that the one

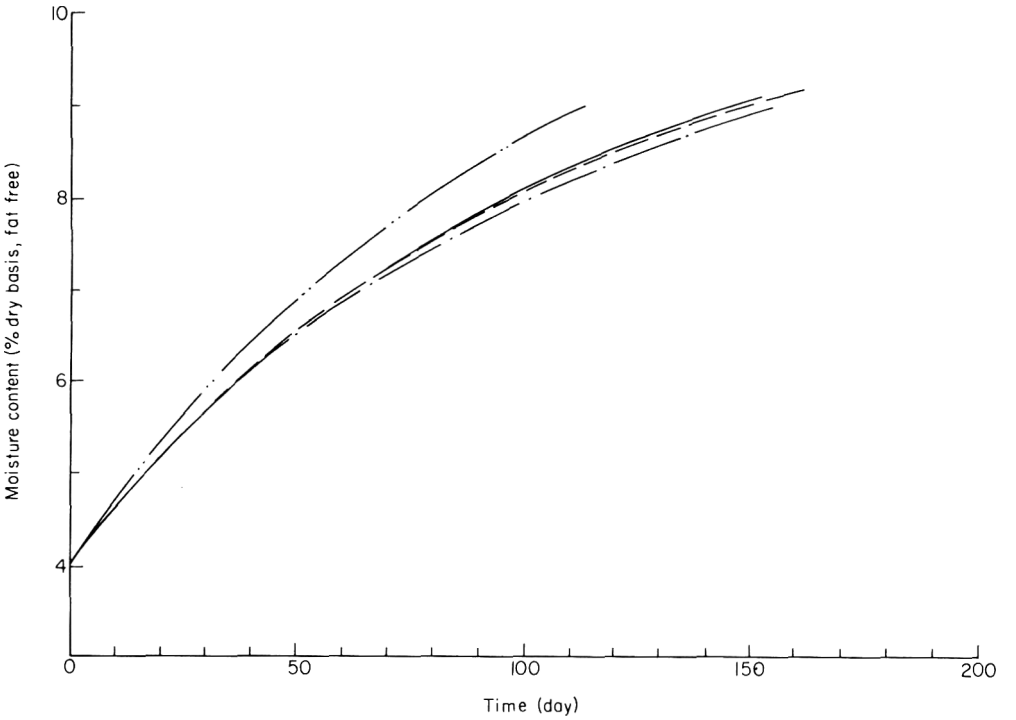

Figure 2. Comparison of predicted moisture gain for dried beef stored at 30°C and 60% r.h. (arbitrary packaging factor) considering Halsey's (—·—) and B.E.T.'s (——) isotherm equations and a straight line simplification (—··—); – – – – 'experimental' isotherm. $P_f = 5 \times 10^{-9}$ g water/sec × g solid (fat-free) × cm Hg.

obtained through Halsey's isotherm. Above 11% moisture content the situation is reversed, and Halsey's based curve show a better fit than B.E.T.'s based one. The straight line approach was not considered in this moisture range because the sigmoidal shape of the isotherm is unlikely fitted by a straight line.

Conclusions

Several factors were evaluated which may help the mathematical prediction of moisture gain by air dried beef packaged in flexible flims. It was experimentally verified that the condition of internal equilibrium (i.e. the absence of significant moisture gradients) in packaged dried beef is easy to attain. This condition of equilibrium greatly facilitates the mathematical analysis of moisture gain during storage. It was also demonstrated that the effect of drying temperature on the shape of the water sorption isotherm of beef may considerably affect the prediction of the kinetics of moisture gain. This suggests that care must be taken in selecting the proper sorption isotherm in any study for predicting moisture gain by packaged air dried beef. Finally, the type of results

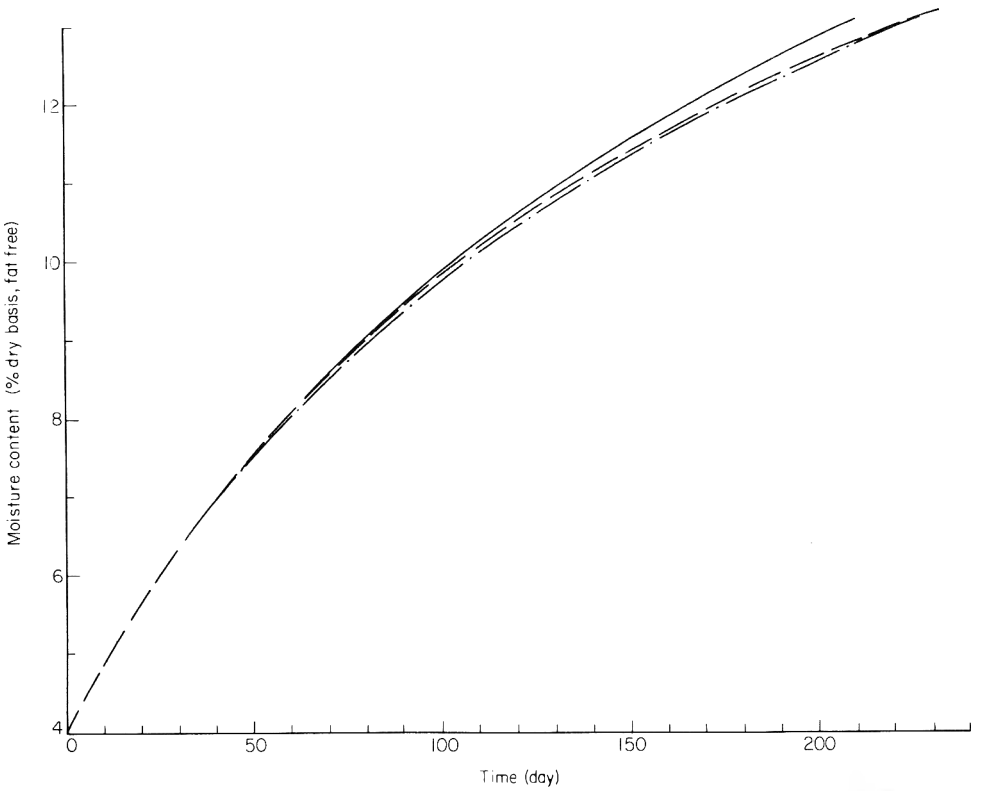

Figure 3. Comparison of predicted moisture gain for dried beef stored at 30°C and 70% r.h. (arbitrary packaging factor) considering Halsey's (— · —) and B.E.T.'s (——) isotherm equations; – – – 'experimental' isotherm. $P_f = 5 \times 10^{-9}$ g water/sec x g solid (fat-free) x cm Hg.

which should be expected when different isotherm equations are used to describe the water sorption behaviour of beef are shown. When a narrow moisture interval is considered (i.e. 4–9%) both B.E.T. and Halsey's based analysis give satisfactory predictions, the former being slightly more accurate. The straight line simplification leads to sizeable errors. For a wider moisture interval, (i.e. 4–13%) a Halsey's based analysis should be preferently made.

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The effect of meat pH and package permeability on putrefaction and greening in vacuum packed beef

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Summary

Beef joints at three pH levels were vacuum packed and stored at 1°C in materials with different gas permeabilities. Dark-cutting, high pH beef (pH 6.2–6.7) developed a green discoloration (greening) in all the packaging materials and had a putrid odour when the packs were opened after 9 weeks. The same type of spoilage occurred at pH 5.9–6.1 in materials of relatively high gas permeability (73 and 92 cm^3 O_2/m^2-day-atm. O_2 at 90% r.h.). Greening and putrefaction did not occur at pH 5.9–6.1 in materials of very low gas permeability (25 and 23 cm^3 O_2/m^2-day-atm. O_2 at 90% r.h.) or in any packs of meat of normal pH (5.4–5.5).

Introduction

It is now common practice in the meat industry to distribute and store chilled beef as large, boneless joints, vacuum packed in materials of relatively low gas permeability. Shelf life is normally limited by the development of sour odours and with good manufacturing practice is at least 2 months at 1°C. However commercial packs of beef sometimes spoil much more rapidly than this, with putrefaction and the appearance of a green discoloration. The main cause of this has been shown to be the high pH of the meat. Greening is due to the pigment sulphmyoglobin, formed by the reaction between myoglobin and hydrogen sulphide produced by bacteria at pH 6.0 and above (Nicol, Shaw & Ledward, 1970). They stated that to avoid greening, meat of a high ultimate pH should not be stored in gas impermeable packages. A similar recommendation was made by Bemm, Hechelmann & Leistner (1976) who found that vacuum packed beef of pH > 6.2 putrefied whilst that of normal pH (5.5–6.0) did not.

Since the hydrogen sulphide in vacuum packed high pH beef is produced by pseudomonads (Nicol *et al.*, 1970), and the growth of *Pseudomonas* spp. is

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inhibited by very low oxygen concentrations (Clark & Burki, 1972), the present experiments attempted to prevent or delay greening and putrefaction by using packaging films of very low oxygen permeability.

Experimental

Meat

Three beef carcasses were selected, 3 days after slaughter, from a commercial abattoir with pH values of 5.6, 6.0 and 6.4 and were designated respectively 'normal', 'intermediate' and 'high' pH. The pH was measured in the eye muscle (*M. longissimus dorsi*) between the 10th and 11th ribs using a Radiometer pH meter Model 29 with a GK2321 electrode. Hindquarters were taken from each carcass and transported under refrigeration to the Meat Research Institute where they were butchered to give four topside, four silverside, and four striploin joints, each weighing approximately 4.5 kg. The pH of the main muscles in each joint was then measured.

To ensure the presence of the appropriate organisms the silverside joints were inoculated with *Pseudomonas* MR 131, a hydrogen sulphide-producing organism isolated in this laboratory from greened vacuum packed beef. The organism was grown in heart infusion broth (Difco) for 24 hr at 25°C, harvested and resuspended to an optical density of 0.285 at 610 μm in Maintenance Medium (MM) containing (w/v) sodium chloride 0.85%, peptone 0.1%; pH 7.0. This suspension was applied at 0.05 ml in^{-2} to Whatman's No. 1 filter paper and the silverside joints inoculated by pressing all their surfaces on to the paper. Topside and striploin joints were not inoculated.

Packaging and storage

Replicate joints were vacuum packed, one in each of four materials the permeabilities of which covered the range of materials commercially available for vacuum packing meat (Table 1). Permeabilities similar to A and B have already been associated with greening; materials C and D were of lower permeability where greening might be prevented. All bags were 25 cm wide and were evacuated and heat sealed using a Swissvac Major vacuum packing machine. None of the bags were heat shrunk and after packing all joints were stored at 1°C for 9 weeks.

Assessment of appearance and odour

Every week during storage the packs were examined in daylight and their appearance recorded. Particular attention was paid to the development of greening, either in the drip or on the meat surface. After 9 weeks when the packs were opened, odour was assessed by a panel of three experts who judged whether the meat would be accepted or rejected by a buyer.

Table 1. The effect of packaging material permeability on the development of greening in vacuum packed beef of different pH values during storage at 1°C

			Packaging material*			
			A	B	C	D
			Oxygen permeability at 90% r.h. (cm^3/m-day-atm. O_2)			
			92	73	25	23
Joint	pH group	pH	Time for development of greening (weeks)			
Silverside	High	6.2–6.7	2	2	2	6
Topside		6.2–6.6	3	3	3	–†
Striploin		6.5–6.6	4	3	4	5
Silverside	Intermediate	5.5–5.9	6	7	–	–
Topside		5.6–6.0	8	8	–	–
Striploin		5.8–6.1	5	5	–	–
Silverside	Normal	5.4–5.5	–	–	–	–
Topside		5.4–5.5	–	–	–	–
Striploin		5.4–5.5	–	–	–	–

* A, Synthene 40T/3627 (Smith & Nephew Plastics Ltd); B, Synthene 38C/3627 (Smith & Nephew Plastics Ltd); C, Synthene 38C/5023 (Smith & Nephew Plastics Ltd); D, Cryovac BB1 (W. R. Grace Ltd).

† No greening at 9 weeks.

Microbiological examination

Each joint was examined microbiologically before packing and after 9 weeks vacuum packed storage by swabbing 100 cm^2 of surface, first with an absorbent cotton wool swab, moistened in 10 ml of diluent (MM) and then with a dry cotton wool swab; the swabs were shaken together with the 10 ml of diluent. Standard drops (0.017 ml) of suitable decimal dilutions were spread on the surface of Plate Count Agar (Oxoid) + 1% NaCl, incubated for 5 days at 25°C to give the total viable count; and Lead Acetate Agar (Difco) containing 0.001% cysteine, incubated in nitrogen + 0.4% oxygen for 2 days at 20°C to count hydrogen sulphide-producing bacteria (Nicol *et al.*, 1970).

Analysis of gas in packs

The volume of gas in the vacuum packs after 9 weeks storage and the concentrations of oxygen, carbon dioxide and nitrogen were estimated, after dilution with helium, on a gas chromatograph (Model 69-552 GOW-MAC Instrument Co., Shannon, Ireland).

Results

There was considerable variation in pH between different muscles and the ranges of pH within each joint are shown in Table 1. It was considered that the higher pH muscles within joints were the most important with respect to putrefaction and greening, and the maximum pH values in joints from the same carcase differed so slightly that it was possible to treat them as groups of 'normal', 'intermediate' or 'high' pH joints according to the carcases from which they were taken.

Appearance and odour

The high pH joints were considerably darker than those of intermediate or normal pH both at time of packing and during storage. At all pH levels the purple colour of the reduced myoglobin pigment, typical of vacuum packed beef was brightest with material D, closely followed by C. With materials A and B the surface colour dulled during storage as metmyoglobin formed from diffusing oxygen but this did not make the colour unacceptable.

Greening developed, first in the free exudate around the meat and then on the surface of the meat (Table 1), in all the high pH joints except the topside packed in material D. At the intermediate pH it occurred only in those packed in materials A and B, and its rate of development was slower than at high pH. Greening did not develop at normal pH, irrespective of packaging material.

After 9 weeks, when the packs were opened, all high pH joints were putrid, smelling strongly of hydrogen sulphide, and would have been rejected by a buyer. Of the intermediate pH joints the silverside and striploin in material A and the striploin in material B would have been rejected for the same reason. The other joints of intermediate pH and all joints of normal pH had no off-odour and were judged acceptable.

Microbiology

The total viable counts on the topside and striploin joints at the time of packing were in the range 1.9×10^2 to 1.1×10^4 cm^{-2}, and hydrogen sulphide producers were undetectable or present in low numbers (up to 20 cm^{-2}): the inoculated silversides had total viable counts between 9.9×10^3 and 7.5×10^4 cm^{-2} and counts of hydrogen sulphide-producing bacteria 3.2×10^3 and 8.2×10^4 cm^{-2}.

After 9 weeks storage, in all packaging materials, total viable counts were highest on the high pH joints and lowest on those of normal pH (Table 2). There was no significant difference ($P < 0.05$) between the total viable counts on joints packed in the different materials.

There was a clear effect of pH on the final numbers of hydrogen sulphide-producing bacteria. Growth had occurred on all high pH joints and numbers were high. On intermediate pH joints growth occurred on all striploins, on three topsides (A, C and D) and on two silversides (A and B) but numbers were

Table 2. The effect of packaging material permeability on bacterial numbers on vacuum packed beef of different pH values after storage for 9 weeks at 1°C

Joint	pH group	Packaging material*							
		A	B	C	D	A	B	C	D
		Total viable count (log_{10}/cm^2)				Count of H_2S-producing bacteria (log_{10}/cm^2)			
Silverside	High	7.6	7.5	7.6	6.9	7.0	6.5	6.9	6.4
Topside		7.2	7.6	6.8	6.8	6.3	6.4	6.2	5.4
Striploin		7.5	7.5	7.5	7.4	6.8	6.9	6.7	6.8
Silverside	Intermediate	6.9	6.6	6.5	6.5	5.5	5.0	3.8	3.2
Topside		6.4	6.0	6.5	6.5	3.2	< 0.0	3.5	4.0
Striploin		7.1	7.4	7.2	7.0	5.4	5.4	4.7	3.3
Silverside	Normal	5.8	5.9	5.8	5.7	1.0	0.5	< 0.0	< 0.0
Topside		5.8	5.7	5.6	5.6	< 0.0	< 0.0	< 0.0	< 0.0
Striploin		6.8	6.8	6.8	6.3	< 0.0	3.0	2.7	2.7

* See Table 1.

lower than on the high pH joints. No hydrogen sulphide-producers were detected on normal pH topsides and on normal pH silversides numbers had fallen to a very low level from the initial count. There had been some growth on normal pH striploins in two of the materials but final numbers were low.

On intermediate pH striploins and silversides, hydrogen sulphide-producers were more common in materials of relatively high permeability (A and B) than in those of low permeability (C and D), but this relationship was not found with intermediate pH topsides. There was therefore some evidence that permeability affected the growth of hydrogen sulphide-producing bacteria.

Internal gas atmospheres

At the end of 9 weeks storage the volume of residual gas in the packs was low, in most cases less than 25 ml. The concentration of oxygen was less than 1% but the withdrawal of a gas sample from such low volumes is difficult and as much oxygen as this could simply be introduced by leakage. The presence or absence of oxygen cannot, therefore, be taken as established. Carbon dioxide concentration was present above 50% in all packs and no consistent difference was noted between bags of different materials, but the concentration was lower in the bags of high pH meat, 50–60% compared with 65–80% with normal and intermediate pH meat.

Discussion

Approximately 150 isolates of hydrogen sulphide-producing bacteria which grew on the high pH meat were identified and the majority belonged to the

family *Pseudomonadaceae*. The optimum pH for growth of this group of organisms is near 7.0. It is possible that they are able to grow and cause greening in beef with pH of 5.9 and above because, at these pH values they can compete better with the lactic acid bacteria which outgrow them on vacuum packed beef of normal pH.

Carbon dioxide is known to inhibit *Pseudomonas* spp. (Haines, 1933). This, however, is unlikely to explain why the hydrogen sulphide-producers grew slowest in materials of very low gas permeability since the concentration of carbon dioxide was similar in all materials. It seems more likely that very low gas permeabilities inhibit the growth of hydrogen sulphide-producers by restricting their oxygen supply.

This paper shows that the putrefaction and greening of vacuum packed high pH beef are not prevented by packing in materials of very low permeability. The shelf-life of this meat, based on the development of greening, was 3–5 weeks at 1°C. Commercially, it may not always be possible to maintain such a low temperature throughout transport and storage, and this would further shorten shelf life. In addition, the retail shelf life in air of joints cut from vacuum packed primals of high pH would be very short once growth of putrefying bacteria had started in the packs. We, therefore, concur with the recommendation that dark-cutting, high pH beef should not be used for vacuum packed storage, irrespective of packaging material.

Nicol *et al.* (1970) suggested that greening could occur at pH 6.0. We have detected it in inoculated joints at pH 5.9, but only with materials of relatively high permeability. In the pH range 5.9–6.1 putrefaction and greening did not occur in materials of very low permeability such as C and D. Such materials cannot be relied upon to do this under commercial conditions, but they may delay putrefaction and greening long enough to obtain a useful shelf life. They could offer an advantage in packing plants where an incidence of dark-cutting carcases indicates that there will be carcases with pH near 6.0. Beef from these carcases might not appear dark enough to be identified as unsuitable for vacuum packing and they could green rapidly if packed in materials of relatively high permeability.

The main cause of greening and putrefaction in vacuum packed beef is the high pH of the meat. Packaging materials of relatively high permeability such as A and B in this study, are not responsible for greening since meat of normal pH packed in them was still acceptable after 9 weeks storage. Such materials remain satisfactory for the vacuum and long-term storage of beef with a pH near 5.6 which includes the vast majority of carcases.

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Rapid thawing of pre-cooked frozen foods in catering

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Summary

The need for a rapid thawing method for use in large cook–freeze projects is described. Following a series of practical tests a machine was developed which thawed products of 20 mm thickness in less than 3 hr.

The findings were compared with calculated theoretical thawing times, and coefficients of heat transfer for the process were derived.

Introduction

The potential advantages of a cook–freeze catering system over conventional systems have already been described (Anon., 1970; Millross *et al.*, 1973) and many establishments now operate in this way. The flow diagrams (Fig.1) illustrate the general principle of two such systems as applied to hospital catering. The direct reheat system relies upon rapid reheating, shortly before use, of frozen foods in some form of container. The size of the container normally used is equivalent to approximately 6–8 portions of food. Using forced convection ovens these containers can be reheated in approximately 30 min. The 'bulk thaw' system differs in that the reheating of the product takes place in two stages. After storage, the required amount of food is allowed to thaw to a few degrees above freezing point before distribution and final reheating to 80°C. The reheating process is then brief and comparatively simple relying on the use of microwaves, radiant heat or forced air convection.

Both of these catering systems offer cost savings through centralized, regular production which is independent of daily variations in demand. However, certain difficulties occur. In the case of the direct reheating system (from −18 to 80°C), provision must be made for the thawing (from −18 to 10°C and no higher) of certain products which are to be served cold, such as sandwiches, some desserts and sliced meats for salads. The alternative bulk

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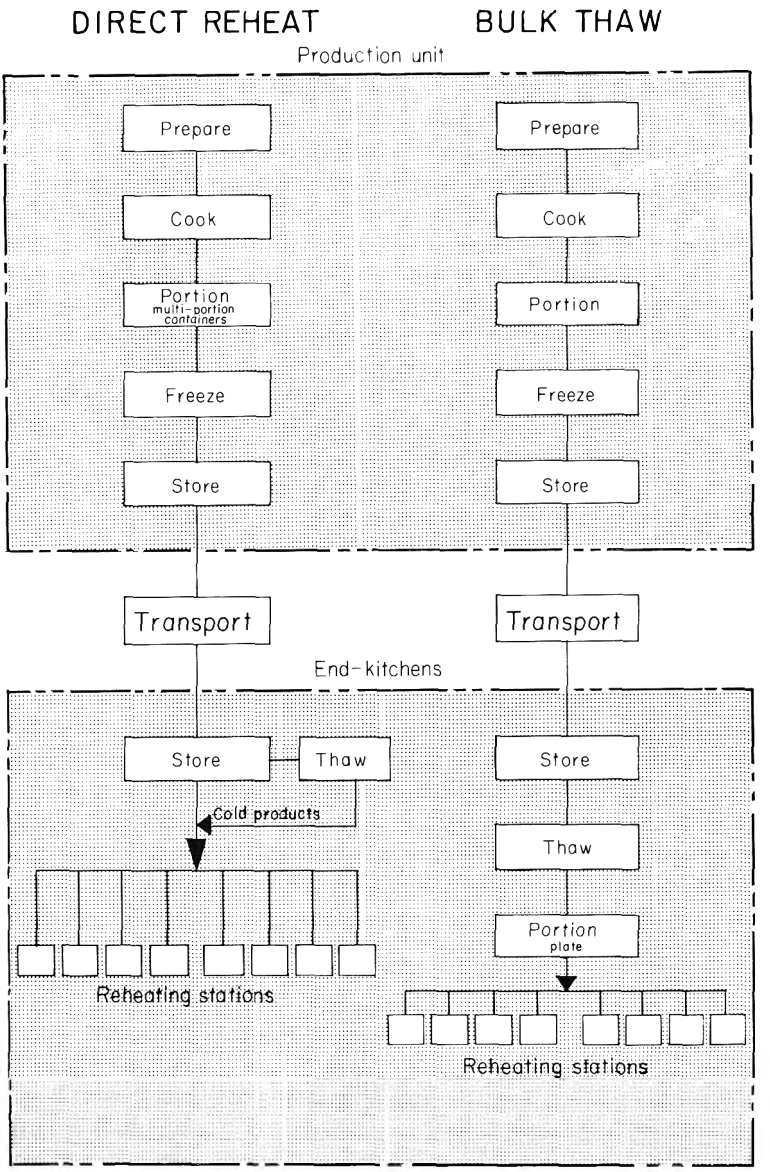

Figure 1. Cook–freeze–thaw systems for use in catering.

thawing system normally has the disadvantage that predictions of demand can be in error due to the long time normally allowed for thawing (12–24 hr or longer at 4°C). In both cases, the catering system requires a long thaw time and a bulk thawing device is required which can potentially be operated on 'same day' ordering procedure.

The caterer has little choice when faced with the problem of thawing frozen food. Periods exceeding 4 hr at room temperature could be hazardous due to the growth of bacteria, whereas under controlled temperatures in a refrigerator

(+4 to +5°C), thawing times can be very extended, though the process is relatively safe.

The subject of thawing has received most attention in the fields of large scale fish and meat processing, the principal methods employed or proposed being:

(A) *Air thawing.* (i) by natural convection, (ii) by forced convection.
(B) *Water thawing.* (i) by immersion, (ii) by spray.
(C) *Plate thawing.*
(D) *Dielectric thawing.*
(E) *Electric resistance thawing.*
(F) *Vacuum heat thawing.*
(G) *Microwave and infra-red thawing.*

The merits of the above methods, for processing blocks of fish and other large scale products have been described elsewhere (Merritt, 1969; Everington, 1971). If adopted in catering, a thawing process would have to be both simple and cheap since many units might be required in a particular establishment, and would probably be operated by unskilled staff. In particular, products would most probably be contained in a package, which might not be fully sealed, and container dimensions might in practice, vary from one product to another. It was decided to investigate further the potential of methods A and B since the remaining alternatives when used in catering establishments were considered to be either too expensive or complex (D, E and F), difficult to control (G) or unsuitable for the form of the product (C).

Initially, simple comparative experiments were carried out to establish approximate thawing times for a typical product in a multiportion container when exposed to:

Natural air convection at 5°C
Natural air convection at 22°C
Forced air convection at 10°C
Cold water at 10°C
Warm water at 54°C

These experiments are reported in Section 1.

A forced air thawing unit was then designed and built, and a further series of experiments carried out, principally using air temperatures of 10°C, these being reported in Section 2.

Section 1. Thawing using air convection and water

Materials and equipment

Food containers. Polypropylene coated board containers measuring 248 × 228 × 45 mm (Mardon Son and Hall Ltd, Bristol) having heat sealed lids of similar material were used. The containers were supplied ready erected, lids were sealed in place using an air operated, electrically heated, lidding machine.

Temperature measurement. Copper-constantan thermocouples, bent and taped to the container bases were set to half the product depth. The extended leads were carefully positioned between the container flange and lid during lid sealing. The leads were then connected to a multipoint recorder (George Kent, Luton) for continuous chart recording of temperature. An ice reference thermocouple was used throughout the experiments, this being immersed in a vacuum flask containing ice and water. Other temperatures, where required, were measured either by mercury in glass thermometers or by thermocouples in conjunction with a direct reading instrument (Comark Ltd, Littlehampton).

Products. For each method tested, three typical cold sweet recipes were used. These were custard, mousse and jelly, all being relatively simple to prepare and self-levelling in the containers. Ten containers of each product were prepared, each fitted with a thermocouple and each being filled to a depth of 25 mm before lidding and blast freezing at −30°C (Southern & Redfern Ltd, Bradford). Care was taken to maintain the containers in a level position during filling and freezing. They were then stored at −20°C until use.

Procedure: Water immersion. The lid seal, even after the heat sealing operation, could not be regarded as fully watertight, therefore only partial immersion was considered. This was achieved in a laboratory water bath which was in turn set at 10 and 54°C. The containers under test were simply floated in the water, the thermocouples connected to the multipoint recorder and the time measured between centre temperatures of −5 and +5°C. The water bath was fitted with a heater and propeller stirrer which maintained water temperature within ± 2°C of the set temperature.

Procedure: Natural air convection. Containers were placed on a stainless steel table top at room temperature (22°C) and connected to the multipoint recorder. The position of the table was chosen to avoid draughts from doors or windows, and heat from other apparatus.

This method was not a true example of thawing by natural convection since conduction between the table surface and the base of the container would occur. It was considered, however, that the method was typical of that which would be used in the practical situation.

For the low temperature test the containers were placed in a domestic refrigerator at 5°C.

Table 1. Thawing times in hours between centre temperatures of −5 and +5°C

Method	Product		
	Custard	Mousse	Jelly
(a) Partial immersion in water at 10°C	1.2	1.0	1.1
(b) Partial immersion in water at 54°C	0.2	0.1	0.2
(c) Natural air convection at 22°C	4.2	3.3	3.6
(d) Natural air convection at 5°C	20.0	14.0	20.0
(e) Forced air convection at 10°C	1.8	1.4	1.8

Procedure: Forced air convection. A laboratory blast freezer (Southern and Redfern Ltd, Bradford) was modified by rewiring through a keyswitch and by addition of a thaw thermostat. This resulted in operation of the refrigeration plant when the internal temperature was above the set value and operation of the electric defrost heaters when the temperature fell below the set value. The air circulation fan operated continuously. This modification was not ideal, and temperatures tended to fluctuate by $\pm 5°C$, but further modifications were ruled out in view of the regular freezing duty required of the machine. Containers were again connected to the multipoint recorder, though in this experiment it was necessary to load unused shelves with empty containers to maintain normal full load air flow. The mean temperature of the air was 10°C, and air velocity was $5\ m\ s^{-1}$.

Results

In all experiments two containers of each product were tested, the results given in Table 1 are the mean of each of these pairs.

Methods (a), (d) and (e) (Table 1) could be regarded as controlled from the product temperature viewpoint. Methods (b) and (c) used a heating medium at a considerably higher temperature than the desired final temperature of the product, and if carelessly used, could result in a bacteriologically unsafe or organoleptically unacceptable product. Clearly method (d) took so long that it could not be considered as a rapid method. The heat gain of the refrigerator compartment was so slow that although the initial setting was 5°C this rapidly fell, as a result of the cold product, to 1–2°C after loading the frozen product.

It was concluded, therefore, that either of methods (a) or (e) promised a reasonable chance of success in the development of a rapid thawing method. In order to minimize the chance of physical spoilage of products, method (e) was selected for further examination.

Section 2. A design for a forced air convection thaw unit

Materials and equipment

Construction of a forced air convection thaw unit. An Electrolux domestic refrigerator ($0.3\ m^3$) was chosen as the basis for the thawing unit since it was foreseen that ultimately such a unit would be required to hold products at a temperature of 2–5°C after the thawing operation. Aluminium ducting and enclosures were added externally to house a centrifugal fan (Smiths Ltd) and water/air heat exchanger (Ford Motor Co., Dagenham). Inside the cabinet, supply and return ducts, tapered to equalize air pressure, were positioned down each side. Slots in the ducts were arranged to distribute air over the top of five container positions. Containers were supported by sheet aluminium removable shelves. A water bath, centrifugal pump (Stuart Turner) and P.V.C. piping were connected to the heater to provide circulation of heating water.

The electrical circuit was connected through a double pole three-position switch to provide 'Thaw', 'Fridge' or 'Off' control. When in the 'Thaw' position the fan ran continuously via a thyrister speed control (Griffin & George Ltd), and the thaw thermostat (Honeywell Ltd) determined via two switch contacts whether by water circulation, or cooling by the refrigerator unit, was required. The thaw thermostat bulb was positioned in the return air stream. When the 'Fridge' position was selected, the fan and pump did not run and the refrigerator operated normally, controlled by the refrigerator thermostat.

The cooling effect was initially found to be marginal, but diversion of part of the air stream towards the evaporator by means of a baffle brought about improvement, and provided that the unit was precooled, air temperatures were quite closely controlled during subsequent experiments and would remain within $\pm 2°C$ of the mean temperature for approximately 85% of the process time. Similarly an external fan motor was found to be essential to minimize the internal heat gain, and this also enabled a stepped pulley vee-belt drive to be fitted. All accessible outside surfaces were insulated with 1 in polystyrene slabs taped in position.

Food containers. Containers similar in materials and construction to those described in Section 1 but measuring 240 × 240 × 40 mm were used. As before, lids were heat sealed in place when required.

Temperature measurement. A direct reading six point recorder (Foster Cambridge) was used in place of the Kent instrument. Normally, the channels were connected to thermocouples placed in ice and water, air supply and return ducts and three product containers. The recorder was internally compensated for 'cold junction'; the thermocouple positioned in the ice and water flask provided a check on instrument calibration. Stainless steel supporting spiders were used to mount the thermocouples within the product (Walker & Glew, 1969).

Air velocity measurement. A modified aluminium container fitted with a miniature anemometer (Furness Controls) was used to measure air velocities on each shelf at various control settings. A full load condition was simulated by placing dummy containers on all remaining shelves during these measurements.

Products. For each experiment a five container full load of custard was used. After preparation to a standard recipe, the containers were filled to the required depth and three fitted with thermocouples set at half depth, and at the geometric centre of the container base. Product depths were measured using a depth gauge and during this operation and the subsequent freezing process, containers were maintained in a level position. All containers were frozen with lids in place for 2 hr at $-30°C$ and 5 m s^{-1} air velocity in a blast freezer. Storage was at $-20°C$.

Procedure. The air velocities at different settings of the thyrister control and using different belt/pulley positions were measured using the anemometer. Three readings on each shelf were taken for each of these settings.

The thaw unit was precooled during the night preceding each experiment with the control set at 'Fridge'. This ensured that residual heat in the structure

would not produce uncontrollable air temperatures. The water bath temperature was maintained at a setting of 15°C throughout all the experiments, and the thaw thermostat maintained at a setting which corresponded to a controlled air temperature of 10°C. Product containers were removed from the cold store, connected to the recorder and placed on the shelves. The unit was then switched to 'Thaw', The time required for the product to pass through the temperature range −5 to +5°C was observed.

Results and discussion

Air velocity measurements for the two control conditions are listed in Table 2. All values are the mean of three readings with all shelves filled, and shelves are numbered from top (1) to bottom (5).

Table 3 gives total process times (−5 to +5°C) in addition to the time

Table 2. Air velocities in thaw unit ($m\,s^{-1}$)

Shelf no.	Setting 1 (Fan 730 r.p.m.)	Setting 2 (Fan 860 r.p.m.)
1	2.1	2.6
2	1.7	2.3
3	1.6	1.9
4	1.5	2.1
5	1.2	1.3
Mean	1.6 $m\,s^{-1}$	2.0 $m\,s^{-1}$

Table 3. Thaw times for custard. Thaw time is the time required to remove latent heat; process time is the time required for the temperature to rise from −5 to +5°C

	Product thickness (mm)	Thaw time (hr)	Process time (hr)	Mean air temperature (°C)
Mean air velocity 1.6 $m\,s^{-1}$				
Lidded	25	4.3	6.1	10.1
	20	3.0	4.3	10.3
	15	2.2	3.6	9.6
	10	0.8	1.3	13.9
Unlidded	25	2.2	3.6	9.7
	20	1.8	3.1	10.1
	15	1.3	2.3	9.2
	10	0.6	1.1	14.9
Mean air velocity 2.0 $m\,s^{-1}$				
Unlidded	25	2.7	3.8	9.4
	20	1.2	2.7	7.6
	15	1.0	1.9	7.2
	10	0.8	1.5	8.4

spent actually thawing which was determined as closely as possible from the recorded charts. In addition, a mean air temperature for each experiment is listed, this having been obtained from the mean of air temperature value at 10 min chart intervals.

A modified version of Planck's equation has been proposed for calculation of thawing times of slab shaped products (Brennan *et al.*, 1969) where:

$$T = [1 + 0.008\,(\theta_2 - \theta_1)] \left[\frac{LP}{\theta_a - \theta_2}\right] \left[\frac{a}{h} + \frac{a^2}{2k_t}\right] \tag{1}$$

where T = process time (hr), θ_2 = product final temperature, θ_1 = product thawing temperature, L = total enthalpy change during process, P = product density, θ_a = temperature of air stream, a = half product thickness, h = overall heat transfer coefficient and k_t = thermal conductivity of thawed food.

This was further modified for thawing operations using multiportion containers by Walker (1970) to:

$$T = [1 + 0.008\,(\theta_2 - \theta_1)] \left[\frac{LP}{\theta_a - \theta_t}\right] \left[\frac{a}{h} + \frac{a^2}{2k_t}\right] \tag{2}$$

where $\theta_2 - \theta_1$ = temperature range of process, θ_t = product thawing temperature and L = product latent heat. Since Walker found good correlation between practical and theoretical results, equation (2) has been used during the following calculations.

Determinations of heat transfer coefficient

For products without lids, heat transfer coefficients should be influenced only by air velocity. Hence, considering unlidded containers initially, and using the example of 20 mm at 1.64 m s^{-1} and air temperature of 10.1°C:

$\theta_1 = -5°C$	$L = 63.6$ kcal kg^{-1} (calculated)*
$\theta_2 = +5°C$	$P = 1080$ kg m^{-3} (measured)
$a = 10 \times 10^{-3}$ m	$k_t = 0.427$ kcal/m hr °C (calculated)*
$\theta_a = 10.1°C$	h = heat transfer coefficient
$\theta_t = -1°C$	$T = 3.1$ hr

Substituting in equation (2)

$$1 + 0.008\,(\theta_2 - \theta_1) = 1 + 0.008\,[+5 - (-5)] = 1.08$$

$$\frac{LP}{\theta_a - \theta_t} = \frac{63.6 \times 1080}{[10.1 - (-1)]} = 6.19 \times 10^3$$

$$\frac{a}{h} + \frac{a^2}{2k_t} = \frac{10 \times 10^{-3}}{h} + \frac{100 \times 10^{-6}}{2 \times 0.427} = 10^{-2}\left[\frac{1}{h} + 0.012\right]$$

* Calculated from empirical formulae using percentage water content of the prepared recipe (Earle, 1966).

Hence:

$$3.1 = (1.08)(6.19 \times 10^3)\left[10^{-2}\left(\frac{1}{h} + 0.012\right)\right]$$

and

$$3.1 = 10 \times 6.68\left(\frac{1}{h} + 0.012\right)$$

and

$$h = 29 \text{ kcal/m}^2 \text{ hr } ^\circ\text{C}.$$

The Planck equation gives the rate of freezing for a solid through which heat is transmitted by pure conduction, and where this heat is removed by pure convection at the surface. In the case of food in a container with a lid the pattern of heat transfer will be modified quite considerably due to the thermal resistance of the lid and any air trapped beneath. Thus, if the value of k used in the equation is that of the foodstuff, then the calculated value of h will be that for convection from air to the container surface, through the lid, and through any static air present. The h values referred to then, in the context of these experiments, depend very much upon the particular configuration of food containers used and are considered to be comparative numbers rather than absolute values. Higher values would be expected for unlidded containers since the static layer of air has been removed.

The value of k, the product thermal conductivity, has been calculated from an empirical formula based upon water content. This method is far from ideal since the structure of different foods can vary considerably even though water content may be similar. Errors in the value of k would result in appreciable

Table 4. Calculated values of h

	Product thickness (mm)	Mean air velocity ($m\,s^{-1}$)	Mean air temperature (°C)	Process times (hr)	Calculated h ($kcal/m^2\,hr\,^\circ C$)
Lidded	25	1.6	10.1	6.1	17
	20	1.6	10.3	4.3	19
	15	1.6	9.6	3.6	17
	10	1.6	13.9	1.3	21
Unlidded	25	1.6	9.7	3.6	38
	20	1.6	10.1	3.1	29
	15	1.6	9.2	2.3	30
	10	1.6	14.9	1.1	23
Unlidded	25	2.0	9.4	3.8	36
	20	2.0	7.6	2.7	51
	15	2.0	7.2	1.9	54
	10	2.0	8.4	1.5	33

variation in the calculated value of h. For this reason all values of h have been expressed as whole numbers. A summary of experimental data and calculated h values is shown in Table 4.

The mean value of h then for custard in board containers was found to be:

at 1.6 m s^{-1} air velocity Lidded 19 kcal/m^2 hr °C Unlidded 30 kcal/m^2 hr °C
at 2.0 m s^{-1} air velocity Unlidded 43 kcal/m^2 hr °C.

Figure 2 shows the calculated thawing curves for three methods of thawing assuming a fluid temperature of 10°C. On the same axes are plotted the results obtained in the experiments described. The calculated curves are based on published values of h for slab shaped products (Earle, 1966), namely:

Still air	5 kcal/m^2 hr °C
Forced air (2.5 m s^{-1})	13.5 kcal/m^2 hr °C
Liquid immersion	500 kcal/m^2 hr °C

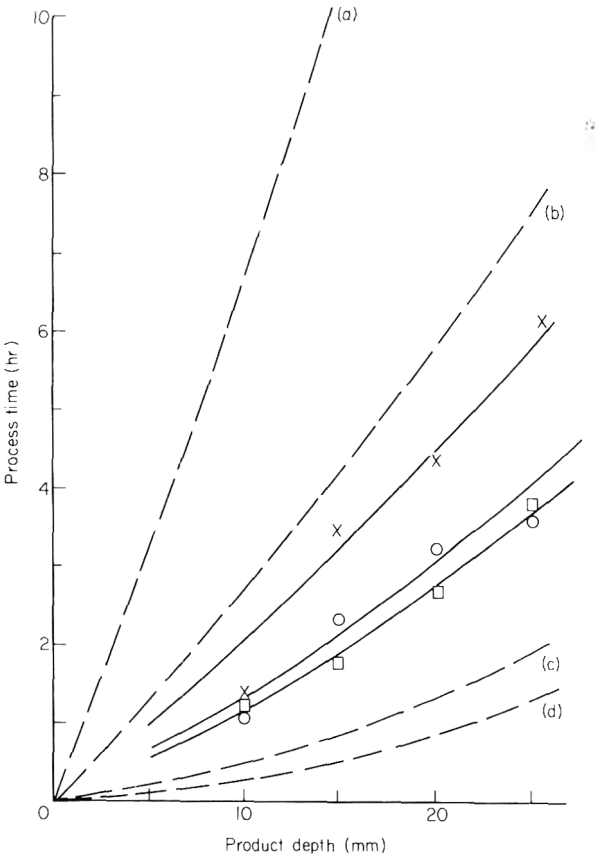

Figure 2. Theoretical and experimental thawing curves. Theoretical curves are, (a) = still air; (b) = forced air convection at 2.5 m s^{-1}; (c) = plate contact; (d) = liquid immersion. Experimental curves are X——X, lidded containers at air velocity 1.6 m s^{-1}; ○——○, unlidded containers at air velocity of 1.6 m s^{-1}; □——□, unlidded containers at air velocity 2.0 m s^{-1}.

The results obtained for unlidded containers are uniformly better than would have been expected under simple forced convection heat transfer theory. This is thought to be a peculiarity of the type of container used, which, on initial thawing would conform, due to its flexibility, to the flat plate supporting shelves and thus obtain heat by intimate contact with a conducting plate. The result has been a modification to the overall value of h as discussed previously, which now combines convection heat transfer on the upper surfaces with low pressure plate contact on the lower surfaces of the container. A value of h quoted for heat exchange in a plate freezer is 125 kcal/m² hr °C (Earle, 1966). The increase in h value for unlidded containers with an increase in air velocity was substantially greater than was expected. Normally the value of h for forced convection varies in proportion to $v^{0.8}$ where v is the air velocity. This would have produced a difference of approximately 5 kcal/m² hr °C over the range of velocities used. The explanation for this discrepancy could be the effect of the container walls on air flow over the upper surfaces of the product. At lower velocities the shielding effect of the walls would appear to have been more pronounced than at higher velocities.

Conclusions

The modification of a simple domestic refrigerator has resulted in a device which will thaw precooked frozen multiportion food packs under controlled temperature conditions in a shorter time than other available methods. A production version of the prototype would probably be simpler, perhaps with an internal fan and electric heating, and could be built as a variation of a standard 'roll-in' type commercial refrigerator. Such a unit could have a dual purpose, being used as a refrigerator when not required for thawing duties.

The particular pack used in these experiments is thought to have produced better results than would, say, a ribbed base foil container or rigid aluminium container. For these an open wire grid shelf would be more suitable and performance would be expected to be similar to the theoretical forced convection thawing curve. It is hoped that future work will result in consolidation of these predictions, and in a more detailed analysis of the effects of container configuration upon heat transfer.

In the application of thawing to the freeze–thaw catering system described in the introduction, a close study of the bacteriological and organoleptic aspects of the complete system would be necessary, even though such catering systems are known to exist elsewhere.

Acknowledgments

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The iron content of curry powders and some of their constituent spices

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Summary

The total iron content of fourteen different spices and eight powders was measured by atomic absorption spectrometry. Some spices were analysed before and after commercial grinding, and it was found in all cases that the iron content had risen significantly. The nature of this iron was demonstrated to be particulate, presumably originating from the grinding machinery.

Introduction

Curry powders are reported to contain very high concentrations of iron, in the order of 750 μg/g (McCance & Widdowson, 1960). McCance & Widdowson point out that there is more than three times as much iron in curry powder as that in any other food. Since 'curry powder' is an unspecified mixture of a variety of spices, it was decided to investigate the source of this high concentration of iron by examining the range of spices commonly used in the preparation of various types of curry powders.

Materials and methods

Whole and commercially-ground spices were purchased from a number of retailers and kindly supplied by the British Pepper and Spice Company. The latter comprised samples from the same batch of spice before and after commercial grinding on mills of mild steel construction.

A curry powder was formulated from whole spices, which were ground in the Moulinex grinder, from the following recipe:

Coriander seed (*Coriandrum sativum*)	3 parts
Turmeric (*Curcuma longa*)	3 parts

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Cardamom (*Elettaria cardamomum*)	2 parts
Cinnamon (*Cinnamomum verum*)	2 parts
Cumin seed (*Cuminum cyminum*)	2 parts
Fenugreek (*Trigonella foenumgraecum*)	2 parts
Ginger (*Zingiber officinale*)	2 parts
Mustard (*Brassica juncea*)	2 parts
Chilli (*Capsicum frutescens*)	1 part
Cloves (*Eugenia caryophyllus*)	1 part
Pepper (*Piper nigrum*)	1 part

Samples were prepared for analysis by atomic absorption spectrometry as follows. The whole spices were ground to a fine powder in an aluminium Moulinex grinder with a tungsten-carbide blade and plastic cover. Aliquots (0.5 g) of each spice were weighed out in duplicate into acid washed 100 ml Kjeldahl flasks and boiled with 25 ml 6 N Analar hydrochloric acid for 20 min (Premi & Cornfield, 1968). The solutions were filtered hot into 50 ml volumetric flasks, made up to volume with distilled water and transferred to plastic containers. Blanks were prepared in the same manner.

A range of standards were prepared in 3 N Aristar hydrochloric acid using appropriate volumes of stock iron solution for atom absorption spectrometer (British Drug Houses Ltd).

Iron analysis was carried out using a Pye Unicam SP190 single beam atomic absorption spectrometer with an air-acetylene flame with the following settings: wavelength 248.3 nm; bandpass 0.2 nm; burner height 10 mm; lamp current 10 mA; air flow 5 litre min^{-1}; acetylene flow 1.5 litre min^{-1}; clear aperture stop.

The results obtained have a range of ± 3% due to sampling and experimental errors.

Several of the spices supplied by the British Pepper and Spice Company which were found to have a marked increase in iron content after commercial grinding were tested for particulate iron using an electromagnet. The Gouy method was employed (Lewis & Wilkins, 1964) whereby the forces developed under a magnetic field were measured on a chemical balance.

One spice, whole coriander (British Pepper and Spice Company) contained unusually high amounts of iron and this was investigated further. The whole seeds were washed quantitatively with water and both the dust thus removed and the washed spice were analysed for iron by atomic absorption spectrometry.

Results

The iron content of the spices, both whole and commercially-ground, supplied by the British Pepper and Spice Company, is shown in Table 1. With the exception of unwashed coriander seed, the iron content increased considerably after grinding. Washing of the coriander seeds removed dust and grit which was

Table 1. Iron content of spices ($\mu g\,g^{-1}$) (British Pepper and Spice Company)

Spice	Whole	Commercially ground
Coriander	2475	640
Coriander – washed	70	
Coriander dust	35 242	
Cumin	950	2613
Fennel	123	1375
Fenugreek	210	995
Turmeric	243	352

Table 2. Iron content of spices ($\mu g\,g^{-1}$)

Spice		Whole	Commercially ground
Cardamon (*Elettaria cardamomum*)	Brand A	71	
	Brand B		1864
Chilli (*Capsicum frutescens*)	Brand B	92	
	Brand C		318
	Brand D		437
	Brand E		457
	Brand F		153
Cinnamon (*Cinnamomum verum*)	Brand B	44	
Cloves (*Eugenia caryophyllus*)	Brand G	32	
Coriander (*Coriandrum sativum*)	Brand B	89	
	Brand C		599
	Brand D		282
Cumin (*Cuminum cyminum*)	Brand B	430	
	Brand C		522
Fenugreek (*Trigonella foenumgraecum*)	Brand B	352	
	Brand F		357
Ginger (*Zingiber officinale*)	Brand G		407
	Brand H		912
	Brand I		974
	Brand K	451	
Mustard (*Brassica juncea*)	Brand J		80
	Brand L	185	
Pepper (*Piper nigrum*)	Brand F	73	
	Brand M		105
Turmeric (*Curcuma longa*)	Brand B	166	
	Brand C		346
	Brand D		531

shown to be extremely high in iron, and the coriander seeds much lower in iron after washing. Spices are cleaned and dust removed prior to commercial grinding. The grinding process is similar for all spices, the only difference being the mesh size of the screen that is fitted which determines the particle size of the product. The only exception is turmeric which is ground on an edge-runner mill, of granite construction, which has a crushing action rather than the shearing action of the other mills.

The iron content of other spices is shown in Table 2. Although ground samples were not obtained from the same source as the whole spices so that direct comparison is not possible, the ground samples generally have very much higher iron content than the whole spices.

The iron content of various commercial curry powders of unspecified composition is shown in Table 3. Results vary from 263 $\mu g\,g^{-1}$ for a curry powder ground and formulated in India, to 735 $\mu g\,g^{-1}$ for the British Pepper and Spice Company curry powder. The curry powder formulated from whole spices has slightly less iron; the calculated figure of 236 $\mu g\,g^{-1}$ agrees very well with the experimental figure of 237 $\mu g\,g^{-1}$.

Table 3. Iron content of curry powders ($\mu g\,g^{-1}$)

Own formulation	237
Brand B	646
Brand D	263
Brand F	735
Brand G	359
Brand H	733
Brand N	495
Brand N	524

The investigation into the nature of the iron added during commercial grinding showed that it was, indeed, particulate. The spices, when whole, or ground in the Moulinex grinder, were diamagnetic, thereby exerting a negative force on a chemical balance when placed in a magnetic field. The commercially-ground spices, from the same batches, were ferromagnetic and exerted a positive force on the balance. Therefore, although attempts to remove the iron particles with a small magnet failed, the Gouy method proved that the iron added by commercial grinding is particulate in nature, and presumably originates from the metal milling machinery.

Discussion

Seven curry powders of varying and unspecified composition all contain appreciable amounts of iron as is generally stated in Food Composition Tables. This iron originates from two sources, (1) as a natural ingredient of one of the

spices and (2) extraneous iron derived, presumably, from the grinding equipment.

The only spice among those analysed that was naturally rich in iron was cumin which, at $950\,\mu g\,g^{-1}$ was five times as concentrated a source as most of the other spices. All the other spices except one showed a marked increase in iron content after grinding in mild steel equipment, and commercial samples of ground spices were higher in iron content than whole samples. The particulate nature of the iron in the powdered material and the fact that it was ferromagnetic confirms the likelihood that iron is derived from the grinding equipment.

Figures for the iron content of various spices published elsewhere (Gopalan, Rama Sastri & Balasubramanian, 1974; Christensen, Beckman & Birdsall, 1968) show a marked variation in iron content between different samples of the same spice presumably as a result of differing origins and grinding procedures.

The one sample that was rich in iron in the whole, unground state and lower when obtained ready-ground, namely coriander, was heavily contaminated with iron-rich dust. This adds an additional, third, source to the iron found in curry powders.

The range of values of the seven curry powders examined, 263 to $735\,\mu g\,g^{-1}$ (mean 536) is lower than the value of $750\,\mu g\,g^{-1}$ given in the standard food tables (McCance & Widdowson, 1960) but the formulations of all these are unknown and may comprise any number of the different spices examined here and in varying proportions.

Since a curry may contain 5–10 g of curry powder per portion this would supply 2.5–5 mg which is a significant contribution towards the total daily recommended intake. No information is available as to what proportion of the iron from these three sources (naturally present, particulate iron and iron dust) is biologically available.

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Storage behaviour of freeze dried fruit juice powders

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Summary

The stability of nutrients and chemical changes in freeze dehydrated juice powders based on a few of the locally available fruits, during storage at ambient temperature (24–28°C) and also at 37°C were studied. Single strength juice and ready-to-serve beverages, fortified with sugar to raise the brix to 15 and 20 respectively prepared from these fruits were taken for freeze drying and further storage studies. The sugar fortified juice powders of 15° and 20° brix had a longer shelf life compared to their single strength counterpart. Of the two varieties of mango studied, *Badami* had a longer shelf life compared to *Raspuri*. Comparing mosambi, pineapple and mango, mosambi retained maximum amount of ascorbic acid at both the temperatures.

Introduction

Amongst the various drying techniques to make fruit juice powders, freeze dehydration is perhaps the best because minimum damage is done to the product during processing. In addition, freeze dried foods retain a maximum of volatile constituents including flavouring materials. The dried product is porous and reconstitutes readily even with cold water simulating original quality.

Freeze drying of orange juice has been reported by Ole Moller (1970), Foda, Hamed & Abd Allah (1970), Jabarit (1970a, b), Monzini & Maltini (1969) and Saint Hilaire & Solmo (1973). Berry & Froscher (1969) have compared the retention of volatiles of orange powders prepared by freeze drying and foam mat drying. Moy (1971) studied vacuum puff freeze drying of mango, pineapple, etc. The effect of storage temperature on development of browning, retention of ascorbic and other factors in pineapple juice powder (Notter, Taylor & Walker, 1958) and orange powder (Schroeder & Cotton, 1948) has also been studied. Notter *et al.* (1955) have reported on the storage behaviour of vacuum puff dried lemonade powder prepared by adding sucrose and citric acid to lemon juice.

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The present work is a study of the storage behaviour of freeze dried powders obtained from fruit juices (both as single strength, natural, and fortified with sugar) of tropical fruits like mango (*Mangigera indica*), pineapple (*Ananas sativus syn Ananas comosos*) and tight skinned orange (*Citrus sinensis*) known locally as mosambi.

Materials and methods

Sample preparation

Samples of mango, varieties *Badami* and *Raspuri*, pineapple and mosambi obtained from the local market were washed, peeled and the juice extracted. A ready-to-serve beverage (RSB) sample was prepared with 35% single strength juice with an acidity of 0.25% measured as anhydrous citric acid and the final concentration adjusted to 15° or 20° brix with added cane sugar, conforming to the Fruit Products Order (1955) (as amended up to 31 December 1974).

Freeze drying

Single strength juice/RSB was frozen into blocks of 25 × 25 × 12 mm size in a blast freezer at a temperature of −30°C as described by Subramanian, Sharma & Nath (1976). Frozen blocks were dehydrated in a Socaltra freeze drier at a chamber pressure of 350 μm and a maximum surface temperature of the product of 55°C. On completion of the cycle, the vacuum was broken with commercial nitrogen gas (purity 99.5%). All the samples were dried to a final moisture content of 1–1.5%.

Storage studies

The freeze dried powder was packed in cans under nitrogen (residual oxygen less than 2%). Samples were stored at ambient (24–28°C) (RT) and at 37°C and drawn for analysis at 45 and 30-day intervals, respectively.

The moisture content of the samples was determined by drying under vacuum at 60°C to constant weight; acidity and reducing sugar were determined by the A.O.A.C. (1970) method and ascorbic acid by the method of Roe & Kuether (1966). β-carotene was estimated by the modified method of Booth (Susantha, 1973) and when there was slight browning in the sample, by chromatography using an alumina column (A.O.A.C., 1970). The degree of browning was estimated by optical density (O.D.) reading of an alcohol extract at 420 μm for mango and at 410 μm for pineapple and mosambi (Hendel, Bailey & Taylor, 1950).

Table 1. Changes in acidity of the samples stored at ambient temperature and 37°C* (g citric acid/100 g powder)

Storage in months –	Ambient temperature (24–28°C)									37°C							
	0	1½	3	4½	6	7½	9	10½	12	0	1	2	3	4	5	6	7
Mango *Badami*																	
Natural	1.54	1.56	1.56	1.56	1.57	1.59	1.59	1.59	1.60	1.54	1.56	1.60	1.68	–	1.6†		
15° brix	1.59	1.59	1.60	1.60	1.62	1.62	1.63	1.64	1.66	1.59	1.59	1.60	1.62	1.64	–	1.68	1.36†
20° brix	1.21	1.21	1.21	1.22	1.24	1.23	1.23	1.27	1.28	1.21	1.22	1.23	1.25	–	1.25	1.27	1.13†
Mango *Raspuri*																	
Natural	3.23	3.23	3.31	3.34	3.35	–	3.36	3.38	3.40	3.23	3.30	3.18	3.18†				
15° brix	1.49	1.49	1.49	1.49	1.52	1.51	1.51	1.53	1.54	1.49	1.49	–	1.52	1.38†			
20° brix	0.94	0.95	–	0.96	0.96	0.99	0.99	0.99	1.03	0.94	0.99	1.08	–	0.95†			
Pineapple																	
Natural	6.85	6.85	6.89	6.90	7.03	7.03	7.04	–	7.05	6.85	6.95	6.83†					
15° brix	2.33	2.33	2.34	2.34	2.37	2.38	2.38	2.40	2.41	2.33	2.34	2.35	2.32	2.26†			
20° brix	1.53	1.54	1.54	1.54	1.55	1.55	–	1.57	1.58	1.53	1.55	–	1.30†				

	0	1½	3	4½	6	7½	Storage in months
Mosambi							
Natural	4.38	4.48	4.59	4.66	4.70	4.00†	
15° brix	1.39	1.41	1.43	1.48	1.51	1.28†	
20° brix	0.99	1.02	1.03	0.97†			

* Results averaged from at least duplicate determinations on each sample.

† Sample browned completely.

– Not done.

Results and discussion

Acidity and pH

In all the cases and at both the temperatures of storage it was seen that the change in pH was insignificant. It was almost constant throughout the storage except when the samples became unacceptable due to browning. At 37°C in the dark brown samples a very slight increase in pH was observed.

During storage at 37°C even though the completely browned samples had a lower acidity than the initial product, a very slow increase in acidity was observed during the initial stages of storage until browning occurred. The acidity later on decreased after the onset of browning (Table 1). Acidity in the case of single strength mango *Badami* powder rose from 1.54 to 1.68 within 3 months and the spoiled sample which was dark brown in colour (5 months) recorded a value of 1.6. The same trend was followed by pineapple, mosambi and mango *Raspuri* natural and their fortified juice powders. Except for pineapple, mango *Raspuri* and mosambi natural powders, the initial acidity of the other products which contained added sucrose was as low as 1.2 to 1.5 g citric acid per 100 g of the powder.

In the samples stored at room temperature a very slight increase in acidity was observed throughout the storage period. There was neither browning nor an ultimate decrease in acidity.

Reducing sugars

Table 2 gives the changes in reducing sugar content during storage at both temperatures. There was a linear increase in the reducing sugar content during storage. It was found that the reducing sugar content at 37°C for *Badami* natural, 15° and 20° brix at the time of spoilage had increased by 4.35, 14.71 and 23.2% respectively. Corresponding increases for *Raspuri* natural, 15° and 20° brix were 7.13, 15.0 and 22.30%; for pineapple natural, 15° and 20° brix it was 4.26, 10.0 and 15.60%; for mosambi natural, 15° and 20° brix it was 4.7, 6.05 and 12.54%.

During storage at room temperature the pattern of increase in the reducing sugar content for different brix was the same as for samples stored at 37°C, although the total increment in reducing sugars was less.

The increase in reducing sugars on storage is known to be due to the hydrolysis of sucrose in the presence of citric acid (Schoebel, Tannenbaum & Labuza, 1969). Model systems containing sucrose as the only carbohydrate are also reported to have shown browning (Karel & Labuza, 1968) due to the hydrolysis of sucrose to reducing sugars. When acid was not present, the model system remained stable. The acidity of *Raspuri* mango was more than that of *Badami* and consequently the rate of inversion in *Raspuri* was faster than *Badami*. Hence earlier spoilage of products from the *Raspuri* variety was observed (Andrabi *et al.*, 1956). Draudt & Huang (1966) found an increase in reducing sugar from 13.2 to 20.1% within 175 days storage at 28°C in the case

Table 2. Changes in reducing sugar of the samples at ambient temperature and 37°C* (g/100 g powder)

Storage in months	Ambient temperature (24–28°C)									37°C							
	0	1½	3	4½	6	7½	9	10½	12	0	1	2	3	4	5	6	7
Mango *Badami*																	
Natural	18.40	18.41	18.62	18.64	18.70	–	18.93	19.17	19.40	18.40	18.54	18.79	18.95	19.14	19.20†		
15° brix	13.26	13.26	13.42	13.51	–	13.54	–	13.64	13.78	13.26	13.61	–	13.70	14.70	–	–	14.96†
20° brix	10.71	10.76	10.83	11.09	–	11.23	11.40	11.34	11.50	10.71	10.84	10.96	–	12.25	13.00	–	13.2†
Mango *Raspuri*																	
Natural	18.00	18.14	18.50	18.55	18.79	18.87	19.31	19.41	19.53	18.00	18.46	18.80	19.30†				
15° brix	9.10	9.12	9.25	9.37	–	9.68	-	9.82	9.88	9.10	9.44	9.58	9.75	10.40†			
20° brix	6.15	6.26	6.50	6.52	–	6.57	6.64	6.71	6.72	6.15	6.44	7.06	–	7.40†			
Pineapple																	
Natural	28.40	28.51	28.58	29.20	29.63	29.80	–	29.45	30.35	28.40	28.85	29.61†					
15° brix	8.50	8.51	8.52	8.54	8.60	–	–	8.62	8.64	8.50	8.53	8.81	–	9.33†			
20° brix	7.18	7.20	7.27	7.40	–	7.40	7.70	7.75	7.90	7.18	7.92	–	8.30†				

	0	1½	3	4½	6	7½	Storage in months
Mosambi							
Natural	26.80	27.02	27.25	27.45	–	28.06†	
15° brix	18.69	18.91	19.04	19.49	–	19.82†	
20° brix	5.70	6.07	–	6.42†			

* Results averaged from at least duplicate determinations on each sample.
† Sample browned completely.
– Not done.

of freeze dried bananas of 8% moisture. At higher moisture levels the increase was more.

The percentage increase of reducing sugar before the material became spoiled at 37°C was more in the ready-to-serve beverages than the natural powder. This might be due to the larger quantity of sucrose available for hydrolysis in the case of the former samples.

A slight decrease in total sugar content found during spoilage at 37°C showed that as sucrose was hydrolysed some of the reducing sugar formed took part in the browning reactions, or it might be due to the breakdown of sugar to furfurals at a higher temperature (Braverman, 1963).

Browning

The extent of browning in the case of mango was measured without eliminating the carotenoids. Baloch, Buckle & Edwards (1973) determined browning of dehydrated carrots by precipitating the interfering pigments which gave increased absorption of the browning extracts. Mango samples showed a decrease in absorption of the extracts as long as there was only loss of β-carotene; with increase in browning, the absorption increased.

Extracts of samples stored at room temperature did not give an increase in

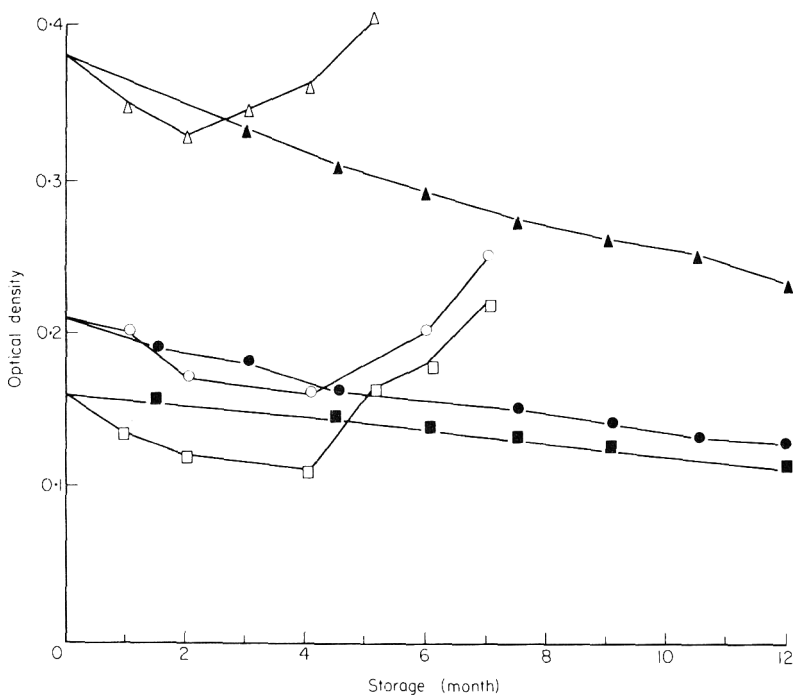

Figure 1. Optical density of alcoholic extract at 420 μm for mango *Badami*. Single strength at 37°C, △——△; single strength at RT, ▲——▲; 15° brix at 37°C, ○——○; 15° brix at RT, ●——●; 20° brix at 37°C, □——□; 20° brix at RT, ■——■.

optical density (O.D.). Only after 10.5 to 12 months storage, the O.D. of the single strength powders of mosambi and pineapple showed a slight tendency to increase but there was no visible browning. In the case of mango *Badami* and *Raspuri* there was a gradual decrease in O.D. (Figs 1 and 2). All the samples were acceptable even after 12 months storage.

At 37°C it may be observed (Figs 1 and 2) that for mango *Badami* and *Raspuri* the curve goes down and after a few months it comes up due to browning of the product. The first indication of increase in the absorption of browning extract (after an initial drop due to loss of carotene) at 420 μm was observed after a storage period of 2 months for *Badami* natural and 4 months for *Badami* 15° and 20° brix. In the case of *Raspuri*, the increase was observed after 1 month for the single strength powder and 2 months for 15° and 20° brix. As expected there was no initial drop in absorption for mosambi and pineapple juice powders and the increase in absorption at 410 μm followed a linear pattern (Fig. 3).

The single strength powder of all the three fruits showed browning development earlier than RSB. Draudt & Huang (1964) detected the browning intermediates to be sugars in combination with amino acids. Some of the non-enzymic compounds originated from acid catalysed degradation of fructose (Shaw, Tatum & Berry, 1967) and few others from degradation of hexoses by amino acids (Spark, 1969). Due to the added sucrose the concentration of the reducing sugars and amino acids already present decreased to a greater extent in

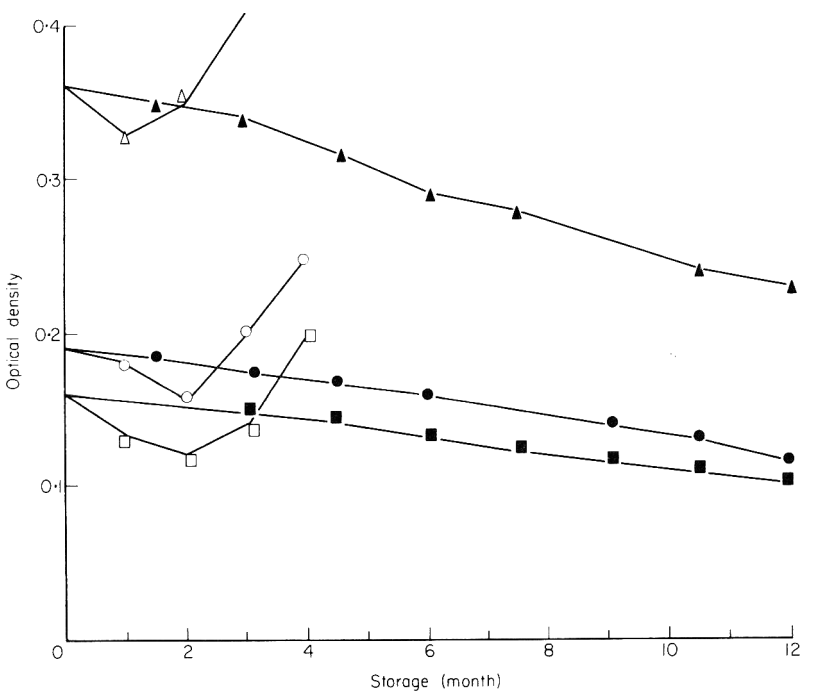

Figure 2. Optical density of alcoholic extract at 420 μm for mango *Raspuri*. Key as in Fig. 1.

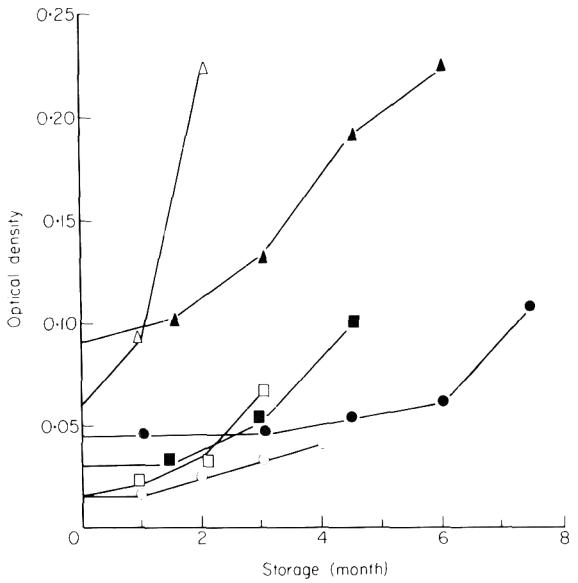

Figure 3. Optical density of alcoholic extract at 410 μm of samples stored at 37°C. Pineapple: single strength, △——△; 15° brix, ○——○; 20° brix, □——□; *Mosambi*: single strength, ▲——▲; 15° brix, ●——●; 20° brix, ■——■.

the beverage powders. This might be the reason for the delay in browning in the RSB powders. Another reason for browning and caking at 37°C might be the instability of the juice solids at that temperature (Notter *et al.*, 1958; Tressler & Joslyn, 1961). Mango *Raspuri*, pineapple and mosambi natural powder had high acidity (4.38, 6.85 and 3.23 g citric acid/100 g powder). The accelerating effect of citrate ion on the browning reaction (Melville, Naoki & Derek, 1974) may be the reason for the earlier browning of these products.

Ascorbic acid.

The percentage retention of ascorbic acid during processing, due to the very low temperature maintained throughout drying, was very high. There was no significant difference among the different samples in retention of ascorbic acid during processing. All the samples retained from 96.5 to 99% of the original ascorbic acid and the samples enriched with ascorbic acid did not show any deviation. During storage ascorbic acid loss was considerable.

At room temperature mango *Badami* retained 57.68, 77.52 and 61.0% and *Raspuri* 39.73, 61.0 and 47.86% respectively, for natural 15° and 20° brix at the end of 12 months storage. In both the cases the level of ascorbic acid in 15° and 20° brix products had been increased by adding pure ascorbic acid. Pineapple retained 49.63, 50.69 and 47.24%, but in the case of mosambi natural, 15° and 20° brix the retention was quite high (84–89%). Mosambi and pineapple 15° and 20° brix were prepared without adding ascorbic acid. Foda *et al.*

(1970) found high retention of ascorbic acid in the case of freeze dried orange juice powder.

At 37°C before the samples were discarded mango *Badami* natural, 15° and 20° brix retained 35.38, 53.8 and 50.63% of their initial ascorbic acid, the corresponding figures for *Raspuri* preparations were 30.52, 44.14 and 46.74%. In the case of pineapple the retention was 50, 52 and 54.16% for natural, 15° and 20° brix respectively. Mosambi preparations retained 60–64% of their ascorbic acid content.

In mango *Badami* ascorbic acid retention was better than in *Raspuri*. It was also seen that the retention was better in the case of 15° and 20° brix. This might be either due to the dilution of the reducing sugar and amino acids with which ascorbic acid can react under aerobic and anaerobic conditions to produce brown pigments (Joslyn, 1957) or due to better retention of the ascorbic acid, added to raise the level. Sucrose is also known to help in the better retention of colour, flavour and ascorbic acid (Shaltyko & Veksler, 1955). In the case of pineapple and mosambi there was not much difference between the retention among the three different concentrations. Thus the higher percentage retention figures for ascorbic acid for 15° and 20° brix mango preparations as compared to the natural counterpart appear to be mainly due to extra ascorbic acid added.

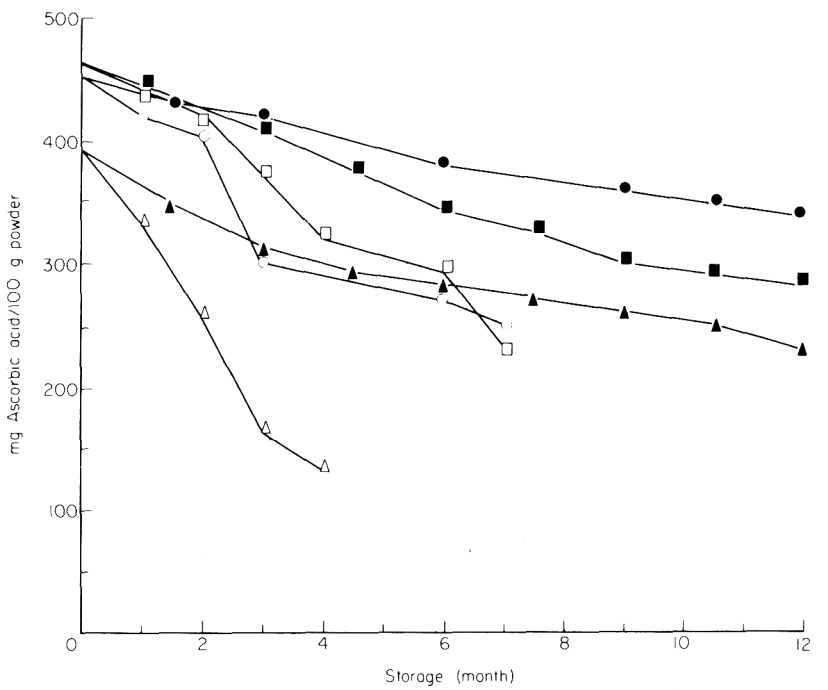

Figure 4. Changes in ascorbic acid content of mango *Badami*. Single strength at 37°C, △——△; single strength at RT, ▲——▲; 15° brix at 37°C, ○——○; 15° brix at RT, ●——●; 20° brix at 37°C, □——□; 20° brix at RT, ■——■.

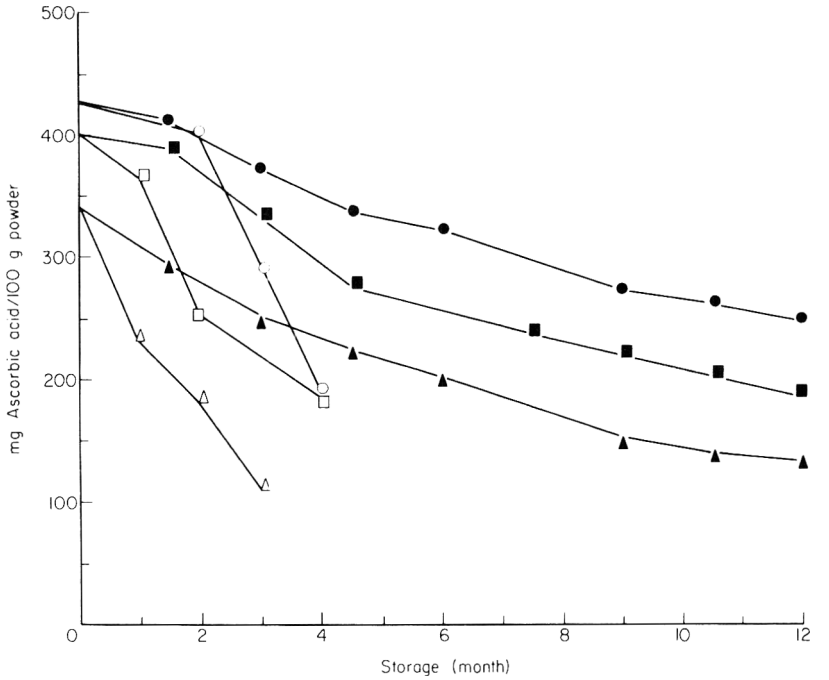

Figure 5. Changes in ascorbic acid content of mango *Raspuri*. Key as in Fig. 4.

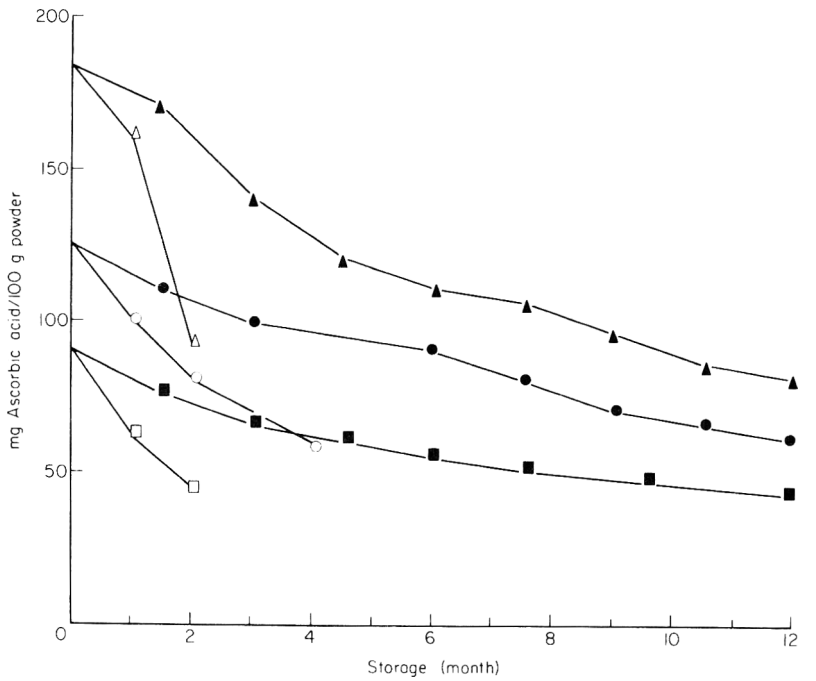

Figure 6. Changes in ascorbic acid content of pineapple. Key as in Fig. 4.

Table 3. Changes in β-carotene during storage at ambient temperature and 37°C* (mg/100 g powder)

Storage (month)	Ambient temperature (24–28°C)						Storage (month)	37°C					
	Mango *Badami*			Mango *Raspuri*				Mango *Badami*			Mango *Raspuri*		
	Natural	15° brix	20° brix	Natural	15° brix	20° brix		Natural	15° brix	20° brix	Natural	15° brix	20° brix
0	50.27	21.10	15.09	19.06	11.50	5.68	0	50.27	21.10	15.09	19.06	11.50	5.68
1.5	–	20.14	–	18.40	10.61	–	1	46.70	18.99	–	15.40	10.46	5.63
3	47.12	18.44	14.69	17.52	8.05	–	2	44.70	16.69	13.17	14.48	9.15	5.03
4.5	46.77	17.56	–	16.20	6.41	5.18	3	41.04	–	12.60	11.27†	–	3.50
6	40.64	16.18	13.60	15.40	6.20	4.68	4	36.80	15.30	11.62		6.00†	3.00†
7.5	36.82	–	12.20	13.60	5.90	3.52	5	30.50†	–	11.13			
9	35.41	–	10.78	12.30	–	–	6		14.24	–			
10.5	32.07	13.46	10.50	10.96	5.81	3.08	7		13.20†	8.67†			
12	30.52	13.02	9.35	8.93	5.40	2.74							

* Results averaged from at least duplicate determinations of each sample.

† Sample browned completely.

– Not done.

At 37°C the decrease in ascorbic acid was very rapid during browning and the product was spoiled, whereas at room temperature the rate of decrease was very sharp at the initial stages and the long tail of the curve denotes very slow rate of decrease during the final stages of storage (Figs 4, 5 and 6). This might be due to the loss of ascorbic oxidase over a period of time (Draudt & Huang, 1966). In potatoes, Leichsenring, Norris & Pilcher (1957) found that ascorbic acid losses were most rapid during the first 6 weeks.

Loss of ascorbic acid and non-enzymatic browning are correlated (Joslyn, 1957; Clegg, 1964). By comparing Figs 1, 2 and 3 with Figs 4, 5 and 6 one can find that a sharp increase in browning is associated with a sharp decrease in ascorbic acid. Browning due to sugar amino reaction (Melville *et al.*, 1974), appears to be very much less compared to browning due to the degradation products of ascorbic acid. Few of the intermediate compounds detected by Shaw *et al.* (1970) were acid catalysed ascorbic acid degradation products (Tatum, Shaw & Berry, 1969) in ascorbic acid model system. The increased level of reducing sugar also confirmed that browning was mainly due to degradation products of ascorbic acid.

β-Carotene

Fading of the colour associated with decrease in β-carotene was observed throughout storage in the case of mango powders and it was quite considerable at both the temperatures. After browning occurred, the modified Booth's method gave erroneously high results since it involved only extraction of the colour with petroleum ether-acetone mixture. Therefore column chromatography using alumina (A.O.A.C., 1970) was employed to determine β-carotene. At room temperature loss of β-carotene did not give rise to off flavour, but at 37°C off flavour was observed even before 50% of the β-carotene was destroyed. This might be due to the browning compounds, though off flavour has also been correlated with loss of carotene (Falcones, 1964).

Mango *Badami* natural, 15° and 20° brix at the time of spoilage retained 60.61, 62.56 and 57.81% of the β-carotene, whereas *Raspuri* retained 59.16, 52.18 and 52.82% for natural, 15° and 20° brix. The same samples at room temperature retained 60.71, 61.69, 61.94% and 47.0, 46.5 and 48.29% for *Badami* and *Raspuri* natural, 15° and 20° brix respectively. There was not much difference in the percentage retention of β-carotene for the three different concentrations.

Conclusion

Pineapple natural spoiled with dark browning and lump formation within 2 months at 37°C storage and mango *Raspuri* after 3 months storage. The 15° and 20° brix of pineapple and *Raspuri* remained acceptable for 1 or 2 months more, pineapple 15° and 20° brix up to 4 and 3 months respectively and

Raspuri 15° and 20° brix up to 4 months. Addition of sucrose increased the shelf life of the fruit juice powders. Pineapple and mosambi juice powder of 15° brix had a longer shelf life than that of 20° brix. At 37°C storage, dissolution of the excess sugar resulted in a transparent clump formation. Comparing *Badami* and *Raspuri, Badami* had a longer shelf life. It was seen in these studies that an optimum sugar concentration was required if the samples were to be stored for a longer period at a higher temperature.

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J. Fd Technol. (1977) **12**, 555–557

Variation in vitamin C content of cashew apple with maturity

S. R. MUDAMBI* AND M. V. RAJAGOPAL

Introduction

Cashew apple (*Annacardium occidentale*) has been shown to be a rich source of vitamin C (Mudambi & Rajagopal, 1977). However, no studies have been reported on the variation in vitamin C content of the fruit with its maturity. It has been suggested that the juice from the cashew apple could be processed in view of its high vitamin C content (Mudambi & Rajagopal, 1977). If this were to be done, it would be necessary to know at what stage of maturity the cashew apple has maximum vitamin C. This work was therefore, undertaken to supply these data.

Materials and methods

Samples of cashew apples were collected from the same tree in the Nsukka campus of the University of Nigeria: Maturity of the samples was judged on the bases of their length, breadth, weight, colour and firmness. The length and breadth of each fruit was taken as shown in Fig. 1. At least four readings of the breadth of each fruit were taken from top to bottom and the average then recorded as the breadth.

The yield of juice was found from some fruits by extracting the juice with a plastic, hand juice extractor.

Dry weight of the fruit was determined by drying weighed quantities of fresh fruit in a hot air oven at 105°C.

After sorting the fruits into seven groups on the above basis, at least five fruits were taken from each group and extracted with metaphosphoric acid, so that the final percentage of the metaphosphoric acid in the extract was 3%. The vitamin C was estimated in the extract as outlined by the Association of Vitamin Chemists (1966).

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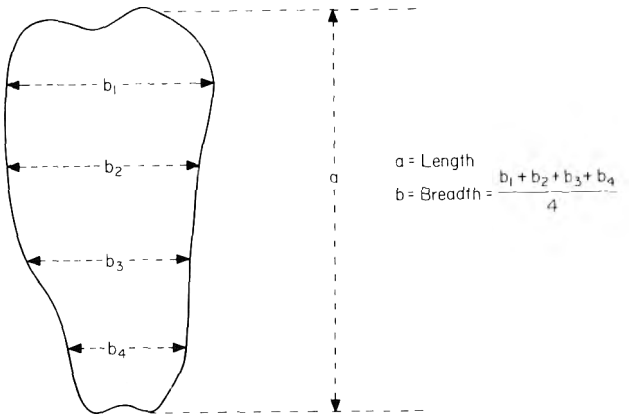

Figure 1. Diagram showing how length and breadth of the cashew apple was measured.

Results and discussion

Table 1 gives the lengths, breadths and the vitamin C contents of an average fruit in each group and the vitamin C content in mg/100 g. It is observed that the vitamin C content increases with increase in size and maturity till the half ripe stage. Between this stage and full maturity the vitamin C content increases by more than 100%. With further increase in maturity there is a substantial drop in the vitamin C content; if the cashew apple juice is to be processed for its high vitamin C content, the fruits would have to be collected first before they are ripe and the juice extracted when the fruits are just ripe. The juice in the fruits was found to vary from 33 to 40%. In view of the fact that large quantities of the fruit go to waste as the cashew processors are only interested in the kernel, it would be worthwhile to look into the use of the apple for processing and packing cashew apple juice as a source of vitamin C. After the extraction of the juice, the remaining solids (the dry weight of the fruit was

Table 1. Size, weight and colour of cashew apple with their vitamin C content

Colour	Length (cm)	Breadth (cm)	Av. wt of one fruit (g)	Vit. C in one fruit (mg)	Vit. C (mg/100 g)	Grade and texture
Green	4.10	1.78	10.0	6.9	68.7	Very immature and firm
Green	5.40	2.48	22.9	30.2	130.9	Immature and firm
Green	5.90	3.16	36.5	63.7	174.7	Immature and firm
Greenish-yellow	6.80	4.10	65.6	129.0	196.4	Half ripe and firm
Yellow	6.00	3.84	57.2	248.0	434.0	Ripe and firm
Yellow	6.80	4.35	71.3	144.4	202.4	More ripe and soft
Yellow	5.80	3.50	53.5	130.2	243.4	Over ripe and mushy

found to vary from 85.5 to 90.2%) could be used as a source of carbohydrate for the manufacture of microbial protein.

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Book reviews

Amine und nitrosamine. By Ahmed Askar.
Berlin: Technischen Universitat, 1000 Berlin 12, 1976. Pp. v + 222. DM 10.50.

This modest paperback pocketbook is one of a series of four (so far) on progress in food science under the general editorial direction of Professor H. J. Bielig and is concerned with the occurrence, properties and metabolism of amines in food, the formation and occurrence of trace nitrosamines and methods of analysis for all of these. The main text (139 pp.), which includes literature references, relates to amines with a short chapter (11 pp.) on nitrosamines; the closing chapter on general analytical techniques and techniques more specific to trimethylamine in fish and to nitrosamines (61 pp.) is followed by a seven-page index.

The collection of data on amines relates to a wide range of foods, including fish, wines, fruit, vegetables and fungi and represents a useful assemblage of this information, particularly in the context of potential nitrosamine formation by interaction between secondary amines and nitrites. The nitrosamines review itself is very brief. The methodology section also is mainly concerned with amines and does not, for nitrosamines, emphasize the need for mass spectrometric confirmation in trace analysis. The volume is however a useful introduction to the subject of free amine compounds in food, their role and their significance in relation to the trace nitrosamine compounds which in some circumstances may be formed.

H. Egan

Food Engineering Operations, 2nd edn. By J. G. Brennan, J. R. Butters, N. D. Cowell and A. E. V. Lilly.
London: Applied Science Publishers, 1976. Pp. xiv + 532. £15.

This comprehensive and well produced book covers the very wide field of work with which those engaged in food engineering may have to be familiar at one time or another. The authors deal with each topic in such a way that the essential features are outlined clearly. A liberal bibliography at the end of each chapter gives the reader a guide to more detailed texts. To anyone involved for the first time in a particular area of food engineering a quick reference to the appropriate section gives a concise appreciation of the subject, its theory and practice. To those who have more experience the text will prove refreshingly up-to-date. Units are given in SI throughout, with their equivalent in more conventional terms where needed.

There are nineteen chapters, four appendices and a very workable index. The book begins with the raw materials of the food industry and their physical properties. It goes on to show how these interact with the engineering of modern production. From then on, by selection, grading, separation, filtration, centrifugation, extraction, crystallization and heat treatment the material comes naturally to the preservation operations of heat processing, evaporation, dehydration, freezing, irradiation and storage. The last section of the book gives useful guidance on the ancillary aspects of food engineering that are more than ever important today: plant hygiene, the hygienic design of equipment, water supply, and effluent disposal. A final chapter deals with materials handling by conveyors, elevators, fork trucks, etc. Throughout there are many excellent diagrams and illustrations.

Engineering in the food industry is very much more diverse than in many other fields of industrial processing. The variability of the raw material and the fact that it is ultimately for human consumption and enjoyment brings a greater constraint on the process engineering. The authors have set out to cover this broad field and the result is a book that not only food engineers but others concerned with the food industry would find useful to have on their book shelf for ready reference.

Alexander S. Alison

Commercial Processing of Vegetables. Food Technology Review No. 27. By L. P. Hanson.
New Jersey: Noyes Data Corporation, 1975. Pp. xii + 449. US$36.00.

In this book, 226 processes covered by 237 patents issued since 1965 are reviewed. The term 'vegetable' has been taken to mean any herbaceous plant whose fruit, seeds, roots, tubers, bulbs, stem, leaves or flower parts are used as foods. This definition includes the grains, but these have been omitted from the Review as have processes which convert vegetables into starches, sugar or protein commodities. Mushrooms, however, have been included.

In addition to the conventional processes of canning, freezing or dehydration, preparative procedures for those vegetables destined for the 'fresh' market have also been reviewed. This is a welcome inclusion and serves to remind the food scientist or technologist of a very important aspect of his subject.

One of the most noteworthy aspects of the book is that the enormous amount of work carried out on the potato is well reviewed. This section is useful for active workers in the field, but those wishing to become acquainted with potato processing would do well to acquire basic knowledge before consulting this section.

The discussion of other vegetables follows generally the classification referred to in the definition above. There are sections on bulbs, stems, leaves and flowerheads, seeds, tomatoes and other vegetable fruits and mushrooms. The three indices at the end cover companies, Inventors and U.S. Patent Numbers.

There are one or two minor complaints such as the varying depth of critical assessment, which is never very deep, of the processes, also why do we have grams, ounces, Fahrenheit and Centigrade in the same text?

Despite these criticisms, which do annoy, the book should be a very useful reference for anyone who is concerned with vegetable processing. Not only will new ideas be introduced to the reader, but perhaps existing vegetable lines will be given a critical re-examination.

J. D. Henshall

Fiber in Human Nutrition. Ed. by G. A. Spiller and R. J. Amen.
New York: Plenum Press, 1976, Pp. xvii + 278. US$29.40.

To read all the publications currently appearing in connection with dietary fibre would be a full-time occupation. This previously neglected non-absorbable component of the diet is now receiving as much attention as established nutrients such as protein and vitamins.

In fact, *Fiber in Human Nutrition* carries a similar format to a textbook on protein. The first chapter defines the chemical compounds which comprise fibre and could be considered analogous to variations in amino acid composition. The following three chapters elucidate the chemistry, methods of analysis, and physical properties of dietary fibre. The analogy with protein becomes less tenuous in subsequent chapters because fibre exerts an indirect, rather than a direct, influence on the well being of the monogastric animal; lipid metabolism and colon function are affected by the physical properties of dietary fibre and, as with other dietary components, a deficiency can lead to disease. These, and other, aspects of dietary fibre have been reviewed by established British and American experts. The food technologist wishing to familiarize himself with this fashionable dietary constituent will find that the extensively referenced *Fiber in Human Nutrition* brings together much of the current knowledge originally published in a diversity of scientific and medical journals.

K. Mary Clegg

Books received

The Science of Life. Contribution of Biology to Human Welfare. Ed. by K. D. Fisher and A. O. Nixon.
New York: Plenum Publishing Corporation, 1977. Pp. xxiv + 358. Paperback US$9.00.

A re-issue of a collection of monographs showing the influence of biological research on human welfare. Amongst the subjects are Food, Environmental Hazards and Natural Resources.

Some Aspects of Human Nutrition. World Review of Nutrition and Dietetics, Vol 27. Ed. by G. H. Bourne.
Basle: S. Karger, 1977. Pp. x + 180. US$44.00.

The volume contains the following review papers: Nutrition of the Pre-school Child in India, Dietary Fibre in Human Nutrition and Pangamic Acid (Vitamin B_{15}).

Cane Sugar Handbook. 10th edn. By G. P. Meade and J. C. P. Chen.
Chichester: John Wiley, 1977. Pp. xviii + 947. £35.00.

A manual for cane sugar manufacturers and their chemists.

Animals for Man. By J. C. Bowman.
London: Edward Arnold, 1977. Pp. ii + 69. Paperback £1.60.

This is an elementary monograph in the series '*Studies in Biology*' sponsored by the Institute of Biology.

The Journal of General Microbiology

Partial contents of Vol. 100, Part 1, May 1977

Partial contents of Vol. 101, Part 2, August 1977

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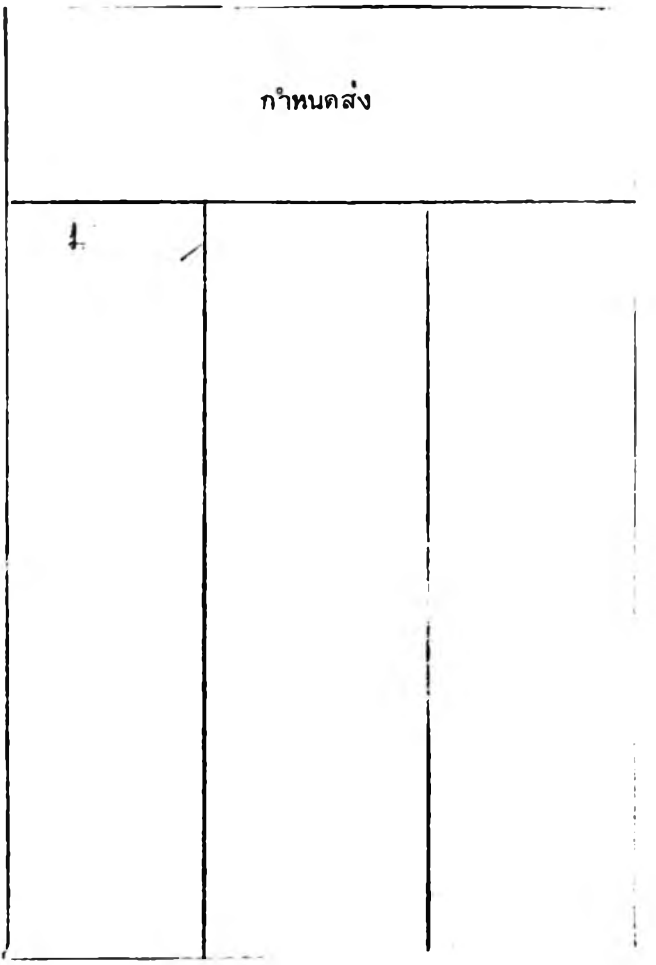
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Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted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

SI UNITS

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

NON SI UNITS

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

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

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