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Protein recovery from waste effluents of potato processing plants

DIETRICH KNORR*

Summary

Potatoes presently supply an important caloric need in high latitude countries where the crop is adapted. They are one of the most efficient suppliers of calories per acre and provide the world with 6 million metric tons of protein per year. Potato protein quality is superior to most major plant proteins, and is close to the quality of whole egg protein. Because of the quantity and quality of potato protein, and the importance of reducing waste effluents, wastes from potato processing plants should be utilized for recovering food, feed and useful byproducts. Methods used for the recovery process of potato proteins are described herein. Problems concerned with product utilization such as protein quality and acceptability (e.g. risk factors, functional properties) are discussed as well as potential uses of the protein products. It is likely that potato protein recovered from waste effluents will find a place in food and feed markets of the future as supplements to existing products, or as a protein source of fabricated foods.

Introduction

This review summarizes the state of the art in protein recovery from potato processing waste effluents. The purpose is to provide reference source material, and to hopefully stimulate other workers in this or similar fields, particularly in protein recovery for human consumption. Conceivably it may propose a viable means of waste effluent treatment in potato processing plants.

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Research and development needs

The report of a working conference entitled *Research to meet U.S. and world food needs* (Anon., 1975) recommends:

> 'Potatoes presently supply an important caloric need for the United States, West Germany, Poland, the U.S.S.R., and other high latitude countries where the crop is adapted. Because they are one of the most efficient suppliers of calories per acre, prospects are good for using potatoes to supply more of the world food needs. Potato research needs to include the development of improved varieties, better cultural practices and disease control, and improved storage methods. An increasing percentage of the U.S. potato crop is being used for processed products (frozen fries, chips, etc.). This produces waste. This waste should be used as food, feed, and byproducts.'

The following statements quote four of the nine top rated food R & D needs for the future as suggested in a recent USDA study (Krochta, Rumsey & Farkas, 1975):

'– Plant and equipment design and operation for greater reduction and recycling of water, and for reducing waste and pollution;
– Alternative sources of fertilizer to replace petrochemical fertilizers;
– Methods for converting food processing-plant effluents into feeds, fertilizers, energy useful chemicals, etc.;
– Systems for recovering, purifying, and utilizing plant proteins to give functional and acceptable (nutrients, flavour, texture, cost) engineered food for human consumption.'

These statements serve to underline the importance of reducing wastes and converting effluents into useful products, and for recovering plant proteins for human consumption. These principles are incorporated into this paper.

Importance of potato protein

The potato tuber, henceforth referred to as potato(es), is one of the most efficient crops in food production, with a yield of 2.3 kJ of energy output per kJ of input (Pimentel *et al.*, 1975). The average energy production per day and per hectare ranges from about 144 to 193×10^3 kJ (Van der Zaag, 1976). Potatoes contain an average of 2.1% crude protein (N × 6.25) on a fresh weight basis, and, worldwide, provide about 6 million metric tons of protein (Markakis, 1975).

Potato yield, in terms of calories and protein per hectare, is considerably higher than that of wheat and rice, currently the two leading world food grains. Consequently the potato has definite potential in alleviating food shortages, especially in the developing countries (Bennet, 1975). A comparison of protein production per day and per hectare for some food crops is given in Table 1.

Table 1. Comparison between protein production per hectare per day for some food crops and potatoes grown in America, Africa, Asian countries between 30° N Lat. and 30° S Lat. and all countries between 30° N Lat. and 30° S Lat. (after Van der Zaag, 1976)

Crop	Protein production (kg) per hectare per day			
	America	Africa	Asia*	All countries*
Potato	1.0	0.9	1.2	1.1
Cassava	0.2	0.1	0.2	0.2
Sweet potatoes	0.9	0.4	0.6	0.5
Yams	0.7	0.6	0.3	0.6
Dry beans	0.9	0.6	0.5	0.6
Chick peas	1.1	0.7	0.9	0.9
Rice	0.6	0.4	0.6	0.6
Corn	0.9	0.9	0.8	0.8

* Between 30° N Lat. and 30° S Lat.

Potato protein is of higher nutritional quality than legume or cereal protein due to its overall superior amino acid balance, particularly methionine (legumes) and lysine (cereals) (Rexen, 1976). Nitrogen balance studies with human adults have shown potato protein to be superior to most major plant proteins, approaching the value of whole egg (Kofranyi & Jekat, 1965; Meister & Thompson, 1976b). This is exemplified in Table 2 which shows the average minimum quantities of different protein sources in g/kg body weight/day required to maintain nitrogen balance in human adults. In a separate

Table 2. Minimum quantity of protein (g/kg body weight/day) to maintain nitrogen balance in human adults (after Kofranyi & Jekat, 1965)

Protein	Average minimum quantity of protein (g/kg body weight/day)
100% egg	0.509
100% milk	0.568
100% wheat	0.901
100% beans	0.667
100% corn	0.651*
100% potato	0.550
75% egg + 25% potato	0.424*
50% egg + 50% potato	0.399
35% egg + 65% potato	0.380
25% egg + 75% potato	0.428

* Single experiment only.

Table 3. Essential amino acid composition of potatoes (mg per g total nitrogen)

Essential amino acid	FAO (1970)		Markakis (1975)*		Schuphan (1960)†		Kapoor, Desborough & Li (1975)	K. G. DeNoord (personal communication) In coagulable potato protein	Becker (1963) Potato in relation to egg (%)
	CM‡	MM§	A¶	CV**	A¶	CV**			
Isoleucine	236	303	257	27.6	431	15.8	261	394	80
Leucine	377	361	362	30.2	418	24.3	499	681	80
Lysine	299	351	342	43.5	388	16.6	441	512	60
Methionine	81	106	92	27.9	100	46.3	120	138	45
Cystine	37	67	55	77.2	50	47.5	–	113	–
Phenylalanine	251	293	280	24.8	281	23.6	231	431	70
Threonine	235	204	233	24.3	256	13.9	204	–	80
Tryptophan	–	103	85	33.4	100	38.1	84	81	100
Valine	292	331	323	22.0	331	10.4	293	403	70

* Combination of own and reported data (FAO data included).
† 258–260 estimations (only 93 for cystine).
‡ CM = Chemical methods.
§ MM = Microbiological methods.
¶ A = Average.
** CV = Coefficient of variation (%).

experiment, a mixture of potato and lactabumin was the most favourable diet having a minimum requirement of 0.374 g protein per kg body weight, and a biological value of 134 (egg protein = 100) (Jekat & Kofranyi, 1970). The essential amino acid compositions reported for potato protein are shown in Table 3. The limiting essential amino acids in potatoes appear to be methionine and cystine. Scrimshaw & Young (1976) have reported that the essential amino acid content of potatoes was adequate in the case of tryptophan but poor in the case of methionine and cystine, whereas Rexen (1976) reported that the limiting amino acid was either methionine or isoleucine.

An impressive example of the importance of potatoes as a protein source was given by Pimentel *et al.* (1975): 'The people of Ireland from the 17th through the 19th Century relied primarily on potatoes for both calories and protein. During this period, the daily diet for the adult working peasant was 4.5 kg of potatoes and about half a litre of milk. This provided about 16 100 kJ and 64 grams of protein per day, of which 45 grams was from potatoes.' Kon & Klein (1928) described an experiment in which two adults lived over a period of 167 days in nitrogen equilibrium and in good health on a diet in which the nitrogen was almost totally derived from potatoes.

Elsewhere, it has been shown in feeding studies that potato protein effectively replaced fishmeal in a chick diet (Kaindelstorfer, 1975), or soybean and fishmeal in a swine diet (N. Zwatz, personal communication). The potential for direct use of concentrated proteinaceous wastewater as an animal feed was found to be very limited, therefore it appears likely that drying (Della Monica, Huttanen & Strolle, 1975; Borud, 1971) will be required for optimum performance. Kirt *et al.* (1974) have discussed the use of concentrated protein water mixed with dehydrated cellulose as a fodder concentrate. Dijkstra (1958) has investigated ensiling of protein rich potato pulp.

Protein recovery

The review directs most of its attention to potato starch waste effluents because of their predominance in most European countries, and because this effluent represents a potentially large source of valuable protein and other nutrients (Adler, 1966; Strolle, Cording & Aceto, 1973).

The following quantities of components were shown by Peters (1972) to be annually discharged together in waste water from potato starch mills in the Netherlands: 15 000 tons of protein, 15 000 tons of non-polymeric nitrogen compounds, including amino acids, 5000 tons of sugar, 15 000 tons of minerals, mainly K_2HPO_4. Three years later, De Noord (1975) stated that 25 000 tons of protein could be produced annually from the potato starch mills of the Netherlands. Meister & Thompson (1976a) reported that a process for recovery of protein from waste effluent of potato chip processing should be economical if combined with starch recovery. Heisler *et al.* (1959) reported two benefits resulting by recovering useful nitrogenous compounds from

Table 4. Comparison of alternative methods for processing potato starch effluents (after Stabile *et al.*, 1971)

Alternative method	Resulting product	Use	Fixed capital cost (US$)	Total operating cost (US$/year)	Sales (US$/year)
Concentration by evaporation	Concentrate with protein	Feed	514 000	148 500	186 700
Protein recovery + biological treatment	Protein	Feed/food	807 000	198 100	44 000
Protein recovery + concentration by evaporation	(A) Protein (B) Concentrate without protein	Feed/food Feed	881 000	281 500	165 000
Protein recovery + ion exchange + biological treatment	(A) Protein (B) Amino acid mixture (C) Organic acid mixture (D) K_2SO_4–$(NH_4)_2SO_4$ (E) $(NH_4)_2SO_4$	Feed Feed/food Beverages Fertilizer Fertilizer	2 550 000	755 750	444 000

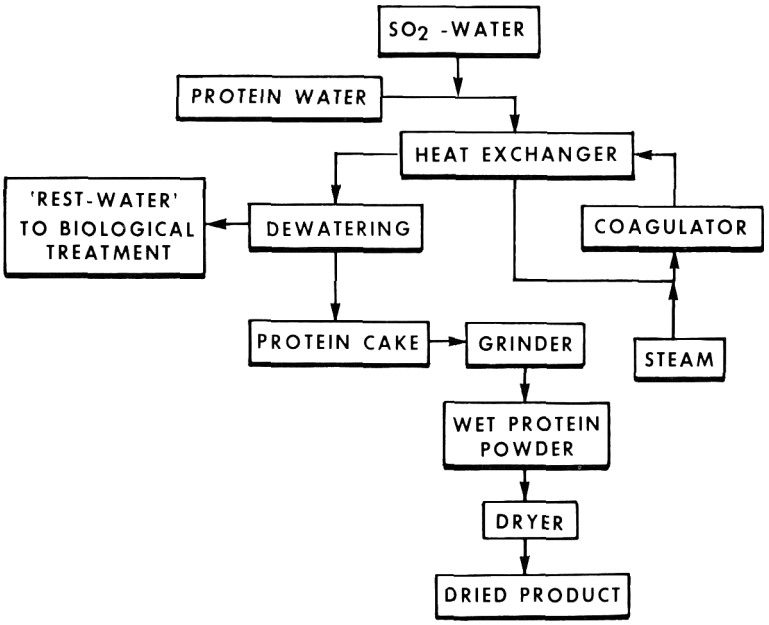

Figure 1. Scheme of potato protein plant (after Peters, 1972).

potato starch factory waste water; first, the pollution effect of the protein water* would be reduced, and second, the economics of potato starch processing would be improved through the creation of marketable byproducts.

In 1971 Stabile, Turkot & Aceto presented an economic analysis of alternative methods for processing potato starch effluents (Table 4). At the time of this study only concentration of effluents by evaporation appeared economically feasible.

A process proposed to recover protein from potato processing water includes three main steps: (1) protein coagulation (precipitation); (2) separation (dewatering) and (3) drying. Sometimes a concentration step is also included. Byproducts like amino acids, organic acids, potassium phosphates, etc. which remain in the protein water after protein coagulation have also been recovered.

Protein coagulation

The most common way to coagulate potato protein is by heating (Figs 1 and 2). As shown in Table 5, steam injection is normally used to heat the protein water. The temperatures used for heat coagulation range from 75 to 120°C, but most reports are between 95 and 100°C. The pH value varied from

* The 'protein water' (fruit water) is one of the wastes from potato starch factories which contains all the water soluble constituents of the potato. It is a dilute solution containing about 5% solids. About 1.5% of the solids can be precipitated by heat treatment. The volume of protein water ranges from about 0.7 to 7.0 m^3/t processed potatoes.

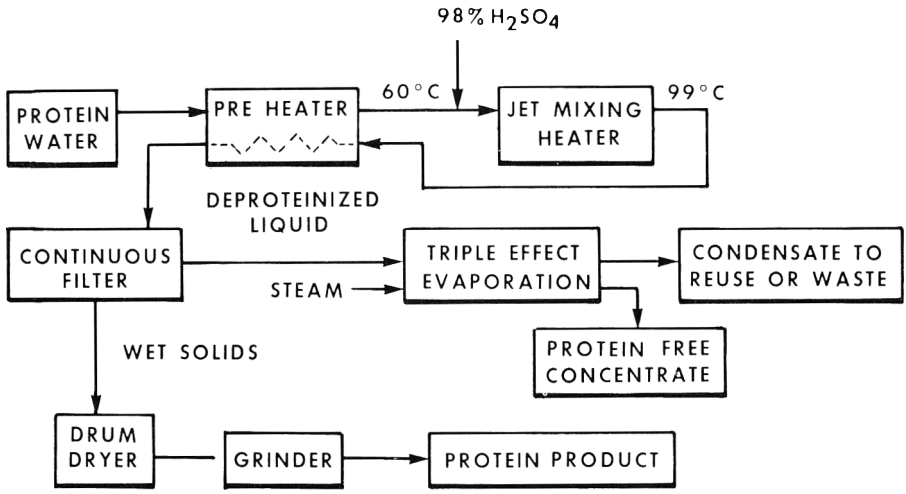

Figure 2. Protein recovery from protein water and concentration of protein free waste from processing potato starch plant (after Stabile *et al.*, 1971).

3.5 to the initial pH of the protein water which ranges from about 5.8 to 6.2 (Strolle *et al.*, 1973).

The influence of pH (1–7) and the heat (23–98°C) on precipitation of protein from waste effluents of potato chip processing has been documented by Meister & Thompson (1976a). The highest temperature was most effective in recovering protein at all but the low pH values. In these experiments an optimal pH range from 3.5 to 4.5 was indicated. At lower pH, room temperature (23°C) gave better recoveries than the heat treatments. Heating to 80°C or acidification to pH 3.0 or slightly below did not give optimal results. Heat (80–90°C) at pH values of 4.0 to 4.5 was most effective for protein recovery. The data indicate that at low pH results were achieved at room temperatures where protein recovery was essentially the same as that reached by heat coagulation, but reclamation of total solids was much higher at low pH (~3.5). Heat coagulation has been proposed by several other authors (Vlasblom & Peters, 1958; Borud, 1971; Strolle *et al.*, 1973). In some cases a combination of heat and pressure treatment have been applied (Landwirtschaftliche Kartoffelverwertungs AG, 1957).

Potato proteins can be also precipitated by physico-chemical procedures. Precipitation of protein concentrates from potato processing water by using polyphosphoric acid has been described by Finley & Hautala (1976). Meister & Thompson (1976a) compared $FeCl_3$ with HCl, and HCl with H_3PO_4 as coagulants; the same workers attempted to raise the pH with lime (CaO) followed by lowering the pH value with either H_3PO_4 or $FeCl_3$ for recovery of proteins from waste effluents of potato chip processing. When HCl was substituted for H_3PO_4, results were almost identical. Meister & Thompson (1976a) also showed that $FeCl_3$ compared favourably with hydrochloric acid. From a cost point of view as well as considerations of adding H_3PO_4 to public

Table 5. Some conditions for heat coagulation of protein from potato processing plant effluents

Heating	Temperature (°C)	pH-value	Remarks	Reference
Steam	95–105	4.8–5.0	Presence of SO_2	Huchette & Fleche (1975)
Steam	95	–	–	Vlasblom & Peters (1958)
	90–100	–	Adding H_2SO_4 or $Ca(OH)_2$	Alton & Tobias (1976)
	75–95	–	–	Xander & Hoover (1959)
Waterbath	80–90	4.0–4.5	Laboratory scale	Meister & Thompson (1976a)
Steam	100	3.5	Sulphuric acid added	Stabile *et al.* (1971)
Steam + heat exchanger	80	4.5	Presence of SO_2	Peters (1972)
Heat exchanger	–	4.0–4.5	–	Strolle *et al.* (1973)
Steam	99	5.5 or less	Optimum conditions found	Anon. (1968); Masters (1972)
Heat exchanger	120	Initial pH of protein water	Combined heat and pressure treatment	Landwirtschaftliche Kartoffelverwertungs AG (1951)
Heat exchanger	120	5.0	Preheating 40–50°C, then 120°C and pressure	Landwirtschaftliche Kartoffelverwertungs AG (1957)
Steam	98–99	4.8	Presence of SO_2	Knorr *et al.* (1977)

water, HCl would be preferred. Ferric chloride is one of the principal coagulants in sewage work; it is relatively inexpensive, has acidic properties, and the trivalent iron ion is a good nucleating site for large floc formation. Another advantage of ferric chloride is that no heating is required. The iron recovered with the precipitated protein could add to its nutritional significance (Knorr, Hoess & Klaushofer, 1976). Protein recovery was satisfactory when a combination of lime treatment followed by H_3PO_4 or $FeCl_3$ treatment was used. However, because of the high amount of calcium in the precipitated material, the usefulness of such protein product is questionable. A serious disadvantage of lime and H_3PO_4 treatment is that water must be neutralized before being discharged. Moreover, although phosphate can be readily precipitated with lime above pH 11.8, its solubility is increased below pH 9 (Meister & Thompson, 1976a). Finley & Hautala (1976) used calcium chloride and removed the precipitated calcium phosphate by centrifugation. The phosphorus content (as P_2O_5) of the dried potato protein concentrate prepared by this method was 1.2%. Potato protein from starch manufacture wastes was prepared by Alton & Tobias (1976) by adding H_2SO_4 or $Ca(OH)_2$. Jackson

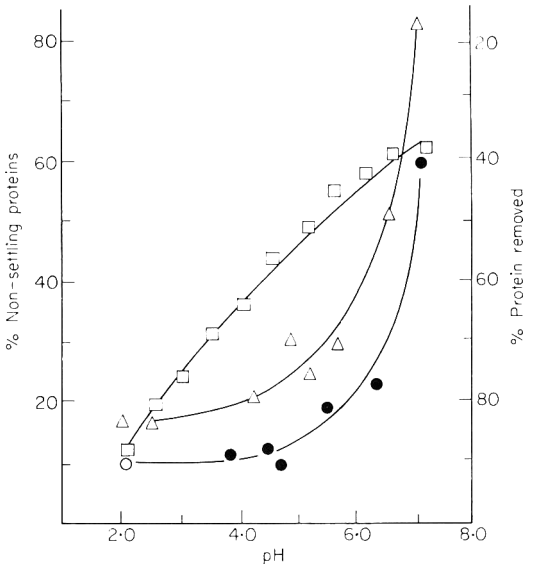

Figure 3. Effect of pH on protein recovery at different solids content. ●, △, Percentage non-settling proteins (after Strolle *et al.*, 1973), 2.8 and 1.5% solids respectively; □, percentage protein removed, 2.2% solids (Finley & Hautala, 1976).

(1962) stated that acid precipitation was preferable to heat precipitation because it preserved the ascorbic acid, and gave a more desirable product on drying, and inhibited foaming.

Finley & Hautala (1976) used 0.1% polyphosphoric acid to precipitate protein from potato cut water and reported that varying the pH level also effected protein removal from waste stream effluents. Their results indicated that as the pH decreased, more protein was removed from aqueous solutions. A pH of 2.0 appeared to be the lowest practical level because of the large quantities of acid required to further lower pH and little protein remains in solution at pH 2.0 (Fig. 3).

Concentration

Meister & Thompson (1976a) studied the effect of concentration and heat treatment on settling of the precipitate. In an experiment where residual crude protein was measured over a time span of 80 min, sedimentation was faster in heat treated samples than in samples treated at room temperature but differences beyond 60 min were not significant. The initial sedimentation in low protein concentrates was faster but the percentage of protein settled during the entire period was lower. Strolle *et al.* (1973) also investigated the influence of pH and concentration of solids on the settling of coagulated protein (Fig. 3), and came to the same conclusion. They showed that with slight acidification the amount of nonsettling proteins decreases significantly and then continues

to decrease slightly over the pH range 5.0 to 2.0. The curves showed that increasing the concentration (1.5–2.8% solids) also decreases the amount of nonsettling proteins. These data suggest that the efficiency of protein recovery could be increased if higher waste strength could be obtained. Water usage should be reduced if possible and the waste effluent recycled.

Recovery of byproducts

The protein and other nitrogen containing substances remaining in the protein water after protein coagulation could be recovered by ion exchange; ascorbic acid, for example, was recoverable (Jackson, 1962). Heisler *et al.* (1959, 1962) and Heisler, Siciliano & Krulick (1972) have investigated the recovery of amino acids, proteins and potassium. Schwartz, Krulick & Porter (1972) have studied the recovery of organic acids and phosphate. Xander & Hoover (1959) precipitated potato protein by heat treatment and contacted the resultant protein free liquid with a strongly basic anion exchanger to absorb amino acids and amides (Fig. 4). Starch laden wastes from potato processing plants have been treated by the 'Symba Process' which converted starches into single cell protein (Anon., 1976; Skogman, 1976).

Reverse osmosis treatment of wastes was investigated by Porter *et al.* (1970), but difficulties were encountered in a similar pilot plant study with potato chip effluents (Meister & Thompson, 1976a). The use of ultrafiltration (after defoaming of the protein water) was discussed by Oosten (1976), Schmidt, Gieseman & Snijder (1976) and Putter (1976). Oosten (1976) described

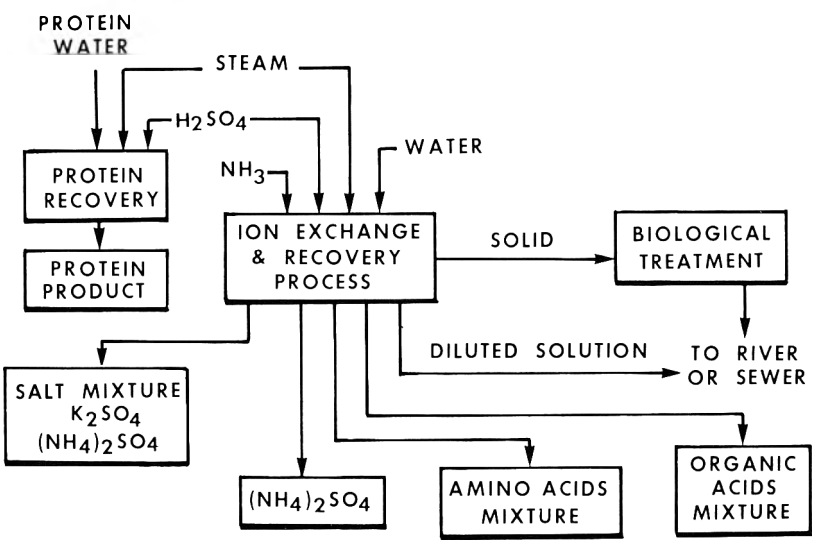

Figure 4. Combination protein recovery, ion exchange treatment and biological waste treatment of processing potato starch plant effluents (after Stabile *et al.*, 1971).

coagulation of the protein in the ultrafiltration concentrate by mixing the concentrate with steam. Schmidt *et al.* (1976) reported spray drying of the ultrafiltration concentrate as well as acid precipitation of the protein followed by diluting and spray drying of the precipitate. Results of experiments in treating potato peeling water by hyperfiltration and ultrafiltration were presented by Minturn (1975).

Separation and drying

The precipitated proteins can be separated by using a continuous rotary filter (Strolle *et al.*, 1973; Stabile *et al.*, 1971), plate and frame type filter press (Strolle *et al.*, 1973), by gravity settling (Meister & Thompson, 1976a; Peters, 1972), or by centrifugation using continuous conveyor-discharge centrifuges (Meister & Thompson, 1976a; Huchette & Fleche, 1975; Peters, 1972). Strolle *et al.* (1973) have compared filtration and centrifugation methods (plate frame filter press, continuous rotary filter and a bench type air driven 'Sharples' super centrifuge) and recommended the use of the plate frame filter press. After dewatering by filtration, they further recommended drum drying or freeze drying and after (solid bowl) centrifugation, air drying, freeze drying or the use of a drying system for heavy paste dispersions as described by Baran (1964). The filter cake can be disintegrated and dried in a pneumatic dryer and the liquid concentrate from a separator can be worked up in a spray dryer (Peters, 1972).

Air drying studies (Strolle *et al.*, 1973) with wastes from potato starch manufacturing in a conventional tray drier gave a dried product which was black, hard, horn like and very difficult to grind; double drum drying gave a fairly soft, greyish product. Freeze drying gave a white soft product; however, this method is undoubtedly uneconomical (Strolle *et al.*, 1973). Spray drying was reported by Anon. (1968), Masters (1972), Huchette & Fleche (1975)

Table 6. Typical composition of a commercial potato protein product (after Knorr *et al.*, 1976)

	Concentration (% w/w)
Moisture	9.2
Crude protein (N X 6.25)	81.5
Crude protein (N X 7.50)*	97.9
Pure protein	79.7
Digestible protein	56.6
Ash	3.58
Fat	0.96
Sugar	0.28

* After Desborough & Weiser (1974).

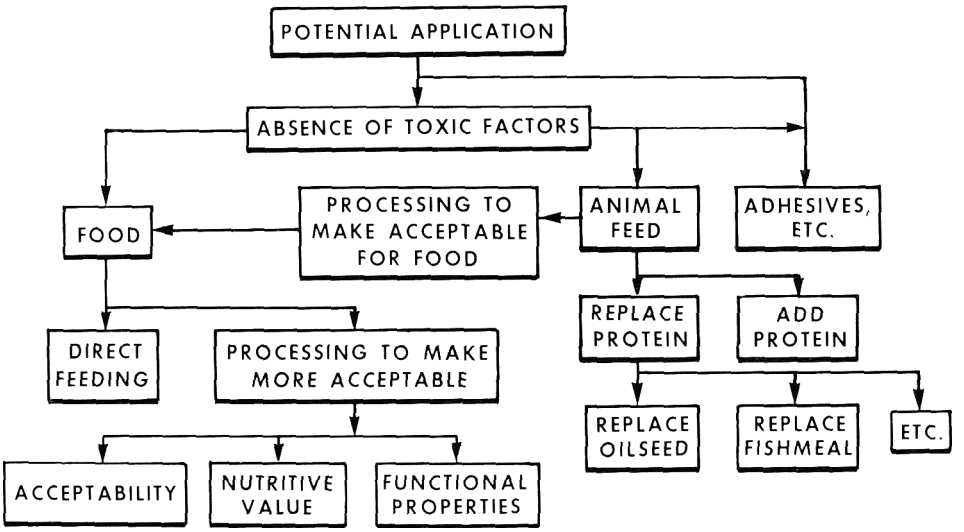

Figure 5. Potential application for potato protein products.

and Schmidt *et al.* (1976) to yield soft, yellowish products (Giokas, 1976) with superior water solubility than equivalent drum dried products (Schmidt *et al.*, 1976). The crude protein content of the dried product ranged from about 70 to 80% (N × 6.25) (Huchette & Fleche, 1975; Putter, 1976; Finley & Hautala, 1976).

The factor 7.5 has recently been proposed for converting Kjeldahl nitrogen to total protein in potatoes (Desborough & Weiser, 1974). A composition of typical commercial European potato protein product, which is now used as a feed, is listed in Table 6.

Product utilization

Major applications of potato protein products considered in this review include the following:

(a) direct use in human food as a major ingredient or supplement;
(b) application of food science to enhance product nutritive value, product functionality* and acceptability†;
(c) use in animal feeds as a substitute for other proteins;
(d) use in industrial applications such as adhesives, coating in paper processing, etc.

De Willigen (1973) recommended preparation of a curd or cheese type product for human consumption prepared from precipitated potato protein.

* This term is used as defined by Kinsella (1976).

† This term is used as defined by Lipinsky & Litchfield (1970) and includes toxic factors, organoleptic factors, gastrointestinal factors, processability, stability and price as major factors affecting acceptability.

As a food supplement potato protein could find possible use in protein enrichment of bread, snacks, etc. Actual experiments describing protein enrichment of bread with potato protein have been published (Knorr, 1977). The influence of partial replacement (5–20%) of wheat flour by a protein product recovered from potato starch waste effluents on loaf volume, moisture loss and deformation of bread was studied during 96 hr of storage time on breads baked at different baking temperatures. The results showed a strong relationship between moisture loss and storage time, and low relationships in the cases of storage time versus deformation and specific loaf volume. Specific loaf volume and deformation decreased with increasing potato protein concentration. Different baking temperatures (~200–240°C) had no influence on those results.

Protein quality

The nutritive value of protein precipitated from simulated waste effluents of potato chip processing was biologically and microbiologically assessed using weanling voles (*Microtus pennsylvanicus*) and *Streptococcus zymogenus* (Meister & Thompson, 1976b). The nutritive value was dependent on the method of recovery. No differences were found between the biological value of protein obtained by heat or acid precipitation but, biological values of proteins treated with CaO and H_3PO_4 or $FeCl_3$ were all lower (Table 7). The effect of heat treatment (heating temperature: 60, 90, 120 and 150°C; heating time: 1, 2, 3, 4 hr) on protein quality in commercial potato protein products

Table 7. Protein Efficiency Index (PEI) of diets tested in vole feeding trial (after Meister & Thompson, 1976b)

Diet	PIE* (Average of six voles)
Casein	3.02a
Burbank tubers	1.89a
Heat precipitate	2.19c
Acid precipitate	2.07c
Sebago tubers	2.51b
Heat precipitate	2.21bc
Acid precipitate	2.96ab
Unknown tuber	1.81e
Heat precipitate	2.09c
Acid precipitate	2.18c

* Studentized range test: values with same letter are not significantly different at 5% level.

recovered from potato starch effluents was assessed microbiologically using a protozoa, *Tetrahymena pyriformis* W (D. Knorr & C. Parajsz, unpublished data). Within these products significant differences existed only between 3 and 4 hr of heating time. The influence of heating temperature was significant at each temperature increment.

Acceptability

Risk factors

Little information is published concerning risk factors and/or toxic factors within potato protein products. For example, concentration of residues such as heavy minerals or pesticides in potato protein products is not described. Heat treatment with several heating systems during the precipitation process can conceivably cause some destruction of amino acids (Huchette & Fleche, 1975). These same workers also found dried protein products with a sulphur dioxide content of 500 ppm and a solanin content of 1000 to 1500 ppm. Treatments with 5% acetic acid at 100°C for 3 hr or with citric acid for 1 hr were necessary to reduce the solanin content to 150 ppm.

Functional properties

The earthy, raw potato flavour of commercial potato proteins was significantly reduced by using a method which combined extraction with diethylether:ethanol:water, and steam treatment, or an extraction with hydrochloric acid and diethylether:ethanol:water (Knorr *et al.*, 1976). The aroma of the resulting spray dried potato protein products was not influenced by drying conditions such as inlet/outlet temperature (120–125/40–42 to 190–195/70–72°C) or drying pH value (4, 6, 8) (Giokas, 1976).

The colour of potato protein is influenced by use of SO_2 or SO_3^{2-} added to the potatoes or to the protein water (Huchette & Fleche, 1975). Antioxidants such as butylhydroxytoluene, ascorbic acid, α-tocopherol, etc. are also claimed to influence the colour of the recovered protein (Huchette & Fleche, 1975). The influence of different drying methods on product colour was described by Strolle *et al.* (1973) and is mentioned above. The effect of pH (4, 6, 8) during drying on the colour of a spray dried product was studied by Giokas (1976). The lightest coloured product was obtained at the lowest pH value.

Nitrogen solubility of a protein concentrate recovered from potato cut water by using polyphosphoric acid was 98.2% (Finley & Hautala, 1976), whereas Giokas (1976) found that protein solubility of a product derived from potato starch waste effluents was significantly influenced by the drying temperature and pH value during spray drying. Schmidt *et al.* (1976) have stated that spray dried protein products showed better water solubility than did drum dried products.

The fat emulsifying capacity of recovered potato protein indicated that potato protein appears to have potential as a fat stabilizer or emulsifier (Finley & Hautala, 1976). When potato protein was used as an emulsifier, Bakel & Breene (1976) showed that stable oil-in-water emulsions were formed up to 25 parts aqueous phase to 75 parts oil. Heat treatment produced more stable emulsions. These oil-in-water emulsions were formed up to 25 parts aqueous phase to 75 parts oil. Heat treatment produced more stable emulsions. These oil-in-water emulsions were unstable. Levels of potato protein used were up to 2.5% (Russo, 1976; Bakel & Breene, 1976).

Conclusions

In general, potatoes are an important protein source and potato protein products recovered from food processing plants could become important also. The energy and protein derived from potatoes per unit area per unit time is high in the tropics in comparison with the main tropical food crops (Van der Zaag, 1976). The potato could improve diets and could improve food production. In countries like the U.S.A., U.S.S.R., Germany, Poland, the Netherlands, Austria and others, protein recovered from potato processing plant waste effluents can become an important contribution to the reduction of water pollution. Problems associated with the economic viability could be solved, especially if the pollution factor is included. In combination with starch recovery and/or recovery of amino acids, potassium, organic acids, phosphate, etc. the recovery process of potato protein could possibly be economical. Research is needed especially for the total recovery process. We have little information about the problems associated with the product, especially those associated with toxic factors, acceptability and functional properties, but it is likely that potato protein recovered from waste effluents will find a place in food and feed markets of the future.

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References

Anon. (1968) *Proc. Biochem.* **3**, 5, 51.

Anon. (1975) *Research to Meet U.S. and World Food Needs.* Agricultural Research Policy Advisory Committee (ARPAC), Kansas City, Missouri, U.S.A.

Anon. (1976) *Fd Eng.* **48**, 66.

Alton, L.V. & Tobias, V. (1976) *Chem. Abstracts.* **85**, 92421u.
Adler, G. (1966) *The Production, Use and Disposal of Potatoes in West Germany. Proceedings Int. Symp. Utilization Disposal Potato Wastes,* Research and Productivity Council, Fredericton, New Brunswick, Canada.
Bakel, J.T. & Breene, W.M. (1976) *36th Annual Meeting of the Institute of Food Technologists, I.F.T.,* Anaheim, California, U.S.A.
Baran, S.J. (1964) *Ind. & Eng. Chem.* **56**, 10, 34.
Becker, G. (1963) *Züchter,* **33**, 313.
Bennet, T.A. (1975) *Canad. Farm Econ.* **10**, 1.
Borud, O.J. (1971) *Die Staerke.* **23**, 172.
Della Monica, E.S., Huttanen, C.N. & Strolle, E.O. (1975) *J. Sci. Fd Agric.* **26**, 617.
De Noord, K.G. (1975) *Voedingsmiddelentechnol.* **8**, 23.
Desborough, S.L. & Weiser, C.J. (1974) *Am. Potato J.* **51**, 185.
De Willingen, A.H.A. (1973) *Die Staerke.* **25**, 213.
Dijkstra, N.D. (1958) *Landbouwkundig Tijdschrift.* **70**, 821.
FAO (1970) *Amino Acid Content of Foods and Biological Data on Proteins.* Rome.
Finley, J.W. & Hautala, E. (1976) *Fd Prod. Develop.* **10**, 4, 92.
Giokas, S. (1976) University of Agriculture, Vienna. Master's Thesis.
Heisler, E.G., Siciliano, J. & Krulick, S. (1972) *J. Sci. Fd Agric.* **23**, 745.
Heisler, E.G., Siciliano, J., Treadway, R.H. & Woodward, C.F. (1959) *Am. Potato J.* **36**, 1.
Heisler, E.G., Siciliano, J., Treadway, R.H. & Woodward, C.F. (1962) *Am. Potato J.* **39**, 78.
Huchette, M. & Fleche, G. (1975) *BRD Offenlegungsschrift.* **25**, 200.
Jackson, M.L. (1962) *Ind. & Eng. Chem.* **54**, 2, 50.
Jekat, F. & Kofranyi, E. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* **351**, 47.
Kaindelstorfer (1975) University of Agriculture, Vienna. Thesis.
Kapoor, A. C., Desborough, S.L. & Li, P.H. (1975) *Potato Res.* **18**, 469.
Kinsella, J.E. (1976) *CRC Critical Rev. Fd Sci. Nutr.* **7**, 219.
Kirt, E.E., Ilus, A.E., Vel'ner, K.A. & Maastik, A.A. (1974) *Sakharnaya Promyshlenost.* **48**, 4, 68.
Knorr, D. (1977) *J. Fd. Sci.* **42**, (in press).
Knorr, D., Hoess, W. & Klaushofer, H. (1976) *Confructa.* **21**, 166.
Knorr, D., Kohler, G.O. & Betschart, A.A. (1977) *J. Fd Proc. & Pres.* **1**, (in press).
Kofranyi, E. & Jekat, F. (1965) *Die biologische Wertigkeit von Kartoffelproteinen.* Westdeutscher Verlag, Köln.
Kon, S.K. & Klein, A. (1928) *Biochem. J.* **22**, 258.
Krochta, J.M., Rumsey, T.R. & Farkas, D.F. (1975) *Fd Technol. Champaign,* **29**, 10, 74.
Landwirtschaftliche Kartoffelverwertungs AG (1951) *Austrian Patent* No. 170903.
Landwirtschaftliche Kartoffelverwertungs AG (1957) *Austrian Patent* No. 193704.
Lipinsky, E.S. & Litchfield, J.H. (1970) *CRC Critical Rev. Fd Technol.* **1**, 581.
Markakis, P. (1975) *Protein Nutritional Quality of Foods and Feeds,* Part II (Ed. by M. Friedman). Marcel Dekker, New York.
Masters, K. (1972) *Spray Drying.* CRC Press, London.
Meister, E. & Thompson, N.R. (1976a) *J. Agric. Fd Chem.* **24**, 919.
Meister, E. & Thompson, N.R. (1976b) *J. Agric. Fd Chem.* **24**, 924.
Minturn, R.E. (1975) *Advanced Techniques for Aqueous Processing and Pollution Abatement.* Oak Ridge Natl. Lab. ORNL–NSF-EP.
Oosten, B.J. (1976) *Die Staerke.* **28**, 135.
Peters, H. (1972) *Pure and Applied Chem.* **29**, 1–3, 129.
Pimentel, D., Dritschilo, W., Krummel, J. & Kutzman, J. (1975) *Science, N.Y.* **190**, 754.
Porter, W.L., Siciliano, J., Krulick, S. & Heisler, E.G. (1970) *Membrane Science and Technology* (Ed. by J.E. Flinn). Plenum Press, New York.
Putter, W. (1976) *Offenlegungsschrift.* **25**, 33, 762.
Rexen, B. (1976) *Pot. Res.* **19**, 189.

Russo, J.R. (1976) *Fd Eng.* **48**, 8, 19.
Schmidt, P. Gieseman, H. & Snijder, H.H. (1976) *BRD Offenlegungsschrift.* **25**, 42, 155.
Schuphan, W. (1960) *Qual. Plant. Mat. Veg.* **6**, 16.
Schwartz, J.H., Krulick, S. & Porter, W.L. (1972) *J. Sci. Fd Agric.* **23**, 977.
Scrimshaw, N.S. & Young, V.R. (1976) *Sci. Am.* **235**, 64.
Skogman, H. (1976) *Food from Waste* (Ed. by G.G. Birch, K.J. Parker and J.T. Worgan). Applied Science Publishers, London.
Stabile, R.L., Turkot, V.A. & Aceto, N.C. (1971) *Economic Analysis of Alternative Methods for Processing Potato Starch Plant Effluents.* Second National Symp. on Food Processing Wastes, Denver.
Strolle, E.O., Cording, J. & Aceto, N.C. (1973) *J. Agric. Fd Chem.* **21**, 974.
Vlasblom, M. & Peters, H. (1958) *Dutch Patent* No. 87,150.
Xander, P.A. & Hoover, E.F. (1959) *U.S. Patent* No. 2,879,264.
Van der Zaag, D.E. (1976) *Pot. Res.* **19**, 37.

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A chemical method for the estimation of mould in tomato products

B. JARVIS

Summary

An objective chemical method has been used to assess the level of fungal contamination of tomato juices and purées. The method is based on estimation of chitin, a cell wall constituent of fungi, which is not found in higher plants. Hydrolysis of the chitin yields a degradation product 'chitosan' which can be quantitatively recovered and deaminated to give an aldehyde, 2,5–anhydro-mannose, which can be estimated colorimetrically. Results with spiked samples showed recoveries better than 95% of the expected level of fungal glucosamine added. Comparison with the Howard Mould Count showed a high correlation coefficient for non-homogenized juices and purées, but a low correlation for homogenized juices. The advantages and disadvantages of the chemical method are discussed in relation to microscopic and viable estimation methods for mould in food.

Introduction

Estimates of fungal contamination of raw and manufactured food products have long been known to be very imprecise and, in some cases, are very subjective. The results obtained by microscopic methods, such as the Howard Mould Count for tomato and other fruit products (Howard, 1911; Troy, 1968; AOAC, 1970) are influenced by many factors including the species of mould, the degree of comminution or homogenization of the product, the degree of standardization of the method and the expertise of the microscopist. Vas *et al.* (1959) and Dakin, Smith & Taylor (1964) observed standard deviations for Howard Mould Count estimates on tomato products of ± 7.43 and ± 7.35% respectively. Assuming a Poisson distribution for mould in a tomato juice, a true mean level of 40% positive fields would be expected to give a count of less than 25% or more than 55% in one test out of twenty. Such a degree of imprecision limits the value of the test for quality control or legal enforcement

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purposes (Williams, 1968). Nonetheless, the Howard Mould Count has been of considerable value in improving the standards of tomato products over the years since its inception in 1916 as a legal standard for pastes and purées in the U.S.A.

Estimates of viable mould propagules by cultural techniques provide an opportunity to isolate specific fungal contaminants by the use of appropriate culture media (Pitt, 1975; Jarvis, 1977). Such methods can be used to estimate the level of fungal contaminants in freshly processed or in dried foods (e.g. spices, flour) but will give no information about the mycological history of a sample which has been subjected to some microbiocidal process. Furthermore, interpretation of quantitative estimates of mould propagules is beset with difficulties for any sample in which fungal proliferation might have occurred, since sample preparation techniques (e.g. homogenization) will fragment individual pieces of mycelium and spore structures into a variable number of viable propagules.

For such reasons, and also because the extent of fungal colonization of food materials may give an indication of possible mycotoxin hazards (Jarvis, 1975), there is a need for more objective methods of analysis. One such method is based on the occurrence of a complex polysaccharide, chitin, in cell walls of micro-fungi, but not in higher plants. Chitin can be chemically or enzymically hydrolysed to yield a polyglucosamine which can be determined quantitatively by a colorimetric reaction (Ride & Drysdale, 1971, 1972; Swift, 1973). This paper presents results of investigations with one such method for estimating chitin in mould-contaminated tomato products and compares the results obtained with Howard Mould Count estimates on the same samples.

Materials and methods

Tomato samples

Tomato purée, paste and juice samples were purchased from local supermarkets or were supplied by manufacturers. Samples of reconstituted juices, before and after homogenization, were supplied by manufacturers or were drawn from laboratory-prepared samples formulated to a commercial recipe. Samples of commercial 'straight' juice were also homogenized in the laboratory. Unless otherwise stated all laboratory homogenization was carried out using a two-stage APV homogenizer (Model 15V–8BA) operated at 1200 ± 100 lbf in^{-2} (8.274 ± 0.689 MPa).

Fungal cultures

Cultures of *Aspergillus terreus*, *A. versicolor*, *Rhizopus nigricans* and *Geotrichum* spp. were grown on slopes of malt agar (Oxoid) at 25°C. Mycelium was harvested by scraping from the agar using a sterile scalpel. Mycelium was

collected also from the surface of rotting tomatoes; no attempt was made to identify the moulds responsible but considerable care was exercised to ensure that the isolated mould was not contaminated with any tomato skin or tissue. Mycelial samples were suspended in sterile distilled water and homogenized at 12 000 r.p.m. for 5 min at 2°C using an MSE top drive homogenizer fitted with a microhomogenizer attachment. The macerated mycelium was washed twice with distilled water, by centrifugation, and was stored as a freeze-dried pellet. For use the mycelium was reconstituted with distilled water and diluted as required in water or tomato juice.

Analytical methods

Howard Mould Count. The method was used as described by AOAC (1970). Pastes and purées were diluted to 8% soluble solids (refractive index 1.3438–1.3445 at 25°C) before testing; juices were tested without dilution. At least 100 fields were examined for each sample tested using a standard Howard Mould Count cell (Watson & Co. Ltd).

Total solids. These were determined by drying a sample to constant weight *in vacuo* at 70°C.

Soluble solids. These were determined by refractive index measurements using an Abbé refractometer (Bellingham & Stanley Ltd).

Fungal chitin. This was estimated essentially as described by Ride & Drysdale (1972). Portions of mycelial suspension in water or tomato juice, and unadulterated tomato samples (2 g), were diluted with nine volumes of anhydrous acetone. After centriguation the pellet was resuspended in acetone and after recentrifugation the pellet was dried *in vacuo*, and then suspended in 4 ml 10 N KOH. The reaction mixture was hydrolysed *in vacuo* for 1.5 hr at 120°C and chitosan was precipitated by the addition of 2.5 volumes of 75% ethanol at 0°C. The subsequent stages were as described by Ride & Drysdale (1972), the analytical method of Tsuji, Kinoshita & Hoshino (1969) being used to determine the aldehyde 2,5–dehydromannose produced by nitrous acid deamination of glucosamine residues in the chitosan. Standard solutions of glucosamine were always tested concurrently. The method was linear over the range 0 to 50 μg glucosamine/tube.

Results

Chitin content of various fungi

The results in Table 1 summarize the mean fungal chitin levels (expressed as fungal glucosamine) of several species of fungi tested. Results of other workers (Ride & Drysdale, 1971, 1972; Swift, 1973) are included for purposes of comparison. The glucosamine content of 'tomato mould' mycelium is similar to that of several laboratory strains of mould tested.

Table 1. Glucosamine content of various fungal mycelium homogenates

Organism	Fungal glucosamine (μg/mg dry wt) after hydrolysis by:		
	Enzyme	Acid	Alkali
*Fusarium oxysporum**†	80	59	76
Coriolus versicolor‡	–	11.6	–
Verticillium albo-atrum†	–	30	24
Botrytis fabae†	–	34	38
Asochyta pisi†	–	47	20
Aspergillus versicolor	–	–	43
A. terreus	–	–	15
Rhizopus nigricans	–	–	29
Geotrichum spp.	–	–	25
'Tomato' mould A	–	–	24
'Tomato' mould B	–	–	27

* Ride & Drysdale (1971), † Ride & Drysdale (1972), ‡ Swift (1973).

Recovery of added fungal chitin and glucosamine from tomato products

Samples of tomato juice and of a five-fold concentrate were prepared in the laboratory from fresh, unblemished tomatoes. There was no microscopic evidence for the presence of any fungal mycelium and no glucosamine was detectable either in hydrolysed or unhydrolysed tomato samples tested. Addition of various levels of fungal mycelium or of glucosamine before hydrolysis gave recoveries ranging from 92.0 to 104.0% of the expected level (Table 2).

Table 2. Recovery of glucosamine from spiked 'fresh' tomato juice and purée

Sample	Type and amount of 'spike'	Mean glucosamine level μg/g sample		Recovery (%)
		Expected	Observed	
Fresh juice	None	0	<0.01	–
	Mould, 0.4 mg d. wt*	17.2	17.4	101.1
	Mould, 0.9 mg d. wt*	38.7	37.6	97.3
	Mould, 1.3 mg d. wt*	55.9	51.4	92.0
	Mould, 1.7 mg d. wt*	73.1	76.4	104.4
Fresh purée	None	0	<0.01	–
	Mould, 1.7 mg d. wt*	73.1	75.9	103.8
	Glucosamine	25.0	24.2	96.8
	Mould + glucosamine	98.1	100.6	102.4

* 1 mg dry weight (d. wt) macerated mycelium ≡ 43 μg glucosamine.

Table 3. Fungal glucosamine and Howard Mould Count in various tomato juices and purées

Brand	Product type	No. samples tested	Total solids (g/100 g)	Howard Mould Count (% + fields)		Fungal glucosamine		
				No. tests	Mean ± S.E.	No. tests	μg/sample Mean ± S.E.	μg/g solids Mean ± S.E.
A	Purée	1	20.4	2	23 ± 3.8	2	23.1 ± 4.3	113 ± 21.1
B	Paste	1	26.2	2	2 ± 1.0	2	2.7 ± 0.2	10 ± 0.8
C	Paste	2	26.5	9	52 ± 3.1	6	98.2 ± 6.5	370 ± 24.5
D	Paste	2	25.4	9	9 ± 2.5	7	14.6 ± 0.9	58 ± 3.4
W1	Juice (NH, R)*	5	6.00	19	42 ± 2.8	22	7.7 ± 0.5	128 ± 8.3
W2	Juice (H, R)	5	5.98	20	57 ± 4.2	22	9.1 ± 0.7	152 ± 11.2
X	Juice (NH, F)	4	5.65	5	29 ± 2.4	8	6.0 ± 0.6	106 ± 10.2
Y	Juice (NH, F)	2	6.30	11	15 ± 2.5	4	2.5 ± 0.1	40 ± 1.6
Z	Juice (H, F)	2	5.94	8	27 ± 2.4	4	2.7 ± 0.1	46 ± 0.8

* NH, not homogenized; H, homogenized; R, reconstituted from paste; F, 'straight' juice.

Table 4. Effect of homogenization of tomato juices on the Howard Mould Counts and the fungal glucosamine contents

Sample	Location prepared and homogenized	No. tests	Mean Howard Mould Count (% + fields)			Mean fungal glucosamine (μg/g sample)		
			NH†	H†	Difference	NH†	H†	Difference
S1	Industry	4	39	54	15*	12.5	13.7	1.2
S2	Laboratory	3	58	89	31**	19.0	15.7	−3.3
T1	Industry	4	14	54	40**	3.5	3.9	0.4
T2	Laboratory	3	8	16	8	3.1	3.5	0.4
W	Industry	22	42	57	15*	7.7	9.1	1.4
X	Laboratory	4	15	26	11*	2.5	2.9	0.4
Y	Laboratory‡	4	27	30	3	2.7	2.7	0.0
Z	Laboratory (Silverson mixer)	2	58	5	−53**	5.8	5.9	0.1

* Difference significant at $P = 0.05$ but not at $P = 0.01$; ** difference significant at $P = 0.01$; no symbol, difference not significant at $P = 0.05$.

† NH, sampled before homogenization; H, sampled after homogenization.

‡ Sample homogenized during manufacture and rehomogenized in laboratory.

Howard Mould Count and fungal chitin levels in commercial tomato juices and purées

A number of typical commercial samples of tomato juice (both 'straight' and 'reconstituted') and of tomato pastes and purées was analysed by both Howard Mould Count and chitin assay methods. The results are shown in Table 3, together with the standard error of the mean values for the various replicate analyses. A general association between high Howard Mould Count and high fungal glucosamine is apparent.

Homogenization of juices produced a statistically significant, but quantitatively variable, increase in Howard Mould Count in all cases except three (Table 4).

These were a commercially homogenized juice which was rehomogenized, a low count juice where the count increased by 100%, but the increase was not statistically significant and a juice which was extensively homogenized with a Silverson mixer. In the former case the Howard Mould Count only increased marginally and in the latter case the Howard Mould Count was reduced as a result of total destruction of mycelial filaments. The increase in Howard Mould Count consequent upon homogenization has been recognized for many

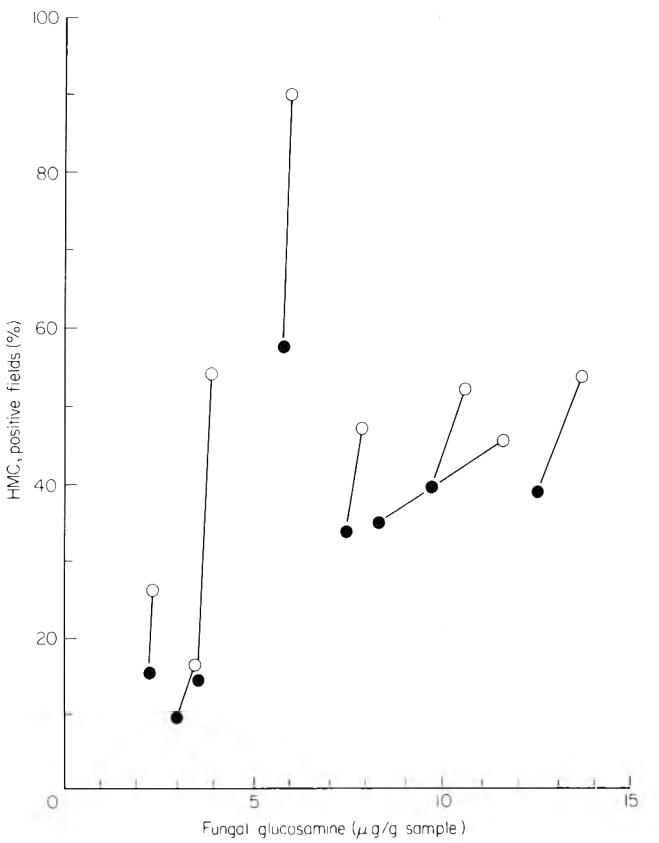

Figure 1. Effects of homogenization on the Howard Mould Count and fungal glucosamine level for paired homogenized (○) and non-homogenized (●) juices.

years to be a major variable in assessing results on homogenized or comminuted products (Eisenberg, 1968; Troy, 1968). By contrast, little increase in the fungal glucosamine level of paired samples occurred as a result of homogenization and such differences as did occur were not statistically significant. It is believed that the small increases seen probably result from mechanical damage to the mycelial walls, thereby making the cell-wall chitin more readily available for hydrolysis. The effects of homogenization on several individual samples are illustrated graphically in Fig. 1.

Discussion

It is widely accepted that most presently available methods of analysis for fungal contamination of food commodities are subjective and produce results which are often difficult to interpret. An objective chemical method would have many advantages for both industrial and legislative control purposes.

Estimation of glucosamine following hydrolysis of fungal chitin could provide an appropriate objective method for quantitative analysis of mould in food, which is both sensitive and potentially suitable for automation. Such an approach has been used previously in studies of plant pathogens (Ride & Drysdale, 1971, 1972) and in the biodeterioration of wood (Swift, 1973). The present study has shown that alkaline hydrolysis of chitin in tomato juice gives a recovery which is consistently greater than 95% of the added level. For non-homogenized tomato juices a highly significant coefficient of correlation

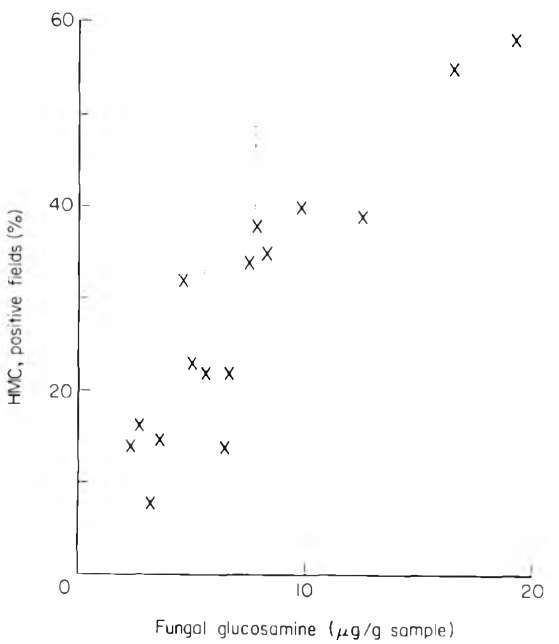

Figure 2. Relationship between Howard Mould Count and fungal glucosamine; non-homogenized juice.

(0.94 with 15 d.f.) was found between fungal glucosamine level and the Howard Mould Count, but a much lower coefficient of correlation (0.42 with 16 d.f.) was found for homogenized juices (Figs 2 and 3). Since the degree of precision of the chitin assay is similar for both homogenized and non-homogenized products, the low correlation coefficient observed for homogenized juices demonstrates once again the illogicality of using the Howard Mould Count method for homogenized products. Calculation of glucosamine on a total solids rather than on a sample weight basis permits direct comparison between juices, purées and pastes (Fig. 4) and it is notable that the results for the few samples of purée and paste tested fit closely with the results for non-homogenized juices. The fit is yet closer if a correction factor is used to convert the Howard Mould Count results for pastes made on 8% soluble solids dilutions to a 6.5% soluble solids level typical of juices.

The precision of the fungal glucosamine assay as an index of fungal contamination is reasonably high for a single operator working with one type of sample material. The precision could undoubtedly be improved by analysing larger samples and by a high degree of comminution before sampling. Modification of the method to make it suitable for automated analysis would also

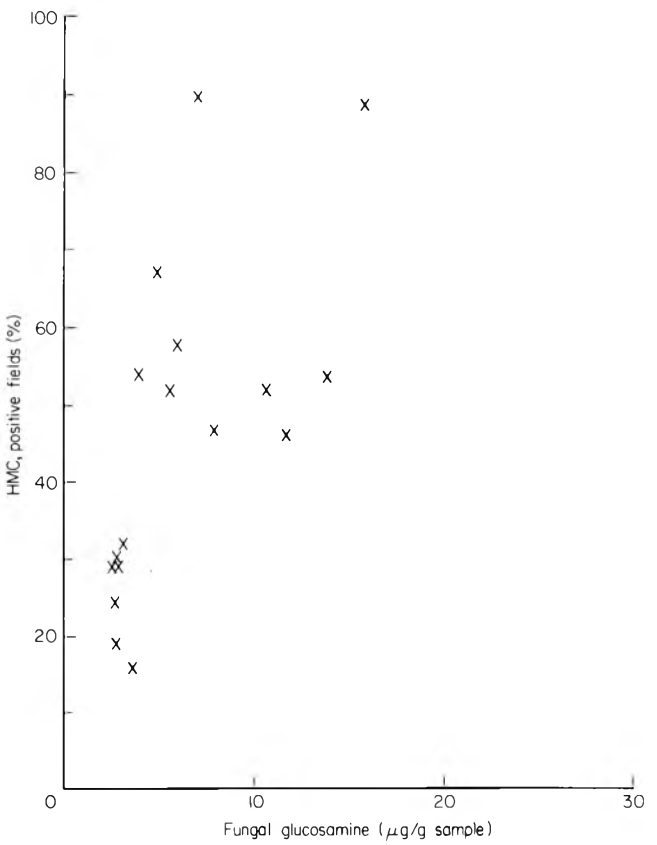

Figure 3. Relationship between Howard Mould Count and fungal glucosamine; homogenized juice.

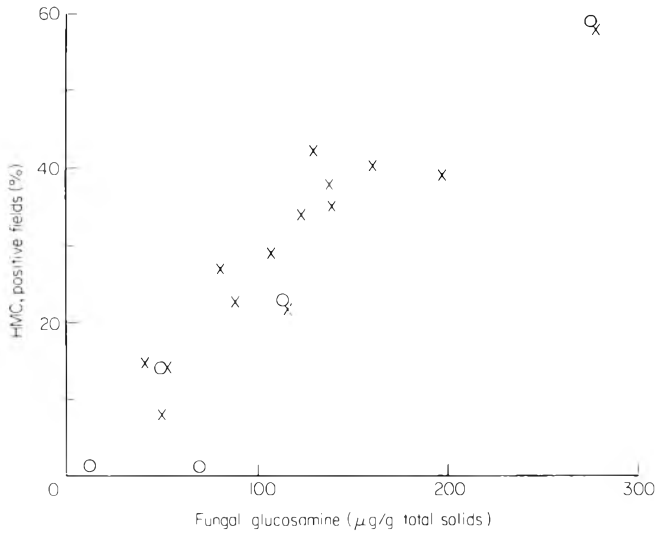

Figure 4. Relationship between Howard Mould Count and fungal glucosamine (calculated on total solids) for non-homogenized tomato juices (×) and purées (○).

improve reproducibility and precision since the many manual stages involved in the analysis would be removed. Since further work on the method is required it is inappropriate to talk specifically in terms of acceptable and unacceptable levels of mould as estimated by chitin analysis. However, the results obtained in the present study suggest that a fungal glucosamine level of about 200 μg/g total solids would be equivalent to a Howard Mould Count of 50% on pastes and purées diluted to 8% soluble solids. This would be equivalent to about 5–6 mg dry weight of fungal mycelium/g total solids. For a tomato juice containing 6.5% total solids, this would be equivalent to 4.2–4.9 mg/g juice. For many foods supporting microbial growth, spoilage is frequently initiated at levels of 10^6–10^7 organisms/g. For yeasts this would be equivalent to a mean level of about 5 μg dry weight yeast cells/g sample, a level not excessively dissimilar to that suggested above for a possible upper limit for fungal mycelium.

This approach towards assessment of fungal contamination could readily be applied to many other food commodities which are subject to fungal spoilage (e.g. cereal grains, oilseeds, etc.). Indeed, preliminary studies by Austwick (personal communication, 1976) have shown that the method can be applied to cereals, but that because of the sensitivity of the method a high degree of sample comminution is required to obtain reproducible results. For some foods the natural occurrence of amino sugars such as glucosamine and galactosamine would affect the baseline and would require the analysis of unhydrolysed samples to provide a zero correction factor. Insect contamination could also give rise to widely misleading results since chitin comprises a major component of the exoskeleton. Further work is needed also to assess whether, and if so to what extent, the chitin content of common food spoilage fungi is modified by

growth under different cultural conditions and on different substrates. However, the present study has demonstrated that chitin assay provides the basis for an objective method which might find wide applicability in food analysis.

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References

A.O.A.C. (1970) *Official Methods of Analysis*, 11th Edn. Association of Official Agricultural Chemists, Washington, D.C.

Dakin, J.C., Smith, D.J. & Taylor, A.McM. (1964) *Variance in the Howard Mould Count arising from observer and distribution differences.* B.F.M.I.R.A. Tech. Circ. No. 259.

Eisenberg, W.V. (1968) *Q. Bull. Ass. Fd Drug Off. U.S.*, **32**, 173–9.

Howard, B.J. (1911) *Tomato ketchup under the microscope.* U.S. Dept. of Agriculture, Bureau of Chemistry, Circular No. 68. Washington D.C., U.S.A.

Jarvis, B. (1975) *Microbiological Trends in Agriculture, Fisheries and Foods* (Ed. by F.A. Skinner and J.G. Carr) p. 251. Academic Press, London.

Jarvis, B. (1977) *Food and Beverage Mycology* (Ed. by L.R. Beuchat). AVI Publishing Co. Inc., Westport, Conn., U.S.A.

Pitt, J.I. (1975) *Water Relations of Foods* (Ed. by R.B. Duckworth), pp. 273–307. Academic Press, London.

Ride, J.P. & Drysdale, R.B. (1971) *Physiol. Plant Pathol.* **1**, 409.

Ride, J.P. & Drysdale, R.B. (1972) *Physiol. Plant Pathol.* **2**, 7.

Swift, M.J. (1973) *Soil Biol. Biochem.* **5**, 321.

Troy, V.S. (1968) *Mould Counting of Tomato Products.* Continental Can Co. Inc., Chicago, U.S.A.

Tsuji, A., Kinoshita, T. & Hoshino, M. (1969) *Chem. Pharm. Bull.* **17**, 1505.

Vas, K., Fabri, I., Kutz, N., Lang, A., Orbanyi, T. & Szabo, G. (1959) *Fd Technol., Champaign*, **13**, 318.

Williams, H.A. (1968) *J. Ass. Pub. Analysts*, 6, 69.

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Rheology of fresh and frozen okra dispersions

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Summary

Rheological properties of fresh and frozen okra dispersions were studied at 20–80°C. The dispersions were pseudoplastic with both consistency coefficient (m) and flow behaviour index (n) influenced by temperature. Fresh sample m was significantly higher than frozen sample m at 20 and 50°C but the reverse was true at 80°C. Flow behaviour indices of fresh and frozen okra dispersions were not significantly different at any of the temperatures used. The results indicated that the viscous properties of soup prepared from frozen okra would not be inferior to those of the fresh product.

Introduction

Okra is an important tropical crop particularly in Africa and Asia where it is made into soup or simply boiled and consumed as vegetable. A recent report (Constantinides, 1976) has indicated okra could be a significant protein source.

The shelf life of okra is limited by its high respiratory rates at warm temperatures (Scholz, Johnson & Buford, 1963; Woodroof & Shelor, 1958) and hence it is usual to process them into canned or dehydrated products soon after harvesting to avoid rapid deterioration. Some storage at 7–10°C and r.h. of 90–95% is also practised (Lutz & Hardenburg, 1968) to hold the fresh fruit for marketing or processing. Frozen storage is also common although the quality of soup from frozen okra is said to be inferior to that made from fresh okra. Similar comments have also been made of preparations from processed okra.

The stringy, gum-like consistency of okra products is an important quality attribute; however, to the authors' knowledge, no rheological data describing the flow behaviour of okra dispersions are presently available. This paper

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describes a study of the viscous properties of dispersions prepared from fresh and frozen okra.

Materials and methods

Fresh okra in very good condition were obtained from a retail outlet in Vancouver, Canada. The okra had been flown in from Fiji Island under refrigeration at 8°C and 95% r.h., and were obtained 3–4 days from harvest.

Part of the okra was frozen in polyethylene film bags and stored at −15°C for 48 hr prior to use while the rest was cut into smaller bits and dispersed in an equal weight of boiling water. The mixture was heated to boiling, then macerated at high speed in a Waring blendor for 10 sec. The frozen samples were weighed before thawing, then treated in a similar way. After blending, the dispersions were allowed to stand at room temperature for 30 min prior to viscometric investigation. About 90 ml of the mixture were used for measurements obtained with the Haake Rotovisko Model RV1 (described by Van Wazer *et al.*, 1963) using the MV1 spindle (gap width, 0.96 mm). The samples were tested at 20, 30, 50, 70 and 80°C using a Kryomat constant temperature bath connected to the water jacket that surrounded the sample holder. During each determination the spindle rotation speed was varied stepwise from maximum to minimum. Shear rates ranged from 13 700 to 8.5 sec^{-1} as calculated using the manufacturer's formula, $\dot{\gamma} = B/U$, where $\dot{\gamma}$ is the shear rate (sec^{-1}), B is the shear rate constant for the spindle and U is the gear setting. The torsion signal due to viscous drag in the fluid at known shear rates was displayed on a 25 cm strip chart recorder.

Flow behaviour curves were constructed using shear rate and apparent viscosity data derived from the viscometric test. The well known Power Law flow model was fitted to the data using the equation:

$$\eta = m\dot{\gamma}^{n-1}$$

where η is apparent viscosity (poise), m is the consistency coefficient (dyne sec^n cm^{-2}) and n is the flow behaviour index (no units). The flow parameters m and n were evaluated with a computer using the method of least squares and a non-linear curve fitting technique. This procedure included the evaluation of statistical parameters that would indicate the accuracy with which the flow model fitted the data.

Preliminary examination of the data indicated that the dispersions deviated considerably from Newtonian flow, which could result in errors in the calculated shear rates using the manufacturer's formula (Haugen & Tung, 1976). Thus, shear rates were recalculated with a correction for Power Law flow (Krieger, 1968) using:

$$\dot{\gamma} = \frac{2\Omega}{n} \cdot \frac{R_c^{2/n}}{R_c^{2/n} - R_b^{2/n}}$$

where Ω is the spindle angular velocity (rad sec^{-1}) and R_b and R_c are the spindle and cup radii respectively. With the corrected shear rates, the Power Law flow model was fitted to the data as previously described. This rheogram correction procedure modified the consistency coefficient but did not change the flow behaviour index.

The viscometric data from the fresh or frozen samples at different temperatures were compared using a covariance test (Snedecor, 1965) of a linear form of the Power Law equation. This method tested for differences of slope $(n - 1)$ and level (m) of the rheograms plotted as $\log \eta$ *v.* $\log \dot{\gamma}$. Similar comparisons were carried out for the fresh and frozen sample data at each temperature.

Results and discussion

Viscometric data from both fresh and frozen samples were fitted accurately by the Power Law flow model with 0.997 the average coefficient of determination. Results are presented in Tables 1 and 2. All flow behaviour index (n)

Table 1. Comparison of Power Law flow model parameters for fresh okra dispersions

Temperature (°C)	Consistency coefficient, m (dyne sec^n/cm^2)*	Flow behaviour index, n*
20	213^{e}	0.153^{x}
30	185^{d}	0.151^{x}
50	128^{b}	0.186^{xy}
70	93.7^{a}	0.212^{y}
80	152^{c}	0.166^{x}

* Parameters with the same superscripts are not significantly different at $P \leq 0.05$.

Table 2. Comparison of Power Law flow model parameters for frozen okra dispersions

Temperature (°C)	Consistency coefficient, m (dyne sec^n/cm^2)*	Flow behaviour index, n*
20	178^{c}	0.168^{xy}
30	162^{c}	0.176^{xy}
50	104^{a}	0.198^{y}
70	124^{a}	0.168^{xy}
80	155^{b}	0.146^{x}

* Parameters with the same superscripts are not significantly different at $P \leq 0.05$.

Table 3. Comparison of Power Law flow model parameters for dispersions of fresh and frozen okra*

Temperature (°C)	State of okra	Consistency coefficient, m (dyne sec^n/cm^2)*	Flow behaviour index, n*
20	Fresh	213^b	0.153^x
	Frozen	178^a	0.168^x
30	Fresh	185^a	0.151^x
	Frozen	162^a	0.176^x
50	Fresh	128^b	0.186^x
	Frozen	104^a	0.198^x
70	Fresh	93.7^a	0.212^x
	Frozen	124^a	0.168^x
80	Fresh	152^a	0.166^x
	Frozen	155^b	0.146^x

* For each determination temperature, parameters with the same superscripts are not significantly different at $P \leqslant 0.05$.

values were less than 1 thus the okra dispersions in water exhibited pseudoplastic (shear rate thinning) behaviour over a relatively wide range of temperatures.

For both treatments, consistency coefficient (m) initially decreased as the temperature was raised while the flow behaviour index increased slightly. This pattern continued up to 70°C in the fresh samples and 50°C in the frozen samples with a reverse trend at higher temperatures. Direct comparison of the data from the fresh and frozen samples (Table 3) showed that the consistency coefficients of the fresh and frozen samples differed significantly at 20, 50 and 80°C. Interestingly, the fresh sample m values were higher than those of the frozen sample at the lower temperatures while the reverse was true at higher temperatures. Fresh and frozen sample flow behaviour indices were not significantly different ($P > 0.05$) at any of the temperatures used.

These effects may be clarified by examination of the apparent viscosities of the fresh and frozen okra dispersions (Table 4) at shear rates pertinent to manual stirring, pouring from a container and oral evaluation (Shama & Sherman, 1973). The apparent viscosities were taken from the rheograms at shear rates of 10 100 and 1000 sec^{-1}. The fresh okra dispersions tended to be slightly more viscous at low temperatures but this is not the case at the higher temperatures. It should be noted that these differences were small in comparison to the product thinning which occurred as the temperature was raised to 70°C. The increase in consistency coefficients and apparent viscosities of both product types at 80°C may be the result of protein coagulation, a reflection of incomplete cooking prior to blending in the sample preparation.

Table 4. Apparent viscosity of fresh and frozen okra dispersions at various temperatures and shear rates

Shear rate/ (sec^{-1})	Temperature (°C)	Apparent viscosity (poise)	
		Fresh okra	Frozen okra
10	20	30.3	26.2
	30	26.1	24.3
	50	19.6	16.5
	70	15.3	18.3
	80	22.2	21.7
100	20	4.31	3.85
	30	3.70	3.64
	50	3.01	2.60
	70	2.49	2.69
	80	3.25	3.03
1000	20	0.613	0.567
	30	0.523	0.547
	50	0.461	0.409
	70	0.405	0.396
	80	0.476	0.424

While certainly there were some differences in the viscometric behaviour of fresh and frozen okra dispersions, these relatively small and temperature dependent differences do not appear to support any claim that soup made from previously frozen okra would be inferior in viscometric properties to that made from fresh okra.

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References

Constantinides, S. (1976) *Time Magazine* (Can. edn), May 31 issue.
Haugen, P. & Tung, M.A. (1976) *Can. Inst. Fd Sci. Technol. J.* **9**, 98.
Krieger, I.M. (1968) *Trans. Soc. Rheol.* **12**, 5.
Lutz, J.M. & Hardenburg, R.E. (1968) *The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks*, U.S.D.A. Handbook No. 66, Washington.

Scholz, E.W., Johnson, H.B. & Buford, W.R. (1963) *Rio Grande Valley Hort. Soc. J.* **17,** 170.

Shama, F. & Sherman, P. (1973) *J. Texture Stud.* **4,** 111.

Snedecor, G.W. (1965) *Statistical Methods,* 5th edn. Iowa State Univ. Press, Ames, Iowa.

Van Wazer, J.R., Lyons, J.W., Kim, K.Y. & Colwell, R.E. (1963) *Viscosity and Flow Measurement.* Wiley-Interscience, New York.

Woodroof, J.G. & Shelor, E. (1958) *Okra for Processing.* Georgia Agr. Expt. Station Bull. 56.

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Effect of selection on the ratings of taste panel assessors*

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Summary

The combination of preference tests with difference tests in taste panel procedure is shown to be free from bias. This combination is used both to select assessors and also to obtain their assessments at the same session. The influence of the selection procedure on the results obtained is considered, and found to be only moderate.

Introduction

The combination of a difference (taste) test with a preference or quality judgement has been deprecated (Peryam, 1958), apparently on the basis of a single report: Schutz & Bradley (1954) found that when a preference judgement was required, following a triangle test (Amerine, Pangborn & Roessler, 1965; ASTM Committee E-18, 1968) on the same samples, assessors tended to prefer the paired samples. This conclusion was now tested.

For the purpose of conducting taste panel assessments of food quality, it is accepted practice to reject potential assessors who have demonstrated an inability to discriminate between samples of the particular product under investigation (ASTM Committee E-18, 1968; Wittes & Turk, 1968; Basker, 1976). The statistical significance level used in making this decision is commonly $P \leqslant 0.25$ (Wittes & Turk, 1968). The samples used to determine discrimination ability are normally not those specifically used in the later assessments (Wittes & Turk, 1968; *cf.* Basker, 1976). Notwithstanding, it does not appear to have been demonstrated experimentally to what extent any real advantage accrues from the use of selected assessors (British Standard, 1975).

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Experimental

Four series (A to D), two of eight and two of seven taste panel sessions, were held over a 109-day period (1974/75), using segments and juice from fresh and stored oranges. At each session the assessors compared the three pairs possible amongst three samples ($\alpha\beta$, $\alpha\gamma$, $\beta\gamma$) by the triangle test (ASTM Committee E-18, 1968); each sample participated in the triangle tests once as the pair and once as the singlet. The assessors recorded their preference judgements and also rated each sample on a five-point hedonic scale, *viz.*

Excellent
Very good
Good: usual quality
Poor
Unacceptable

The procedure was repeated during the following season (1975/76) on four series (E to H) each of twelve taste panel sessions, over a 144-day period.

For mathematical analysis, the ratings were assigned intuitive numerical scores (Kramer & Twigg, 1970): +2, +1, 0, −1 and −2 respectively. The frequencies with which these scores were recorded were 2.7, 22.9, 52.3, 20.3 and 1.8% respectively.

When an assessor correctly identified the odd (singlet) sample, his ratings of both the pair and the singlet have meaning. When the odd (singlet) sample was not correctly identified, the assessor had 'differentiated' (in, say, the $\alpha\alpha\beta$ triangle) between $\alpha\beta$ on the one hand and α on the other; thus the only rating with any meaning was that of the latter α: the rating given to the $\alpha\beta$ 'pair' is not assignable to either α or β.

Results and discussion

The results of the preference tests are summarized in Table 1. No statistically significant difference was found either in the number of preference tests favouring the paired samples ($\chi^2 = 0.039$, d.f. = 1, $0.80 < P < 0.90$) or in the number of assessors favouring these samples ($\chi^2 = 1.281$, d.f. = 1, $0.20 < P < 0.30$).

Now put:

N_1 = number of assessors who correctly identify the odd (singlet) sample;
N_2 = number of assessors who fail to identify the odd sample;
N_3 = number of assessors who identify the odd sample other than by chance (deliberate assessors);
N_4 = number of assessors who identify the odd sample by chance alone (chance assessors);
N_5 = total number of assessors.

Table 1. Preferences for paired and for 'odd' samples

Series	Number of preference tests			Number of assessors	
	Preferring paired samples	Preferring odd samples	Preferences tied	Preferring paired samples	Preferring odd samples
A	10	11	3	109	109
B	12	10	2	117	118
C	9	9	3	91	83
D	7	13	1	91	106
E	17	16	3	204	190
F	17	16	3	205	201
G	19	16	1	217	187
H	18	15	3	218	202
Totals	109	106	19	1252	1196

When a significant difference exists between the samples presented to the taste panel, a contingency table may be drawn up (Table 2), given that the deliberate assessors all correctly identify the odd sample. The proportion N_3/N_5 depends on the product under investigation, the similarity between the samples, and the population of assessors. The quality ratings of the chance assessors are expected to be identical for both samples in the triangle, in mean and in variance. The variance of the quality ratings of the chance assessors is expected to be greater than that of the deliberate assessors: in failing to distinguish any finite difference between the samples, the chance assessors are expected to decrease the precision of their quality ratings. Thus the variance of the N_2 assessors is expected to be greater than that of the N_1 assessors.

When an *in*significant difference exists between the samples presented to the taste panel, the identifications of the deliberate assessors are distributed in the same proportion (1 : 2) as those of the chance assessors. Thus, no difference is expected in this case between the variances of the N_1 and the N_2 assessors.

The three triangle tests of each session may also be considered as a means of selecting assessors (Wittes & Turk, 1968; Basker, 1976). At forty-six of the forty-eight sessions in 1975/76, all three odd samples were identified by at least one assessor. The ratings of the N_1 assessors by definition exclude

Table 2. Contingent numbers of assessors: significantly different samples

	Correct identification	Incorrect identification	Totals
Deliberate assessors	N_3	0	N_3
Chance assessors	$N_2/2$	N_2	N_4
Totals	N_1	N_2	N_5

Table 3. Score variances when undiscriminating assessors are rejected at $P = 0.18$ level

Statistical significance of triangle identification	Number of samples	Number of N_1 assessors	Number of N_2 assessors	Score variance for N_1 assessors	Score variance for N_2 assessors	Variance ratio S_2^2/S_1^2	Statistical significance of S_2^2/S_1^2
$P > 0.10$	73	623	1027	0.5796	0.6166	1.0638	$P > 0.05$
$0.05 < P < 0.10$	19	196	221	0.6371	0.7420	1.1647	$P > 0.05$
$0.01 < P < 0.05$	22	261	245	0.5642	0.7083	1.2554	$P \simeq 0.05$
$0.001 < P < 0.01$	15	200	137	0.5275	0.6840	1.2967	$P \simeq 0.05$
$(0.005 < P < 0.01)$	(9)	(113)	(86)	(0.5009)	(0.6348)	(1.2673)	$(P > 0.05)$
$(0.001 < P < 0.005)$	(6)	(87)	(51)	(0.5616)	(0.7683)	(1.3681)	$(P > 0.05)$
$P < 0.001$	15	242	108	0.6092	0.6152	1.0098	$P > 0.05$
$(0.0002 < P < 0.001)$	(6)	(88)	(52)	(0.5739)	(0.6226)	(1.0849)	$(P > 0.05)$
$(P < 0.0002)$	(9)	(154)	(56)	(0.6291)	(0.6079)	(1/1.0349)	$(P > 0.05)$
Total	144	1522	1738				

N_1 = Number of assessors who correctly identified the 'odd' samples.
N_2 = Number of assessors who failed to identify the 'odd' samples.
S_1^2 = Score variance for N_1 assessors.
S_2^2 = Score variance for N_2 assessors.

instances of zero correct identification: this is equivalent to rejecting undiscriminating assessors at the $P = 0.18$ level (Wittes & Turk, 1968; Basker, 1976). (At the remaining two out of forty-eight sessions, the equivalent rejection level is $P = 0.66$; Wittes & Turk, 1968; Basker, 1976.)

The results obtained during 1975/76 at various significance levels of triangle identification are shown in Table 3. The score variances for the different groups of asssessors were obtained by pooling the sample variances (Dixon & Massey, 1957). (The results at the highest significance levels of triangle identification have also each been divided into two sub-groups. The sub-groups show that the changes in the variances and their ratios discussed below constitute a true trend change. The smaller numbers of assessors in each sub-group, however, do not permit conclusions regarding appreciable statistical significance.) The mean value of N_5 ranged from about 22 to 23. As expected, the variance ratio S_2^2/S_1^2 was not statistically significant where the triangle identification was not significant ($P > 0.10$). The variance ratio (and its significance) increased as the triangle identification significance increased, and then decreased abruptly: at this stage, therefore, the differences between the samples were sufficiently great so as to enable otherwise chance assessors to make the correct identification.

The taste panel results are now reconsidered at the $P = 0.66$ rejection level of undiscriminating assessors ($P = 1.00$ for two out of forty-eight sessions) (Wittes & Turk, 1968; Basker, 1976). Put:

N_6 = number of assessors who correctly identified the odd sample in at least two of the three triangles.

The results obtained are shown in Table 4. No significant improvement (decrease) was obtained in the score variances.

Table 4. Score variances when undiscriminating assessors are rejected at $P = 0.66$ level

Statistical significance of triangle identification	Number of N_6 assessors	Score variance for N_6 assessors	Variance ratio S_1^2/S_6^2	Statistical significance of S_1^2/S_6^2
$P > 0.10$	—	—	—	—
$0.05 < P < 0.10$	146	0.6532	1/1.0253	$P > 0.05$
$0.01 < P < 0.05$	202	0.5976	1/1.0592	$P > 0.05$
$0.001 < P < 0.01$	135	0.4499	1.1725	$P > 0.05$
$(0.005 < P < 0.01)$	(80)	(0.4158)	(1.2047)	$(P > 0.05)$
$(0.001 < P < 0.005)$	(55)	(0.4995)	(1.1243)	$(P > 0.05)$
$P < 0.001$	185	0.6218	1/1.0207	$P > 0.05$
$(0.0002 < P < 0.001)$	(68)	(0.6082)	(1/1.0598)	$(P > 0.05)$
$(P < 0.0002)$	(117)	(0.6297)	(1/1.0010)	$(P > 0.05)$

N_6 = Number of assessors who correctly identified the 'odd' sample in at least two of the three triangles.

S_1^2 = Score variance for assessors who correctly identified the 'odd' sample in at least one of the three triangles, i.e. for N_1 assessors.

S_6^2 = Score variance for N_6 assessors.

Conclusions

No bias was found to be present when preference tests followed difference tests. The two procedures may therefore be legitimately combined.

When assessors are selected at the $P = 0.18$ rejection level of non-discrimination, significant decreases of score variance over those of rejected assessors were obtained only when the statistical significance of the triangle identification was about $P = 0.01$. No further significant decrease was obtained when the rejection level was more stringent ($P = 0.66$). The significance level at which the triangle identification will be obtained cannot be known beforehand: in these sessions $(15 + 22)/144$, or about one-quarter, lay in the range $0.001 < P < 0.05$. This is considered to be sufficient to justify the use of a selection system. In the aggregate, however, the influence of the selection process was only moderate, and might have been nullified in its influence on the standard effort of a mean score because of the reduction in the number of scores considered.

References

Amerine, M.A., Pangborn, R. M. & Roessler, E.B. (1965) *Principles of Sensory Evaluation of Food.* Academic Press, New York.

ASTM Committee E-18 on Sensory Evaluation of Materials and Products (1968) *Manual on Sensory Testing Methods,* American Society for Testing and Materials, Philadelphia, Pa. ASTM Special Technical Publication 434.

Basker, D. (1976) *Chem. Senses Flav.* **2,** 209.

British Standard (1975) *Glossary of Terms relating to Sensory Analysis of Food,* B.S. 5098.

Dixon, W.J. & Massey, F.J. (1957) *Introduction to Statistical Analysis,* 2nd edn. McGraw-Hill, New York.

Kramer, A. & Twigg, B.A. (1970) *Quality Control for the Food Industry,* 3rd edn. Avi Publishing Company, Westport, Conn.

Peryam, D.R. (1958) *Fd Technol., Champaign,* **12,** 231.

Schultz, H.G. & Bradley, J. (1954) in: *Food Acceptance Testing Methodology.* (Ed. by D.R. Peryam, F.J. Pilgrim and M.S. Peterson). Quartermaster Food and Container Institute, Chicago, Ill.

Wittes, J. & Turk, A. (1968) in: *Correlation of Subjective–Objective Methods in the Study of Odors and Taste,* (Chairman, W.J. Stahl), American Society for Testing and Materials, Philadelphia, Pa. ASTM Special Technical Publication 440, pp. 49–70.

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Some characteristics of the heat of water vapour sorption in dried foodstuffs

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Summary

Differential heat curves (obtained from adsorption and desorption isotherms) previously reported, were utilized for calculating 'integral' heats of water vapour sorption in dried foods. Foods examined amounted to almost thirty and included fruits, meats, vegetables and spices. 'Integral' heats were calculated by integrating the area under each differential heat curve in the moisture range of more practical interest. Calculated heats may be useful for estimating the heat requirements during dehydration. Average values of the heats of sorption were also reported which may be useful for the analysis of sorption rates.

An empirical equation was obtained for expressing with very high accuracy, the differential (isoteric) heats of desorption as a function of moisture content.

Introduction

Recently, Iglesias & Chirife (1976a) reported isosteric heats of water vapour adsorption and desorption in a great variety of foodstuffs. Differential heats were calculated by applying the Clausius–Clapeyron equation to adsorption or desorption isotherms at different temperatures. Literature sorption data were used by Iglesias & Chirife (1976a) for their calculations, and the results were reported as plots of heat of sorption against the moisture content. The main aspects of the differential heat curves were discussed in terms of the mechanistic interpretation of the process of water sorption. In a companion paper, Iglesias & Chirife (1976b) compared the Clausius–Clapeyron heats with those obtained from the application of B.E.T. theory to the water sorption

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data, and also discussed the phenomenon of hysteresis in connection with the heats of adsorption and desorption.

The present paper contains the results of further work on the characterization of the energetics of water vapour sorption in dried foodstuffs and is based on calculations carried out on the differential heat curves reported by Iglesias & Chirife (1976a). It consists of, (a) the calculation of 'integral' heats of adsorption and desorption, (b) the evaluation of average heats of sorption for the range of moisture contents covered, and (c) the development of an analytical expression to relate heats of desorption to moisture content in several foods.

Results and discussion

(a) Calculation of 'integral' heats of sorption

Although the isosteric heat of sorption, which is a differential heat, is a useful parameter, for some purposes it is more useful to have the value of the integral heat of sorption. The relationship of the integral heat of sorption, Q, to the more common quantity, the differential heat of sorption, q^{st}, is made clear (Gregg & Sing, 1967) by reference to any typical plot of heat of sorption against moisture content, as for example those reported by Iglesias & Chirife (1976a). Q is given by summing the area under the curve since,

$$Q = \int_0^X q^{st} dX \tag{1}$$

where X is the moisture content. In the present work, the 'integral' heat of sorption was defined as,

$$Q' = \int_{X_m}^{X_f} q^{st} dX \tag{2}$$

where X_m = monolayer moisture content (g water per 100 g of fat-free dry matter), X_f = moisture content at which the heat of sorption is 'close' to the heat of vaporization of pure water and has the same units as X_m.

Thus, the 'integral' heat defined by (2) represents the total heat evolved (or required) when a food goes from the monolayer moisture content up to a moisture condition at which the heat of sorption is 'close' (the term 'close' will be defined later) to the heat of vaporization of pure water or vice-versa. For a desorption or drying analysis this 'integral' heat may be used to estimate the extra heat of dehydration, which is defined as the additional heat above the normal heat of vaporization required to reduce the moisture content from near saturation to the monolayer moisture content, and is expressed in kcal per 100 g of dry food (fat-free). The monolayer value, calculated from the B.E.T. equation (Labuza, 1968), was selected as the lower moisture limit because it

has been suggested that it may represent an adequate end point of the drying process. This is because the dehydrated food is considered to have an improved overall storage stability at this moisture value (Labuza, 1975). Monolayer moisture contents were taken from the results reported by Iglesias & Chirife (1976c), and correspond to the average temperature at which the heats were calculated. By inspecting the heat curves reported by Iglesias & Chirife (1976a) it can be seen that they were not drawn up to the point where they fall in line with the heat of vaporization of water. Most of them end at moisture contents at which the heat of sorption is still about 0.30–0.50 kcal/g mole (1.25–2.09 kJ/g mole) over the value of the heat of vaporization of water. This was due to the uncertainty in the determination of the isosteric heat of sorption in the low energy range, as thoroughly discussed by Iglesias & Chirife (1976a). By this reason the integration was carried out only up to the last point drawn in the curves, even if it does not correspond exactly with the point at which the heat of sorption falls in line with the heat of vaporization. However, by inspecting the shape of the curves in the low-energy range it can be seen that the area neglected with this procedure is relatively small; in fact it was estimated that for most of the curves analysed this procedure may lead to an underestimation of usually less than 10% in the net 'integral' heats. Consequently, the 'integral' heats here reported applied strictly for the moisture ranges indicated, although also may represent, in some cases, approximately the total heat evolved or required in going from the monolayer up to saturation or vice-versa.

The calculations of 'integral' heats of sorption were performed on the differential heat curves reported by Iglesias & Chirife (1976a). As these authors reported adsorption and desorption heat curves, the calculations were done on both type of curves. Table 1 shows the results of the calculations performed on the adsorption heat curves. The results are given in kcal per 100 g of dry food (fat-free). Table 1 also shows the net 'integral' heat of adsorption, Q'_{nA}, and the percent of the total 'integral' heat, Q'_A, which correspond to the net heat. It is important to notice that the net heat is the difference between the total heat evolved and the heat of vaporization of pure water. It can be seen that, for the moisture ranges covered, the contribution of the net 'integral' heat of absorption over the total heat evolved, varies widely. It ranges from about 5% for sugared items such as banana and pineapple to about 30% for winter savoury, although for most of the items it is 10–20%.

Iglesias & Chirife (1976a) also reported differential heat curves calculated from desorption isotherms, and noted that for several foods the adsorption and desorption heat curves did not agree. Table 2 shows the comparison between net 'integral' heats (over the moisture ranges indicated) calculated on the adsorption and desorption differential heat curves. It can be seen that for all the foods examined the net desorption 'integral' heats are larger than the adsorption ones. The percent differences between adsorption and desorption net 'integral' heats ($Q'_{nD}-Q'_{nA}/Q'_{nA} \cdot 100$) may amount to about 130%. Iglesias & Chirife (1976b) discussed the possible relationship between hysteresis and the discrepancy between heats of adsorption and desorption and also analysed

Table 1. 'Integral' heats of sorption calculated from adsorption differential heat curves; reference temperature: 35°C

Product	Q'_A (kcal/100 g d.m. f.f.)*	Q'_{nA} (kcal/100 g d.m. f.f.)*	$Q'_{nA}/Q'_A \cdot 100$ (%)	Moisture range covered (% dry basis, $X_m - X_f$)
Fruits				
Banana	12.42	0.66	5.2	3.6–24
Pineapple	12.71	0.66	5.2	9.1–30
Meats				
Chicken, cooked	9.33	1.83	19.6	4.0–17
Chicken, raw	9.47	1.63	17.2	4.4–18
Trout, cooked	7.69	1.23	16.0	3.8–15
Trout, raw	7.06	1.23	17.4	3.9–14
Spices				
Aniseed	3.82	0.36	9.4	4.0–10
Cardamom	7.70	1.20	15.6	4.8–16
Cinnamon	6.23	1.33	21.3	5.4–14
Chamomile	8.40	1.50	17.9	5.1–17
Cloves	4.96	0.75	15.1	3.7–11
Coriander	6.20	0.95	15.3	4.9–14
Ginger	6.72	1.47	21.9	5.9–15
Laurel	7.90	1.20	15.2	3.4–15
Nutmeg	4.78	0.80	16.7	4.1–11
Peppermint	8.11	1.76	21.7	5.0–16
Sweet marjoram	7.24	1.36	18.8	3.8–14
Thyme	7.26	1.55	21.3	4.1–14
Winter savoury	8.27	2.56	30.9	5.1–15
Vegetables				
Avocado	7.31	1.08	14.8	2.2–13
Beetroot	8.07	1.38	17.1	5.4–17
Celery	9.17	1.27	13.8	4.3–18
Chives	7.32	1.61	22.0	6.1–16
Aubergine	9.27	2.12	22.9	4.6–17
Lentil	7.85	1.51	19.2	6.0–17
Mushrooms (*Boletus*)	9.00	0.99	11.0	4.1–18
Paranut	3.09	0.62	20.1	1.7–6.0
Salsify	8.35	1.73	20.7	4.7–16
Tapioca	6.40	0.69	10.8	6.1–16

* To convert kcal/100 g dry matter (fat-free) to kJ/100 g dry matter (fat-free) multiply by 4.185.

the validity of applying the Clausius–Clapeyron equation to isotherms showing hysteresis.

(b) Evaluation of average heats of sorption

In the analysis of rates of moisture adsorption or desorption in dried foodstuffs, heat transfer should usually be considered along with mass transfer. This

Table 2. Comparison between adsorption (Q'_{nA}) and desorption (Q'_{nD}) integral net heats

Product	Q'_{nA} (kcal/100 g d.m. f.f.)*	Q'_{nD} (kcal/100 d.m. f.f.)*	Diff. Q'_{nA} and Q'_{nD}† (%)	Moisture range covered (% dry basis f.f.)
Meats				
Chicken, cooked	1.17	1.86	59.0	5.0–16
Chicken, raw	1.51	2.17	43.7	4.5–17
Trout, cooked	1.10	1.74	58.2	4.4–15
Spices				
Aniseed	0.22	0.29	31.8	5.0–10
Cinnamon	1.36	1.38	1.5	6.2–13.9
Chamomile	1.16	1.22	5.2	6.0–17
Coriander	0.95	1.41	47.9	5.2–14
Ginger	1.20	1.47	22.5	7.0–15
Nutmeg	0.74	0.87	17.3	4.3–11
Thyme	1.04	1.11	6.7	6.5–14
Sweet marjoram	1.08	1.57	45.4	4.5–14
Winter savoury	1.34	2.11	57.5	7.5–15
Vegetables				
Paranut	0.261	0.350	34.1	2.5–6.0
Tapioca	0.317	0.722	127.8	7.8–16

* To convert kcal/100 g dry matter (fat-free) to kJ/100 g dry matter (fat-free) multiply by 4.185.

† $(Q'_{nD} - Q'_{nA})/Q'_{nA} \cdot 100$.

is because any adsorption or desorption of moisture requires release or consumption of heat and in certain cases heat transfer can slow mass transfer rates or even control the adsorption or desorption rate completely (King, 1968). For vapour phase diffusion in dried foods the effective diffusivity, D_{eff}, is given by (King, 1968; Bluestein & Labuza, 1972),

$$D_{eff} = \frac{\text{M.W.}}{\rho_s} \, b \left(\frac{\partial a_W}{\partial X}\right)_T p^0 \, \frac{\alpha}{1+\alpha} \tag{3}$$

$$\alpha = \frac{R T^2 k}{b a_W p^0 q^{st^2}} \tag{4}$$

where M.W. = molecular weight
ρ_s = bulk density
b = vapour space permeability
a_W = water activity, p/p^0
T = absolute temperature
p^0 = vapour pressure of water
k = thermal conductivity of the food sample and
R = gas constant.

The term $\alpha/(1+\alpha)$ determines the degree of mass or heat transfer control. If $\alpha \gg 1$ the process is totally mass transfer controlled; if $\alpha \ll 1$ the process is totally heat transfer controlled. King (1968) noted that one of the most critical assumptions in his analysis was the constancy of the heat of sorption with moisture content and suggested that when the heat varies with moisture one may use an average over the moisture range considered. The differential heat curves reported by Iglesias & Chirife (1976a) showed that the assumption of constancy of q^{st} with moisture is usually far from reality, and the use of an average value becomes necessary. The average heat of sorption is given by,

$$\bar{q}^{st} = \frac{1}{X_f - X_m} \int_{X_m}^{X_f} q^{st}\, dX \tag{5}$$

Table 3. Average value of heat of sorption, $\bar{q}^{st}$, calculated from adsorption differential heat curves; reference temperature: 35°C; moisture range covered: $X_m - X_f$ (same as indicated in Table 1)

Product	$\bar{q}^{st}$ (kcal/g mole)	$X_{\bar{q}}$ % dry basis (fat-free)
Winter savoury	15.0	9.0
Aubergine	13.5	9.4
Ginger	13.3	10.0
Peppermint	13.3	9.7
Salsify	13.3	9.1
Chives	13.3	9.7
Cinnamon	13.2	9.3
Thyme	13.2	6.0
Paranut	12.9	2.9
Chicken, cooked	12.9	9.7
Lentil	12.8	10.8
Sweet marjoram	12.8	7.2
Chamomile	12.7	9.4
Trout, raw	12.6	8.4
Beetroot	12.5	11.5
Chicken, raw	12.5	10.3
Nutmeg	12.5	6.9
Trout, cooked	12.4	8.6
Cardamom	12.4	9.3
Coriander	12.3	8.8
Laurel	12.3	7.8
Cloves	12.2	6.6
Avocado	12.2	6.2
Celery	12.1	9.9
Mushrooms, *Boletus*	11.7	9.1
Tapioca	11.5	10.5
Aniseed	11.5	6.3
Banana	11.0	17.2
Pineapple	11.0	14.0

Table 3 shows the calculated values obtained by applying eqn (5) to the differential heat curves corresponding to the adsorption process and the moisture range (beginning at the monolayer) covered. It can be seen that the values of average heats range from 11 to 15 kcal/g mole. Table 3 also shows the moisture content, $X_{\bar{q}}$, at which the isosteric heat of adsorption is equal to the average heat (defined by eqn (5)).

According to what we have seen, desorption heats are generally higher than adsorption heats. Depending on the confirmation of this fact, which should be through direct calorimetric determinations, it may have some influence on the rates of moisture adsorption and desorption. For other conditions similar, α values would be smaller for the adsorption process than for the desorption one, as indicated by eqn (4). For α values not far from 1 (mixed mass and heat transfer control) this could lead to rates of adsorption faster than the desorption ones, as indicated by eqn (3).

(c) Development of an empirical equation to correlate heats of desorption with moisture content

It was found that the following equation,

$$q_D^{st} = 10.38 + a \cdot X'^{b} \cdot \exp(cX') \qquad (6)$$

where q_D^{st} = isosteric heat of desorption (kcal/g mole), X' = moisture content (g water/g fat-free dry matter) and a, b, c = parameters, describes very well the desorption heat curves in the moisture ranges here studied. The factor $\exp(cX')$ in eqn (6) ($c < 0$) provides a quick convergence of q_D^{st} to the value of the heat of vaporization of pure water (10.38 kcal/g mole) when the moisture content is of the order of about 0.4 g water per g of dry matter. This agrees with the fact that at about this moisture value, water is present in most foods as unbound water (Duckworth, 1971) and consequently the net heat of desorption approaches zero. As is obvious, eqn (6) may be integrated between the monolayer value, calculated with the desorption branch of the isotherm, and any desired moisture level to obtain, either, 'integral' heats of desorption or average differential heats. The factor $\exp(cX')$ also allows the integral to converge whatever the limits. The factor X'^{b}, which does not destroy the convergence of the integral, was included to fit the data at low moisture values. The parameters a, b, c were calculated under the condition,

$$\sum_{i=1}^{n} [\ln(q_i^{st} - 10.38) - \ln a - b \ln X_i' - cX_i']^2 = \text{minimum}$$

which implies unweighted least squares after a logarithmic transformation. Weighted least squares were not used because they would have assigned more weight to the data corresponding to low moisture contents. This was not desired because at low moisture contents the asymptotic behaviour is

Table 4. Calculated parameters, a, b, c, (eqn (6)) for desorption differential heat curves (valid from the monolayer value calculated on the desorption branch of the isotherm); reference temperature: 35°C

Product	a	b	c	$\bar{D}$ (%)
Cardamom*	1.172×10^4	1.952	−35.89	0.57
Chamomile	2.498	−0.7229	−18.89	0.75
Chicken, cooked	3.633×10^2	0.4709	−38.47	0.28
Chicken, raw	8.211	−0.2090	−14.81	0.61
Coriander	1.110×10^7	3.550	−73.02	0.64
Cinnamon	1.518×10^2	0.7448	−23.58	0.23
Ginger	2.292×10^3	1.179	−37.85	0.15
Mushrooms, *Boletus*	0.2383	−0.8055	−3.478	0.30
Nutmeg	2.806×10^2	0.5345	−48.09	0.51
Paranut	1.054×10^4	1.311	−111.70	0.31
Tapioca	1.449×10^4	2.351	−33.33	0.28
Thyme	0.602×10^{-2}	−2.516	−6.755	0.37
Trout, raw*	4.090×10^2	0.5726	−21.56	0.78

* Differential heat (desorption) curves for these products were not reported by Iglesias & Chirife (1976a), but were calculated from desorption isotherms reported by Wolf *et al.* (1973). Monolayer values for these products were reported by Iglesias & Chirife (1976c).

uncertain. Table 4 shows the calculated parameters for the desorption curves of several foods. In order to have an idea of the goodness of fit of eqn (6) as applied to the experimental data, the following percentage mean deviation, $\bar{D}$, was defined:

$$\bar{D} = \frac{1}{n} \sum_{i=1}^{n} \frac{|(q_{iE}^{st} - q_{iC}^{st})|}{(q_{iE}^{st} + q_{iC}^{st})/2} \cdot 100$$

where n = number of experimental points (usually seven to ten), E: experimental and C: calculated.

As it is defined, the deviation $\bar{D}$ also gives the maximum percentage error of the 'integral' heat obtained by integration of eqn (6). The results of $\bar{D}$ are also included in Table 4. It can be seen that the fitness of the proposed equation may be considered excellent, as all deviations, $\bar{D}$, calculated were usually well below 1%. As mentioned, the main value of eqn (6) is that it may be used to calculate 'integral' or average desorption heats between any desired moisture limits (between the range proposed). The integral of eqn (6) between finite limits is an Incomplete Gamma Function, which has no analytical solution, but the factor, exp (cX') ($c < 0$) leads to a quick convergence so it is easy to perform a numerical integration. Equation (5) also makes possible extrapolation in the high moisture side, and eventually would permit computation of the small 'integral' heat neglected in the graphical integrations. However, the

extrapolation has also some risk because the behaviour of the differential heat curves cannot be guaranteed.

Conclusions

'Integral' heats of water sorption, adsorption and desorption, were obtained from previously reported differential heat curves. In the moisture ranges analysed the contribution of the net 'integral' heat of adsorption over the total heat evolved ranges between 5 and 30%. 'Integral' heats of desorption were always found to be higher than the corresponding 'integral' heats of adsorption. Average heats of adsorption were also calculated. An empirical equation was used to relate differential (isosteric) heats of desorption to moisture content. The use of an empirical equation is justified because at present no theoretical model exists to describe quantitatively the variation of the heat of sorption with moisture content in dried foods.

Some suggestions can be made about future research in this area. For example, calorimetric determinations of heats of sorption, ad- and desorption, are needed to confirm the results obtained through the use of the Clausius–Clapeyron equation. In some drying operations it is necessary to dry the food to very low moisture contents, 1–3% dry basis. The determination of heats of sorption in these moisture ranges becomes necessary in view of the high values suggested by the nature of some differential heat curves (Iglesias & Chirife, 1976a).

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References

Bluestein, P.M. & Labuza, T.P. (1972) *AIChE J.*, **18**, 706.
Duckworth, R.B. (1971) *J. Fd Technol.* **6**, 317.
Gregg, S.J. & Sing, K.S.W. (1967) *Adsorption, Surface Area and Porosity*. Academic Press, New York.
Iglesias, H.A. & Chirife, J. (1976a) *Lebensm.-Wiss. u-Technol.* **9**, 116.
Iglesias, H.A. & Chirife, J. (1976b) *Lebensm.-Wiss. u-Technol.* **9**, 123.
Iglesias, H.A. & Chirife, J. (1976c) *Lebensm.-Wiss. u-Technol.* **9**, 107.
King, C.J. (1968) *Fd Technol., Champaign,* **22**, 165.
Labuza, T.P. (1968) *Fd Technol., Champaign,* **22**, 263.
Labuza, T.P. (1975) *Water Relations of Foods* (Ed. by R.B. Duckworth). Academic Press, New York.
Wolf, W., Spiess, W.E.L. & Jung, G. (1973) *Lebensm.-Wiss. u-Technol.* **6**, 94.

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Drum dried beet powder

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Summary

Pilot plant scale studies on drum drying of beet slurry indicate that such process is feasible for the production of beet powder. Compared to the conventional dice air dehydration drum drying has much faster rates and better steam efficiency. Moisture was reduced to the desired level of 3.8–4.0% using a double drum drier (60 cm length by 35 cm diameter) operated at 123°C with 18 sec drying retention time. Colour stability of the drum dried powder during storage (25, 31, 35, 40 and 45°C) was slightly better than that of the powder obtained in the conventional tunnel dehydration.

Introduction

Interest in natural food colourants has increased markedly in the past few years particularly because of intensified consumer awareness to the health aspects of some artificial dyes. In general terms, dehydrated red beets and beet juice concentrates are permitted in the U.S. and elsewhere as colourants (1960 Color Additive Amendment Food Drug and Cosmetic Act of 1938) and have been shown to be applicable as water soluble pigments in food systems (von Elbe & Maing, 1973; Pasch *et al.*, 1975).

The bulk of the beet crops for commercial processing is diverted to dehydration plants. Dehydrated beets may be produced as slices, cubes or stripes. An important use of dehydrated beets is in the form of beet powder added as a red colourant to products such as dressing sauces, dry casserole mixes and tomato products not covered by standards of identity.

Beet powder is prepared by grinding beet dice to pass through a U.S. #60 sieve. Beet dice, slices or strip-cut are usually dehydrated in tunnel driers, continuous conveyor-belt dehydrators or belt-through driers (93–99°C) down to 11% moisture and may be finished in a bin drier (63°C) to final moisture content of 5% (Luh, Somoggi & Meehan, 1975).

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Conventional air dehydration of diced, sliced or strip-cut vegetable is a relatively slow rate process with a poor thermal efficiency. Thus when the final product is beet powder, the practice of the slow air dehydration of beet dice should be examined and ought to be compared with faster and more efficient dehydration techniques, such as drum drying of beet slurry.

The purpose of this investigation is to propose and to evaluate the drum drying of beet slurry to beet powder, and to compare such process with the conventional air dehydration.

Materials and methods

Drum drying

The drum drying experiments were carried out in two types of driers: (a) laboratory double drum drier (Mitchel, England) and (b) pilot plant double drum drier (Escher Wyss, W. Germany – Type ZA 130). The specifications and the operational conditions of the driers are given below:

	Laboratory model (Mitchel)	Pilot plant (Escher Wyss)
Drum diameter (cm)	16.5	35.0
Drum length (cm)	15.0	60.0
Drying temperature (°C)	120	123
Retention time (sec)	11	18
Working gap between drums (mm)	0.110	0.137

Beet slurry was prepared by the following procedure: washed trimmed beet roots (Detroit) were diced (0.5 × 0.5 × 0.5 cm). The diced beet passed a grinding machine (Hobart, A-200) and finally through a corundum stone mill (Fryma, KMR) yielding a fine particles slurry (~ 5 μm). The slurry was continuously fed to the drum drier by a suitable positive displacement pump. Vapours evolved during drying were removed by means of a hood and overhead exhaust fan. To ease product removal cold air (7–8°C) was directed near the doctor blades.

Conventional air dehydration

The conventional dehydration was carried out in a temperature controlled (± 0.5°C) cabinet on 0.5 × 0.5 × 0.5 cm beet dices, with 250 f.p.m. air velocity over the trays. The dehydration cycle was carried out in three stages (USDA, 1944) as follows: 93°C for weight reduced to three times dry weight; following by 77°C for weight reduced to two times dry weight and finishing with 68°C

air temperature down to the final 4% moisture. Overall cycle time was ~ 2.5 hr. The dried beet dice was ground to pass through a U.S. #60 sieve.

Analytical methods

Moisture content was determined by drying in a vacuum oven for 6 hr at 70°C.

Sorption studies were conducted on drum dried beet powder. Water vapour sorption was determined gravimetrically at 25°C using a Cahn Gram Electrobalance (Ventron Inst. Calif., Model RG).

Colour measurements (expressed as betanin concentration) were determined in accordance with the method described by Saguy, Kopelman & Mizrahi (1977). The procedure is based on computer aided non-linear curve fitting of the visible spectrum of the beet solution, with a predicted function of the individual beet pigments.

Storage studies, up to 200 days period, were carried on beet powder placed in 25, 31, 35, 40 and 45°C thermostatically controlled (± 0.25°C) cabinets. Samples were drawn periodically and were analysed for colour content.

Data were processed and analysed on the Technion IBM 370/168 computer. Regression lines and related statistical coefficients were obtained by BMD02R (Dixon, 1971).

Results and discussion

Our studies on the drum drying of beet indicate that such process is feasible for the production of beet powder. Few difficulties were encountered in drum drying beet down to 3.8–4.0% moisture, which is also the desired moisture level of beet powder normally obtained by dehydration of diced beet.

At the end of the drying process the two different dehydration techniques showed no difference in the concentration of the major pigment responsible for the red-purple colour (6.80 mg betanine per g powder). The above indicates that, compared to the low-temperature long-time conventional drying, the short-time high-temperature drum drying has no detrimental effect upon colour retention in the course of the drying process.

Colour stability studies of the drum and the conventional dehydration beet powder, conducted at various storage temperatures (Figs 1 and 2), showed that both products maintain a first order reaction, i.e.

$$a/a_0 = \exp(-k_1 t) \quad (1)$$

where: a = betanine concentration at time, t; a_0 = initial betanine concentration; k_1 = rate constant; t = time.

The degradation rate constants, k, or its alternative format, the half life time, $T_{1/2}$, are listed in Table 1. The rate constant and the half life time values

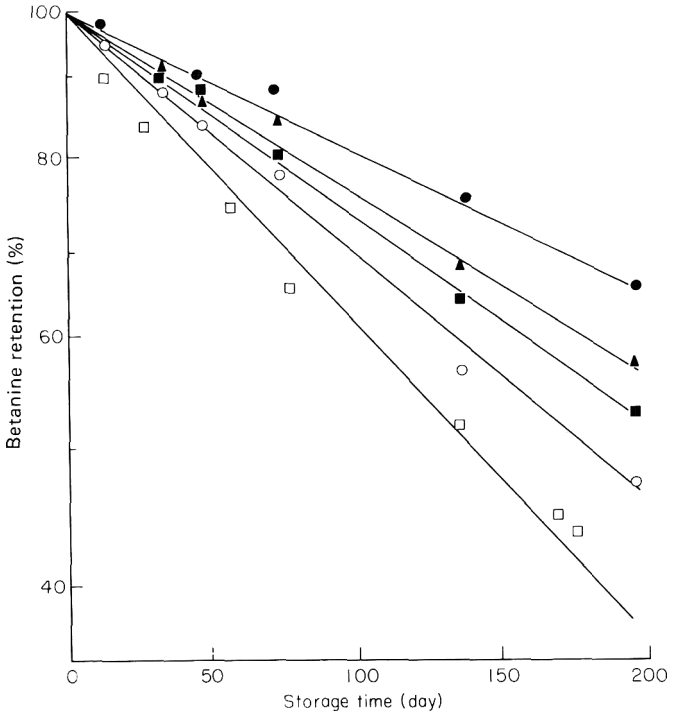

Figure 1. Colour retention (betanine) during storage of drum dried beet powder. ●, 25°C; ▲, 31°C; ■, 35°C; ○, 40°C; □, 45°C.

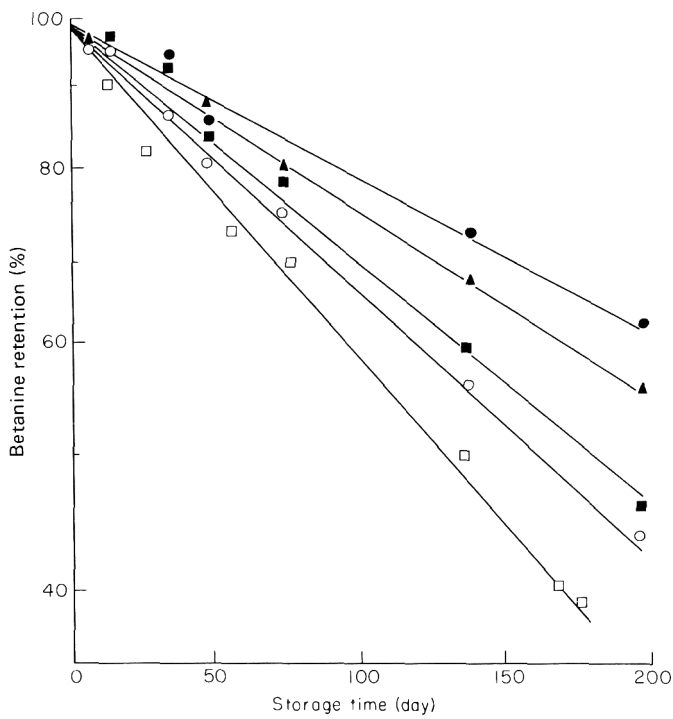

Figure 2. Colour retention (betanine) during storage of conventional air dehydrated beet powder; symbols as in Fig. 1.

indicate that the betanine retention during storage is slightly better in the drum dried powder as compared to the conventional air dehydration product. The degradation rate constants (Table 1) can be fitted into Arrehenius temperature coefficient pattern (Fig. 3) yielding an energy of activation of 7.00 and 8.00 kcal/mole for drum and air dehydrated powders, respectively.

Table 1. Betanine degradation rate constants of beet powders

Temperature (°C)	Drum		Cabinet	
	$k_1 \times 10^3$ (day^{-1})	$T_{½}$ (day)	$k_1 \times 10^3$ (day^{-1})	$T_{½}$ (day)
25	2.34	296.9	2.65	261.5
31	2.86	242.4	3.02	229.5
35	3.41	203.3	4.02	172.4
40	3.81	181.9	4.72	146.8
45	4.37	159.7	5.02	138.1

Sorption isotherm (at 25°C) of the drum dried powder is shown in Fig. 4. The sorption is lacking any typical turning point and is similar in nature to the type III pattern (Gregg & Sing, 1967), which normally describes dried vegetables isotherms such as carrots, green pepper, peas, cabbage and onion

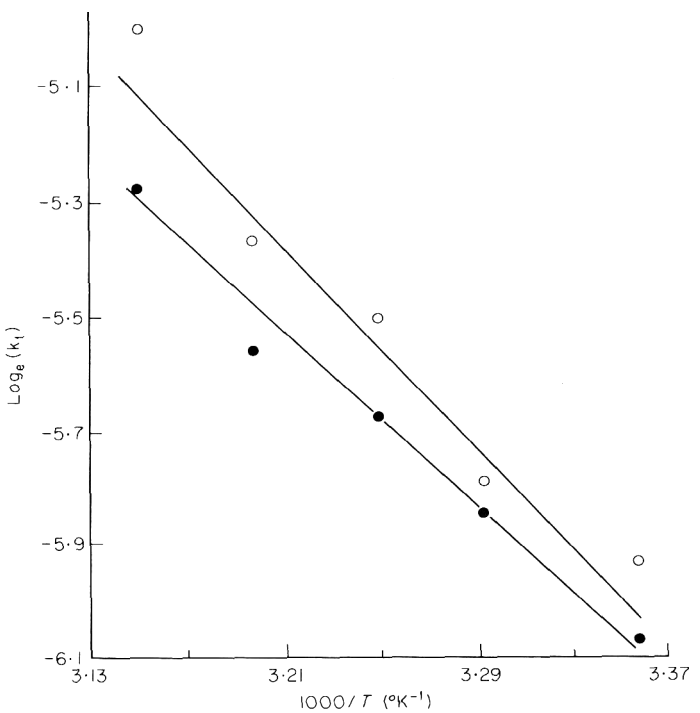

Figure 3. Effect of temperature upon colour (betanine) degradation rate constant (k_1) of beet powder. ○, cabinet; ●, drum.

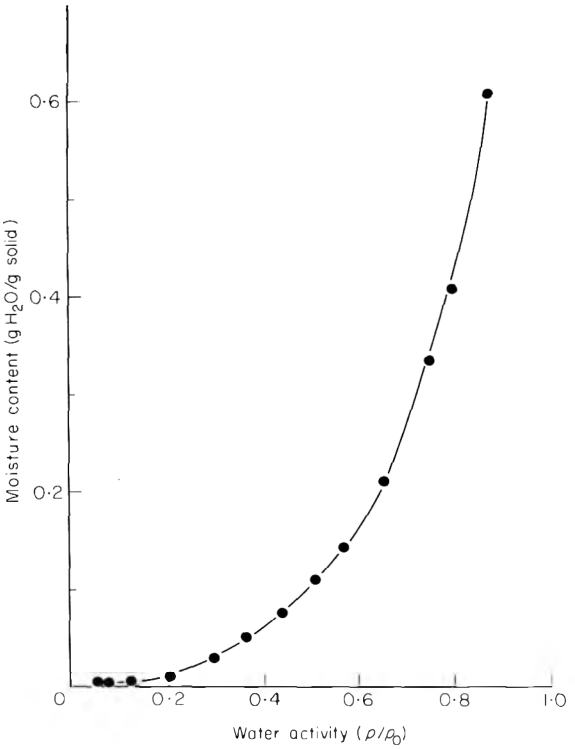

Figure 4. Sorption isotherm at 25°C of drum dried beet powder.

(Salwin, 1963). Water sorption isotherm data (Fig. 4) were fitted into a multilayer adsorption equation (Halsey, 1948), which recently was successfully applied for several foods (Iglesias, Chirife & Lombardi, 1975). At the water activities tested (0.07–0.80) such linear fitting yielded the following format (correlation coefficient, $r = 0.979$):

$$\ln [\ln (p_0/p)] = -0.451 \ln (X) - 1.525 \quad (2)$$

where: p/p_0 = water activity and X = moisture content (g water per g solids).

In conclusion, it can be seen that producing beet powder by drum drying is a feasible process. Compared to the conventional dice dehydration, drum drying has much faster dehydration rates and better steam efficiency.

Further investigations for improving and optimization of the process are being carried out.

References

Dixon, W.J. (1971) *Biomedical Computer Programs.* Univ. of California Press, Berkeley, Calif.

von Elbe, J.H. & Maing, I.Y. (1973) *Cereal Sci. Today,* **18,** 263.

Gregg, S.J. & Sing, K.S.W. (1967) *Adsorption, Surface Area and Porosity.* Academic Press, London.

Halsey, G. (1948) *J. Chem. Phys.* **16**, 931.
Iglesias, H.A., Chirife, J. & Lombardi, J.L. (1975) *J. Fd Technol.* **10**, 289.
Luh, B.S., Somoggi, L.P. & Meehan, J.J. (1975) *Commercial Vegetable Processing* (Ed. by B.S. Luh and J.G. Woodroof), Ch. 9. Avi Publishing Co., Wesport, Conn.
Pasch, J.H., von Elbe, J.H. & Sell, R.H. (1975) *J. Milk Fd Tech.*, 38, 25.
Saguy, I., Kopelman, I.J. & Mizrahi, S. (1977) *J. Fd Sci.* (In press).
Salwin, H. (1963) *Fd Technol., Champaign*, **17**, 1114.
United States Department of Agriculture (1944) *Vegetable and Fruit Dehydration, A Manual for Plant Operators.* Pub. No. 540, Washington, D.C.

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Total folate activity in Brussels sprouts: the effects of storage, processing, cooking and ascorbic acid content

J. D. MALIN

Summary

The total folate activity of Brussels sprouts has been assayed with *Lactobacillus casei* after preliminary digestion with chicken pancreas conjugase. The effects of storage, processing and cooking on the total folate activity was investigated. The observed relative stability of the total folate was attributed to the presence of considerable quantities of antioxidative ascorbic acid and to the low surface area/volume ratio in comparison to many other green vegetables. The dietary folate contribution of cooked sprouts is discussed and compared with other foods.

Introduction

The difficulties and limitations of assay methods for naturally occurring monoglutamate and polyglutamate folates are reflected in the variations of reported levels for this vitamin in foodstuffs, and for the effects of processing and cooking operations on these levels.

Microbiological assays with *Lactobacillus casei* have been commonly used to measure the free and total folate activity of raw and cooked foodstuffs, i.e. the folate activity before and after treatment of the food extract with conjugase (γ-glutamyl peptidase) preparations (Hoppner, Lampi & Perrin, 1973; Toguchi *et al.*, 1973; Chanarin, 1975). However, it has been shown (Malin, 1976) that the free folate activity of foods may be over-estimated due to the presence of naturally occurring conjugases within the extract of the sample; only the total folate activity is recommended for an indicator of the vitamin level and for any subsequent changes in the levels brought about by processing or cooking operations.

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The total folate activity of Brussels sprouts, and the effect of storage, processing, cooking and ascorbic acid content on this activity, have been investigated.

Materials and methods

Preparation of sprout extracts

The procedure was designed to achieve maximal folate extraction with minimal destruction of labile folate derivatives. Test samples were macerated in buffer containing ascorbic acid antioxidant and the resulting homogenate was heat treated to disrupt cellular material and to inactivate the indigenous enzyme systems.

Buffer (pH 6.0) was prepared by mixing $Na_2HPO_4 \cdot 2H_2O$ (0.1 M) and NaH_2PO_4 (0.1 M) in the ratio 18:4 before the addition of L-ascorbic acid (0.15% w/v). A weighed sample of longitudinally quartered sprouts of known moisture content was macerated for 1–3 min in a blender with 15–20 volumes of buffer. The homogenate was quantitatively transferred with buffer washings to a Pyrex conical flask, sealed with aluminium foil, and rapidly autoclaved for 10 min at 115°C. After rapid cooling to 20°C, the homogenate was washed into a volumetric flask and diluted to volume with buffer. Portions (50 ml) were heat sealed in polyethylene pouches and stored in the dark at −21°C. Before microbiological assay, the frozen homogenate was thawed and filtered through Whatman No. 1 paper using a low vacuum to produce an optically clear extract.

Treatment of extracts to release total folate activity

A fresh enzyme preparation was made for each sample digestion. Desiccated chicken pancreas (Difco Labs, U.S.A.) was suspended in water (5 mg/ml), thoroughly mixed with a glass rod, and incubated at 37°C for 45 min. After filtration of the slurry through Whatman No. 1 paper, the clear filtrate was used immediately. The thawed sprout extracts containing 50 g sprout tissue/litre were adjusted from pH 6.0 to 8.0 (Malin, 1974) by the addition of a few drops of sodium hydroxide (50% w/v) and a portion (10 ml) pipetted into a test tube containing chicken pancreas conjugase preparation (2 ml). Toluene preservative (0.6%) was added and the sample tubes, plus enzyme blanks (10 ml water + 2 ml enzyme preparation), were incubated at 37°C for 16 hr. After incubation, the enzymically digested samples were investigated for signs of microbial contamination (odour, gassing turbidity) and if no spoilage was detected the samples were diluted appropriately and assayed with *L. casei*.

Total folate assay with L. casei

The assay organism chosen was *L. casei* var. *rhamnosus* (NCIB 6375). Assays were carried out in rimless Pyrex tubes (150 × 16 mm) closed with sterilizable polypropylene stoppers.

A standard response curve for synthetic folic acid, i.e. pteroylglutamic acid (PGA), was prepared by diluting a stock solution containing 100 μg PGA/ml (Scientific Hospital Supplies, Liverpool) to a working solution of 0.4 ng/ml. Triplicate tubes containing between 0 and 0.8 ng PGA/tube were prepared and diluted to 2 ml with glass distilled water. Triplicate sample tubes containing suitable dilutions of conjugase treated extract were prepared simultaneously and diluted to 2 ml with water. To each sample and standard tube was added 2 ml basal medium (Becton and Dickinson, U.S.A.) containing ascorbic acid (3 mg/ml); all tubes were stoppered and sterilized at 115°C for 5 min, rapidly cooled to 37°C in a stirred water bath, and inoculated with the prepared assay organism.

The inoculum was prepared in the following way: on the evening before an assay, a loopful of culture was transferred from a maintenance stab of liver medium (Cooperman, 1967) to a bottle of the liver broth and incubated at 37°C overnight. The following morning, a loopful of the overnight culture was transferred to a fresh bottle of broth and incubated at 37°C for 8 hr. The culture was centrifuged at 2500 rpm for 5 min and the spent broth decanted. Sterile saline (0.9% w/v) was added and the bottle shaken to re-suspend the cells. The process of washing was repeated five times and the cells finally suspended in saline to give an absorbance of 0.100 ± 0.02 when measured at 600 nm against a saline reference in a 1 cm glass cuvette (Pye/Unicam SP 700 Spectrophotometer). One drop of inoculum was added to all tubes except the sterile blanks. All tubes were then incubated for 40 hr at 37°C. The microbiological purity of each inoculum was checked by colonial appearance and Gram staining after growth on Liver medium and Plate Count Agar (Oxoid Ltd, London) for 30 hr at 37°C.

After the 40 hr incubation period, the triplicate standard PGA tubes and sample tubes were thoroughly mixed on a vortex mixer ('Whirlimix' Fisons, England). A sample (2 ml) of the cell suspension was pipetted into a clean tube and diluted with water (10 ml). After thorough mixing, the turbidity of each tube was determined at 600 nm in a 1 cm glass cuvette using a similarly diluted sterile blank as the reference. The absorbance of each tube was read not less than 30 sec after pouring into the cuvette, to allow the flow birefringence effect of the bacterial suspension to equilibrate (Pearson, 1967). Non-inoculated sample tubes were also read to determine the level of background absorbance (if any).

The most linear range of the PGA standard curve was between 0.05 and 0.6 ng PGA/tube although this tended to vary between assays due, primarily, to the growth response of the particular inoculum in the folate-free basal medium. The turbidity of sample dilutions falling within the most useful part

of the standard curve were used to calculate the equivalent PGA activity (total folate activity); after allowing for the folate activity of the enzyme blanks, the results were expressed in μg per 100 g of original sprout sample on both a wet weight (WB) and dry weight (DB) basis. The total folate activities were recorded as the mean, with the standard deviation given in parentheses, and were obtained from the results of all sample dilutions from three separate assays of individual extracts.

Moisture determination

The moisture content of longitudinally quartered sprout samples (10–20 g) was determined gravimetrically by oven drying for 16 hr at 80° C.

Ascorbic acid determination

A semi-micro volumetric method was used for the determination of ascorbic acid in Brussels sprouts. Before titration with standardized 2:6-dichlorophenol-indophenol, the sprout homogenate was adjusted to pH 0.6 and treated with formaldehyde to remove possible interference from sulphur compounds.

Results

Total folate activity of fresh sprouts

Relatively large samples (not less than 3 kg) of fresh sprouts were obtained from various sources throughout the season. After size selection (spherical diameter), the moisture content and total folate activity was determined (Table 1). The results showed variation in the total folate activity of the three samples, particularly when calculated on a dry weight basis.

Table 1. Total folate activity of samples of fresh sprouts

Variety	Moisture (%)	Diameter (cm)	*L. casei* total folate activity (μg/100 g)	
			WB*	DB*
Lancelot (Ayrshire) November	83.68	2.0–3.5	175 (9)†	1072 (55)
Frigostar (Ayrshire) December	84.14	2.5–3.5	211 (15)	1330 (94)
English Grade 1. (chain store) February	87.55	2.0–3.5	208 (23)	1670 (185)

*WB = wet weight basis; DB = dry weight basis.

† Figures given in parentheses are standard deviations.

Table 2. Total folate activity and ascorbic acid content of fresh sprouts stored at −21°C

−21°C (day)	Moisture (%)	Ascorbic acid content (mg/100 g)		Total folate activity (μg/100 g)	
		WB*	DB	WB	DB
0	84.14	107.1	675.3	211 (15)†	1330 (94)
31	84.33	68.1	436.5	195 (20)	1244 (127)
67	83.37	23.6	141.9	167 (9)	1004 (54)
107	83.74	18.6	113.1	160 (10)	984 (61)
188	83.48	16.1	97.4	124 (15)	750 (91)

* WB = wet weight basis; DB = dry weight basis.
† Figures given in parentheses are standard deviations.

The storage of fresh sprouts at −21°C

Fresh sprouts of the Frigostar variety were graded (2.5–3.5 cm diameter) and a sample (4 kg) stored on aluminium trays in a cold store at −21 ± 2°C. The total folate activity and ascorbic acid content were monitored over a period of 188 days (Table 2).

Losses of total folate activity and ascorbic acid occurred during storage at −21°C, the greatest rate of loss occurring during the first 67 days. After 6 months storage, the average loss (DB) of total folate and ascorbic acid contents was 48% and 86%, respectively.

Total folate activity and ascorbic acid content of fresh sprouts after blanching, blast freezing, frozen storage and cooking

The procedure is illustrated in Table 3; at each sampling stage (1–6), the moisture content and ascorbic acid content were determined immediately and homogenates of sprout tissue were prepared for subsequent total folate assay. No statistically significant changes (Table 4) were found in the total folate activity of sprouts at any of the sampling points. The most noticeable loss of ascorbic acid occurred at the blanching stage, although significant losses also occurred during subsequent storage and cooking. A loss of 46% ascorbic acid was recorded in the final cooked product compared with the original level.

Total folate activity of boiled and pressure-cooked sprouts

A portion (400 g) of English grade 1 sprouts was withdrawn for moisture determination and total folate assay and three similar portions were added separately to boiling water (1 litre volume), brought back to the boil and then

Table 3. Processing and cooking of blast-frozen sprouts

		Sampling stage
FRESH SPROUTS (5 kg Frigostar)		
Graded 2–4 cm, washed in 35 litre water, drained		
WASHED SPROUTS	→	1
Steam-blanched 5 min, water cooled, drained		
BLANCHED SPROUTS	→	2
Blast-frozen −22°C/135 min		
BLAST-FROZEN SPROUTS	→	3
Stored at −21°C in polyethylene pouches (500 g)		
STORED FROZEN SPROUTS	14 days →	4
	180 days →	5
400 g (−21°C/380 days) + 1 litre boiling water, simmered 10 min, drained		
COOKED FROZEN SPROUTS	→	6

boiled for 10, 20 and 30 min, respectively. The total folate activity of the strained sprouts and the cooking liquor was determined (Table 5).

No significant decrease in total folate activity could be detected after boiling the sprouts; apparent increases of 7 and 2% total folate in sprouts boiled for 10 and 20 min, respectively were not statistically significant. However, total folate activity was detected in the cooking water; consideration of this activity combined with that of the cooked sprouts indicated that a statistically significant increase in total folate activity had occurred as a result of boiling.

The total folate activity of a sample of fresh sprouts (Lancelot variety, size 2–3.5 cm) was determined before and after pressure-cooking at 115°C for 5 min in a laboratory autoclave. No statistically significant change in the total folate activity was detected after pressure-cooking, Table 5.

Total folate activity of cooked, commercially frozen sprouts

Samples of two major brands of frozen sprouts were purchased from a local supermarket and cooked according to the recommended directions. The total folate activities on a dry weight basis were 1375 μg/100 g and 1448 μg/100 g.

Table 4. Total folate activity and ascorbic acid content of fresh sprouts after blast freezing, frozen storage and cooking

Sample*	Moisture (%)	Ascorbic acid content (mg/100 g)		Total folate activity (μg/100 g)	
		WB†	DB	WB	DB
1	86.45	98.3	725.4	187 (23)‡	1380 (170)
2	87.68	71.1	577.1	181 (23)	1469 (186)
3	87.38	72.9	577.6	192 (25)	1521 (198)
4	87.40	70.7	561.1	182 (16)	1444 (123)
5	87.03	57.1	440.2	178 (35)	1372 (270)
6	88.11	46.3	391.0	184 (24)	1554 (202)

* 1, Fresh, washed sprouts; 2, blanched washed sprouts; 3, blast frozen washed sprouts; 4, 14 days at −21°C; 5, 180 days at −21°C; 6, cooked sprouts after 380 days at −21°C.
† WB = wet weight basis; DB = dry weight basis.
‡ Figures given in parentheses are standard deviations.

Table 5. Total folate activity of cooked sprouts and cooking water

Cooking time (min)	Moisture (%)	Total folate activity of sprouts (μg/100 g)		Total folate activity of cooking water (μg)	Leaching of total folate activity* (%)
		WB†	DB		
0	87.55	208 (23)‡	1670 (184)	–	–
100°C/10	90.02	179 (12)	1793 (120)	100 (12)	12.0
20	91.05	153 (3)	1709 (33)	147 (14)	17.6
30	89.88	163 (20)	1610 (196)	152 (19)	18.2
0	83.68	175 (9)	1072 (55)	–	–
115°C/5	83.61	184 (12)	1122 (73)	–	–

* Expressed as a percentage of total folate in 400 g uncooked sprout (WB).
† WB = wet weight basis; DB = dry weight basis.
‡ Figures given in parentheses are standard deviations.

which were values similar to those for sprouts frozen, stored and cooked in the laboratory (Table 4).

Total folate activity and ascorbic acid content of commercially canned sprouts

A sample of locally purchased canned sprouts (Dutch product) showed very poor quality with respect to odour, colour, texture and taste when compared with fresh or frozen products. The moisture content and ascorbic acid content of the drained sprouts (1.5–2.0 cm diameter) were determined immediately the can was opened and tissue homogenates were prepared for subsequent

assay with *L. casei*. On a dry weight basis, the canned sprouts contained 966 μg total folate activity/100 g and 210 mg ascorbic acid/100 g.

Discussion

The *L. casei* total folate activity of three varieties of fresh raw sprouts ranged from 175–211 μg/100 g on a wet weight basis. The levels are considerably higher than those of Hoppner, Lampi & Perrin (1972) who assayed four samples of sprouts from a local retail outlet and reported a total folate activity of 53–91 μg/100 g. Chanarin (1975) found the *L. casei* total folate activity of one sample of fresh sprouts to be 130 μg/100 g. The range of results is indicative of natural variation of the samples due to varietal differences, seasonal influence, maturity, post-harvest history, and also to variations in the methods of assay used. With respect to the current investigations, the higher folate activities than previously reported can be attributed to:

(1) Higher concentrations of antioxidative ascorbic acid in extraction buffers, i.e. 2.25–2.50 g ascorbic acid/100 g sprouts compared with 0.4–0.8 g/100 g (Hoppner *et al.*, 1972).

(2) Less severe folate extraction procedures, i.e. the autoclaving of sprouts homogenates at 115°C for 10 min compared with 120°C for 15 min (Chanarin, 1975).

(3) More efficient conjugase treatment of sprout extracts, i.e. chicken pancreas digestion at pH 8.0 (Malin, 1974) compared with the pH 6.0 procedure used by Hoppner *et al.* (1972).

The *L. casei* total folate activity found for fresh Brussels sprouts is comparable to that of the reported values of other green vegetables, especially cabbage (Butterfield & Calloway, 1972).

When freshly picked sprouts were stored at −21°C without a blanching pre-treatment, significant losses of total folate activity and ascorbic acid content were observed. The rate of loss of total folate activity was less than that of ascorbic acid, indicating the greater susceptibility of ascorbic acid to oxidative loss. This observation is in agreement with the established concept that ascorbic acid can act as a folate antioxidant. Ford *et al.* (1968) and Ford, Porter & Thompson (1974) have shown that both indigenous and added ascorbic acid exhibit a protective effect on the folate activity of UHT milk and that added ascorbic acid (60 mg/litre) was sufficient to protect the folate in milk during UHT processing and subsequent storage at 20°C for 60 days. Brussels sprouts contain between 80–140 mg ascorbic acid/100 g sprout tissue (Abrams, 1975); thus, the natural vitamin appears to be sufficient to exert an antioxidative effect on the folate activity.

In this investigation, changes (if any) in the folate activity of sprouts after storage trials, or processing and cooking operations, were calculated on the

basis of dry weight. Published data concerning the effects of cooking on folate activity (Chanarin, 1975) have been calculated on a wet weight basis. Studies with sprouts revealed that, during boiling or steaming, increases in moisture content of up to 3.5% could occur in the vegetable (Table 5) thereby effectively reducing the initial solid content by up to 20%; therefore, vitamin 'losses' calculated on a wet weight basis cannot be attributed solely to destructive or leaching mechanisms since dilution of the sprout tissue has also occurred.

No significant losses in the total folate activity of sprouts could be detected after any permutation of processing and cooking treatments. On the contrary, after boiling, the activity found in the cooking water added to the amount in the cooked sprouts confirmed a statistically significant net increase in total folate. The result was in agreement with Suckewer, Bartnik & Secomska (1970) who found varying increases in the total folate activity of peas and French beans in a commercial canning process, and with Chanarin (1975) who reported increases in total folate activity of 279 and 12% during the boiling of fresh cabbage and potatoes, respectively.

One possible explanation for these apparently anomalous results is that polyglutamate folates, predominating in cooked sprouts (Malin, 1976), are marginally less susceptible to losses during extraction than smaller folate molecules which form as a result of partial conjugase hydrolysis during the maceration of uncooked sprout tissue.

The apparent stability of the total folate activity of Brussels sprouts may possibly be attributed to two main factors:

(1) The presence of significant quantities of antioxidative ascorbic acid.

(2) The relatively small surface area compared to other folate-rich sources, e.g. spinach, broccoli and cabbage, which effectively reduces the amount of folate leached into the cooking water. Toguchi *et al.* (1973) reported that between 20–90% of the total folate activity of nineteen foods studied was lost into the cooking water after only 5 min boiling; the current investigation showed that, after boiling for 10 min, only 12% of the total folate of sprouts was present in the cooking water.

The significant losses of total folate activity observed in fresh unblanched sprouts stored at $-21°C$ in comparison with the stability of blanched sprouts would indicate possible enzymic degradation. In addition, the disruption of the cellular structure by freezing and the loss of ascorbic acid by either direct or enzymic oxidation could also have contributed.

To date, the most satisfactory routine method of folate analysis for naturally occurring compounds is the *L. casei* microbiological assay. It must be emphasized, however, that the nutritional significance of *L. casei* total folate activity is not clearly understood although it is generally agreed that a loss in total folate activity reflects a loss in nutritional value to man. The FAO/WHO (1970) and WHO Expert Group (1972) have suggested recommended daily intakes in terms of free folate. However, more recent work has indicated

that free folate activity may be considerably influenced by the assay technique and is susceptible to over-estimation (Malin, 1976).

Despite the lack of understanding of the nutritional significance of *L. casei* total folate activity, it is still a useful method for the comparison of various foods. In three separate surveys (Hurdle, 1967; Cooper, Contlie & Brunton, 1970; Chanarin, 1975) the daily dietary intake of total folate was found to be 161–297, 240 and 129–300 μg, respectively. When the total folate activities of the range of cooked sprouts analysed in this investigation were calculated for 60 g portions, they all contained 92–110 μg, regardless of fresh or frozen origins, or method of cooking. (The canned product was the exception with only 57 μg total folate/60 g without reheating.) In other words, an average helping of sprouts could provide almost half of the average daily intake of total folate found in the three independent surveys. The folate activity in Brussels sprouts and its relative stability to cooking and processing operations would appear to qualify the vegetable as a valuable source of this dietary nutrient.

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References

Abrams, C.I. (1975) *J. Fd Technol.* **10**, 203.
Butterfield, S. & Calloway, D.H. (1972) *J. Am. diet Assoc.* **60**, 310.
Chanarin, I. (1975) *Getting the Most out of Food*, 10th Series. Van den Berghs and Jurgens, London.
Cooper, B.A., Contlie, G.S.D. & Brunton, L. (1970) *Am. J. clin. Nutr.* **23**, 848.
Cooperman, J.M. (1967) *Am. J. clin. Nutr.* **20**, 1015.
FAO/WHO (1970) *FAO Nutr. Meetings.* Rep. Ser. No. 47.
Ford, J.E., Porter, J.W.G., Thompson, S.Y., Toothill, K. & Edward-Webb, J. (1968) *Proc. Nutr. Soc.* **27**, 60A.
Ford, J.E., Porter, J.W.G. & Thompson, S.Y. (1974) *Int. Dairy Cong.* **1**E, 567.
Hoppner, K., Lampi, B. & Perrin, D.E. (1972) *J. Inst. Can. Sci. Technol. Aliment.* **5**, 60.
Hoppner, K., Lampi, B. & Perrin, D.E. (1973) *J. Am. diet. Assoc.* **63**, 536.
Hurdle, A.D.F. (1967) M.D. thesis, University of London.
Malin, J.D. (1974) *J. Sci. Fd Agric.* **25**, 1051.
Malin, J.D. (1976) *Proc. Nutr. Soc.* **35**, 143A.
Pearson, W.N. (1967) *The Vitamins* (Ed. by P. Gyorgy and W.N. Pearson), Vol. VII, p. 19. Academic Press, New York.
Suckewer, A., Bartnik, J. & Secomska, B. (1970) *Roczniki Pontswowego Zakladu Higieny*, **21**, 619.
Toguchi, H., Hora, K., Hasei, T. & Sonada, H. (1973) *Vitamin (Bitamin)*, **47**, 21.
WHO Expert Group (1972) *Nutritional Anaemias.* Tech. Report Series No. 503.

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Technical note: Microbial protein from corn waste

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Introduction

Corn is grown in large quantities in Nigeria. At present, after the corn has been removed from the cobs they are thrown away as refuse. The quantity of corn harvested in Nigeria during 1968–69 was more than one million tonnes and so the amount of corn cob waste thrown away as waste adds up to a considerable quantity. Some work has been reported on the use of corn canning waste as a medium for the growth of fungi for use as animal feed in the U.S.A. (Church, Nash & Brosz, 1972). They found that *Trichoderma viride* grew well on the corn waste. But this work was limited to the use of liquid waste from canning factories which had a high starch content. Updegraff *et al.* (1973) grew moulds on wastes from the coffee and rum distilling industries and found that *Aspergillus oryzae* could produce significantly more protein than other organisms. Updegraff (1971) also noted that *Aspergillus fumigatus* gave high rates of protein synthesis when newsprint was used as a source of carbohydrate. As the corn cob waste has a high cellulose content, the present work was undertaken to see how far the corn cob waste could be utilized as a source of carbohydrate for the growth of mould mycelium. *A. fumigatus* and three other aspergilli were used as growth organisms.

Materials and methods

Cultures of *Aspergillus fumigatus, A. flavus, A. niger* and *A. tamarri* were obtained from the culture collection of the Department of Microbiology, University of Nigeria.

Corn cob waste was obtained from a local mill in Nsukka. The corn cob waste was oven-dried at 100°C for 24 hr, cut into small pieces and ground into fine powder by means of a ball mill. This was used as a sole source of carbohydrate. The effects of added nitrogen in the form of $(NH_4)_2SO_4$ and of phosphorus as Na_2HPO_4 and of pH and temperature were explored in pre-

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liminary experiments in 50 ml shake flasks. All four fungi grew optimally at 30°C and at pH values from 3.5 to 6.0, pH 3.5 was chosen for the fermentation because it could help suppress bacterial contamination during future scale-up experiments.

Inoculum cultures were grown in 100 ml batches (on the same culture medium as in the fermentors) in shake flasks with a heavily sporulated stock agar slant on potato dextrose agar (Difco). Incubation was at 30°C and the shaking at 350 r.p.m. for 2 days after which cultures showed abundant mycelial growth.

Fermentation of unhydrolysed corn cob waste

Fermentation was carried out in 2 litre Erlenmeyer flasks. Each flask was charged with 500 ml of fermentation medium consisting of 50 g corn cob waste/litre, $(NH_4)_2SO_4$ 10 g/litre, and Na_2HPO_4 2 g/litre. The contents were *not* sterilized before introduction of the inoculum. The incubation was at 30°C and aeration rates were adjusted from 3.5 to 4.7 litre/min by the use of a mechanical pump. The pH of the medium was adjusted to 3.5. A 2% inoculum was used for each species. Fermentation was carried out for 14 days for all species of aspergilli in the first experiment. In the second experiment, the fermentation period was extended to 19 days. As further extension of the period of fermentation did not show any increase in protein content, in all subsequent experiments fermentation period was limited to 19 days.

Fermentation of hydrolysed corn cob waste. The corn cob waste was hydrolysed by the addition of 50 ml of 1N HCl to 450 ml of the fermentation medium containing 25 g of corn cob waste and autoclaving. The pH was adjusted with sterile NaOH after autoclaving to 3.5. This was used as the fermentation medium and fermentation carried out for 19 days at 30°C in the same way as described above by the inoculation of four species of aspergilli into separate flasks.

Analyses. After the fermentation period, the mycelia of each species was harvested and dried in an oven at 105°C and analysed for protein and moisture by the AOAC method (1970). Protein was calculated by determining the Kjeldahl nitrogen and multiplying by 6.25 as described in the AOAC method (1970). Total organic soluble carbon (TOC) was measured on the clear supernatant of a centrifuged sample by the method of Van Hall & Stenger (1967).

Results and discussion

The data from the various batch type fermentations are summarized in Table 1. It is seen that all the fungi gave moderately good conversion of carbohydrate into protein, the percentage of protein ranging from 9.28 to 14.63%. The concentration of the initial suspended solids was always less than that obtained

Table 1. Summary of data from batch type fermentations

	A. flavus			*A. niger*			*A. fumigatus*			*A. tamarri*		
	Unhyd.	Unhyd.	Hyd.	Unhyd.	Unhyd.	Hyd.	Unhyd.	Unhyd.	Hyd.	Unhyd.	Unhyd.	Hyd.
Fermentation data												
Initial TOC	14.5	13.6	12.8	13.5	14.3	11.5	13.4	14.5	12.5	14.6	13.8	12.3
Final TOC	5.8	6.5	4.3	4.9	5.3	3.9	4.7	5.6	3.2	5.1	4.9	3.0
Length of ferm. (day)	14	19	19	14	19	19	14	19	19	14	19	19
Initial suspended solids (g/litre)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Suspended solids recovered (g/litre)	38.4	35.0	28.2	42.6	41.2	32.4	41.2	40.0	24.0	43.6	42.8	33.6
Foaming problems	Mod.	Mod.	Mod.	Mod.	Mod./ heavy	Mod./ neavy	Mod.	Mod.	Mod.	Mod.	Mod.	Mod.
Initial pH	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Inoculum age (day)	2	2	2	2	2	2	2	2	2	2	2	2
Inoculum pH	7.0	7.2	7.1	7.3	7.1	7.15	7.2	7.1	7.2	7.1	7.3	7.2
Dried products data												
Kjeldahl nitrogen (%)	1.817	1.892	2.340	1.485	1.643	2.290	1.706	1.751	2.380	1.505	1.802	2.210
*Protein – Kjeldahl N_2 x 6.25 (%)	11.36	11.82	14.63	9.28	10.27	14.35	10.67	12.19	14.87	9.41	11.26	13.82
Moisture	5.58	5.61	6.27	5.16	5.72	5.86	5.83	5.93	5.35	4.94	6.24	6.33

* The protein content of the corn cob waste was found to be 2.25%.

Table 2. Overall yields of total solids recovered and percentage of protein yield

Name of organism	No. of days fermented	Type of substrate	Total solids recovered (%)	Total protein obtained (%)
A. flavus	14	Unhydrolysed	76.8	8.72
	19	Unhydrolysed	70.0	8.27
	19	Hydrolysed	56.4	8.25
A. niger	14	Unhydrolysed	85.2	7.90
	19	Unhydrolysed	82.5	8.47
	19	Hydrolysed	64.8	9.30
A. fumigatus	14	Unhydrolysed	82.4	8.79
	19	Unhydrolysed	80.0	9.75
	19	Hydrolysed	48.0	7.13
A. tamarri	14	Unhydrolysed	87.2	8.20
	19	Unhydrolysed	85.6	9.63
	19	Hydrolysed	67.2	9.28

after fermentation indicating that the moulds consumed the waste corn cob solids and converted part of them into mycelium solids and part into soluble metabolites. *A. flavus* gave the highest protein yield when unhydrolysed corn cob waste was used and *A. fumigatus* the highest yield when hydrolysed corn cob waste was used as the carbohydrate source.

In Table 2 are given the overall yields of the solids recovered in each of the batch fermentations. The percentage total protein yield as available from the total solids recovered is also given. It can be seen that while the protein in the recovered total solids is high when *A. fumigatus* is used, the overall protein yield in terms of the total solids recovered is low. With *A. niger*, on the contrary, hydrolysis of the corn cob waste has helped in improving the overall yield of protein even though the total solids recovered is only 64.8%. In *A. tamarri*, and in *A. flavus* the yield of protein using hydrolysed corn cob waste is about the same as using unhydrolysed corn cob waste. It seems from these experiments that the microorganism to be used for converting waste carbohydrate (such as cellulose) into protein would have to be examined in both hydrolysed and unhydrolysed substrates to find out which gives better yields. It would, however, be more advantageous to use an organism which does not need hydrolysed carbohydrate and at the same time gives good yields. The results obtained here indicate that *A. fumigatus* and *A. tamarri* show good promise. This is in conformity with Updegraff (1971) in his work on organisms capable of attacking cellulose, who found *A. fumigatus* to give good yields. Though Updegraff *et al.* (1973) have shown that in coffee wastes *A. oryzae* gave good yields of protein there seems to be no report on the use of *A. tamarri*.

The percentage of total organic soluble carbon has decreased in all the

fermentation indicating that all these organisms could be used for treating corn cob wastes. The results are, however, not conclusive enough to single out any organism.

It remains to be seen how far the protein recovered from these fungal fermentations are free from mycotoxins. Preliminary qualitative fluorometric determinations did not show any presence of aflatoxin. It is planned, however, to carry out feeding trials with animals to determine whether this could serve as a dietary source of protein and whether there is any indication of toxin formation.

Large scale trials on a pilot plant scale would have also to be carried out once a promising microorganism has been found to determine the problems that one would encounter if this process is to be industrially used.

Acknowledgment

The technical assistance of Mr F. E. Ofoegbu is gratefully acknowledged.

References

A.O.A.C. (1970) Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th edn. Washington, D.C.

Church, B.D., Nash, H.A. & Brosz, W. (1972) *Dev. Ind. Microbiol.* **13**, 30.

Updegraff, D.M. (1971) *Biotechnol. Bioeng.* **13**, 77–97.

Updegraff, D.M., Espinosa, R., Griffin, L. & King, J. (1973) *Dev. Ind. Microbiol.* **14**, 317.

Van Hall, C.E. & Stenger, V.A. (1967) *Anal. Chem.* **39**, 503.

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Technical note: Effect of storage on ascorbic acid content of oranges

SUMATI R. MUDAMBI

Introduction

The main source of ascorbic acid in the Nigerian diet is the orange. Next to banana, orange is the largest grown fruit crop in Nigeria. It is sold in markets, bus stops, near offices and schools. It is thus widely eaten in the country. Even though orange is a seasonal fruit, it is available in the markets throughout the year but at a slightly higher price during off-season. Oranges are stored in the markets without refrigeration for several hours before being sold. They are purchased weekly in most of the houses and stored at room temperature due to lack of refrigeration facilities. While a considerable amount of work has been done elsewhere on the seasonal variation of ascorbic acid in oranges (Merril, 1964; Sinclair, 1961), variation in the ascorbic acid content due to varietal difference (Maugalam & Mudambi, 1972), and the effect of various storage temperatures on the stability of ascorbic acid in oranges (Lopez, Krehl & Good, 1967; Eaks & Masias, 1965) there is no work in Nigeria reported on these aspects. Oyenuga (1968) and Woot Tsuen Wu Leung, Busson & Jardin (1968) have, however, given the ascorbic acid content of Nigerian oranges.

Another aspect peculiar to Nigeria is that peeled oranges are sold in motor parks all over the country. These peeled oranges which are consumed by customers by sucking the juice are exposed to the hot tropical sun for hours. There are no data available on the stability of ascorbic acid in these peeled oranges exposed to this harsh treatment.

This work was, therefore, undertaken to find out the variation of ascorbic acid with season, and on the change of ascorbic acid content of the orange when stored at room temperature as compared to storage at refrigeration temperatures and when peeled and exposed to the sun and stored in the shade.

Materials and methods

Fresh oranges were bought from the Nsukka local market in the months of December and February. In December, there is usually some rain and it is

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considered to be the season for oranges. February is a dry month and oranges are out of season.

Sampling

Lots of forty oranges each were used for studying changes in ascorbic acid content with temperature during December and February. In February, lots of forty peeled and whole oranges each were used for determining changes in ascorbic acid content when stored in the sun and in shade.

Storage

During December, the lots of forty oranges were stored at (a) room temperature – 27°C and (b) 17°C. During February lots of forty oranges each were stored at (a) room temperature – 27°C, (b) 5°C and (c) 0°C. Ascorbic acid from the juice of these oranges held at these various temperatures was determined at the end of 0, 1, 2, 3, 4, 5, 6 and 7 days.

Again during February, two lots of peeled and whole oranges were stored (a) in the sun and (b) in the shade and the juice from these analysed for their ascorbic acid content at the end of 0, 3, 6 and 9 hr.

To get a representative sample, juice of five oranges of each lot given the above treatments was mixed and an aliquot of 100 ml was taken for analysis. All juice was expressed from the oranges by means of a plastic hand juice extractor.

Estimation of ascorbic acid

After expressing the juice from five oranges, the volume was recorded. The juice was rendered acidic by the addition of equal volumes of 6% metaphosphoric acid. Ascorbic acid was estimated in the extracts in triplicate by titrating against a standardized solution of 2,6-dichlorophenol indophenol (A.V.C. 1966).

Results and discussion

Table 1 gives the volume of juice per 100 g of fruit and the ascorbic acid content per 100 ml as found in December and February at 27°C from 0 to 7 days of storage. It is observed that there is an increase of 5% in the ascorbic acid content after 7 days of storage in December and a decrease of 7.5% during February for the same period of storage. There was also an increase in the juice content for the oranges stored in December and a decrease in the juice

Table 1. Juice volume and ascorbic acid content of oranges stored at 27°C in December and February

Days	December oranges				February oranges			
	Sample 1		Sample 2		Sample 3		Sample 4	
	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml
0	46.0	54.0	35.6	54.0	43.8	53.2	43.8	53.2
1	46.2	54.0	36.3	54.6	42.7	53.2	44.1	53.2
2	46.5	54.6	46.5	54.8	43.3	53.2	44.1	53.2
3	46.9	55.0	39.6	56.6	42.5	53.2	37.9	53.2
4	47.0	55.6	42.5	56.6	42.2	50.6	41.7	50.6
5	48.9	56.0	45.6	56.6	42.0	50.6	40.5	50.6
6	49.0	56.0	47.0	56.6	38.3	49.2	38.8	49.2
7	49.4	56.0	47.9	56.6	32.3	48.1	32.9	48.2
Mean	–	55.2	–	55.9	–	51.1	–	51.1
Standard deviation		0.52		1.05		2.39		2.39

A.A., ascorbic acid.

Table 2. Juice volume and ascorbic acid content of oranges stored at 17°C in December and 0 and 5°C in February

Days	December oranges (stored at 17°C)				February oranges			
	Sample 1		Sample 2		Sample 3 (stored at 5°C)		Sample 4 (stored at 0°C)	
	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml	ml juice per 100 g	mg A.A. per 100 ml
0	46.0	54.0	35.6	54.0	43.8	53.2	43.8	53.2
1	46.8	54.0	46.9	54.6	44.0	53.2	44.1	53.2
2	47.4	55.0	51.5	55.4	44.4	53.2	44.5	53.2
3	47.0	55.6	49.7	56.8	42.7	53.2	41.9	53.2
4	48.0	56.0	44.4	57.4	44.3	53.2	43.3	53.2
5	48.1	56.6	46.2	57.4	42.2	53.2	42.5	53.2
6	48.9	57.0	47.2	57.4	40.9	53.2	44.4	53.2
7	49.0	57.0	50.0	57.4	44.2	53.2	43.7	53.2
Mean	–	55.7	–	56.3	–	53.2	–	–

A.A., ascorbic acid.

content for oranges stored in February. This could be because the oranges plucked in February were fully ripe. That oranges could ripen during storage with increase in juice and ascorbic acid content has been shown by Bartholomew & Sinclair (1951). Moreover, it was observed that most of the oranges bought in December were green in colour and slowly turned to yellow during the storage period. There was a highly significant difference between the values obtained for ascorbic acid during December and February when stored at 27°C from 1 to 7 days.

Ascorbic acid content of oranges stored at 17°C in December and at 5 and 0°C in February are shown in Table 2. It can be seen that even at 17°C there is an increase in juice content after storing for 7 days. (This shows that respiration continued in oranges even at 17°C.) But at 0 and 5°C there is no change in juice and in the ascorbic acid content.

Significant variations in values of ascorbic acid were obtained for samples stored at 27°C and those at 0 and 5°C. (Tables 1 and 2). The results show that the higher the temperature of storage the more the variations in ascorbic acid content during storage. At 0 and 5°C there is no change in ascorbic acid content during storage.

The change in the concentration of ascorbic acid when peeled and (a) stored in the sun and (b) stored in the shade, is shown in Table 3. In this table the ascorbic acid content of unpeeled oranges also exposed to similar treatment is given. It is seen that peeled oranges show a decrease of 40% of their ascorbic acid content when exposed to the sun for 9 hr. In the shade there is only a loss of 21% after 9 hr exposure. The reduction in ascorbic acid is proportional to the time of storage. On the contrary unpeeled oranges stored in the sun for 9 hr showed marked stability in their ascorbic acid content. This could be due to their thick waxy skin. There was no drying and shrinkage in size after 9 hr of storage. The volume of juice in the unpeeled oranges was found to increase with the time of exposure to the sun. Eaks & Masias (1965) have also reported

Table 3. Juice volume and ascorbic acid content of peeled and whole orange in February

	In sun		In shade		
Type of sample	Juice of storage	ml juice per 100 g	mg A.A. per 100 ml	mg A.A. per 100 ml	Decrease
Peeled oranges	0	49.5	53.0	53.0	
	3	40.9	46.0	50.0	6
	6	39.8	40.0	48.0	9
	9	38.6	32.0	42.0	21
Whole oranges	0	29.2	50.0	50.0	
	3	32.0	50.0	50.0	
	6	32.6	50.0	50.0	
	9	35.0	50.0	50.0	

A.A., ascorbic acid.

Table 4. Juice volume and ascorbic acid content per peeled and whole orange in February

Type of sample	Time of storage (hr)	In sun			In shade		
		ml juice per orange	mg A.A. per orange	Decrease (%)	ml juice per orange	mg A.A. per orange	Decrease (%)
Peeled oranges	0	99.0	52.5		99.0	52.5	
	3	72.0	33.1	37.9	80.0	40.0	24
	6	69.0	27.6	47.0	72.0	34.6	34
	9	60.0	19.2	63	64.0	24.8	56
Whole oranges	0	70	35.0	0	70	35.0	–
	3	77	38.5	–	73	36.5	–
	6	80	40.0	–	78	39.0	–
	9	70	35.0	–	75	37.0	–

A.A., ascorbic acid.

an increase in the juice after exposure of lines to a higher temperature for a short period of time.

As oranges are normally purchased and consumed in units, it is important to know how much ascorbic acid one gets if one orange is eaten. The mean range of ascorbic acid per orange was 43.0 to 57.2 mg in December while in the dry season (February) it was 36.3 to 37.0 mg at 27°C (Table 4). Even in the dry season one orange will supply the daily requirement of an adult man or woman and two oranges will meet the need of pregnant and lactating mothers. Again when the loss of ascorbic acid of peeled oranges when exposed to the sun is considered per orange, it is more pronounced. When exposed to the sun, the loss per orange of the peeled orange is 61% whereas the loss per orange in the shade is 56% both after 9 hr of storage. These are factors which should be taken into consideration when oranges are used to meet the nutritional needs of individuals for ascorbic acid.

Acknowledgment

The technical assistance of Miss E. I. Etteh is gratefully acknowledged.

References

Association of Vitamin Chemists (1966) *Method of Vitamin Assay* 3rd edn. Interscience Publishers, New York.

Bartholomew, W.T. & Sinclair, W. (1951) *The Lemon.* University of California Press, Los Angeles.

Eaks, I. & Masias, E. (1965) *J. Fd Sci.* **30**, 509.

Lopez, A., Krehl, W.A. & Good, E. (1967) *J. Am. Diet. Assoc.* **50**, 308.

Maugalam, M. & Mudambi, S.R. (1972) *Ind. Fd Packer,* **26**, 20.

Merrill, A. (1964) *J. Diet. Assoc.* **44**, 264.

Oyenuga, V.A. (1968) *Nigerian Food and Feeding Stuff.* Ibadan University Press, Ibadan, Nigeria.

Woot Tsuen Wu Leung, Busson, T. & Jardin, C. (1968) *Food Composition Table for Use in Africa.* FAO, Rome.

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Book reviews

Wastewater Renovation and Re-Use. Ed. by Frank M. D'Itri.
New York: Marcel Dekker Inc., 1977. Pp. xxxi + 705. SFr 130.

This book contains the proceedings of the international conference on renovation and re-use of wastewater through aquatic and terrestrial systems, which was organized by the Institute of Water Research, Michigan State University, and was held in 1975. The conference presented views from Canada, Denmark, Hungary, Israel, Mexico, the Netherlands, the United Kingdom, the United States and West Germany.

The subjects covered under the seven main headings were: Water re-use mainly for agricultural purposes, Re-use for forestry, Use of treated and untreated sewage on sewage farms, Aquaculture-growing marine or freshwater plants or fish, Toxic elements and pathogens, Public health aspects, and Research needs.

Throughout the proceedings reports on established systems of water re-use as well as on experimental work are given, and the factors limiting re-use, such as accumulation of potentially hazardous and pathogenic factors, receive detailed attention.

Public health problems were clearly highlighted as needing further research urgently. Other areas requiring study are identified in engineering, management, hydrology, as well as problems in the political, economic and social spheres. Questions concerning the energy relationships between wastewater nutrients and chemical fertilizers were considered also to have high priority.

This book is a fascinating and very readable collection of papers 'bringing together the many talents required to solve this multifaceted problem', to quote the preface. In it will be found historical accounts, current results of research and proposed programmes for future research and further study.

A. H. Potten

Freeze Drying Processes for the Food Industry: Food Technology Review No. 30. By M. H. Gutcho, The Campden Food Preservation Research Association, Chipping Campden, Glos.
New Jersey: Noyes Data Corporation, 1977. Pp. xii + 401. US$39.00.

This review covers the U.S. Patent literature from the early 1960s to October 1976. The number of patents covered is 192 of which seventy-six have previously been discussed in *Food Technology Review* No. 1, 1968. The book is divided into three parts: the first deals with freezing operations, i.e. heat

transfer mechanisms, maintaining optimum conditions for sublimation by using inert gases or modifying the pressure regime, de-icing, and monitoring the freeze drying cycle; the second deals with equipment for freeze drying, i.e. containers, modification to apparatus and continuous freeze drying equipment; the third and final part includes the freeze drying of specific foodstuffs, e.g. coffee, meats, milk products, potatoes, vegetables, fruit, tea and a number of miscellaneous products.

Those who are interested in freeze drying will find a vast amount of detailed technical information given in this book. It will serve as a monument to the amount of research and development work which has been put into this process. The book is attractively produced, but is relatively expensive.

S. D. Holdsworth

The Science & Technology of Gelatin. Ed. by A. G. Ward and A. Courts. London, New York and San Francisco: Academic Press, 1977. Pp. xv + 564. £18.00.

This book offers a most comprehensive and up-to-date coverage of gelatin and its raw material collagen. It will undoubtedly serve as a standard text and reference manual for all concerned with the topic. Indeed, it could well become the definitive work in this field.

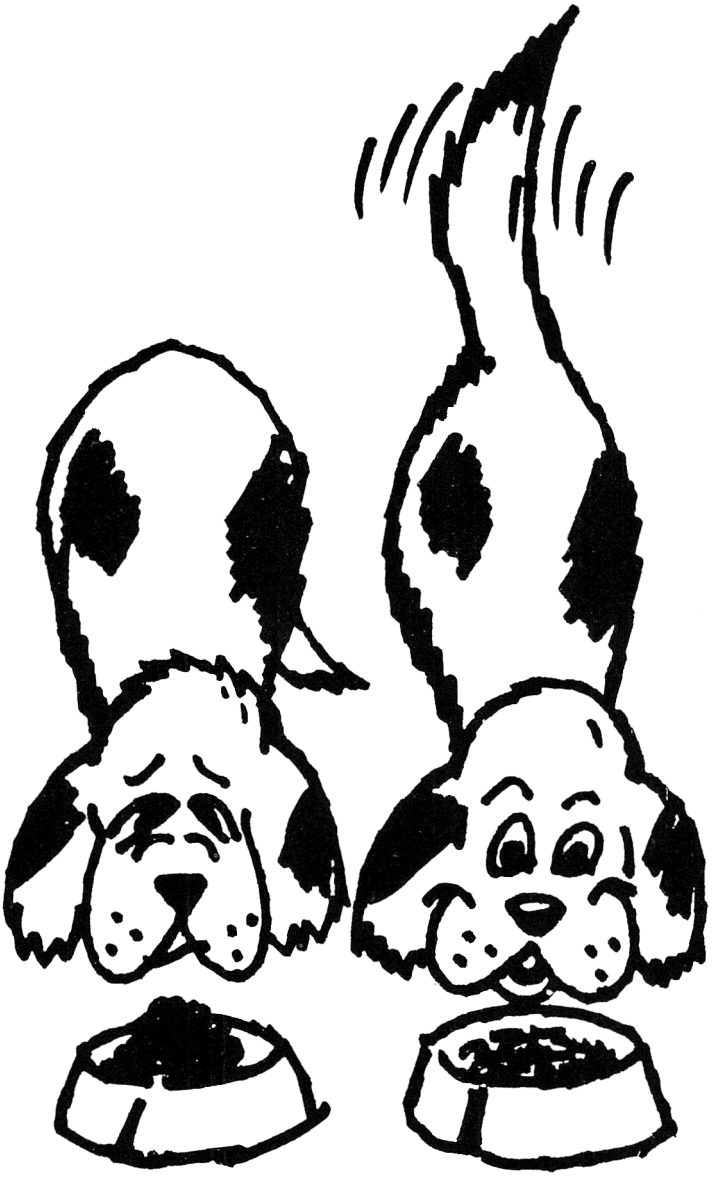
The scope covered is perhaps wider than would at first be expected. It will find application in academic, food, photographic, pharmaceutical and leather fields. The text is pitched at a level which will be useful to the specialist scientist yet not incomprehensible to the undergraduate. There are relatively few comprehensive books on gelatin and this one fills a very undesirable gap in our literature of this highly distinctive field. It will prove invaluable to researchers and users of gelatin who previously had to scour the journals for much of the data. General workers in the field of colloids will also find the book of interest because gelatin serves as an admirable model for so much of the technology.

Coverage comprises the structure and properties of gelatin, collagen and collagen-containing tissues; the technology of gelatin manufacture and the uses of gelatin in all its varied applications are included and also the examination and testing of gelatin and gelatin products. The book is a very good review but, because of the extremely wide fields which are covered, the detail is sometimes limited. Also, of course, the book freely admits that a proportion of the science and technology of gelatin lies in private companies' confidential files. I was somewhat surprised to find an appreciable portion of the book devoted to the science and technology of collagen but, of course, this is all entirely relevant to the subject. For me it is a pity that the book cannot also cover the commercial and legal aspects of gelatin, but these are clearly beyond the range of the title and indeed the stated aims of the authors.

Fifteen authors have between them composed the sixteen large chapters in this book and their differing styles may be considered an advantage or a disadvantage depending upon one's view-point. Certainly I found the book rather heavy to read but this is primarily because its wealth of facts requires time to assimilate. Fortunately, great effort has been made to standardize the units employed. Diagrams, charts and graphs have been used freely and to good effect, although the book contains very few photographs. I was particularly pleased with the extremely large number of recent references which in themselves will be invaluable. The contents of papers when quoted are usually accompanied with sound comment and reappraisal.

Expectations were fully met with this volume. The two editors, being former research directors of the Gelatin and Glue Research Association, were admirably fitted to the task of preparing this book for publication. The authors must be congratulated on the text which is thoroughly worthwhile and certain to be widely read throughout the world.

Eric T. Best



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References. Only papers closely related to the authors' work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

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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'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2.** Each table must have a caption in small letters. Vertical lines should not be used.

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