EDITORIAL

The publication of a new journal has been considered by some people to be of doubtful merit in these days of rising costs and consequent economies in many directions. However, a few minutes' consideration and a run through the scope of *Food Chemistry*, to be found on the inside front cover, will prove that it will provide an outlet for papers which will not easily find a place in other journals.

The whole emphasis is to be on the *chemistry* of food. The Concise Oxford Dictionary defines 'Chemistry' as the 'Science of the elements and their laws of combination and behaviour under various conditions'. When carbon, hydrogen, oxygen, nitrogen and perhaps sulphur, combine in special ways, proteins are obtained; when nitrogen and sulphur are absent, the first three elements may form carbohydrates or fats; and foods are, after all, either conglomerates of protein, carbohydrate and fat, or just one of these complexes alone. However, that is not the whole story, for water and trace elements play their part, probably in ways which are not fully understood by the chemist; and other elements or complexes, alien to food, may play a rôle in destroying the good which, in their absence, the food might do.

Food, of course, is ingested not only by humans, but by plants, animals or bacteria, to name but a few, and these, themselves, may become the food of humans. Indeed, the survival of men often depends on their ability to convert otherwise inedible material into a form acceptable to the human digestive system. There are, too, untold cycles of anabolic and catabolic processes interwoven with each other contributing to the sustenance of man.

It is becoming increasingly difficult to conduct useful scientific investigations without interdisciplinary involvement, and many scientists believe that attempts to confine work within one discipline must result only in poor results. It is, for instance, scarcely possible today to be a competent analyst without either a knowledge of electronics or the assistance of an expert in that science. Thus, it is to be hoped that *Food Chemistry* will be a means of drawing the inter-related

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disciplines together in work of a complementary character. It is not adequate, for instance, to know the percentage of carbohydrate in a food, without also acquiring some idea of the character of the carbohydrate, how and to what it is metabolised, its source and the effect of the soil on which it has been grown, or the trace elements which accompany it. One of the great needs is to draw such data together, and to see them as a picture which, even then, may require a few more brush strokes to complete. It is, indeed, disturbing that some canvases seem prepared with only a few preliminary daubs.

With the concern there is today for the feeding of the world population, the chemistry of food is of vital consideration. Whereas, for instance, until relatively recently it was considered adequate to use chemical fertilisers, today the pendulum has swung back, and natural fertilisers are being advocated. The chemistry involved may be very similar in both instances, but the means whereby the chemical processes are implemented are quite different and it may be that the end-results are not the same. The chemical repercussions of such action are tremendous, involving, as they do, the possible erosion of mineral resources, the cycling of natural products, and the quality of the food so produced.

The utilisation of waste from numerous industries is now being directed towards food cultivation and manufacture. This is but harnessing processes which have always been functioning. Who has not walked through a wood and seen an old tree sprouting fungi? The ramifications of waste utilisation are vast and every step of the way involves chemistry.

It has been noted earlier how the elements are brought together to form proteins, carbohydrates and fats. Natural syntheses basically use the sun as the source of energy. It was not until 1932 that vitamin C was isolated as the factor responsible for preventing scurvy, and shortly afterwards its chemical structure was elucidated. Today this vitamin is readily available in synthetic form at any pharmacy. This is but one example of the organic chemist's ability to build up complexes as food for man, but ascorbic acid is a relatively simple molecule compared with, say, a protein.

Chemists have been searching for years for a sweetener to replace sucrose; their labours were intensified when the latter became scarce and expensive. Here we see an interplay of research endeavour and economic desirability. On the one hand the financial support for such research may come from industrial concerns hoping to cash in on the 'sweetness world'; on the other there is the not unsurprising reaction of those already in that environment to a fellow bed-mate. Chemists used to tend to look at themselves as people apart from such mundane considerations, but sophistication and instrumentation in methods has led to more and more expensive equipment, and the chemist must cease to be an ostrich. Unfortunately, this results in a tendency to explain away failures by blaming a faulty switch or some such unchemical contrivance. One of the large London hospitals possesses a 24-channel autoanalyser which is employed to monitor regularly the biochemical character-

istics of the patients. On being asked what happened to the system if the machine were to break down, the reply made it clear that none of the technicians could undertake the manual determinations!

Besides taking a fairly liberal view of what papers may be acceptable under the title *Food Chemistry*, the Journal will include reviews of suitable books and correspondence, not of the acrimonious kind, but constructive and critical in character. It may be possible, also, to prepare a special issue dealing with some particular topic.

We launch *Food Chemistry* in the confidence that it will fill a gap and will offer a suitable receptor for those who are seeking to extend our knowledge of the chemistry involved in daily sustenance.

STUDIES ON PARBOILED RICE: PART 1—COMPARISON OF THE CHARACTERISTICS OF RAW AND PARBOILED RICE

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ABSTRACT

Various properties of raw and parboiled rice were compared in an effort to elucidate the factors responsible for the changes induced by parboiling.

The parboiled rice was less prone to disintegration on cooking, the kernels remaining well separated and less sticky than the non-parboiled sample. The solids leached into the cooking water and the extent of solubilisation of the kernels on cooking were both considerably lowered by parboiling. Amylograms of flour prepared from the rice revealed that this was due to the resistance of the starch in the parboiled rice to swelling and solubilisation in hot water.

From the results of X-ray diffraction spectra it was concluded that the behaviour of parboiled rice is influenced by the presence of an insoluble helical amylose complex and not, as is generally assumed, by retrogradation.

INTRODUCTION

Parboiling usually results in a product which, when cooked, is firmer and less cohesive than untreated rice. Most consumers regard these properties as desirable.

The most obvious physico-chemical change occurring during parboiling is gelatinisation of the starch. The present study was undertaken in order to investigate the influence of starch on the behaviour of parboiled rice.

Several investigations have been carried out in the past but have not produced conclusive results. De *et al.* (1966) reported that parboiling caused a decrease in the intensity and peak wavelength of the starch–iodine complex of some rice varieties and an increase in others. They attributed this to enzymic interconversion of the starch fractions, which is a rather unlikely explanation.

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Varietal differences in the colour change with iodine from blue to pink in sections of parboiled rice have been reported (Quadrat-i-Khuda *et al.*, 1962). This reduced staining was also observed by Rao & Juliano (1970), who also reported that, after parboiling, the temperature of initial viscosity increase of rice flour in the amylograph was generally higher, particularly with high amylose varieties.

The most popular view is that the properties of parboiled rice are influenced mainly by retrogradation. In the opinion of Rao & Juliano (1970) retrogradation can be used to explain the effect of parboiling on iodine staining, amylograph characteristics, resistance to breakdown during cooking and reduced solubility of the starch. Studies of amylose solubility and hydration characteristics of parboiled rice led Ali & Bhattacharya (1972) to a similar conclusion.

In view of the absence of conclusive data confirming the role of retrogradation in parboiled rice it was considered desirable to investigate the validity of this claim.

MATERIALS AND METHODS

Materials

The rice used was Ribe, a medium grain Italian variety, obtained from Brooke Bond Liebig Ltd who also supplied a sample of commercial parboiled rice, Riso Flora, produced from the same variety.

Methods

Apparent solubility and cookwater loss: This method allows the solubility properties of rice to be studied while maintaining the kernel integrity during the cooking process (Priestley, 1974).

One gramme of rice is added to 20 ml boiling distilled water in a boiling tube fitted with a condenser. After the desired heating period the cooking water is drained, its volume measured and the grains rinsed with 20 ml cold distilled water. This is also drained and added to the cooking water.

The volume of drained cooking water is replaced by an equal volume of cold distilled water. The grains are then macerated for 20 sec using a tissue homogeniser (Ultra-Turrax, Janke and Kunkel) operating at 20,000 rpm and the dispersion transferred to a 50 ml centrifuge tube with 15 ml water used for rinsing.

The dispersion is then shaken and centrifuged at 3000 rpm for 15 min (Minor, MSE). A 20 ml aliquot of the supernatant is evaporated on a steam bath and dried for 4 h at 105°C. The cooking water and rinsings are dried in a similar manner.

The weight of soluble material released by macerating the kernels and the cookwater loss are calculated as a percentage of the dry weight of the sample. The term 'apparant solubility' is used to designate the solubilised material in the kernels since, under these conditions, starch does not form a true solution.

Amylograms: A Brabender amylograph was used with flour suspensions containing 13% dry solids.

X-ray diffraction spectra: These were obtained using a Philips generator type PW 1010 with nickel-filtered Cu Kα radiation operating at 40 KV 20 mA. An internal standard of 10% calcium carbonate was added.

RESULTS AND DISCUSSION

The response of the two samples to cooking for 40 min in boiling water is shown in Fig. 1. Whereas the parboiled rice maintained its kernel integrity the non-parboiled sample was extensively disintegrated and much stickier.

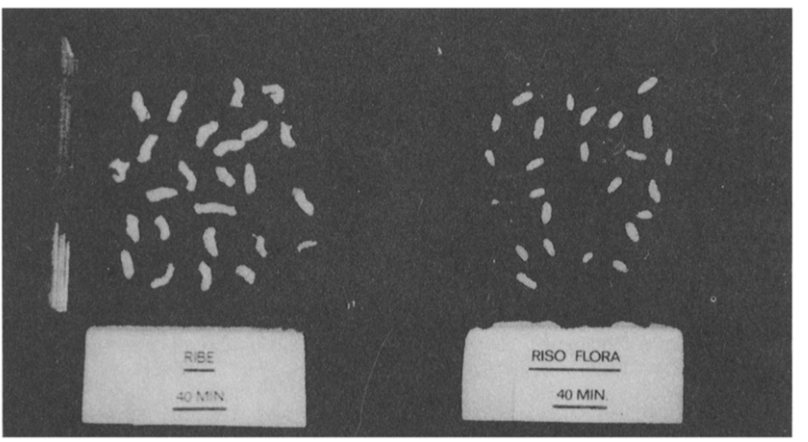

Fig. 1. Raw rice (Ribe) and parboiled rice (Riso Flora) after cooking for 40 min.

The loss of solids into the cooking water is compared in Fig. 2. During the initial stages of cooking more solids were leached from the parboiled rice, presumably due to its gelatinised nature. Although cookwater losses have been used as an index of cooking quality on many occasions, precision is very poor (Bhattacharya & Subba Rao, 1966). Considering the length of the diffusion path necessary for starch to leach into the cooking water there is unlikely to be an equilibrium set up between the soluble starch in the kernel and the released solids. More useful information can be gained by measurement of the apparent solubility of the kernels which reflects changes in solubility of the major component, starch, in the cooked kernel itself (Priestley, 1974).

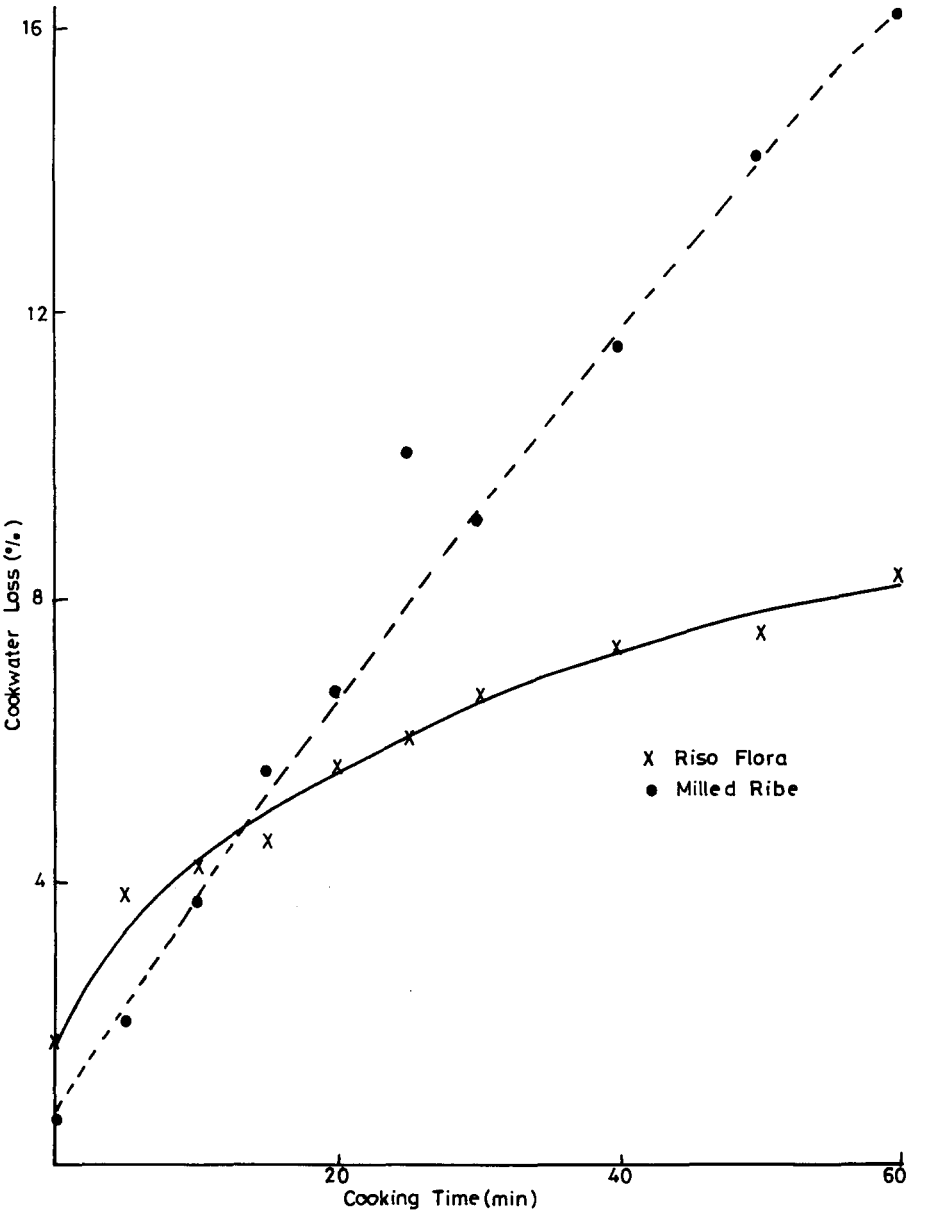

Fig. 2. Cookwater losses of raw rice (Ribe) and parboiled rice (Riso Flora) during cooking.

The changes in apparent solubility of the two samples are compared in Fig. 3. The raw rice was rapidly solubilised during the early stages of cooking, reaching a maximum apparent solubility around 50% after 30 min. The parboiled rice, however, was only slowly solubilised, no maximum being reached even after cooking for 1 h. This indicated that the starch in parboiled rice had been stabilised against solubilisation by hot water. However, since parboiling is known to produce a more compact arrangement of starch in the kernel (Rao & Juliano, 1970) this could affect solubilisation by restricting the rate of water penetration. In order to examine the response of the starch to hot water without the influence of the physical structure of the kernel, flours were prepared and pasted in the amylograph.

The amylograms are shown in Fig. 4. The raw rice flour exhibited a curve fairly typical of native cereal starch, its viscosity increasing rapidly as heating was continued beyond its gelatinisation temperature of 60°C up to a maximum hot-paste viscosity of 2900 BU at 74°C. Heating beyond this point caused a rapid reduction in viscosity as the starch granules ruptured and collapsed due to heating and the mechanical action of the stirrer, this reduction continuing as the temperature was held at 95°C. The paste from the parboiled rice, however, did not show a significant increase in viscosity until 80 to 85°C when a gradual rise occurred and continued as the temperature was raised to, and held at, 95°C. An ultimate peak viscosity was not reached even after constant heating at 95°C for 30 min.

The resistance of the parboiled rice paste to swelling and solubilisation provided strong evidence of the presence of associative bonding in the starch. The most likely nature of this bonding was either retrogradation or the formation of an insoluble amylose complex. The most useful means of distinguishing between these crystallisation phenomena is by X-ray diffraction.

The X-ray diffraction spectrum of raw rice (Fig. 5) shows the typical A-type pattern of cereal starch with peaks at 3·84 Å and 5·85 Å and an unresolved doublet at 4·85 Å (Zobel, 1964). After parboiling, the original A-type pattern disappeared and was replaced by a V-type pattern (Fig. 6) with strong diffraction lines at 6·80 Å and 4·42 Å, confirming the presence of a helical amylose complex (Rundle & Edwards, 1943). These peaks were designated V1 and V2, respectively as an aid to identification in later quantitative studies. There was no evidence of a B-type pattern, characteristic of retrograded starch, which would be expected to give diffraction peaks around 15·8 Å and 5·2 Å (Zobel, 1964).

X-ray diffraction patterns of stale bread give a strong B-type pattern (Wright, 1971). Considering the strength of the association indicated in this study, and that staling can be reversed by heating at 50°C (Schoch & French, 1947), the absence of a B-type pattern indicates that retrogradation has no significant influence on the behaviour of parboiled rice.

It is well known that amylose can form complexes with molecules capable of entering and stabilising the helix, such as fatty acids (Mikus *et al.*, 1946) and lysolecithin (Nakamura *et al.*, 1958), which are both present in the rice grain. In

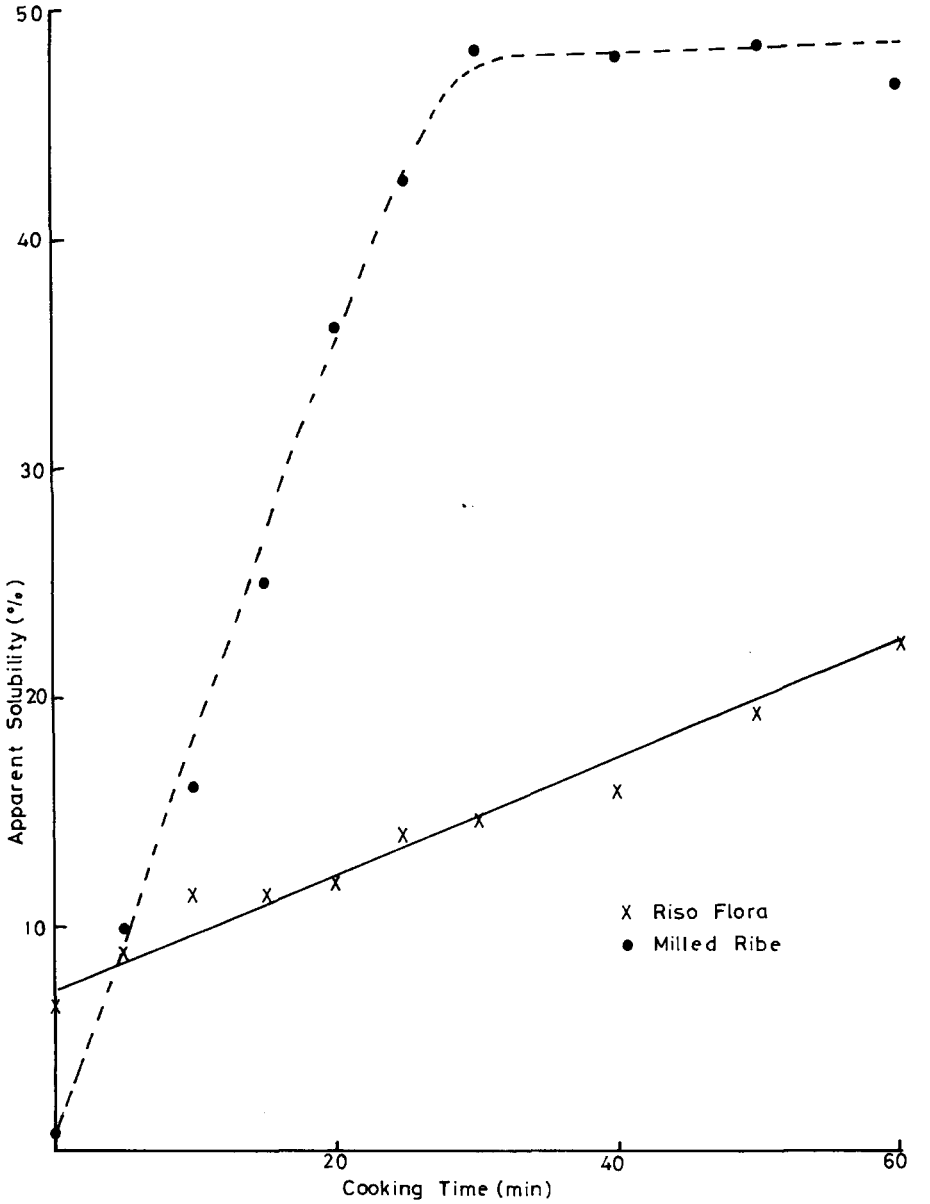

Fig. 3. Apparent solubility of raw and parboiled rice during cooking.

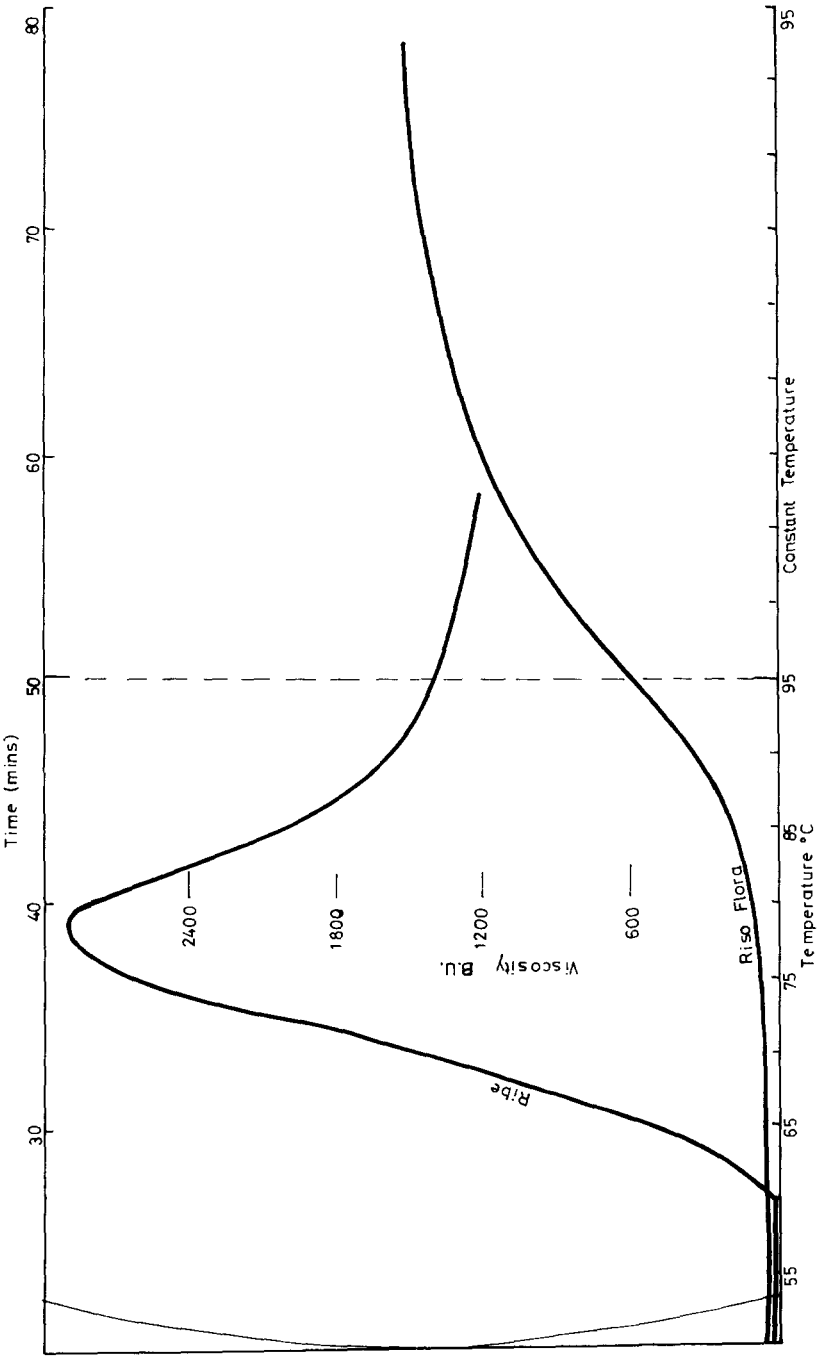

Fig. 4. Amylograms of raw and parboiled rice flour.

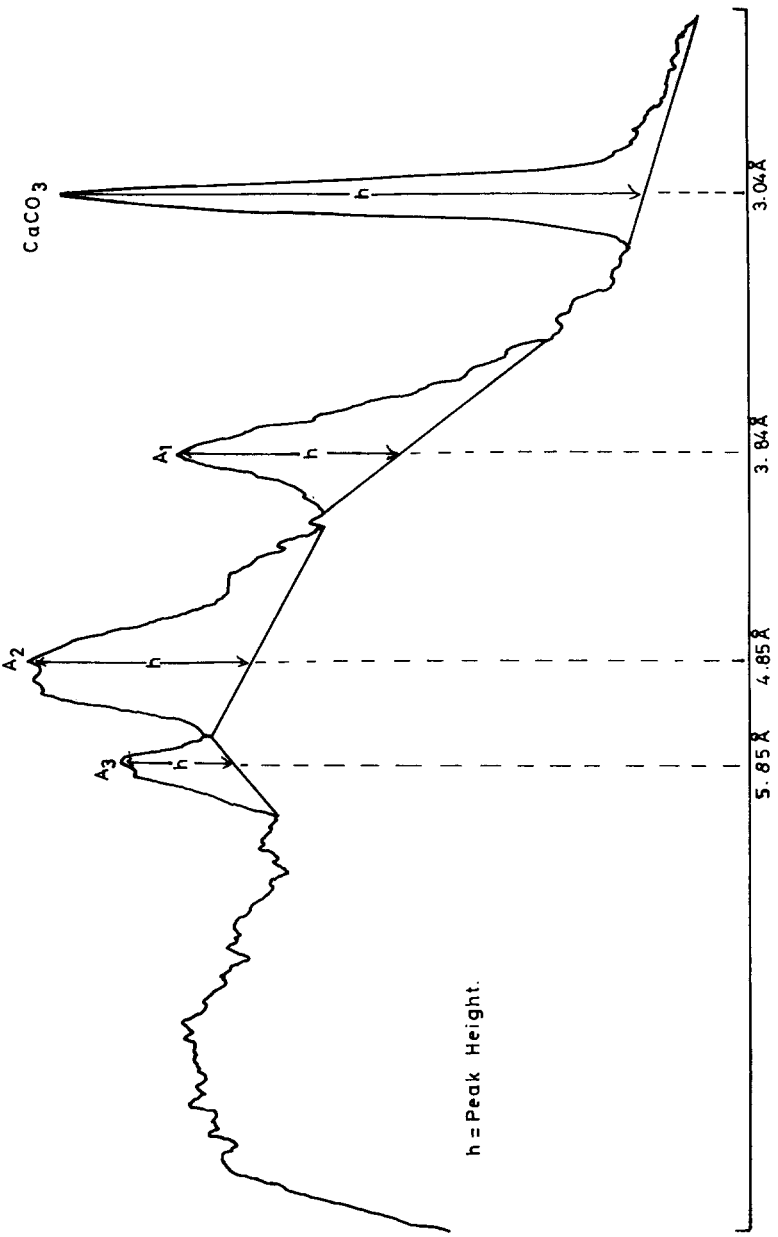

Fig. 5. X-ray diffraction spectrum of raw rice flour.

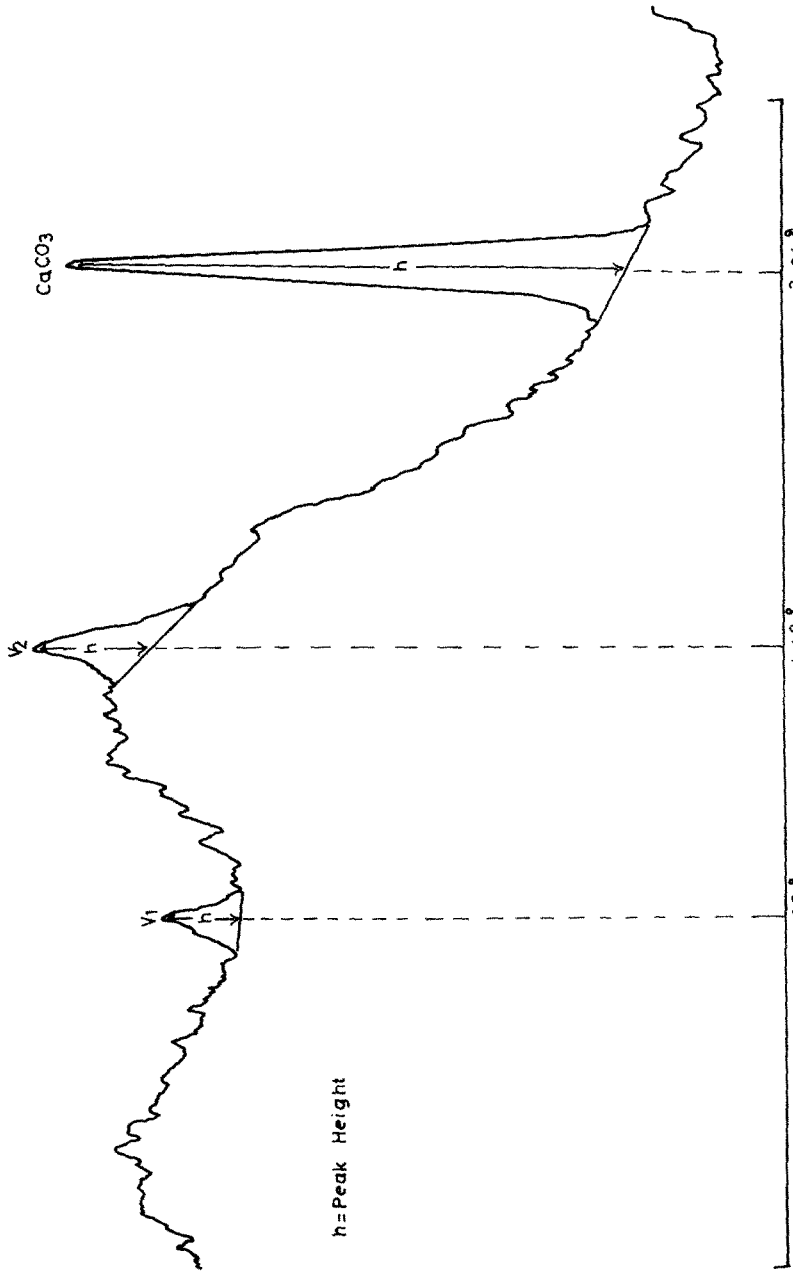

Fig. 6. X-ray diffraction spectrum of parboiled rice flour.

such cases the complex would be insoluble and stable at relatively high temperatures. The formation of such complexes is reported to restrict swelling and solubilisation of starch by stabilising the granule structure (Gray & Schoch, 1962).

This stabilising effect readily accounts for the characteristics of parboiled rice such as lowered cookwater loss and apparent solubility, maintenance of kernel integrity during cooking and the response of its flour to heating in the amylograph. The reduced solubility of the starch is likely to be a significant factor in determining the texture of parboiled rice. Since iodine is not capable of displacing fatty acid from the amylose helix (Osman *et al.*, 1961) this would explain the reduced staining of rice starch after parboiling.

CONCLUSIONS

The presence of an insoluble amylose complex appears to be responsible for the reported characteristics of parboiled rice. Since retrogradation and complex formation may both result in decreased swelling, solubility and iodine-staining properties of starch, previous investigators relying on these criteria erroneously concluded that retrogradation was more important.

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THE VARIATIONS OF ASCORBIC ACID CONTENT IN VEGETABLE PROCESSING

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ABSTRACT

The variations of ascorbic acid in fresh and canned vegetables were studied in an attempt to correlate variability due to the variety, grower, size of the product and the effect of each processing operation in a commercial operation, which would result in a guide for better control of nutrients and nutritional labelling. It was found that variations in ascorbic acid content due to the variety, grower and size of product were dependent upon individual crop. However, in most cases, the heating processes such as blanching and retorting had a significant detrimental effect on the ascorbic acid content in all crops studied.

INTRODUCTION

The nutrient composition of processed food has been a matter of public interest for many years. More recently, this concern over nutrition and nutrients in our food has given impetus to requirements for nutritional labelling of food products in the United States. Nutritional labelling poses a great difficulty to the processors of natural products due to the variability of nutrient in raw material, effects of processing and instability of nutrients in complex biological systems. The results of many earlier investigations (Clifcorn, 1944; Cameron, 1955; Cain, 1967; Elkins *et al.*, 1972 and 1973) on the content of vitamins in processed vegetables show a rather wide variation in the amounts found in different samplings of the same product. Whether such variations were due to differences in variety of raw materials used, to differences in cultural practices under which the products were grown, to differences in analytical methods, to differences in processing techniques employed in canning, or to a combination of all of these factors could not be ascertained from the data published previously.

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The fact that the vitamin content of canned food is adversely affected by various conditions encountered previous to and during processing is known to a certain extent. There have been many reports on the effects of certain cultural practices on ascorbic acid content in vegetables (Harmer & Sherman, 1944; Gunn *et al.*, 1945; Lyon & Besson, 1943; Eheart *et al.*, 1955). An extensive review of the influence of climate and fertiliser practices upon the ascorbic acid content of vegetables was made by Sommers & Besson (1948). Recently, Lee (1974) reviewed variations in some nutrients of processing vegetables due to the cultural practices. However, most reports are inconsistent and inconclusive, because many agricultural variables such as variety, location, soil, climate, maturity at harvest, and the type and amount of fertiliser, pesticide, herbicide, etc., are interrelated and difficult to control. Therefore, because of this variable complexity, it is difficult to draw any general conclusions.

The influence of processing on the nutritional content of vegetables has been reviewed by Feaster (1960), Bender (1966) and Lee (1958). Some changes in the vitamin content of vegetables resulting from major operations of the canning process and some variations due to the equipment were studied by Guerrant *et al.* (1946), Lamb *et al.* (1947) and Wagner *et al.* (1947). The retention of ascorbic acid has been taken as an index to the retention of the original nutritive and quality values of processed foods. It seems reasonable that, if important variables could be ascertained, it would be conducive to the processors to control raw materials and to modify some of the processing techniques, which would result in a guide for better control of nutrients and nutritional labelling. This research was designed to study the various effects encountered routinely in commercial canning plants on the ascorbic acid content of processed vegetables.

MATERIALS AND METHODS

Sampling

Vegetables selected for this study were peas, corn, table beets, wax beans and green beans. Four canning plants located in Western New York State were chosen for sampling. Sampling was carried out at each major operation from the receiving dock to the warehouse during the regular processing season of 1974. Canning operations were examined with respect to each operation with each product. In each case an effort was made to secure data concerning the previous history of the raw material with special reference to variety, location, grower, date of planting and harvesting, and growing conditions. Product flow through the plant was followed where duplicate 1 kg samples were taken in sequence. Samples were placed immediately in an ice box and transferred to the laboratory for analysis. Cans of the finished product were collected from the same lot on which the raw product sampling was carried out. Analysis was done within 24 h.

Analysis

All analysis was carried out by duplicate determination on each duplicate sample. Representative samples for moisture determination were ground in a mortar and dried in an oven at 60°C under vacuum for 48 h. The photometric method of indophenol–xylene extraction technique using formaldehyde modification procedure to correct for the action of reductones by Robinson & Stotz (1945) was used for the ascorbic acid analysis. Samples which were kept overnight were stored in metaphosphate buffer solution for the analysis of ascorbic acid. Since appreciable amounts of water-soluble substances are frequently lost during canning, ascorbic acid value expressed on dry weight may not be meaningful. Therefore, it is expressed 'as assayed' in this paper. However, since there also were moisture additions or losses during the various stages of canning, ascorbic acid calculated on the 'as assayed' basis is not necessarily a true measure of retention. Statistical analyses on ascorbic acid contents among varieties, among growers, sizes and sampling stations within a variety, and on final products among varieties were conducted.

RESULTS AND DISCUSSION

All of the detailed data could not be included here, therefore, condensation and deletions were essential. The most important data, such as those on variety, grower, size of product and sampling stations, are included.

Peas

Three varieties from four growers were investigated. Average ascorbic acid content in fresh peas (mixed size) of Early Sweet, Nugget and Medalist varied but not significantly. However, the Nugget variety grown by growers C and D did show significant difference in ascorbic acid content (Table 1). Nugget variety grown by grower C contained 30% more ascorbic acid than that of grower D.

Table 2 shows the ascorbic acid content of pea samples collected during processing according to their sieve sizes. Blanching time and temperature was based on grade size. Size 2 peas were blanched for 3 min at 85°C in a pipe blancher while size 5 peas were blanched for 3 min at 90°C in a drum blancher. The peas were then

TABLE 1
ASCORBIC ACID CONTENT IN PEAS EXPRESSED BY VARIETY AND GROWER

Variety	*Grower*	*Ascorbic acid (mg/100 g)*	*Significance of F-value*
Early Sweet	Grower A	21·80	ns
	Grower B	23·86	ns
Nugget	Grower C	27·40	*
	Grower D	20·55	*

* Statistically significant at 5% level.

packed in No. 303 cans, filled with brine and sealed. Heat processing was done in a FMC automatic cooker at 115°C for 35–40 min and cooled. After blanching, the ascorbic acid retention ranged from 83–96%. When peas were blanched in distilled water at 97°C for 1 min, Birch *et al.* (1974) observed 28% loss of the initial ascorbic acid in the pea. Ascorbic acid retention in the final product ranged from 38% to 60%. Statistical analysis showed that size within and between varieties, and sampling station all significantly affected ascorbic acid content. It might be expected that more ascorbic acid content would be measured in small peas (size 2) than in large peas (size 5), since it has been reported that ascorbic acid content in peas decreases

TABLE 2
CHANGES OF ASCORBIC ACID CONTENT IN PEAS DURING PROCESSING

Variety	*Size*	*Station of sampling*	*Ascorbic acid (mg/100 g)*	*Original ascorbic acid retention (%)*
Early Sweet	2	After grading	18·87	100
		After blanching	16·33	86·5
		After retorting	8·65	45·8
	5	After grading	20·56	100
		After blanching	19·78	96·2
		After retorting	10·51	51·1
Medalist	2	After grading	19·19	100
		After blanching	17·18	89·5
		After retorting	7·36	38·3
	5	After grading	17·49	100
		After blanching	14·64	83·7
		After retorting	5·59	31·9
Nugget	2	After grading	21·78	100
		After blanching	18·98	87·1
		After retorting	13·06	59·9
	5	After grading	24·60	100
		After blanching	21·94	89·1
		After retorting	14·03	57·0

with the increase in maturity (McKee *et al.*, 1955; Morrison, 1974). However, it was found that there was no direct relationship between the ascorbic acid content and the size of peas in this study. In general, the retorting process appeared to be the major cause of ascorbic acid losses. Canned peas of the Nugget variety contained almost twice the amount of ascorbic acid as the Medalist variety in both sizes. An important fact to point out is that the more ascorbic acid that there is in fresh peas, the more ascorbic acid there is in the final canned product. Among the three varieties, the Medalist variety retained the least amount of ascorbic acid.

Corn

Two varieties of corn from three growers were investigated. Fresh corn of the Commandeer variety contained a slightly higher amount of ascorbic acid than the Style-Pack variety but not significantly higher. Unlike peas, there were no significant differences in ascorbic acid content of corn grown by the different growers.

Processing significantly affected ascorbic acid contents. Fresh corn was passed through a vibrating desilker and a steam tunnel to wilt leaves, transferred to a husker, cutter, flotation washer and then filled into No. 303 cans with brine, sealed and heat processed in a FMC continuous cooker at 126°C for 10 min. As shown in Table 3, approximately 40% of the ascorbic acid was retained in the final product and the heating process appeared to be the major influence on ascorbic acid losses.

TABLE 3
CHANGES OF ASCORBIC ACID CONTENT IN CORN, BEETS, WAX BEANS AND GREEN BEANS DURING PROCESSING

Product	*Variety*	*Station of sampling*	*Ascorbic acid (mg/100 g)*	*Original ascorbic acid retention (%)*
Corn	Style-Pack	From truck	9·33	100
		After cutting	8·87	95·0
		After washing	7·97	85·4
		After retorting	4·03	43·1
	Commandeer	From truck	9·68	100
		After cutting	9·02	93·1
		After washing	7·86	81·2
		After retorting	3·86	39·8
Beet	Ruby Queen	From truck	9·28	100
		After peeling	4·70	50·6
		After retorting	2·15	23·1
Wax bean	Early Wax	From truck	19·36	100
		After washing	16·84	86·9
		After cutting	16·13	83·3
		After blanching	1·47	7·6
	Midas	From truck	17·26	100
		After washing	16·53	95·5
		After cutting	14·52	84·1
		After blanching	1·06	6·0
Green bean	Slender White	From truck	18·19	100
		After washing	15·86	87·2
		After cutting	13·32	73·2
		After blanching	2·65	14·5
	Early Galatan	From truck	15·09	100
		After washing	14·02	92·9
		After cutting	11·77	77·9
		After blanching	5·13	33·9

Beets

Only one variety of beets, Ruby Queen, and three different dates of processing were studied. Samples from the three different harvest dates did not show any significant difference in ascorbic acid content. Beets were washed, graded, blanched for 10 to 15 min at 100°C, immersed in a lye bath (12% NaOH) for 10 min at 88°C and then peeled in an abrasive peeler. Peeled beets were sliced, filled into cans with brine, sealed and heat processed at 125°C for 13 min. Sampling was carried out at the unloading dock, after peeling, and after heat processing. Approximately 50%

of the original ascorbic acid content was lost during blanching and peeling operations, and another 50% loss followed during heat processing. Finished canned beets retained only 23% of the original ascorbic acid content.

Wax bean and green bean

Two varieties of wax beans from three growers and two varieties of green beans from four growers were studied. Ascorbic acid contents in the two varieties of each bean (wax and green) were not significantly different. However, grower effects on Midas wax bean and on Slender White green bean did show statistical significance in their different ascorbic acid contents (Table 4).

TABLE 4
ASCORBIC ACID CONTENT IN FRESH BEANS EXPRESSED BY GROWER

Variety	*Grower*	*Ascorbic acid (mg/100 g)*		*Significance of F-value*
Wax bean				
Midas	Grower A	15·84		*
	Grower B	18·70		*
Green bean				
Slender White	Grower C	19·48	ns	*
	Grower D	20·14	ns	*
	Grower E	14·97	ns	*

* Statistically significant at 5% level.

Beans were washed, cut, blanched at 77°C for 45 sec and then filled into cans, sealed and finally heat processed for 13 min at 120°C in a FMC continuous cooker and cooled. Samplings were carried out at the truck, washer, cutter, blancher and after heat processing. Ascorbic acid content in the final products was not included in the table because some food additives added during the canning process interfered with the true values. Ascorbic acid contents of beans from different sampling stations were significantly different. The major cause of ascorbic acid losses appeared to be in the blanching operation. Wax beans retained only 6–7·6% of original ascorbic acid after blanching. However, green beans retained more ascorbic acid than wax beans. Slender White retained 14·5% of ascorbic acid, while Early Galatan, which is a late variety, retained almost 34% of ascorbic acid. Between these two varieties, the differences in ascorbic acid content of the blanched beans were statistically significant. Wagner *et al.* (1947) and Moyer *et al.* (1952) showed that ascorbic acid oxidase required at least 82°C for complete inactivation. The lower ascorbic acid retention of blanched beans in this study may be rationalised by assuming that beans were under-blanched at this particular plant. Since considerable losses of ascorbic acid were observed during blanching on the cut beans as compared with other vegetables studied, a laboratory blanching experiment on whole beans and cut beans was conducted. It was found that, under the same conditions, whole beans retained approximately 30% more ascorbic acid

than cut beans. Therefore, for higher ascorbic acid retention, it is desirable to blanch beans before cutting.

SUMMARY

Studies were made of the ascorbic acid content and its retention in canned vegetables in an attempt to correlate variability due to the variety, grower, size of the product and effect of each processing operation on ascorbic acid in canned food as produced in commercial canning plants. Five vegetables with a total of 72 different samplings of products were subjected to ascorbic acid analysis. In general, as is well known, blanching and heat processing operations in the canning process were the major causes of ascorbic acid losses. Cut beans lost an especially large percentage of ascorbic acid during blanching. However, the cultural practices of the different growers did affect the ascorbic acid content in some fresh peas and beans. Pea size was also a factor causing a different level of ascorbic acid in peas. Varietal averages of ascorbic acid content of the canned peas were significantly different. However, corn and beets did not show any significant difference on variety, harvest date and grower.

It should be noted that blanching times and temperatures, as well as types of equipment and raw products, will be different from cannery to cannery. Therefore, some observations described here may not be true for all canneries. However, some of the results show the general direction in which processors should be moving to control the problems. Supplementary studies are being carried out in order to find the effects of variables in raw product and processing on the other nutrients and shelf life.

In conclusion, it is suggested that a closer monitoring of raw material and unit operations in the canning process be a prerequisite to reliable and realistic nutritional labelling of processed vegetables.

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VITAMIN C AND PLASMA TRIGLYCERIDES IN EXPERIMENTAL ANIMALS AND IN HUMANS

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ABSTRACT

A chronic latent vitamin C deficiency in guinea pigs enhances triglyceride concentration in blood plasma but does not markedly alter the composition of fatty acids in serum and hepatal triglycerides. An addition of 0·5% *ascorbic acid to the diet of golden hamsters tends to normalise alimentary hypertriglyceridemia provoked by a high-glucose fat-free diet not containing vitamin C. A daily administration of* 2 × 500 *mg of an effervescent ascorbic acid for three months to subjects aged between* 50 *and* 75 *who were in a state of a seasonal vitamin C deficiency resulted in a significant depression of triglyceridemia. The hypotriglyceridemic effect of ascorbic acid depended on the starting plasma concentration of triglycerides: the higher the initial triglyceridemia, the more striking the hypotriglyceridemic effect. An administration of* 2 × 500 *mg of effervescent ascorbic acid every* 24 *h to a selected group of subjects with an initial triglyceridemia above* 200 *mg* % *for the duration of one year brought about a very significant decline of triglyceridemia, and this still persisted six weeks following cessation of ascorbic acid administration. An intervention of vitamin C at the level of triglyceride splitting enzymes is presumed.*

INTRODUCTION

The majority of studies dealing with the effect of vitamin C on blood lipids have been concerned with the relationship ascorbic acid–cholesterol (Ginter, 1975) despite the fact that the level of plasma triglycerides is also of significance for the pathogenesis of atherosclerosis (Albrink, 1973).

Sokoloff *et al.* (1967) described a striking hypotriglyceridemic effect of ascorbic acid in a large group of rabbits and rats receiving cholesterol. As the results they obtained in patients were ambiguous the question as to whether the triglyceride

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decline noted in some of the patients had been caused by some other factor remains an open one. The present work summarises the results of our studies on the effect of ascorbic acid on plasma triglycerides in two species of laboratory animals and in humans. Elderly people in whom the occurrence of latent vitamin C deficiency was supposed and where the ascorbic acid intake could be controlled were involved in the experiment.

METHODS

Guinea pigs

Adolescent male guinea pigs (Breeding Station of the Pharmacological Institute, Slovak Academy of Sciences), with initial weights of approximately 400 g, in which the effect of a chronic latent vitamin C deficiency had been followed on plasma triglyceride concentration and the composition of triglycerides isolated from the liver, blood serum and epididymal fat were used. All the animals were fed a basal scorbutogenic diet (Ginter *et al.*, 1969). Control guinea pigs received 10 mg of ascorbic acid per animal in 24 h. In the experimental group, hypovitaminosis C was induced in the manner currently employed (Ginter, 1975). Between weeks 16 and 20 of hypovitaminosis C, some of the control and deficient guinea pigs were decapitated following a 17-h starvation and the composition of fatty acids in triglycerides, isolated by thin layer chromatography, was determined in their blood serum, liver and epididymal fat by gas chromatography (Ginter *et al.*, 1969). In further groups of controls and hypovitaminous animals, triglyceride concentration in blood plasma was determined at 6·5 and 11 months of hypovitaminosis C (Fletcher, 1968).

Golden hamsters

Male golden hamsters (Velaz, Prague), with initial weights of approximately 60 g, were used to follow the effect of ascorbic acid on alimentary hypertriglyceridemia provoked by the administration of a fat-free high-glucose diet without ascorbic acid. Some of the animals were sacrificed at the start of the experiment when still being fed on a normal laboratory diet (cereals, vegetables), to obtain starting normal triglyceride values. Then the remaining animals were put on a moderately modified lithogenic diet according to Dam (1964), one group being fed this diet without any addition, the other with an addition of 0·5% of ascorbic acid. After 3 and 4 weeks of the experiment, the animals of both groups were decapitated, without any previous withdrawal of food, triglyceride concentration was determined in their blood plasma (Fletcher, 1968) and vitamin C concentration in their liver (Roe & Kuether, 1943).

Humans

The effect on humans of effervescent tablets containing 500 mg of ascorbic acid (Celaskon effervescens, manufactured by Slovakofarma Hlohovec, Czechoslovakia) was followed on triglyceridemia in 82 subjects of both sexes, aged 50 to 75. The experiment was carried out in the Pensioners' Home at Stupava (District of Bratislava), where a control on the consumption of the vitamin drug was assured. The experiment was initiated in February 1974, at a time of year when a seasonal occurrence of vitamin C deficiency could be expected in the majority of the subjects. All the subjects were given one tablet each of Celaskon effervescens at lunch and with their evening meal, *i.e.* 1000 mg of ascorbic acid every 24 h. With such a dosage, approximately 620 mg of ascorbate was absorbed daily (Mayersohn, 1972), that is a dose presenting no danger of oxalate stone formation (Lewin, 1973). Immediately before initiating the ascorbic acid treatment, vitamin C concentration was determined in whole blood (Roe & Kuether, 1943) and triglyceride level in blood plasma (Fletcher, 1968). Blood was drawn from a vein with subjects either fasting, or having consumed only a very light breakfast (tea and a roll, without any fat). The nursing personnel were instructed to see that the nutritional regime in the experimental subjects should remain unchanged. After three months, triglyceridemia and ascorbemia were again determined. The subsequent course of the experiment is described below and the results presented.

The results of experiments on animals and humans were statistically processed (Student's *t*-test, linear correlation) by computer (Olivetti Programma 101).

RESULTS AND DISCUSSION

Guinea pigs

Figure 1 makes it clear that in a protracted latent vitamin C deficiency, triglyceridemia in hypovitaminous guinea pigs receiving 0·5 mg of ascorbate daily is higher by about 50% than that in controls fed the same diet but with the addition of 10 mg ascorbate every 24 h. Triglyceridemia increases with the duration of the experiment, but the relative 50% ratio between the controls and the hypovitaminous group remains approximately unchanged. It should be emphasised that this difference cannot be ascribed to any differences in weight increments since the pattern of weight curves in the two groups is identical. Fujinami *et al.* (1971) also reported an increase in triglyceridemia in guinea pigs suffering from acute vitamin C deficiency, but the difference between the controls and the deficient animals in this case was substantially smaller than in our experiments lasting over a much longer period.

Table 1 shows the composition of triglycerides isolated from blood serum, liver

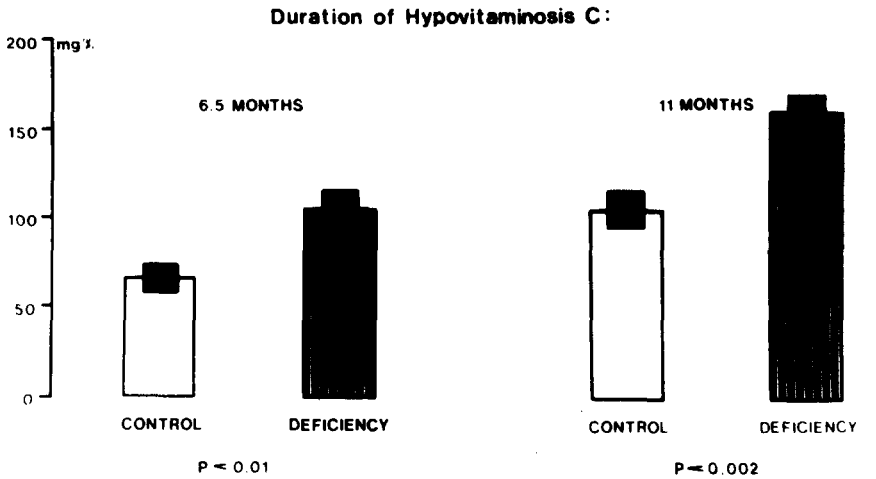

Fig. 1. Effect of chronic latent vitamin C deficiency on plasma triglyceride concentration in guinea pigs. Means from 8–15 animals. Black columns indicate ± standard error of the mean.

and epididymal fat to have been but moderately affected by chronic hypovitaminosis C. A point of interest is the statistically highly significant increase in saturated palmitic acid and a decline of essential linoleic acid in the epididymal fat of hypovitaminous animals but physiologically the significance of these changes remains open.

Golden hamsters

From Fig. 2 it is evident that the golden hamster is a suitable test animal for provoking alimentary hypertriglyceridemia. Hypertriglyceridemia may be induced

TABLE 1

THE INFLUENCE OF CHRONIC LATENT VITAMIN C DEFICIENCY ON FATTY ACID COMPOSITION OF TRIGLYCERIDES ISOLATED FROM BLOOD SERUM, LIVER AND EPIDIDYMAL FAT OF GUINEA PIGS. IN THE TABLE MEANS FROM 9–12 ANIMALS ± SEM ARE GIVEN

Fraction	*Blood serum*		*Liver*		*Epididymal fat*	
	Control	*Deficiency*	*Control*	*Deficiency*	*Control*	*Deficiency*
C_{12}	4·3 ± 0·3	5·4 ± 0·4	6·2 ± 0·4	5·2 ± 0·03*	8·3 ± 0·3	8·1 ± 0·2
C_{14}	1·3 ± 0·1	1·1 ± 0·1	1·6 ± 0·1	1·3 ± 0·1	1·4 ± 0·1	1·2 ± 0·1
$C_{14:1}$	1·1 ± 0·1	1·3 ± 0·1	1·5 ± 0·1	1·4 ± 0·1	1·5 ± 0·1	1·4 ± 0·1
C_{16}	26·8 ± 1·1	27·4 ± 0·7	28·8 ± 0·5	27·8 ± 0·4	25·5 ± 0·5	28·6 ± 0·4*
$C_{16:1}$	3·3 ± 0·2	3·1 ± 0·2	4·3 ± 0·2	4·0 ± 0·2	3·9 ± 0·2	3·4 ± 0·2
C_{18}	6·3 ± 0·2	6·5 ± 0·3	3·6 ± 0·2	4·3 ± 0·2	4·7 ± 0·2	5·0 ± 0·1
$C_{18:1}$	28·5 ± 0·8	27·0 ± 0·3	26·0 ± 0·6	28·5 ± 0·5*	27·7 ± 0·7	28·5 ± 1·0
$C_{18:2}$	18·4 ± 0·5	18·3 ± 0·7	19·9 ± 0·4	20·0 ± 0·5	18·9 ± 0·4	16·3 ± 0·6*
$C_{18:3}$	1·5 ± 0·1	1·5 ± 0·1	1·3 ± 0·1	1·2 ± 0·1	0·8 ± 0·1	0·8 ± 0·1

* Significantly different from the control group ($P < 0·01$–$0·001$).

in this species within a few weeks by keeping the animals on a synthetic fat-free high-glucose diet without any ascorbic acid. In comparison with the controls receiving a natural diet, triglyceridemia increases to more than twice the initial values within three weeks and to more than three times those values within four weeks of feeding on this diet. If, however, 0·5% of ascorbate is added to this diet, the plasma triglyceride level persists practically at the initial value. Hence, under extreme nutritional conditions, vitamin C also exerts a hypotriglyceridemic action in animals synthesising ascorbic acid. The physiologically unbalanced diet used in this study has probably provoked a partial ascorbic acid deficiency in the hamsters, for their liver concentration of vitamin C was found to have dropped to one-third

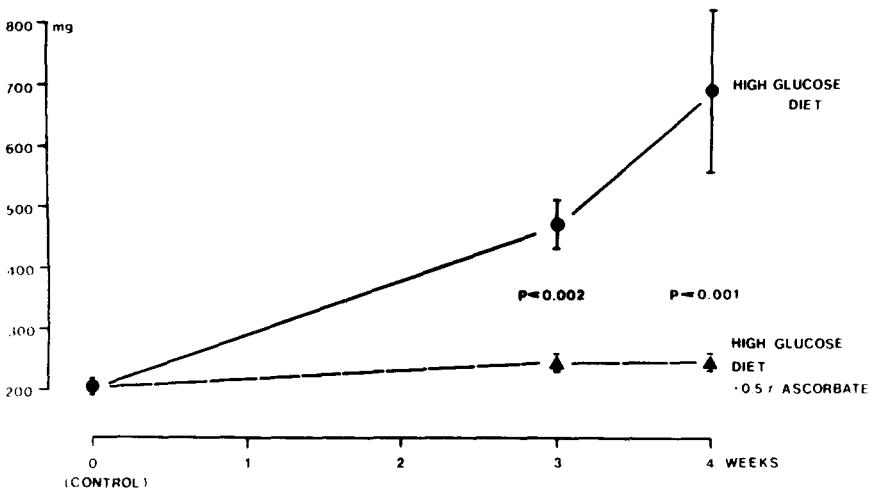

Fig. 2. Effect of 0·5% of ascorbic acid added to the diet on alimentary hypertriglyceridemia induced by a high-glucose fat-free diet in golden hamsters. Means from 8–12 animals. Vertical bars for each value indicate ± SEM.

of the values in normal animals (*i.e.* 36·7 ± 2·0 mg % in hamsters on natural diet, 12·1 ± 2·1 mg % in those on a high-glucose diet without addition of ascorbate). The effect of an addition of 0·5% ascorbate to this diet was to normalise the vitamin C level in the liver (40·3 ± 0·6 mg %). The weight curves of hamsters fed the basal synthetic diet and those having the same diet with 0·5% ascorbate were identical.

Humans

After three months of ingestion of 1000 mg ascorbic acid daily, the subjects showed a significant decrease in triglyceridemia (initial values 230 ± 11 mg %, after three months 168 ± 8 mg %, $P < 0·001$). A more thorough analysis of the

results showed the hypotriglyceridemic action of ascorbic acid to depend on the initial triglyceridemia. Figure 3 expresses the change in triglyceridemia following a three-month administration of ascorbic acid as a function of the starting triglyceride concentration in blood plasma prior to the initiation of the experiment. A statistical analysis of this relationship has shown that a relatively close linear correlation is involved: in subjects with an initial triglyceridemia below 200 mg %, the plus and minus changes are practically matched. With initial values around 100 mg %, plus changes tend to prevail (*i.e.* towards an increased triglyceridemia). However, in subjects with an initial value above 200 mg %, minus changes clearly predominate, with triglyceridemia decreasing approximately linearly with an increasing initial concentration of plasma triglycerides.

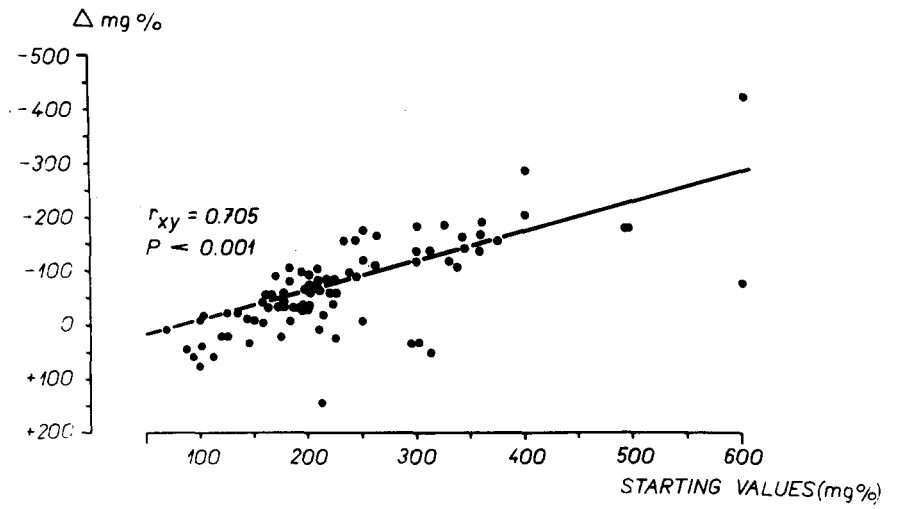

Fig. 3. Linear correlation between initial plasma triglyceride concentration and change in triglyceridemia after three months of administration of 2 × 500 mg of ascorbic acid every 24 h in subjects aged 50 to 75.

In the light of these results, our experimental group was narrowed down to subjects with an initial triglyceridemia above 200 mg % (n = 24, of whom 18 were females). Subjects included in this study continued to receive the same dose of effervescent ascorbic acid (*i.e.* 2 × 500 mg every 24 h) for a year. Plasma triglyceride concentration was determined every three months. The results are presented in Fig. 4. A striking decline of triglyceridemia is already evident after three months of administration of high vitamin C doses, and this trend becomes still more pronounced in the subsequent stages of the experiment. After a year of daily doses of 1000 mg ascorbate, the triglyceridemia was found to have dropped from the mean initial value of 331 mg % to 188 mg %, and it may be observed

that in the majority of subjects, even when evaluated individually, there is also a clinically significant decline. Six weeks after termination of ascorbic acid administration, triglyceridemia was again determined in all subjects. The mean value of 200 mg % then found showed that the hypotriglyceridemic action of ascorbate still persisted.

Simultaneously with the experimental group, we also followed in parallel time intervals triglyceridemia in a group of controls of similar mean age who received no extra ascorbic acid. Triglyceridemia in these subjects showed no major seasonal deviations, so that the changes noted in the experimental group cannot be accounted for by seasonal variations, and as the body weights in our experimental subjects did not undergo any substantial alterations, the changes in triglyceridemia cannot be ascribed to this factor, either.

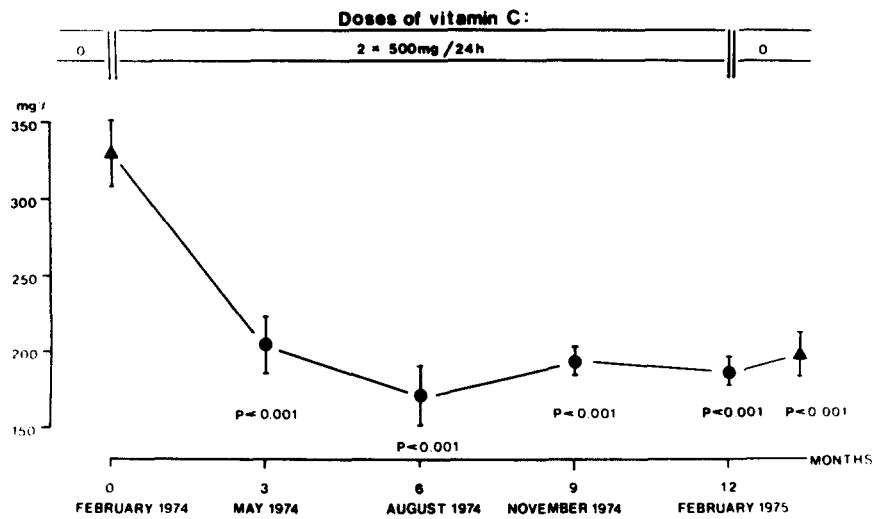

Fig. 4. Effect of vitamin C on plasma triglyceride concentration in hypertriglyceridemic subjects. Means from 24 subjects. Vitamin C dosage is given in the upper part of the figure. Vertical bars for each value indicate ± SEM. Statistical significance of decline in comparison with the initial state is given at each value.

The striking hypotriglyceridemic effect of ascorbic acid which, in contrast to the findings of Peterson *et al.* (1975), we have found in our group may be explained by two factors: (a) the selection of subjects with an initial triglyceridemia above 200 mg %; (b) the fact that the study had been made on subjects in a state of latent ascorbic acid deficiency. The mean initial vitamin C concentration in whole blood in our group amounted to 0·41 ± 0·03 mg %. After three months of ascorbic acid administration it rose to 1·42 ± 0·07 mg %, but even this value was lower than the

initial plasma ascorbic acid concentration in Peterson *et al.*'s subjects. The present results cannot be interpreted in the sense that high doses of ascorbic acid will, under all circumstances, tend to depress triglyceridemia. On the contrary, administration of ascorbic acid to 'vitamin C-saturated' subjects will, in all probability, remain ineffective. Our conception lays stress on the preventive aspect of the problem. Accordingly, the latent chronic vitamin C deficiency should be considered as *one of numerous factors* inducing hypertriglyceridemia. It seems probable that hypertriglyceridemia produced on this basis will respond to the action of high ascorbate doses by an evident decline.

The mechanism of action of vitamin C deficiency on the levels of plasma triglycerides has not yet been elucidated. Ascorbate deficiency does not appear to affect the composition of the metabolic pool from which fatty acids incorporated into hepatal and plasma triglycerides are derived, since the composition of triglycerides isolated from the liver and blood plasma is practically identical in the controls and the hypovitaminous guinea pigs. A more probable factor is an intervention at the level of enzymes catalysing triglyceride splitting. The moderate increase of triglyceridemia observed in acutely deficient guinea pigs was associated with a decline in the lipoprotein lipase activity in the blood of scorbutic animals (Fujinami *et al.*, 1971). The hypotriglyceridemic action of ascorbate in rabbits and rats receiving cholesterol was associated with an increase in lipoprotein lipase activity in the blood (Sokoloff *et al.*, 1967). Ascorbic acid also enhanced the lipolytic activity of the aorta in white rats (Milenkov & Mitkov, 1969). On the other hand, an inhibitory effect of ascorbate on the lipolytic activity of the myocardium and adipose tissue was reported (Kotzé *et al.*, 1974; Tsai *et al.*, 1973). This finding has led to considerations (Anon., 1974), according to which high ascorbate doses in humans might inhibit mobilisation of non-esterified fatty acids from depot tissues and thus lead to obesity. However, *in vivo* experiments have shown that, on the contrary, an inhibition of mobilisation of non-esterified fatty acids occurs under a vitamin C deficiency (Mueller & Cardon, 1961). Scorbutic guinea pigs fail to respond even to adrenaline administration by a mobilisation of non-esterified fatty acids (Mueller, 1962). It would be desirable to further process these complex problems for they may be associated with the presumed antiatherogenic action of vitamin C.

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LEAD CONTENT OF ROADSIDE FRUIT AND BERRIES

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ABSTRACT

Blackberries, elderberries, hawthorn berries, holly berries and rose hips have been examined for their lead content, which has been shown to be directly related to the proximity of the growing fruit and berries to roads, the traffic density and the time of exposure. The maximum levels found (in ppm for undried fruit and berries) were blackberries 0·85, *elderberries* 6·77, *hawthorn berries* 23·8, *holly berries* 3·5 *and rose hips* 1·45. *Very thorough washing with water removed* 40–60% *of the lead from heavily contaminated fruit and berries. When elderberries were used for winemaking over* 60% *of the lead was extracted and remained in solution in the wine.*

INTRODUCTION

It is well known that various industries can cause substantial contamination of nearby soil and vegetation, particularly that upwind from the industrial plants. Thus United Kingdom studies have demonstrated substantial contamination of vegetation by cadmium, zinc and lead from metal smelters in the Avonmouth (Burkitt *et al.*, 1972; Little & Martin, 1972) and Swansea (Goodman & Roberts, 1971) areas, and lead fall-out from a lead tetraethyl plant near Manchester has been demonstrated (Lee, 1972). The contamination is comparatively local, however, and at Avonmouth, upwind from the factory, the lead level of grass fell from 275 ppm (dry weight) at 0·2 miles (321 m) to 23 ppm at 7·9 miles (12·7 km). Although such contamination is substantial, the localities are fairly clearly defined and appropriate precautions may be taken.

Contamination of vegetation by lead from automobile exhausts is much more general, and in recent years there have been reports of contamination of grasses, mosses and leaves of trees close to motorways in the United Kingdom (Davies & Holmes, 1972), the United States (Smith, 1972, 1973; Chow, 1970; Hopkinson *et al.*, 1972) and a number of European countries (Horak & Huber, 1973; Majerus & Denaeyer, 1973; Impens *et al.*, 1973*a*, 1973*b*; Quinche *et al.*, 1969; Zuber *et al.*,

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1970; Fidora, 1972; Sommer *et al.*, 1971; Wiklander, 1971; Hoegger, 1973). To a more limited extent similar contamination has been observed for such leafy vegetables as lettuce and spinach (Impens *et al.*, 1973*b*; Rabinowitz, 1972), and, on the basis of their observations of the lead content of grass growing near major roads in Birmingham, Davies & Holmes have recommended that vegetables should not be grown within 50 m of busy roads. As might be expected, the amount of lead taken onto the surface of the vegetation depends on the amount of traffic, the distance from the road and the length of exposure; the contamination varies considerably from one species to another.

While the problem is now well documented, and it is generally recognised that fodder immediately adjacent to busy motorways should not be used to feed cattle, there appears to be little information about the degree of contamination of roadside fruits and berries, apart from an observation by Pertoldi Marletta *et al.* (1973) that grapes growing near a busy road contained up to ten times the amount found in similar grapes growing well away from the road; the maximum levels of lead were around 0·5 ppm for undried grapes.

The present work was undertaken to establish the levels of lead in soft fruit (*e.g.* blackberries and elderberries) and berries (*e.g.* hawthorn berries and rose hips) growing wild on busy roadsides, since they might constitute a hazard if picked and eaten by members of the unsuspecting public. Thus, children, who are especially susceptible to small amounts of lead, might well pick and eat blackberries, while other fruits and berries are frequently processed into jams, jellies and wine. As there are more than one million home winemakers in the United Kingdom, it would seem sensible to determine typical lead levels in some of the fruit and berries they use to see if there is a significant health hazard.

In referring to risk, it is worth remembering that the Lead in Food Regulations (1961) set an upper limit of 2 ppm for lead in (undried) fruit and vegetables, apart from apples and pears where the limit is 3 ppm in order to accommodate possible lead uptake from lead arsenate sprays. Even more valuable is the comment of the FAO/WHO Expert Committee on Food Additives (1972) that for adults the 'tolerable weekly intake' of lead from both food and water is 3 mg. For children this limit must be reduced substantially, partly because of the lower body weight and partly because lead is absorbed from the gut about five times as effectively as in adults (Alexander *et al.*, 1973). The limit of ingested lead per day for children is therefore likely to be around 100 μg; King (1971) has quoted 300 μg per day as being potentially hazardous to children.

MATERIALS AND METHODS

Sampling of fruit and berries

Berries and fruit were picked from all parts of convenient bushes and trees,

TABLE 1
LEAD CONTENT OF BLACKBERRIES

Sample	*Lead[a] content (ppm)*	*Location (map reference)*	*Traffic scale (0–5)*	*Mean distance from road (m)*
B1	0·85[a]	720782; A4009	3	2
B2	0·60	684872; A423	3	3
B3	0·57	745678; A327	3	6
B4	0·56	738687[b]	1–2	2
B5	0·54	765746	2	2
B6	0·47	699906; B481	2	5
B7	0·46	772888	1	3
B8a	0·28	746630	0	30
B8b	0·32	746630[c]	0	30
B9	0·27	695825	0	15
B10	0·21	752888	1	2
B11	0·16	744625	1	3
B12	0·15	737717	0	60
B13	0·14	757707	1–2	2
B14	0·10	814733	1	6
B15	0·08	760613	1	5

[a] Sample washed by decantation contained 0·78 ppm lead.
[b] Same road as sample B13, but within 80 m of M4.
[c] Sample B8b taken two weeks later from same spot as B8a.
[d] Expressed as Pb.

TABLE 2
LEAD CONTENT OF ELDERBERRIES

Sample	*Lead content[e]*		*Location (map reference)*	*Date picked*	*Traffic scale (0–5)*	*Mean distance from road (m)*
	Unwashed	*Washed[a]*				
E1	6·77	—	805780; A4	3.10.74	4	1
E2a	2·79	1·48	805780; A4[b]	3.10.74	4	1
E2b	4·77	2·84	805780; A4[b]	10.10.74	4	1
E2c	4·74	2·66	805780; A4[b]	13.10.74	4	1
E2d	4·63	—	805780; A4[b]	17.10.74	4	1
E3	2·58	1·52	A41	24.10.74	3	1
E4	2·14	1·30	A423	24.10.74	3	1
E5	1·70	0·81	395461; B3400	13.10.74	2–3	1
E6a	1·66	—	799777; A321	3.10.74	2–3	2
E6b	0·80	—	799777; A321[d]	3.10.74	2–3	2
E7	1·63	0·74	322642; A338	13.10.74	3	1
E8	1·61	—	745765; A4155	17.10.74	3	1
E9	1·56	0·95	580662; A4	13.10.74	4	4
E10	1·47	—	735758	17.10.74	2	2
E11	1·31	0·58	482682; A4	13.10.74	4	6
E12	0·96	0·45	475482; B3400	13.10.74	2–3	3
E13	0·84	0·47	372521; A343	13.10.74	3	3
E14	0·33	0·20	294607	13.10.74	1	1

[a] Continuous washing for one hour.
[b] Sample E2 a–d picked at same location as E1 but from adjacent bush; E2a less ripe than E1.
[c] East side of road.
[d] West side of road.
[e] Expressed as Pb.

TABLE 3
LEAD CONTENT OF HAWTHORN BERRIES, HOLLY BERRIES AND ROSE HIPS

Sample	*Lead content*[c] *Unwashed*	*Washed*	*Location (Map reference)*	*Date picked*	*Traffic scale (0–5)*	*Mean distance from road (m)*
Hawthorn berries						
HA1	23·8	9·9[a]	783768; A4	9.1.75	4	2
HA2	13·4	—	720722; A33	2.2.75	3	3
HA3	11·7	—	725750	23.2.75	3	2
HA4	10·5	—	785785; A423	16.2.75	3	2
HA5	9·4	—	715665; A33	2.2.75	3	3
HA6	4·8	3·8[a]	745765; A4155	9.1.75	3	1
HA7	4·5	—	768805; A4155	16.2.75	3	2
HA8	2·8	—	768805; A4155	16.2.75	3	6
HA9	0	—	740647	2.2.75	1	2
Holly berries						
HO1	3·5	—	725750; A4155	16.2.75	3	4
HO2	2·0	1·1[b]	660815; B479	23.2.75	2–3	1
HO3	1·6	—	660815; B479	23.2.75	2–3	20
Rose hips						
RH1	1·45	—	708732	5.7.75	2–3	2

[a] Continuous washing for 1 h.
[b] Three washes by decantation.
[c] Expressed as Pb.

and a representative sample taken by the usual quartering procedure. The distances quoted in Tables 1, 2 and 3 are the mean distance from the edge of the road tarmacadam.

Washing procedure

The fruit was placed in a nylon sieve immersed in continuously running cold tap water for one hour, drained, and surface dried gently with absorbent tissue.

Analytical procedure

Fifty grammes of fruit or berries were treated with 40 cm^3 of 0·5M HNO_3 (AR) and simmered gently for 15 min. After it had cooled, the solution was filtered under suction through Type A/E (Gelman) glass fibre filters, the residue washed with distilled water and the filtrate made up to 50 cm^3. The filtrate was examined for its lead content by the standard atomic absorption procedure using the Shandon Southern A 3300 (carbon rod) instrument. This procedure extracted all of the lead from the fruit or berries, inasmuch as the same results were obtained when the mixture was wet oxidised with $HNO_3/HClO_4$ prior to filtration. Reagent blanks were prepared and allowance made (<0·02 ppm).

Traffic scale

Locations were placed on an arbitrary 0–5 scale, point 5 being motorways, point 4

very busy A roads (*e.g.* A4), down to point 0 being countryside with no regular traffic within 50 m.

RESULTS AND DISCUSSION

The blackberries, which were picked in September, 1974 from a range of sites, both busy and quiet, had lead levels between 0·08 and 0·85 ppm (undried fruit). While the higher levels were always found on busy roadsides (point 3 on an arbitrary 0–5 scale) levels of up to 0·46 ppm were detected in fruit in relatively quiet areas. The sample having the highest level (0·85 ppm) was thoroughly washed by decantation but this only reduced lead to 0·78 ppm. This highest level is appreciably below the regulation limit of 2 ppm, but some caution is necessary to safeguard against children picking and eating blackberries from busy roads since 100 g of the 0·85 ppm lead berries would yield 85 μg of lead.

TABLE 4
LEAD LEVELS IN WINES MADE FROM ELDERBERRIES

Lead[a] in elderberries (ppm)	*Amount of fruit used (g/litre)*	*Lead[a] in wine (ppm)*	*% of lead extracted*	*Comments*
1·45	450	0·40	62	Well washed berries
4·63	400	1·23	66	Unwashed berries

[a] Expressed as Pb.

As the blackberry season is quite short, however, the danger is not great; a longer term source of lead would be jam made from such berries since the jam would contain above 0·5 ppm of lead.

The elderberries contained more lead than blackberries, probably because they had been exposed to exhaust fumes for a much longer period. Most of the samples were taken from reasonably busy A category roads, and most of the berries had lead levels in the 1–2·5 ppm range, but on very busy stretches of the A4 the berries acquired levels approaching 7 ppm. When the elderberries were washed very thoroughly in running water over a period of an hour around half of the lead could be removed. Elderberries are used extensively for home winemaking, so experiments were carried out to see how much lead was extracted into the wine. Each wine was made from a basic solution containing 200 g dextrose monohydrate, 4 g tartaric acid and 2 g diammonium hydrogen phosphate per litre of diluted water, together with 400 or 450 g of elderberries; fermentation was effected with a commercial yeast suspension (Grey Owl, general purpose). The results (Table 4) show that over 60% of the lead in the fruit is extracted into the wine. Further tests were carried out on the wines after two months and four months, to see whether the

lead remained in solution or was gradually removed as the usual deposition of tannin-based materials occurred, and in each case the level remained unchanged. These experiments indicate that homemakers of elderberry wine should be careful not to pick berries on busy roadsides, since the regular consumption of wine with levels of around 1 ppm would contribute a significant amount of lead to the system. It is worth noting that the maximum permitted lead in German wines is 0·4 ppm.

In order to establish the extent of the contamination of vegetation on long-term exposure, tests were made on hawthorn berries, holly berries and rose hips picked in January or February, 1975. The hawthorn berries picked along the A4 had very high levels of lead (23·8 ppm) and almost 10 ppm remained even after very thorough washing. It is generally believed that the lead contamination is largely on the surface, rather than distributed through the vegetation, and an attempt was made to separate the hawthorn berry skins from the fleshy interiors. The separation was only partial since some flesh remained attached to the skins, and vice versa, but as analysis of the 'skin' and 'flesh' sections gave 105 and 5 ppm lead, respectively, it is clear that the lead was mainly surface. Once again, wine is made from hawthorn berries and these results show that such wines could be a real health hazard if the berries were picked late in the season from busy roadsides.

Finally, it is not unreasonable to deduce that vegetables such as broccoli and cauliflower that are grown close to high density traffic roads are likely to have quite high lead levels, and although much of this is likely to be removed in cooking, the 'vegetable water' remaining will retain the lead and should not be used for making soup stock, etc.

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THE CHEMICAL COMPOSITION OF NIGERIAN ONIONS (*ALLIUM CEPA*, LINN)

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ABSTRACT

Four Nigerian cultivars of Allium cepa *were analysed for the proximate chemical composition,* i.e. *carbohydrate constituents, mineral make-up and the amino acid pattern to provide basic information on their nutritive value. Crude protein ranged from* 6·4% *in 'Kano' to* 19·6% *in 'Oyo', ether extract from* 1·7% *in 'Kano' to* 35·2% *in 'Oyo', nitrogen-free extract (nfe) from* 18·2% *in 'Oyo' to* 85·6% *in 'Kano'. Sugars identified in* 80% *ethanolic extracts of samples were glucose, fructose, sucrose and an oligosaccharide containing a ketose portion. Total sugars figures ranged from* 13·2% *in 'Oyo' to* 71·8% *in 'Kano'; hemicellulose, from* 8·5% *in 'Zaria' to* 13·2% *in 'Oyo'; cellulose from* 10·9% *in 'Kano' to* 24·6% *in 'Oyo'. Paper chromatography of hemicellulose hydrolysates of samples revealed the presence of galactose, arabinose, xylose, rhamnose and ribose. Nitrogen-free extract overestimated available carbohydrates by* 19·26% *to* 40·04%. *The onions were rich in calcium, magnesium and potassium; 'Oyo' was higher in iron* (8·1 *mg*/100 *g*) *and the essential amino acids than other cultivars.*

INTRODUCTION

As in many other countries, onions are used in Nigeria as a condiment in daily cooking and as a salad. Their medicinal properties are also harnessed to treat ailments such as fever, and to relieve earache (Ikram, 1971). These onions are produced mainly in the Northern States of Nigeria and, after curing, are transported to the South by road and rail, for sale. Estimated onion production in the country increased from 68,000 metric tons in 1959/60 to 86,000 metric tons in 1969/70 (Olayide *et al.*, 1972). Although the literature is replete with data on the chemical composition of many cultivars of *Allium cepa* (Bacon, 1957; Bhatti &

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Asghar, 1965; Quereshi *et al.*, 1968; Flam & Mitiska, 1971; Ikram, 1971), no such data are available on Nigerian onions. This work was carried out to fill the information gap with analytical data on the carbohydrate constituents, mineral make-up and amino acid composition of 'Kano', 'Sokoto', 'Zaria' and 'Oyo' for the future appraisal of their nutritive value.

EXPERIMENTAL

Materials

The four cultivars analysed are usually referred to according to their major areas of production. The bulbs of 'Kano', 'Zaria' and 'Sokoto' and the foliage leaves and false stem of 'Oyo' are harvested normally for consumption. These were the respective portions analysed. After the removal of the scales and roots, the samples were chopped into pieces and representative portions were taken for dry matter estimation. The remaining pieces were freeze-dried and then milled for subsequent analysis.

Analytical methods

The proximate chemical composition was determined using the Official Methods of Analysis of the AOAC (1970). Sugars were extracted with 80% ethanol, the extract concentrated *in vacuo*, deionised and subjected to paper chromatography, using *n*-butanol, acetic acid and water in the ratio of 4:1:1 (v/v) as the irrigating solvent. Identification of sugars was achieved with silver nitrate (Trevelyan *et al.*, 1950), trichloroacetic acid and naphthoresorcinol (Partridge, 1948) and *p*-anisidine hydrochloride (Lewis *et al.*, 1972). Amounts of identified sugars were estimated using the phenol–sulphuric acid method of Dubois *et al.* (1951) and the corresponding sugar standard curve.

Absence of starch was confirmed using the conventional iodine test.

The acid-hydrolysis method of Bailey (1964) was employed on sugar-free residues for hemicellulose estimation, whilst the sugars present in the hydrolysates were identified by chromatography.

Cellulose was estimated using the Crampton and Maynard method (Crampton & Maynard, 1938).

For mineral determination, atomic absorption spectrophotometry was employed.

For amino acid content, 0·1 g of each sample was hydrolysed at 110°C for 24 h with 6N of HCl in an atmosphere of nitrogen in a sealed test-tube. Five millilitres of the filtered hydrolysate were dried *in vacuo* and then reconstituted with pH 2·2 buffer solution. One millilitre of this new solution was injected into each of the two columns containing Resin 3105 in an Hitachi Amino Acid Analyzer Model KLA-3B (Paulissen, 1972).

RESULTS AND DISCUSSIONS

Proximate chemical composition

From Table 1, it is apparent that only 'Oyo' cultivar was rich in crude protein (19·6%) and ether extract (35%), respectively. The latter might partly be attributed to the extraction of chlorophyll and other ether soluble pigments. The bulbs of other cultivars analysed were devoid of chlorophyll. A crude fibre content of 14·5% appeared to reflect the bulky nature of the edible portion of 'Oyo' cultivar. While nfe, which conventionally represents the readily digestible carbohydrate fraction of foods, accounted for more than 78% of the dry matter of 'Zaria', 'Sokoto' and 'Kano', this figure was only 18·2% in 'Oyo'.

TABLE 1
THE PROXIMATE CHEMICAL COMPOSITION OF ONION CULTIVARS IN g/100 g SAMPLE (dry matter basis)

	'Sokoto'	*'Zaria'*	*'Oyo'*	*'Kano'*
Crude protein	8·32	11·46	19·61	6·35
Ether extract	2·49	3·11	35·22	1·69
Crude fibre	4·98	2·31	14·51	3·14
Ash	4·48	4·85	12·42	3·19
Nitrogen-free extract (nfe)	79·73	78·27	18·24	85·63
Dry matter	8·33	8·45	9·00	7·70

Carbohydrate constituents

Table 2 gives the amounts and types of major carbohydrates in the edible portions of the onions analysed. They all contained sucrose, glucose, fructose and an oligosaccharide which was more abundant in 'Zaria', 'Kano' and 'Sokoto' than in 'Oyo'. The sum of these sugar figures (total sugars) for each cultivar was regarded as its available carbohydrate (McCance & Lawrence, 1929).

The observed difference between the total sugars figures of either 'Kano', 'Zaria' or 'Sokoto' and that of 'Oyo' might be attributed partly to the physiological

TABLE 2
CARBOHYDRATE CONTENT OF ONION CULTIVARS IN g/100 g SAMPLE (dry matter basis)

Constituents	*'Sokoto'*	*'Zaria'*	*'Oyo'*	*'Kano'*
Oligosaccharide containing a ketose portion	2·55	7·66	1·06	3·99
Sucrose	20·63	21·15	0·62	13·55
Fructose	13·71	16·82	6·96	28·38
Glucose	20·04	18·48	4·57	25·88
Total sugars	56·93	64·11	13·21	71·80
Total hemicellulose	8·83	8·46	13·20	9·66
Cellulose	11·60	11·13	24·57	10·88
Total carbohydrates	77·36	83·70	50·98	92·34

TABLE 3
COMPARISON OF NFE AND AVAILABLE CARBOHYDRATES, AND CRUDE FIBRE AND UNAVAILABLE CARBOHYDRATES

	nfe (*a*)	*Available carbohydrates* (*b*)	$\left(\frac{a-b}{b}\right)\%$	*Crude fibre*	*Unavailable carbohydrates*
'Sokoto'	79·73	56·93	40·05	4·98	20·43
'Zaria'	78·27	64·11	22·09	2·31	19·59
'Oyo'	18·23	13·21	38·01	14·51	37·77
'Kano'	85·63	71·80	19·26	3·14	20·54

roles of the portions harvested in the live plants. The foliage leaves of 'Oyo' are photosynthetic whilst the bulbs of 'Kano', 'Zaria' and 'Sokoto' act as storage organs for products of photosynthesis transported from the green portions of their plants. Nutritionally, all these sugars are available for use as supplementary energy sources since all the cultivars are often consumed fresh. In addition, when these onions are added to give flavour to meat which is being boiled preliminary to stew preparation, usually the cooking water is not discarded, thereby utilising the dissolved sugars.

These onions did not contain any starch. This further confirms an earlier observation of Jones & Mann (1963) that starch is generally absent from all *Allium* species. Bennett (1941), and Joslyn & Peterson (1958) did not report any starch in the cultivars they analysed, but Quereshi *et al.* (1968) reported a starch content of 5·1 % for 'white' cultivar in Pakistan. They did not, however, carry out any qualitative test for starch.

Available carbohydrate fraction formed about 77·8 %, 73·6 %, 76·6 % and 25·9 % of the total carbohydrates of 'Kano', 'Sokoto', 'Zaria' and 'Oyo', respectively.

'Oyo' was richer in structural carbohydrates, *i.e.* hemicellulose and cellulose, than other cultivars. Paper chromatography of the hemicellulose hydrolysates of these onions revealed the presence of galactose, which was present in the largest amount, and arabinose, xylose, rhamnose and ribose.

In Table 3, it is apparent that the amounts of available carbohydrates in these onions were highly inflated by the classical nfe by a percentage ranging from 19·26 in 'Kano' to 40·05 in 'Sokoto'. The crude fibre figures also grossly underestimated the magnitude of the unavailable carbohydrate fraction (Southgate, 1969). In nutritional studies where the amount of available energy contributed by different foodstuffs to that of the diet must be estimated, carbohydrate fractions of such foodstuffs obtained by the conventional Moglin (Van Soest & McQueen, 1973) method are multiplied by the physiological energy value of 15·69 kJ/g. Using the nfe and crude fibre figures, the amounts of available energy derivable from the edible portions of these cultivars would have been inflated, whilst that of the unavailable energy would have been underestimated. Less emphasis should be placed on nfe and crude fibre as reliable representations of available and unavailable carbohydrate portions of some human foods, as earlier suggested by Southgate & Durnin (1970).

TABLE 4
MINERAL COMPOSITION OF ONION CULTIVARS IN mg/100 g SAMPLE (dry matter basis)

Minerals	*'Sokoto'*	*'Zaria'*	*'Oyo'*	*'Kano'*
Phosphorus	3·40	3·90	5·50	3·30
Calcium	20·16	22·60	62·10	15·96
Magnesium	7·72	8·79	10·14	7·55
Potassium	151·20	180·80	316·25	142·50
Sodium	7·60	8·36	13·34	5·93
Manganese	0·22	0·56	1·38	0·23
Iron	1·03	0·97	8·07	0·34
Zinc	7·66	0·79	1·04	4·47

Mineral composition

'Oyo' cultivar was found to be higher than others in minerals, except in zinc (Table 4). If consumed in adequate quantities, its calcium content of 62·1 mg/100 g may make it a useful source of calcium. All the onions were rich in magnesium. The observed difference between the magnesium figure for 'Oyo' and that of any other cultivar might be due to the presence of chlorophyll pigment in the former. The relatively high potassium and low sodium content of these onions may make them a useful diet supplement in some cardiac disorders (Falase, 1975). 'Oyo's' potassium content of 316·25 mg/100 g made it outstanding in this respect. 'Oyo', too, was very rich in iron (8·1 mg/100 g). Future work, however, is desirable to ascertain the form in which this element exists before its nutritional significance can be appraised.

Amino acids

The amino acids identified in the protein hydrolysates of the onion samples are

TABLE 5
AMINO ACID CONTENT OF ONION CULTIVARS IN mg/100 g SAMPLE
(fresh weight basis)

Amino acid	*'Sokoto'*	*'Zaria'*	*'Oyo'*	*'Kano'*
Valine	21	21	47	17
Leucine	20	23	69	16
Isoleucine	12	14	47	11
Threonine	10	11	33	7
Methionine	13	13	19	9
Phenylalanine	18	22	49	17
Lysine	16	23	53	15
Histidine	7	10	23	23
Arginine	31	35	52	29
Aspartic acid	41	41	67	36
Serine	12	12	31	9
Glutamic acid	81	84	90	64
Proline	10	13	35	10
Glycine	21	20	35	17
Alanine	20	20	51	15
Tyrosine	12	12	34	10
Total	345	374	735	305

listed in Table 5. They were all found to be relatively poor in essential amino acids; tryptophan was destroyed during the hydrolysis. The protein of 'Oyo' contained the highest amounts of the individual amino acids except for histidine in 'Kano'. The total amounts of essential amino acids, excluding tryptophan, formed 35%, 37%, 48% and 33% of the total amino acid contents of 'Sokoto', 'Zaria', 'Oyo' and 'Kano', respectively.

The figures presented in Table 5 compared favourably with previous data compiled by Orr & Watt (1968). Furthermore, 'Oyo' contained about 50% of the amounts of lysine, threonine, valine, leucine, tyrosine, more than two-thirds of the amounts of phenylalanine and isoleucine, and about the same amount of arginine and histidine as are present in human milk. The proportions of glycine and alanine were even higher. Further research work on the medicinal properties of these onions would be useful to supplement the data presented in this paper on their nutritive value.

ACKNOWLEDGEMENT

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UREA DISC-GEL ELECTROPHORESIS OF RABBIT AND BOVINE MYOSIN

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ABSTRACT

Numerous procedures for improving the entry of aggregates and of large myosin subunits into disc gels were investigated. Both rabbit and bovine myosin behaved essentially the same during electrophoresis in urea. Aggregates of myosin and large and small subunits were found to enter and migrate in a 2·5% acrylamide gel in 7M urea on using a continuous buffer system. Washing with low ionic strength buffer (0·04M KCl) prior to the addition of urea increased the proportion of migrating myosin. Storage of myosin aggregates in urea for periods up to three weeks increased the yield of electrophoretically migrating protein. The possible significance of these findings with regard to the properties of meat is discussed.

INTRODUCTION

Since myosin plays a most important role in the contraction of living muscle and in the development of *rigor mortis* following death, its significance in the properties of meat is evident. Thus, studies on the properties and interactions of myosin may help in explaining its functions. One method of studying myosin involves dissociation of subunits and their subsequent analysis. The current concept of the myosin molecule indicates that it is composed of two large subunits with molecular weights of approximately 200,000 and two or three small subunits with molecular weights of a lesser magnitude (Lowey *et al.*, 1969; Dreizen & Gershman, 1970; Gazith *et al.*, 1970; Weeds & Lowey, 1971).

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Electrophoretically whole myosin has been limited to examination in liquid matrix systems due to its large molecular weight. Whole myosin has a limited mobility in gel matrix systems. Myosin small subunits have been separated from the large subunits, using mild separatory conditions, and subjected to electrophoresis in conventional systems (Locker & Hagyard, 1967*a*,*b*; Oppenheimer *et al.*, 1967; Gaetjens *et al.*, 1968; Weeds, 1969; Sarkar *et al.*, 1971; Dow & Stracher, 1971).

Myosin large subunits have been successfully subjected to electrophoresis in gel systems containing dissociating agents. Small *et al.* (1961) have demonstrated that myosin large subunits exist in a dissociated state in 12M urea at 40°C. They stated that high urea concentrations (6 to 10M) favour the dispersal of myosin aggregates into monomeric chains. Both Small *et al.* (1961) and Heywood *et al.* (1967) used a polyacrylamide gel matrix and a 12M urea solvent for electrophoresis of myosin. The resultant single band was interpreted as representing myosin large subunits. Florini & Brivio (1969) carried out electrophoresis of myosin dissociated in 9M urea on acrylamide gels; however, results were difficult to interpret. Parsons *et al.* (1969) carried out electrophoresis of myosin in urea on a starch matrix.

Myosin large subunits were shown by Weber & Osborn (1969) and Paterson & Strohman (1970) to migrate in polyacrylamide gel and sodium dodecyl sulphate (SDS) buffer as monomeric (approximate molecular weight 200,000) polypeptide chains. The myosin large subunits migrated as one band in the SDS complex.

In the present investigation other approaches for dissociating myosin were explored, and the present paper describes some parameters influencing behaviour of the large subunits upon electrophoresis in acrylamide gels. The effect of aggregation on the electrophoretic behaviour of myosin is described, and it is shown that the aggregational state can be changed to favour formation of large subunits in 7M urea, which migrate under electrophoretic conditions.

METHODS

Preparation of myosin

Myosin was prepared from beef and rabbit muscle according to the method of Richards *et al.* (1967). Myosin was chromatographed on a Sephadex DEAE A-50 column with a pyrophosphate buffer. The leading portion of the myosin peak was discarded, and the remainder was concentrated by dialysis against 1M sucrose containing 0·5M KCl and 0·05M Tris-HCl (pH 7·4).

Electrophoresis

Samples were prepared for electrophoresis by dialysing 24 h against 8M urea. Once the sample was equilibrated with 8M urea, it was quite stable and could be held for five days at 3°C or warmed to room temperature for a short time without significant effect upon the electrophoretic pattern.

Disc-gel electrophoresis was carried out by the method of Davis (1964) utilising a 5% running gel and 3% spacer gel. Preparation of the stock solutions was described by Jolley *et al.* (1967). Disc-gel electrophoresis of myosin was also accomplished in a continuous buffer system utilising a 2·5% cyanogum without a spacer gel. Both the above gel systems contained 7M urea.

Disc gel tubes 0·5 mm by 5·0 mm were utilised. A current of 2 ma per tube was maintained during electrophoresis. Electrophoresis was terminated when the bromphenol blue dye had traversed the gel. The resultant gels were removed and stained with coomassie blue by the method of Chrambach *et al.* (1967). The R_m values were determined relative to the migration of the bromphenol blue dye and the surface of the running gel.

Electrophoretic patterns were checked for artifacts due to persulphate oxidation. This was accomplished by comparing electrophoretic properties of gels polymerised by persulphate with those of gels polymerised by riboflavin. Identical results with and without persulphate indicated that this type of oxidation was not present. DDT (dithiothreitol) was used in the tank buffer at a concentration of 1 mM to maintain the myosin aggregates and subunits in a reduced condition, thereby resulting in less likelihood of oxidation of the SH-groups.

The protein concentration of all preparations was determined by the biuret method (Gornall *et al.*, 1949). Deionisation of 10M urea was accomplished by passing the solution through an Amberlite MB-3 mixed bed resin. The chemicals used in forming the gel matrix were purified by the methods of Loening (1967).

RESULTS AND DISCUSSION

Many attempts to improve entry of the large myosin subunits into the 5% disc gels were unsuccessful and included the following treatments in both 0·6M KCl and 7M urea: (1) chelation of divalent ions with 0·05M EDTA (pH 6·5); (2) dissociation of possible contaminating actomyosin by treatment with pyrophosphate in the presence of trace Mg^{2+}; (3) substitution of free amino groups by acetylation and carboxmethylation (Locker & Hagyard, 1967*b*), trinitrophenylation (Tokuyama & Tonomura, 1967), methylation or ethylation (Means & Feeney, 1968); (4) elimination of difficult materials such as protein–nucleic acid complexes (Loening, 1967) by treating with sodium dodecyl sulphate, streptomycin sulphate (Little, 1967) or ribonuclease (McDonald, 1955); (5) treatment with hydroxylamine to remove possible ester linkages between subunit chains of myosin (Gallop *et al.*, 1959); (6) treatment with sodium sulphite (Bailey, 1967); (7) reduction with β-mercaptoethanol and (8) N-ethyl maleimide alkylation.

Urea disc-gel electrophoresis was performed on skeletal muscle myosin from four rabbits and one bovine preparation. No major differences in the behaviour of the different preparations were apparent upon electrophoresis in urea. However, relatively fresh preparations stored for not more than one to two days in 0·5M KCl

(pH 7·0) generally gave a more intense band between the running and spacer gels. Some variation persisted, even in samples from similar sources prepared by identical methods and at the same protein concentrations. The denser band from fresh preparations is supported by the results of Rossomando & Piez (1970) who

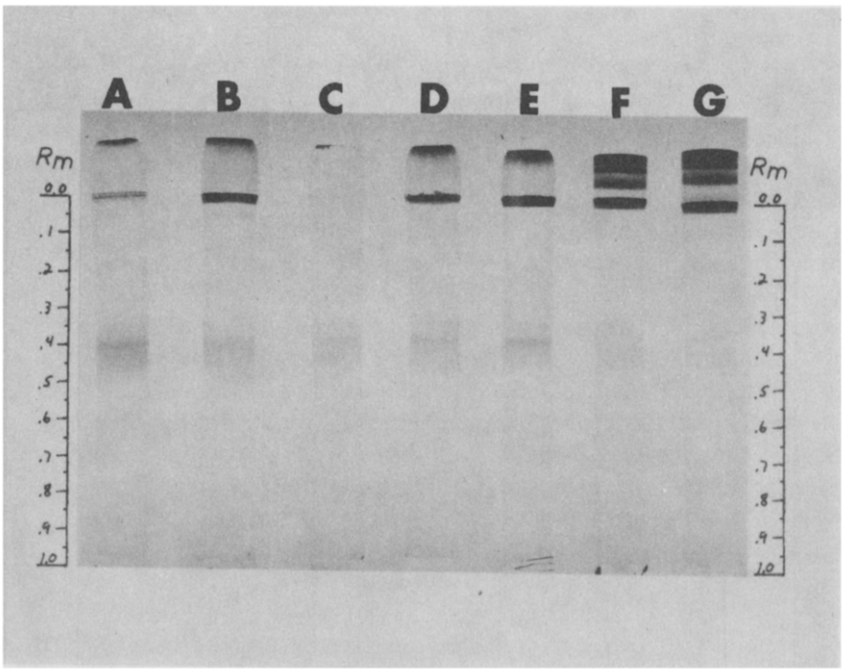

Fig. 1. Disc electrophoresis of myosin using a 5% running gel and 3% spacer gel; in the presence of 7M urea.
A = 0·03 ml of beef myosin preparation containing 3·0 mg protein/ml.
B = 0·03 ml of beef myosin preparation containing 3·2 mg protein/ml.
C = 0·03 ml of aggregated beef myosin preparation containing 4·6 mg protein/ml.
D = 0·04 ml of aggregated beef myosin preparation after washing three times in 0·04M KCl (pH 7·0), dissolving in 0·5M KCl and dialysing against 8M urea. Sample for electrophoresis contained 3·5 mg protein/ml.
E = 0·04 ml of aggregated beef myosin preparation after washing three times in 0·04M NaAC (pH 7·0), dissolving in 0·5M KCl and dialysing against 8M urea. Sample for electrophoresis contained 4·2 mg protein/ml.
F = 0·04 ml of Sample D (above) after dialysing for three weeks against 8M urea at 3°.
G = 0·03 ml of Sample E (above) after three weeks in 8M urea at 3°.

have shown with molecular sieve chromatography on agarose columns that ageing of myosin results in aggregates of up to 30% of the total.

In all preparations of myosin, several faint bands with R_m values of 0·35 and 0·39 were found. These bands (Fig. 1(A) and (B)) plus another common band at R_m = 0·42 appear to correspond to myosin small subunits. Gazith *et al.* (1970)

have shown that low molarity urea is capable of dissociating myosin small subunits from the large subunits. Florini & Brivio (1969) obtained several fast migrating bands in a similar system, but at higher urea concentrations. Perrie & Perry (1970) have carried out extensive investigations on myosin small subunits in acrylamide gel with 8M urea solvent.

Further electrophoretic analysis in 7M urea indicated that these small bands had R_m values of approximately 0·50 after acetylation of whole myosin. The acetylated small subunits can be removed by chromatographing the treated myosin preparation on a DEAE-Sephadex matrix with phosphate buffer.

Figure 1 shows the disc-gel pattern of whole myosin on a 5% running gel with a 3% acrylamide spacer gel using a 7M urea solvent. In all cases, the myosin large subunits or aggregated large subunits failed to migrate into the running gel.

Gel C (Fig. 1) shows myosin prepared in 0·6M KCl, dialysed against 8M urea and subject to electrophoresis. The myosin large subunits are in a high degree of aggregation, as exemplified by their failure to migrate through the spacer gel. Gels D and E (Fig. 1) utilised the same protein preparations. However, the samples were washed three times with 0·04M neutral KCl and the resultant precipitate was redissolved in neutral 0·6M KCl, dialysed against 8M urea and subject to electrophoresis. The resultant band patterns indicated that this treatment produced a smaller amount of aggregated protein that could not migrate. Gels F and G (Fig. 1) show the 0·04M KCl washed samples, which were left in 8M urea for two to three weeks. These gels show that myosin aggregates are further dissociated with increased time in 8M urea, since less protein remained at the gel origin than in gels D and E (Fig. 1).

Figure 2 shows myosin subject to electrophoresis in the continuous buffer system on a 2·5% acrylamide matrix in 7M urea solvent. These gels were used to separate myosin subunits and its aggregates. The samples in Fig. 2 correspond to those shown in Fig. 1. They allow further observation of the myosin aggregates (gel C), the effects of washing with dilute buffer (gels D, E) and the further dissociated aggregates obtained upon ageing of samples in 8M urea (gels F, G). These gels show that the continuous 2·5% acrylamide gel and 7M urea treatment enable myosin large subunit aggregates to migrate electrophoretically.

This study showed two methods for increasing the amount of migrating protein. First, washing the myosin in 0·04M KCl three times prior to dialysis against urea resulted in the electrophoretic migration of a larger percentage of the myosin. Second, it was found that prolonged storage of myosin in 8M urea (one to three weeks) resulted in more migrating protein. The mechanism of increasing the amount of migrating protein by prolonged exposure to urea could be due to a time-dependent variable during the dissociation process.

As shown in Fig. 2, the myosin large subunits and aggregates form well-focused bands on using 7M urea for dissociation during electrophoresis on 2·5% acrylamide with a continuous buffer system. This system of electrophoresis for myosin would

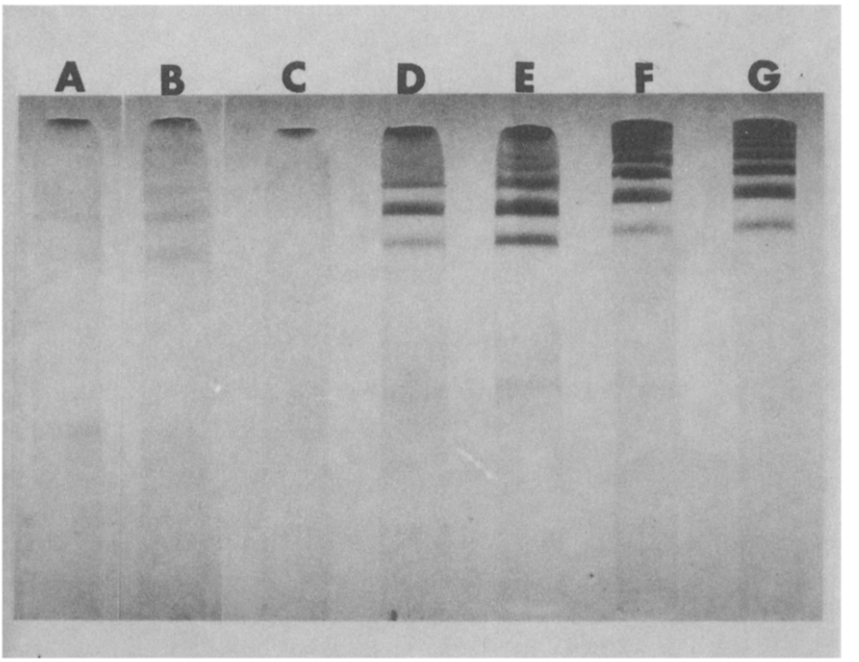

Fig. 2. Electrophoresis of myosin in a 2·5% acrylamide running gel without a spacer gel in the presence of 7M urea, using a continuous buffer system. Legends and protein concentrations for each sample are the same as those given for Fig. 1.

appear to be useful for separating myosin into its subunits and studying the functions of these fractions on the various physical properties of meat. These could include such parameters as water binding, tenderness and emulsifying capacity. The behaviour of myosin aggregates in the presence of urea could also be useful in explaining changes in protein solubility of meat products, such as occur during freezing, cooking and other processing procedures.

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TRANSFORMATION OF CARBOHYDRATE AND NITROGEN COMPOUNDS IN POTATO TUBERS DURING DRYING: PART 1—THE INFLUENCE OF TRITOX APPLIED IN CULTIVATION OF POTATOES UPON TRANSFORMATIONS OF CARBOHYDRATE AND NITROGEN COMPOUNDS DURING POTATO DRYING

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ABSTRACT

The influence of tritox on the concentrations of starch, saccharose, reducing sugars and nitrogen compounds, is investigated in fresh and dried potato tubers. Tritox hinders carbohydrate transformations and enhances nitrogenous transformations. These effects may be enzyme-mediated.

INTRODUCTION

It has been found, in researches carried out so far, that an increase of sugars at the expense of starch occurs during the drying of potato tubers (Kamienobrodzki & Lisinska, 1967, 1968*a*,*b*; Leszczynski *et al.*, 1976). The content of particular nitrogen compounds also changes under such conditions. The amount of transformation of carbohydrate and nitrogen compounds depends on many factors, such as temperature of drying, type of drying gas and time of drying (Lisinska, 1971).

Among other factors influencing the speed and direction of these transformations, the chemicals capable of stopping the germination of potatoes should be mentioned.

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Kamienobrodzki & Lisinska (1968*a*) asserted that Hormonit, Akanit and Depon,* used in autumn, did not stop all the biological changes in tubers being either dried or cooled, because the change starch $\rightleftharpoons$ sugars was even more intensive than in the tubers of potatoes which were not sprinkled with these chemicals.

Zaleski (1964) maintained that the level of boron in soil influences the content of carbohydrate compounds in potatoes, during their storage at low temperatures (+1°C). From Kamienobrodzki & Lisinska's (1967) work it appears that transformations of carbohydrate compounds in potato tubers, both stored at about 0°C and dried at room temperature, go in the same direction. Thus we can expect that Zaleski's suggestion may also apply to the dried tubers.

Taking this into consideration, some new factors relating to the cultivation of potatoes have now been studied. These new factors are: chemicals used during fertilisation and fight against diseases, and noxious insects, as well as the degree of plant virus infection. All such factors can influence the direction and speed of carbohydrate and nitrogen compound transformation in tubers during their drying.

The aim of this work was to study the influence of tritox,* applied in the cultivation of potatoes against potato-beetle, on the changes in content of carbohydrate and nitrogen compounds during the drying of sliced potato tubers at room temperature.

MATERIALS AND METHODS

Potatoes of the 'Lenino' variety coming from the experimental fields of the Institute of Plant Protection at the Agricultural University of Wrocław from the vegetative seasons 1971 and 1972 were used. A suspended preparation of tritox* was used for the cultivation of the potatoes. The detailed data† concerning dose and time of the preparations used were given in a previous work (Leszczynski *et al.*, 1976).

Every year three samples of potato tubers were received from the fields treated or *not* treated with tritox (*controls*). Fifty tubers were taken out of each sample. They were cut into two parts lengthways after they had been washed and cleaned; one part was used for the analysis of fresh potatoes, the other for drying potato slices 3–4 mm thick at room temperature.

In fresh and dried material after a suitable crushing the following compounds were determined:

Dry matter by drying at 105°C up to constant weight.

* Hormonit—5% naphthylacetic acid methyl ester.
Akanit, Depon } West German preparations: chemical formulae reserved by manufacturer.
Suspended tritox extra 50%—17% DDT + 8% lindane + 25% DMDT
DDT = 1,1,1-trichloro-2,2-di-(*p*-chlorophenyl)ethane
lindane = γ-BHC = benzene hexachloride = 1,2,3,4,5,6-hexachlorocyclohexane
DMDT = 1,1,1-trichloro-2,2-di-(*p*-methoxyphenyl)ethane.

† Dose of tritox 2 kg/1 ha = 0·8 kg/acre = 1·77 lb/acre. Applied in July, 1971 and 1972 after hatching of the larvae of potato-beetles.

Starch by Ewers-Grossfeld's (1935) and Kamienobrodzki's (1957) method.
Reducing sugars by the method of Jarosz *et al.* (1955).
Sugars in general by Jarosz *et al.* (1955) method.
Total nitrogen by Kjeldahl's method.
Protein and amide nitrogen by Dobrzycki *et al.* (1958) method.
Amino acid nitrogen by Sörensen's method.
The content of sugars by paper chromatography (solvent of propanol:ethyl acetate:water, 7:1:2, and benzidine reagent for visualisation).
The content of free amino acids by two-dimensional chromatography (Block & Durrum, 1958). Solvent 1—propanol:water (7:3) and solvent 2—phenol: water (7:3) and 0·2% solution ninhydrin in acetone as a spray reagent (Opienska-Blauth *et al.*, 1956). The content of free amino acids was quantitatively determined on a spectrophotometer 'Spekol'.

All the analyses were made in two parallel repetitions, and the average results are shown in the accompanying tables, diagrams and photographs.

RESULTS AND DISCUSSION

In Table 1 the results of starch content, total sugars, reducing sugars and saccharose are shown. These were converted to dry matter of fresh and dried potato tubers, the samples, either treated or not treated with tritox, coming from the vegetative seasons of 1971 and 1972. The content of starch in all the dried samples was lower than the samples before drying. Moreover, the decrease of starch content during drying of the tubers treated with tritox was, in both cases, lower than in tubers coming from the control fields.

The amount of sugars and saccharose increases in inverse proportion to the change of starch content. The changes in the content of reducing sugars in the dried

TABLE 1

THE CONTENT OF CARBOHYDRATES IN FRESH AND DRIED POTATO TUBERS TREATED AND NOT TREATED WITH TRITOX IN THE VEGETATIVE SEASONS 1971 AND 1972 (MEANS OF THREE DETERMINATIONS)

Vegetative season	*Sample type*	*Material*	*Dry matter in %*	*Starch*	*Sugars (as invert)*		
					Total	*Reducing*	*Saccharose*
					In % of dry matter		
1	2	3	4	5	6	7	8
	The control	fresh tubers	23·3	79·5	2·00	0·67	1·33
		dried tubers	88·7	67·7	7·54	0·70	6·84
1971	The sample treated	fresh tubers	25·0	79·3	1·80	0·39	1·40
	with tritox	dried tubers	88·8	69·2	6·83	9·51	6·42
	The control	fresh tubers	18·5	73·9	3·62	2·78	0·84
		dried tubers	90·1	70·5	6·68	2·76	3·92
1972	The sample treated	fresh tubers	22·2	76·0	3·27	1·11	2·16
	with tritox	dried tubers	90·1	74·0	5·00	1·14	3·86

tubers are inconsiderable. Figure 1 shows the decrease of starch content and the increase of sugars in the dried potatoes compared with their concentration in fresh material for the samples either treated or not treated with tritox (and from the vegetative seasons of 1971–72). Figure 2 shows nitrogen changes.

Figures 3 and 4 show chromatograms of sugars contained in the extract of fresh and dried potato tubers treated with tritox (strips 4–6) and those not so treated (strips 1–3). The content of sugars in fresh potato tubers in both samples is the same. Before drying, the tubers contained only three sugars: saccharose, glucose

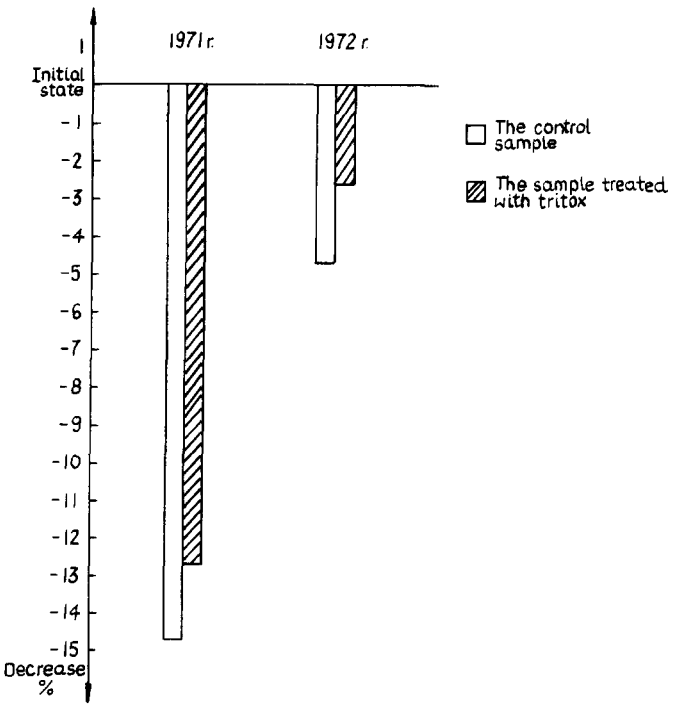

Fig. 1. Changes in the content of starch in dried potatoes, expressed in % in relation to the starch content in fresh material.

and fructose. After drying a number of other sugars occurred in all the samples. One has been identified as maltose. The occurrence of maltose in the tubers under some abnormal conditions has already been discovered in previous work (Lisinska, 1973). In addition, other authors (Habib & Brown, 1957; Schick & Klinkowski, 1961) assume that the occurrence of this sugar in potato tubers is likely.

Changes in the content of nitrogen compounds in tubers before and after drying (Table 2 and Fig. 5) differed from the changes in the content of carbohydrates.

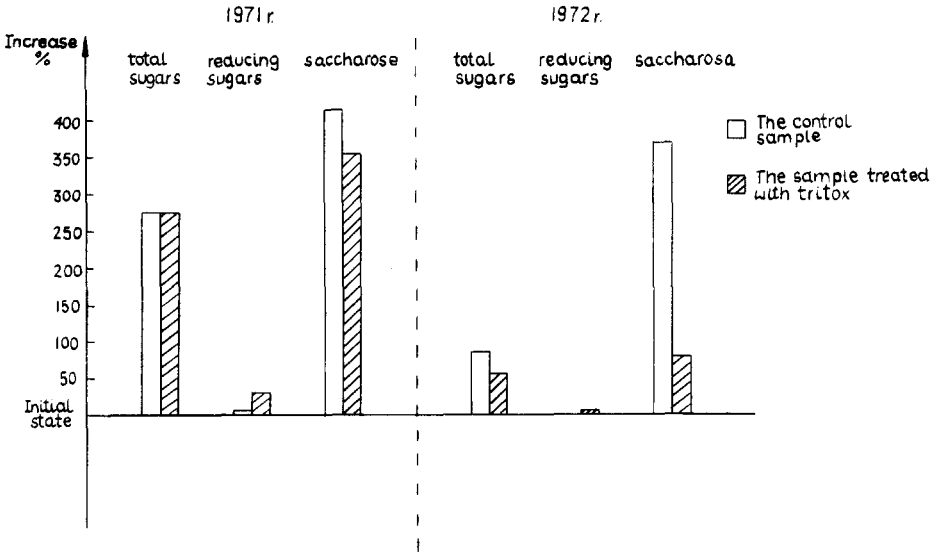

Fig. 2. Change in the content of sugars in dried potatoes, expressed in % in relation to the contents of these sugars in fresh material.

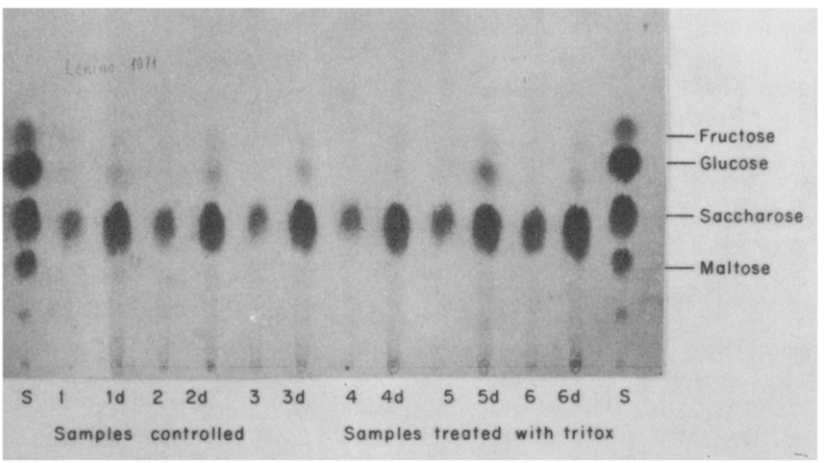

Fig. 3. Chromatogram of sugars contained in extracts from fresh and dried potato tubers treated and not treated with tritox in the vegetative season 1971. S, standard; 1–6, fresh potatoes; 1d–6d, dried potatoes.

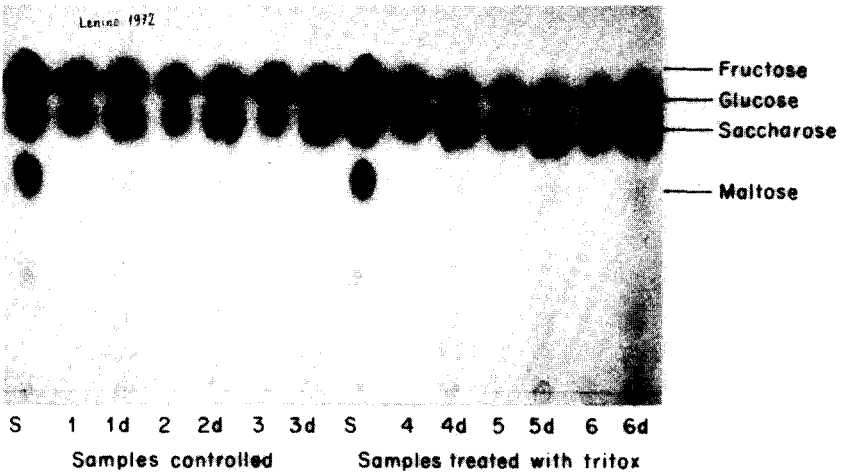

Fig. 4. Chromatogram of sugars contained in extracts from fresh and dried potato tubers treated and not treated with tritox in the vegetative season 1972. S, standard; 1–6, fresh potatoes; 1d–6d, dried potatoes.

They were bigger in the samples treated with tritox than in the control samples.

The content of protein nitrogen in the tubers of the samples coming from 1971 decreased 15% during drying and those from 1972 2%, whereas in the tubers sprinkled with tritox the protein nitrogen content was 18% and 10%, respectively. The content of amide nitrogen was different because the quantity of that compound increased in the dried tubers as compared with the quantity in the fresh ones. The increase in the samples treated with tritox was 12% in 1971 and 43% in 1972, and 14% in the samples of tubers controlled coming from 1971. Only in the tubers controlled in the vegetative season of 1971 did the quantity of amide nitrogen decrease during the process of potato drying.

TABLE 2

THE CONTENT OF NITROGEN COMPOUNDS IN FRESH AND DRIED POTATO TUBERS TREATED AND NOT TREATED WITH TRITOX IN THE VEGETATIVE SEASONS 1971 AND 1972 (MEANS OF THREE DETERMINATIONS)

Vegetative season	*Sample type*	*Material*	*Nitrogen*			
			Total	*Protein*	*Amide*	*Amino acid*
			In mg/100 g of dry matter			
1	2	3	4	5	6	7
1971	The control sample	fresh tubers	1560	1150	95	265
		dried tubers	1590	980	90	215
	The sample treated with tritox	fresh tubers	1540	1020	83	280
		dried tubers	1540	840	93	245
1972	The control sample	fresh tubers	1265	575	85	180
		dried tubers	1350	565	95	170
	The sample treated with tritox	fresh tubers	1230	630	65	190
		dried tubers	1315	565	95	195

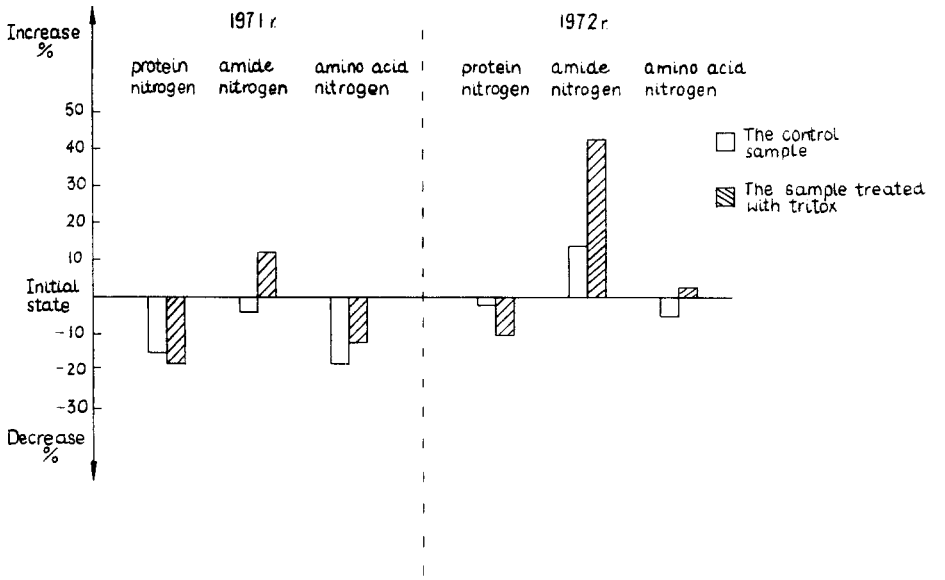

Fig. 5. Change in the content of nitrogen compounds in dried potatoes, expressed in % in relation to the contents of these compounds in fresh material.

The content of amino acid nitrogen changed a little in the dried tubers compared with fresh potatoes.

Table 3 shows the results of quantitative chromatographic analysis of some amino acids and amides in fresh and dried potato tubers either treated or not treated with tritox (coming from the two vegetative seasons). The chromatographic analysis confirms the chemical determinations of amide and amino acids contents obtained. The

TABLE 3

THE CONTENT OF SOME AMINO ACIDS AND AMIDES IN FRESH AND DRIED POTATO TUBERS TREATED AND NOT TREATED WITH TRITOX IN THE VEGETATIVE SEASONS 1971 AND 1972 (MEANS OF THREE DETERMINATIONS)

Vegetative season	*Sample type*	*Material*	*Aspartic acid*	*Glutamic acid*	*Asparagine*	*Glutamine*	*Serine*
			In mg/100 g of dry matter				
1	2	3	4	5	6	7	8
1971	The control sample	fresh tubers	274	144	415	30	48
		dried tubers	103	72	504	133	41
	The sample treated with tritox	fresh tubers	160	134	371	32	76
		dried tubers	112	76	480	182	48
1972	The control sample	fresh tubers	134	441	389	trace	21
		dried tubers	147	164	497	56	38
	The sample treated with tritox	fresh tubers	126	479	425	trace	18
		dried tubers	245	205	432	93	46

content of glutamine and asparagine increased during the drying of tubers while the quantity of acids of these two substances decreased only in the tubers from 1971.

DISCUSSION

On the basis of this study we can state that the potato tubers coming from the experimental fields sprinkled with tritox had a different chemical content than tubers from the control fields. The results shown in the accompanying tables and diagrams show that this preparation applied in the cultivation of potatoes influenced the speed and direction of carbohydrates and nitrogen transformations in the tubers during drying.

A more intensive accumulation of sugars, as well as decrease of starch content, has been observed during the drying of potato tubers coming from control fields as compared with the tubers sprinkled with tritox. On the other hand, the changes of compounds during the drying of tubers which actually gave a decreased amount of protein nitrogen and an increased content of amide nitrogen, were bigger in potatoes coming from the fields sprinkled with tritox.

The observed phenomenon of different intensity in the course of reactions in the two groups of compounds must have been caused by tritox.

The influence of this preparation on the enzymic complex catalysing the carbohydrate and nitrogen transformations could have been initiated during the vegetative period of potatoes. There is also a possibility that in mature potato tubers a residue of an applied pesticide (Drygas & Kroczynski, 1968) occurred, stopping or stimulating the reactions of starch and protein decomposition in the dried tubers.

Bogdarina (1961) assumes the possibility of HCH (hexachloro-cyclohexane) and DDT's (*i.e.* dichloro-diphenyl-trichloroethane) influence in changes of biochemical processes in growing plants, and has noted the increase of non-protein nitrogen content at the expense of protein nitrogen in potatoes after spraying them with insecticides. These preparations cause a loss of balance in biochemical changes of the growing potatoes, and even in the process of drying the tubers, as the experiments show.

The detailed experiments in this sphere will help us to explain the changes observed in the content of potato tubers' components under the influence of the chemicals applied in their cultivation.

CONCLUSIONS

The following conclusions can be drawn on the basis of the results obtained in the course of this study:

1. Tritox applied in the cultivation of potatoes influenced the biochemical processes taking place during the drying of tubers.

2. Changes in the content of carbohydrates were smaller in those potato tubers in which tritox was applied.
3. The content of nitrogen compounds underwent greater changes during the drying of potato tubers from fields sprinkled with tritox than during the drying of the tubers from control fields.

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A COMPARISON OF TWO GLUCOSE SYRUP DRINKS WITH D-GLUCOSE MONOHYDRATE SOLUTIONS AS TEST-LOADS IN THE GLUCOSE TOLERANCE TEST

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ABSTRACT

A solution of D-*glucose monohydrate is a commonly used test-load in the Glucose Tolerance Test, but it is also permissible to use a solution of glucose syrup (WHO,* 1965). *However, since* 1965, *a greater variety of glucose syrups has become available and efforts have been made to define the Glucose Tolerance Test more rigidly. Thus, a considerable section of this paper is devoted, by way of introduction, to clarifying and standardising various details of this test.*

INTRODUCTION

Definitions

Glucose Syrup is defined as a 'purified concentrated aqueous solution of nutritive saccharides obtained from starch' (ISO, 1973*a*) and, in the *Codex Alimentarius*, the main specification gives a minimum Dextrose Equivalent (*see* below) of 20% on a dry basis. It will be noted that the relatively recently developed so-called 'Fructose Syrups' or 'Fructose-bearing Syrups' can be included in this definition. However, at the time of writing, the desirability of having such an embracive definition for glucose syrups is in question. 'No official definition exists for products less than 20% DE (Dextrose Equivalent), but these (materials) are universally referred to as "maltodextrins".' (Wood, 1970).

The reducing power of a carbohydrate is the percentage by mass of reducing sugars, expressed in terms of anhydrous D-glucose, but, when this is expressed in terms of grammes of reducing sugars (expressed as anhydrous D-glucose) per 100 g

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of dry substance, it is known as the Dextrose Equivalent (DE). An over-simplification is that starch ≈ 0 DE whilst anhydrous D-glucose = 100 DE, but, in a glucose syrup, DE is not a measure of the amount of D-glucose present, but rather of the total reducing substances. Table 1 shows a typical pair of glucose syrups illustrating this.

There are two varieties of glucose syrup, each with a DE of 42, one produced solely by acid hydrolysis of starch and the other by acid, followed by enzymic hydrolysis. In the former instance, the DE will be produced mainly by the 18·5% D-glucose and 14·5% maltose whereas, in the latter, it will be produced by the 5·5% D-glucose and 42·0% maltose. In each, additional contributions of less and less magnitude will be made to the reducing power by the tri-saccharides through to the highest saccharides present.

The most reliable method of determining the reducing power is to titrate a test solution at the boiling point against a specified volume of Fehling's solution, using methylene blue as internal indicator. In such a method, standardised conditions are most important, and these have been laid down in a draft proposal for an International Standard soon to be published.

TABLE 1
SACCHARIDES (% W/W DRY BASIS) OF TWO GLUCOSE SYRUPS POSSESSING THE SAME DE

DE	*Type of conversion*	*Mono-*	*Di-*	*Tri-*	*Higher*
42	Acid	18·5	14·5	12·0	55·0
42	Acid-enzyme	5·5	42·0	15·0	37·5

When starch is hydrolysed by acid, the spectrum of sugars will depend on the degree of hydrolysis, but the mixture, when the hydrolysis is stopped to produce glucose syrup, will consist of D-glucose $C_6H_{12}O_6$; maltose, $C_{12}H_{22}O_{11}$, *i.e.* $2C_6H_{12}O_6–H_2O$; maltotriose, $C_{18}H_{32}O_{16}$, *i.e.* $3C_6H_{12}O_6–2H_2O$ and so on, to the very high molecular weight ingredients. A typical 40 DE syrup composition profile is shown in Fig. 1.

With the increase of commercially available enzymes, it is now possible to manufacture glucose syrups of almost any pre-defined composition, but the important points to establish in the context of this paper are that, first, the fructose-bearing syrups are not considered, and, second, that all the glucose syrups are metabolised completely to D-glucose (*see* below).

The word 'glucose' is 'a term not to be used without qualification (for example, D-glucose)' (ISO, 1973*b*); on the other hand, 'Dextrose' is defined as being 'obtained by the complete hydrolysis of a starch, by purification and crystallisation' and can 'be obtained in either anhydrous or monohydrate form' (ISO, 1973*c*). Both the anhydrous and monohydrate forms are the subjects of a monograph in the *British*

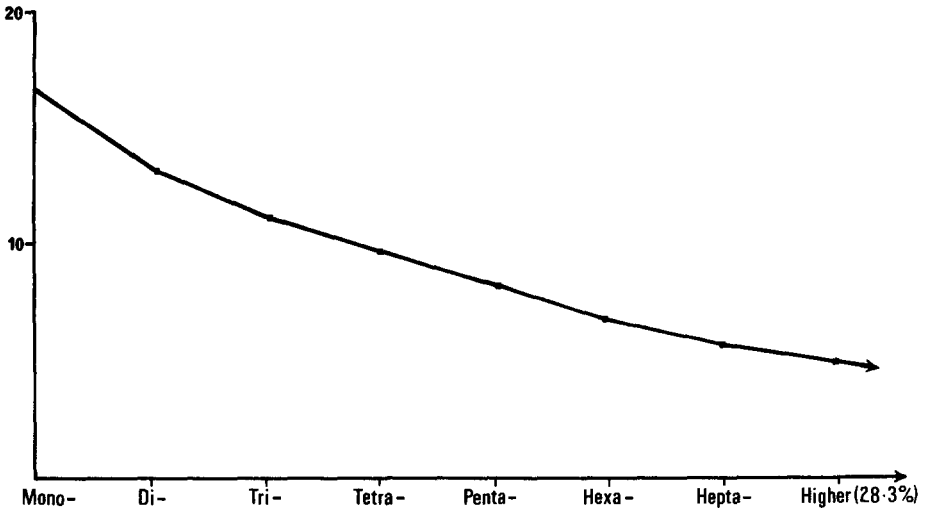

Fig. 1. Typical composition profile of 40 DE glucose syrup (CDT, 1969). Ordinate: % w/w dry basis of each saccharide.

Pharmacopoeia (BP, 1973) and it is interesting to note that the earlier *Pharmacopoeia* (BP, 1968), which also had monographs on both, had the term 'Medicinal Glucose' as an official synonym for the monohydrate. This term has been dropped as a synonym in the most recent edition; nevertheless, the form in which pharmacists and the medical profession usually encounter it is as the monohydrate and, in fact, there is a paragraph in the BP which states: 'When Medicinal Glucose or Purified Glucose is prescribed or demanded, Dextrose shall be dispensed or supplied'.

The Glucose Tolerance Test

When many carbohydrates are ingested, they are metabolised to D-glucose and this is evidenced by a rise in the blood glucose level. (The term 'blood glucose' is commonly used medically and means 'D-glucose in the blood'.) However, in normal conditions, humans also secrete insulin which lowers blood glucose, and this secretion is stimulated by the presence of the blood glucose. In the Glucose Tolerance Test a challenge is given to this control mechanism by the ingestion of a standard quantity of carbohydrate and the blood glucose determined at intervals after the ingestion.

The conditions under which this test is applied are very important and attempts have been made to standardise it (Meinert, 1972). A set of criteria was drawn up as follows:

Subject should fast for 12 h overnight.

Subject should sit quietly for $\frac{1}{2}$ h before blood is sampled.

Blood sampling should be capillary or venous.
Subject should remain seated or reclined throughout, and refrain from smoking.
The oral load (*see* below) should be drunk within five minutes from the moment the subject starts drinking.
Two loads are acceptable, 50 g D-glucose monohydrate given as 10–20% solution, or 100 g D-glucose monohydrate in 400 ml water. Fifty grammes of D-glucose monohydrate may be replaced by an equivalent carbohydrate load (45·4 g) of Liquid Glucose (WHO, 1965).

Liquid Glucose was the subject of a monograph in the *British Pharmaceutical Codex* (BPC, 1963) and *The Pharmacopoeia of the United States* (USP, 1960) but, as noted above, the internationally accepted name for this material, now, is 'Glucose Syrup'.

Work has been done to establish that glucose syrup gives a similar glucose tolerance curve to D-glucose (Butterfield, 1964) and a solution of glucose syrup (as 'Lucozade') has been used regularly in this test (Jarrett & Keen, 1969; Jarrett *et al.*, 1972). The Glucose Tolerance Test usually calls for the determination of blood glucose concentrations after fasting, then ½ h and 2 h after ingestion of a standard carbohydrate load. When criteria on these bases have been set for determining whether a subject is diabetic or not, the Glucose Tolerance Test can be used as a fairly quick sorting test, but doubtful cases may require more careful examination.

The WHO report previously cited recommends that the D-glucose monohydrate solutions shall contain a 'non-caloric flavouring where possible to avoid nausea', but it is common experience that some people, even so, find D-glucose solutions nauseating. No such symptoms have been encountered using glucose syrup solutions.

The standard test load of carbohydrate

As has already been mentioned, either 50 g or 100 g D-glucose monohydrate is recommended, but may be replaced by what was originally known as 'liquid glucose'. This term meant material described in the current *Pharmaceutical Codex* and was, in fact, made by the acid-hydrolysis of starch. Its definition was not particularly specific. As has also been noted earlier, the embracive name today is 'glucose syrup' and, as the ability to analyse these by chromatographic and other methods has improved, it has been possible to define such syrups more closely. Such detailed knowledge of their composition is desirable in order that their equivalence, in terms of D-glucose monohydrate after ingestion, may be calculated. The amount of glucose syrup to be used for the Glucose Tolerance Test can be calculated as carbohydrate solids equivalent to a stated D-glucose monohydrate load, or the amount can be calculated as D-glucose monohydrate *after metabolism* equivalent to a stated load. The volumes of drinks used in this work have been calculated in the second way. It is important to note that the glucose tolerance load is defined in terms of the *monohydrate*.

The method of determination

There have been many methods devised allegedly to determine D-glucose in whole blood, but many of these, in fact, determined total reducing substances, and results were higher than the true D-glucose content. With the development of enzymic methods of determination, it is now possible to use a method which will determine D-glucose specifically. This may be done manually or by autoanalyser. The method depends on a double enzymic reaction:

(i) Glucose oxidase

$$\text{D-glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2$$

(ii) Peroxidase

$$H_2O_2 + \text{phenol} + \text{4-aminophenazone} \rightarrow \text{red dye} + 2H_2O$$

Because glycose syrups are metabolised to D-glucose, the problems presented in the determination of D-glucose in glucose syrups *themselves* do not arise, but it is worth making the point that the glucose oxidase/peroxidase method is not applicable to them. In view of earlier comments about DE, it will be evident that such a determination will not be a measure of the total D-glucose residues present. A further difficulty is that, not uncommonly, glucose oxidase contains maltase and, hence, any maltose present will be determined as glucose. This danger is not present in biological determinations when only D-glucose is present.

METHOD

Six male and six female subjects were tested. The following conditions were imposed:

1. Subjects were apparently healthy and non-diabetic.
2. For three days prior to test at least 510 kcal (136 g) as carbohydrates were ingested daily.
3. Subjects fasted from 21.00 hours on the day prior to test until 09.00 hours on the test-day itself.
4. During the fasting and test period, neither smoking nor excessive exercise was permitted.
5. Samples of blood were taken via cannula. In order to obtain a satisfactory estimate of fasting value, the cannula was inserted intravenously as soon as the subject reported and, soon afterwards, three blood samples were drawn off at 15 min intervals.
6. After the fasting sample had been taken and the test load had been ingested, blood samples were taken every 15 min thereafter for a period of 2½ h.
7. The test load consisted of either 50 g D-glucose monohydrate made up to 250 ml with water or 235 ml glucose syrup drink I (GSD I); or 100 g D-glucose monohydrate made up to 400 ml with water (Lemon acid/flavoured) or 140 ml glucose syrup drink II (GSD II) diluted to 400 ml with water. GSD I was a commercial preparation known as 'Lucozade' manu-

factured by Beecham Products. GSD II was a commercial preparation known as 'Hycal' manufactured by Beecham Products.

8. Each occasion of test on the same subject was at least six weeks apart.
9. Blood samples were taken via the cannula into a disposable syringe, transferred immediately into a disposable plastic tube containing lithium heparin

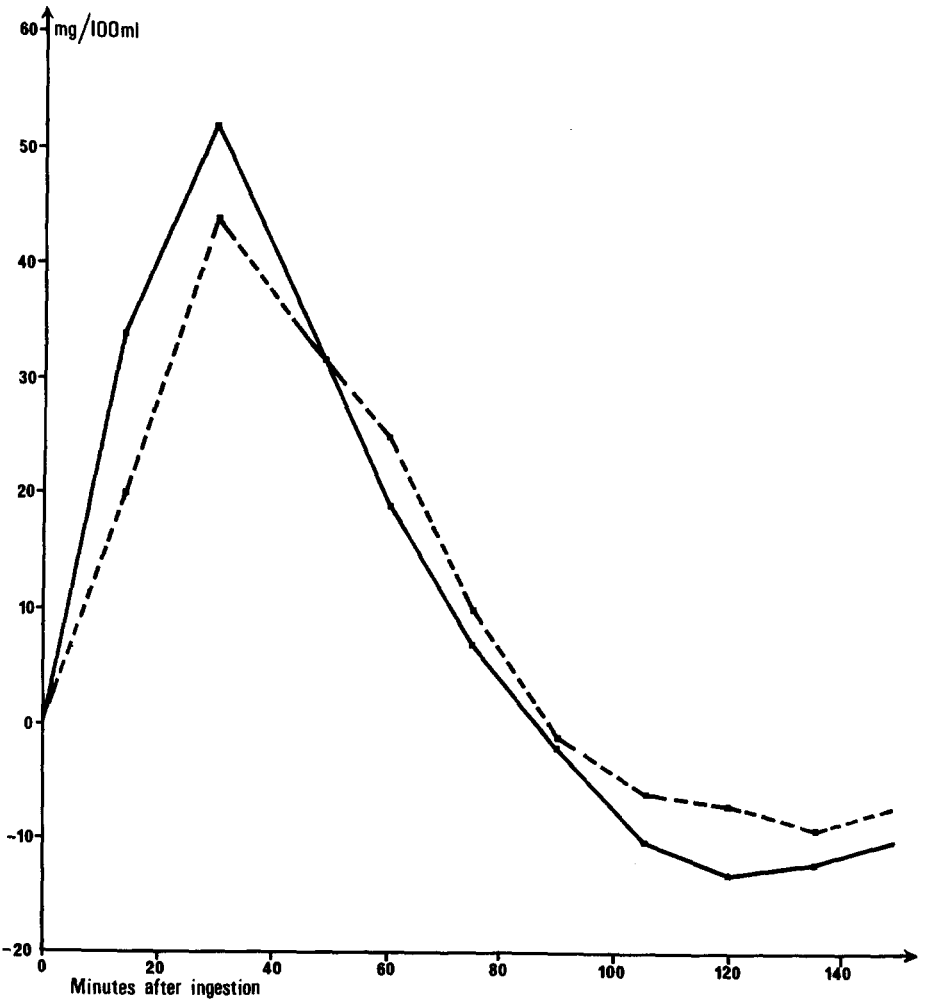

Fig. 2. Plasma glucose differences from fasting concentrations (6 male subjects). ——— 50 g/250 ml D-glucose monohydrate. - - - - 235 ml GSD I.

anticoagulant and mixed gently. To reduce the risk of glycolysis (Overfield *et al.*, 1972) the samples were immediately centrifuged for 5 min at 2000 rpm and the plasma immediately transferred to a clean, disposable plastics tube. This was stoppered and placed in deep-freeze until the glucose determinations were performed by the method of Sokoloff modified for use with Autonalyser II (Werner *et al.*, 1970).

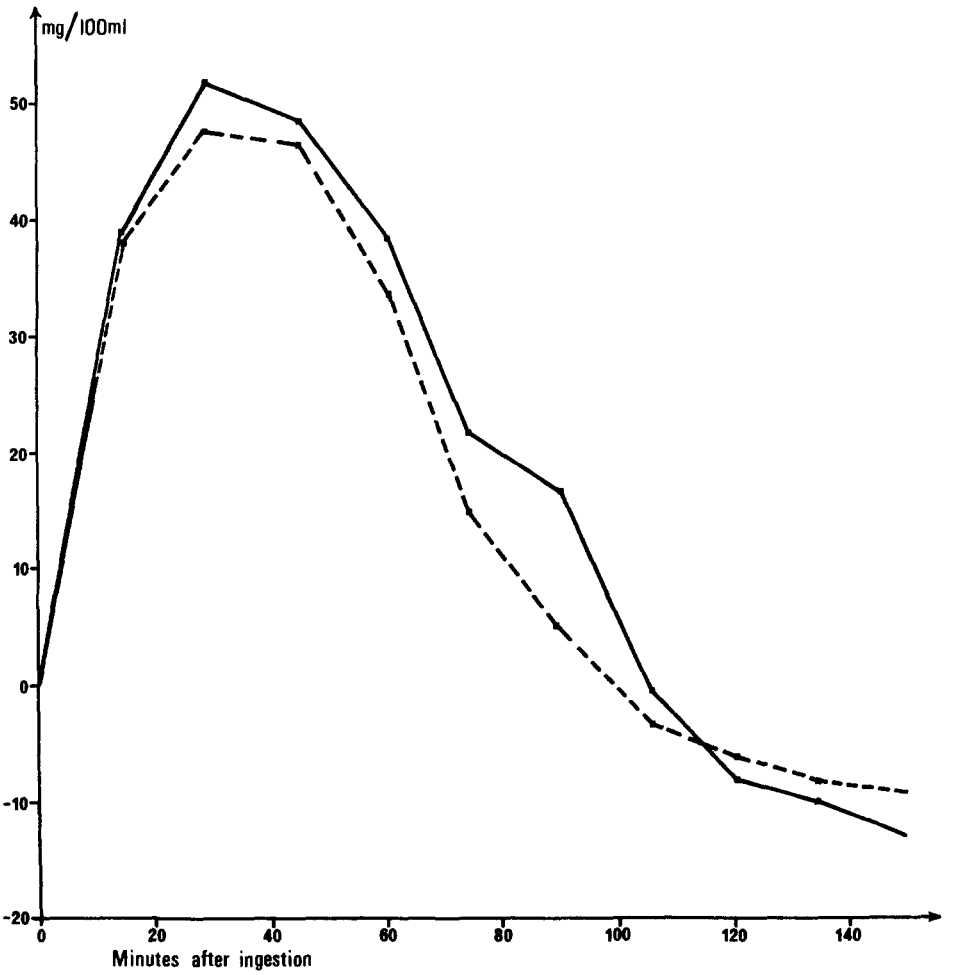

Fig. 3. Plasma glucose differences from fasting concentrations (6 female subjects). ——— 50 g/250 ml D-glucose monohydrate. - - - - 235 ml GSD I.

RESULTS

The data for the 6 male subjects were expressed as differences from mean plasma-fasting concentrations in respect to 50 g D-glucose monohydrate and 235 ml GSD I, and 100 g D-glucose monohydrate and 140 ml GSD II. The data for the 6 female subjects were similarly expressed (in the female comparison of GSD II only 4 females were available). These sets of data are shown in Figs. 2 to 5.

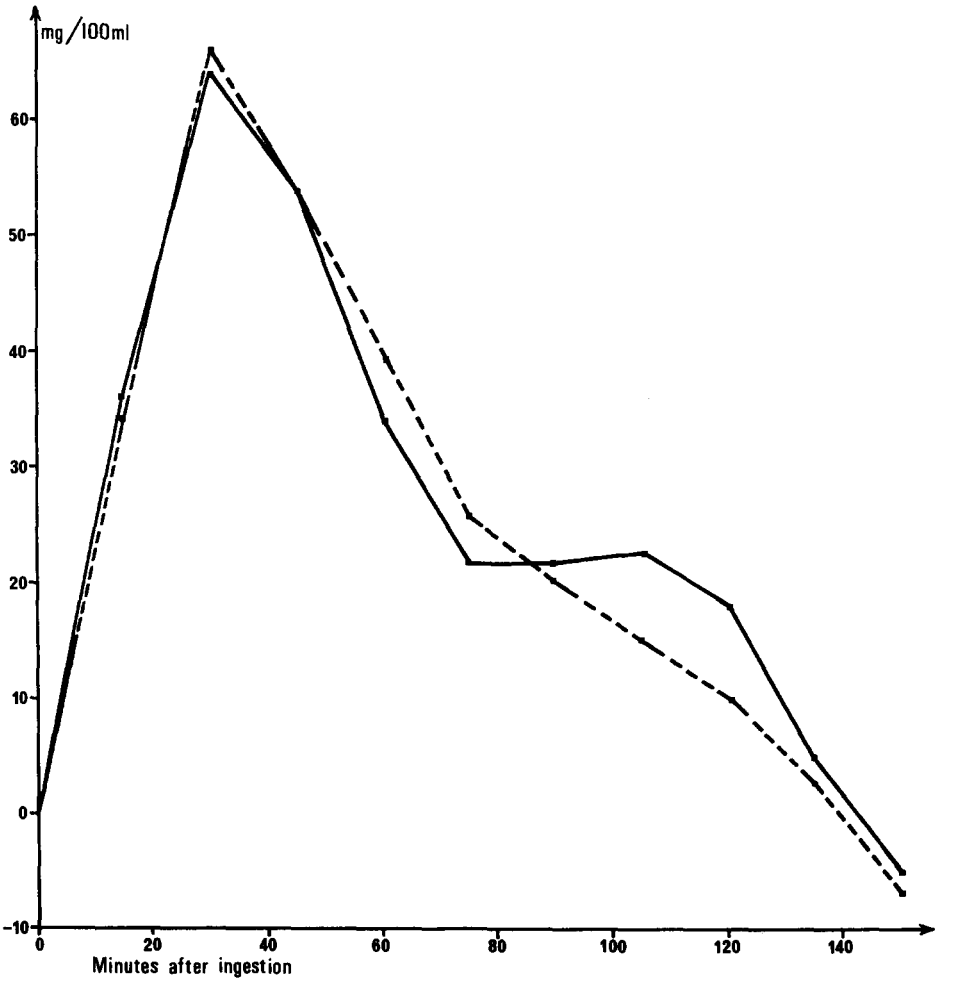

Fig. 4. Plasma glucose differences from fasting concentrations (6 male subjects). ——— 100 g/140 ml D-glucose monohydrate. - - - - 140 ml GSD II.

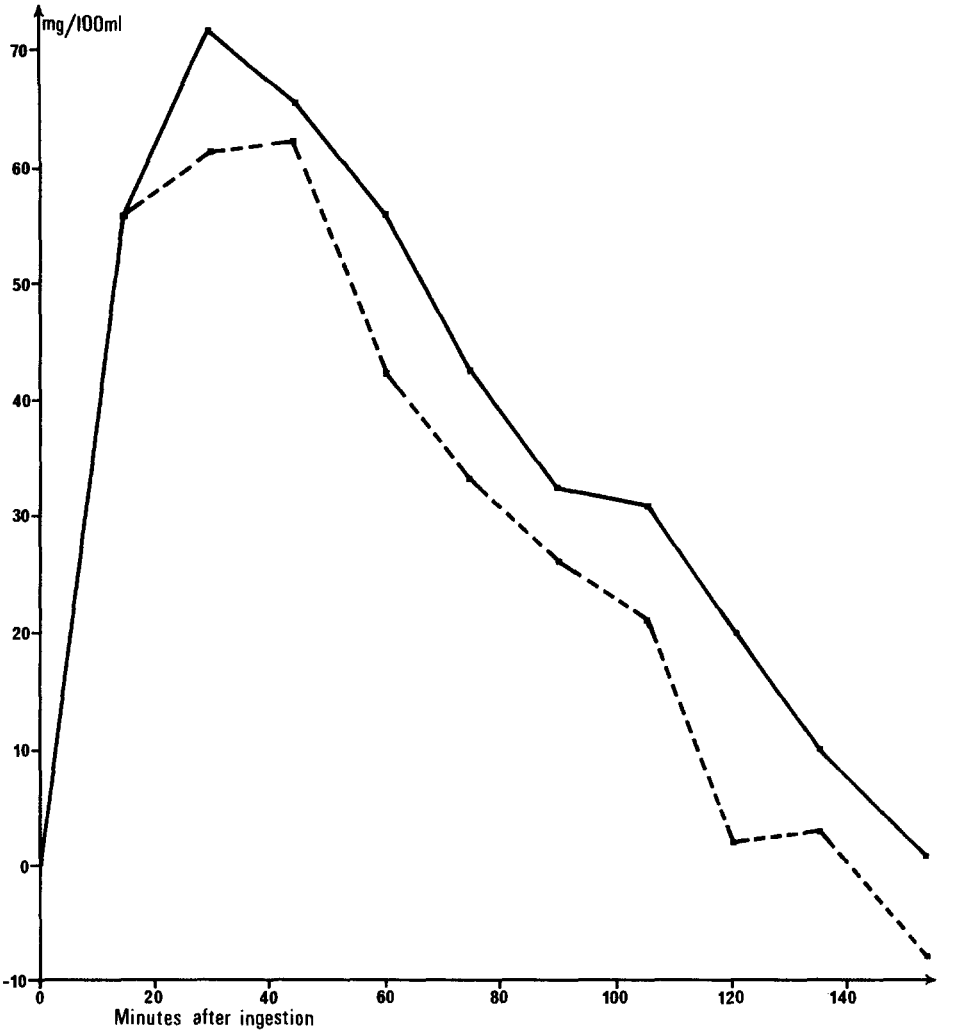

Fig. 5. Plasma glucose differences from fasting concentrations (4 female subjects). ——— 100 g/140 ml D-glucose monohydrate. - - - - 140 ml GSD II.

DISCUSSION

Several workers have reported the variability that can exist between duplicate glucose tolerance curves using D-glucose monohydrate as the test-load (Unger, 1957;

Wast *et al.*, 1964; McDonald & Fischer, 1965) and our results confirm this within-individual variation. There is also a between-individual variability. In the data presented, the D-glucose monohydrate curves are the means of duplicate runs on the individuals tested, whereas the data on the glucose syrups are single runs on the same individuals. Due to the between-individual variation, comparisons were made by relating concentrations to fasting concentrations.

The plasma glucose concentration of a series of duplicate samples was determined covering a wide range of concentrations. The precision of the method was established as $\pm 1{\cdot}4$ mg/100 ml (99% confidence limits). Values were rounded off to the nearest whole number.

In general, subjects preferred taking the glucose syrup drinks and this was particularly noticeable when 100 g D-glucose monohydrate was offered.

For each set of data (Figs 2 to 5) the time (in minutes) to peak plasma glucose concentration and actual peak height (difference from fasting value, mg/100 ml) were considered. Non-parametric statistical tests were used to compare the former, and analyses of variance for the latter. In each case, no statistically significant (95%) difference was shown.

TABLE 2
PERCENTAGE (W/W) SACCHARIDES EXPRESSED ON DRY BASIS

Type of drink	*Mono-*	*Di-*	*Tri-*	*Higher*
GSD I	28·0	22·0	10·1	39·9
GSD II	19·4	14·2	11·8	54·6

Glucose syrups have a number of special characteristics which cannot be matched by equal concentrations of other carbohydrates. They are also available, now, as spray-dried powders and in physical forms achieved by other procedures. The food chemist has before him, therefore, a wide choice. In order to confer certain desirable properties on each product, the two glucose syrup drinks (GSD I and GSD II) under test were manufactured from different glucose syrups. Their differences are shown in Table 2.

This work has shown that with such a range of differences, the blood glucose profiles engendered by their ingestion will be equivalent to those given by D-glucose monohydrate.

ACKNOWLEDGEMENT

This work was done at various Beecham locations and suitable acknowledgement is made to Dr M. Brook, Beecham Applied Research Laboratories, Leatherhead, for permission to publish. GSD I and GSD II were 'Lucozade' and 'Hycal', respectively, kindly supplied by Beecham.

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BOOK REVIEWS

The Role of Vitamin C in Cholesterol Metabolism and Atherogenesis. By E. Ginter. Treatises on Biology of Slovak Academy of Sciences, Vol. 21. 1975. 100 pp.

This monograph is written by a well-known scientist who has for several years been investigating the relationship between ascorbic acid and cholesterol metabolism. That such a relationship exists in the guinea pig seems beyond reasonable doubt, as the monograph states. However, there are two queries which it is not possible to answer at this time. One is whether the findings in guinea pigs apply to man (both need ascorbic acid in their diets) and the other is whether the relationship between ascorbic acid and cholesterol metabolism does, in fact, influence atherogenesis in the human rather than the contrived animal situation.

The monograph describes several animal studies and discusses the kinetics of the cholesterol pools and the author is obviously aware of the limitations of his findings. It is a useful publication for the person involved in this area of metabolism but is of more limited value for the general reader.

IAN MACDONALD

Sugars in Nutrition. Edited by H. L. Sipple and K. W. McNutt. Academic Press, New York, San Francisco, London.

Research in nutrition, especially in that of humans, has not been developed very extensively and I was pleased to find that almost every chapter in this book indicated where research could be applied rewardingly. The book is a set of papers based on an International Conference on Sugars in Nutrition held in 1972 at Vanderbilt University School of Medicine, Nashville, Tenn., USA. There are twelve sections, four of which are devoted to disorders; a further section deals with therapeutic uses, thus making 284 out of a total of 719 pages which are of specialised interest only.

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The remaining sections are concerned with the psychology of sweetness, discussing the biochemistry of chemoreceptors; and food sugars, although mentioning only the common ones, whereas the rarer sugars could have been discussed usefully. The technological developments section (four chapters) deals particularly with glucose syrups containing fructose, materials which, since this Conference and the shortage of sucrose, have achieved commercial and scientific importance. The subject of the digestion and absorption of sugars occupies four chapters, one of which deals with enzyme deficiencies. This section, perhaps above all the others, highlights how much research is still required. It is doubtful whether polyols can be called carbohydrates, yet three of the six chapters on the metabolism of sugars are devoted to these compounds. The last section deals with dental problems, giving them rather less emphasis than they perhaps merit.

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L. F. GREEN

Starch and its Components. By W. Banks and C. T. Greenwood. Edinburgh University Press. 1975. ix + 342 pp.

This book is concerned mostly with chemical and physical studies of starch and the action of various degrading and debranching enzymes which act on starch. The authors are undoubtedly world authorities of unequalled status in this field, and their approach is thorough, fundamental and imaginative. In their introduction they claim interest for those concerned with the many applications of starch, yet admit that the technological and industrial applications of starch are outside the scope of the book. Thus the previous outstanding works of Radley and Knight in these areas are ignored, although that by Kerr is alluded to. The authors naturally emphasise their own extensive works but offer a balanced and reliable criticism of

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all the most significant fundamental advances in starch chemistry. Hence their recurrent tendency to pass judgement on the work of other researchers, far from irritating the reader, serves rather to enliven what might otherwise be an intellectually dense subject. The book presents the most up-to-date knowledge of the two components of starch, amylose and amylopectin. Amylose, which was originally supposed to be a linear polymer, is now known to be lightly branched, and the glucose residues in both polymers are in the preferred and expected $^4C_1$ conformation. The latest evidence for the double helix is also interestingly presented. This may consist of separate chains which are parallel or antiparallel or folded chains in which the double helix must be antiparallel. The account of the relative susceptibility of amylose and amylopectin to acid hydrolysis is logical and focuses (p. 253) on false arguments in the literature which do not take account of the relative number of molecular species of the two polymers. Thus arguments which seek to prove that the bonds in amylopectin are more susceptible to acid hydrolysis than those in amylose are invalidated. As far as enzyme hydrolysis is concerned, mathematical treatment of the action patterns of the amylases is thorough but discussion of the number of glucose residues involved with the active site of the enzyme appears to be lacking. Perhaps this underlines the fundamental chemical approach of the authors because, again, although the chemical modification of amylose is thoroughly dealt with, the treatment is thematically directed towards the structural elucidation of starch, and not the application of the products. The size and shape of starch granules are so distinctive that microscopical examination is often sufficient for diagnosis of botanical origin. The book presents an intriguing and beautiful collection of scanning electron micrographs of starch granules but the biochemical cause of their morphological characteristics is still obscure. This text fulfils its declared objectives and is undoubtedly the best fundamental account of starch chemistry yet produced.

G. G. BIRCH

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An International Journal

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Style for headings

BIODEGRADATION OF PIG WASTE: BREAKDOWN OF SOLUBLE NITROGEN COMPOUNDS AND THE EFFECT OF COPPER

K. ROBINSON, S. R. DRAPER & A. L. GELMAN

School of Agriculture, Aberdeen, Scotland, Great Britain

MATERIALS AND METHODS

Source of materials

Style for references

BOBROV, R. A. (1952). The effect of smog on the anatomy of oat leaves. *Phytopathology*, **42**, 558–69.

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Style for tables

TABLE 1

SOIL PH AND LEVELS OF COPPER, CHROMIUM AND ZINC (DRY SOIL BASIS) EXTRACTED BY 0·1 N-EDTA-NA (PH 4·0) AFTER SIX WEEKS OF INCUBATION OF SOIL TREATED WITH VARYING LEVELS OF DRIED SEWAGE SLUDGE

Treatment	*pH*	*Copper ppm*	*Chromium ppm*	*Zinc ppm*
Control	7·3	9·6	0·1	34
0·5% sewage sludge	7·4	11·2	0·1	40
1·0% sewage sludge	7·6	11·5	0·1	46
2·0% sewage sludge	7·8	11·8	0·1	54

FACTORS INFLUENCING THE METABOLIC EFFECTS OF DIETARY FRUCTOSE*

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ABSTRACT

Dietary fructose, either as such or in sucrose, raises the level of triglyceride in the fasting blood. Several factors can modify this response and among these are the sensitivity and sex of the consumer, the type of dietary fat accompanying the fructose, and whether the fructose is taken as such or in sucrose.

INTRODUCTION

The metabolic effects of fructose are different from those of glucose, notably in the response of the triglyceride level of fasting serum (Macdonald, 1966*a*). The triglyceride in fasting blood is mainly endogenous triglyceride and is a risk factor in coronary artery disease (Carlson & Bottiger, 1972). Patients with hypertriglyceridaemia show a further rise in this lipid fraction when given additional fructose and especially sucrose (Nikkila & Kekki, 1972). It may therefore be of clinical interest to discuss some of the factors that can modify the metabolic response to dietary fructose—taken either as such, or in the disaccharide sucrose.

SEX OF THE CONSUMER

The first suggestion that the lipid response to sucrose was not the same for young women as for men was reported in 1964 (Beveridge *et al.*, 1964). It was found that

* Based on a paper read at a symposium on 'Sugars in Nutrition' held at Nashville, Tenn., USA, in 1972.

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a high sucrose diet raised the fasting serum triglyceride concentration in men but not in young women. This was confirmed soon after (Macdonald, 1965) and was taken a step further by the finding that the triglyceride response of post-menopausal women to dietary sucrose was similar to that of men (Macdonald, 1966*b*). In a more detailed study the component of sucrose responsible for the different effects on serum triglyceride levels was found to be fructose (Macdonald, 1966*a*).

It was obviously of interest to discover which hormone or hormones were responsible for the different lipid response of young women to fructose and to learn whether the difference lay in absorption or in metabolism. Some evidence to suggest that the difference did not lie in the processes of absorption was the fact that when fructose or glucose were given intravenously to non-human primates, a sex difference in the immediate triglyceride response was apparent (Jourdan, 1969).

The difference in the triglyceride response between males and young females to sucrose by mouth was found in monkeys and the rise in fasting levels of serum triglyceride in the males could be prevented by the administration of oestrogen. On the other hand, giving testosterone to the female animals did not result in any rise in triglyceride levels while on a high sucrose diet (Coltart & Macdonald, 1971). These findings would suggest that oestrogen prevents the increase in the triglyceride level in response to sucrose (and, presumably, fructose).

However, this finding is contrary to that found in pregnanacy where there is not only hypertriglyceridaemia but also hypervolaemia (Svanborg & Vikrot, 1965) and also to the finding of a rise in fasting triglyceride level during oral contraceptive therapy (Wynn *et al.*, 1966). A resolution to this apparent conflict has recently been suggested by the preliminary findings that in male rats, the lipid response to sucrose is affected not only by oestrogen and progesterone, but when the two hormones are given together to an animal, the triglyceride response in the liver seems to be opposite to that of either hormone given alone (Jeffreys & White, 1972).

An augmentation effect is seen when the oral contraceptive is given to monkeys on a high sucrose diet. Under these circumstances the level of endogenous serum triglyceride rises more than when either hormone is given alone (Stovin & Macdonald, 1975).

It is for future research to decide where and how the sex hormones operate in dietary fructose–lipid metabolism. The administration of glycerol both acutely and chronically leads to a marked increase in serum triglyceride in men, but a much less marked response is seen in young women (Macdonald, 1970). As α glycerol phosphate is an intermediate in fructose metabolism these findings would suggest that the sex hormones interfere at this stage or later in the formation of the glycerol moiety of triglyceride. On the other hand, Nikkila & Kekki (1971) have shown that young women clear triglyceride from the serum more effectively. As the level of any constituent of the serum is a balance between input and output, it is possible that both factors could be playing a part.

TYPE OF DIETARY FAT ACCOMPANYING THE FRUCTOSE

Although it is frequently necessary, when studying the metabolic effects of a dietary constituent, to give that constituent in large quantities—or remove it altogether—this is not usually comparable with the physiological levels of ingestion. Hence it would seem appropriate to study the effects of dietary fructose in conjunction with fat, especially as it is known that the nature of dietary fat can have profound effects on some aspects of cholesterol metabolism. Endogenous triglycerides are no exception and the increased serum concentration of this lipid fraction brought about by sucrose can be reversed, both in normal subjects (Macdonald, 1967) and in hyperlipidaemia (Antar *et al.*, 1970). The fasting level of serum triglyceride, normally raised by a diet high in fructose, is substantially reduced when the fructose diet contains sunflower seed oil, although the fall is not so marked as in fructose-free diets (Macdonald, 1972). It seems, therefore, that the nature of the dietary fat could be a more important factor than the nature of the dietary carbohydrate, even possibly—although this has not been shown—in the so-called carbohydrate-induced hyperlipidaemia.

EFFECT OF DIETARY NITROGEN ACCOMPANYING THE FRUCTOSE

Although no experiments using dietary fructose with various forms of dietary protein have been carried out, this is not so with sucrose. A rise in endogenous serum triglyceride occurs in man in diets very high in sucrose, whether the protein is calcium caseinate, sodium caseinate, egg albumen or gelatin, but when an amino acid mixture replaces the protein the rise in fasting serum triglyceride levels is very much greater. It can be deduced that this response is in some way connected with fructose because, in the amino acid diet with the fructose replaced by glucose syrup, no such rise in serum triglyceride occurs (Coles & Macdonald, 1972).

STATE OF INGESTED FRUCTOSE

The bulk of the fructose normally consumed by humans is in the disaccharide (with glucose) sucrose. Dietary fructose in the monosaccharide form occurs mainly in honey and fruit. It is therefore of interest to learn whether there are any metabolic differences which depend on whether fructose is consumed as a disaccharide with glucose or *per se*. Certainly the osmotic effects of fructose become apparent in adults when 70–100 g fructose are taken by mouth, whereas no osmotic consequences occur when the same amount of fructose is eaten in twice the amount of sucrose. Consistent with this is the finding in men that the serum fructose level is

significantly higher after ingesting sucrose than after equimolecular amounts of glucose and fructose (Macdonald & Turner, 1968). Any direct effect that fructose has on metabolism can presumably be modified according to whether it is consumed as such or in the form of sucrose.

OTHER FACTORS THAT MAY MODIFY THE METABOLIC EFFECTS OF DIETARY FRUCTOSE

It has been established that the frequency of eating can change the metabolic response (Fabry, 1967) and that the frequency of consuming sucrose affects the rate of increase of triglyceride in fasting serum (Macdonald *et al.*, 1970), a rise that is probably due to fructose.

There is a marked individual variation in the metabolic response to fructose, and this applies not only to triglyceride levels but also to other metabolites such as uric acid (Heuckenkamp & Zollner, 1971) and lactate (Pereira & Jangaard, 1971). There is also a within-individual variation in the response to fructose, because endogenous hypertriglyceridaemia seems to be more frequent in middle-aged than in young men.

Serum fructose levels after ingesting sucrose are higher in men with peripheral vascular disease than in aged-matched controls (Macdonald & Turner, 1971) although it is not known in this instance which is the cart and which the horse.

CONCLUSIONS

There are doubtless many as yet unknown factors that modify the metabolic response to fructose, but those that are known would suggest that not only is it important to know how the body responds to a single constituent of the diet, but it is of equal importance to learn in what way the other constituents of the diet and the nature of the consumer modify this response.

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THE RELATIONSHIP BETWEEN CARBOHYDRATES AND SERUM LIPIDS

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ABSTRACT

Some of the recent work relating to carbohydrate–lipid metabolic relationships is reviewed. Both type and concentration of dietary carbohydrate influence metabolic conversion to serum, hepatic and skin lipids. Total lipids and individual components of lipids may be affected in this way.

INTRODUCTION

Conclusions about the influence of different carbohydrates on lipid metabolism in man and in experimental animals are not unequivocal, despite the ever increasing number of publications on the subject (Anon., 1965, 1966).

The differences in the physiological response to various carbohydrates are due to differences in the chemical composition of the carbohydrates, and some authors consider a high intake of sucrose in the diet to be an important factor in the genesis of cardiovascular diseases.

Sucrose, compared with starch, is a more powerful lipogenetic stimulus, although this conclusion is mainly based on changes in serum lipids (Ahrens, 1957; Hodges & Krehl, 1965; Kaufmann *et al.*, 1966; Macdonald, 1967 and others).

Akinyansjku *et al.* (1967) reported that the substitution of D-glucose for starch in the diet had been shown to raise plasma triglycerides and Fitch and Chaikoff (1960) reported an increase in the activities of several enzymes involved in lipogenesis in the liver.

Cohen & Teitelbaum (1964); Macdonald (1964, 1966), Macdonald & Roberts (1966); Nikkila & Pelkonen (1966), and many others reported that the higher lipogenetic effect of some simple sugars, including sucrose, may be due to the fact that they are absorbed more rapidly and are also more potent stimuli for insulin release, than are others.

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Togel *et al.* (1913) showed that in both animals and man the metabolism of dietary fructose is different from that of glucose.

Nikkari (1965) and Wiese *et al.* (1966) examined the influence of diet on the components of the skin surface fats in animals; Macdonald (1964) reported that a high carbohydrate diet altered the fatty acid pattern and that the nature of this alteration was dependent on the type of carbohydrate eaten. Rosenfeld (1906); Birk (1909); Kuznitsky (1913) and Korolev (1965) investigated the effect of excess fat or excess carbohydrate in the diet upon the amount of the skin surface lipid.

GENERAL METABOLISM

Fatty acid synthesis is largely dependent on glucose oxidation. Glucose can be broken down either by the Embden–Meyerhof route, or by the hexose monophosphate shunt. In diabetes mellitus, since glucose metabolism is retarded the patient is unable to use this shunt.

This is reflected in a reduction of triphosphatepyridine nucleotide dehydrogenaze (TPNH) and reduced glutathione. The hexose monophosphate shunt is more important in the synthesis of lipids since the formation of reduced triphosphopyridine nucleotide (TPN) is mediated via this mechanism and since TPNH is an essential co-factor in the synthesis of fatty acids.

Sipperstein & Fagan (1958) suggested that stimulation of the hexose monophosphate shunt markedly accelerated lipogenesis and cholesterol synthesis.

Cohn & Joseph (1959) force-fed adult male rats a light carbohydrate diet for six to seven weeks, after which they killed the rats and studied their hepatic and epidermal fat tissues. 6-Phosphoglucodehydrogenase activity indicated that, under the force-feeding, the hexose monophosphate shunt was used more extensively. It is possible that feeding excessive calories causes the hexose monophosphate shunt to become an accessory mechanism for converting carbohydrate into lipid.

The metabolism of dietary fructose is different from that of glucose (Togel *et al.*, 1913). In the view of Higgins (1916), fructose shows a tendency to change to fat in the body, whereas glucose tends to change to glycogen and be stored as such.

The presence of fructose in the sucrose molecule would appear to be the cause of increasing lipid metabolism. Hers (1955) and Adelman *et al.* (1967) reported that in rats, uptake of fructose by muscle and adipose tissue is very slow, and most fructose is utilised by the kidney for glucogenesis only when the rat is in a hypoglycaemic state, as reported by Salomon *et al.* (1961).

Feeding rats with fructose enhances the activity of some liver enzymes (Fitch & Chaikoff, 1960) associated with lipid formation, *e.g.* the activity of 6-phosphogluconate dehydrogenase. This generates nicotinamide-adenine dinucleotide phosphate dehydrogenase (NADPH) in the pentose cycle, which is needed for the biosynthesis of fatty acids and cholesterol or the activity of α-glycerophosphate

dehydrogenase involved in the formation of the glycerol component of the triglycerides. Macdonald (1970) also suggested that fructose may increase the production of α-glycerophosphate and this stimulates triglyceride synthesis.

The most tempting theory suggests that a large load of readily absorbable carbohydrates results in temporary hyperglycaemia and this might overload the pathway, necessitating greater use of the hexose monophosphate shunt; and we know that the excessive use of the hexose monophosphate shunt increases the TPNH which, in turn, may enhance the formation of fatty acids and cholesterol.

Atherosclerosis is a metabolic disease which is characterised by alterations in the cholesterol lipid-β-lipo-protein concentrations in the serum. This means that the levels of circulating cholesterol lipids and β-lipoproteins are a sign of the metabolic abnormalities associated with atherogenesis (Katz *et al.*, 1958). The bulk of epidemiological evidence and much current investigation strongly suggest that elevated lipid levels associated with atherosclerosis are related to prolonged habitual consumption of excessive calories, saturated fats, cholesterol and refined carbohydrates.

The evidence for some involvement of sucrose metabolism in atherosclerosis is very strong and it has been concluded that 'a substantial part of ingested sucrose does not reach the systemic blood as monosaccharides but is diverted to the liver, presumably for fat synthesis, and that hyperlipidaemia in human volunteers consuming sucrose-rich diets may also be explained on this basis' (Naismith, 1971).

It has been suggested that the dietary change most likely to be an aetiological factor in the increased incidence of ischaemic heart disease is the replacement, in 'civilised' diets, of a large proportion of starch by sucrose (Yudkin, 1963). Yudkin (1963) also believes that persons consuming large amounts of the latter have a greatly increased chance of developing myocardial infarction (Yudkin & Morland, 1966) and more especially those who consume twice as much sucrose as normal (Yudkin & Roddy, 1964).

Cohen *et al.* (1961) postulated that consumption of sucrose may be responsible for the increased prevalence of diabetes and atherosclerosis in the general populations of western countries.

Starke (1950) fed the 'Kempner' (mainly rice and fruit, low salt, low protein for hypertensive and renal patients) diet to 154 patients with hypertensive cardiovascular disease for 240 days and found that after treatment with the diet the cholesterol values were significantly lower than before treatment.

Keys *et al.* (1960) fed male volunteers with sucrose and simple carbohydrates and they developed high serum cholesterol levels, but when the dietary carbohydrates were replaced by complex carbohydrates derived from vegetables and fruits, serum cholesterol levels decreased.

Staub *et al.* (1967) studied the influence of various dietary carbohydrates on serum cholesterol in rats. Experiments with sucrose, pregelatinised potato starch, glucose and fructose confirmed that animals fed sucrose had higher serum

cholesterol levels than those fed starch and the animals fed glucose did not vary significantly from those fed starch or carbohydrates of various chain lengths.

Winitz *et al.* (1964) reported that when subjects were fed with D-glucose, the serum cholesterol levels fell and when the carbohydrate composition of the diet was changed to 75% glucose and 25% sucrose, cholesterol levels rose and, finally, when the carbohydrate content of the diet was again exclusively glucose, cholesterol levels again fell.

Keys *et al.* (1961) fed middle-aged men with pectin and cellulose for three weeks and in those given pectin there was a significant decrease in the blood cholesterol concentration but no effect in those given cellulose.

Anderson *et al.* (1963) reported the results of two experiments designed to obtain information about the effect on serum lipid of glucose, sucrose, lactose and corn oil, interchanged isoenergetically. When 233 g of glucose per day were substituted for sucrose there was no significant change in serum cholesterol or phospholipid levels but there was a slight increase in triglycerides.

Interchanging 104 g of glucose and lactose resulted in no change in the serum lipids. When 100 g of corn oil was replaced by 233 g of sucrose, serum cholesterol, phospholipids and triglycerides increased by 27, 40 and 87 mg per 100 ml respectively.

It is known that raised levels of cholesterol and triglycerides in the serum are associated with an increase in arterial disease.

Ahrens *et al.* (1961) reported that a raised serum triglyceride level was usually due to the conversion of dietary carbohydrate to fat. Not all people that consume carbohydrate have a raised serum triglyceride level, but it is estimated that approximately one out of every seven males with ischaemic heart disease suffers from induced hypertriglyceridaemia (Nestel, 1966).

Ahrens *et al.* (1961) introduced the concept of carbohydrate-induced lipaemia and included all lipaemic states in which the administration of a diet rich in carbohydrates resulted in a rise in serum triglyceride levels.

Kuo *et al.* (1967) suggested that hypertriglyceridaemia decreased when starch was the main dietary carbohydrate and increased when sucrose was the main dietary carbohydrate. Beveridge *et al.* (1964) reported that the sex of the consumer affected the response to fructose or sucrose in the diet. In young women these dietary carbohydrates are associated with a change or a fall in the serum triglyceride level, but in men and postmenopausal women a rise is seen. Since we know that coronary arterial disease is rare before the menopause this observation may possibly have clinical relevance.

Lang *et al.* (1972) studied the serum lipid levels and atherosclerosis results from diets containing 66% of either simple or complex carbohydrates in male monkeys *Cebus albifrons*, *Macoca arctoides* and *Macoca mulatta*, for a period of 16 months. Although increases of serum cholesterol and serum triglyceride levels were observed in all species, the effects were different in each. In *Macoca mulatta*, the dextrin diet

led to significantly higher levels of serum cholesterol and coronary arterial atherosclerosis, whereas the sucrose diet was associated with significantly reduced glucose tolerance. In *Cebus albifrons*, the dextrin-fed animals had significantly more intimal proliferation of the coronary arteries than those fed sucrose. Both diets were equally atherogenic in *Macoca arctoides*.

The fate of carbohydrates taken with a meal but in excess of immediate energetic needs is an interesting physiological question. It is well accepted that carbohydrates are converted to fat, and this may explain the variations in serum lipids resulting from feeding different carbohydrates. These differences may be related to rates of carbohydrate absorption, utilisation and conversion into fat.

Macdonald & Braithwaite (1964) studied the influence of dietary carbohydrates on the lipid pattern in serum and in adipose tissue. Seven men were fed a basic diet consisting of lean fish, meat, green vegetables, 100 ml skim milk, and as much raw fruit and water as they wished. Each man also consumed 500 g of either raw corn starch or sucrose per day. The study was divided into three twenty-five day periods. The first began with feeding either sugar or starch, the second was a free choice diet period. The final period was a repetition of the first but the alternate carbohydrate was fed. The diets were considered to be adequate. There was a decrease in the total serum lipids and also in the concentration of sterol esters and phospholipids during the starch diet, and an increase in total serum lipids and in the concentration of triglycerides during the sucrose diet. There was a modest decrease in the serum cholesterol level with the starch diet, but no change with the sucrose diet. Hypercholesterolaemia was not a characteristic in these subjects. Both types of carbohydrate diets were associated with an increase in the concentration of palmitic and oleic acids and a corresponding decrease in the concentration of linoleic acid in the serum.

The serum concentration of myristic acid was higher with the sucrose diet, and stearic acid was lower with the starch diet. The effect of varying the source of carbohydrates was most pronounced on the serum lipids which decreased with the starch diet and increased with the sucrose diet. These values were even higher than those obtained with free choice diets.

These observations suggest that dietary sucrose may involve different metabolic processes from starch and that there may be differences in rates of absorption. Portmann *et al.* (1956); Kokatnur *et al.* (1958); Kritchevsky *et al.* (1958); Grant & Fahrenbach (1959), all reported that the occurrence of atherosclerosis depended on the feeding of diets containing generous quantities of both cholesterol and fat, in addition to sugar.

Macdonald (1962) studied the effects of diets on liver and depot fat in rabbits. The experimental diets used were low in protein, high in carbohydrates and had varying amounts of fat. This was intended to stimulate the clinical condition known as kwashiorkor which develops in children who consume excessive amounts of carbohydrate and insufficient amounts of protein. In rabbits so fed, hepatic lipids

accumulated in direct proportion to the mean daily intake of carbohydrates. Fat accumulation was greater when sucrose was fed than when starch was fed. Similarly, the quantity of sterol esters, triglycerides, diglycerides, monoglycerides and phospholipids in the liver all increased when sucrose consumption increased. The metabolic rate seemed to be related to the mean daily intake of carbohydrate. This was reflected in a greater weight loss when more carbohydrate was fed. The fats in the liver contained more myristic and palmitic acids and less linoleic acid as the intake of carbohydrates increased. Linoleic concentrations decreased more rapidly when sucrose was fed. The decrease was not the result of deficient intake of linoleic acid. Thus the lipid response in rabbits to dietary carbohydrate is not only related to the level of intake, but also to the type of carbohydrate consumed.

Birch & Etheridge (1976), reported that in five-day periods, with D-glucose, low molecular weight fractions of glucose syrup (MWFGS) and intermediate MWFGS, serum cholesterol dropped with D-glucose, and low MWFGS, but there was a larger drop with the intermediate MWFGS than with the two others. (Intermediate MWFGS more closely resembles starch than the two others.) Serum phospholipid levels dropped on D-glucose, low MWFGS and intermediate MWFGS, but there was no distinction between the D-glucose and the low MWFGS, and the intermediate MWFGS gave less of a drop in phospholipid concentration In D-glucose and low MWFGS diets, serum triglyceride levels increase, but decrease in the intermediate MWFGS diet. Macdonald (1962) also reported similar findings. Naismith *et al.* (1974) fed weaning rats for 50 days with starch, glucose, maltose, fructose and sucrose. When compared with the effects of the high starch diet, the diets containing the sugars promoted a substantial and similar rise in plasma lipids. Hepatic lipogenesis was increased by all sugars, sucrose and maltose having an equal effect. Lipogenesis in adipose tissue was, however, depressed by feeding sucrose and fructose. The sugars were digested and absorbed much more rapidly than starch. The rate of digestion and absorption of the different carbohydrates might be a major factor in determining their effect on tissue lipogenesis and thus on plasma lipid concentrations. It is concluded that the hyperlipidaemic effect of sucrose is due primarily to its rapid rate of hydrolysis and absorption, and that any differences that may exist between the metabolism of glucose and fructose are of secondary importance.

Fabry *et al.* (1968) examined the effect of feeding frequency and type of dietary carbohydrate on hepatic lipogenesis in albino rats. The rats were fed diets containing 70% starch and sucrose.

Some of them were fed *ad libitum*, while others had access to food only 2 h a day during the six-month period. Fabry *et al.* reported that feeding sucrose, as compared with a starch diet, markedly enhanced lipogenesis of hepatic tissue, assessed by the incorporation of labelled acetate into the total lipids and cholesterol. In restricted feeding of rats there was a higher incorporation of acetate into the total lipids and cholesterol. The highest lipogenic activity of liver slices was thus found in rats where the effect of the sucrose diet and 2 h feeding were combined.

Tepperman *et al.* (1943) reported that the respiratory quotient of rats fed for 1 h/day is higher on a sucrose diet than on a standard diet. Wertheimer & Beutor (1965) found in undernourished rats a greater lipogenesis in the liver and adipose tissue when starch was replaced by sucrose.

SKIN LIPID

Llewellyn (1967) studied the effects in healthy subjects of the ingestion of carbohydrates on skin surface lipid over a three-week period using 18% protein and starch, 18% protein and sucrose and 9% protein and sucrose. Carbohydrate was 82% of the energetic intake, 7 g/kg. He found that in the 18% protein and sucrose diet there was an increase in the total lipid, triglyceride and squaline fraction, and with the 18% protein and starch diet, there was a decrease in the total lipid and triglycerides and an increase in the squaline fraction. In the 9% protein and sucrose diet there was an increase in the total lipid and triglyceride levels, but no significant change in the squaline fraction.

Birch & Etheridge (1976) reported that total skin lipids increase with the D-glucose and low molecular weight glucose fraction diets and drop in the intermediate molecular weight glucose syrup fraction diets. However, the percentage of triglycerides and cholesterol in the total skin surface lipids drop with the D-glucose diet and increase with the intermediate molecular weight glucose syrup fraction diets. There was less change with the low molecular weight glucose syrup fraction diets.

Nikkari (1965) reported that a high carbohydrate diet increases the amount of skin surface lipids in rats.

Rebello & Macdonald (1974) studied the effects of diets containing glucose or fructose on the skin lipogenesis and found that in the consumer, compared with the control, there was an increased incorporation of ^{14}C glucose in the total lipids. A similar effect was also seen using ^{14}C fructose in the fructose diet. There was a significant increase in the incorporation of radioactivity in free cholesterol in the fructose diet, but not in the glucose diet. There was an increased incorporation of radioactivity in the sterol and wax esters in the glucose diet. Both diets showed an increase in the triglyceride fraction which fell after a few weeks.

SUMMARY

(1) The lipid response to dietary carbohydrate is not only related to the level of intake but also to the type of carbohydrate consumed.

(2) Comparing the polysaccharides (such as starch) with disaccharides (*e.g.* sucrose), serum cholesterol, serum triglyceride and serum phospholipid, levels drop with starch and increase with sucrose. Liver lipids such as sterol, tri-, di- and

monoglycerides, phospholipid and myristic acid increase and liver linoleic acid levels drop with sucrose diets, whereas the liver sterols decrease with starch diets.

(3) Serum triglyceride levels increase with glucose and decrease with starch.
(4) Serum cholesterol levels decrease with glucose and increase with sucrose.
(5) Vegetables, fruits and pectin are capable of lowering serum cholesterol levels.
(6) The degree of conversion of carbohydrates into glycerides and free sterols

is greater after feeding sucrose and fructose than after feeding glucose. The amount of glucose converted into liver, and serum phospholipids, is significantly lower than that of sucrose and fructose.

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SULPHUR DIOXIDE AND FOOD PRESERVATION—A REVIEW

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ABSTRACT

The general chemistry, technological properties, toxicity and legislative aspects of sulphur dioxide in food are reviewed. The bulk of evidence supports its continued use.

INTRODUCTION

Elemental sulphur occurs in the earth's crust, especially in the oil-fields of Texas, Louisiana and the volcanic regions of Italy and Japan, to the extent of up to about 0·05%. Sulphur also occurs naturally as sulphates and sulphides (Sidgwick, 1952*a*).

One of the methods of making sulphur dioxide is by burning sulphur, and this has been practised for many centuries. Homer refers to the use of sulphur dioxide fumes as a disinfectant (Kidney, 1973) and it is still used in this way and for the bleaching of various materials such as silk, wool and straw (Kingzett, 1966).

The textbook description of sulphur dioxide is 'a colourless gas, with a suffocating smell . . . readily soluble in water' (Durrant & Durrant, 1972). It can be detected by odour as low as 3 ppm, give rise to throat irritation at 8 ppm and cause coughing and eye irritation at 20 ppm. Some people can detect its presence in certain foods quite easily by its rather metallic flavour, by odour, by a sensation in the nose similar to the onset of a cold, or by sneezing when the food is ingested.

It is intriguing to find, therefore, that this compound has come to be one of the few permitted food preservatives. Nevertheless, it has been used in this way for a long time and 'is particularly valuable because a number of effects are achieved with the one compound where otherwise two or more additives might be required' (ARC/MRC, 1950). Sulphur dioxide is, indeed, used in foods for reasons other than preservation in the anti-microbial sense. For instance, it is used extensively in

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California to preserve dried apricots, where it helps to preserve their ascorbic acid and carotene contents and inhibits the browning which occurs during drying and storage (Stafford *et al.*, 1972). In preserving dried fruits with sulphur dioxide, there must be enough present to maintain physical and nutritional properties, but not so much as to affect flavour (Bolin & Boyle, 1972). It is effective in preventing both types of browning, the non-enzymic (Burton *et al.*, 1963; McWeeney & Burton, 1963) and that due to enzymes, *e.g.* in potatoes (Mapson, 1962). However, it can also have adverse effects, such as the destruction of thiamine activity (ARC/MRC, 1950).

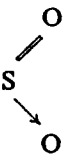
An additive may be intentional or incidental (Food Additives, 1961). Sulphur dioxide may fall into either or both of these categories. For instance, the level of sulphur dioxide in fruit pulp is not to exceed 350 ppm. When a jam manufacturer boils his fruit plus sugar, it is likely that only a small proportion of the sulphur dioxide will remain in the boil. This would be an incidental portion. However, the manufacturer may wish to take advantage of the permitted level of 100 ppm in jam, and add further quantities of sulphur dioxide. This part of the total content would be the intentional portion.

Frequently, in foods, there are present materials containing aldehydic or keto groups with which sulphur dioxide will combine to form complex compounds and some 'sulphited foods have been found to be toxic after storage' (ARC/MRC, 1950).

There are, therefore, good reasons for a food manufacturer to consider carefully whether the addition of sulphur dioxide or its equivalent is the best way to preserve his product. Not only so, but he must give consideration to the possibility of desorption, transfer from a preserved material to an unpreserved material, and losses through packaging materials (Bolin & Boyle, 1972).

Far too often, sulphur dioxide, either as such or as a salt, has been added indiscriminately to food with little understanding of how it acts, its reaction with other ingredients, the effect of compounds formed from it, or its ultimate fate. However, 'foods containing sulphur dioxide . . . have been consumed by man for centuries . . . and a study of sulphur dioxide metabolism in man will probably provide reassurance of its safety in use at levels higher than those currently recommended by FAO/WHO' (Allen & Brook, 1970).

CHEMISTRY

The structure of sulphur dioxide is  (Sidgwick, 1952*b*). It dissolves readily in

water to produce sulphurous acid, H_2SO_3, which is a fairly strong acid, and in alkalis to produce sulphites, hydrogen sulphites and metabisulphites. The alternative forms which may be used legally, with their theoretical and minimum sulphur dioxide contents, are given in Table 1 (Stat. Ins., 1974) but, generally, in this paper, the term 'sulphur dioxide' is used to include sulphites, hydrogen sulphites and metabisulphites.

TABLE 1

CHEMICALS YIELDING SULPHUR DIOXIDE WHICH ARE PERMITTED TO BE USED IN THE PRESERVATIVES IN FOOD REGULATIONS, 1974

Chemical	*Formula*	*Theoretical yield of SO_2, %*	*Min. %*[a]	*Approximate sol'y (g/100 ml)*
Sulphur dioxide	SO_2	100·00	95	11 at 20°C
Sodium sulphite anhydrous	Na_2SO_3	50·82	48	28 at 40°C
Sodium sulphite (heptahydrate)	$Na_2SO_3 \cdot 7H_2O$	25·41	24	24 at 25°C
Sodium hydrogen sulphite	$NaHSO_3$[b]	61·56	60	300 at 20°C
Sodium metabisulphite	$Na_2S_2O_5$	67·39	64	54 at 20°C 82 at 100°C
Potassium metabisulphite	$K_2S_2O_5$	57·60	—	25 at 0°C
Calcium hydrogen sulphite	$Ca(HSO_3)_2$	Solution only[c]	—	—

[a] FAO, 1962.

[b] There is some doubt whether true sodium hydrogen sulphite exists in the solid state and that the material so described is either sodium metabisulphite or a mixture of bisulphite and metabisulphite (Smith & Stevens, 1972).

[c] Defined such that 'not less than 6 and not more than 8 per centum (weight/volume) sulphur dioxide . . . corresponding to not less than 10 and not more than 14 per centum (weight/volume) of calcium hydrogen sulphite, $Ca(HSO_3)_2$' (FAO, 1962).

In view of the alternatives, some care may be necessary in deciding the most appropriate chemical to use in preserving a food.

The general equilibria of sulphur dioxide with water are expressed thus:

$$H_2O + SO_2 \rightleftharpoons H_2SO_3 \rightleftharpoons HSO_3^- + H^+ \rightleftharpoons SO_3^{--} + 2H^+$$

$\longleftarrow$ decrease — pH — increase $\longrightarrow$

HSO_3^- is at a maximum at pH = 4, but $\frac{2}{3}$ or more of free SO_2 is in this form for almost the whole of the pH range 2–5; H_2SO_3 is at a maximum at very low pH values, as shown in Table 2.

Neither the part played by the four species SO_2, H_2SO_3, HSO_3^- and SO_3^{--}, nor their inhibitory concentrations in relation to specific organisms, is known, although combined sulphur dioxide is said to give little or no preservative action (Ingram, 1948; Schroeter, 1966*a*), and this will be considered later in this paper.

TABLE 2
PERCENTAGE FREE SULPHUR DIOXIDE AT DIFFERENT pH

pH	*% Free SO_2*
1	86
2	37
2·5	16
3	6
4	0·5

Sulphur dioxide *can* act as an oxidising agent, being then reduced to elemental sulphur, *e.g.*

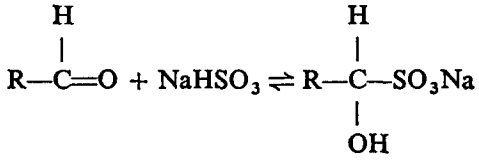
$$2FeSO_4 + 2SO_2 = Fe_2(SO_4)_3 + S$$

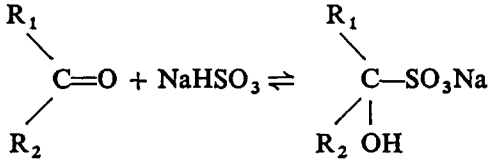
but a more pertinent example is the enhancement of the oxidation of orange oil by sulphur dioxide (Beard *et al.*, 1972). It can, however, itself be oxidised to produce sulphate:

$$2Na_2SO_3 + O_2 = 2Na_2SO_4$$

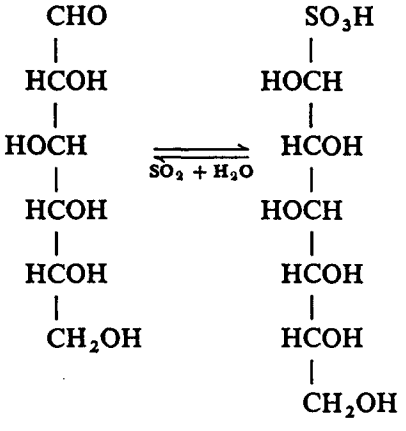
a property which becomes an important consideration in product formulation and packaging (*vide post*), but the reverse process may take place during fermentation and may account for 16–125 ppm sulphur dioxide (Wurdig & Schlotter, 1967).

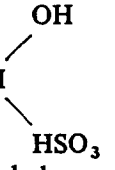
A large number of addition compounds has been established, both with salts and with organic substances, the most important being, perhaps, its combination with aldehydes and ketones:





Many aldehydes, such as citral, $(CH_3)_2.C:CH.CH_2.CH_2.C(CH_3):CH.CHO$, may be present in food flavourings. One of the most important aldehydic materials present in some foodstuffs is D-glucose, either as such or added as one of the constituents of glucose syrup or derived from sucrose by acid-inversion. The following reaction occurs when sulphur dioxide reacts with the open-chain of D-glucose:



and, in this reaction, the maximum proportion of  occurs at

pH = 4·75, but a significant proportion occurs through the whole range of pH 2 to 6. Below pH = 2 and above pH = 6, a large proportion of sulphur is free.

The characteristics of the D-glucose/sulphite system may be summarised as follows:

At near neutrality (say pH = 7·0)

(i) High proportion of SO_2 is in free form.
(ii) Very fast attainment of equilibrium between D-glucose and SO_2.
(iii) 'Free' SO_2 predominates in the SO_3^{--} form with about 2% as HSO_3^-, and none as H_2SO_3.

At pH = 4 to 5

(i) Maximum amount of SO_2 in combined form.
(ii) Relatively fast attainment of equilibrium between D-glucose and SO_2.
(iii) 'Free' SO_2 predominantly in the HSO_3^{--} form, with less than 1% as H_2SO_3, and the remainder as SO_3^{--}.

At pH = 2 to 2·5

(i) Proportion of combined SO_2 still significant.
(ii) Attainment of equilibrium retarded.
(iii) 'Free' SO_2 still predominantly in the HSO_3^{--} form, but 15–35% as H_2SO_3.

At pH = 1

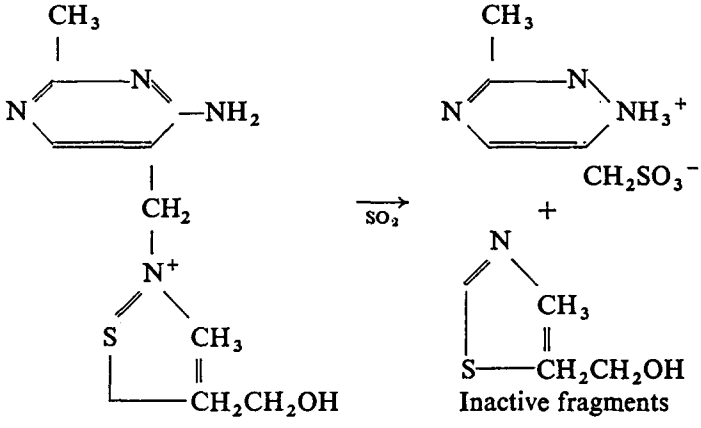
(i) 75% SO_2 in the 'free' form.

(ii) Attainment of equilibrium very slow.

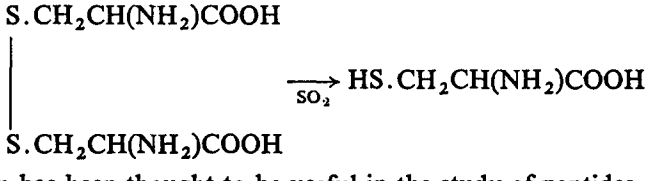
(iii) 'Free' SO_2 predominantly (86%) in the H_2SO_3 form.

A detailed study of the equilibrium between D-glucose and sulphurous acid has been made (Vas, 1949) and the combination of sulphur dioxide with concentrated orange juice has been reported (Ingram & Vas, 1950*a*; Ingram & Vas, 1950*b*), but little information appears to be available about other systems, although aldehydes of lower molecular weight than D-glucose tend to combine more strongly with sulphur dioxide and reach equilibrium more quickly. The effects of sulphite addition compounds, organoleptically and otherwise, are discussed in more detail below.

When sulphur dioxide is added to food containing thiamine, the latter reacts (Schroeter, 1966*b*):



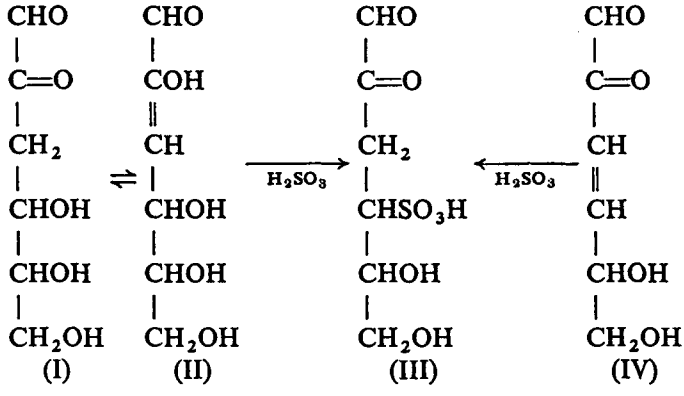
It can also react with cystine to give cysteine and β-amino-β-carboxethylthiosulphate



This reaction has been thought to be useful in the study of peptides and proteins containing cysteine or cystine, but it is also an important consideration from the point of view of food chemistry and the use of sulphur dioxide as a food preservative (Swan, 1957).

Both enzymic and non-enzymic browning have already been mentioned. An example of the former is the browning of an apple when cut. This is due to the action of phenolases which are widely distributed in fruit and vegetables. It is likely that sulphur dioxide inactivates the phenolases (Schroeter, 1966*c*).

The chemistry of non-enzymic browning (such as the Maillard reaction between amino acids and sugars) is a very complicated matter and subject to considerable controversy, but sulphiting 'appears to be a generally effective procedure for the prevention of browning in foods and beverages' (Schroeter, 1966*d*) although it has been stated that 'sulphur dioxide, sulphites and bisulphites thus exert their inhibitory effect by breaking the chain of chemical reactions that leads to the formation of brown pigments' (Ingles, 1966). One mechanism that has been suggested is the formation of very stable sulphonic acid derivatives, as when sulphurous acid combines with D-glucose.



The sulphonic acid so formed (III) has been thought to arise by substitution in 3-deoxyglucose (I) or by adding on to the unsaturated osone (Anet & Ingles, 1964; Ingles, 1966; Ingles, 1962 and Lindberg *et al.*, 1964). Compounds (I) and (III) have both been found in browning systems. It is also interesting to note that these sulphonic acid derivatives do not break down to yield sulphur dioxide in the Monier-Williams distillation, and their formation explains the loss of sulphur dioxide during storage (Ingles, 1966).

The chemistry of non-enzymic browning in foods and the value of sulphites in its control have been reviewed in detail (McWeeny *et al.*, 1974). These authors have also considered the reactions of sulphites, the importance of their observations to food products and what may be the effect of eating such products. They conclude: 'there is still much to be learned about the chemistry of sulphite in food but it . . . is a very versatile product . . . this versatility also implies reactivity and it is important that the implications as well as the applications of this reactivity in food processing are taken into account'.

TECHNOLOGY

In good manufacturing practice 'the use of preservative should not (i) result in deception; (ii) adversely affect the nutritive value of the food or (iii) permit

continued growth of food poisoning organisms whilst suppressing growth of others that would make spoilage evident' (Moses & Pippin, 1970) and it is in these three areas that the food technologist needs to consider, especially, the possible effects of adding sulphur dioxide.

Addition should not result in deception

This desideratum requires considerable thought because the question can be asked: What is deception? When sultanas are heavily sulphited, they are bleached to a light, brownish yellow. Hence, they can be bought either as a darkish-brown fruit containing low sulphur dioxide content, or as a light brownish-yellow variety containing high sulphur dioxide content (permitted up to 2000 ppm). Most of this will be lost when the fruit is cooked, but, interestingly, this variety usually commands a higher price than the darker variety because this is how people like to buy them! In a sense, this is a deception because it is presenting the fruit in an unnatural colour-state, but this is not the implication of this phrase.

When sulphur dioxide is sprayed on minced beef, it achieves a bright-red, fresh looking appearance. However, the instant the preservative disappears, putrefaction proceeds apace. This is deception within the meaning of the phrase, because it presents the meat in an apparently natural state. These two illustrations contrast two practices. True deception is when there is an attempt to present something as harmless when, in fact, it is far from that.

Addition should not affect the nutritive value of the food

Here the food technologist enters a vast area which is largely uncharted. When considering the chemistry of the reactions of sulphur dioxide with other materials, certain well-documented data were given, but the practicalities of such information need to be highlighted. First, however, the food technologist may be concerned not only with the nutritive value of the food—he may not even *be* concerned with this aspect—but also with the organoleptic impact of his product, for, after all is said and done, if the public do not *like* the product they are unlikely to buy it more than once.

Sulphur dioxide forms additive compounds with aldehydes and ketones which are the chief flavouring agents in citrus products, essences and so on. What is the effect on flavour properties when sulphur dioxide is added? This judgement is made more difficult by the taste-threshold value of sulphur dioxide in the material being tested. Most of the work in this area appears to have been done for the wine industry (Schroeter, 1966*e*). Are such addition compounds toxic? The field of enquiry is gigantic although, in general terms, the answer to that question appears to be 'No'.

In the realm of flavouring aldehydes and ketones, the nutritive value has little importance, but when the special instance of an aldose sugar such as D-glucose is to be considered, a change in nutritive value could be important. This is, perhaps,

especially true when thinking about glucose syrups, for these are commonly preserved with up to 400 ppm sulphur dioxide for manufacturing purposes.

When a food is manufactured to contain thiamine, it is clear that the technologist must avoid preservation with sulphur dioxide and will have to have recourse to benzoic acid or to a sophisticated method of manufacture which does not call for a preservative at all. If sulphur dioxide is added to food containing enzyme-protein, then the additive compound must be relatively unstable and the possibility of toxic residues from decomposition must be eliminated (Wyss, 1948).

The addition of sulphur dioxide has been found to be advantageous in preventing or deterring discoloration in a large variety of materials. The nature of such discoloration is not always clear, but, in some instances, may be due to non-enzymic browning. Such materials as a variety of fruits, coconut, preserves, sugars, peppers, tomato purees, cabbage, pumpkins, beans, peas, potatoes, carrots, flour, starch and beer have been listed (Schroeter, 1966*f*).

When the reaction of amino-compounds with carbohydrate has to be considered, the former are often present only in trace quantities. The addition of sulphur dioxide may hold back the reaction causing darkening for sufficient time to ensure an adequate shelf-life to the product, but, again, it is clear that the other consequences of such an addition must have consideration.

When nutritive values are being considered, it is important to know the metabolic fate of sulphur dioxide itself. This has been shown to oxidise to sulphate in the bodies of dogs (Wilkins *et al.*, 1968) but it would be useful to establish the rate of conversion in man (Allen & Brook, 1970). The food preserver cannot shut his eyes to nutrition and his involvement in it. The mechanism of sulphur dioxide oxidation has been studied in diluted catalytic solutions (Junge & Ryan, 1958) but much more is required to be known about the composition of foods (Cook, 1974) and hence the possible interaction between the constituents and sulphur dioxide. Addition should not permit continued growth of food-poisoning organisms yet suppress the growth of other organisms that would make spoilage evident.

The practice (already noted), that used to be prevalent, of spraying minced beef with sulphite liquor in order to retain its bright red colour, is also illustrative of this third requisite.

Sulphur dioxide is not a universal antibacteriocide or antifungicide, and more will be said about this later; but it is sufficient to note here that it is quite possible for organisms to develop an immunity to sulphur dioxide, say, during storage of one ingredient of the food. When this ingredient is incorporated into the food, because of the changed environment, that organism may then start developing and may cause deterioration of the product or the formation of toxic materials.

A food technologist has to consider not only the finished product in its manufactured state, but how it will store. The conditions of storage given to products are not always those advised by the manufacturer, and variations of temperature and the amount of light (*e.g.* in a shop window or on a shop shelf) may play havoc with

the finely balanced chemical equilibria of the original factory-fresh product. For instance, in a soft drink, there may be present, amongst other ingredients, sulphur dioxide, ascorbic acid, colourings, essences, glucose syrup, sucrose, acids and dissolved oxygen—the complexities of such a mixture are almost infinite!

Certain changes can be considered: sucrose may invert to D-glucose and laevulose. Sulphur dioxide does not form an additive compound with sucrose, but it does with D-glucose (an aldose) and with laevulose (a ketone). The process of inversion will depend on the pH of the product, the conditions of storage and, possibly, other factors. A second change that may occur is an alteration in acid concentration due, perhaps, to the interaction of the acid with another ingredient. This means a change of pH and hence a change in the equilibrium and, therefore, of preserving power.

Most citrus drinks have some ascorbic acid present; some have a declared quantity. On the other hand, during a bottling process, it is likely that sufficient oxygen will be included to oxidise at least some of the ascorbic acid. The presence of sulphur dioxide acts as a competitor for this oxygen, thereby leaving the ascorbic acid unchanged (Diemair *et al.*, 1961). However, the sulphur dioxide, in doing so, will change to sulphate and hence lose its preserving power. A nice balance must, therefore, be drawn between such ingredients. Some of these changes may catalyse one or other or both of the reversible reactions:

$$\text{D-glucose} \rightleftharpoons \text{D-glucose sulphite}$$

$$SO_2 \rightleftharpoons SO_4^{--}$$

It has been stated that 'a full insight into the reasons for the specificity of different preservatives against several species of microbes can only be obtained through a thorough study of their metabolism' (Schelhorn, 1953). This must also apply to their susceptibility to preservatives.

'FREE' AND 'BOUND' SULPHUR DIOXIDE

The terms 'free' and 'bound' sulphur dioxide are used in various places in this paper and it should be made clear what is implied by them. In some situations, sulphur dioxide combines to form such compounds as sulphonic acid derivatives. Such compounds are extremely stable to either alkali or acid, and the sulphur dioxide is bound very securely; nevertheless, the term 'bound sulphur dioxide' is not used in relation to such compounds but is reserved for the less stable addition compounds which are affected by pH changes and from which sulphur dioxide can be liberated.

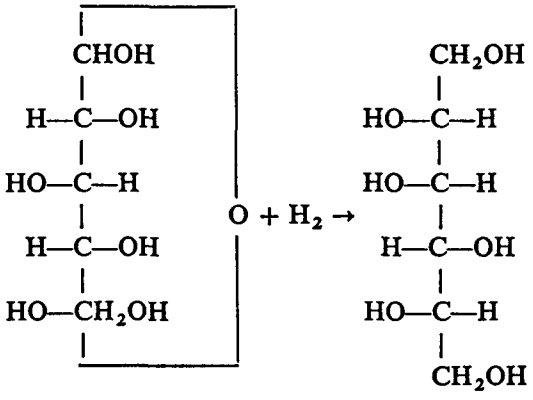
Thus, when sulphur dioxide is added to a food, it may disappear by combination to form a stable compound, or by oxidation; in both instances, it will not be determined by the official Monier–Williams method (Pub. Health, 1927). The sulphur dioxide may also combine in the looser way described above, when it *will*

be determined by the Monier–Williams method. However, such loosely combined compounds do not possess preservative properties, so that it is quite possible to have a large proportion of total sulphur dioxide, as determined by the Monier–Williams method, combined so as to have no preserving action. The extent to which such loose combinations occur depends, as already described, upon pH, so that the terms 'free' and 'bound' sulphur dioxide describe the proportions which are not or are combined in this loose fashion.

The subject has been studied quantitatively in cider. Five compounds were found which formed bisulphite compounds: D-xylose, acetaldehyde, L-xylosone, D-galacturonic acid, pyruvic acid and L-ketoglutonic acid, and their separate contributions to the SO_2-binding power of cider were able to be calculated (Burroughs, 1964).

It is possible, therefore, to have a foodstuff with a high proportion of its determinable sulphur dioxide present in the 'bound' state, and hence not acting as a preservative. This seems to have been recognised in the Labelling of Food Regulations 1970 (Stat. Ins., 1970) where it is stated: 'where any permitted preservative . . . is present in *an ingredient* . . . the presence of that preservative . . . need not be stated' (author's italics).

When glucose syrup is hydrogenated so that the end-chain groups are reduced, D-glucose, for instance, is reduced to sorbitol:



Thus, the degree of reduction lowers the proportion of aldose available for other reactions and could conceivably reduce the total sulphur dioxide requirement without decreasing its preserving power, because there would be less positions on which it could bind.

In general terms, the food technologist's problem is to ensure that the amount of sulphur dioxide added to a material is sufficient adequately to prevent the growth of yeasts, moulds and bacteria, but not so excessive that it will cause the organisms to become resistant to the preservative or, as it were, to force the organisms into a state of dormancy.

ANALYTICAL

It has been stated that 'no entirely satisfactory general procedure has yet been devised' for the determination of sulphur dioxide in foods (Schuller & Veen, 1967); nevertheless, the statutorily accepted method for determining sulphur dioxide in Great Britain is by what is frequently known as the 'Monier–Williams' method (Pub. Health, 1927). This has also been adopted by FAO/WHO (*e.g.* in Recommended International Standard for Glucose Syrup, CAC/RS, 9-1969). However, in practice, there are many variations of the Monier–Williams method and it is probably only in the case of a legal controversy that the strict details of the official method would be followed.

Quantitative methods fall into two groups: those involving distillation and those which do not. The most common of the latter, used for rapid, routine determination, when accuracy is not the chief criterion, is a 'straight' titration with a solution of iodine. Polarographic methods have also been described. When levels are below about 40 ppm, with some materials the weight of sample required to be taken for a distillation method is too great. Under such circumstances, the determination of sulphur dioxide using pararosaniline hydrochloride has been described (King & Pruden, 1969). The method using rosaniline hydrochloride, on which this is based, is, at present, described for the determination of sulphur dioxide in white sugar (Carruthers *et al.*, 1965).

As noted above, the basic distillation method is that of Monier–Williams, but the determination of sulphur dioxide after distillation has been divided into iodometric methods, alkalimetric methods and colorimetric methods (Schuller & Veen, 1967). Many of the modifications of the Monier–Williams method use more sophisticated apparatus than was available in the originators' day (*e.g.* Gothard & Michel, 1972).

A method devised for a quick check for the presence of sulphur dioxide (*e.g.* in minced meat) is to distil in a stream of carbon dioxide engendered by adding hydrochloric acid and marble lumps to the sample in a conical flask, and raising to the boil. The evolving gases are allowed to pass through a mixture of hydrogen peroxide and barium chloride solution. A cloudiness indicates the presence of sulphur dioxide. The method has also been used quantitatively (Parkes, 1926).

Thin-layer chromatographic methods have been applied to preservatives but neither sulphur dioxide nor sulphites are mentioned (Notes, 1971; Tjan & Konter, 1972).

A procedure which appears to be quite reliable for the determination of 'free' and 'bound' sulphur dioxide in soft drinks has been published (Lloyd & Cowle, 1963).

The presence of sulphur dioxide may interfere with the determination of other materials such as cyclamates (Saturley, 1971), a point that may be of importance in the future should these sweeteners become legally permitted again.

MICROBIOLOGY

All three main groups of organism—bacteria, moulds and yeasts—attack food. Bacteria may merely cause deterioration or they may be damaging to human health (*e.g.* botulinus). In some instances, bacteria are deliberately introduced to secure a particular effect, *e.g.* in Yoghourt. Moulds also may be allowed for a particular reason, *e.g.* penicillium in Gorgonzola cheese, but more likely they will cause foods to appear unsightly and, by their own metabolic processes, may cause the food to become toxic. Yeasts are used in fermentation, and produce, in finality, alcoholic beverages, but the adventitious presence of yeasts in food can also cause unwanted fermentation, acid and carbon dioxide formation, foreign to the requirements of the final product.

In a study of soft drinks purchased in 11 European countries, the percentage of drinks containing 10 cultivable yeasts per millilitre was highest (37%) in those bought in France (which contained no preservative), and lowest in Scandinavian products (8%) which did contain preservative. Nearly half the yeasts found were Candida, followed by Saccharomyces. Torulopsis, Hansenila, Crytococcus, Pichia and Rhodotorula were rare. No relationship was found between the bacterial strain and its sensitivity to preservatives, although it is clear that sulphur dioxide was not included in the study (Naarden, 1968). Thus, apart from any effect on the nutrient value and wholesomeness of the food, wastage on a large scale can occur. Whilst sterilisation, refrigeration and heat pasteurisation are amongst the ways that can be adopted to maintain foodstuffs safe for consumption, these may not be adequate or practicable and a degree of chemical preservation will be desirable. However, this should not be used to mask inefficient production and storage procedures.

It is frequently possible to control at a low level the number of organisms in a finished product by care in the manufacturing process. The cleaning of machinery, especially such items as pipelines, pumps and storage vessels, requires special skills and regular attention. It is not uncommon to find that it is better to keep pipelines filled rather than to empty and attempt to clean them at the end of each day's production. Another common practice is to flush out pipelines, etc., with bactericides. There are problems here, too. A universally satisfactory compound for this purpose has not been found; repetitive contact by some organisms with a bactericide can cause them to develop an immune strain. Probably the best procedure is to alternate the type of sterilising solution about every three days.

It is not always realised that food factories do not usually aim to produce *sterile* products. Their aim is to produce food which will not deteriorate; in other words, there will be some organisms present, but they will be inocuous. There are, of course, some food products which have special inherent dangers, such as salmonella in chicken. Frequently, in production control, a bacterial count—not in itself

important because the presence of pathogens is excluded by the character of the food—can be indicative of the state of the plant, and a rise in the usual low level may suggest that cleaning processes have been neglected. If such conditions are allowed to continue, it may not be long before spoilage organisms, moulds and/or yeasts, appear. Because of these facts, there has been emphasis on the impact of chemical engineering on preservation of food (Holdsworth, 1971) and refinements of food preservation have been reviewed (Review, 1968).

When sulphur dioxide is considered as a preservative, especially in processes where much of it may be lost during manufacture, all too frequently there can be a tendency to add increasing quantities to raw materials. This practice is a sad commentary on the precautions taken during manufacture and can have unfortunate repercussions. It is not always appreciated that moulds and yeasts can develop an immunity to sulphur dioxide, or they may spore and remain dormant, even at ever-increasing high levels of preservative. A microbiological examination of such material, on a three-day incubation, may not show a count and the material consequently will be judged satisfactory. Had a longer incubation period been given, the organisms would have become acclimatised to the new environment of the plating medium, and would have developed. When such raw materials *are* passed as satisfactory and used to manufacture a product where, clearly, conditions are different, the organisms will develop and cause spoilage.

Another problem which arises in the microbiological examination of foods is the inhibiting effect of sugar concentration on the growth of such micro-organisms as osmophilic yeasts. It is advisable to dilute such samples before examination.

Sulphur dioxide is not specific for all organisms, and not a lot of data is available. The following have been noted:

(i) Undissociated acid is the inhibiting species for yeasts and is 100–500 times as efficient as benzoic acid against all micro-organisms tested (Rahn & Conn, 1944; Schelhorn, 1953).
(ii) The bisulphite ion is the inhibiting species for B. Coli (Rahn & Conn, 1944).
(iii) The undissociated H_2SO_3 concentration is the controlling factor (Pollard, 1961) and, because of this, mixed preservatives may be preferred.

This third point is recognised in the preservatives regulations current in Great Britain where 'permitted preservative' means 'any preservative specified . . . or any mixture of two or more preservatives' (Stat. Ins., 1974) and may be illustrated by soft drinks for consumption without dilution, where either 70 ppm sulphur dioxide or 160 ppm benzoic acid is permitted. If 40 ppm sulphur dioxide (4/7th of permitted limit) were present, then only 3/7th of 160 ppm benzoic acid would be allowed.

Attempts have been made to express microbiological effects quantitatively. Two

relationships have been postulated:

(i) $C^n t = k$

where C = concentration of germicidal compound, t = time for a certain extent of germicidal effect; k = a constant and n = concentration coefficient.

(ii) $C_0 = 2vt_{\frac{1}{2}} - 1{\cdot}386k$

where C_0 = initial concentration; $t_{\frac{1}{2}}$ = time required for 50% decrease and v and k are constants.

It has been suggested that suppression of spore swelling, which is characteristic of developing spores, can provide an early indication of the potential value of a material as a preservative, but the work was done mainly on the esters of hydroxybenzoic acid (McCafferty & Parker, 1970).

When sulphurous acid combines with carbonyl compounds to produce hydroxysulphonates (l.s.t.) the antimicrobial action is decreased, but the intensity of decrease does not always correspond with the binding relationship. This is because the hydroxy-sulphonates, themselves, may have an antimicrobial action (Rehm, 1964). The same author has shown that sulphurous acid may form an addition compound with the NAD (nicotinamide-adenine-dinucleotide) of organisms, thereby inhibiting their fermenting properties. He has also listed seven 'parts of effects' in the mechanism of the antimicrobial action of sulphurous acid (Rehm, 1973). Free sulphur dioxide has been shown to have 30 times the antimicrobial power of bound sulphur dioxide (Bioletti & Cruess, 1912), but later work suggests the latter is minimal (Schroeter, 1966*a*).

TOXICOLOGY

The possible toxicity of sulphur dioxide in foods was first raised in 1896 (Kionka, 1896); just because a compound is a good preservative, there is no guarantee that it will not be a health hazard. It may, in itself, be toxic, or it may have an effect on the food so as to produce a toxic substance. The metabolic fate of sulphites and sulphurous acid themselves is rapid oxidation to sulphate and elimination in the urine. Adult humans excrete about 2·4 g of sulphate in the urine each day and, in animals, there is a capacity to oxidise sulphites to sulphates (MacLeod *et al.*, 1961). Sulphur-containing compounds such as cystine and methionine appear to be converted to produce sulphites which, in turn, are converted by means of sulphite-oxidase to sulphate, and then excreted (Mudd *et al.*, 1967; Irreverre *et al.*, 1967). The possible risks must be assessed in relation to the likely use of a particular material. For instance, the presence of sulphur dioxide in a food in extensive high use could be a far greater hazard than its presence in one infrequently eaten in small quantities. For this reason, only propionic acid is allowed to be added to bread and no preservatives are allowed to be added to milk; 2-(thiazol-4-yl) benzimidazole is permitted in bananas at not more than 3 ppm; biphenyl, 2-hydroxybiphenyl or 2-(thiazol-4-yl) benzimidazole at not more than 70, 12 or 6 ppm, respectively in

citrus fruit. Dehydrated cabbage, raw, peeled potatoes, dehydrated potatoes and dehydrated vegetables (other than cabbage and potato) may contain up to 2500, 50, 550 and 2000 ppm, respectively of sulphur dioxide, but these limits take into consideration that all these foods are cooked before being eaten, and hence lose most—if not all—of their sulphur dioxide (Stat. Ins., 1974).

Almost every substance is toxic in large enough quantities, hence the levels likely to be consumed have been studied (HMSO, 1959). It is, perhaps, worth commenting that social customs change as to how people treat and eat their food, so that such studies should be brought up to date regularly. It has been reported that 'a large single dose of sodium sulphite can produce irritation of the stomach and intestine, followed by vomitting, diarrhoea and circulatory disturbances' (1959 report) but rats fed on diets containing about 300 ppm for long periods suffered no toxic effects. In such trials it must be appreciated that, as sulphur dioxide destroys thiamine, there is a likely secondary effect that the test animals will suffer or die from thiamine deficiency. When sodium metabisulphite was fed to pigs at a 0·35% level for 48 weeks, no effect was observed (Til *et al.*, 1972*a*). Rats fed sulphite-containing diets could safely ingest about 14 mg/kg body-weight per day (Fitzhugh *et al.*, 1946). When sodium metabisulphite was added to the drinking water of rats, it was found that at least 72 mg SO_2/kg body-weight/day could be safely included (Til *et al.*, 1972*b*). When sulphur dioxide was administered orally to aneurine-deficient rats no influence on the rate of growth of the rats was noted (Lockett, 1957).

The combination of sulphur dioxide with aldehydes, ketones and sugars has already been mentioned. These additive compounds appear to be no more toxic than free sulphurous acid. They behave like the free acid as regards the symptoms they produce, but disassociation of the sulphur dioxide from the parent compound will determine the intensity of action (Rost & Franz, 1904).

It has been demonstrated that sulphites can react with nucleic acid components, and mutations have been produced in Escherichia coli and phagelambda by exposure to *strong* solutions (Mukai *et al.*, 1970) but lower concentrations had no significant effect (Valencia *et al.*, 1972).

A review of the current work on the interactions between sulphites and *medicinal* substances has been made (Smith & Stevens, 1972) and recent toxicological and basic biological studies have also been reviewed briefly (Art., 1973) but the 'immediate relevance of such work to SO_2 and SO_3^{--} concerned in the diet is open to question' and the point is made that 'there are several gaps in our knowledge'.

LEGISLATION

The development of a considered view on the use of sulphur dioxide as a preservative in food tended to progress nationally, but, with the formation of the European

Economic Community and the increasing opportunities for international trade, it became increasingly important to reach common agreement on permitted levels. Some of these negotiations have been dogged by strong partisanship—for instance, the desire of the wine industry to leave as much scope as possible for the addition of sulphur dioxide to wine by reducing the permitted levels in other foods.

The United States Department of Agriculture began studying food adulteration as early as 1883. In 1902, Dr H. W. Wiley fed male volunteers measured amounts of preservatives to determine their effect on the health of the subjects. He concluded that chemicals were often used unnecessarily; that they were deleterious in large amounts and might be injurious even in small amounts, although these levels were not defined; but, in 1907, it was stated that no food manufacturer would be prosecuted if food contained sulphur dioxide at 350 mg/kg provided there was a label declaration of its presence. The present USA food additives regulations list preservatives that are 'generally recognised as safe' (GRAS); sulphur dioxide is one of these (Moses & Pippin, 1970).

In 1924, in Great Britain, a Department Committee on the Use of Preservatives and Colouring Matters in Foods reported:

> '(i) that it is undesirable to add to articles of food any material not of the nature, substance and quality of food;
> (ii) that if for commercial or other reasons the addition is necessary, it should be limited to the minimum required to effect its purpose;
> (iii) that if it can be shown that some of these materials are less undesirable than others, preference should be given to the employment of the less undesirable materials' (HMSO, 1959).

The report concluded that only two preservatives, of which sulphur dioxide was one, should be permitted in food—and then only in a limited number of foods.

In order to estimate the intake of sulphur dioxide, data were obtained from 22 Public Analysts covering the previous two or three years of their duties. From this information, the Committee calculated the approximate quantity of sulphur dioxide likely to be consumed (*see* Table 3).

It was appreciated that cooking considerably reduces the sulphur dioxide content of some foods (for instance, sausages may lose up to 50% of their original content) but it was estimated, on the basis of the then existing regulations and the average weight of food consumed per day, that the intake was about 15 mg per day. The corresponding figure commonly used in the USA (SSS, 1975*a*), but excluding alcoholic beverages, has been quoted as 2 mg, but it is probable that the majority of people take in 10–15 mg sulphur dioxide per day (SSS, 1975*b*). In 1962, the FAO/WHO recommendation for a conditional acceptable daily intake was 0·35–1·5 mg/kg body-weight, *i.e.* about 23–100 mg/man/day (FAO/WHO, 1962). The levels that were permitted as a result of the British enquiries became law (Stat. Ins., 1962). Extra amounts can, of course, be taken by exposure to airborne

sulphur dioxide from various sources (SSS, 1975*b*). In these regulations, the word 'preservative' was defined as 'any substance which is capable of inhibiting or arresting the process of ferment action, acidification or other deterioration of food or of masking any of the evidence of putrefaction' and the definition goes on to make 13 exclusions.

TABLE 3

Food	*mg/day*
Sausages and sausage meat	1·3
Dried fruit	0·3
Raisins and sultanas	1·0
Jam	0·2
Crystallised fruits, etc.	—
Fruit and fruit pulp	1·0
Sugar	0·6
Cornflour	0·3
Fruit juices	0·1
Food eaten in catering establishments (+10%)	0·5
Wine	0·2
Cider	1·4
Beer	4·7
Soft drinks	3·1
Jelly tablets	0·2
Total	14·9

The 1962 Preservatives in Food Regulations were reviewed by The Food Additives and Contaminants Committee whose report came out in 1972 (HMSO, 1972). This Committee kept five considerations in mind during its deliberations:

'(i) most foods are subject to attack by bacteria, yeasts and moulds at ordinary temperatures;

(ii) food manufacture and distribution should be so organised as to ensure that food reaches the consumer in a wholesome, nutritious and palatable state;

(iii) good hygiene and modern packaging are important in the production and distribution of food and help to prevent spoilage and to keep wastage to a minimum;

(iv) both in production and in storage at all levels of distribution, preservation should, as far as possible, be by physical means such as sterilisation, pasteurisation and refrigeration;

(v) the use of specific chemical preservatives, even when safe-in-use and within the limits of any allocated acceptable daily intake, should be allowed only when the desired effect cannot be obtained by manufacturing practices which are economically and technologically satisfactory and where the advantages to be derived from such use would clearly outweigh any possible disadvantage'.

The Committee placed the preservatives considered in three categories:

Group A: Additives that the available evidence suggests are acceptable for use in food.

Group B: Additives that on the available evidence may be regarded meantime as provisionally acceptable for use in food, but about which further information is necessary and which must be reviewed within a specified time.

Group C: Additives for which the available evidence was inadequate to enable an opinion to be expressed as to their suitability for use in food'.

After very careful review of 'sulphur dioxide and sulphurous acid and its sodium, potassium and calcium salts' the Committee concluded: 'The calculated average daily intake of sulphur dioxide per person is thus very near the estimated acceptable daily intake. Because of the uncertainties in estimating this actual dietary intake we would regard it as desirable to see the results of total diet studies on sulphur dioxide in food as consumed by the time of the next review. Information on the effect of ingested sulphite on the various physiological parameters of human respiration is also desirable in view of the sensitivity of chronic bronchitics to atmospheric sulphur dioxide. Nevertheless we are prepared to advise exceptionally that sulphur dioxide and sulphurous acid and its sodium, potassium and calcium salts should remain classified into Group A.'

The new set of regulations, based on this report, came into force on 1 August 1974 (Stat. Ins., 1974). In these, the definition of a preservative was altered to 'any substance which is capable of inhibiting, retarding or arresting the growth of micro-organisms or of masking the evidence of any such deterioration', this time with 15 exclusions. There were a few alterations in the description of some specified foods and one or two additional items, but, broadly speaking, the permitted levels of sulphur dioxide remained unaltered.

The trend internationally has been to review the permitted levels of sulphur dioxide with considerable care and stringency. This has been prompted not necessarily for toxicological or other hazardous reasons, but rather on the argument that its low level in some ingredients will permit a higher level in others. It can also be argued that stringent permissive low levels place a much higher requirement on methods of manufacture to be near-commercially sterile.

As early as 1956, the FAO/WHO recommended to 'collect and disseminate information . . . on the physical, chemical, biochemical, pharmacological, toxicological and other biological properties of individual food additives with particular regard to their absorption, metabolism, excretion, acute and chronic toxicity, and carcinogenic effects in animals, as well as methods of and reasons for their use or the reasons for their limitations or prohibition', and it was agreed to give second priority to preservatives, antimicrobial agents and antioxidants (FAO/WHO, 1956). Later, procedures for the evaluation of the safety in use of food additives were laid

down (WHO, 1958) and amongst the summary and conclusions it was noted that 'the establishment of a uniform set of experimental procedures that would be standardised and obligatory . . . raises many difficulties'. An outline of the procedures for acute short-term and long-term toxicity studies was given.

'An adequate knowledge of the metabolic and biochemical effects of a food additive may provide in some cases a satisfactory background for recommendations on safety in use'.

Because of the tendency to reduce product levels and for FAO/WHO and the EEC to wish to quote analytical methods in commodity standards, a fresh examination of methods of analysis is demanded. It is, however, important to note that 'although there are statutory limits on the amounts of measurable sulphite allowable in foods there is no specific legislative control of the amounts of the compounds formed from the sulphites added to food' (McWeeney *et al.*, 1974). This statement must be related to whether sulphur dioxide is liberated from such compounds by the treatment embodied in the method of determination.

CONCLUSION

That a chemical such as sulphur dioxide cannot be used in food preparation is illogical because such a statement suggests that it is more undesirable to the body than a naturally occurring substance, which is patently not so. It is the physiological action which is of importance. 'The only danger in the use of SO_2 or sulphites as preservatives is in the consumption of high concentrations of them, and provided an adequate margin of safety is allowed—as has in fact been the case—in fixing the permitted concentrations, the danger is remote. The deleterious effect which SO_2 would have upon the flavour and colour of foods if used in disproportionate amounts is an added safeguard against the indiscriminate use' (Banfield, 1952). Although these words were written 23 years ago, they remain equally true today.

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PHYSICO-CHEMICAL CHANGES IN 'SABA' BANANAS DURING NORMAL AND ACETYLENE-INDUCED RIPENING

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ABSTRACT

Physico-chemical changes in 'Saba' bananas, a variety widely used as a raw material for several products in the Philippine food industry, were analysed under conditions of normal and acetylene-induced ripening.

Starch degradation, the formation of reducing and non-reducing sugars and an increase in the moisture content, pectins and acidity of the pulp, occurred during ripening. Acetylene accelerated the rate of chemical change, without significantly affecting the final levels at which chemical constituents were present in the ripe pulp.

Peel colour and pulp texture were both found to be useful indices of the stage of ripeness of the pulp after harvest. Changes in these physical parameters correlated well with the starch and sugar content of the pulp, irrespective of the rate of ripening.

INTRODUCTION

The stage of ripeness of fruit after harvest is an important factor in determining its suitability for processing into various types of product. The major reason for this is that its chemical composition is altered by biochemical processes that accompany post-harvest ripening.

Because most of the food-processing plants in the Philippines are not supply integrated, fruits normally reach the processor at varying stages of ripeness. Under such circumstances, optimum utilisation of a given supply of raw materials requires a food technologist, skilled in product development. Knowledge of the nature of chemical changes in fruits at various stages of the ripening process can provide the basic information necessary in the development of new and better products from many of our tropical fruits.

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Information on chemical changes during post-harvest storage of the banana is extensive only for the two important varieties traded widely in the world market: the Gros Michel and Cavendish varieties. Extensive reviews of the chemical composition and post-harvest physiology of the banana were made by Von Loesecke (1950), Simmonds (1966) and, more recently, by Palmer (1970). Biochemical changes, specifically organic acids, during the ripening of four local banana varieties, namely: the 'Saba', 'Lacatan', 'Latundan' and 'Bungulan' have been reported by Camurungan (1970). Bondad (1972) reported on the post-harvest ripening and degreening of 'Lacatan' banana with the use of 2-chloro-ethyl-phosphonic acid. A report on the chemical composition of unripe (green) and ripe plantain bananas was made by Ketiku (1973). Charles & Tung (1973) investigated the physical and rheological properties of Valery bananas during ripening.

The 'Saba' banana is one of several types known to grow in the Philippines. It is never eaten raw but only after boiling, frying or syruping. In the local food industry the fruit has become an important raw material for essentially starch-based food products like banana chips, banana sauce (known locally as banana ketchup), fried bananas in syrup (or Banana-Q) and bottled boiled bananas in syrup. In general, these products are of good quality but require considerable improvement in order to compete effectively with similar products, marketed locally or abroad.

The use of acetylene, in the form of calcium carbide, to accelerate the ripening of bananas is a wide-spread local practice. Because of this, it was decided to study ripening changes in the fruit under both normal and acetylene-induced ripening conditions.

EXPERIMENTAL

(*a*) *Procurement of bananas*

'Saba' bananas, the variety known as 'Saba' Leyte, were used in this study. Three hundred fingers of commercially mature fruits were harvested from a farm in Trece Martires, Cavite. Commercial maturity was determined with the help of a farmer who could make the assessment by visual observation. The fruit was considered mature when its four angular corners had lost prominence due to increased fullness of the pulp.

(*b*) *Method of ripening*

Two methods of ripening were used: one which we will term normal ripening, and the other, 'acetylene-induced' ripening. Normal ripening was made to occur under conditions existing in the laboratory, 28–32°C, and 64–77% relative humidity. 'Acetylene-induced' ripening was done following the conditions recommended by Guerrero (1969) for the calcium carbide treatment of fruits. Immediately after harvest, the bananas were mixed with 6·0 g of calcium carbide per 20 litres air

space and exposed to the resulting acetylene gas for 48 h. At the end of the 48-h treatment, the bananas were removed from the carbide-containing box and allowed to continue to ripen under the same conditions as in normal ripening.

(*c*) *Physico-chemical analysis*

(*i*) *Sampling and preparation of sample:* Ten fingers were chosen randomly from the batch of 300 fingers. Physical analyses were performed on these ten fingers, after which they were sliced crosswise into thin pieces. Twenty to fifty grammes of the sliced fruit were separated for starch analysis. The rest was further comminuted in a Waring blender. From this, all samples for chemical analysis, with the exception of starch and sugars, were taken.

Sample preparation for starch involved separation of 20–50 g of the sliced fruit above and drying in a forced draught oven at 70°C until a moisture content of about 10% was reached. For sugars, a separate sampling of two fingers per batch was necessary because non-reducing sugars were found to be rapidly converted to reducing sugars as soon as the fruit was cut.

The results shown in the accompanying tables were obtained from duplicate analyses of samples from two ripening trials.

(*ii*) *Starch determination:* Dried material (2·5 g) was extracted with 5–10 ml portions of anhydrous ethyl ether, washed with 150 ml of 10% ethanol (USP 95% by vol) and then with a little stronger ethanol. The mixture was filtered, and starch in the residue hydrolysed according to the method described in Browne & Zerban (1941). The insoluble residue was heated for two-and-a-half hours with 10 ml concentrated hydrochloric acid, cooled and neutralised with 10% sodium hydroxide. It was made up to volume with 200 ml of water. After filtration, a 2-ml aliquot was used to determine glucose by the Folin-Wu method (AOAC, 1970).

(*iii*) *Sugars:* Two fingers chosen randomly from the batch of 300 fingers were peeled, weighed and immediately immersed in 100 ml of water whose pH had been adjusted to 7·5 with 0·1N sodium hydroxide. The sample was boiled for 1 h, macerated in a mortar and pestle, quantitatively transferred to a 500-ml volumetric flask and diluted to the mark with water. Further clarification of the extract and determination of total sugars and of reducing and non-reducing sugars, by the Lane & Eynon volumetric method, was done according to the procedure described by Ruck (1963).

(*iv*) *Pectins:* Twenty to fifty grammes of the blended mixture described in (i) were analysed for pectins by the Carre-Haynes method, as described in Joslyn (1950). The only modification adapted was that the suspension produced by adding 500 ml of water to the blended mixture described in (i) above was filtered to obtain a clear extract, prior to the addition of alkali and precipitation of the pectins as calcium pectate.

(*v*) *Moisture:* Moisture content was determined by drying 5 g of the blended mixture in a vacuum oven at 70°C and 30 psig vacuum for 16 h.

(*vi*) *Protein:* Percent protein was determined on a 5-g sample of the blended mixture by Kjeldahl digestion.
(*vii*) *Fats:* Fat content was determined on the residue left after moisture determination by extraction with ether for 16 h in a Soxhlet apparatus.
(*viii*) *pH:* A Fisher pH meter (Accumet Model 220) was used in the determination of pH.
(*ix*) *Physical properties:* Texture was determined with the use of a precision penetrometer (Precision Scientific Co., Chicago, Illinois, USA), on the peeled pulp. Colour index of the peel was based on a standard colour chart for bananas (Von Loesecke, 1950).

RESULTS

The fruit

The 'Saba' bananas used were thick, relatively short and with angular corners. The length varied from 11·9 to 13·0 cm and width from 4·3 to 5·0 cm. The fruit had an average weight of 60–70 g per finger, of which 30 to 40% constituted the peel.

The peel was completely green when unripe and turned into a dull brownish-yellow hue on ripening rather than to the bright yellow colour typical of most ripe bananas. The pulp was hard and white when unripe and soft and light yellowish on ripening. The seeds were small, well embedded in the centre of the pulp and were normally consumed with the fruit. In some 'Saba' varieties the seeds are larger.

Chemical analysis

Chemical analysis of the pulp and peel of ripe and unripe 'Saba' banana is shown in Table 1. The pulp contained primarily carbohydrates and water. Starch,

TABLE 1
PULP AND PEEL COMPOSITION OF UNRIPE AND RIPE 'SABA' BANANA

Constituents	*Analysis (as % fresh weight)*			
	Pulp		*Peel*	
	Unripe[a]	*Ripe*[b]	*Unripe*[a]	*Ripe*[b]
Starch	31·7	22·1	4·4	2·1
Total sugars	—[c]	11·6	—	4·9
Non-reducing sugars	—	8·2	—	4·1
Reducing sugars	—	3·4	—	0·8
Pectins	0·14	0·31	0·64	0·06
Water	57·68	57·54	82·45	78·80
Protein	1·24	1·17	0·96	1·29
Fat	0·12	0·15	1·44	2·66
Crude fibre	0·54	0·42	2·08	1·93
Ash	0·98	0·91	1·51	2·08

[a] One day after harvest.
[b] Five days after harvest, fruit is ripe enough for cooking.
[c] Presence indicated, but concentration too low to be measured.

the principal carbohydrate, was present at a concentration of 31·7% at the unripe stage. This is considerably higher than that of other banana varieties reported in the literature (Palmer, 1970). Sugar content was significant only in the ripe pulp. The moisture content of the pulp (52·4% when ripe), was lower than the range of moisture contents reported in the literature for bananas (Palmer, 1970).

For the peel, the major constituent was water, 82·45% and 78·80% in the unripe and ripe fruit, respectively. No other constituent was present in significant amounts on a fresh weight basis.

In general, the pulp had significantly higher levels of starch and sugars while the peel contained more of the minor constituents—protein, fat, ash and crude fibre—besides having a higher moisture content.

PHYSICO-CHEMICAL CHANGES DURING NORMAL AND 'ACETYLENE-INDUCED' RIPENING

Table 2 shows the changes in the physico-chemical properties of 'Saba' bananas during normal ripening. Corresponding data for fruits induced to ripen under the influence of 'acetylene' are shown in Table 3.

TABLE 2

CHANGES TO THE PHYSICO-CHEMICAL PROPERTIES OF THE 'SABA' BANANA DURING NORMAL RIPENING

Physico-chemical properties	*Days after harvest* 1	2	3	4	5	6	7	8
PULP[a]								
Starch	31·7	28·4	26·8	24·4	22·0	16·3	9·7	14·4
Total sugars	—[b]	—	1·9	4·6	11·6	18·2	18·8	20·1
Non-reducing sugars	—	—	1·94	4·6	8·2	12·6	11·6	11·6
Reducing sugars	—	—	—	—	3·4	5·6	7·2	8·5
Pectins	0·14	0·19	0·21	0·28	0·31	0·36	0·43	0·92
Moisture	57·68	57·65	57·81	61·22	57·54	58·76	59·01	60·01
Protein	1·24	—	—	—	1·17	—	1·22	—
Fat	0·12	—	—	—	0·15	—	0·13	—
pH	6·4	6·2	6·2	5·6	5·0	4·6	4·6	4·6
Texturometer reading (mm)	25·8	25·5	33·0	31·7	68·8	133·0	147·4	148·2
Texture	firm	firm	firm	firm	soft	soft	very soft	very soft
PEEL								
Colour index	1·0	1·0	1·0	1·5	3·5	4·5	5·5	7·5
Colour	green	green	green but turning yellow green	same	greenish yellow	yellow with green tips	yellow brown flecks	yellow brown flecks
PULP/PEEL RATIO (w/w)	1·16	1·11	1·06	1·70	1·18	1·18	1·44	1·59

[a] Expressed as percentage of fresh pulp.
[b] Presence indicated but concentration too low to be measured.

TABLE 3

CHANGES IN PHYSICO-CHEMICAL PROPERTIES OF THE 'SABA' BANANA DURING ACETYLENE-INDUCED RIPENING

Physico-chemical properties	*Days after harvest* 1	2	3	4	5
PULP[a]					
Starch	33·5	24·2	14·3	12·2	10·0
Total sugars	1·9	10·2	17·7	20·1	21·5
Non-reducing sugars	1·9	7·9	12·9	12·0	12·1
Reducing sugars	—[b]	2·4	4·9	7·9	9·4
Pectins	0·10	0·20	0·38	0·40	0·41
Moisture	56·57	59·57	60·69	60·08	61·98
Protein	1·20	—	—	—	1·24
Fat	0·21	—	—	—	0·15
pH	6·1	5·0	4·7	4·7	4·6
Texturometer reading (mm)	24·5	77·9	132·2	136·8	152·6
Texture	firm	soft	soft	soft	very soft
PEEL					
Colour index	1·0	3·0	4·5	6·0	7·0
Colour	green	yellowish	yellow with green tips	yellow with brown flecks	yellow with brown flecks
PULP/PEEL RATIO (w/w)	0·97	1·00	1·20	1·35	1·35

[a] Expressed as percentage of fresh pulp.
[b] Presence indicated but concentration too low to be measured.

(*a*) *Starch*

Rapid hydrolysis of starch, the major respiratory substrate, is evident in both Tables 2 and 3. The total starch content decreased from 31·7% in the unripe pulp to 14·4% after eight days of normal ripening and from 33·5% to 10·0% after five days of 'acetylene-induced' ripening. 'Acetylene' thus accelerated the rate of starch degradation without significantly affecting the magnitude of the overall change in starch content during the ripening process.

The exact role of acetylene in fruit ripening has not been very much studied. It is known to exhibit changes similar to those which are produced by the better-known hydrocarbon gas, ethylene, at lower concentrations (Palmer, 1970). The latter is presently believed to act primarily as a ripening hormone, triggering, in some still unknown way, the biochemical reactions responsible for the overall changes that occur in the fruit during the ripening process (Palmer, 1970). It is possible that the 'acetylene' used in this experiment contained traces of ethylene.

(*b*) *Sugars* (*Total, reducing and non-reducing*)

Sugars are known to be formed in bananas during ripening from the hydrolysis of starches. Table 2 shows that the non-reducing and reducing sugars in 'Saba' bananas increased from very low levels to 11·6 and 8·5%, respectively during normal ripening. Sugars were present even in newly harvested pulp but rapid formation did not take place until after the fourth day of harvest. This period corresponds to the

time immediately following the reported climacteric rise in bananas (Palmer, 1970; Bondad, 1972).

On treatment with acetylene the increase in sugar content took place immediately and at a much faster rate. As shown in Table 3, the final total sugar content attained at the end of the ripening period was only slightly higher for the 'acetylene-ripened' pulp compared with that not similarly treated.

(*c*) *Pectins*

Total pectins increased progressively from 0·14% to 0·92% in eight days of normal ripening and from 0·10 to 0·41% in five days, after acetylene-induced ripening. The difference in final levels of pectins obtained could be due to slight differences in the stage of ripeness of the pulp. As can be seen from Tables 2 and 3, the pectin content of the normally ripened pulp, when taken on the seventh day after harvest, is very similar to that of the 'acetylene-induced' ripened pulp on the fifth day after harvest.

(*d*) *Moisture*

The moisture content of the pulp of the normally ripened and 'acetylene-induced' ripened 'Saba' increased over the same range (Tables 2 and 3).

This increase in moisture content is expected, as water arising from the osmotic withdrawal of moisture from the peel is reported to exceed the net water lost in bananas due to transpiration (Von Loesecke, 1950).

Unlike other studies on bananas, however, which report a gradually increasing moisture content of the pulp on ripening, the pattern of moisture changes observed for 'Saba' was initially erratic and started to increase regularly only after the fifth day of ripening, when significant amounts of sugars had already been formed in the pulp (*i.e.* on the fifth day). This could indicate that osmotic withdrawal of moisture from the peel had a very significant effect on the net increase in moisture content of the pulp.

(*e*) *pH*

The pH of the pulp of normally ripened banana decreased from 6·4 to 4·6 and from 6·1 to 4·6 when exposed to acetylene. This increase in organic acidity is due to the expected formation of Krebs cycle acids. Specifically for the 'Saba' banana, malic and citric acid is reported to increase three to four times its original value (Camurungan, 1970). For the normally ripened fruits, an abrupt decrease in pH occurred on the fourth to sixth days of post-harvest storage (Table 3) which coincide with the period immediately following the onset of the sudden outburst in sugar content in the ripening pulp.

In 'acetylene-induced' ripened 'Saba', the decrease in pH took place immediately (Table 3), just like other chemical changes already previously noted. Acetylene treatment appears to have had no effect on final acidity.

(f) Colour and texture

As can be seen in Tables 2 and 3 and in Figs 1 and 2, the expected degreening and softening of 'Saba' on ripening started to occur after the fourth day in the normally ripened bananas and after the first day in the acetylene-induced ripened samples. As can be seen in Figs 1, 2 and 3, the patterns of peel colour and textural changes are remarkably similar to the pattern of sugar formation in the normally-ripened pulp, in that significant amounts of sugar did not occur until after the

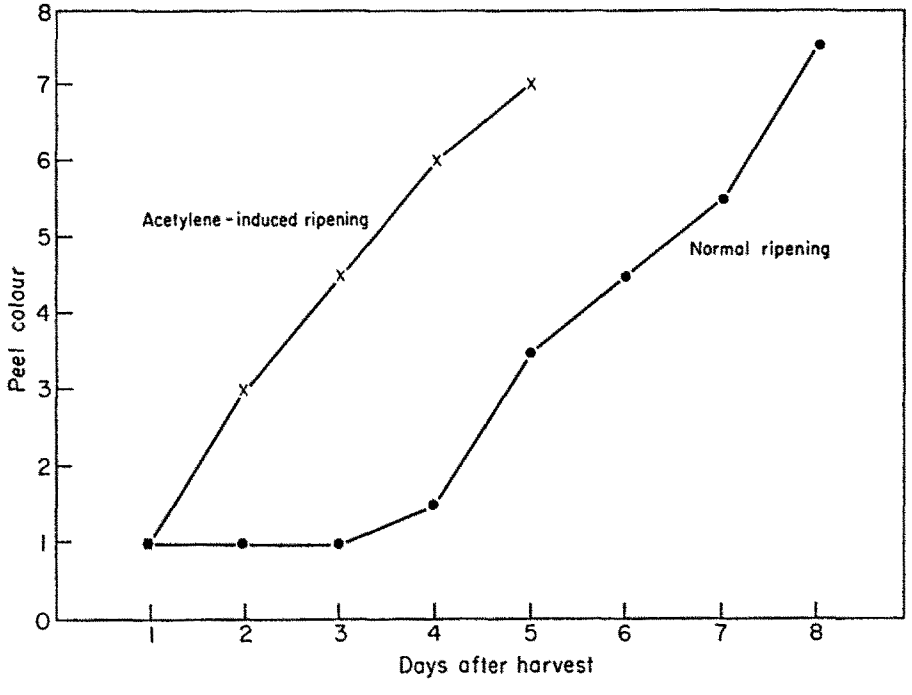

Fig. 1. Peel colour changes in 'Saba' bananas during normal and 'acetylene-induced' ripening.

fourth day of harvest. Marked changes in peel colour and pulp texture also coincided with the time during which marked changes in other chemical constituents took place in the pulp.

Correlation coefficients calculated between starch and sugar content and colour and textural changes were high for both normal and 'acetylene-induced' ripened samples. Values of 0·95 and 0·98 were obtained for the correlation coefficient between texture and starch and total sugars, respectively and of 0·89 and 0·95 for the correlation coefficient between colour and starch and total sugars. Camurungan (1970) has reported that textural changes are useful indices of the stage of ripeness

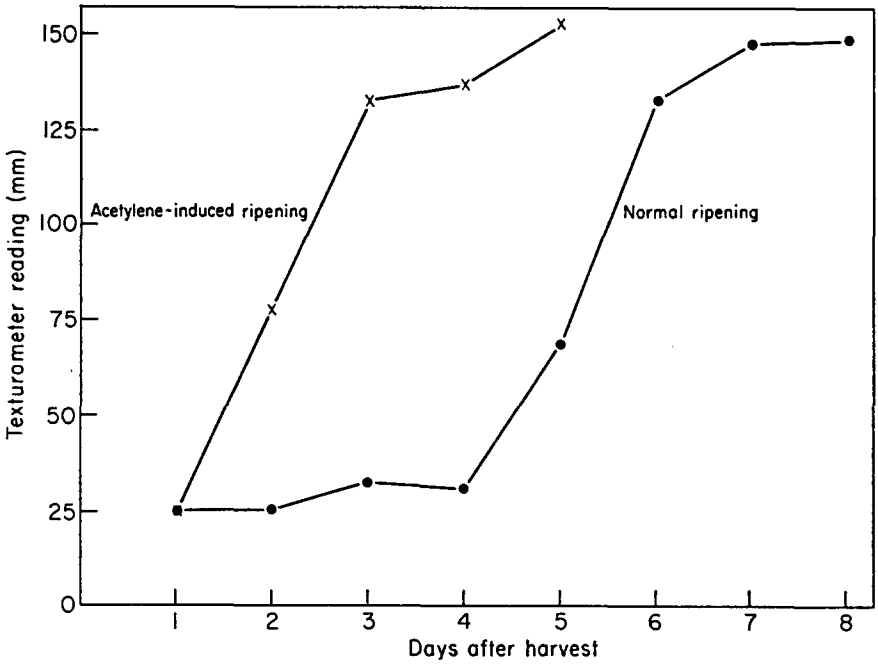

Fig. 2. Texture changes in 'Saba' bananas during normal and 'acetylene-induced' ripening.

of fruits after harvest. In this work we found that both texture and colour changes can serve as useful indices of chemical changes during ripening.

(*g*) *Pulp/peel ratio*

As shown in Tables 2 and 3, the pulp/peel ratio was initially erratic and showed a progressive increase only after significant amounts of sugar had been found in the pulp. This pattern of change closely resembled that for changes in the moisture content of the pulp.

DISCUSSION

Typical of other banana varieties, carbohydrates formed the major constituent of 'Saba' banana pulp. Starch content was very high when unripe—31·7%—while sugar content reached a maximum of 20·1% when ripe. As expected, during the ripening process there was marked degradation of starches, formation of sugars and increases in acidity and total pectins.

Acetylene treatment after harvest, which is a widespread local practice, accelerated changes involved during ripening, without significantly affecting the final level of chemical constituents present in the pulp, after it ripened.

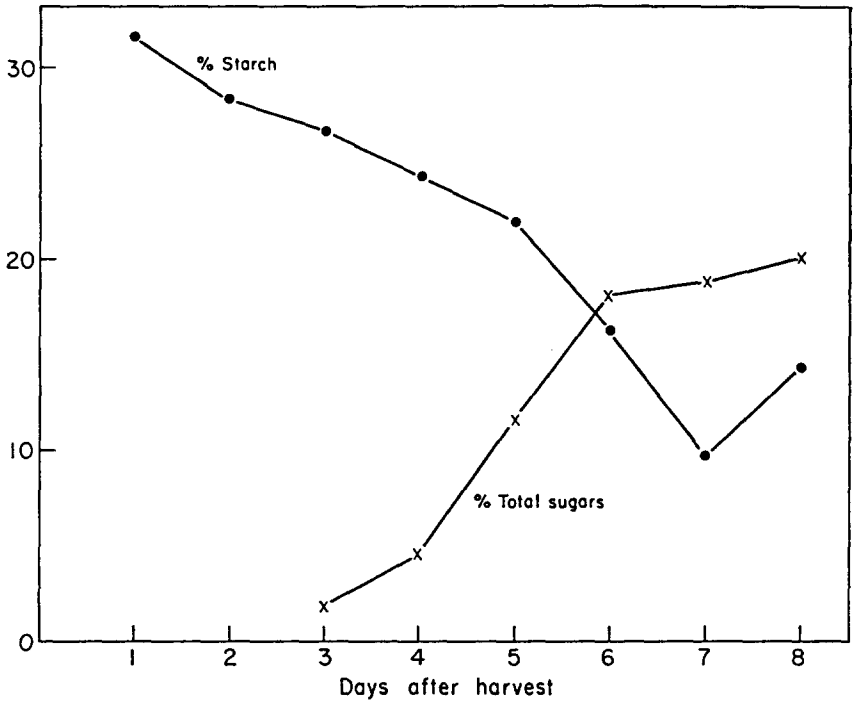

Fig. 3. Changes in starch and sugar content of 'Saba' bananas during normal ripening.

Starch content of the fruit, as seen in Fig. 3, decreased progressively from the first day after harvest. Parallel to this, the formation of sugars occurred largely on the fifth day after harvest, and reached a maximum of 20·1% at the overripe stage. For 'acetylene-induced' ripening, starch degradation and sugar formation took place immediately after harvest (Fig. 4).

While these changes are typical of gross biochemical changes known to occur in bananas during ripening, they are reported for the first time for 'Saba' bananas. The nature and magnitude of these chemical changes provide important basic information for product development.

For products such as banana sauce, banana flour and banana chips, for which a high starch content is desired during processing, and for some of which a high sugar content would create undesirable processing problems, the fifth day after harvest would represent the fruits' maximum desired level of ripeness. For other products such as candied banana products, for which the full banana flavour is essential, maximum ripeness, during which maximum sugar formation occurs, must be attained. Sugars appears to be important to the overall flavour of 'Saba' banana, as the fruit is relatively more bland than other banana varieties.

The fruit was found to be low in pectins. Although the latter increased during ripening, the final levels obtained do not compare with those of other local fruits which are known to be good sources of pectins.

Fats and proteins are minor constituents and, as expected, remained as such during the ripening process.

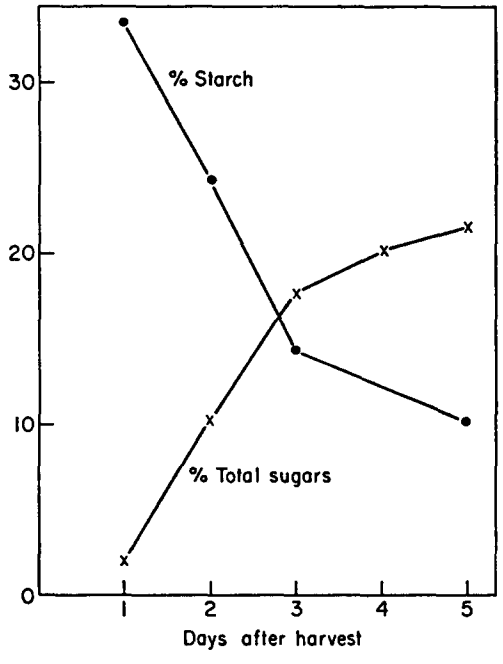

Fig. 4. Changes in starch and sugar content of 'Saba' bananas during 'acetylene-induced' ripening.

The acidity of the pulp increased with increasing level of ripeness. The final acidity obtained at the ripe stage, however, still classifies the banana in the category of low-acid foods.

Chemical changes acquire little practical importance unless they can be correlated with physical parameters that can be more readily measured. This is especially significant when one considers that slight changes in maturity can nullify the use of 'days after harvest' as a convenient index of the stage of ripeness, and thus of the level of chemical constituents in the pulp, at any prescribed stage of ripeness. Thus attempts were made to correlate texture and colour of unpeeled banana with the level of its two major constituents—starch and sugars. The correlations were found to be highly significant, indicating the possible usefulness of physical indices in gauging the chemical composition of the pulp.

SUMMARY

Analysis of the physico-chemical constituents of 'Saba' bananas during normal and 'acetylene-induced' ripening indicated the following:

(1) The pulp of 'Saba' bananas contained primarily carbohydrates and water. In the unripe pulp, the principal carbohydrate was starch (31·7%) and in the ripe pulp, sugars (20·1%). The non-reducing sugars were present at higher concentration than reducing sugars.

(2) The pulp contained more starch and sugars than the peel, which contained higher levels of the minor constituents: fat, crude fibre and ash.

(3) Chemical changes accompanying ripening consisted of the conversion of starch to sugars and an increase in the pectin content, acidity and moisture content of the pulp.

(4) Moisture content of the pulp increased during ripening, whilst that of the peel decreased. Osmotic withdrawal of moisture from the peel appeared to play a major role in the increase in moisture content of the pulp on ripening.

(5) Treatment of the fruit with acetylene was found to bring about ripening changes similar to that produced during ripening, by the better known hydrocarbon gas, ethylene. The mechanism of action for the process, which at present is still unknown, could very well be similar for the two gases or indeed could be due to small concentrations of ethylene in the acetylene used. Acetylene treatment accelerated the rate of ripening, without significantly affecting the final starch and sugar content of the ripe pulp.

(6) Peel colour and pulp texture correlated well with changes in starch and sugar content of the pulp under both conditions of ripening. These physical parameters can thus serve as useful indices of the stage of ripeness of the fruits after harvest.

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STUDIES ON PARBOILED RICE—PART 2: QUANTITATIVE STUDY OF THE EFFECTS OF STEAMING ON VARIOUS PROPERTIES OF PARBOILED RICE*

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ABSTRACT

A study was made of the effect of steaming on the solubilisation of the starch in paddy rice and the relative crystallinity and milling yields of the dried product.

The rates of gelatinisation and solubilisation of the starch were extremely dependent on steaming pressure. Solubilisation continued long after gelatinisation was completed and was a more useful index of processing treatment. The relative crystallinity due to amylose complex formation was highly significantly and positively correlated ($r = 0{\cdot}960$) *with the extent of solubilisation achieved during steaming. Slight steaming markedly increased milling breakage and yields were only improved in relation to unprocessed rice after the starch had been completely gelatinised.*

INTRODUCTION

During the soaking stage of parboiling, rice paddy is hydrated so that sufficient water is present to allow subsequent gelatinisation. Soaking is usually carried out at a temperature below that of starch gelatinisation in order to minimise splitting of the kernels.

During the steaming stage gelatinisation and solubilisation of the starch take place but these changes have not been followed quantitatively. Previous investigators have relied on the cold or warm water solubility of starch or amylose in the dried, powdered product (Roberts *et al.*, 1954; Dimopoulos & Muller, 1972) which gives erratic results (Ali & Bhattacharya, 1972). The effectiveness of parboiling in reducing milling breakage is well known but has not been related quantitatively to either gelatinisation or solubilisation achieved during steaming.

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The first part of this paper demonstrated the presence of an insoluble amylose complex in parboiled rice. The present study was carried out to obtain quantitative data on the degree of gelatinisation and solubilisation of the starch achieved during steaming, and to ascertain their relationship to amylose complex formation and milling breakage.

MATERIALS AND METHODS

Materials

Paddy rice, variety Ribe, was used. The amylose content of its starch was 18·1%.

Methods

Parboiling procedure: Fifty grammes of paddy were soaked in 500 ml of distilled water at 50°C for 4 h and drained. It was then steamed at the desired pressure in a Prestige pressure cooker and cooled rapidly with 250 ml of cold water. After draining, the steamed rice was dried at 30–35°C for 48 h in a forced circulation air oven.

Dehusking was carried out manually and milling was achieved using an experimental mill constructed according to the design of Hogan *et al.* (1964). The kernels were milled to approximately 5% polish.

Milling yield: This was estimated by milling 500 kernels and counting the number remaining unbroken. Since the milling action was relatively violent, commercial units would be expected to give higher yields.

Apparent solubility: Immediately after steaming, 5 g of the paddy were used for moisture determination and thirty-five kernels for determination of apparent solubility, as described in Part 1 of this paper. The results obtained are not directly comparable with those obtained from milled rice since the paddy contains more non-starch material in the form of husk and bran layers.

Degree of gelatinisation: This was measured using the method of Birch & Priestley (1973) with slight modification (Priestley, 1974).

Relative crystallinity: This was estimated by measuring the height of the X-ray diffraction peaks above the background scatter at 6·80 Å and 4·42 Å (designated peaks V_1 and V_2 respectively). Correction was made for the height of the peak of the internal standard, 10% calcium carbonate, at 3·04 Å, and the moisture content of the sample. Since the peak heights V_1 and V_2 bore a constant relation to each other, only the results for peak V_1 are presented.

RESULTS AND DISCUSSION

The moisture content of the paddy rose from 13 to 35% after soaking. During steaming this rose to about 45%, regardless of the pressure employed.

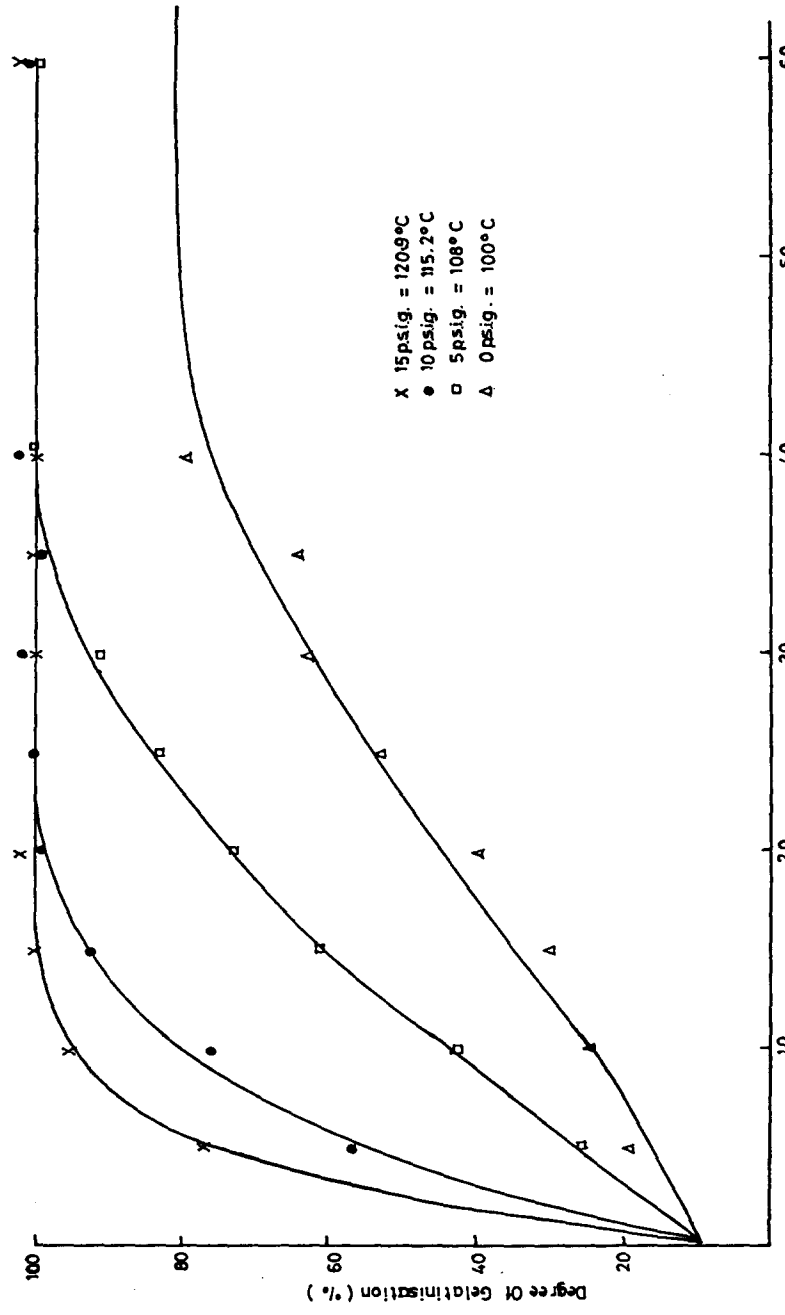

Fig. 1. Rate of gelatinisation of soaked paddy rice at various steaming pressures.

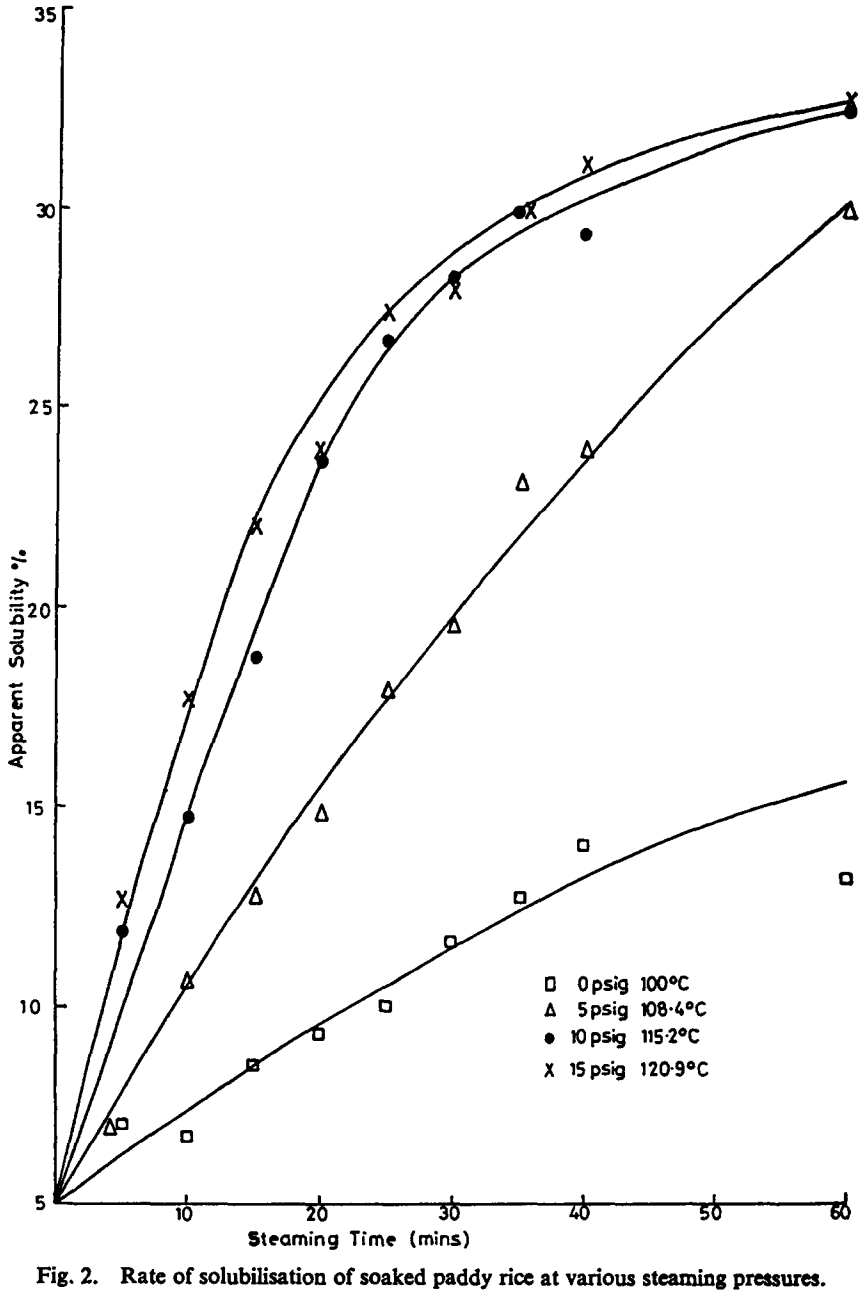

Fig. 2. Rate of solubilisation of soaked paddy rice at various steaming pressures.

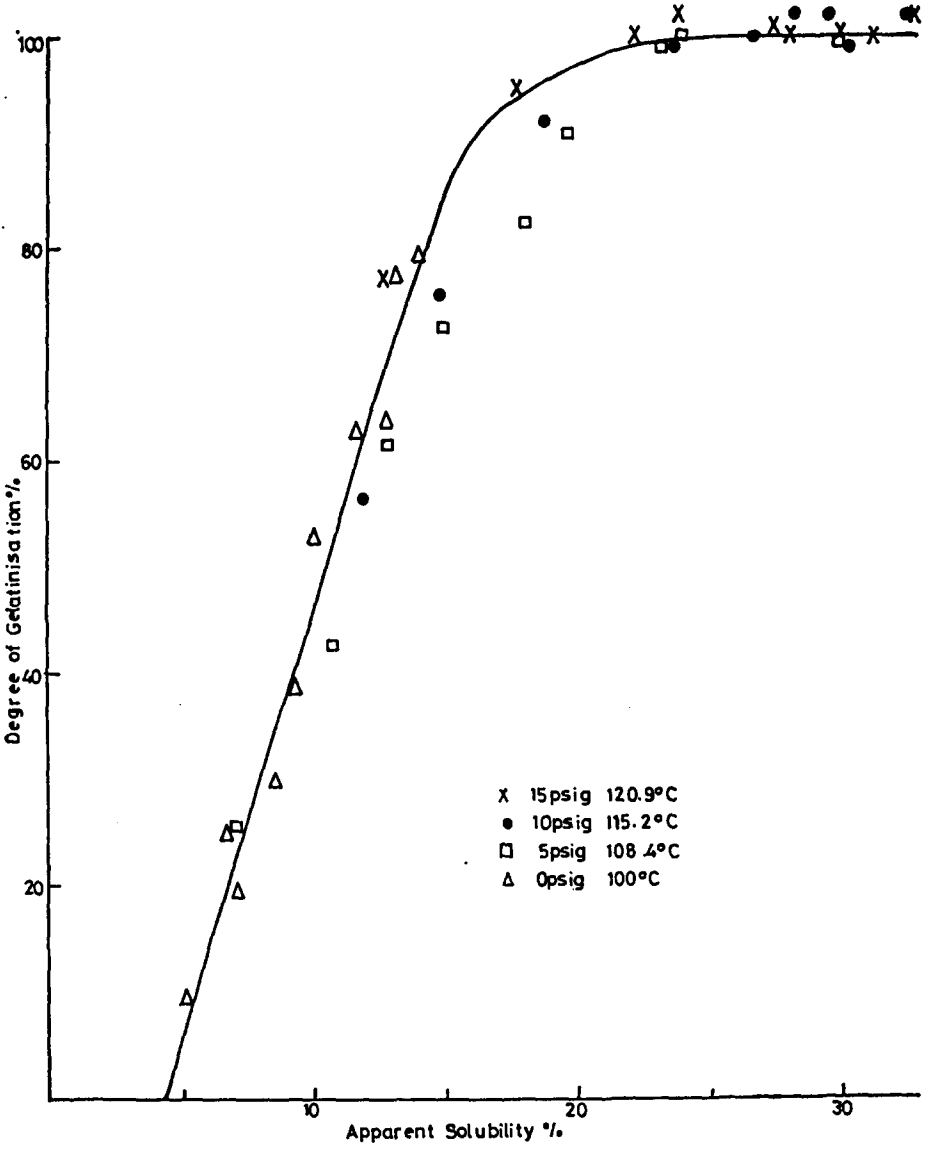

Fig. 3. Apparent solubility versus degree of gelatinisation of rice paddy at various steaming pressures.

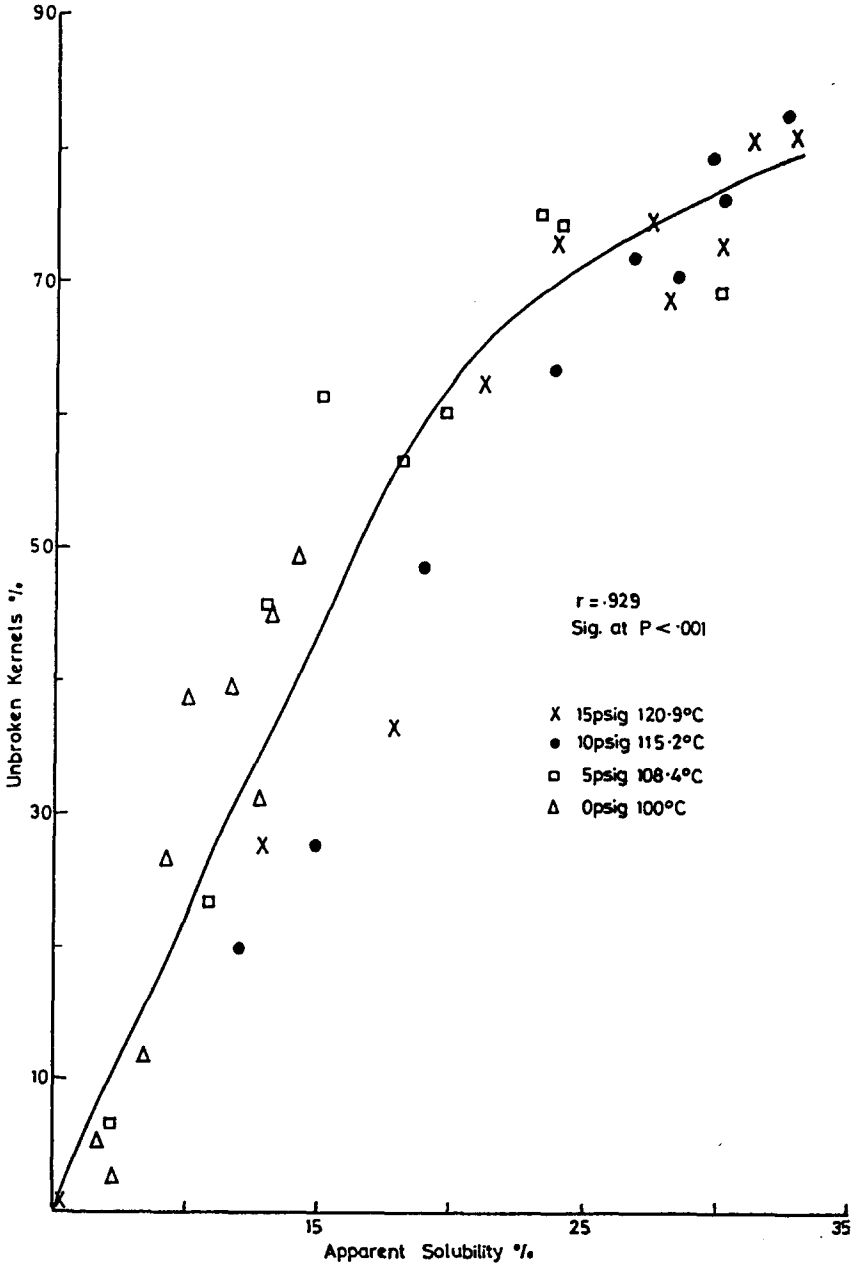

Fig. 4. Effect of apparent solubility achieved during steaming on milling yield.

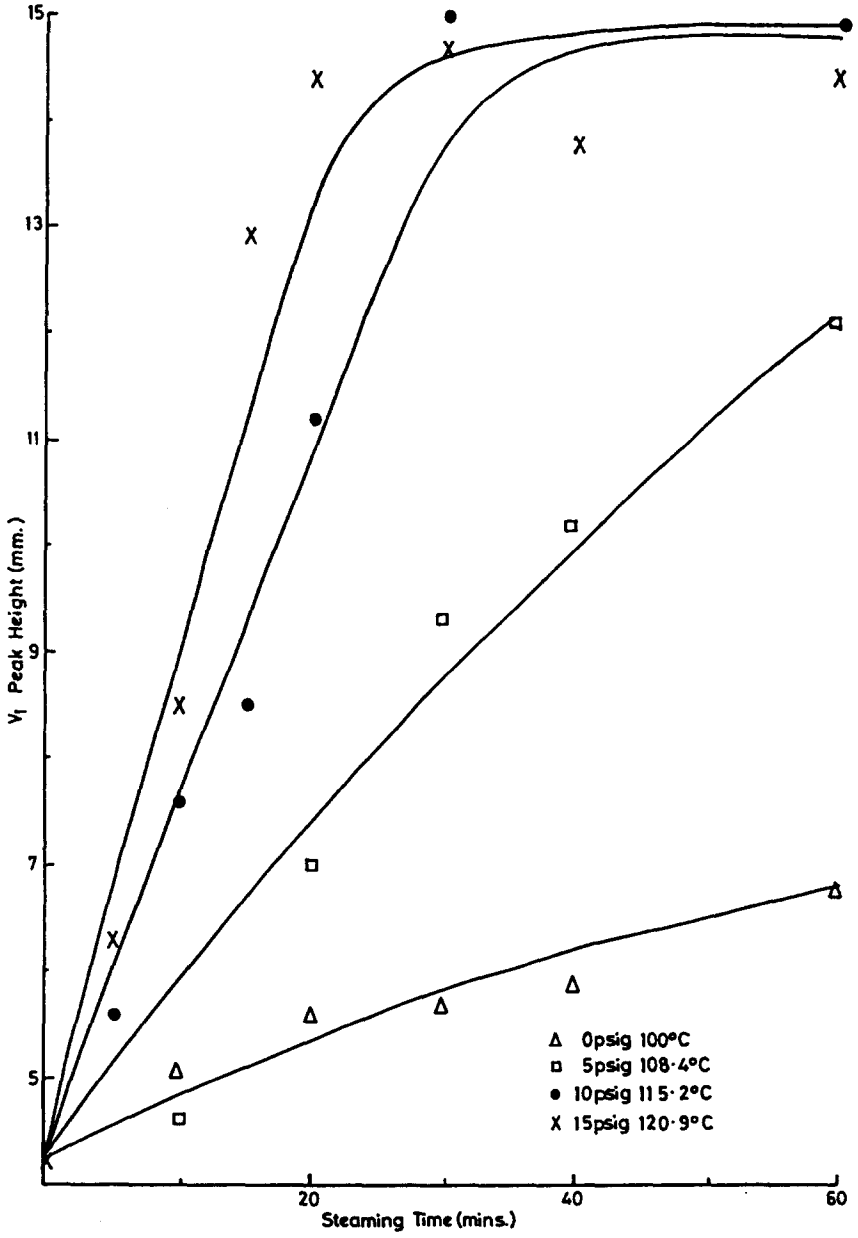

Fig. 5. Peak height of V_1 X-ray diffraction peak after various steam treatments.

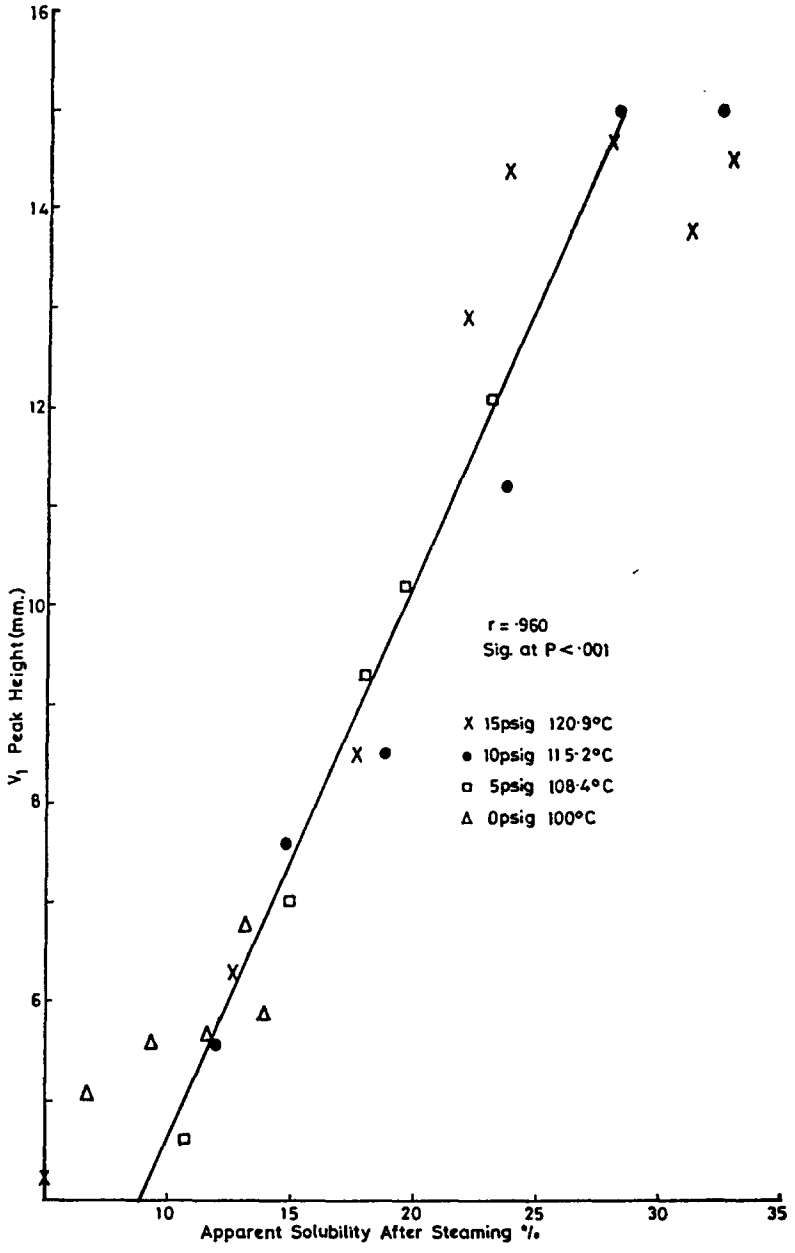

Fig. 6. V_1 peak height versus apparent solubility after steaming.

The rate of gelatinisation is extremely dependent on steaming pressure (Fig. 1). Gelatinisation was complete in 20 min at 10 psig while 60 min was necessary in order to achieve 80% gelatinisation at 0 psig. The rate of solubilisation is also very pressure-dependent. But, whereas gelatinisation was completed in 20 min at 10 psig, the kernels had not reached a maximum apparent solubility after 60 min at the same steaming pressure. The rate of solubilisation is also very pressure-dependent (Fig. 2). Apparent solubility is plotted against degree of gelatinisation in Fig. 3. The relationship is linear up to the point where gelatinisation is complete, after which solubilisation can still continue. Thus, degree of gelatinisation is a useful index of processing only under less severe steaming conditions whereas apparent solubility can be used over the entire range of processing conditions investigated. For this reason, other measured parameters gave lower correlations with degree of gelatinisation than with apparent solubility and are not presented.

The relationship between apparent solubility achieved during steaming and the milling yield of the dried rice is shown in Fig. 4. The corresponding yield using raw rice was 66%. It is evident, therefore, that merely soaking the paddy results in almost complete breakage. It was not until the apparent solubility exceeded 20%, corresponding to complete gelatinisation, that any improvement occurred. It is well known that soaking rice in water induces the formation of cracks (Desikachar & Subrahmanyan, 1961). Apparently, sufficient solubilisation must take place in order to 'cement' these cracks together, thus strengthening the kernel.

The relative extent of amylose complex formation under the various processing conditions, as indicated by the height of the V_1 X-ray diffraction peak, is shown in Fig. 5. This, again, is extremely dependent on steaming pressure.

The highly significant positive linear relationship ($r = 0{\cdot}960$) between the V_1 peak height and apparent solubility (Fig. 6) indicates that, in order for complex formation to occur, the starch must first be solubilised. This would allow the amylose freedom to adopt a helical configuration around the complexing agent.

CONCLUSIONS

Measurement of the apparent solubility of steamed paddy offers a useful index of the severity of its processing treatment. Degree of gelatinisation is a useful index only for less severe conditions. The extent of solubilisation achieved during the steaming stage appears to govern the resistance of the rice to milling breakage and the formation of complexed amylose.

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PURIFICATION AND CHARACTERISATION OF A MOLLUSC β-D-(1→3)-GLUCAN HYDROLASE

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ABSTRACT

Two β-D-(1→3)-glucan hydrolase enzymes of the crystalline style of the surf clam, Spisula solidissima, *have been purified and characterised. Both enzymes are predominantly* exo*-hydrolases and have similar properties, suggesting that one is formed from the other. Purification and characterisation were effected to establish the optimum conditions for hydrolytic activity. This is a prerequisite for the determination of their potential use in the food industry, such as for lytic activity on microorganism cell walls. For example, one ultimate aim of this work is the formulation of an enzyme preparation capable of releasing protein from yeasts and fungi for human consumption.*

INTRODUCTION

Due to high protein content and rapid growth, microorganisms have attracted considerable attention as a source of protein for human food. The nutritional value of single-cell protein depends on the availability of the intracellular protein to the digestive system and it has been shown that the presence of an impervious bacterial cell wall limits access to its cell protein (Tannenbaum & Miller, 1967). Mechanical, chemical and enzymic methods for the degradation of cell walls are possible (Lindblom, 1974; Tannenbaum, 1971), although the use of enzymes which attack specific bonds in the cell wall structure is limited by economic considerations (Hughes *et al.*, 1971). However, β-D-glucan hydrolases from the digestive tract of *Helix pomatia* have been shown to attack yeast cell walls (Eddy & Williamson, 1957) and the cell walls of *Saccharomyces cerevisiae* have been successfully ruptured by a combined chemical and enzymatic process for the purpose of protein recovery (Phaff, 1975).

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Cell walls of *Saccharomyces cerevisiae* contain an outer layer of a mannan-protein-phosphate complex which is soluble in alkali. The inner wall contains at least three glucans, one of which is alkali soluble. The major component of the alkali-insoluble glucan (85%) is a polysaccharide with predominantly glucosido-β-D-(1→3)-linkages with occasional β-D-(1→6)-linkages. The minor component (15%) is a highly branched β-D-(1→6)-glucan with β-D-(1→3) interchain and interresidue linkages (Phaff, 1975). Enzymes capable of hydrolysing these glucans occur in the digestive fluid of *Helix pomatia*, but this snail has limited availability. Yeast glucanases are obtainable only in a limited number of species and only at limited periods of the cell cycle. We have previously reported (Shallenberger *et al.*, 1974) that the major carbohydrase enzyme of the surf clam, *Spisula solidissima*, is a β-D-(1→3)-glucan hydrolase, laminarinase [EC 3.2.1.6]. Although clams can be found in most waters of the world, the major processing areas are the east and west coasts of the United States. The stomach of the clam contains an anatomical structure, the 'crystalline' style, which is the source of the digestive enzymes. It is considered waste material by the clam processing industry. Thus, this β-D-(1→3) glucan hydrolase is readily available from a source not presently utilised by the industry. Before the lytic and hydrolytic potential of this β-D-(1→3)-glucanase can be satisfactorily determined, the enzyme must be concentrated by purification, and characterised in order to be able to assess optimum conditions for activity.

In the present paper we report the purification and characterisation of two laminarinase components in the crystalline style. The efficacy of the preparation as a lytic enzyme and for the other food chemistry uses will be reported subsequently.

MATERIALS AND METHODS

Enzyme source

Crystalline styles of the surf clam, *Spisula solidissima*, were obtained from the Shelter Island Oyster Company, Long Island, New York. After removal from the clam stomach, styles were frozen and stored at −10°C until used.

Laminarin

Cold water insoluble laminarin was purchased from ICN Pharmaceuticals Inc., Ohio. It was readily soluble in appropriate buffers at 50°C and remained in solution during incubation for enzyme activity assays.

Buffer solutions

Buffer A: ammonium hydroxide (0·3 ml, 30% as NH_3), acetic acid (0·3 ml, 99%) to 1000 ml with distilled water: pH 5·4.

Buffer B: ammonium hydroxide (30 ml, 30% as NH_3), acetic acid (30 ml, 99%) to 1000 ml with distilled water: pH 5·4.

Column chromatography

SP Sephadex C-50 (Pharmacia Fine Chemicals, New Jersey) was swollen in water and treated successively with 1·0M NaOH, 0·5M HCl and 1·0M NaOH. After removal of alkali with an extensive water wash, the gel was equilibrated in Buffer A for 24 h.

Gel filtration

Sephadex G-100, 40–120 μ (Pharmacia Fine Chemicals), was swollen in Buffer A for 5 h at 100°C and equilibrated in the same buffer for 48 h.

Analyses

Total reducing sugars in enzymic digests were determined as glucose by Nelson's (1944) colorimetric method. Total protein was determined colorimetrically at 700 nm with the Folin-phenol reagent (Lowry *et al.*, 1951) using bovine serum albumin as standard.

Enzyme assays

Enzyme activity of column fractions was determined by the release of reducing sugars from laminarin as follows: laminarin (0·9 ml, 0·2%) in Buffer A (pH 5·4) was incubated with column fractions (0·1 ml) at 37°C for 30 min. After inactivation of the enzyme by addition of the Somogyi-Nelson reagents, total reducing sugars released were determined as glucose. Results are recorded as units of enzyme activity per millilitre fraction, where one unit of activity is defined as that amount which releases 1 μ mole reducing sugar (as glucose) per minute under the conditions of incubation described.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed on 6% acrylamide gels as described by Davis (1964). Laminarinase activity was located according to the technique of Gabriel & Wang (1969). Sodium dodecyl sulphate (SDS) gel electrophoresis was carried out following the procedure of Fairbanks *et al.* (1971).

Paper chromatographic procedures

The hydrolytic products from laminarin were chromatographed on Whatman No. 1 paper by descending irrigation with isopropanol:ethyl acetate:water (6:1:3, v/v). Hydrolysis products were located with alkaline silver nitrate.

Molecular weight estimation

The molecular weights of the two preparations were estimated by molecular-sieve chromatography on a column of Sephadex G-100 (1·5 × 90 cm). Buffer A was the eluting buffer, fractions of 2 ml being collected every 10 min. Calibration of the column was effected using proteins of known molecular weight, namely ribonuclease, chymotrypsinogen and ovalbumin.

RESULTS AND DISCUSSION

Purification of laminarinases

A typical enzyme purification scheme is shown in Table 1.

Step I: Crystalline styles of the surf clam (314 mg dry weight) were dissolved directly in Buffer A (15 ml) and layered on an SP-Sephadex C-50 column (2·5 × 90 cm, initial dimensions). Elution was carried out by gradient addition of Buffer B

TABLE 1

PURIFICATION OF *Spisula solidissima* β-D-(1→3)-glucan hydrolases I and II

Step		*Total activity units*	*Total protein (mg)*	*Specific activity (units/mg)*	*Activity coefficient*	*Yield (%)*
1. Starting material:		174	314	0·55	1·0	100
2. SP-Sephadex C-50 chromatography:	I	90	17·4	5·17	9·4	51
	II	45	4·4	10·22	18·6	25·8
3. Sephadex G-100 chromatography:	I	41	3·3	12·42	22·6	23·6
	II	18·8	0·9	20·9	38·0	10·8

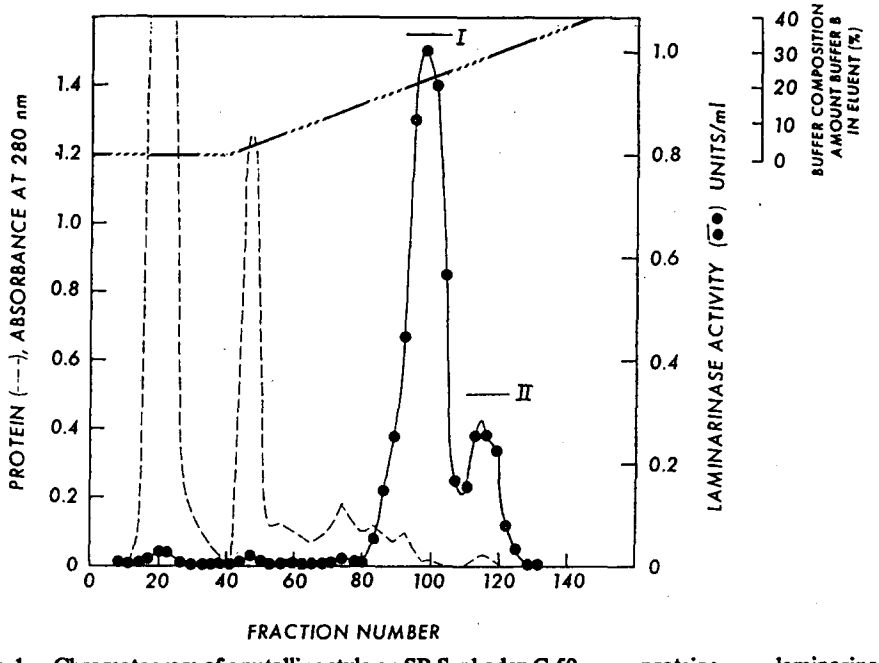

Fig. 1. Chromatogram of crystalline style on SP-Sephadex C-50. – – –, protein; ——, laminarinase activity; – · · · –, composition of eluting buffer. Fractions of 8 ml were collected every 12 min and assayed for laminarinase activity as described in the text. The solid bar represents the fractions collected.

to Buffer A. Fractions of 8 ml were collected every 12 min over a 24-h period. The majority of the protein was unadsorbed and readily passed through the column. Two peaks having β-D-(1→3)-glucan hydrolase activity were obtained (Fig. 1). Fractions 92–106 (designated laminarinase I) and fractions 110–122 (designated laminarinase II) were pooled and subsequently treated separately.

Step II: Active fractions were dialysed against 10 volumes of distilled water overnight and lyophilised to yield 17·4 mg laminarinase I and 4·4 mg laminarinase II.

Step III: The chromatography of laminarinases I and II on a Sephadex G-100 column (2·5 × 90 cm) with Buffer A is shown in Fig. 2(a) and (b). Fractions of

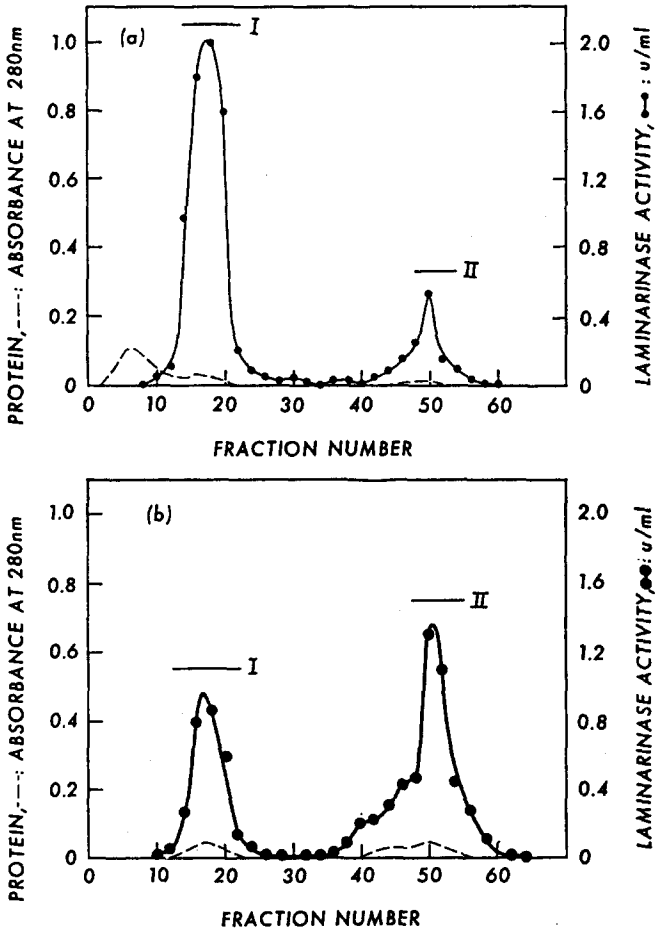

Fig. 2. Elution patterns of laminarinase I (a) and laminarinase II (b) from a Sephadex G-100 column. – – –, protein; ——, laminarinase activity. Fractions of 2 ml were collected every 5 min. The solid bar represents the fractions collected.

2 ml were collected every 5 min. In this way, laminarinases I and II were separated into two discrete components. The broad shape of the laminarinase II peak suggests that it is composed of a number of components of similar molecular weight, all having laminarinase activity. The yields of the two laminarinases were 3·3 mg (I) and 0·9 mg (II) with laminarinase I purified 23-fold and laminarinase II purified 38-fold.

Homogeneity of the enzyme preparations

Neither preparation was found to be electrophoretically homogeneous. Laminarinase I contained at least four proteins, as shown by polyacrylamide gel electrophoresis (Davis, 1964) and SDS electrophoresis (Fairbanks *et al.*, 1971), and only two possessed laminarinase activity. Laminarinase II contained three proteins, two of which had laminarinase activity.

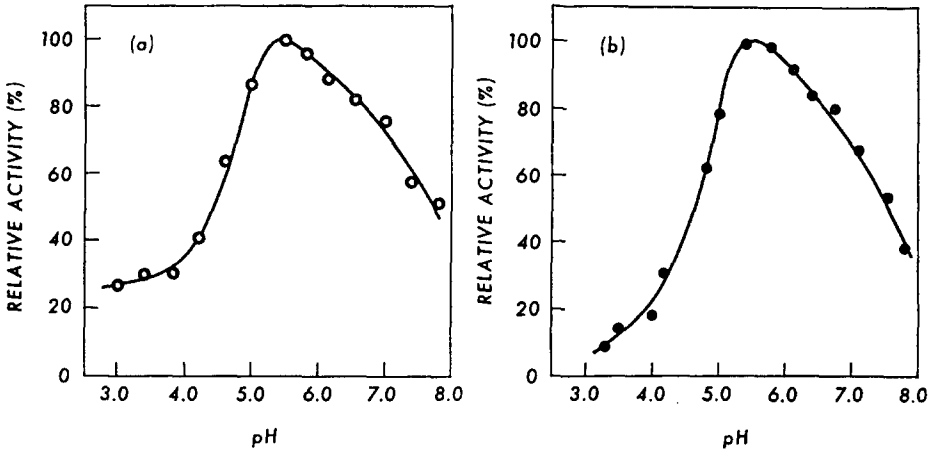

Fig. 3. pH-activity relationships for laminarinase I (○—○ (a)) and laminarinase II (●—● (b)). The laminarin concentration was 0·5% (w/v) and the temperature of incubation 37°C.

Dependence of enzyme activity on pH

The activity of laminarinase preparations I and II was determined at various pH values using laminarin (0·5%, w/v) as substrate in a ten-fold dilution of McIlvaine's citrate-phosphate buffer. Maximal activity of both laminarinases I and II was observed at pH 5·5 (Fig. 3(a) and (b)).

Dependence of enzyme activity and stability on temperature

Maximal temperature activity of laminarinase preparations I and II was determined using laminarin (0·5%, w/v) as substrate in ten-fold diluted McIlvaine's

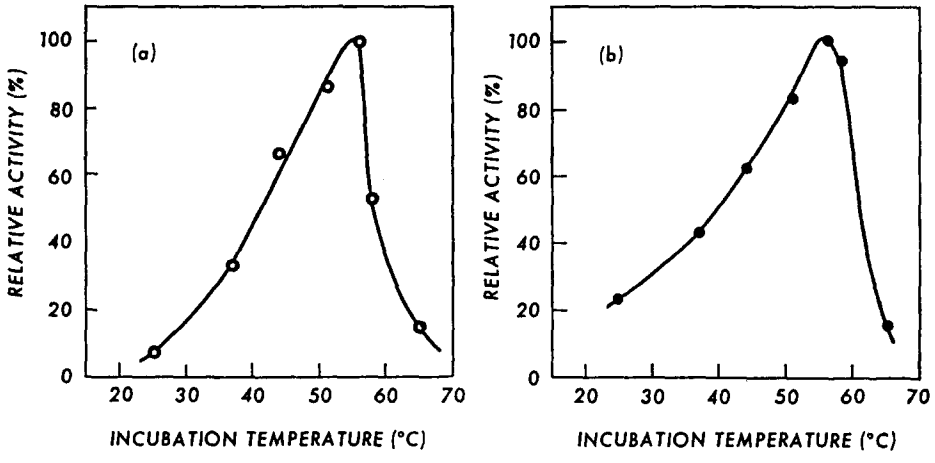

Fig. 4. Effect of temperature on the activity of laminarinase I (○—○ (a)) and laminarinase II (●—● (b)). Enzyme activity was assayed at pH 5·4. Incubation time was 30 min.

buffer at pH 5·5. The variation of activity with temperature is shown in Fig. 4(a) and (b). Maximal activity of laminarinase I and II after a 30 min incubation was at 55°C. The high temperature optimum is surprising for a marine organism, but it should be noted that the temperature stability studies indicate that, with a longer incubation period, the optimum would be lower.

The temperature stability of the preparations was determined in the presence and absence of substrate at 37°C and 55°C. In the presence of laminarin (0·5%,

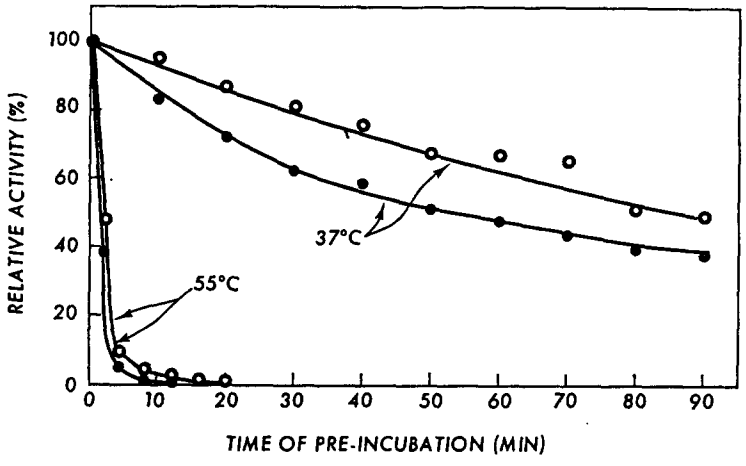

Fig. 5. Thermal stability curve. After incubation for the time specified at 37°C or 55°C, the remaining activity was assayed by the standard method at 37°C. (○—○ (a)) Laminarinase I; (●—● (b)) laminarinase II.

w/v), and at both temperatures, laminarinase I generated reducing sugars at a constant rate for 2 h, the time limit of the assay. Under the same conditions, laminarinase II generated reducing sugars at a constant rate for 60 min at 55°C and 75 min at 37°C. Inactivation or end product inhibition then occurred (data not shown).

The stability of I and II in the absence of substrate is shown in Fig. 5. After heat treatment for various times at 37°C and 55°C, activity remaining was assayed at pH 5·5 using laminarin (0·5%, w/v) as substrate by incubating for 30 min at 37°C. Heat treatment at 55°C for 8 min resulted in almost complete loss of activity, laminarinase I being marginally more stable than laminarinase II. At 37°C, the loss of activity was more gradual, and again laminarinase I was found to be more stable than laminarinase II.

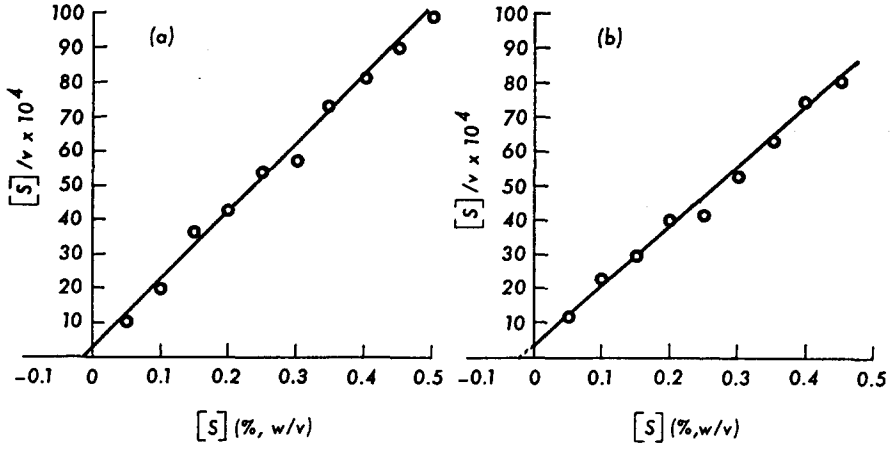

Fig. 6. Reaction velocity data for the action of enzyme preparations I (a) and II (b) on laminarin. After incubation at 37°C for 30 min at pH 5·4 with a series of concentrations of laminarin from 0·05% (w/v) to 0·5% (w/v), the reducing sugars produced were assayed (as glucose) as described in the text.

Kinetic properties

The reaction velocity data for the incubation of laminarinase preparations I and II with a range of laminarin concentrations is presented in Fig. 6(a) and (b) as a plot of [S]/v against [S] (Wilkinson, 1961). The Km (laminarin) of laminarinase I was found to be 0·013% (w/v) and that of laminarinase II 0·022% (w/v).

Molecular weight estimation

Using the calibrated Sephadex G-100 column (*see* Materials and Methods), laminarinase I had a V_e/V_0 ratio (ratio of elution volume to void volume) of 1·75

and laminarinase II a V_e/V_0 ratio of 3·58. A plot (Fig. 7) of V_e/V_0 against log (molecular weight) showed that these values correspond to molecular weights of approximately 36,100 daltons (I) and 11,100 daltons (II).

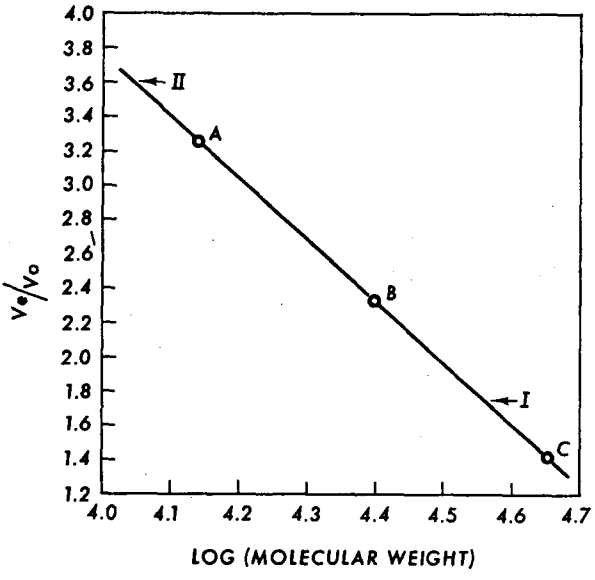

Fig. 7. Calibration of a Sephadex G-100 column with proteins of known molecular weight (A = ribonuclease, B = chymotrypsinogen and C = ovalbumin). The arrows indicate the positions of laminarinase I and laminarinase II elution.

Analysis of the enzymic hydrolysate of laminarin

Laminarin (100 mg) was dissolved in Buffer A (5 ml) and to 1 ml samples of this solution were added laminarinase I (100 μg) and laminarinase II (100 μg). The mixtures were incubated at 37°C for 1 h. After inactivation of the enzymes by boiling for 5 min, 25 μl samples were chromatographed as previously described. The results are shown in Table 2. The products of hydrolysis of each enzyme preparation were found to be identical. The following two types of β-D-(1→3)-glucanases are known: (a) that which fragments laminarin in a random fashion

TABLE 2

CHROMATOGRAPHIC IDENTIFICATION OF LAMINARIN HYDROLYSIS PRODUCTS

Enzyme	*Glucose*	*Laminaribiose*	*Laminaritriose*	*Higher molecular weight sugars*
Laminarinase I	+++	tr	tr	tr
Laminarinase II	+++	tr	tr	tr

+++, intense spot.
tr, trace amount indicated.

yielding laminaribiose and higher oligosaccharides, and (b) that which releases glucose from the end of the polysaccharide chains. If the β-D-(1→3)-glucanases of *Spisula solidissima* are single entities, they are of the latter, *exo*-hydrolytic, form. However, the possibility cannot yet be excluded that our preparations contain a mixture of both types, as indicated by the trace amounts of biose, triose and higher oligosaccharides found.

CONCLUSIONS

The presence of an active β-D-(1→3)-glucan hydrolase in bivalve crystalline styles was demonstrated by Sova *et al.* (1970). The results reported here confirm the presence of two β-D-(1→3)-glucan hydrolases in the crystalline style of *Spisula solidissima*. Because of the marked similarity in the properties of these latter two laminarinases, it is probable that laminarinase II is in some way derived from laminarinase I. The fact that the laminarin hydrolysis products of the two enzymes are the same (Table 2) is the major supporting evidence for this proposal. Either II is present *in situ* or is a product of the isolation procedures.

In their present state of purification, the laminarinase preparations are suitable for assay as potential lytic enzymes. However, since yeast and fungal cell walls contain chitin, we anticipate that our laminarinase preparations may require supplementation with a chitinase for efficient action.

ACKNOWLEDGEMENTS

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