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The Growth of Food-poisoning and

IT IS not possible in a short article to discuss fully the many factors which affect the growth of bacteria in foods, but some of the basic principles underlying methods of preventing food spoilage and bacterial food poisoning will be dealt with. It will be necessary to omit consideration of the heat processing of canned foods.

It is known that a great variety of bacteria may gain access to various foods and that among them are certain pathogens which give rise to bacterial food poisoning. Of these the two most common groups are the salmonellae and staphylococci. Food poisoning by the *Salmonella* organisms depends on the ingestion of the living organisms, usually in considerable numbers. Staphylococcal food poisoning, on the other hand, follows the ingestion of the enterotoxin which is formed by certain strains of *Staphylococcus aureus*. Significant amounts of the enterotoxin are found only when large numbers of these bacteria are present and many millions per gram would be needed to form sufficient toxin to elicit the usual symptoms. As the enterotoxin is more heat-stable than the staphylococci which produce it, the toxin may persist in foods which have been cooked sufficiently to destroy the staphylococci.

For both groups of organisms, therefore, the control of food poisoning has two separate aspects. Firstly, there is the question of exclusion of the organisms or prevention of contamination, and secondly there is the need to prevent growth of bacteria which have gained access to the foods.

CONTROL OF CONTAMINATION

Contamination may be important from both a qualitative and quantitative point of view. Of the many organisms which gain entry to food products very few can cause food poisoning, and of the remainder only a few are important spoilage organisms. In England, the salmonellae are the most important food-poisoning organisms; in 55 per cent. of the outbreaks investigated in 1951 and 1952 these organisms were the causal agent. In the United States, however, staphylococcal food poisoning is more common; from 1945 to 1947 about 82 per cent. of the outbreaks in which the agent was determined were due to staphylococci.

The greater the number of organisms present in a product, the shorter is its storage life. During growth each organism divides to give two new organisms: in 10 divisions a single organism will give rise to approximately 1000 descendants; in 20, to one million; in 30, to 1000 million. Spoilage will become evident at the level of about 10^8 organisms or more per gram of product. Thus the storage life can be expressed as the number of divisions to reach a spoilage level. If the initial contamination is 10^6 organisms per gram in one case and 10^3 per gram in another, the second has a better storage life by 10 divisions. This is equivalent to 5 hours, if each division takes half an hour, or to 5 days if 12 hours are needed for each division.

Since it is extremely difficult to exclude all organisms from food, control measures must aim at excluding pathogenic organisms and minimizing the entry of others.

By W. G. Murrell

Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

Food-spoilage Bacteria in Foods

The measures which may be used to reduce the contamination level are:

- Good sanitation.
- Use of easily-cleaned equipment.
- Prevention of dust.
- Handling methods which avoid the carry-over of material from previous batches.

Additional methods for preventing the entry of pathogens are:

- Strict attention to personal hygiene by food handlers.
- Use of equipment to avoid handling of food by human beings.
- Installation of fly screens.
- Control of rodents.
- Exclusion of sources of infection such as carriers.

Contact between human beings and food in a processing establishment is most undesirable. In numerous cases of food poisoning, the causal organism has been traced to food handlers.

Epidemiological studies of food-poisoning outbreaks using serological and bacteriophage typing methods for identification of the organisms have in many cases enabled the source of the infection to be defined, and frequently one person has been shown to be responsible for carrying the food-poisoning organisms.

Staphylococci have been shown to persist for months in the noses of certain individuals; in fact, the nose may be regarded as their natural environment. If these organisms are in the nasal passages they will most probably be present on the hands and clothing as well. Some studies suggest that dust from clothing may be a source of infection of food as important as sneezing. Persons with skin infections and purulent lesions should not be permitted to prepare food or handle materials connected with its manufacture.

CONTROL OF GROWTH OF ORGANISMS IN FOODS

Many factors affect the growth of organisms. They include the type of organism, the chemical and physical properties of the food, and the storage conditions, including temperature. Of these, temperature is the most important. The effect of temperature on growth is twofold. It determines which organisms can grow and their rate of growth.

Bacteria are able to grow at temperatures ranging from about 20 °F to 160 °F. Some organisms grow best at the higher temperatures whereas others will grow only at the lower temperatures (Fig. 1). Pathogens have a high minimum growth temperature. Chilling to temperatures of 40 °F or lower prevents the growth of pathogens and food-poisoning bacteria, and results in a predominance of organisms suited to these temperatures. Organisms of the latter type are species of *Achromobacter* and *Pseudomonas* which are important in chilled meat storage. The organisms which cause proteolytic spoilage of milk instead of the usual souring are another example. They cause spoilage of milk if it is kept too long in the refrigerator.

The higher the temperature within the growth range, the greater is the possible growth rate. The growth rate of organisms in the high-temperature regions may be five

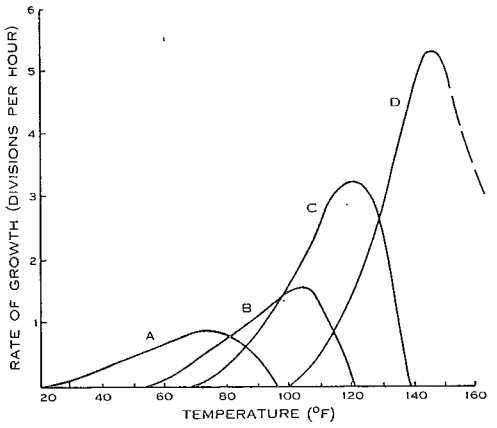

Fig. 1.—*The effect of temperature on the growth rate of four organisms* (A, B, C, D).

or six divisions per hour, whereas the organisms growing at 40 °F may divide only once in four hours. This reduction in growth rate with temperature is well illustrated by the effect of temperature on the time for development of slime on meat (Fig. 2). It means in practice that when chilling near the freezing point it becomes increasingly important to strive for every degree of reduction in temperature. Suppose the surface of the meat contains 50,000 organisms per square centimetre, and detectable slime occurs when the numbers increase to 50 million. This one-thousandfold increase takes about 10 divisions. If the growth rates of organisms are as indicated in the table on page 65, a reduction in temperature from 80 °F to 40 °F will increase the time for 10 divisions by 42·5 hours; in other words, the storage life will be extended by nearly two days. Over half this increase results from the reduction of the temperature from 50 °F to 40 °F.

The greatly increased rate of growth at the higher temperatures is very important when prepared and cooked foods are kept warm for a long time before consumption. It is particularly important that these practices be avoided in canteens and cafeterias where large batches of food are handled.

Control of growth, therefore, involves rapid cooling of foods, and holding at temperatures as low as practicable. Intervals during handling and between steps in the processing line should be reduced to a minimum. It is notable that food-poisoning outbreaks are particularly common with products such as pressed meats in the processing of which there is much handling and the factors of time and temperature favour growth of organisms.

Heat treatments are of great value in eliminating pathogens and for reducing the numbers of viable organisms at various stages in a processing line, e.g. milk pasteurization and rebaking of filled pastries. The final heat treatment or cooking process of products that are consumed without further heating can extend the storage life considerably. However, heat treatment should not be regarded as an alternative to the application of the principles of hygiene.

CONCLUSION

The methods outlined above for the prevention of food spoilage and food poisoning are based on the control of contamination and a reduction in the growth of organisms. They are simply good housekeeping methods. Despite this, reports from some countries indicate that though manufacturers (more

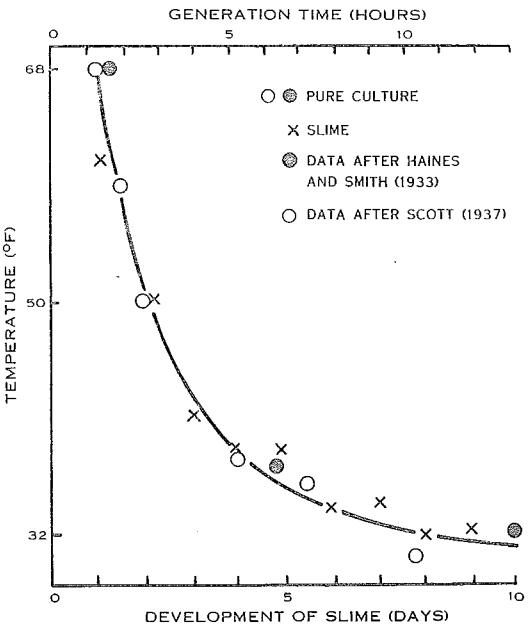

Fig. 2.—*Rate of development of bacterial slime on beef at various temperatures compared with the growth in pure culture of* Achromobacter *spp. from the slime* (*after Ingram* 1951).

particularly the smaller manufacturers of meat products) are willing to cooperate in improving standards, they still have very little knowledge of the steps to be taken. Obviously this suggests that there is still need for food inspectors to advise food manufacturers, caterers, and retailers on the practical application of technical knowledge.

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Spoilage Organisms on Fresh Beef

Illustrating the effect of temperature on the generation time of the organisms and the time to produce slime on the beef

Temperature (°F)	Generation Time (hr)	Time to Produce Slime (hr)	Increase in Time to Produce Slime* for Each Fall of 10 °F (hr)
80	0·75	7·5	—
70	1·0	10	2·5
60	1·5	15	5
50	2·75	27·5	12·5
40	5·0	50	22·5
30	12·0	120	70

* i.e. safe keeping time.

Australian Pea Processors' Conference

OFFICERS of the Division of Food Preservation and Transport played a prominent part at a conference held in Melbourne on July 27 and 28, 1955, on the canning, freezing, and dehydration of green peas. The conference, which was for the purpose of discussing the technological problems of the industry and for hearing addresses by officers of C.S.I.R.O. and State Departments of Agriculture, was arranged by the Vegetable Processors' Section of the Victorian Chamber of Manufactures.

The opening session was addressed by Dr. S. H. Bastow, member of the C.S.I.R.O. Executive, who stressed the need for research into many phases of primary and secondary industry in Australia.

Mr. R. O. Kefford, of the Victorian Department of Agriculture, told the conference of the areas in Victoria which had a relatively long season for harvesting peas for processing, and referred to a number of problems associated with production. He emphasized the need for more experimental work, but pointed out the limitations imposed by shortages of staff and finance.

Mr. R. S. Mitchell, of the Division of Food Preservation and Transport, C.S.I.R.O., outlined the investigations which led to the procedure now used for predicting the optimal harvest time of pea crops for processing. Mr. L. J. Lynch, also of C.S.I.R.O., gave an account of related investigations which had been carried out since the Pea Canning Conference in Melbourne in 1952. He also discussed the effect of a number of factors on the accuracy of the maturometer.

Mr. H. R. Twilley, of Gordon Edgell and Sons Ltd., Tasmania, spoke on the place of the quality control and production departments in a cannery. Mr. R. Pryse-Jones, from the same company in New South Wales, gave an informative talk on the problems encountered in the processing of peas, from the acquisition of the seed to the storage of the canned product in a warehouse. Other speakers referred to the freezing and dehydration of peas.

Considerable discussion followed, and it was resolved to:

- Seek improvements in the supply of pea seed.
- Consider means of obtaining more finance for research.
- Establish an Australian Vegetable Processors' Convention.

The conference was brought to a close with an official dinner at which Mr. M. G. Edgell presided and Mr. D. Bingham was the guest speaker.

Ozone is widely used as a deodorizing agent because of its ability to oxidize many objectionable odours and gases into non-objectionable products.

THE USE OF OZONE

OZONE is readily generated, and in very dilute concentrations in air has a pleasant, fresh smell and decomposes into oxygen. However, in concentrations greater than about 0·5 p.p.m. it is irritating and may cause headache and nausea. It is considered dangerous to workers in cold stores at concentrations greater than one part per million. Other disadvantages are: it will rapidly oxidize some materials, e.g. rubber; it causes rancidity in fats; it is unstable; it has low penetrating power; and some fruits, e.g. apples, peaches, and bananas, are injured by very low concentrations of the order of 1-2 p.p.m.

Nevertheless, ozone has been used for many years in food stores in Europe and America. Its use has been discussed in several papers by Ewell (1938, 1941, 1942, 1945, 1946). In egg and cheese stores, in concentrations of 1-1·5 p.p.m., it is used as a deodorant and to reduce superficial growth of moulds and bacteria. In higher concentrations it is used to remove odours from empty cold stores.

FRUIT AND VEGETABLE STORES

The use of ozone in fruit and vegetable stores has given rise to conflicting reports and opinions, particularly from commercial operators. One of the first reports of investigations into the effect of ozone in fruit stores was by Baker (1933). He concluded that ozone as used commercially in a number of apple stores kept the atmosphere in the stores free from fruit and other odours, but did not affect the keeping quality of the fruit. Scupin (1938) reported that in Germany ozone satisfactorily controlled fungal rotting in fruit, and that it did not increase the rate of ripening.

Smock and Watson (1941) found that 1-2 p.p.m. of ozone used for 1-2 hours each day controlled surface moulds on fruit and equipment but failed to reduce rots. The ozone removed only some of the odours present. Watson (1943) concluded that ozone must be generated continuously in the store in order to reduce surface mould. Similar results have been reported by other workers in America and Canada. In later investigations Uota and Smock (1948) and Smock (1949) found that, although ozone undoubtedly could control surface moulds and associated odours, it did not control other odours. Air purification with activated carbon was needed to remove odours completely. An investigation of the use of ozone in apple cool stores was carried out by Schomer and McColloch (1948). They found that about 2 p.p.m. of ozone for 1-2 hours daily inhibited surface mould but did not necessarily kill it. Concentrations of even 3.25 p.p.m. for several hours a day during storage failed to check decay. These concentrations reduced superficial scald, but not to a commercially significant extent. At the same time they caused serious lenticel injury, the surface of some varieties became sticky and varnish-like, and the flavour of most varieties was adversely affected. The authors concluded that ozone at a concentration of 3·25 p.p.m. does not affect ripening. It is of interest that Smock and Watson (1941) found that 1-2 p.p.m. of ozone for only 1-2 hours a day caused blackening around the lenticels in several varieties of apples, giving the appearance of a fine lenticel spot.

It has long been considered that accumulation of ethylene in fruit stores reduces the storage life of the fruit. Ethylene, which is a

By E. G. Hall

Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

IN FRUIT STORES

major constituent of "apple volatiles", is well known as a stimulator of ripening at higher temperatures, but there is little sound evidence that it affects the behaviour of fruit under cool storage conditions. The value of air purification for apple storage has recently been carefully investigated by Fisher, Porritt, and Edge (1953). They were unable to demonstrate that the removal of ethylene and other volatiles from the atmosphere during low-temperature storage had any effect on a number of varieties of apples. A similar conclusion has been reached for pears by Hansen (unpublished data).

No detailed work has been done in Australia on the use of ozone in apple stores, but there is little doubt that the above conclusions apply. Surface mould is often troublesome in stores with a high humidity (which condition is desirable to minimize shrinkage); it may be desirable to introduce 0·5-1·0 p.p.m. of ozone into such stores for an hour or two daily. It is clear, however, that ozone will not increase the storage life of apples and pears.

Little information is available on the effects of ozone on other fruits. Ewell (1946) considered that 2-3 p.p.m. for several hours daily would increase the life of grapes, small fruits, and berries. He also claimed that exposure to low concentrations of ozone enhanced the flavour and aroma of aromatic fruits such as strawberries.

Removal of ethylene appears to be beneficial in the storage of bananas. Gane *et al.* (1953) have shown that under gas storage, ethylene accumulates and hastens the ripening of the bananas. Ozone effectively removed the ethylene and increased storage life. To avoid injury to the fruit by the ozone it was necessary to circulate the air through an external ozone chamber and filter it through activated carbon, which removes the ozone and many non-ethylenic volatiles. The use of ozone for continuous removal of low concentrations of ethylene from the atmosphere has been investigated in detail by Colbert (1952), who considered it to be commercially feasible.

The susceptibility of fruits to injury by ozone varies widely, although more than 2 p.p.m. is often damaging. Gane (1936) found that bananas were sensitive and were slightly injured by 1·5 p.p.m., apples were unaffected by 2 p.p.m. and pears by 3 p.p.m., whereas higher concentrations caused skin injury. Oranges withstood concentrations of ozone as high as 40 p.p.m. for short periods.

If fruit in cool store is exposed to ozone continuously, or almost continuously, the concentration of the gas must be strictly controlled. As this presents many difficulties the chief use of ozone is likely to be for deodorizing empty rooms. Concentrations as high as 25 p.p.m. are required for this purpose, but the gas is unstable and disappears quickly.

Some foodstuffs, particularly eggs and fatty foods such as butter, cheese, and meats, are often tainted by volatiles from fruit. Citrus fruits are a common source of taints: they give off a strong odour, especially if moulds are developing in them. Ozone is widely used to remove the odour of citrus fruit from the atmosphere and inner surfaces of cold rooms and refrigerated cargo spaces aboard ships.

There are some indications in the literature that ozone can prevent the tainting of different types of fruit when they are stored together, but there is not enough information to warrant a recommendation.

PRODUCTION OF OZONE

Ozone is usually produced by a suitably controlled electrical discharge at high voltage. It is important that the generator should not give off oxides of nitrogen. This occurs in some types of generators, in which there is insufficient dielectric between the electrodes. It is also important to know at what rate ozone is produced by the machine; this can be varied over wide limits by altering the applied voltages. Ozone can also be generated by ultraviolet lamps (Ewell 1945, 1946) which emit light with a wave-length of 1850 Angstrom units. The concentration of ozone in the atmosphere can be determined fairly readily. Wylie (1947) has compiled a list of selected references to papers dealing with the more usual colorimetric methods of determination. However, precise production and measurement of low concentrations, such as might be used during the storage of fruit, have, more recently, been found to be rather difficult. The techniques are being investigated at the Brisbane laboratory of the Division of Food Preservation and Transport.

CONCLUSION

The value of ozone in fruit stores has recently been discussed by Kuprianoff (1954), who considers, despite the paucity of reliable experimental data, that it would be advantageous to use it more widely. It seems clear that ozone will under suitable conditions remove many odours and control surface mould, especially on walls and boxes, but it will not reduce rotting of the fruit or have any marked effect on apple scald or other storage disorders. Nevertheless, it is likely to be useful for improving storage when the removal of ethylene is specifically required.

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The brassicas most commonly preserved by freezing are broccoli, Brussels sprouts, and cauliflower. Provided the raw material is of good quality, the frozen product is attractive in appearance and valuable nutritionally.

Preservation of Brassicas by Freezing

By S. M. Sykes
Department of Agriculture, N.S.W.

and I. J. Tinsley
Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

THE preservation of brassicas is of advantage to both growers and processors. It provides the former with an outlet for a winter crop, and the latter with an opportunity to utilize plant when it might otherwise be idle.

HARVESTING AND HANDLING

The vegetables are harvested by hand at about the stage of maturity regarded as normal for the fresh market.

The sprouting or non-heading types of broccoli are favoured for freezing. Several cuttings of shoots are made from one crop, the smaller side shoots being preferred to the large central ones; in U.S.A. the practice is to cut out the young central shoot early in the growth of the plant. The shoots are cut when the buds are at the point of opening to disclose the yellow flowers, and gathered in baskets.

Aphis is a serious pest which must be controlled in the field, for it is almost impossible to remove the insects from the shoots by washing in the factory.

Brussels sprouts are harvested by making several pickings from the same crop. The sprouts are picked when they are of reasonable size but are still compact, tender, and sweet.

Cauliflowers should be cut when the curd has grown to full size but is still compact and of a good white colour. The curd must be protected against injury and discoloration during growing and harvesting. Varieties in which the curd is protected by leaves are therefore preferred to more open types, but in addition the leaves are often tied above the curd.

All three vegetables should be processed promptly after harvesting. Broccoli in particular is subject to deterioration during handling and loses quality very rapidly when held in boxes or bags at ordinary temperatures. Brussels sprouts may lose sugar and become tough when held for more than one or two days. Cauliflower is probably less subject to deterioration, but it must be handled without too much delay to obtain the best-quality product.

It is frequently necessary in commercial operations to hold the vegetables overnight, in which case they should be cooled as rapidly as possible with cold air or water, and then held at a temperature within the range 32-40 °F.

PROCESSING

The methods of preparation of the three vegetables before freezing are similar. The vegetables are trimmed and subdivided by hand and then placed on a moving belt which discharges the pieces into a washer. Broccoli is sometimes given an initial spray with cold water to firm the tissue for trimming and cutting. A mechanical cutter (e.g. an asparagus cutter) is sometimes used to cut the broccoli into pieces of standard length (e.g. 4 or 5 inches). Cauliflower is usually

cut into pieces about 2 inches long and 1½ inches in diameter. Brussels sprouts are carefully trimmed to eliminate any yellow or blemished outer leaves.

The washing operation involves complete immersion of the pieces and vigorous agitation of the water. The usual type of washer has high-pressure water jets discharging into a bath in which the vegetable is carried downwards and forward by the force of the water.

The vegetables are lifted from the washer and moved to the blancher by means of an elevator. Steam blanching is usually preferred to hot-water blanching because it causes slightly smaller losses of soluble solids such as sugars and ascorbic acid.

The completion of blanching is determined by testing for inactivation of the enzymes. Blanching commonly lasts three to five minutes for broccoli and cauliflower, and three to six minutes for Brussels sprouts. Cooling of the pieces after blanching may be carried out by spraying, dipping in a bath, or in flumes of cold water.

After being inspected on a moving belt the vegetables are collected in metal pans and taken to the packaging line.

PACKAGING AND STORAGE

The vegetables are hand-packed into suitable containers. A side-opening container is preferred to an "end-fill" type of carton or bag, because of the difficulty of packing neatly through a narrow opening. "End-fill" containers are suitable for institutional packs, since neatness of packing is not then so important.

The closed packs of vegetables are then frozen in a suitable freezer. Sometimes, however, the vegetables are frozen in metal moulds lined with a loose layer of material proof against water vapour, and the packaging is completed after freezing. The packages are usually placed in master containers which are stored at 0° to −5 °F.

RESEARCH WORK

In 1951, freezing trials of the above vegetables were carried out mainly with the object of studying the effect of variety on quality after freezing. The effects of certain other factors, such as the maturity of the buds in broccoli and the method of blanching in Brussels sprouts and cauliflowers, were also noted. The results of this work, which was a cooperative project of the C.S.I.R.O. Division of Food Preservation and Transport and the N.S.W. Department of Agriculture, are summarized below.

Samples of a number of varieties and selections were prepared, frozen in a pilot plant, and stored for various periods at 0 °F. The samples, on removal from storage, were cooked and presented to a panel of trained tasters who scored the samples for colour, texture, and flavour. The results, which are not presented in detail here, were examined statistically. Ascorbic acid determinations were made after periods of storage of approximately 1, 4, and 9 months.

Broccoli

Varieties.—The following varieties and selections were tested:

Green Sprouting
Californian—Strain Early
De Cicco
Calabrese
Early Calabrese
Californian—Strain Midseason
Medium
Early Medium
Green Sprouting—Grower Selection

With the exception of Green Sprouting—Grower Selection, which came from Windsor, N.S.W., all the varieties were grown at the Bathurst Experiment Farm.

Apart from the inferior colour of one strain, Early Medium, all samples gave a good frozen product. Other small differences in quality were observed but they were not significant. It seems that the suitability of these varieties for commercial freezing would depend more on their agronomic features than on their quality after processing.

Ascorbic Acid.—Ascorbic acid determinations were carried out on samples of each variety after storage.

The differences between varieties could not be regarded as significant because of the variation between samples within each variety. A general downward trend in ascorbic acid content was observed during storage. The ascorbic acid content of these varieties after storage for one month at 0 °F ranged from 73 to 112 mg per 100 g of frozen material.

Other Factors.—In conjunction with the variety trial, the effect of the colour of the original material and of the bud size on the quality of the processed product was investigated in one variety. The difference in the quality of the processed product prepared from side shoots and centre heads was also investigated.

There was some indication that broccoli with loosely packed buds of a large size was inferior to more compact material with buds of smaller size. Very young material with closely packed small buds did not differ in acceptability from material of normal picking maturity. The presence of a purplish colour in raw broccoli had no effect on the frozen cooked material.

Brussels Sprouts

Varieties.—The following varieties grown at the Bathurst Experiment Farm were tested:

California Half Dwarf	Carter's Market Gardener
Grower 7	Zwaan's Early Morn
Hercules	Long Island Improved

All varieties gave a product which compared favourably with cooked fresh Brussels sprouts. One variety, Californian Half Dwarf, was rated above the other varieties on all factors. The other five varieties gave frozen products which were acceptable and did not differ significantly from each other.

After storage for one month, the ascorbic acid values in samples from each variety ranged from 93 to 114 mg per 100 g. Because of package-to-package variation and the limited quantities of raw material, it was impossible to draw any conclusion regarding the influence of varietal differences on ascorbic acid content. Lower values were observed after storage for eight months.

Blanching.—There were no significant differences between samples blanched for 3½ minutes in steam and samples blanched for the same time in boiling water. This may have been due to the large amount of variation observed between individual packages in the one batch of material.

Cauliflowers

Varieties.—The following varieties were tested:

Zwaan's Early Snowball	Super Snowball
Snowball X	Snowball 16
Snowball M4086	Russian 2A
Codania Snowball	Phenomenal—5 months
Early Snowball	Phenomenal—4 months
	RN3

The varieties used included some which have been recommended overseas for freezing, and others grown locally for the fresh market. With the exception of the variety Phenomenal—5 months, which was grown at Windsor, all were obtained from the Experiment Farm of the N.S.W. Department of Agriculture at Bathurst.

The Snowball types, in the fresh state, had characteristic small compact white curds. Early Snowball, M4086, and RN3 seemed to have a greater number of small leaves within the curd than other Snowball types. The heads of Russian 2A were not quite as compact as the Snowball types, but were readily cut into small attractive pieces. Phenomenal—5 months was not very compact and showed some green in the curd stalks.

The quality assessment of the frozen products of 11 varieties of cauliflower was complicated by the large amount of variation between individual heads of any one variety, this variation being reflected in package-to-package differences. All varieties gave a very satisfactory frozen product. The highest scores were given to the varieties Zwaan's Early Snowball, Snowball X, Snowball M4086, and Russian 2A.

Ascorbic Acid.—Ascorbic acid values ranged from 33 to 69 mg per 100 g after storage for one month at 0 °F. The average rate of loss under storage was equivalent to about 27 per cent. over a period of 12 months.

Blanching.—Samples of a batch of frozen cauliflower prepared after blanching in hot water for three minutes were compared by means of tasting tests with one in which the raw material was steam blanched for the same time. No significant differences were detected.

ACKNOWLEDGMENTS

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Solids Content in

Earlier articles in this series appeared in **C.S.I.R.O. Food Preservation Quarterly**, *Vol. 13 (1953), pp. 3-8, 21-31; Vol. 14 (1954), pp. 8-18, 26-31, 46-52, 74-6; and Vol. 15 (1955), pp. 28-32, 52-7.*

IN Part VIII of this series (Kefford 1955*b*) a number of indirect methods for the determination of solids content in foods were discussed. It is now appropriate to indicate the application of these methods to specific canned foods, notably those foods for which solids content is commonly specified in regulations, specifications, or contracts.

FRUIT JUICES

The Commonwealth Food Specifications (1952*c*) for apple juice, apple and black-currant juice, pineapple juice, citrus juices, and citrus juice cordials specify solids content in terms of density in degrees Brix. The determination is made directly with Brix spindles (Royal Australian Chemical Institute 1952*a*; Association of Official Agricultural Chemists 1950*b*) or indirectly from the refractive index. For black-currant syrup and berry fruit pulps (Commonwealth Food Specifications 1952*a*) limits are specified for total soluble solids content, expressed as sucrose, to be determined on the undiluted product by a refractometer reading at 20 °C or corrected to 20 °C (R.A.C.I. 1952*b*).

It should be noted that the refractometric procedure may lead to significant errors when considerable amounts of non-sugar soluble solids, e.g. citric acid, are present (Joslyn 1950). Stevens and Baier (1939) have tabulated corrections to be applied to refractometer sugar-scale readings, according to the citric acid content determined by titration, for the purpose of converting them to density in degrees Brix or to true soluble solids content. The corrections are unimportant for most single-strength juices but become significant for lemon juices and citrus concentrates.

SYRUPS IN CANNED FRUITS

The Commonwealth Food Specifications (1952*b*) for canned cherries, pineapple, and fruit salads specify limits for the cut-out Brix of the syrup. Again the determination is made with Brix spindles (R.A.C.I. 1952*c*; A.O.A.C. 1950*b*) or alternatively with the refractometer. For other canned fruits only the fill-in Brix of the syrup is specified.

The solids content of the covering liquor in sweet pickles is also specified in terms of degrees Brix (Commonwealth Food Specifications 1952*f*) to be determined in the same way (R.A.C.I. 1952*e*).

JAMS AND HONEY

Limits for soluble solids content in jams, jellies, marmalades, and honey are included in Commonwealth Food Specifications (1952*e*). The soluble solids content, expressed as sucrose, is determined on the undiluted sample by a refractometer reading at 20 °C or corrected to 20 °C (R.A.C.I. 1952*d*; A.O.A.C. 1950*c*). Champlin (1943) has devised a useful chart showing refractive indices of sucrose solutions in the concentration range 64-72 per cent. and over the temperature range 10-30 °C.

A number of authors (Meschter 1950; Cheftel, Frichet, and Estang 1951; Martin 1955) have discussed the precautions to be observed in refractometric measurements on jams.

When examining jams for determination of the finishing point, care is necessary to

Critical comments on the procedures described, and suggestions for modified or alternative methods found to be useful in practice, will be welcomed.

Specific Canned Foods

By J. F. Kefford

Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

obtain a representative sample from the hot batch. It is then essential to avoid loss of moisture from the sample during cooling and transfer to the refractometer prism. The sample of hot jam should be poured into a "Pyrex" test-tube or a suitable metal container, which is stoppered immediately and cooled by shaking in running water. When the jam temperature approaches room temperature the container is dried, and a sample of jam transferred expeditiously to the refractometer prism by means of a glass rod.

Care is again needed to obtain a representative sample from finished, packaged jams. A surface sample may be unreliable because of condensation in the headspace and subsequent dilution of the jam. Further, large pieces of fruit in jam may initially have a lower solids content than the liquid phase, and several days may be required to attain equilibrium (cf. Mapes and Kennedy-Ripon 1954). A jam under examination should, therefore, be thoroughly mixed until homogeneous, using a mincer or blender if necessary to comminute large pieces of fruit (A.O.A.C. 1950*a*).

TOMATO PRODUCTS

In the marketing and utilization of tomato products, solids content assumes particular importance. A more detailed discussion of its determination and significance is therefore appropriate.

The screened juice from whole tomatoes has an approximate composition usually within the following limits:

Total solids	5-7%, of which 11-14% is insoluble solids and 1-2% is salt (cf. Morpeth 1951)
Soluble solids	4-6%, of which 50-55% is sugars and 9-10% is acids
Insoluble solids	1%
Sugars	2-3%, practically all invert
Acids	0·3-0·6%, mainly citric with a little malic (cf. Van Dame 1953)
Soluble protein	0·8-1·2%
Mineral constituents	0·3-0·6%
Salt	0·05-0·1%, calc. as sodium chloride

The figures given are derived from American studies (Bigelow, Smith, and Greenleaf 1950*a*) but they are generally in line with Australian experience. Tomato pulps made in part from tomato residues may depart from the ranges of composition shown particularly in having more insoluble solids and less acid. Morpeth (1948, 1951) has tabulated the composition of a large number of samples of concentrated tomato purées of American and European origin.

A number of methods are applicable to the estimation of solids content in tomato products, e.g. drying in air or in vacuum at various temperatures (Kefford 1955*a*), specific gravity measurements on the whole pulp or the filtrate from the pulp, and refractive index measurements on the filtrate from the pulp. However, Horner (1940) has demonstrated that these different methods may give widely different values. Choice of method thus becomes to some extent arbitrary.

Refractometric Methods

Most workers now favour the refractive index measurement as a measure of solids content, particularly on the grounds of simplicity and convenience (Horner 1940; Cheftel, Frichet, and Estang 1951; Marcuse 1952; Bertozzi, Preti, and Wiesner 1953). Horner recommends filtering the mixed sample through a dry filter paper and setting aside the filtrate for at least 10 minutes before making the refractometer reading. He found, however, that filtered samples gave slightly lower refractive indices than corresponding centrifuged samples, probably because of adsorption of some soluble constituents on the filter paper.

In the procedure of Cheftel, Frichet, and Estang (1951), the sample is filtered through cloth of specified characteristics, the first few drops being discarded. Nylon fabric is preferred for thick purées.

When the solids content of the sample is greater than about 20 per cent. it is often difficult to filter off even the few drops required for a refractometer sample. It may then be advisable to dilute a weighed amount of the sample with an equal weight or twice the weight of distilled water.

In factory control examinations, filtration can often be avoided by using the techniques suggested by Kefford (1955*b*).

When examining samples of hot tomato pulp the most important source of error is evaporation during handling of the sample (Gurley 1946). Preferably the pulp is sampled with a 10-ml pipette having a large opening at the tip, and a piece of rubber tubing with a screw clip at the suction end. The sample is retained by tightening the screw clip and the pipette is cooled throughout its length by holding for $\frac{1}{2}$-1 minute in a stream of cold water or a deep vessel of cold water, e.g. a measuring cylinder standing in the sink. The pipette is withdrawn and wiped dry, and the lower portion of the sample rejected. A drop or two from the remainder is placed on the refractometer prism, where it quickly assumes the temperature of the prism, and the reading is made immediately.

The refractive index of a tomato product is a measure of the *soluble solids content*, which may be expressed as sucrose by reference to the International Sucrose Scale (A.O.A.C. 1950*e*). The refractive index may also, however, be used as a measure of *total solids content* by reference to tables which have been constructed on the basis of analyses of raw and concentrated tomato products (Bigelow, Smith, and Greenleaf 1950*d*; Sipple 1936). These tables relate the refractive index to the total solids content determined by vacuum drying. It is assumed that the relation between total solids content and soluble solids content in tomato products is substantially constant. But this assumption is not necessarily valid in relation to tomato products from different varieties, canneries, or growing areas.

The Commonwealth Food Specifications (1952*d*) specify minimum contents of soluble, salt-free tomato solids in tomato juice (5 per cent.), pulp (6 per cent.), purée (10 per cent.), and paste (22 per cent.), to be determined (R.A.C.I. 1952*g*) by refractometric measurements at 20 °C, or corrected to 20 °C, and expressed as sucrose using the International Sucrose Scale (A.O.A.C. 1950*e*). The South African Bureau of Standards (1950) also specifies a minimum content of 5 per cent. salt-free soluble solids in tomato juice, to be determined in the same way.

Specific Gravity Methods

The Food Technology Association of N.S.W. (1952) in a standard specification for tomato pulp specifies that "the soluble solids content is at least 6 per cent. as indicated by the specific gravity of a clear filtrate from the pulp being at least 1·025 at 20 °C". This form of specification was adopted because collaborative studies between different laboratories showed better agreement between specific gravity measurements than between refractometric measurements.

To determine the specific gravity, the pulp sample is filtered through a dry filter paper to give a substantially clear filtrate which is then weighed at 20 °C in a specific gravity bottle calibrated with distilled water at 20 °C. The specific gravity may also be determined by hydrometry and the time for a determination may be shortened to 5-7 minutes by using special short-stemmed hydrometers which float in a small volume of filtrate (Bigelow, Smith, and Greenleaf 1950*c*).

Salt Corrections

When a salt-free solids content is specified and the product contains added salt, a correction is applied for the salt content; thus, for each 0·1 per cent. of sodium chloride:

- Subtract 0·00017 from Abbé refractive index readings,
- Subtract 0·12 from Abbé refractometer sucrose-scale readings, or
- Subtract 0·0007 from specific gravity values.

Tomatoes contain only low amounts (0·05-0·1 per cent.) of natural chloride.

The salt content is determined by the following Mohr procedure (R.A.C.I. 1952*g*). With tomato juice samples, filter and take 20 ml of filtrate. With samples of tomato pulp, purée, or paste, take 3-5 g, dilute with distilled water to 100 ml, and take a 20-ml aliquot. Neutralize the aliquot with excess chloride-free calcium carbonate. Titrate with 0·1N silver nitrate solution using as an indicator a 5 per cent. solution of potassium chromate. Then 1 ml 0·1N $AgNO_3$ ≡ 0·00584 g NaCl.

Townsend *et al.* (1954*b*) recommend neutralizing with dilute sodium hydroxide solution to pH 5-7, or to the methyl orange colour change from orange to yellow. They also describe an alternative electrometric procedure using a silver electrode and a pH meter as potentiometer.

The A.O.A.C. (1950*d*) official method uses the Volhard technique of back titration with thiocyanate (cf. De Francesco and Giovannini 1953).

Wiesner (1953) has constructed tables and nomograms for the calculation of added salt, and also total solids, acidity, and reducing sugars, in tomato products.

Tomato Sauce

The Commonwealth Food Specifications (1952*g*) lay down minimum limits for specific gravity (1·11) and total soluble solids content (25 per cent.) in tomato sauce.

The specific gravity is determined by weighing in any suitable specific gravity bottle (R.A.C.I. 1952*f*; A.O.A.C. 1950*c*). The National Canners' Association has designed special specific gravity bottles for tomato products (Bigelow, Smith, and Greenleaf 1950*b*; Townsend *et al.* 1954*a*), but quite satisfactory bottles can be "home-made" for this purpose.

Good-quality sample bottles of about 4 oz capacity are required, having polished bottoms, smooth sides, and sloping shoulders to permit ready escape of air bubbles. Babcock milk testing bottles with the neck removed may also be used. The tops are ground perfectly flat and the bottles are calibrated with distilled water at 20 °C.

When a tomato sauce sample is examined, a weighed dry bottle is filled almost full. Air bubbles are removed by centrifuging for several minutes at about 1000 r.p.m. Then the bottle is filled to the top and centrifuged again. Finally the bottle is slightly overfilled and the product is levelled off with a straight edge, e.g. a spatula or a microscope slide. The bottle is washed on the outside, wiped dry, and weighed to the nearest 0·005 g. Then a thermometer is inserted and the temperature read. This temperature should be in the range 10-30 °C (50-86 °F) and for greatest accuracy close to 20° C (68 °F). The apparent specific gravity is calculated and corrected to 20 °C by reference to tables (Bigelow, Smith, and Greenleaf 1950*f*; Townsend *et al.* 1954*c*). In routine work, the calculations may be simplified by preparing a table or graph for each specific gravity bottle, showing the specific gravity corresponding to each weight of bottle plus sample.

The soluble solids content of tomato sauce is determined on the undiluted sample by a refractometer reading at 20 °C, or corrected to 20 °C (R.A.C.I. 1952*f*), and presumably expressed as sucrose. The South African Bureau of Standards (1951) specifies for tomato sauce the refractive index as such, the minimum limit being 1·3780.

Bigelow, Smith, and Greenleaf (1950*e*) set out tables relating the total solids content of tomato catsups in the range 16-40 per cent. to the specific gravity and the Abbé refractometer reading. The total solids contents are one per cent. higher than those of sucrose solutions of the same refractive index. These tables, however, are only approximate since tomato sauces contain ingredients, such as onions, spices, vinegar, and salt, which complicate the relations between solids content and physical properties.

Gurley (1946) compared the two methods described for determining the solids content of tomato catsup. He concluded that both methods give the same result when applied to cold samples, and that the same refractive index is shown by the whole product and the filtrate.

Hot sauces should be cooled for examination by the procedure suggested above for tomato pulps.

Tomato Content

With the object of maintaining standards of composition and identity in tomato sauces, attempts have been made to specify and to estimate the amount of true tomato ingredient present. For instance, the New South Wales Pure Food Act (1908) specifies a content of "non-sugar organic tomato solids", which is estimated by subtracting from the percentage of total solids, determined by drying at atmospheric pressure for four hours at 98-100 °C, the sum of the percentage of total sugars and the percentage of ash. It is assumed that the organic solids present, other than sugars, are derived wholly from the tomato ingredient of the sauce.

Morpeth (1948, 1951) describes methods for the estimation of the tomato content of tomato sauces based on the determination of free acids, combined acids, total acids, potash content, and lead number. The determination of the red pigment, lycopene, by absorption spectroscopy has also been used to estimate tomato content in tomato sauces (Stock 1950) and canned tomatoes (Kramer 1952).

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ANSWERS TO INQUIRIES

STERILIZATION OF FISH BOXES

What are the most effective methods of sterilizing fish boxes?

The two simplest methods are the use of hot water or steam, and the application of suitable disinfectants.

The boxes should first be cleaned with cold water by hosing or high-pressure spraying to get rid of loose debris, fish scales, and slime. Badly soiled boxes should also be scrubbed. Hot water or steam may be applied from sprays or hoses, or the boxes may be immersed in hot water or exposed to steam in tunnels.

Where large numbers of boxes are to be heated, a box-washing and sterilizing machine similar to those used at Grimsby, England, for the treatment of metal fish boxes is recommended. This machine* has a conveyor belt system along which the boxes are carried through a three-phase cleaning process. Dirt and slime are first removed by high-pressure steam; the boxes are then rinsed in hot fresh water, and later in cold water.

* ANON. (1951).—Grimsby gives fish a clean start. *Fish. Tr. Gaz.* No. 3554: 9.

The most effective disinfectants for using on fish boxes from which slime and dirt have been removed are chlorine-containing substances and some of the new quaternary ammonium compounds. The former may be sprayed on, or the boxes immersed in a solution. Directions for use of the disinfectants always accompany the containers in which they are carried. It is not necessary to rinse the boxes after treatment with the chlorine disinfectants, as the chlorine will disappear fairly rapidly on exposure to air, but it *is* necessary when quaternary ammonium compounds are used. Tanks for immersion treatment must be made of non-corrosive materials such as concrete. The strength of the solution must be maintained by the addition of further amounts of the disinfectant from time to time.

Some of the new wooden fish boxes used in England are now pre-treated by spraying with a formalin-casein mixture which impregnates the surfaces and makes them less liable to soiling. It is claimed that boxes treated in this way are readily cleaned.

NEWS from the Division of Food Preservation and Transport

WORK OF THE FRUIT STORAGE SECTION

Investigations into the storage and transport of fresh fruits have been carried out at the Divisional Headquarters at Homebush since it was opened in 1938. At the present time the staff engaged on this work consists of three Research Officers (including one from the N.S.W. Department of Agriculture), one Technical Officer, and two Technical Assistants. Most of the work, in both the laboratory and the field, is carried out in co-operation with the New South Wales Department of Agriculture under the general direction of an Advisory Committee consisting of representatives of the Division, the Department of Agriculture, and the Botany Department of the University of Sydney.

A branch laboratory at Gosford, N.S.W., was established in 1948 to investigate problems of wastage in citrus fruits. Its staff comprises two Research Officers and one Assistant who are officers of the New South Wales Department of Agriculture.

The Fruit Storage Section has accumulated data on the storage behaviour of the principal Australian varieties of apples, pears, stone fruits, and citrus fruits, and has defined optimum conditions for their storage. Refinements of recommended practices are being developed as research continues.

As fresh fruits are actively living material, variability is a characteristic feature of their behaviour. Differences have been found between fruit from adjacent trees in an orchard, and even from different positions on the one tree. A special study of the extent and causes of this variability has been undertaken with apples, and the effects of root-stock, fertilizers, and other cultural factors on the storage quality of both apples and oranges are being investigated. In addition to the above factors, variability in the storage behaviour of fruit may be attributed to seasonal conditions and to pre-storage handling.

Refrigerated gas storage, or controlled atmosphere storage, can increase greatly the storage life of many varieties of apples and pears. A study is being made, with the Granny Smith variety of apples, of the effects of various concentrations of oxygen and carbon dioxide in the storage atmosphere. Longer investigations include examination of the factors underlying the normal ripening of sound pears and the failure of over-stored fruit to ripen. Basic studies are in progress on the physiology of the development and maturation of oranges.

The development of an effective and safe post-harvest treatment of fruit to kill the eggs and larvae of the Queensland fruit fly is proceeding in cooperation with entomologists of the N.S.W. Department of Agriculture. The reaction of various fruits to low-temperature treatment and to fumigation with ethylene dibromide has been investigated. An examination is being made of the effects of these treatments on oranges from the main producing centres in Australia. If the treatments do not damage the fruit yet kill the larvae and eggs of the fruit fly, it may be possible to re-establish markets in New Zealand for oranges from districts now subject to quarantine on account of fruit-fly outbreaks.

From time to time investigations are carried out on specific problems such as substitute packing materials for grapes, mould wastage in pears, the waxing of bananas, and the storage quality of potato varieties. A valuable colour print library of fruit and vegetable storage disorders is being built up with the help of the photographic unit at Homebush. Much assistance is rendered to the fruit and vegetable industry by answering inquiries on the storage, packaging, and transport of fresh fruit and vegetables.

Research in fruit storage is often slow. Because of the variable nature of fruit, particularly its seasonal variability, storage experiments must be repeated for several years. The experiments must also be statistically planned, and the large amount of data must be analysed by a statistician before sound conclusions can be reached.

PERSONAL

Dr. R. N. ROBERTSON, Chief Research Officer, is leaving Sydney on R.M.S. *Strathaird* in December 1955 for a six-month visit overseas. Dr. Robertson will renew his contacts with research workers on plant physiology in the United Kingdom, where he will spend several months at Cambridge, on the continent of Europe, and in the United States of America.

Dr. A. B. HOPE, Research Officer at the Division's Plant Physiology Unit in Sydney since January 1953, left Australia in September to take up a C.S.I.R.O. studentship for two years at the Botany Department, University of Cambridge. Dr. Hope will undertake biophysical investigations under Professor G. E. Briggs, F.R.S., and will also spend some time with Professor A. L. Hodgkins at the Physiology School, Cambridge.

Mr. G. M. ROSTOS, a graduate in engineering of the University of Karlsruhe, who has been on the research staff of the Division since 1945, has transferred to the Organization's Central Experimental Workshops at Maribyrnong, Vic.

Mr. REUBEN ALLAN, well known to those who have sought the advice of C.S.I.R.O. on fish preservation, will retire from the Organization in January 1956. Mr. Allan, who joined C.S.I.R.O. as a Technical Officer in 1939, has worked in the Division of Fisheries and in the Division of Food Preservation and Transport. Mr. Allan brought from his native Aberdeen a great enthusiasm for all things pertaining to fish, and a fine Scotch accent. With his wide practical knowledge of fish and his keen business acumen he has been a great help to those engaged in fish processing in Australia. The staff of the Division wish Mr. Allan a long and happy retirement, and much successful angling.

DONATION OF EQUIPMENT

Messrs. John Heine and Sons, machine manufacturers, Leichhardt, N.S.W., have presented the Division of Food Preservation and Transport with a can-end embossing machine worth over £100. Some time ago they donated a Campden manometer valued at £200. The Division is deeply appreciative of these generous contributions to its research facilities.

PUBLICATIONS BY STAFF

WHEN TO HARVEST CANNING PEAS. *C.S.I.R.O. Aust. Div. Food Pres. Transp., Circ. No. 5-P. 8 pp. (1955).*

A method for predicting the exact time at which peas should be harvested for canning has been evolved by Mr. L. J. Lynch and Mr. R. S. Mitchell, of the C.S.I.R.O. Division of Food Preservation and Transport.

The importance of knowing the precise date for harvesting peas has long been recognized by both research workers and canners. The quality of canned peas is closely related to the age at which they are harvested, and peas are at their best maturity for only a very short period. Until comparatively recently an approximate prediction of the time of harvest was based on the number of days the particular variety took to reach maturity. Before the stated time had elapsed, a field officer examined the crop and the desirable day of harvest was decided by personal judgment. In the last few years the heat unit system has been adopted, particularly in America, but its indications are so approximate that a final crop evaluation in one form or another is still necessary.

The circular outlines a simple and practical method of predicting the date of harvest by means of a portable instrument designed by C.S.I.R.O. workers. This instrument, known as the maturometer, is described and illustrated in the circular.

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RECENT ADDITIONS TO KNOWLEDGE OF THE CHEMISTRY OF CITRUS FRUITS. *J. F. Kefford. Rev. Pure Appl. Chem. 5: 77-98 (1955).*

This paper reviews the literature published from 1948 to 1954 on the chemical composition of citrus fruits.

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IMINO-ACIDS IN SANTALUM LEAVES. *H. S. McKee and G. Urbach. Nature 175: 470 (1955).*

Extracts of leaves from two Australian species of *Santalum* were examined by paper chromatography and found to contain proline, hydroxyproline, and glutamine.

WATER-SOLUBLE CONSTITUENTS OF FRUIT. II. THE SEPARATION OF ACIDS ON ANION-EXCHANGE RESINS: THE ISOLATION OF l-QUINIC ACID FROM APRICOTS. *E. F. L. J. Anet and T. M. Reynolds. Aust. J. Chem. 8: 267-75 (1955).*

An abstract of Part I of this series has already been published (*C.S.I.R.O. Food Pres. Quart.* **15**: 39). Displacement chromatography on columns of strongly basic anion-exchange resins has been used to separate, isolate, and purify some water-soluble organic acids. The order of emergence of 27 acids from these columns is recorded. Using this method, pure *l*-quinic, succinic, L-malic, and citric acids were isolated from the flesh of the apricot fruit. The method was also used for the purification of hydroxy-acids.

The detection of acids on paper chromatograms with a silver nitrate reagent is described; silver nitrate-sodium hydroxide was used to detect acids having a *vic.*-glycol or α-keto group. The R_F values for 30 acids in two solvent systems are recorded.

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WATER-SOLUBLE CONSTITUENTS OF FRUIT. III. AN EXAMINATION OF THE SUGARS AND POLYOLS OF APRICOTS, PEACHES, PEARS, AND APPLES BY PAPER CHROMATOGRAPHY. *A. S. F. Ash and T. M. Reynolds. Aust. J. Chem. 8: 276-9 (1955).*

The sugars and polyols in 80 per cent. ethanolic extracts of apricots, peaches, pears, and apples were examined by paper chromatography using several solvents. Xylose, fructose, glucose, sucrose, sorbitol, a cyclitol (probably *meso*inositol), and one or more ketose oligosaccharides were detected in all the fruits. Galactose was detected in pears and may also be present, in trace amounts, in peaches and apples. Apricots, peaches, and pears picked in several seasons were examined.

WATER-SOLUBLE CONSTITUENTS OF FRUIT. IV. THE ORGANIC ACIDS IN PEACHES. *E. F. L. J. Anet and T. M. Reynolds. Aust. J. Chem. 8: 280-4 (1955).*

The acids in several varieties of peaches were separated by displacement chromatography on strongly basic anion-exchange resins. *l*-Quinic, L-malic, and citric acids were the three main acids, any one of them being the predominant acid depending on the variety, season, and maturity of the fruit. Mucic acid was found in small quantity in all samples; galacturonic acid was present only in fruit picked at commercial maturity and ripened at 20 °C. The effect of maturity on the three major acids was studied for one crop of Blackburn Elberta peaches; the immature fruit contained only traces of citric acid.

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PLANT MITOCHONDRIA AND SALT ACCUMULATION. *R. N. Robertson, Marjorie J. Wilkins, and A. B. Hope. Nature 175: 640 (1955).*

Mitochondria maintain a higher internal concentration of both mobile cations and mobile anions than the external solution in which they are held. This letter presents evidence that the accumulation of chloride against the concentration gradient is achieved by a mechanism dependent on oxygen uptake.

Copies of the papers mentioned above may be obtained from the Librarian, Division of Food Preservation and Transport, Private Bag, P.O., Homebush, N.S.W. (Telephone: UM 8431, UM 6782.)