CXCVIII. THE ROLE OF SORBITOL IN THE CARBON-METABOLISM OF THE KELSEY PLUM

I. CHANGES IN CHEMICAL COMPOSITION DURING GROWTH AND STORAGE

By IZAK DONEN

From the Chemistry Department, University of Capetown

(*Received 14 August 1939*)

MUCH of the C lost as CO_2 by respiring fruit may usually be accounted for in terms of loss of sugar and acid. But this does not appear to be true for mature Kelsey plums which, though they contain no starch, were found on storage at 13 or 25° to show no loss of sugar at all, and under certain conditions of storage even showed an increase in sugar content.

On investigation it was found that Kelsey plums contain a considerable amount of sorbitol, in some instances as much as 4·5 % of the fresh weight in the mature fruit. Quite a number of the fruits of the Rosaceae are now known to contain sorbitol [Reif, 1934; Strain, 1937], but little is known of the role of hexitols in the metabolism of higher plants. That sorbitol is a probable source of sugar for respiration has been indicated by some investigators [Nuccoroni & Bartoli, 1932; Martin, 1937]. In the Kelsey plum there appears to be also a definite relationship between the initial sorbitol content and the fruit's susceptibility to low temperature injury in store [Donen, 1939]. The part played by sorbitol thus appears to be not only of theoretical but also of some commercial importance.

The investigation described in this paper deals with the accumulation of sorbitol in the Kelsey plum during growth, and traces the course of change in the respirable material of the fruit when stored at 13 and 25° and the extent to which this is modified by a previous exposure of the fruit to 1°. The quantitative relation between loss of C as CO_2 and C as carbohydrate and acid will be discussed in a later communication.

EXPERIMENTAL PROCEDURE

Plan of experiments. Changes during growth were estimated by collecting weekly samples of plums over a period of 77 days before the commercial gathering of the fruit in the 1938–9 season. At each weekly picking three extra samples were taken for storage at 25°, and these were analysed at fortnightly intervals. The general effect of maturity on loss of sorbitol and sugar in store was thus determined. These data were used to corroborate more detailed studies on chemical changes in plums stored at 13° carried out in 1937–8 on three series of samples (S 1, S 2 and S 3). These series were chosen to represent fruit of contrasting sorbitol and sugar contents, the picking being made at 90, 120 and 154 days after petal-fall, when the mean weights per fruit for the three series were 22·4, 50·2 and 80·0 g. respectively. The last pick (S 3) represented fully mature Kelsey plums.

In addition two further series of samples (S 4 and S 5) were picked in 1937–8 for storage at 1°. S 4 was similar to S 3, but S 5 consisted of plums from a different group of trees, on which, in contrast to the first group, the crop had not been thinned and the plums were expected to be of very low sorbitol content.

Method of sampling. Each sample was picked separately by a method of random selection. Plums for S1, S2, S3 and S4 were picked off the same 16 trees on a farm in Somerset West, Cape. S5 was taken from a different group of trees in the same orchard. The samples for estimation of changes during growth and storage at 25° were taken from a group of 20 trees on a farm in Banhoek. A sample usually consisted of 48 plums, but for mature fruit it was reduced to 32. Each tree contributed the same number of plums to every sample.

The trees on both farms were well grown, about 11 years old, well irrigated and fertilized and carrying a moderately heavy crop.

Preparation of sample. Immediately after picking the samples were weighed and taken to the laboratory. The initial samples of each series were analysed immediately, the others stored in constant temperature chambers maintained at high R.H. (90–95 %). Loss of weight was estimated by weighing the fruit again just before analysis.

For analysis the plums were quartered, stones removed, and two opposite quarters of each plum put through a mincing machine. The pulp was thoroughly mixed and 250 g. taken for extraction with 75 % alcohol [Donen, 1937]. After extraction the alcohol was evaporated *in vacuo* at 30° and the aqueous solution made up to 250 ml. and filtered through kieselguhr. Tests showed that filtration through kieselguhr in no way affected the results of analysis.

Methods of analysis

Alcohol-insoluble residue (A.I.R.) was obtained by collecting the residue from alcohol extraction and drying at 80° for 100 hr.

Dry weight was estimated on a portion of pulp dried at 50° for 84 hr. in a well-ventilated oven.

Soluble solids (i.e. solids which are soluble both in 75 % alcohol and in water). 5 ml. of extract as obtained above were dried in a shallow, flat-bottomed dish at 50° for 84 hr. Excellent agreement was obtained between values of dry weight and the sum of soluble solids plus A.I.R.

Acidity. 5 ml. of extract were diluted to 250 ml. with distilled water and titrated with $N/10$ NaOH, with phenolphthalein as indicator. Results were expressed in terms of malic acid.

Residual soluble solids (R.S.S.) were obtained by calculation:

R.S.S. = soluble solids − sugar − acid − sorbitol.

Sugar analysis. Harding and Downs modified copper reagent was used, and the procedure adopted for the determination of reducing sugar, sucrose and fructose was exactly as laid down by van der Plank [1936]. The original extracts were not clarified with Pb acetate or charcoal; baker's yeast was employed instead, and the reducing value of the extracts after fermentation was used as a correction for interference by substances other than sugar. This correction was small, usually 0·2 %, as compared with the total sugar value of 5–10 % of fresh weight. Results obtained in this manner agreed well with those obtained on extracts previously clarified with dibasic Pb acetate.

Sucrose was hydrolysed by adding 2·5 ml. of $5N$ H_2SO_4 to 25 ml. of approximately 0·2 % sugar solution and then heating at 70° for 12 min. On cooling the acid was neutralized with a previously estimated quantity of $10N$ NaOH. Tests were carried out on 13 plum extracts to compare the results of inversion of sucrose by the above method and by using invertase. Close agreement was obtained, although acid hydrolysis usually gave slightly higher results. The mean difference in 13 tests was $+1\cdot1\,\%$ and the highest difference $1\cdot8\,\%$. As a further check on the above method, estimations were made of increase in fructose after hydrolysis with acid and after inversion with invertase. Identical results were obtained. Calculation of the ratio of increase of fructose to increase of glucose on hydrolysis gave a value of $1\cdot039 \pm 0\cdot021$ (mean of 10 determinations). As this calculation carried the error of four separate sugar determinations, the result is most satisfactory and leaves no doubt that only sucrose is hydrolysed by treatment with H_2SO_4.

Isolation and identification of sorbitol. 100 ml. of plum extract were fermented with 2·5 g. of yeast at 37° for 72 hr. The yeast was removed by centrifuging and then by filtration through kieselguhr. A portion equivalent to 5 ml. of original extract was evaporated in a 100 ml. beaker to a syrup, cooled and treated with 0·5 ml. of benzaldehyde and 1 ml. of 50 % (by vol.) H_2SO_4. On stirring for a few minutes a buff-coloured syrupy mass was obtained which, on storing at 1°, set to a cake within a few hours. The procedure followed thereafter was the same as described by Martin [1937] for determination of sorbitol. All his precautions were observed. A whitish granular powder was obtained, M.P. 172–174°. This corresponds to the M.P. of dibenzalsorbitol as commonly reported. The benzal derivative was hydrolysed with HCl, and the acid and benzaldehyde removed by steam distillation. The residue was acetylated and recrystallized as described by Tutin [1925]. A small crop of well-formed crystals was obtained which melted at 97° and was found to be identical with hexa-acetylsorbitol.

A portion of the fermented extract was evaporated to dryness and treated with pyridine as described by Strain [1934]. A large crop of white crystals was obtained. This was recrystallized several times from pyridine, then dried *in vacuo* over conc. H_2SO_4 for several weeks and finally exposed to the air of the laboratory for 7 days. By then the crystals had lost all their pyridine and a white granular cake remained which melted at 91–92° and was found to be identical with anhydrous sorbitol [Strain, 1934].

Estimation of sorbitol. That sorbitol may be obtained in best yields as the dibenzal derivative is well known. Martin [1937] claims that by adopting certain precautions and slightly modifying Werder's original procedure [1932] for estimation of sorbitol, nearly theoretical yields of dibenzalsorbitol may be obtained. Furthermore he claims that sugar does not interfere with the determination.

Martin's claims were substantiated by tests on pure sorbitol and on plum extracts. Good replication was obtained. Thus: taken 0·1906 g. sorbitol, found (by the dibenzal method) $0\cdot1937 \pm 0\cdot0054$ g. (mean of six determinations). This represents a yield of $101\cdot6 \pm 2\cdot8\,\%$. Since some of the available plum material had been previously dried at 50°, tests were also made to find out whether drying of plum pulp at that temperature affected the subsequent yield of sorbitol. 5 g. of the dried material were extracted five times with 75 % ethyl alcohol at 60° and the alcohol removed by evaporation *in vacuo*. Portions of the aqueous solution were used for sorbitol determinations. The results (Table I) indicate that drying

Table I

Sample	Fresh pulp. Sugar removed by fermentation	Fresh pulp. Sugar not removed	Dried material. Sugar not removed
1	0·97	—	0·89
2	1·99	—	1·91
3	2·56	—	2·51
4	2·04	2·06	1·97
5	2·71	—	2·68
6	1·50	1·52	—

of the pulp at 50° and the presence of sugar in the extract did not materially affect the yield of sorbitol.

In this study sorbitol was determined as the dibenzal derivative on alcoholic extracts of the dried material or, when available, on that of fresh pulp. Martin's procedure was adhered to, but the precipitates were dried at 80° instead of 100°.

Presentation of results and error of estimation. All results are expressed as percentage of original fresh weight (O.F.W.) and are presented in a series of graphs. In Figs. 4–7 curves of best fit were calculated to the experimental points. Most of these curves are straight lines, but some, e.g. the sorbitol curve in Fig. 6, are exponential in form. Eight samples for initial analysis were taken with each series of storage samples and the results used for calculation of standard error and significant differences. Standard deviation was calculated as $\sigma = \sqrt{\frac{ev^2}{n-1}}$ where v = deviation from mean and n = number of samples. Significant differences were taken as $2 \cdot 31\sigma$ and are indicated by lines along the appropriate curves in the figures given in the text.

Discussion of results

Sorbitol accumulation during growth

The chief point of interest in the accumulation of sugar-alcohol in Kelsey plums during growth is its relationship to sugar intake. Even very young fruits contain as much as 1 % of sorbitol when total concentration of sugar is only 4 % and when sucrose is almost entirely absent (Fig. 1). Rapid accumulation of the sugar-alcohol begins only during the period of cell enlargement just after stone growth is completed. The beginning of this period usually coincides with the minimum concentration of dry weight of the fruit [Donen, 1936].

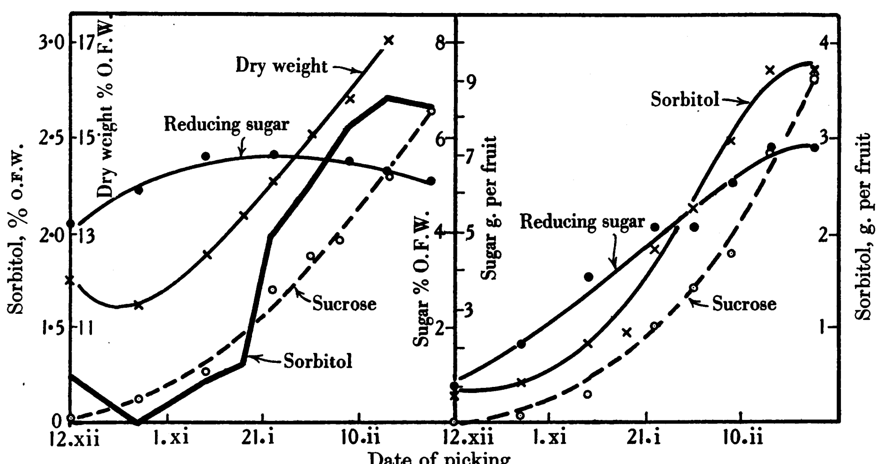

Fig. 1. Fig 2.

Fig. 1. Change in concentration of dry weight, sugars and sorbitol in the Kelsey plum during growth.

Fig. 2. Accumulation of sugar and sorbitol in the Kelsey plum during growth.

A possible interrelationship between sorbitol and the hexoses, but not sucrose, is strongly suggested by the concentration curves shown in Fig. 1. The shapes of the curves for reducing sugar and sucrose are typical for these sugars and have been confirmed over a number of seasons and for fruit from different orchards [Donen, 1936]. It is significant, therefore, that any notable increase in sorbitol concentration should occur only when the concentration of reducing sugars has reached a constant level. This suggests that sorbitol is stored instead of hexoses when the latter have reached their maximum concentration. If hexoses are then accumulated at a greater rate than the rate of growth of the plum, i.e. in excess of the amounts needed to maintain the hexose concentration, sorbitol will be formed. Towards the end of the growth period, when hexose accumulation (g./fruit, Fig. 2) declines, sorbitol formation also stops. On the other hand sucrose continues to accumulate so long as the plum remains on the tree [Donen, 1936].

That the fall in intake of sorbitol towards the end of the growth period is genuine and not due to sampling error was confirmed by analysis of a series of samples of plums from different orchards picked just at the first appearance of colour in the fruit and again about a fortnight later when the plums were showing considerable yellowing and reddening—the usual signs of advanced ripening. The results (Table II) indicate that sorbitol concentration shows little change towards the end of the growth cycle of the plum.

Table II

Sample	1	2	3	4	5	6	7
Sorbital concentration as % O.F.W.:							
Fruit with 1st tinge of red	2·23	1·50	1·61	3·15	2·10	1·87	2·92
Ripe fruit	2·11	1·45	1·56	3·24	2·30	1·98	2·60

Sources of respirable material

The mature Kelsey plum contains, on the average, about 16·65 g. of dry weight material per 100 g. fresh weight.[1] Of this 1·5 g. represent the alcohol-insoluble residue which contains no starch and consists mostly of hemicelluloses and pectic materials. Widdowson [1932] found no substantial evidence that the hemicelluloses present in the apple are a source of reserve carbohydrate in that fruit. It is probable that this is also true for the plum, for the observed loss of A.I.R. in the plum on storage is very small and only just larger than the calculated significant difference (Figs. 4–6).

The 15·15 g. of alcohol-soluble solids consist of 10·48 g. of sugar, 2·82 g. of sorbitol and 0·835 g. of acid (expressed as malic). This leaves 1·01 g. of residual soluble solids which should be almost completely accounted for by soluble nitrogenous compounds (equivalent to 0·07 g. N, i.e. very approximately 0·35 g. organic material) and soluble mineral salts (equivalent to 0·4 g. of ash). The changes in R.S.S. fraction in the Kelsey plum on storage are very small (Figs. 4–6) and not significant. The general tendency of the R.S.S. curves to show an increase and for those of A.I.R. to show a decrease during storage might be partly due to the progressive hydrolysis of proteins [Donen, 1936] and also to the increase in the soluble pectin fraction in stored plums. Under the conditions of alcoholic extraction employed, it became increasingly difficult to precipitate all of the soluble pectin.

It thus appears that in the plum the likely source of respirable material during storage is confined to sugars, acid and sorbitol. This is borne out by results given in Table III which show that total loss in dry weight is very nearly equal to the sum of losses in acid, sugar and sorbitol.

[1] The figures quoted are average results obtained by taking mean values of 44 analyses of commercially ripe fruit from different orchards.

Table III. *Losses of respirable material from stored plums and the probable errors of the various estimates*

All results in g. of original fresh wt.

Population	Days	Loss of dry wt.	Loss of sorbitol So	Loss of acid A	Loss of sugar S_T	Total loss of So + A + S_T	Loss of So + A + S_T as % loss of dry wt.
S1	64	1·92 ± 0·10	0·39 ± 0·03	0·70 ± 0·08	0·81 ± 0·09	1·90 ± 0·12	99 ± 9
S2	102	4·16 ± 0·15	0·89 ± 0·03	1·22 ± 0·03	1·86 ± 0·08	3·97 ± 0·09	95·5 ± 4
S3	55	2·80 ± 0·14	2·14 ± 0·11	0·46 ± 0·03	0·00 ± 0·15	2·60 ± 0·17	93 ± 8
S4 (1–20°)	49	2·09 ± 0·14	1·53 ± 0·12	0·35 ± 0·03	− 0·09 ± 0·15	1·79 ± 0·20	86 ± 12
S4 (1–7·5°)	68	1·68 ± 0·14	1·46 ± 0·12	0·52 ± 0·03	− 0·33 ± 0·15	1·65 ± 0·20	98 ± 15
S5 (1–20°)	49	3·09 ± 0·13	0·89 ± 0·04	0·53 ± 0·02	1·48 ± 0·10	2·90 ± 0·11	94 ± 6
S5 (1–20°)	68	2·35 ± 0·13	1·09 ± 0·04	0·51 ± 0·02	0·74 ± 0·10	2·33 ± 0·11	99 ± 8

Loss of respirable material

The loss of sorbitol from plums stored at 25 or 13° is very rapid and the rate of initial loss increases markedly with maturity (Fig. 3). On prolonged storage the sorbitol content reaches a very low and almost constant value, and usually at this point there is a notable increase in the rate of loss of total sugar (Figs. 3–7). This interrelationship between sugar and sorbitol is shown in all series of samples. The young fruits of S1, which contained very little sugar-alcohol, show an immediate loss of sugar; plums with a relatively low sorbitol content (S2) first lose practically all of their sorbitol before any marked fall in sugar content is observed;[1] whilst plums with a high concentration of sorbitol (S3) show a rapid loss of sugar-alcohol but hardly any loss of sugar. This contrast between fruits of high and low sorbitol contents is again shown by the S4 and S5 plums (Fig. 7).

The rate of acid loss shows no marked relation to maturity and does not in any way reflect the rate of loss of either sugar or sorbitol. The changes in acid in all series follow, however, the dry weight curves which are quite a good measure of change in total respirable material (Table III).

The total sugar values obtained in samples 7, 8 and 9 (Fig. 3) and those for S3 (Fig. 6) strongly suggest that in mature plums stored at 13 and 25° sugar content remains constant if sorbitol concentration is high. A straight line has therefore been fitted to the total sugar curve in S3. The mean value for total sugar in all stored samples of this series is 10·15 ± 0·22 and the value calculated for eight initial samples is 10·10 ± 0·15. No significant loss of sugar has therefore taken place.

Effect of storage at low temperature

When Kelsey plums are first stored at low temperature (0 or 1°) for 21–25 days and then raised to higher temperatures (above 7·5°) they show an increase of as

[1] The greatly increased rate of sugar and acid loss which occurs in S2 (Fig. 5) on, say, day 40 is not entirely due to depletion of sorbitol. The onset of the respiratory climacteric occurred at that stage, and the marked loss of acid and sugar as well as of dry weight is a reflexion of the increase in rate of respiration. Sorbitol exhaustion does not normally coincide with onset of the climacteric.

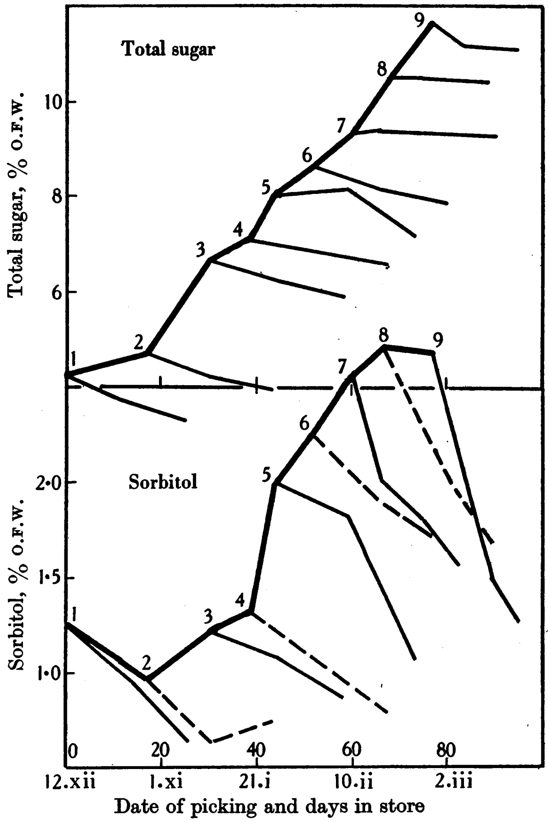

Fig. 3.

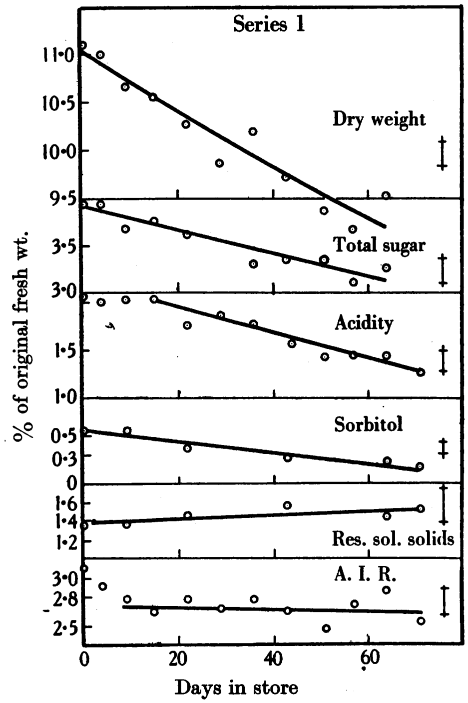

Fig. 4.

Fig. 3. Effect of maturity on loss of total sugar and sorbitol in Kelsey plums stored at 25°. Heavy lines refer to initial analyses on picking, thin lines to analysis in store. Numbers along the heavy lines indicate serial number of picking.

Fig. 4. Chemical changes in Kelsey plums stored at 13°. S1, vertical lines alongside curves indicate significant differences.

much as 10–15% of total sugar. Some typical results are given in Table IV; the observed increase in sugar is always accompanied by a slightly greater decrease in sorbitol.

Table IV. *Changes in total sugar and sorbitol after exposure to low temperature*

Sample	Initial analysis		25 days at 0°		25 days at 0° and 10 days at 25°		25 days at 0° and 20 days at 25°	
	Sugar	Sorbitol	Sugar	Sorbitol	Sugar	Sorbitol	Sugar	Sorbitol
1	10·68	3·42	10·76	—	11·28	2·20	11·04	1·74
2	10·43	3·79	—	—	10·80	—	11·67	2·11
3	11·28	3·44	11·58	2·63	12·35	2·47	11·96	2·14
4	10·63	3·52	—	—	11·37	2·55	11·81	2·35
5	9·93	3·27	—	—	11·15	—	10·14	2·07
6	10·88	3·20	—	—	12·20	1·89	11·72	2·08
7	10·89	2·67	—	—	11·82	1·43	10·18	0·99

The relationship between sorbitol content and sugar increase was studied by storing two series of plums (S4, high in sorbitol; and S5, low in sorbitol) at first for 25 days at 1° and then one set of each series at 20° and another set at 7·5° (Fig. 7). The increase in sugar begins in the plum during the period of exposure to low temperature and the extent of the increase depends upon the initial sorbitol content and the rate of its depletion. When the plums are transferred from low to

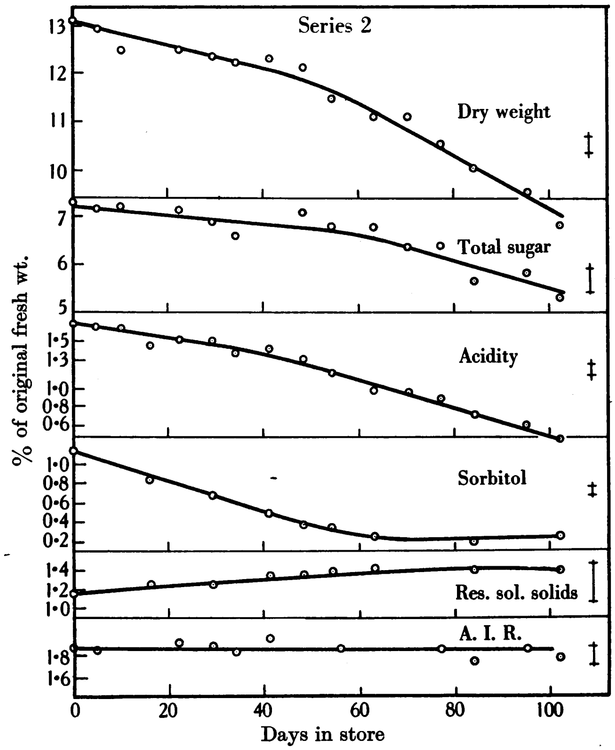

Fig. 5.

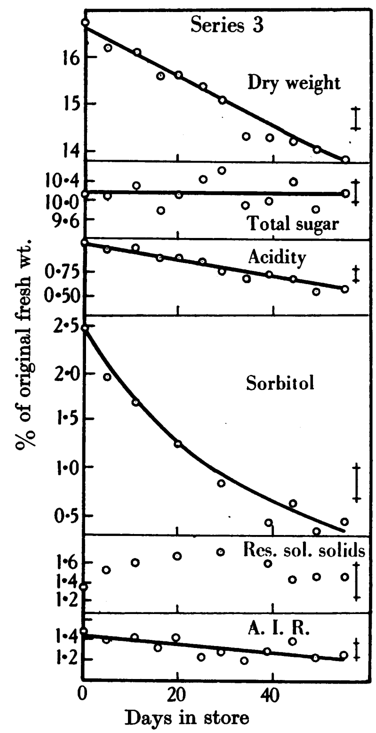

Fig. 6.

Fig. 5. Chemical changes in Kelsey plums stored at 13°. S2, vertical lines alongside curves indicate significant differences.

Fig. 6. Chemical changes in Kelsey plums stored at 13°. S3, vertical lines alongside curves indicate significant differences.

higher temperatures the rate of sorbitol loss is greatly increased. The increase in sugar continues, however, until the concentration of sorbitol has become very low. After that, rapid loss of sugar may occur, as in S5, where the sorbitol reached a very low and almost constant level. Plums of S5, containing only 1·3 % sorbitol, showed much less increase in sugar than the S4 fruit which initially had 2·5 % of sugar alcohol.

Martin [1937] reported that Bosc pears when ripened at 67° F. lost sorbitol and showed an increase of sugar. He did not emphasize that his pears were originally stored at 30–31° F. for about 9 weeks, and apparently assumed that sorbitol gave rise directly to sugars. If that is also true for the Kelsey plum the observed constant value for total sugar in the S3 would then be merely a reflexion of the balance between sugar lost in respiration and sugar replaced by sorbitol.

In the next communication it will be shown, however, that total loss of C as sugar, acid and sorbitol (and occasionally as sorbitol alone) considerably exceeds the observed loss of C as CO_2 during the first 30 or 40 days of storage of plums at 25 or 13°. Under these conditions accumulation of sugar should have been observed. Increase in sugar was observed, however, only in those plums which had been previously kept for some time at low temperature, whilst good agreement between loss of C as CO_2 and C lost as sorbitol + sugar + acid was obtained only if the plums were kept in store for some considerable time (60–100 days).

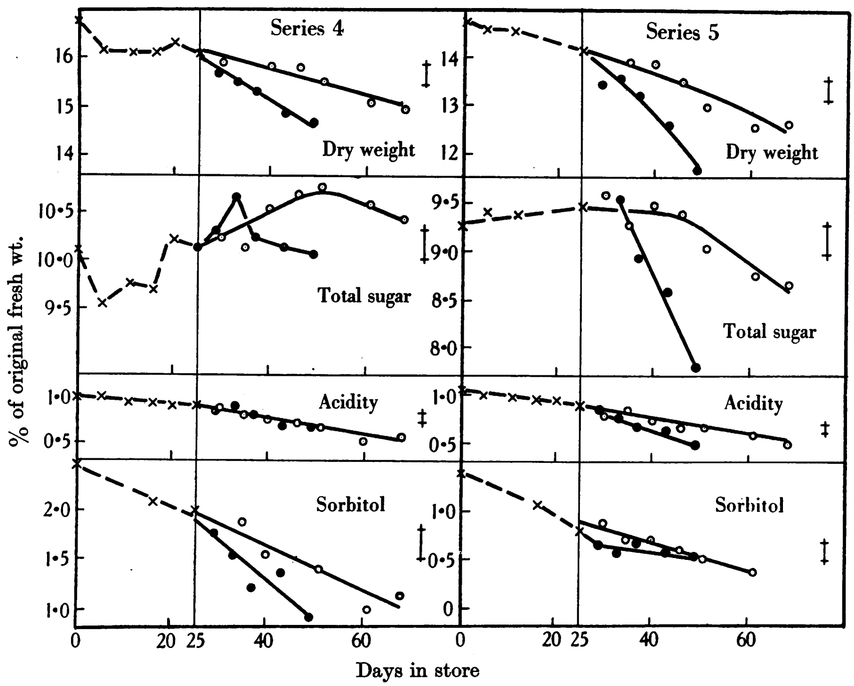

Fig. 7. Chemical changes in Kelsey plums stored for 25 days at 1° and then transferred to 20 and 7·5°: —×——×— 1°, o—o 7·5°, •—• 20°. Vertical lines alongside the curves indicate significant differences.

This suggests that possibly sorbitol first breaks down to intermediate compounds, which under certain conditions (e.g. exposure to low temperature) are rapidly converted into sugars.

Summary

1. Sorbitol has been identified as a constituent of the Kelsey plum.

2. Experiments are described tracing the accumulation of sorbitol during growth and the changes that take place in the respirable material of the plums on storage at 13 and 25°.

3. The mature Kelsey plum contains, on the average, 2·8 % of sorbitol, most of which is accumulated during the later part of its growth cycle. It is suggested that during growth of the plum sorbitol is stored in place of hexoses when the latter have reached a maximum concentration.

4. Loss of respirable material from stored plums can be almost completely accounted for in terms of sorbitol, sugar and acid. Sorbitol is lost rapidly in

store, but the loss of sugar is dependent upon the initial sorbitol concentration. At 13 and 25° mature plums of a high sorbitol content show no significant loss of sugar. Plums of low sorbitol content show marked sugar loss only when most of the sorbitol has disappeared.

5. When plums are stored at 1° for 25 days and then transferred to 7·5 or 20° they show a 10–15 % increase in total sugar. The extent of this sugar increase depends upon the initial sorbitol content and on its rate of exhaustion in store.

REFERENCES

Donen (1936). *Trans. roy. Soc. S. Afr.* **24**, 7.
—— (1937). *Trans. roy. Soc. S. Afr.* **25**, 73.
—— (1939). Unpublished data.
Martin (1937). *Bot. Gaz.* **99**, 48.
Nuccoroni & Bartoli (1932). *Boll. ist. agr. Pisa,* **8**, 277.
Reif (1934). *Z. Untersuch. Lebensm.* **68**, 179.
Strain (1934). *J. Amer. chem. Soc.* **56**, 1756.
—— (1937). *J. Amer. chem. Soc.* **59**, 2264.
Tutin (1925). *Biochem. J.* **19**, 416.
Van der Plank (1936). *Biochem. J.* **30**, 457.
Werder (1932). Handbuch der Pflanzenanalyse, **2**, 767. Vienna.
Widdowson (1932). *Ann. Bot., Lond.,* **46**, 597.