CCXLII. THE ROLE OF SORBITOL IN THE C-METABOLISM OF THE KELSEY PLUM

II. RELATION OF CARBOHYDRATE AND ACID LOSS TO CO₂ PRODUCTION IN STORED FRUIT

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It was shown in a previous communication [Donen, 1939] that, in the Kelsey plum sugars, acid and sorbitol were the most likely sources of C in the respiration of the detached fruit, and that their combined loss accounted satisfactorily for the depletion of dry weight during storage. In this paper an attempt is made to construct a balance sheet relating C lost by stored plums as CO₂ with the C lost as sugar, acid and sorbitol. Such a balance sheet is of some interest, as little information is available of the extent to which compounds other than sugars, or known precursors of sugars, contribute towards the C lost as CO₂ by respiring plant tissue. In the leaves of barley [Yemm, 1935], rhubarb [Vickery & Pucher, 1939], and wheat [Krotkov, 1939] as well as in germinating rice seedlings [Dastur & Desai, 1935] the loss of carbohydrates alone is insufficient to account for the output of CO₂. Proteins or some undetermined carbohydrates have been suggested as the possible sources of the excess CO₂. In the apple the loss of acid and sugar was found to be 17–30 % greater than the C given off as CO₂ [Archbold & Barter, 1934]. Although the metabolisms of the apple and the leaf are possibly not directly comparable, nevertheless this result is the direct opposite of what has been found for detached leaves. In the plum the C lost as CO₂ can be, under certain conditions, almost completely accounted for in terms of losses of sorbitol, acid and sugar, but it will be shown that this conclusion may be greatly modified by the length and storage conditions of the experiment.

EXPERIMENTAL

The data given in this paper are presented in the form of graphs. They deal with rates of loss of C in stored fruit and are based on results of experiments described in Part I of this study [Donen, 1939]. For details of analysis and of chemical change in stored Kelsey plums that communication should be consulted. Here only a brief outline will be given of the scheme of the experiments.

Five different sets of plums were taken for storage. The first three sets (S 1, S 2, S 3) were stored at 13° immediately on picking and represented fruit of different maturity and different chemical composition. S 1 consisted of young fruit containing 4 % total sugar, 2 % acid as malic and only 0.5 % sorbitol. S 2 plums were picked midway in the growth cycle of the fruit and contained 7.3 % sugar, 1.6 % acid and 1.5 % sorbitol. The S 3 group of plums represented mature fruit and contained 10 % sugar, 1 % acid and 2.5 % sorbitol.

The other two sets of plums (S 4 and S 5) consisted of mature plums which were first stored for 21 days at 1° and then ripened at higher temperatures

(7.5 and 20°). S 4 consisted of fruit high in sorbitol and was identical in composition with S 3. In contrast to S 4, S 5 contained relatively little sorbitol (1.4 % in S 5 as compared with 2.5 % in S 4), but the sugar (9.3 %) and acid (1 %) contents were almost the same as in S 4.

The fruit was stored at the requisite temperatures immediately on picking, and the changes in sorbitol, sugar and acid were followed by analyses at 5 to 7 days' intervals. Curves of best fit were then calculated to the experimental points and from these curves corrected values of the estimates were obtained. These corrected values were used for the estimation of average rates of loss shown in Figs. 1-5. Differences between the observed and calculated values were used for the estimation of standard error. Significant differences in estimation of loss of C were then calculated and are shown alongside the appropriate curves.

CO₂ estimation was done on samples chosen from each different set of fruit and stored under the same temperature conditions. The CO₂ drift of S 1 was estimated on a sample of 32 plums. Such a large sample was not found suitable for the more mature series and the number was reduced to 24 for S 2, to 5 for S 3 and S 4, and to 8 for S 5. In selecting the smaller samples for CO₂ estimation, care was taken to choose plums which represented as nearly as possible the average of the population used for the experiment.

The arrangement of the apparatus for CO₂ estimation was briefly as follows. Air was blown by means of a pump through two bubblers containing strong NaOH and then through a trap containing soda-lime to absorb all traces of CO₂. The air stream was then passed through a water trap, through a bubbler containing CaCl₂ solution to adjust humidity and finally into a gas-tight container holding the fruit. The CaCl₂ bubbler and the water-trap were kept inside the constant temperature chamber to allow adjustment of the incoming air to the temperature of the fruit. The water-trap was essential to allow condensation of moisture in the incoming air as its temperature changed from 20° outside the chamber to 13° or 1° inside the chamber.

The CO₂ evolved was absorbed in a series of Pettenkoffer tubes which contained $N/10$ Ba(OH)₂ and were worked by a clock mechanism to allow automatic change of absorption tubes every 3 or 6 hr. Smooth curves were drawn through the CO₂ record thus obtained and the values given in the graphs are readings taken from these smoothed curves.

All results have been expressed in terms of C and the given rates of loss refer to mg. C lost per day per 100 g. of original fresh weight.

Results

Series I. Young plums contain little sorbitol, and in S 1 sugar and acid accounted for 80 % of the total loss of C estimated as sugar + acid + sorbitol (to be referred to as S.A.S.-C). Sugar and acid contributed C in almost equal proportions and the rates of loss of all three components were quite uniform throughout the observed storage period. In Fig. 1 the rates of loss of sugar and acid are represented as continuous straight lines. The fluctuations in the estimations of average rates that were actually obtained may be judged from the lines for total C.

The CO₂ curve showed a rapid fall during the first 7 days of the experiment and only during this period was the rate of loss of CO₂-C significantly higher than the observed rate of loss of S.A.S.-C. During the remaining period, including the slight climacteric rise towards the end, the loss of CO₂-C did not differ significantly from that of S.A.S.-C.

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After 64 days, when the experiment was terminated, the estimated loss of S.A.S.-C was 730 mg. (with a probable error of ± 41 mg.) compared with a CO_2 loss of 721 mg.

Fig. 1. Rate of loss of C in "young" plums (S 1) stored at 13°. AA, acid. BB, acid+sugar. CC, acid+sugar+sorbitol. Black circles, CO_2 .

Fig. 2. Rate of loss of C in half-matured plums (S 2) stored at 13°. AA, acid. BB, acid+sugar. CC, acid+sugar+sorbitol. Black circles, CO_2 .

Series II. The experiment with the fruit of this series lasted for over 100 days. These plums contained a considerable amount of sorbitol and the loss of S.A.S.-C during the first 50 days was completely dominated by the rate of loss of the sugar-alcohol (Fig. 2). The latter contributed almost 50 % of the S.A.S.-C compared with a contribution of about 25 % each by sugar and acid. The CO_2 -C loss during the same time was significantly less than the S.A.S.-C loss and had the experiment been terminated after about 50 days it could have been concluded that the S.A.S.-C loss (594 ± 65 mg.) was 29 % greater than the CO_2 -C loss (458 mg.).

The sorbitol content of the plums in this experiment was almost completely exhausted during the first 50 days of storage, and its contribution to the

S.A.S.-C lost during the remaining period of the experiment was negligible. During the latter stage sugar loss accounted for 61 % of the S.A.S.-C and acid for about 33 %. This last stage was also marked by a rapid rise in the rate of CO_2 evolution which reached the peak of its climacteric on about the 80th day. It is of interest to note that the rate of loss of S.A.S.-C followed to some extent the rising curve of CO_2 -C, but even then the latter was significantly higher than the S.A.S.-C curve. During the last 48 days CO_2 -C loss was 13 % higher than the loss of S.A.S.-C. For the whole period of the experiment (102 days), however, C lost as sugar, acid and sorbitol almost exactly balanced the CO_2 -C. S.A.S.-C loss was 1490 ± 65 mg. CO_2 loss was 1470 mg.

Series III. The mature plums used for this experiment showed no significant change in sugar concentration when stored at 13° , and the C lost was due solely to loss of acid and sorbitol. The rate of acid loss was uniform throughout and it contributed only 17.8 % of the S.A.S.-C.

Fig. 3. Rate of loss of C in mature plums (S 3) stored at 13° . AA, acid. BB, acid + sugar. CC, acid + sugar + sorbitol. Black circles, CO_2 .

The rate of sorbitol loss followed an exponential curve; it was very high at the beginning but fell off rapidly towards the end of the experiment. During the first 20 days the loss of sorbitol-C alone was 57 % higher than the loss of CO_2 -C. If acid loss is included then during that period 78 % more C was lost as S.A.S. than as CO_2 .

The CO_2 curve rose steeply after the 16th day (Fig. 3) and reached the peak of its climacteric 15 days later. During the climacteric period (20th to 55th day) the CO_2 -C loss was far in excess of the loss of S.A.S.-C. The latter was 465 ± 65 mg. whilst the CO_2 evolved amounted to 696 mg. C, i.e. very nearly 50 % higher than the S.A.S.-C loss. This surplus of CO_2 -C was almost exactly balanced by the deficit during the first 20 days of storage so that for the whole duration (55 days) the CO_2 -C loss was 1000 mg. and that for S.A.S.-C 996 ± 65 mg.

It is apparent from the results so far presented that the losses of C measured as loss of sugar, acid and sorbitol were not a true measure of the actual amounts of C eliminated from the plum at all stages of the experiment. The fact that

apparent deficits of C at one stage were balanced by apparent surplus of C at another stage strongly suggests that sorbitol, and possibly even sugar, gave rise to intermediate breakdown products which were not measured by the analytical methods employed, but which were subsequently drawn upon in respiration, thus finally giving rise to CO_2 .

Series IV and V. These experiments were in essence studies of the extent to which the metabolism of Kelsey plums is modified by exposure to low temperatures (1°), and how this is again affected by transferring the fruit back to a higher temperature (20 or 7.5°). The complex series of changes that occur in the rates of loss of sugar, acid and sorbitol are shown in Fig. 4 (for S 4) and in Fig. 5 (for S 5).

Fig. 4. Rate of loss of C in mature plums (S 4) stored for 25 days at 1° and then transferred to 7.5 or 20° . AA, acid. BB, acid + sorbitol. CC, acid + sorbitol + sugar. Black circles, CO_2 . For further explanation see text.

In both graphs the AA line above the zero line, 00, represents rate of loss of acid. The BB line indicates the rate of loss of acid and sorbitol combined, and the CC line that of acid + sorbitol + sugar. The rates of loss of sugar are measured as the distances along the ordinates between BB and CC, and are positive if CC is above BB, and negative (i.e. there is a gain in sugar) if CC is below the line BB.

Reference has already been made [Donen, 1939] to the fact that exposure of the fruit to 1 or 0° introduces effects which are not merely the direct result of retardation of rates of change by low temperature. In Fig. 4 it is shown that an initial loss of sugar in S 4 on storage at 1° was reversed to a gain after 10 days, and that this increase in sugar persisted not only during the remaining period of storage at 1° but also for a considerable time after the fruit was transferred to 20 or 7.5° .

The considerable loss of sugar shown in S 4 during the first 10 days is of interest, for during that period the loss of sorbitol and acid was more than

sufficient to account for the loss of CO_2 -C. S 5 did not show this initial loss of sugar. In another experiment with Kelsey plums, and also in several experiments with Peregrine peaches, it was found, however, that such sugar depletion is the usual occurrence during the first 10–12 days of storage at 1 or 0° . Its possible significance will be referred to in a later section of the paper.

The increase of sugar in cold-stored plums was observed in both S 4 and S 5, as well as in other Kelsey plums similarly treated. It was not observed in plums stored at 13° or at higher temperatures and must be, therefore, an effect peculiar to low temperature storage.

Fig. 5. Rate of loss of C in mature plums (S 5) stored for 25 days at 1° and then transferred to 7.5° or 20° . AA, acid. BB, acid + sorbitol. CC, acid + sorbitol + sugar. Black circles, CO_2 . For further explanation see text.

S 4 plums showed a more pronounced sugar synthesis than S 5, and in both series post-storage at 7.5° was more effective than 20° in prolonging the period of this sugar increase.

The source of C for the synthesis of sugar is of some interest here. In Table I an analysis is given of the amounts of C gained or lost by the various constituents at the moment of maximum increase in sugar in the two sets of plums. The loss of C as acid and sorbitol was sufficient to account both for the loss of CO_2 and for the gain in sugar. But it will be observed that although some of the C for sugar formation might have arisen from acids, even the total C lost as acid was not sufficient to account for all the increase in sugar and a good proportion of the latter must have had sorbitol as its original source. In each case, moreover,

Table I

C-balance at points of maximum rate of sugar increase. All results in mg. C.

	Period	Net sugar gain	Loss of sorbitol	Loss of acid	Loss of S.A.S.-C	Loss of CO ₂ -C	Excess S.A.S.-C over CO ₂ -C %
S 4	25 days at 0° and 8 days at 20°	138	324	82	268	198	35
	25 days at 0° and 26 days at 7.5°	258	427	139	308	294	5
S 5	25 days at 0°	60	205	42	187	114	64
	25 days at 0° and 10 days at 7.5°	106	258	74	226	181	25

there is (considering the whole period) sufficient C from sorbitol breakdown to account for all the increase in sugar, and this, coupled with the observation that low sorbitol fruit (S 5) showed a much less pronounced sugar increase than plums having much sorbitol (S 4), points to the probability of sorbitol being the sole source of synthesized sugar in Kelsey plums stored at low temperatures.

Fig. 6. The variation in excess of C lost as sum of acid, sugar and sorbitol over C lost as CO₂ with time in store. S 1, S 2 and S 3 stored at 13°. S 4 and S 5 stored for 25 days at 1° and then at 7.5°.

Throughout the duration of the S 4 and S 5 experiments the accumulated loss of S.A.S.-C was in considerable excess over the total loss of CO₂-C. As in S 2 and S 3 there were short periods, however, over which the loss of C as CO₂ was in excess of S.A.S.-C loss, thus providing further evidence of accumulation of intermediate breakdown products of sorbitol and sugar. During the period of maximum sugar increase the concurrent loss of sorbitol could not possibly have accounted for the gain in sugar, and intermediate compounds resulting from

previous breakdown of sorbitol must therefore have been utilized in its synthesis. The variation in the amounts of these undetermined breakdown products is shown in Fig. 6. In S 4 and S 5 there was an initial accumulation of undetermined products which were depleted during the stage of sugar synthesis and reached a minimum at the point of maximum sugar concentration.

The rapid breakdown of sugars, observed towards the end of the experiment in both series of plums, is rather curious and not compatible with any theory of mere utilization of carbohydrate for respiration. The transition of sugar synthesis to sugar breakdown occurred at a stage when there was still a considerable amount of C unaccounted for as CO_2 and at a time when, for instance in S 4, sorbitol concentration (1 %) and its rate of loss were still quite high (Fig. 4). When the experiments were terminated the two sets of S 4 plums stored at 20 and 7.5° showed an excess of S.A.S.-C over CO_2 -C equal to 54 and 50 % respectively. The corresponding figures for S 5 were 106 and 100 %. These differences were most marked during the period immediately following the maximum sugar increase. In S 5 plums, post-stored at 20°, S.A.S. loss was 733 mg. C per 100 g. of original fresh weight over a period of 16 days. Sugars contributed 604 mg. of that. The CO_2 lost during the same time amounted to only 295 mg. C.

DISCUSSION

From the foregoing results it is clear that in considering the relationship between respiratory CO_2 and the substrates that give rise to it, low temperature effects should be allowed for. The storage temperature of 13° was chosen for the present experiments because it was known that 13° was outside the range of temperatures at which low temperature breakdown had previously been observed. Parallel experiments at 25° gave results similar to those at 13° and the results at the latter temperature may therefore be assumed to be representative of the normal metabolism of the Kelsey plum.

Maturity of the fruit, with the corresponding variation in sorbitol content, appears to be the most important factor governing the C-balance of the Kelsey plum at high temperatures. In young fruit containing little sorbitol CO_2 -C losses balance quite accurately the losses of acid, sugar and sorbitol at almost any stage of the experiment (e.g. S 1, and see Table II). As the fruit matures and its sorbitol content increases, a C balance may still be obtained but such agreement is, in a sense, accidental, as it depends entirely upon the length of the experiment and on the previous history of the fruit (see S 2 and S 3 and Table II). This is illustrated by the curves shown in Fig. 6 which trace the excess of C lost as

Table II. *Maturity and loss of sugar, acid and sorbitol*

The weight of the fruit in the first column is given as a guide to the maturity of the Kelsey plums on picking. They were then stored for 26 days at 25°. The given losses of C represent total loss after 26 days at 25°.

Wt. per fruit in sample g.	Initial sorbitol content % o.f.w.	Sugar lost in mg. C	Acid lost in mg. C	Sorbitol lost in mg. C	Total C loss S.A.S.-C	Loss of CO_2 -C
24	1.25	368	92	238	698	650
43	0.97	284	32	87	403	429
73	1.31	192	68	202	462	420
93	1.99	288	122	364	774	431
137	2.71	4	86	412	502	382
138	2.66	0	112	556	668	452

sugar, acid and sorbitol over CO_2 -C liberated by the fruit during the course of the various experiments.

The results shown in these curves are readily explained on the hypothesis that when sorbitol is broken down not all of its C is immediately utilized for CO_2 formation, but that some of its breakdown products are accumulated in the fruit. This might happen if the rate of sorbitol breakdown is greater than the rate of CO_2 formation from the intermediate products, or, if sorbitol gives rise to more than one type of intermediate compound and one of these is preferentially respired. Accumulation of intermediate C compounds not recorded either as acid, sugars or sorbitol then takes place and continues until the gradual depletion of sorbitol results in a slowing down of the breakdown of sorbitol to a rate below that of CO_2 formation. The accumulated intermediate C compounds are then rapidly utilized and finally exhausted. If at the same time the sorbitol level becomes very low, more sugar is utilized (as in S 2), but if rate of loss of sorbitol is still considerable, sugars are not used (S 3).

The relation between sorbitol and sugar utilization is not quite clear but possibly the two processes are distinct. It is probable that sorbitol breakdown is an irreversible process, as no increase in sorbitol has been observed in any of the experiments with stored plums. Also, at high temperatures sorbitol either does not give rise to sugars or does so at a very slow rate, for otherwise an increase in sugar should have occurred in S 3 where loss of sorbitol was far in excess of the CO_2 requirements during the first 20 days in store.

It is generally accepted that low temperature may profoundly affect the metabolism of detached plant organs and Barker's work on potatoes [1933] suggests that such effects might last for some considerable time after the return of the organ to higher temperatures. In the plum, low temperature storage affects both the sugar and sorbitol metabolism and results in a series of changes which appear successively as sugar loss, sugar synthesis and finally sugar breakdown once again.

More experimental evidence is needed to clarify this complex process, but a working hypothesis may be ventured here. The observations described above may be pictured as the resultant of two simultaneous processes induced in the fruit by low temperature. The first is a stimulation of sugar breakdown; and this stimulation persists for a considerable time after the return of the fruit to higher temperatures. Such a process would explain the rapid initial sugar breakdown at low temperature as well as the loss of sugar during post-storage at the high temperatures. Superimposed upon the first process is a second one resulting in stimulation of sugar synthesis from sorbitol; it only occurs at low temperatures but its maximum rate is higher than that of the first process of sugar breakdown. When the fruit is returned to high temperatures sugar synthesis from sorbitol is immediately slowed down, more rapidly by a temperature of 20° than by that of 7.5° . This second process would account for the observed change over from initial sugar breakdown to sugar synthesis at low temperature and for the reversal of that on returning the fruit to the higher temperatures. It fits in with the observations that sugar increase ceased in S 4 during post-storage when ample supply of sorbitol was still available and that it did not occur at all in fruit which was not first cold-stored.

It is of interest here that the results obtained with S 4 and S 5 suggests that, like sorbitol, sugars are also capable of giving rise to considerable quantities of intermediate C compounds which are not completely utilized in respiration. This conclusion is in no way incompatible with modern ideas on respiration, but the possible accumulation of such large amounts of breakdown products of sugar has not been previously reported.

To what extent similar sugar breakdown occurs in other plant organs stored at low temperatures is not known, but the results obtained with the Kelsey plum offer an explanation of the findings of Archbold & Barter [1934]. These workers stored their apples for some considerable time at 1° and then used them for respiration tests at 12°. Their observation that more C was lost as sugar and acid than was accounted for by CO₂-C is similar to our observations on Kelsey plums stored at low temperature. It is quite possible that they would have obtained different results if their experimental material had not been cold-stored prior to the beginning of their experiment.

In view of the important part played by sorbitol in the C-metabolism of the Kelsey plum it is essential to investigate what role hexitols in general play in the metabolism of higher plants. Strain [1937] and Reif [1934] have indicated that sorbitol, and probably other higher alcohols, are present in a great number of leaves and fruits. The ordinary methods of sugar analysis do not account for hexitols, and some of the sources of C in respiration which have been ascribed to unknown carbohydrates or to proteins might well have had their origin in compounds of this type.

SUMMARY

A comparison has been made of the C lost as sorbitol, sugar and acid (S.A.S.-C) with the C given off as CO₂ by Kelsey plums stored at 13°.

It is shown that maturity of the fruit, with the corresponding variation in sorbitol content, is the most important factor governing the C-balance in plums stored at high temperatures. In young plums, containing little sorbitol, the loss of S.A.S.-C balanced quite accurately the loss of CO₂-C at almost any stage of the experiment. In mature fruit this balance could also be obtained, but agreement between losses of S.A.S.-C and CO₂-C was accidental and depended entirely upon the length of the experiment.

The results show that loss of S.A.S.-C was not a true measure of the actual amount of C eliminated from the plum at all stages of the experiment and it is suggested that sorbitol and sugars gave rise to intermediate breakdown products which could subsequently be drawn upon in respiration and thus finally gave rise to CO₂.

When plums were stored at 1° and then returned to higher temperatures (7.5 and 20°), effects were induced which profoundly affected both the sugar and sorbitol metabolism of the fruit. A series of changes resulted which appeared successively as sugar loss, sugar synthesis and finally sugar breakdown once again. It is suggested that these changes were the resultant of two simultaneous processes induced in the fruit by low temperature and consisted of sugar breakdown and concurrent sugar synthesis from sorbitol. The S.A.S.-C loss in such plums was always much greater than the corresponding CO₂-C loss.

REFERENCES

- Archbold & Barter (1934). *Ann. Bot., Lond.*, **48**, 957.
Barker (1933). *Proc. roy. Soc. B*, **112**, 312.
Dastur & Desai (1935). *Ann. Bot., Lond.*, **49**, 53.
Donen (1939). *Biochem. J.* **33**, 1611.
Krotkov (1939). *Plant Physiol.* **14**, 203.
Reif (1934). *Z. Untersuch. Lebensm.* **68**, 179.
Strain (1937). *J. Amer. chem. Soc.* **59**, 2264.
Vickery & Pucher (1939). *J. biol. Chem.* **128**, 685.
Yemm (1935). *Proc. roy. Soc. B*, **117**, 504.