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CARBOHYDRATE CHANGES DURING COLD STORAGE OF SOME INULIN-CONTAINING ROOTS AND TUBERS

P. P. RUTHERFORD* and E. W. WESTON

Wye College, University of London, Ashford, Kent

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Abstract—Cold storage of chicory and dandelion roots caused breakdown of inulin and high-molecular-weight oligosaccharides (degree of polymerization, D.P. > 10) to lower D.P. oligosaccharides. These changes were nearly complete within the first 6 weeks of cold storage, and fitted very well an exponential curve. Storage of chicory roots at higher temperatures decreased the rate of sugar breakdown. Cold storage caused less breakdown of inulin and high D.P. oligosaccharides in Jerusalem artichoke tubers.

INTRODUCTION

THE carbohydrates stored in roots and tubers vary during the annual growth cycle and the changes associated with the dormant period, normally in winter, are of particular interest. In general, the polysaccharides are converted into simpler sugars during the colder months. The rate and the extent to which this takes place is governed by a number of factors, temperature probably being the most important.

In our work, attention has been confined to some members of the Compositae which store inulin and a series of oligosaccharides, all derived from sucrose by the addition of 1:2-linked fructofuranoside units.[1]

Attempts have been made to fit the experimental values for the changes in insoluble, soluble and reducing sugars to exponential curves so as to obtain numerical expressions for the storage characteristics of the inulin storing roots and tubers examined.

In an investigation of changes in Jerusalem artichoke (*Helianthus tuberosus* L.) tuber during the winter, Bacon and Loxley[2] sampled tubers from the field so that the storage temperature was variable. Jefford and Edelman[3] extended this study by examining tubers stored at 2° and at 15–20°. Individual sugars were examined only in samples taken after 0 and 7 weeks of storage. In our work a more detailed and controlled examination was carried out on the effects of cold storage on the carbohydrates occurring in Jerusalem artichoke tubers, and also in roots of chicory (*Cichorium intybus* L.) and dandelion (*Taraxacum officinale* Weber) which have similar carbohydrate systems.

RESULTS

The percentage of dry matter in the roots and tubers remained fairly constant during the storage period (Table 1). All values for the sugars in the roots and tubers are expressed as percentages of the amount of dry matter present.

* Present address: School of Biological Sciences, Bath University of Technology, Claverton Down, Bath, Somerset.

1 J. S. D. BACON and J. EDELMAN, *Biochem. J.* **48**, 114 (1951).
2 J. S. D. BACON and R. LOXLEY, *Biochem. J.* **51**, 208 (1952).
3 T. G. JEFFORD and J. EDELMAN, *J. Exptl Botany* **14**, 56 (1963).

TABLE 1. CHANGES OF DRY MATTER IN VARIOUS ROOTS AND TUBERS DURING COLD STORAGE

Time of storage (weeks)	Percentage dry matter		
	J. artichoke	Chicory	Dandelion
0	19·6	—	29·1
1	20·0	—	—
2	—	15·5	29·0
4	—	14·9	30·0
6	—	16·4	—
8	—	14·2	26·9
9	19·5	—	—
10	—	13·5	—
13	17·7	—	—
14	—	—	29·3
16	—	14·6	—
19	17·6	—	—
24	—	14·7	—

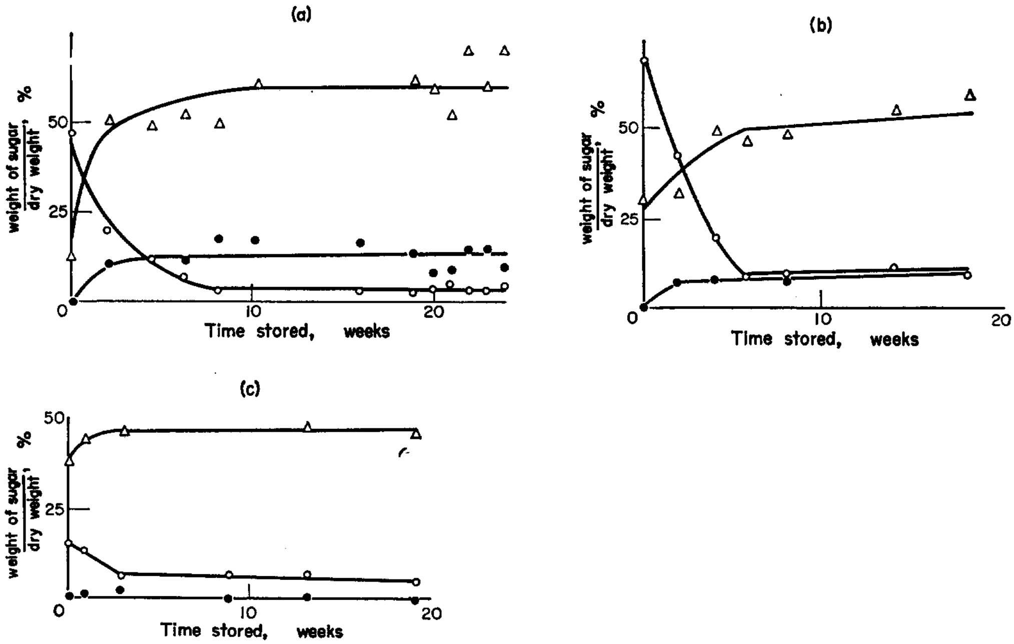

FIG. 1. THE EFFECT OF DURATION OF COLD STORAGE AT 3° ± 1° ON THE INSOLUBLE, TOTAL SOLUBLE AND REDUCING SUGARS PRESENT IN JERUSALEM ARTICHOKE TUBERS, DANDELION AND CHICORY ROOTS.

(a) Chicory roots.
(b) Dandelion roots.
(c) J. artichoke tubers.

○—insoluble carbohydrates.
●—reducing sugars.
△—total soluble sugars.

In general, cold storage caused breakdown of the insoluble polysaccharides accompanied by a corresponding increase in soluble sugars. The greater part of this change occurred during the first 5 weeks of storage and was most marked in chicory and dandelion roots (Figs. 1A

TABLE 2. PERCENTAGE SUGARS (ON A DRY MATTER BASIS) IN CHICORY ROOTS DURING COLD STORAGE

Sugars	Storage period (weeks) 0	2·1	4·3	6·3	10·3	16·0	22·0	25·0
Fructose	0·33	10·47	10·40	11·26	18·30	12·61	11·52	20·41
Glucose	0·17	0·98	1·55	0·75	3·85	1·40	1·56	0
Sucrose	2·53	6·90	7·58	20·44	8·60	14·71	26·74	21·53
Trisaccharide	0·29	4·17	4·86	5·25	5·42	6·03	7·55	10·31
Tetrasaccharide	0·37	3·43	4·25	4·06	5·18	3·03	4·61	7·91
Pentasaccharide	0·11	2·77	3·40	2·55	3·95	2·04	2·68	4·89
Hexasaccharide	0·21	2·24	3·11	1·59	3·02	1·18	1·82	3·62
Heptasaccharide	0·32	1·84	14·60	0·76	2·10	1·60	1·25	2·36
Higher sugars	7·76	7·05		1·18	4·86		1·61	4·30

TABLE 3. PERCENTAGE SUGARS (ON A DRY MATTER BASIS) IN JERUSALEM ARTICHOKE TUBERS DURING COLD STORAGE

Sugars	Storage period (weeks) 0	1	3	8·9	19·3	25·9
Fructose	0·82	0·90	1·54	0·28	0·56	1·13
Glucose		0·20	0·02	1·00	0	0·14
Sucrose	4·85	12·74	14·71	15·67	12·37	12·13
Trisaccharide	5·79	6·19	8·95	10·37	10·72	10·42
Tetrasaccharide	4·42	7·17	9·02	8·58	8·96	7·16
Pentasaccharide	3·93	3·76	5·63	6·13	5·05	4·96
Hexasaccharide	3·40	3·01	4·25	4·44	3·70	3·28
Heptasaccharide	2·71	1·98	2·95	3·00	2·14	1·95
Higher sugars	9·59	4·46	5·50	5·33	2·45	3·51

TABLE 4. PERCENTAGE SUGARS (ON A DRY MATTER BASIS) IN DANDELION ROOTS DURING COLD STORAGE

Sugars	Storage period (weeks) 0	2	4	5·6	7·9	14	18·1
Fructose	0·10	5·82	8·04	8·25	8·00	10·93	8·75
Glucose	0	0·85	1.20	0·69	0·41	1·04	0·95
Sucrose	9·10	6·04	9·49	8·95	8·29	10·26	9·44
Trisaccharide	4·00	3·26	4·94	4·23	4·46	6·44	5·60
Tetrasaccharide	3·41	3·18	3·00	4·68	3·48	7·32	6·45
Pentasaccharide	1·95	2·83	4·15	4·42	3·20	6·34	4·69
Hexasaccharide	1·71	2·51	3·79	3·73	2·78	5·49	4·52
Heptasaccharide	0·92	2·60	3·23	2·92	1·95	3·78	3·80
Higher sugars	10·54	11·39	6·26	7·44	6·06	2·00	16·47

and 1B). Figure 1C shows that cold storage caused comparatively little breakdown of the insoluble polysaccharides present in Jerusalem artichoke tubers.

Percentages of the individual soluble sugars obtained by chromatographic examination of a number of samples, selected at intervals during cold storage, are shown in Tables 2, 3 and 4. Of the three replicates available for Jerusalem artichoke tubers, chicory and dandelion roots at each sampling date, the sample chosen was that containing amounts of reducing, soluble and insoluble sugars which were closest to the average. The amounts of fructose in both chicory and dandelion roots increased markedly during the first few weeks of cold storage and then stabilized to about 12 per cent for chicory and 9 per cent for dandelion. However, in Jerusalem artichoke tubers the percentage of fructose present was much smaller, varying between 0·28 and 1·54 per cent. The amounts of glucose in the three roots and tubers were less than 2 per cent, and showed no obvious trend with duration of cold storage. Cold storage affected the sucrose content in various ways; in chicory the percentage sucrose increased throughout cold storage, in dandelion the sucrose content remained approximately constant, close to 9 per cent, whilst in Jerusalem artichoke there was an initial rapid rise followed by a slight fall in the amount of sucrose present. The changes in the other oligosaccharides were similar for all tissues and to one another. There was a slight increase during the first few weeks of storage, thereafter the percentage sugar slightly increased or remained constant. Apart from the trisaccharide, corresponding sugars were lower in chicory than in dandelion. Jerusalem artichoke tubers always contained a larger amount of these oligosaccharides.

FIG. 2. THE BREAKDOWN IN CHICORY ROOTS OF CARBOHYDRATES INSOLUBLE IN 80 PER CENT ETHANOL AFTER STORAGE FOR VARIOUS PERIODS OF TIME AT 5° AND AT 15–20°.

○ at 5°.
● at 15–20°.

The effect of storing chicory at a higher temperature, varying between 15 and 20° was to reduce the rate at which insoluble carbohydrates were broken down (Fig. 2).

DISCUSSION

The uniform dry matter contents (Table 1), confirmed that storage in moist peat reduced desiccation to a minimum; this made it permissible to express the sugars present in the roots and tubers as percentages of the dry matter.

The sugars occurring in the tissues under examination form a continuous series from the simple hexoses to inulin with about 35 hexose units,[4] thus any division into soluble and insoluble sugars was bound to be arbitrary. It was found impossible to separate individual oligosaccharides with more than 8 hexose units so the method chosen was one which completely extracted all sugars of D.P. 1–8. Although the amount of total soluble sugars must vary according to the proportion of sugars of higher molecular weight extracted, the total amount of these sugars present was so small that any variation was likely to be small compared with the total amount of sugar extracted. Of the various methods of extraction which have

[4] E. L. HIRST, D. I. MCGILVRAY and E. G. V. PERCIVAL, *J. Chem. Soc.* 1927 (1950).

been used,[2, 5–9] continuous extraction with boiling 80 per cent ethanol for 6 hr[9] was the most suitable and so used in all this work.

Variation of the insoluble sugar content in both chicory and dandelion roots with duration of storage indicated a logarithmic decrease. This was examined for chicory by plotting a derived linear equation of the form $\log(y-a) = -kt + \log c$, where y is the percentage insoluble sugar, t the storage time in weeks and c and a constants derived from the initial and final values of y. The lines drawn in Fig. 1A were obtained in this manner. The experimental points for both soluble and reducing sugars do not fit the derived curves as closely as those for the insoluble sugars and it would therefore appear that for these sugars the usefulness of the derived linear equation as a means of calculating parameters is limited. Although none of the other figures was obtained in this manner, those for dandelion are also approximately exponential in shape.

The general pattern of polysaccharide breakdown in all three roots and tubers is similar to that found previously in artichoke[2, 3, 10] and chicory.[11] It was also similar to the changes in starch content found in potato[12, 13] and in hop roots.[14] In all these tissues, storage during dormancy, whether in the field or after lifting, resulted in a conversion of polysaccharide to sugars of lower molecular weight. The detailed changes, however, varied considerably from one species to another and were dependent upon environmental conditions during storage.

Despite some variability the changes in insoluble carbohydrates fit very well an exponential curve which would give a simple numerical expression of the storage characteristics of various root crops. Since many root crops are stored commercially for both animal and human consumption and since carbohydrate status must be an important factor in both nutritional value and palatability, an understanding of the processes involved during storage has practical applications. There is also evidence that the carbohydrate composition of the root has a marked effect on growth during the next season, in particular on those plants which are artificially forced such as rhubarb or chicory.[15]

The conditions controlling chemical reactions involving carbohydrates which take place in roots are not simple as is clear from the fact that higher storage temperatures reduce the conversion of polysaccharides to simple sugars. An attempt is now being made to isolate and examine the enzyme systems involved.

EXPERIMENTAL

The sources of the roots and tubers used, and the methods of storage and sampling have been described earlier.[16] Material was taken from the cold store at intervals over a period of 26 weeks, and six disks together weighing 3 g for Jerusalem artichoke and dandelion and 2·5 g for chicory, were taken for each analysis, three replicate plants being sampled on each occasion. Dry weights were determined by oven drying at 100°.

[5] M. Lemoigne, *Ann. Ferment.* **6**, 193 (1941).
[6] M. Tanret, *Bull. Soc. Chim. France* **9**, 200 (1893).
[7] H. H. Schlubach and H. Knoop, *J. Liebigs Ann. Chem.* **497**, 208 (1932).
[8] R. Dedonder, *Bull. Soc. Chim. Biol.* **34**, 144 (1952).
[9] A. Bevenue and B. Washauer, *J. Assoc. Offic. Agri. Chemists* **33**, 122 (1950).
[10] P. Hanower, *Compt. Rend.* **224**, 604 (1947).
[11] J. Wolff and B. Geslin, *Ann. l'Inst. Pasteur* **32**, 71 (1918).
[12] B. Arreguin-Lozano and J. Bonner, *Plant Physiol.* **24**, 720 (1949).
[13] B. Samotus and S. Schwimmer, *J. Food Sci.* **28**, 163 (1963).
[14] I. H. Williams, J. B. Roberts and J. R. Coley-Smith, *Ann. Report Hop Research Dept.* Wye College, 48 (1960).
[15] P. P. Rutherford and A. A. Jackson, *Ann. Gembloux* **71**, 187 (1965).
[16] P. P. Rutherford, C. M. Griffiths and R. L. Wain, *Ann. Appl. Biol.* **58**, 467 (1966).

Extraction of Sugars

The disks were continuously extracted with 80 per cent ethanol for 6 hr in a Soxhlet. The extract was evaporated to dryness at 30° in a rotary evaporator and the residue was dissolved in water and made up to 50 ml.

Total Soluble Sugars

20 ml of the original alcoholic extract were hydrolysed by boiling under reflux with 20 ml 2 per cent acid for 1 hr and then neutralized with a slight excess of $BaCO_3$ (1·5 g). Separate experiments had shown that this treatment completely hydrolysed all the polysaccharides to glucose and fructose without any destruction of fructose. The mixture was made up to 100 ml, shaken with a little powdered charcoal and filtered. The amounts of reducing sugars present were determined by the method of Somogyi.[17] Using standard hexose solutions it was found that 1 ml of 0·005 N sodium thiosulphate was equivalent to 0·150 mg of hexose, a factor somewhat higher than that of 0·135 mg quoted by Somogyi.

Reducing Sugars

A suitable volume of the original alcoholic extract was analysed directly by Somogyi's method.

Insoluble Sugars

The tissue disks, after extraction with 80 per cent ethanol, were ground with a little sand, refluxed for 2 hr with 50 ml of 1 per cent oxalic acid, cooled, neutralized with a slight excess of $BaCO_3$ (1·5 g) and then made up to 100 ml with water. A little powdered charcoal was added, the solution shaken, filtered and reducing sugars were estimated by the method of Somogyi.

Chromatographic Separation

Ionic substances were removed from the original alcoholic extract by Amberlite IRC-50(H) and IR-4B(OH) resins. The neutral extract after evaporation to dryness was separated chromatographically on Whatman No. 3MM chromatographic paper using n-butanol/ethanol/water (4:1·1:1·9 v/v) for 48 hr at 25°.[18] Marker strips were cut off and the position of the spots revealed by the mixture 4 per cent aniline in acetone, 4 per cent diphenylamine in acetone, and syrupy phosphoric acid 5:5:1 v/v[19] followed by heating at 100° for 10 min. Strips containing the individual sugars were then cut from the main chromatogram and the sugars were eluted with water. After freeze-drying, the sugars, where necessary, were hydrolysed at 100° with 4 ml of 2 per cent oxalic acid for 1 hr, cooled, neutralized with 1 ml NaOH and then determined by Somogyi's method.

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17 M. SOMOGYI, *J. Biol. Chem.* **160**, 61 (1945).
18 L. HOUGH, J. K. N. JONES and W. H. WADMAN, *J. Chem. Soc.* 1702 (1950).
19 J. L. BUCHAN and R. I. SAVAGE, *Analyst* **77**, 401 (1952).