ON THE BREAKDOWN OF SUGARS DURING THE DRYING OF PLANT SAMPLES AND THEIR SUBSEQUENT DRY STORAGE

MAIJA-LIISA SALO

Department of Animal Husbandry, University of Helsinki

Received February 13, 1965

When fresh plant material is investigated, the sampling and preparation of the sample for analysis constitutes a phase of work which deserves attention. In grasses the length of the stubble affects the results because e.g. the fructosan content is different in different parts of the stalk. Weather conditions and time of the day are also significant (11, 8).

When a sample is cut from the plant, enzymatic activity still continues, causing decomposition of carbohydrates. Rapid inactivation of the enzymes is therefore necessary. In investigations concerning the water-soluble carbohydrates usually one of the following procedures is employed in the pretreatment of the samples: 1) drying of the sample in an oven, 2) immersion in boiling ethanol, or 3) freeze-drying.

Oven-drying at 60—100°, previously the most commonly used method, has several advantages. The enzymes are relatively rapidly destroyed by the high temperature, and even large samples can be easily dried in a short time. As a drawback of the method it should be mentioned that chemical changes may be caused in the sample by the heating. WAITE and BOYD (11) dried grass samples for 40 minutes in a forced-draught oven in which air at 100—110° is passed through the grass at the rate of 100 cu.ft. per minute. They maintain that their method prevents any appreciable breakdown of soluble carbohydrates. The present writer finds that if the sample is dehydrated at such high temperature, considerable sugar losses occur even in grass, and more so in cabbage or swede. JARRIGE's (4) method appears more reliable. The food sample is first heated for 15—20 minutes at 100° to stop enzyme action and then for several hours at 40° to complete desiccation. STEGER and PÜSCHEL (10) keep the grass sample in a steam flow for an initial period of 10

Table 1. Sugar contents (% of dry matter) and losses during drying and storage. Leaves and grasses.

Dried sample	Freeze-drying				Vacuum drying at 70° 4½ hours					Vacuum drying at 70° 18 hours					Oven drying at 95–108° 2–3 hours					
	Mono-saccharides	Sucrose	Fructosan	Total a	Mono-saccharides	Sucrose	Fructosan	Total b	Loss a–b % of item a	Mono-saccharides	Sucrose	Fructosan	Total c	Loss a–c % of item a	Mono-saccharides	Sucrose	Fructosan	Total d	Loss a–d % of item a	Loss c–d % of item c
Sugar beet leaves	6.8	1.9		8.7						6.7	1.9		8.6	1.1	5.5	2.2		7.7	11.5	
Rye leaves, sample 1										4.3	4.1	1.8	10.2		2.5	3.6	1.7	7.8		23.5
Rye leaves, sample 2																				
immediately after drying	5.2	12.0	2.7	19.9						5.4	10.5	2.8	18.7	6.0						
stored 4 months at +20°										5.3	9.9	3.2	18.4	6.6						
» » −8°	5.1	12.0	2.6	19.7																
Meadow grass, leaf stage																				
immediately after drying	3.7	4.7	7.2	15.6	3.3	4.4	7.2	14.9	4.5	3.1	4.3	7.2	14.6	6.4						
stored 4 months at +20°					3.4	4.4	7.4	15.2	3.8											
» » −8°	3.7	4.7	7.4	15.8																
Timothy, leaf stage																				
immediately after drying	4.9	5.6	10.6	21.1	4.8	5.0	10.7	20.5	2.8											
stored 4 months at +20°					4.7	4.7	10.8	20.2	5.2											
» » −8°	4.7	5.3	11.3	21.3																

Table 2. Sugar contents (% of dry matter) and losses during drying. Vegetable materials. The losses are calculated as % of the results of freeze-dried samples.

Material	Freeze-drying	Vacuum drying at 40° 24—28 hours		at 50° 24—26 hours		48 hours		at 60° 24 hours		at 67° 21 hours		at 70° 10.5 hours		21—24 hours		Room drying at 20° 72 hours		Oven drying at 40° 72 hours	
	Sugar content	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss	Sugar content	loss
Carrot (Nantes Typ Topp)																			
Sample 1.	53.5													49.5	7.5				
Carrot (Amsterdamer)																			
Sample 1.	47.8													44.3	7.3				
Leek, stalks only (Toftø)																			
Sample 1.	60.8													57.0	6.3				
Leek, entire (Toftø)																			
Sample 1.	46.9													44.4	5.3				
Cabbage (Staup)																			
Sample 1.	50.7													45.7	9.9				
Cabbage (Länsipohja)																			
Sample 1.	47.7							41.4	12.7	40.0	16.5			39.1	18.4				
Sample 2.	48.7	48.3	0.8	46.0	5.5	44.6	8.4									44.4	8.8		
Carrot (Nantes Typ Topp)																			
Sample 2.	55.1							53.3	3.3	51.6	6.4			51.2	7.1				
Sample 3.	55.3	55.1	0.4	54.6	1.3											51.3	7.2		
Cabbage (Faales Blåtopp)																			
Sample 1.	50.9	50.4	1.0	49.9	2.0	49.8	2.3					46.0	9.6	44.8	12.0				
Brussels sprouts (Jade)																			
Sample 1.	23.9	23.1	3.3											19.2	19.7			12.4	48.1
Leek, stalks only (Toftø)																			
Sample 2.	53.2	53.0	0.4											49.4	7.1			34.5	35.2
Celeriac (Aebleformat)																			
Sample 1.	18.3	17.9	1.9											16.5	9.6			1.6	91.2

minutes; they maintain that when the enzymes have been destroyed in this manner, virtually no further losses occur in the course of drying.

Immersion in boiling ethanol immediately after cutting is a suitable method in sugar determinations and is widely used (e.g. 6, 12, 1). The boiling ethanol rapidly penetrates the tissues and immediately destroys the enzymes. Gold ethanol, quickly heated to boiling point, is less to be recommended since it brings about a considerable increase in the quantity of reducing sugars, obviously as a consequence of the enzymatic hydrolysis of sucrose (12).

Freeze-drying has been generally employed in recent times (e.g. 3, 7, 5, 2). According to DAVIES et al. (3), both drying by heat and immersion in boiling ethanol have several disadvantages and therefore a freeze-drying technique is preferable.

Experiments on drying and storage of plant materials

Materials and methods. In the present experiments comparisons were first made between freeze-drying, vacuum drying at 70° and high-temperature (95—100°) drying in a forced-draught oven. Comparisons were later made also between vacuum drying at lower temperatures, room drying (at 20°) and oven drying at 40° without forced-draught. All results are compared to those obtained by freeze-drying which is considered the best method for the preservation of sugars.

Sugar beet leaves from which the central vein was removed and gramineous plants at the leaf stage were chosen for the first experiments (Table 1). Test samples were analysed separately for monosaccharides, sucrose and fructosan. The methods are described in a previous paper (9). The fructosan was extracted with water at 20° and determined as reducing sugars since this method is more accurate than the colorimetric determination. Fructosan in solution was hydrolysed with 0.02 N sulphuric acid in a boiling-water bath for 30 minutes.

In later experiments some vegetables were studied (Table 2). In this case only the total sugar quantity was determined (9). Carrots and celeriac were ground with a vegetable grinder into strips about 1 mm in thickness, while cabbage and leek were cut with a knife into small pieces. These vegetable materials dried more slowly than grasses. In the freeze-drying a fresh sample of 100 g took three days to dry, and even in the vacuum oven at 70° about 20 hours were needed for a 150 g sample. The drying receptacles were plastic trays (13 cm × 13 cm), in which the sample made a 4—5 cm layer.

The dried samples were ground in a Wiley mill using a 40-mesh (0.42 mm) screen. The heat dried materials were kept in the laboratory (about 20°) and the freeze-dried samples in the freezer compartment of a refrigerator (about —8°). The moisture content of the samples was 3—5 %.

Results and discussion. In Table 1 results are shown for sugar beet leaves and gramineous plants. The table also gives the results of the storage tests. In all cases the loss percentages have been calculated only with reference to the total sugar quantities since the writer has observed with gramineous plants that during an extraction period of 5—6 hours some fructosan is dissolved in 90 % ethanol. As the siphoning in individual Soxhlet aggregates is not quite identical,

the quantities of sucrose and fructosan may vary somewhat even in replicate tests, although their total sums agree.

Sugar beet leaves differed from the other materials in that no sugar losses occurred during vacuum drying at 70°. The losses during drying at 100° were 11,5 %. In young rye leaves the losses were nearly 25 % during drying at 100° even when compared to those in vacuum drying at 70°. The highest losses with both materials occurred for the monosaccharides. The second rye sample was taken two weeks later on a sunny day, in consequence its dry matter and sugar contents were much higher than those of the first sample. The sugar losses on vacuum drying were 6 % as compared to freeze-drying; drying at 100° was not employed.

In the next two experiments the samples were kept in the vacuum oven only for the period required for them to dry; in the case of meadow grass a second sample was kept in the oven overnight. This shorter drying period resulted in slightly lower sugar losses. With the longer drying period the losses were the same as had been found with rye leaves. It was concluded from the results that sugar beet leaves and gramineous plants may be dried in the vacuum oven at 65—70° without any appreciable sugar losses. A temperature of 100°, on the other hand, is altogether too high.

It is sometimes necessary to keep dried samples in storage prior to analysis, and the storage tests were therefore undertaken. The results are shown in Table 1. The usual practice was to keep the heat-dried samples in the laboratory and the freeze-dried samples in the freezer compartment of a refrigerator. The results show that the sugar contents after four months of storage were practically the same as those immeadiately after drying.

Laidlaw and Wylam (7) obtained different results in their preservation tests. They kept freeze-dried samples in storage both at room temperature and at 0° and found in each case considerable variations in the carbohydrate content. No direct relationship between the time of keeping and the carbohydrate present could be established; the results varied individually, in some cases more than 100 % from the original value. Complete drying of the samples did not eliminate the variations. According to the writer's experience, some variations occur even in parallel determinations between the monosaccharides, sucrose and fructosan, although the sums agree; this is so particularly when the replicate analyses are made on different days. However, the variations arising in storage are not any greater.

In Table 2 results are given for certain vegetables. In this case only the total sugar quantity was determined. A comparison was made in the first five experiments between freeze-drying and vacuum drying at 70°.

The sugar losses in carrot and leek were approximately the same as in grass, while somewhat higher losses, about 10 %, were recorded for cabbage. The losses were suspected of being caused by enzymatic action since the temperature of the vegetable material was 25—35° during most of the drying period, although the vacuum oven was kept at 70°. Attempts were therefore made to destroy the enzymes by pre-heating the sample, spread out into a thin layer at 100°, but the sugar losses were not reduced. The cause for these losses must lie in the excessive temperature. The lower part of Table 2 shows the results of tests in which the temperature of

the vacuum oven was maintained at 70°, 60°, 50° or 40° respectively. It appears that if the temperature is higher than 40°, the risk of sugar losses is present. The differences between the results of freeze-drying and vacuum drying at 40° would seem to be within the range of analytical error. The table also shows that the sugar losses caused by heating are different with different plant materials. In these tests, cabbage was most susceptible, and among the different varieties the autumn cabbage »Länsipohja» had higher losses than the winter cabbages »Faales Blåtopp» and »Staup». The sensitivity of Brussels sprouts to heating appeared to be about the same as that of autumn cabbage. In a previous study also a third *Brassica* species, a swede, was found to be very heat-sensitive. On comparing two treatments, immersing in boiling ethanol and drying in the vacuum oven at 63°, a sugar loss of 18 % for swede in the latter procedure was obtained.

The high temperature (70°) employed in the first tests was chosen because a rapid destruction of the enzymes was thought indispensable. All the reports read by the writes stress the importance of enzyme inactivation; this was done either by heating or by freezing. According to the present findings, destruction of the enzymes is not necessary when the vacuum is used. Obviously respiration and other sugar-decomposing activities are inhibited when no oxygen is available. As evidently a 100 % dryness is not achieved at 40°, it seems reasonable to determine the moisture content of the fresh plant sample at a higher temperature; the writer has used drying at 55° for 2 days. At this temperature some plant materials undergo a decline in the reducing activity of sugars, the dry matter losses are very small, however, as will be seen later.

The vegetable materials were also dried in thin layers at room temperature as well as in plastic trays at a temperature of 40° in the ordinary drying oven without forced-draught (Table 2). The samples were stirred several times in order to accelerate drying. At room temperature, the samples dried within two days with only low sugar losses (7—9 %). In the oven the samples were in a thicker layer and nearly three days were required to dry 100 g. It may be mentioned that samples of the same size dry in 24 hours without being stirred in the vacuum oven at the same temperature. The oven test showed conclusively that if the drying temperature favours enzymatic action, oxygen must be absent. While the sugar losses in vacuum drying were negligible, the losses in the oven amounted to 35 % for leek, 48 % for Brussels sprouts, and as much as 91 % for celeriac. The celeriac turned dark brown as a result of drying, whereas no such changes occurred in leek or Brussels sprouts.

The preservation of the sugars in samples dried at 40° in the vacuum oven was studied only with some materials. It was observed that there was no appreciable change in the sugar content when the samples with a moisture content of 3—4 % were stored for one month at room temperature.

The effect of organic acids during drying

The writer previously observed that no changes occur in the weight and appearance of pure sugars when dried at 70° in the vacuum oven. When dried in the

oven at 100° fructose melts, turns brown and undergoes a slight loss of weight. The appearance of glucose and sucrose does not change at 100°. When fructose was heated for 20 hours in the vacuum oven at 60 or 70° no change in the reducing activity was noted. It was concluded that some substances present in the plant material were responsible for the decomposition of sugars. The effects of various organic acids were studied, using the following procedure: 200 mg fructose was placed in Erlenmeyer flasks together with anhydrous organic acid at 1—3 % of the fructose quantity. The acids were pipetted from stock solutions of given concentration. Water was added to make a volume of 2 ml, into which the sugar was dissolved. The flasks were heated at 70° or 40° in the vacuum oven, as shown in Table 3. The experiment showed that at 70° considerable decrease in the reducing

Table 3. The effect of organic acids and their salts on the reducing activity and dry matter of fructose when heated in a vacuum oven at 70° or 40°.

Acid or salt and its quantity, % of fructose		Vacuum drying at 70° 20 hours			Vacuum drying at 40° 20 hours		
		Colour	Decrease in reducing activity of fructose, %	Loss of dry matter, %	Colour	Decrease in reducing activity of fructose, %	Apparent loss of dry matter, %
Oxalic acid	1 %	Brown (+)	85.5	6.5	Colourless	18.5	−2.7
»	2 »	» (++)	89.5	9.5	»	22.5	−1.6
»	3 »	» (+++)	92.5	10.0	»	24.5	−1.3
Citric acid	1 »	Colourless	39	2.3	»		
»	2 »	»	37	2.5	»		
»	3 »	»	49.5	2.5	»	2.5	−3.1
Malic acid	1 »	»	21	1.8	»		
»	2 »	Pale yellow	35.5	2.8	»		
»	3 »	»	45	3.2	»	2.0	−3.3
Na-oxalate	10 »	»	1.5				
K-oxalate	10 »	Yellow	2				
Na-citrate	10 »	Brown (+)	5.5				
K-Na-tartrate	10 »	Yellow	2				

activity of fructose was caused by all three acids. Oxalic acid was much more effective than the citric or malic acids. The sugar became darker brown and foamed during the drying process, which did not occur with the other acids. The reducing activity of the fructose was almost completely suppressed. The citric and malic acids lowered the reducing to a lesser degree, and the sugar was only slightly discoloured if at all. For instance, the sample with 3 % citric acid was colourless although the reducing activity of the fructose had decreased by 50 %. Further tests were carried out on the effects of four salts of organic acids at 10 % of the fructose quantity. All four caused some discoloration, but only sodium citrate, which also produced the darkest colour, can be considered to have lowered the reducing activity. This effect was

due to alkalinity. Heating at 40° caused no colour in any of the test members, but in the oxalic acid samples the reducing activity was distinctly lowered. Thus it seems possible that drying even at a temperature as low as 40° may cause sugar losses in some materials. Such materials may be, for instance, various kinds of silage.

In the tests with acids the dry matter losses were determined, calculated as per cent of the combined quantity of fructose and the anhydrous acid. Only for oxalic acid was the loss so great that one may speak of a true error. The water had not completely evaporated from all samples at 40°, although the salts investigated were found to lose their water of crystallisation at this temperature. It appears likely on the basis of these experiments that the original dry matter can be determined by drying at 70° without any appreciable error.

The pH in the juice of the vegetable varied between pH 5.7 and 6.2. No correlation was established between the pH of the juice and the sugar losses caused by heating. In the contrary, the lowest pH was recorded for the leek juice whose sugar losses were nevertheless the lowest. Boiling did not change the pH of the juice.

Summary

In the present studies various drying procedures were investigated and compared to freeze-drying. The persistence of sugars in some of the dried samples was also investigated. In addition, the effect of some organic acids or their salts on fructose during heating was investigated. The following results were obtained:

A drying temperature exceeding 40° caused sugar losses at least in some of the plant materials. *Brassica* species were found to be the most sensitive to heat. The sugar content remained practically the same in vacuum drying at 40° as in freeze-drying. Drying at 40° must be carried out in the absence of oxygen.

During vacuum drying at 70°, oxalic, citric and malic acids — even in concentrations of only 1 % — produced a considerable decrease in the reducing activity of fructose; oxalic acid had the same effect even at 40°. The dry matter loss was not so serious.

When grass samples, vacuum dried at 70°, were subsequently kept at room temperature, and when similar samples, freeze-dried, were kept at about —8°, their sugar contents after 4 months of storage were the same as immediately after drying.

REFERENCES

(1) Bailey, R. W. 1958. Carbohydrates in pasture species. II. The soluble sugars of red clover (Trif. pratense). J. Sci. Food Agric. 9: 748—753.

(2) Bath, I. H. 1960. Analysis of the structural carbohydrates of herbage. Ibid. 11: 560—566.

(3) Davies, A. W. & Evans, R. A. & Evans, W. C. 1948. Studies on the biochemistry of pasture plants. 1. A new technique for the preparation and preservation of herbage samples. J. Brit. Grassl. Soc. 3: 153—158.

(4) Jarrige, R. 1954. Nature and importance of soluble glucides in the growth of fodder plants. O. E. E. C. Grassl. Conf. 1954, p. 270—275.

(5) JOHNS, A. T. 1955. Pasture quality and ruminant digestion. I. Seasonal change in botanical and chemical composition of pasture. N. Z. J. Sci. Tech. 37: 301—311.
(6) LAIDLAW, R. A. & REID, S. G. 1953. Analytical studies on the carbohydrates of grasses and clovers. I. Development of methods for the estimation of the free sugar and fructosan contents. J. Sci. Food Agric. 3: 19—25.
(7) —»— & WYLAM, C. B. 1952. II. The preparation of grass samples for analysis. Ibid. 3: 494—496.
(8) MACKENZIE, D. J. & WYLAM, C. B. 1957. VIII. Changes in carbohydrate composition during the growth of perennial rye-grass. Ibid. 8: 38—45.
(9) SALO, M.-L. 1965 Determination of carbohydrate fractions in animal foods and faeces. Acta agr. fenn. 105: 1—102.
(10) STEGER, H. & PÜSCHEL, F. 1959. Über den Nachweis von leicht hydrolysierbaren Kohlenhydraten in pflanzlichen Material, sowie Vorkommen und Beeinflussung in Grün- and Rauhfuttermitteln. Arch. Tierern. 9: 211—235.
(11) WAITE, R. & BOYD, J. 1953. The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life-cycle. J. Sci. Food Agric. 4: 197—204.
(12) WYLAM, C. B. 1953. Analytical studies on the carbohydrates of grasses and clovers. III. Carbohydrate breakdown during wilting and ensilage. Ibid. 4: 527—531.

SELOSTUS:

SOKEREIDEN SÄILYMINEN KASVINÄYTTEITÄ KUIVATTAESSA JA KUIVANA SÄILYTETTÄESSÄ

MAIJA-LIISA SALO

Kotieläintieteen laitos, Helsingin yliopisto

Kasvinäytteiden kuivatusta koskevissa tutkimuksissa korostetaan entsyymien inaktivoimisen tarpeellisuutta ja ehdotetaan se tehtäväksi joko kuumentamalla tai jäähdyttämällä. Oheisessa tutkimuksessa suoritetut kokeet osoittivat, että sokereita hajottavan entsyymitoiminnan voi ehkäistä myös kuivaamalla hapettomassa tilassa, ts. vaakuumikuivaajassa. Toinen sellainen seikka, johon kuivatuksessa on syytä kiinnittää huomiota, on lämpötila. Jo 60—70° lämpötila aiheuttaa monissa kasviaineksissa huomattavaa sokereiden pelkistyskyvyn alenemista. Tutkimuksessa olleista kasveista olivat *Brassica*-lajit kuumennukselle herkimpiä. 40°:ssa vakuumikuivaajassa kuivattaessa säilyi sokeripitoisuus kaikissa tutkituissa kasveissa käytännöllisesti katsoen samana kuin kylmäkuivatuksessa, mitä sokerimäärityksissä pidetään parhaana kuivaustapana. Tavallisessa kuivauskaapissa 40° lämpötilassa kuivattaessa sokereista häviää suurin osa.

Kasveissa esiintyvien orgaanisten happojen havaittiin lisäävän sokereiden kuumennusherkkyyttä. Yhdenkin prosentin oksaali-, sitruuna- ja omenahappokonsentraatiot aiheuttivat 70°:ssa hyvin suurta fruktoosin pelkistyskyvyn alenemista ja oksaalihappo aiheutti sitä vielä 40°:ssakin. Kuiva-ainehäviö oli pelkistyskyvyn alenemista huomattavasti alempi.

Kuivattujen näytteiden sokeripitoisuudessa ei 1—4 kk:n säilytysaikana todettu tapahtuvan muutoksia. Näytteiden vesipitoisuus oli 3—5 %.