filter. Advantage might be taken of the increased absorbance at 360 mμ and a Beckman Model B spectrophotometer used for the determinations. However, at the lower wave length difficulty might be encountered from impurities absorbing in the ultraviolet region.

The diisopropyl ester of 2,2,2-trichloro-1-hydroxyethylphosphonic acid behaved anomalously on pyrolysis. No reproducible red-color tests were obtained by pyrolysis of this homolog. Most interest has centered about the dimethyl ester (Bayer L 13/59) because of its use in controlling DDT-resistant houseflies in dairy barns. Attempts were made to determine micro quantities of the order of 1 p.p.m. of this ester in cow's milk, but interfering colors were obtained. Although no actual determinations were made of spray residues of this compound, the method is sensitive to 20 γ and hence could be used for this purpose.

A number of chlorine-containing insecticides were tested for their possible interfering effects:

Insecticide, 200 γ	Color	Equivalent to L 13/59, γ
DDT	None	..
Lindane	None	..
Chlordan	Reddish brown	24
Heptachlor	Reddish brown	41
Aldrin	Reddish brown	10
Dieldrin	Reddish brown	9
Endrin	Reddish brown	8
Toxaphene	Reddish brown	14

The trace of reddish brown color resulting from some of the insecticides could well be due to the impurities present in the materials tested.

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POTATO COMPOSITION

Survey of Major and Minor Sugar and Starch Components of the White Potato

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Quantitative analysis and qualitative paper chromatography of the ion-free alcohol-soluble sugars of plants have confirmed the fact that sucrose, fructose, and glucose comprise the major sugars of the white potato. Trace amounts of sugars chromatographically similar in behavior to ketoheptose, melibiose, melezitose, and raffinose, along with a non-moving fructan (fructosan) and a significant quantity of inositol, have been detected in such extracts. The generally accepted pattern of sugar change of potatoes stored at various temperatures was confirmed, and it was found that fructose, of the three major sugars, seemed to be most responsive to temperature changes. Neither the amylose-amylopectin ratio nor the phosphorus content of the starches isolated from the potatoes was affected by storage time and temperature. A study of various starch dispersion procedures has defined optimum conditions of solubilization of potato starch for starch-iodine "blue value" determinations.

The sugar and starch contents of white potatoes in relation to processing (*6*, *15*, *20*, *26*) and to carbohydrate metabolism (*2*, *4*) have been studied extensively since the pioneer work of Müller-Thurgau almost 75 years ago (*13*). Recently, however, chromatographic methods have been designated for the detection of sugar components of the alcohol-soluble, nonionic, nitrogen- and lipide-free carbohydrate fraction of vegetable tissue (*9*, *23*). Williams and Bevenue have reported evidence of the presence of ketoheptose and melibiose in such extracts from potato (*24*). The present paper is primarily concerned with the changes in the sugar and starch components, as revealed by both quantitative chemical and qualitative chromatographic procedures, of several varieties of potatoes subjected to different storage temperatures. The results presented are part of an extensive investigation of compositional factors influencing the browning of processed potato products. Subsequent publications will deal with phosphate and nitrogenous components and their relation to nonenzymatic browning of processed potatoes.

Materials and Methods

Potatoes Most of the results were obtained with two contrasting potato varieties: White Rose potatoes harvested in September 1952 in the

Table I. Weight-Volume Data for 1952 Storage Experiments

Temp., ° F.	Time, Weeks	Specific Gravity		Moisture, %		Relative Weight	
		RB[a]	WR[b]	RB	WR	RB	WR
..	0	1.084	1.083	77.3	78.6	100.0	100.0
40	1	1.088	1.087	78.2	78.7	99.7	99.6
	2	1.084	1.085	77.8	78.7	99.4	99.3
	4	1.084	1.084	77.1	78.6	98.8	98.9
	10	1.087	1.084	77.3	78.5	97.4	97.8
	18	1.085	1.082	77.9	78.5	97.0	96.7
50	1	1.086	1.086	77.7	78.2	98.7	99.4
	2	1.081	1.081	77.5	78.9	99.4	99.2
	4	1.082	1.082	77.5	78.9	99.0	98.8
	10	1.082	1.082	77.9	78.7	98.4	97.9
	18	1.086	1.086	78.4	78.5	97.4	95.7
70	1	1.082	1.082	77.3	77.9	98.8	99.2
	2	1.082	1.082	77.8	79.2	98.4	99.1
	4	1.082	1.082	77.2	80.5	97.5	97.6
	10	1.083	1.083	77.1	77.9	95.4	94.7
	18	1.086	1.086	77.7	77.5	86.4	86.1

[a] Russet Burbank variety. [b] White Rose variety.

Stockton, Calif., area and a similar lot of the Russet Burbank variety harvested in the same month near Rupert, Idaho. The latter was grown on virgin land from certified-seed (Blue Tag) foundation stock of 1951. Zero time controls were taken 2 days after harvest for the White Rose variety and 2 weeks after harvest for the Russet Burbank. Five varieties were used in the 1953 experiments to obtain additional data on sugar composition (Table II).

Storage Conditions and Sampling

Five hundred-pound lots of the 1952 harvest were randomized and separated into three sublots, and each sublot was stored in uncovered galvanized metal containers at 40°, 50°, and 70° F., respectively. The corresponding average relative humidities of the rooms were 95% at both 40° and 50° F. and 40 to 60% at 70° F. Samples were taken for sugar analysis at 0, 1, 2, 4, 10, and 18 weeks. Russet potatoes from storage at 40° after 18 weeks were placed at both 70° and 34° F. for 4 additional weeks, and another lot was placed at 70° F. for another 3 weeks. After 18 weeks at 40°, one lot of White Rose variety was placed at 34° and another lot at 70° F. for 3 weeks. A third lot of White Rose potatoes stored at 40° for 18 weeks was placed at 70° F. for 3 weeks.

In the 1953 storage experiment all varieties were analyzed after 5 weeks at 40° and 70° F. The time between harvest and the start of the experiment varied between 2 and 7 weeks (Table II). All samples were kept at 40° F. before the storage experiment was started.

Extraction, Analysis, Chromatography

At each of the designated intervals 25 pounds of potatoes (50 to 70 tubers) were hand-peeled and diced by means of an Urschel dicer. Exactly 400 grams of the dice were added immediately to boiling alcohol and the procedure of Williams and Bevenue (*23*) was followed. This procedure results in an essentially ion-free mixture of alcohol-soluble carbohydrates. These conditions were found adequate for quantitative extraction of the sugars (*25*). The water-clear sugar solutions from the 1952 study were brought to partial dryness by means of infrared heat followed by lyophilization. To obtain the total solids content of these extracts it was necessary to dry to constant weight in a vacuum oven because of the hygroscopic nature of the sugar mixture. For the 1953 study all extracts were diluted to exactly 1 liter.

A suitable aliquot from the 1952 study was diluted to 100 ml. and employed for analytical studies. Total and reducing sugars were determined by a micro-Somogyi method according to the AOAC procedure described by Williams and Bevenue (*22*). Total fructose was determined by the resorcinol–hydrochloric acid method of Roe (*14*) as described by Umbreit *et al.* (*21*). Recovery studies with mixtures of pure sugars and with sugars and potato extracts revealed that reliable values can be calculated if the total resorcinol-reacting components of the aliquot being measured do not amount to more than an equivalent of 90 γ of fructose. From these three measurements the sucrose, glucose, and fructose contents of the extracts were calculated.

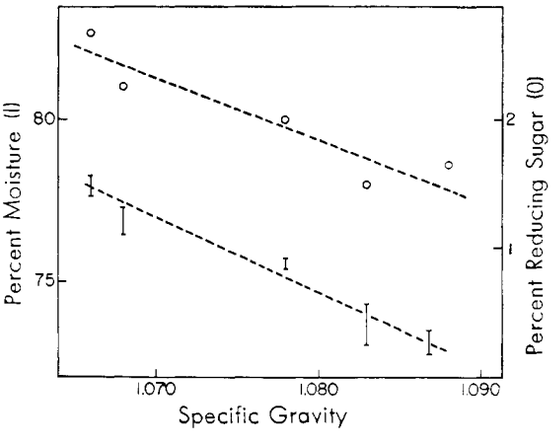

Figure 1. Relationship among specific gravity, moisture percentage, and reducing sugar for potatoes used in 1953 storage experiment

For chromatographic analysis for the 1952 study, 100 to 300 mg. were dissolved in 1 ml. of water, and 3 and 6 μl. were spotted on Whatman No. 1 filter paper. The solutions obtained from the 1953 experiments were concentrated tenfold prior to application. Irrigation was carried out in butyl alcohol–ethyl alcohol–water for 65 hours (*23*). Sprays for identification included silver nitrate, 3,5-dinitrosalicylic acid, aniline phthalate, orcinol, and resorcinol. The silver and orcinol sprays were not employed for the 1953 storage series. Interpretation of such chromatograms has been discussed (*23*). The identification and order of magnitude of the amount of the minor sugars present were ascertained by comparison with authentic sugars and sugar alcohols and by the limits of detection of the sugars with the various sprays. The nonsugar solids of the deionized alcohol carbohydrate fractions

Table II. Data for 1953 Potato Storage Experiment

Variety	Date of Harvest (1953)	Prestorage at 40° F., Weeks	Specific Gravity	Moisture Content after Storage		
				Control	5 Weeks at 40°	5 Weeks at 70°
Chippewa (Maine)	Sept. 10	2	1.068	76.4	77.3	77.3
Kennebec (Maine)	Sept. 10	2	1.083	74.3	73.4	73.0
Katahdin (Maine)	Sept. 10	3	1.078	75.4	75.4	75.6
Russet Burbank (Idaho)	Oct. 3	5	1.086	73.4	72.7	72.9
White Rose (California)	Sept. 15	7	1.066	77.6	78.2	75.2

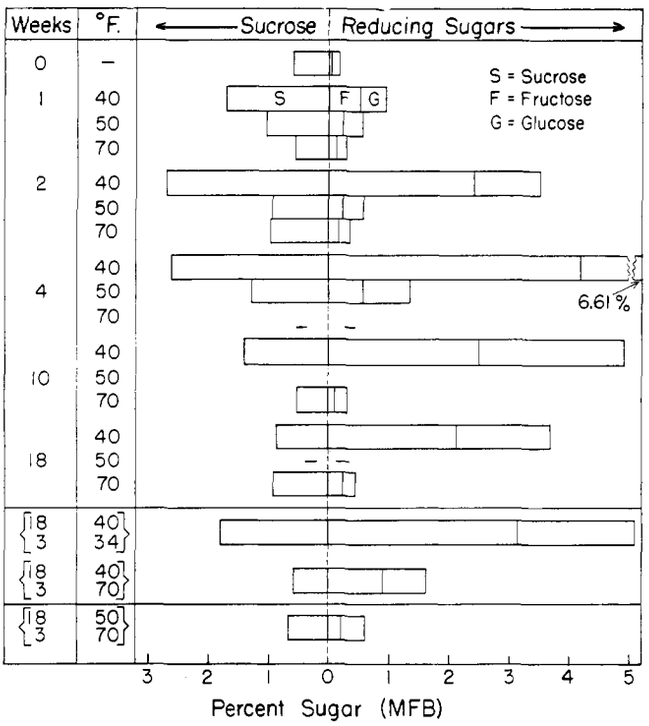

Figure 2. Major sugar components of White Rose potatoes as affected by time and temperature in 1952 storage experiments

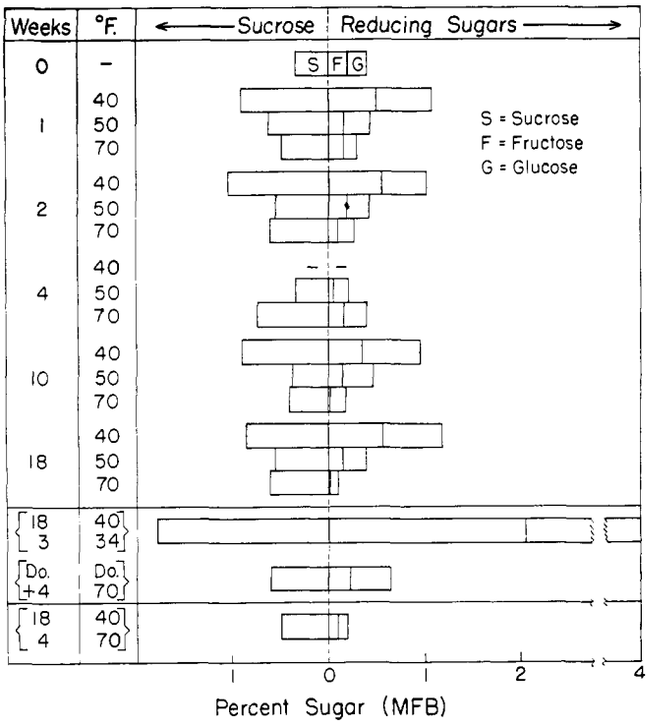

Figure 3. Major sugar components of Russet Burbank potatoes as affected by time and temperature in 1952 storage experiment

in the 1952 experiments were calculated from the difference between the solids content and the total sugar as determined analytically.

Starch and Iodine Blue Values

Starch samples were isolated from the potatoes by blending the diced potato with water at high speed in an electrical blender. The mixture was mashed and screened through a fine nylon cloth to remove the fiber, which was again mixed with water and pressed on the nylon cloth. The starch settled from the filtrates within 2 hours. The supernatant liquid was decanted and the starch purified by four additional settlings from water. The starch was defatted by four successive 3-hour digestions in boiling 85% methanol and then dried in vacuo at 50° C. The method reported (*12*) for determination of the starch-iodine "blue value" was applied to selected starch samples, except that the concentration of the perchloric acid was increased. It was found that the concentration (39% perchloric acid) used previously (*12*) did not completely disperse potato starch when the extraction was carried out in an ice-water bath. Several concentrations of perchloric acid–41, 44, 48, 52, and 56%—were tested; at concentrations at or above 44% the starch was completely dispersed. At 52 and especially at 56% perchloric acid the starch-iodine blue values decreased as time of extraction at 32° F. was increased. At the other concentrations employed the starch-iodine blue values remained constant as the time of dispersion increased to 90 minutes, the maximum time tested.

Some samples were dispersed in sodium hydroxide solution at room temperature and calcium chloride solution (55.2 grams of calcium chloride dihydrate in 100 ml. of solution adjusted to pH 6.1 with 0.8% acetic acid) at 212° F. Blue values of the starch dissolved in alkali were the same as the values obtained with perchloric acid solution. Calcium chloride solutions gave higher blue values, but these were shown to be due to the presence of the calcium chloride. Thus a solution of calcium-free starch gave a blue value of 104, and a value of 109 if the blue color was developed after additional calcium chloride. Autoclaving starch suspensions buffered at pH 6.0 to 6.3 in the presence of calcium chloride decreased the blue value. The perchloric acid method was considered the most convenient to disperse the starch. Starch samples were dissolved with 43% perchloric acid in an ice-water bath as follows: Fifteen milliliters of perchloric acid solution (one volume of 70 to 72% perchloric acid added to one volume of water, cooled in a refrigerator) were added with stirring to a mixture of 0.250 gram of starch and 1 ml. of water, cooled in an ice-water bath. The mixture was allowed to stand with occasional stirring for 30 minutes in the bath. The solution was diluted to 250 ml. and blue value determined (*12*).

For the determination of total starch, 100 grams of diced potato were blended 4 minutes with 95% ethyl alcohol in a blender. The mixture was transferred to a 500-ml. volumetric flask and the volume adjusted with alcohol. Ten milliliters of

Table III. Fructose as Average Percentage of Reducing Sugars at Various Storage Temperatures

Variety	*Storage Expt.*	*34° F.*	*40° F.*	*50° F.*	*70° F.*
Russet Burbank	1952	54	47 ± 4	37 ± 3	36 ± 10
	1953	..	51 ± 3	...	17
White Rose	1952	62	60 ± 4	40 ± 6	47 ● 1
	1953	..	61 ± 3	...	34
Chippewa	1953	..	52 ± 2	...	18
Kennebec	1953	..	50 ± 0	...	22
Katahdin	1953	..	52 ± 5	...	21

the mixture were added to a 40-ml. graduated centrifuge tube containing 1 gram of Celite and 20 ml. of 80% ethyl alcohol. After stirring and heating in a water bath at 80° C. and cooling, the solids were separated by centrifuging. The supernatant was discarded and the residue extracted three additional times with 30 ml. of hot 80% ethyl alcohol. After final extraction, water was added to the residue to make 10 ml., then 13 ml. of perchloric acid (270 ml. of 72% perchloric acid added to 100 ml. of water) were added with constant stirring. This mixture was stirred occasionally for 20 minutes, then 20 ml. of water were added and, after agitation, the solids were removed by centrifuging. The solution was transferred into a 250-ml. volumetric flask, 6.5 ml. of perchloric acid were added to the residue, and after occasional stirring for 20 minutes the contents of the tube were washed into the 250-ml. flask. The volume was adjusted and the mixture filtered. After the first portion of the filtrate had been discarded, the starch was determined in the filtrate by the anthrone–sulfuric acid method as described by McCready *et al.* (*12*). Phosphorus content of the starches was determined by the method of Allen (*1*).

Other Measurements The change in weight (specific gravity, as determined by weight loss in water) of 20 randomly selected tubers from each lot was determined at the appropriate time intervals. The moisture content of the potato dice was determined by a preliminary forced-air drying at 80° C., followed by a final drying in a vacuum oven at 70° C.

Results

Weight-Volume Data A summary of the weight-volume relationships is shown in Table I. Both varieties lost about 3% of their weight over 18 weeks at 40° and 50° F., with a corresponding loss in volume. The rate of loss, as to be expected, was slightly greater at 70° F. during the early stages of storage and considerably greater after 4 weeks. This later loss was due in part to the development of sprouts. In both varieties the potatoes at 70° F. lost 5% of their original weight in 10 weeks and 14% in 18 weeks. The moisture content remained about the same throughout the storage period, averaging 78.5% for White Rose and 77.6% (unexpectedly high) for Russet Burbank.

Low moisture content was reflected in specific gravity, which was found to average the same for both varieties, 1.086, with no discernible trend upon subsequent storage at the various temperatures. Thus, specific gravity can be eliminated as a parameter in interpreting differences between these two varieties in the 1952 storage experiment. The specific gravities fall into the expected pattern in the 1953 experiment (Table II). In general, as has been reported (*16*), the higher the specific gravity, the lower the moisture content (Figure 1). In contrast to the 1952 experiments, the initial lots of potatoes had been placed at 40° F. for varying times after harvest.

Effect of Storage. **Effect on Sugar Components.** Results on the sugar composition of the two varieties in the 1952 experiments are shown in Figures 2 and 3. The freshly harvested potatoes contained very little sugar. Both varieties accumulated reducing sugar at 40° F. the accumulation going through a maximum at about 4 weeks and then dropping to a relatively constant but high level thereafter. Total and reducing sugars were constantly higher in White Rose than in Russet Burbank. In both varieties lowering the temperature of storage to 34° F. caused the total-sugar and especially the reducing-sugar content to increase, whereas conditioning at 70° F. resulted in considerable decreases. The Russets conditioned better than the White Rose in the sense that their total reducing sugars were less. However, the White Rose actually lost more sugar.

With regard to variation in individual content during storage, the sucrose content of Russet Burbanks ran on the average 60% higher at 40° F. than at 70° F. At 70° F. about two thirds of the total sugar was accounted for as sucrose, whereas at 40° F. somewhat less than half was present as sucrose. With White Rose at 70° F. two thirds of the sugar was sucrose, and at 40° F., about one third after prolonged storage. This would indicate that upon storage at low temperatures total reducing sugars accumulate more rapidly than does sucrose and that the differential rate of accumulation is greater for White Rose than for Russet Burbank. However, at early stages of storage (less than 2 weeks) sucrose seemed to develop more rapidly than either of the two reducing sugars (see the last two items in Table IV).

There were differences in accumulation (Figures 2 and 3) in 1952 of the individual reducing sugars upon storage at 40° and 70° F. The relative amounts of fructose for both varieties was found to be greater at 40° F. than at 70° F.

Table V. Nonionic, Nonsugar, Alcohol-Soluble Contents of Potatoes

(Percentage of dry weight)

Temp., ° F.	Storage Time, Weeks 0	1	2	4	10	18	21–25[a]
				White Rose			
40	0.20	0.56	0.43	0.74	0.92	0.76	0.98[b]
50	0.20	0.46	0.09	0.07	...	...	0.57[c]
70	0.20	0.27	0.40	...	0.24	0.80	0.34[d]
				Russet Burbank			
40	0.41	0.45	0.27	0.28	0.41	0.48	0.65[e]
50	0.41	0.53	0.32	0.96	0.72	0.63	0.26[f]
70	0.41	0.36	0.32	0.32	0.49	0.32	0.50[g]

[a] 18 weeks at 40° F.
[b] Plus 3 weeks at 34° F.
[c] Plus 3 weeks at 70° F.
[d] 18 weeks at 50° plus 3 weeks at 70° F.
[e] 18 weeks at 40° F. plus 4 weeks at 34° F.
[f] Plus 3 weeks at 70° F.
[g] Plus 4 weeks at 34° F. and 3 weeks at 70° F.

Table IV. Distribution of Sugar Components Lost or Gained by Changing Storage Temperature

Variety	Storage Expt.	Temp. Change, ° C.	Time, Weeks[a]	Total Sugar Lost, %	Fraction of Sugar Lost: Sucrose	Fructose	Glucose
Chippewa	1953	40 to 70	5	1.53	0.31	0.56	0.13
Kennebec	1953	40 to 70	5	1.19	0.27	0.53	0.20
Katahdin	1953	40 to 70	5	1.94	0.27	0.43	0.30
Russet Burbank	1953	40 to 70	5	0.87	0.31	0.40	0.29
	1952	40 to 70	4	1.29	0.26	0.38	0.37
	1952	34 to 70	3	4.61	0.26	0.40	0.34
White Rose	1953	40 to 70	5	0.87	−0.09	1.00	0.09
	1952	40 to 70	3	2.29	0.09	0.65	0.26
Russet Burbank	1952	70 to 40[b]	1	−1.21	0.45	0.26	0.29
White Rose	1952	70 to 40[b]	1	−1.87	0.58	0.25	0.17

[a] Interval between temperature change and sampling.
[b] First week of storage of 1952 experiment.

White Rose contained up to twice as much fructose as glucose at 40° F., whereas Russets contained about equal amounts of the two sugars. At 70° F. equal amounts of the two sugars were present in the White Rose, whereas the Russets contained between two and three times more glucose than fructose. The values at 50° F. are closer to those at 70° F. than to those at 40° F.

Results of the 1953 experiments are shown in Figure 4. The superior sugar-accumulating capacity of White Rose was again demonstrated. The other varieties used in this study resemble more closely Russet Burbank in sugar-accumulation characteristics than White Rose. Thus, Table III confirms previous evidence that (except for White Rose) the ratio of glucose to fructose approaches unity at low temperatures and is consistently above 2 at higher temperatures. Again, in agreement with previous results, White Rose shows a distinct tendency toward fructose accumulation, in comparison with Russet and the Maine varieties.

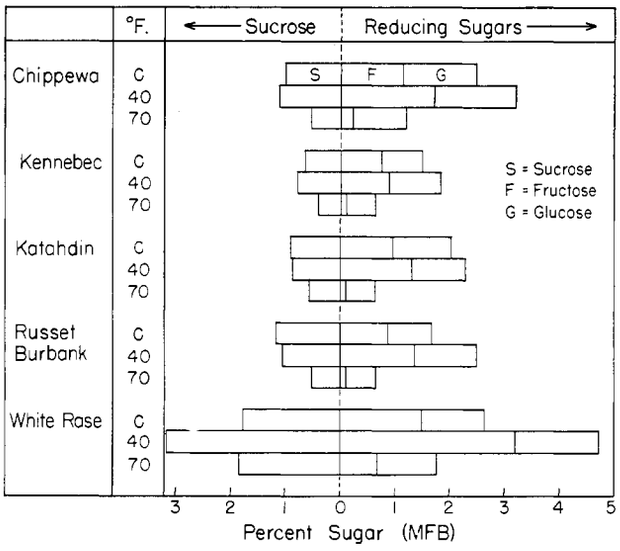

Figure 4. Major sugar components of potato varieties used in 1953 storage experiment

C, control sample at start of experiment. Storage time, 5 weeks at indicated temperatures

Storage at 70° F. in the 1953 experiment was essentially a conditioning experiment, inasmuch as the initial lot had been at 40° F. for a considerable length of time. Calculation of the distribution of sugar lost in these and previous conditioning experiments revealed that a greater proportion of fructose was lost than either glucose or sucrose (Table IV). In contrast to the 1952 experiment, a certain degree of correlation seems to exist among specific gravity, reducing sugar content, and average moisture level (Figure 1).

The percentage range of nonsugar material in the extracts in relation to the dry weight of potatoes (moisture-free basis) lies, for most samples, between 0.2 and 0.6% of the dry weight of the tuber with no discernible trend with respect to temperature, time, or variety (Table V).

Effect on Starch Content Results of determination of total starch are given in Table VI. White Rose potatoes stored at 40° F. decreased in starch content but showed, little, if any, change in starch when stored at 50° and 70° F. Data from Russet Burbank indicate that the starch remained almost constant, except at 18 weeks of storage at 40° F., when a decrease in percentage of starch was noted, and at 18 weeks at 70° F., when starch percentage had increased.

Starch-iodine blue values of starch isolated from potatoes stored at different temperatures indicate that there were no differences in amylose content of starches as affected by storage time or temperature, except for the differences between the two lots. The starch-iodine blue values of starches isolated from White Rose were 100 ± 2 and from Russet Burbank 105 ± 2. Phosphorus content of these starches also did not change upon storage. Starches from White Rose contained 0.08% phosphorus and from Russet Burbank, 0.10% phosphorus.

Results of Chromatography The major constituents of the alcohol-soluble sugars (found chromatographically) of potatoes are glucose, sucrose, and fructose. In general, semiquantitative observations of the spots for these sugars corresponded to the values found by analytical procedures. In addition, trace sugars were detected whose rates of movement and reaction with the sugar sprays corresponded to those of stachyose, raffinose, melezitose, and a heptulose for the 1952 series (Tables VII and VIII) and only one spot corresponded to that of melibiose for the 1953 series (Table IX). As there was a considerable degree of variability in limits of detection of the sugars (Tables VII to IX), it would be hazardous to rule out the presence of any of these sugars as affected by storage conditions. Although inspection of these tables would indicate certain trace sugars are more likely to be found at 70° F. than at 40° F., limits of detection are higher in samples from the latter temperature. All samples tested contained traces of heptulose. Raffinose was found only in extracts from Russets stored at 40° and 50° F. and melezitose in most of the White Rose samples in spite of the relatively high limits of detection at 40° F. The melezitose spot was distinguishable from any possible melibiose not only by its slightly but significantly different rate of movement but also by the fact that it reacted only with the resorcinol ketose spray, and not with the reducing spray, 3,5-dinitrosalicylic acid. On the other hand, the "melibiose" spot found in the 1953 storage series reacted to the aldose but not to the ketose spray. A spot moving at the rate of both cellobiose and inositol was present in many of the samples of the 1952 study (Tables VII and VIII). As this spot did not show up with the reducing sprays other than silver, it has been tentatively identified as inositol. When present, the limits of detection of this spot (if it is inositol) are the same order of magnitude as, but never more than, the percentage of the nonsugar components of the extract.

Interesting changes occurred in the sugar pattern of the 1953 extracts (70° F. storage) which had been subjected to microbial growth after storage for several months at 40° F. The sucrose, fructose, and melibiose decreased or disappeared and two new pentose-containing oligo- and polysaccharides developed (pink spot with aniline reagent), corresponding to R_g values of 0.00 and 0.10 (movement of glucose = 1.00), respectively. R_g values of free pentoses were over 1.00.

Table VI. Starch Content of Potatoes as Affected by Storage Time and Temperature

(Percentage of dry weight)

Temp., ° F.	Storage Time, Weeks 0	1	2	4	10	18	21–25[a]
			White Rose				
40	78.9	75.2	75.2	68.2	72.5	73.8	72.8
50	78.9	77.5	78.2	78.2	79.3	79.0	71.3
70	78.9	77.4	19.3	...	77.7	76.0	76.4
			Russet Burbank				
40	74.4	75.7	78.5	73.7	75.7	71.9	69.9
50	74.4	75.7	75.5	76.0	76.5	78.7	78.4
70	74.4	76.2	77.0	73.7	76.4	79.3	72.3

[a] See Table V.

Table VII. Detection of Trace Sugars in Russet Burbank Potatoes

(1952 storage experiment)

Temp., ° F.	Time, Weeks	Trace Sugars (Limits of Detection, % Dry Weight)[a]				
		Fructan	Raffinose	Heptulose	Melezitose	Inositol
..	0	+0.03	+0.03	+0.02	−0.03	...
40	1	−0.08	+0.08	+0.04	−0.08	−0.4
	2	+0.03	+0.03	+0.02	−0.03	−0.2
	4	−0.04	+0.04	+0.02	−0.04	−0.2
	10	−0.06	−0.06	+0.03	−0.06	−0.3
	18	+0.06	−0.06	+0.03	−0.06	−0.3
50	1	−0.05	−0.05	+0.03	−0.05	−0.3
	2	−0.06	+0.06	+0.03	−0.06	−0.4
	4	+0.07	+0.07	+0.04	−0.07	+0.4
	10	+0.06	−0.06	+0.03	−0.06	+0.3
	18	+0.06	−0.06	+0.03	−0.06	+0.2
70	1	+0.03	−0.03	+0.02	−0.03	+0.2
	2	−0.03	−0.03	−0.02	+0.03	−0.2
	4	−0.03	−0.03	+0.02	−0.03	+0.2
	10	+0.03	−0.03	+0.02	−0.03	+0.1
	18	+0.02	−0.03	+0.02	−0.03	+0.3

[a] +. Detection of sugar by paper chromatography.
−. Absence of sugar.

Discussion

Both the quantitative analytical and qualitative chromatographic results with the essentially ion-free alcohol-soluble constituents of potato confirm observations previously made. They show that potatoes develop sugar when subjected to lowered temperature and lose sugars upon conditioning at higher temperatures (*6*, *7*). When stored at low temperatures, there is generally an initial rise of sucrose followed by the other sugars (*2*, *4*, *5*) and at continued low-temperature storage the sugar development goes through a maximum. That the rate of sugar development will depend not only upon temperature and variety but also upon other factors such as time of harvest (*4*), prestorage conditions (*7*), and soil conditions has been frequently demonstrated.

The chromatographic results demonstrate conclusively that the major sugars of tubers are indeed sucrose, glucose, and fructose. The conclusion that fructose is generally more responsive to temperature change than glucose seems to have validity with respect to data reported here. These observations are in accord with recent observations on involvement of free fructose (but not free glucose) in the synthesis of sucrose (*10*, *18*). It remains to be seen whether this conclusion merits the category of a generalization.

In this survey the assignments of the sugars suggested by the movements and spray reactions of the chromatographic spots must be considered tentative. Thus the nonmoving ketose spot has been designated as fructan, although stachyose fulfills the above-mentioned requirements. Proof can be obtained only by isolation of the sugars and establishment of their identity by co-chromatography with several solvents and eventually by chemical characterization.

The limits of trace sugars detected indicate that these sugars do not seriously affect the analytical values for sugars at high sugar concentrations (low-temperature storage) but may constitute a significant portion of the total sugar when the values for the latter are low. The ubiquitous presence of heptulose in the samples examined points to the operation of the oxidative pathway of carbohydrate metabolism (*3*) and to its possible involvement in sucrose transformations (*19*). Detection of both raffinose and melibiose may imply the presence of invertase activity in the intact tuber, although the potato extract usually displays little or none of this enzyme (*17*, *18*). Similarly, the presence in potatoes of both inositol and phytic acid (*11*) might indicate the presence of phytase (*8*).

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Table VIII. Detection of Trace Sugars in White Rose Potatoes

Temp., ° F.	Time, Weeks	Trace Sugars (Limits of Detection, % Dry Weight)[a]				
		Fructan	Raffinose	Heptulose	Melezitose	Inositol
..	0	−0.06	−0.06	+0.03	+0.06	+0.2
40	1	−0.11	−0.11	+0.06	+0.11	+0.5
	2	−0.02	−0.12	+0.06	+0.12	−0.6
	4	−0.05	−0.15	+0.03	+0.05	−0.9
	10	−0.12	−0.12	+0.05	−0.12	−0.7
	18	−0.08	−0.08	+0.04	+0.08	−0.4
50	1	−0.14	−0.14	+0.07	−0.14	−0.8
	2	−0.06	−0.06	+0.03	−0.06	−0.3
	4	−0.02	−0.07	+0.04	+0.07	−0.4
70	1	+0.02	−0.02	+0.01	+0.02	+0.1
	2	+0.03	−0.03	+0.02	+0.03	+0.2
	10	+0.03	−0.03	+0.02	+0.03	+0.2
	18	+0.02	−0.02	+0.01	+0.02	+0.1

[a] Calculated from percentage total sugar and absolute limits of detection of sugar. +. Detection of sugar by paper chromatography. −. Absence of sugar by paper chromatography.

Table IX. Limits of Detection Melibiose Present in Potatoes[a]

Variety	Control	40° F.	70° F.
Chippewa	−0.09	−0.09	+0.04
Kennebec	−0.08	−0.08	+0.04
Katahdin	−0.11	−0.07	+0.03
Russet Burbank	−0.08	−0.08	+0.03
White Rose	+0.05	−0.09	+0.05

[a] 1953 storage experiment, % dry weight.
+. Detection of sugar.
−. Absence of sugar.

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CEREAL CARBOHYDRATE DETERMINATION

Sulfonated 1-Naphthol and Anthrone Reactions Applied to Sulfuric Acid Extract of Cereals

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Proximate analysis is satisfactory for the estimation of protein, ash, and "crude fat" content of cereals and cereal grains. However, there is need for a simple method for the direct estimation of carbohydrates in the nitrogen-free extract. The sulfonated 1-naphthol reaction (modified Molisch test) not only gives consistent results for the estimation of carbohydrate in the 1.25% sulfuric acid extract, but it also offers a method of estimating pentose in this extract. As the anthrone reaction yields a stable color for hexoses and an unstable color for pentoses, this test can be used as a check for the sulfonated 1-naphthol reaction. These tests can be run on aliquots of the 1.25% sulfuric acid extract when crude fiber is determined.

ONLY THE PROTEIN, ASH, FAT (ether extract), and crude fiber contents are shown by the proximate analysis of cereals. The remainder of the dry material is assumed to be soluble carbohydrate and is called the nitrogen-free extract (NFE). A simple method is needed for determining carbohydrates in this fraction, which may contain some bound water and pentosans which are of very little value in human nutrition.

The absorption band of the colored products from the reaction of sulfonated 1-naphthol and sulfuric acid (modified Molisch test) is more in the violet (*1, 3–5*) for pentoses than for hexoses. Consistent results are obtained for both types of carbohydrates.

The color formed in the anthrone reaction with pentoses fades rapidly (*6–8*), whereas the color with hexoses is comparatively stable.

The objectives of the present work were: to determine the precision of the sulfonated 1-naphthol reaction when it is applied to the sulfuric acid extracts of cereals and cereal products; to study the possibility of using both the anthrone and the sulfonated 1-naphthol reactions for estimating pentoses present in the extract; and to develop a simple rapid procedure for the determination of carbohydrates in the extract, which may be applied when crude fiber is determined.

Methods and Materials

Procedure Extraction Two-hundred milligram samples of ground material were extracted by refluxing with 50 ml. of 1.25% sulfuric acid (1.25 grams per 100 ml.) for 30 minutes. The mixture was cooled, made to 100 ml., and filtered. All extractions were made in triplicate. An aliquot of the filtrate, containing 6 to 8 mg. of carbohydrate, was diluted to 200 ml. These diluted extracts were used for the determinations.

Sulfonated 1-Naphthol Reaction. Five milliliters of concentrated sulfuric acid was rapidly added from a delivery pipet to a test tube (while mixing) containing 1 ml. of sulfonated 1-naphthol reagent (*1*) and 1 ml. of the diluted extract. The time allowed for the pipet to drain was constant (10 seconds) and the hot mixture was vigorously agitated for 15 seconds longer and then placed in a boiling water bath. After a total heating period of 8 minutes the tube was placed in a mixture of ice and water until the spectrophotometric readings were made. When the tubes were cool, the absorbances were measured at 530, 540, 550, 560, 570, and 580 mμ with a Model B Beckman spectrophotometer in 1-cm. cells. The reference mixture contained 1 ml. of distilled water in place of the extract and the standard contained 0.040 mg. of carbohydrate in 1 ml. of water. These were treated the same as the extract.

Anthrone Reaction. Ten milliliters of 0.2% anthrone reagent (0.2 gram of anthrone per 100 ml. of concentrated sulfuric acid) was placed in a 50-ml. Erlenmeyer flask containing 5 ml. of the diluted extract, and the contents of the flask were thoroughly mixed. After standing for 1 hour, the absorbances were measured at 620 and 630 mμ with the Model B Beckman spectrophotometer in 1-cm. cells. The reference mixture contained 5 ml. of distilled water in place of the extract and the standard contained 0.200 mg. of carbohydrate in 5 ml. of water. The standard and the blank (reference mixture) were treated the same as the diluted extract.