.5% (Table V) was not protective. scorbate alone gave some protection in ıe one experiment in which it was tried Table V), but this compound behaves ratically when added to meat, sometimes accelerating rancidity. Hot water ctracts of green onion tops and green eppers had no protective effect.

The combination of tripolyphosphate ıd ascorbate gave excellent protection uring the first few months of storage, ut the treated samples were highly ıncid at 47 weeks (Table I) and 1 ear (Table IV). The increase in lipid xidation is sudden and drastic and may ɔincide with loss of the ascorbate, alough this has not been demonstrated.

BHA was the most effective of the ntioxidants tried in preventing lipid xidation (Table IV). Unfortunately, though no rancid odor was present in ıe BHA-treated samples even after a ear of storage, an unidentified off-odor as evident at least during the last few ıonths of storage. The peculiar medicinal" odor detracted from the alatability of the cured meat. Further ıvestigation of this problem is needed efore BHA can be recommended as an ıtioxidant under these conditions.

Cured meat protected either with HA or with tripolyphosphate plus scorbate (up to the time of the sudden ıcrease in oxidation) showed extremely igh peroxide to TBA ratios. These ıtioxidants appear to prevent peroxide ecomposition. Privett (*9*) has offered vidence that the synergistic activity of scorbate may be due, at least in part, to s ability to delay peroxide decomposiɔn.

Sensory Scores and Pigment hanges. Statistical evaluations of the ɔrrelation between several of the tests sed were obtained using the Spearman ank correlation (*11*). The results are ummarized in Table VII.

The correlation of rancid odor with BA numbers is very high. This is true ɔth for cured and for uncured samples, and the correlation increases further when the total number of cured and uncured samples are ranked together. When it is considered that the organoleptic ratings were made over a period of many months, with no standards for comparison, a correlation coefficient of 0.92 is surprisingly high.

Peroxide number also correlates well with sensory scores in cured samples. The correlation coefficient is lower when uncured samples are ranked and, as might be expected, drops still more when cured and uncured samples are lumped together. Peroxides appear to be a less useful measure of rancidity than the TBA test, especially when sample treatments affect peroxide stability. The difficulty of designating a "threshold" peroxide number for rancid odor is evident when sensory scores are compared with peroxides in cured and uncured meats.

To correlate pigment losses in cured meats with rancidity tests, pigment ratios in Tables II, IV, and V were calculated for ranking purposes as per cent retention, assuming that a ratio of 1.3 represents complete pigment destruction. These results could then be combined with pigment retentions by the Hornsey method (Table I). Pigment loss showed a high degree of correlation with both the TBA and peroxide numbers. Any antioxidant treatment which retarded lipid oxidation also retarded pigment loss.

All samples in Experiment 1 gave positive sulfhydryl tests throughout a year of storage. Freezing has been shown to protect sulfhydryl groups (*4*). Free nitrite was present in the cured sample throughout but rapidly disappeared from the cured sample containing ascorbate. This observation is in agreement with earlier work (*8*).

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'OTATO SUGARS

'he Quantitative Analysis of Glucose ınd Fructose in Potatoes

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THE COLOR DEVELOPMENT in potato chips depends upon the formation of rown pigments during the frying of .e potato slices in an oil bath. Habib ıd Brown (*7*) and Shallenberger (*12*) ɔnclude that this color development results from the reaction between reducing sugars and amino acids. The exact mechanism of this reaction is not known; it is influenced by a number of factors, such as pH, temperature, concentrations of reactants, and catalysts. A number of investigations (*3, 5, 6, 8, 10, 13*) show that the sugar and amino acid contents vary considerably among potato varieties, and are influenced by environmental, cultural, and storage conditions.

To determine the concentrations of sugars in potatoes which had been subjected to diverse growing and storage conditions, a rapid, sensitive, and ac-

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The reducing sugars, glucose and fructose, of potato tubers are extracted with 80% ethanol and measured quantitatively by means of a modification of the ferricyanide reduction method. The resulting ferrocyanide combines with arsenomolybdate and is measured spectrophotometrically at 745 mμ. Glucose and fructose are determined simultaneously through differences in their oxidation rates at 100° and 55° C. This method is accurate for total and individual reducing sugar constituents; the precision in all cases is better than 2%. The ferricyanide method gives results which compare quite favorably with the standard Shaffer-Somogyi method. Several substances, including ascorbic acid, amino acids, and polyphenols, do not produce significant interference when present in the concentration ranges normally found in potatoes.

curate method was required for measuring glucose, fructose, and hydrolyzable sugars. Methods most often used for sugar analyses have been based on the reduction of copper (*2*). These methods have required long periods to perform, and the instability of the reduced copper has been a serious source of error. A limitation of the copper reduction methods, from the standpoint of potato investigations, has been their inability to discriminate between glucose and fructose. An improved method for measuring sugars was developed by Ting (*14*) for the analysis of reducing sugars in citrus juices. His method involved the reduction of alkaline ferricyanide to ferrocyanide, which was converted to its blue-green arsenomolybdate complex and measured colorimetrically at 515 mμ. Glucose and fructose were measured simultaneously through differences in their oxidation rates at 100° and 55° C. Total sugars were determined after inversion. However, the method described by Ting (*14*) lacked the sensitivity and accuracy required for measuring the relatively low concentrations of reducing sugars of potatoes.

The analytical method described in this paper involves the quantitative extraction of reducing sugars from potato tubers and the subsequent simultaneous analysis of glucose and fructose by a modification of Ting's (*14*) ferricyanide-arsenomolybdate method.

Experimental

Apparatus. The absorption spectrum of the ferrocyanide-arsenomolybdate complex was obtained on a Beckman DK-2 recording spectrophotometer. All measurements of absorbance at 745 mμ were made with a Beckman DU spectrophotometer. In all cases, 1.00-cm. silica cells were used.

Reagents. All of the reagents are the same as described by Ting (*14*). Because of the high sensitivity of the method, reagent-grade chemicals must be used throughout.

Procedure. From any given lot of potatoes, 10 tubers are selected randomly and sliced without peeling. These slices are reduced to 1.5-mm. cubes, and a 50-gram subsample is heated with hot alcohol according to the A.O.A.C. method (*2*), reheated for 30 minutes at 75° C., cooled to room temperature, and filtered with suction. The residue is re-extracted two additional times with 100 ml. of 80% alcohol by the above procedure. The extracts are combined, diluted to 500 ml. with 70% alcohol, and stored at 4° C. until analyzed.

Measurement of Reducing Sugars. These reactions are carried out essentially as described by Ting (*14*). The 1-ml. aliquot taken for analysis should contain from 0.10 to 0.45 mg. of reducing sugars. Heating is carried out with the water level of the bath above that in the flasks.

Absorbance measurements are made 30 to 60 minutes after the addition of the arsenomolybdate solution, at a wavelength of 745 mμ; a blank is treated in exactly the same way as the samples. This same procedure may be used for the determination of hydrolyzable sugars, after inversion.

One-milliliter aliquots of glucose and fructose solutions of graduated concentrations (0.01 to 0.05% w./v.) are subjected to the same procedures, and the respective absorptivities are calculated.

Calculations. At 100° C. glucose and fructose, and at 55° C. fructose, reacts at the same rate with ferricyanide, whereas at 55° C. glucose reacts at a much slower rate. Therefore, the absorptivity after oxidation of glucose and fructose at 100° C. and of fructose at 55° C. is the same (designated by a_{100}); a_{55}, the absorptivity resulting from oxidation of glucose at 55° C., is much smaller. Since Beer's law is valid over the concentration range of interest, the concentration of total reducing sugars (glucose and fructose) is found from the value of A_{100}, the measured absorbance from the reaction at 100° C.

$$c_t = c_g + c_f = \frac{A_{100}}{a_{100}} \times D \qquad (1)$$

where c_t is the total concentration of glucose (c_g) and of fructose (c_f) in grams per 100 grams of potato on a wet basis. D is the dilution factor (in most cases, D = 10 since 50 grams of potato are extracte into 500 ml., and 1 ml. of extract taken for analysis). The value a_{100} is the slope of the plot of absorban (A_{100}) versus concentration of gluco or fructose standard solutions. Th concentration of glucose (c_g) in gran per 100 grams of potato on a w basis, is given by

$$c_g = \frac{(A_{100} - A_{55})}{(a_{100} - a_{55})} D \qquad (2$$

where A_{55} is the measured absorban from the reaction at 55° C. Th value of a_{55} is the slope of the plot absorbance (A_{55}) versus concentratio of the glucose standard solution. Th concentration of fructose (c_f) in gran per 100 grams of potato (wet basis) then given by

$$c_f = c_t - c_g \qquad (3$$

Since the cell length is the same for a measurements of absorbance, it omitted from the equations.

Results and Discussion

Prior to the analyses of the reducir sugars in potato tubers, paper chr matographic separation of the pota extract was made to identify the maj reducing sugars present. The separ tion was effected by one-dimensiona ascending flow of the solvent syste methyl ethyl ketone–propionic acid water (75:25:30 v./v.). The suga spots were developed with a 0.5% solu tion of 3,5-dinitrosalicylic acid (mon sodium salt) in 4% aqueous sodiu hydroxide. According to Woodwar and Rabideau (*15*), reducing suga develop a brown color with 3,5 dinitrosalicylic acid, and nonreducin sugars, a green color. The sprayed chromatograms showed only two brow spots, which corresponded in positio to glucose and fructose. This agree with previous reports (*9*, *10*, *13*) tha glucose and fructose were the majo reducing sugars in most varieties potato tubers.

The absorption spectra of the fe rocyanide-arsenomolybdate complex developed following reaction of fe

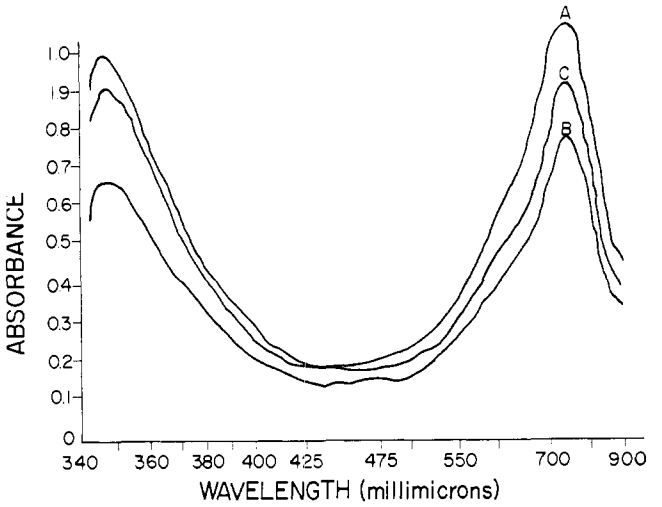

Figure 1. Absorption spectra of ferrocyanide-arsenomolybdate complex (reaction temp. 100° C.)

(A) Glucose, 0.06 gram/100 ml.; (B) Glucose, 0.04 gram/100 ml.; (C) Potato extract

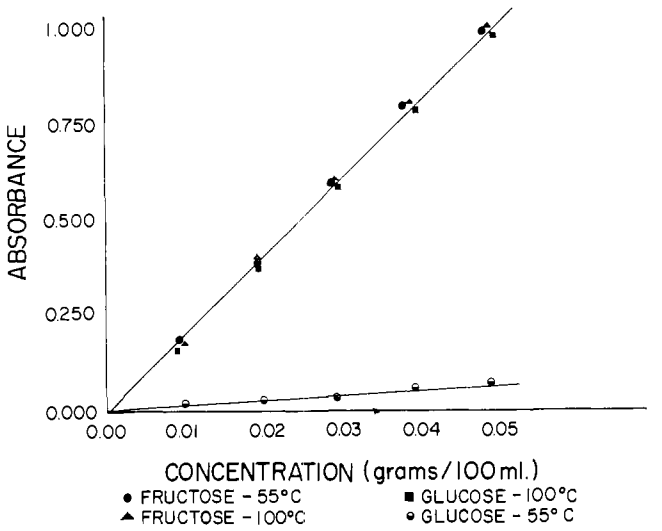

Figure 3. Relationship of reducing sugar concentrations to absorbance of ferrocyanide-arsenomolybdate complex

(Wavelength setting 745 mμ, slit width 0.045 mm.)

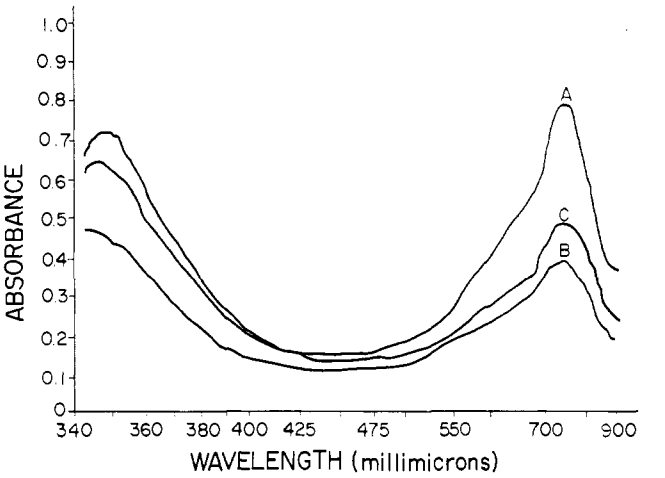

Figure 2. Absorption spectra of ferrocyanide-arsenomolybdate complex (reaction temp. 55° C.)

(A) Fructose, 0.04 gram/100 ml.; (B) Fructose, 0.02 gram/100 ml.; (C) Potato extract

Table I. Precision of Analyses of Total Reducing Sugars by the Ferricyanide-Arsenomolybdate Method

Control Solution, Mg./100 Ml.	Potato Extracts: Extract	Per Cent in Potato: A	Per Cent in Potato: B	Difference between duplicates
9.28	I	0.261	0.259	0.002
9.33	II	0.357	0.356	0.001
9.33	III	0.224	0.228	0.004
9.49				
9.28				
9.38				

Table II. Comparison of Methods for the Determination of Total Reducing Sugars

Sample	Total Reducing Sugars, %: Ferricyanide	Total Reducing Sugars, %: Shaffer-Somogyi (11)
1	0.77	0.90
2	1.10	1.10
3	0.35	0.31
4	0.23	0.29
5	0.44	0.41
6	0.37	0.37

ricyanide with potato extracts and with standard solutions of glucose and fructose are shown in Figures 1 and 2. These spectra all showed absorption maxima in the 740 to 750 mμ region. The untreated potato extract showed no appreciable absorbance between 650 and 900 mμ. By measuring the absorbance at 745 mμ, where absorptivity was maximum, the sensitivity and accuracy of the method were greatly improved over Ting's method (*14*), in which measurements were made at 515 mμ. Accuracy was increased because absorbance values fell within the region of minimum error (0.2 to 0.8) for the recommended concentration range—i.e., 0.01 to 0.045 gram sugar per 100 ml. of solution. The sensitivity was increased more than six times, as determined by the rate of change in absorbance with change in concentration. This rate of change for each 10 mg. of sugar per 100 ml. was 0.2 absorbance unit at 745 mμ and 0.03 at 515 mμ. Because of the relative flatness of the absorption peak from 740 to 750 mμ, measurement at 745 mμ results in a high degree of precision.

Figure 3 shows the relationship of absorbance to concentration obtained with glucose and fructose standard solutions oxidized at temperatures of 100° and 55° C. Fructose and glucose at 100° C. and fructose at 55° C. gave the same slope and thus the same absorptivity, designated a_{100}. At 55° C., however, glucose gave a much smaller absorptivity, designated a_{55}.

Data, presented in Table I, indicate the excellent precision of the method when applied to replicate portions of a synthetic standard solution (control) and of several potato extracts. Values obtained for the control had a relative standard deviation of 0.84%.

The ferrocyanide-arsenomolybdate complex became stable about 15 minutes after its preparation. But after about 1 hour the absorbance decreased gradually. Therefore, all measurements were taken 30 to 60 minutes after the formation of the complex.

In the procedure described here, clarification of the extract with lead acetate and removal of alcohol by evaporation were unnecessary. Deletion of these steps offered distinct advantages over the Shaffer-Somogyi method (*11*). Clarification did not

cause any appreciable changes in reducing sugars as measured by the ferricyanide method, but occlusion of sugars could occur during clarification. The heat employed in the removal of alcohol in the Shaffer-Somogyi method (*11*) favored loss of reducing sugars through reaction with amino acids. Table II shows a comparison of results obtained by the Shaffer-Somogyi and ferricyanide methods. Alcohol did not constitute an interference in the present method; therefore, evaporation was unnecessary unless the sugar level was below 10 mg. per 100 ml. The use of more than 1 ml. of extract per 5 ml. of ferricyanide solution resulted in less-than-normal color development. Increasing the volume of ferricyanide proportionately yielded more accurate results, but the blanks were inordinately high.

The effects of several possible sources of interference were also evaluated. Ting (*14*) found that ascorbic acid would interfere only if present in concentrations greater than 100 mg. per 100 ml. The ascorbic acid concentration found in potatoes was much less than this value (*13*) and so should be unimportant. Other possible interfering substances, which proved to be unreactive, were citric acid and several amino acids. However, polyphenols constituted a definite interference if present in significant concentration. Solutions of catechin and chlorogenic acid, representative of polyphenols, were selected to determine their reactivity with ferricyanide under the conditions of the method. These were found to constitute a definite interference—e.g., 0.01% chlorogenic acid and 0.04% catechin had ferricyanide-reducing powers equivalent to 0.011 and 0.08% reducing sugars, respectively. However, analyses of alcohol extracts of potato tubers by the method of Arnow (*1*), in which a red complex was developed if polyphenols were present, showed no detectable amounts of these substances. This was confirmed by a negative chromatographic analysis of potato extracts for polyphenols. In this study, therefore, no corrections of reducing sugar values for interferences were needed. Since polyphenol concentrations of 20 mg. per 100 grams of potato or higher may occur in some cases (*4*), the Arnow method (*1*) should be applied routinely. Whenever the polyphenol content of alcoholic extracts exceeds 0.5% of the sugar concentration, commensurate corrections based on chlorogenic acid should be determined and applied to sugar values.

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POTATO AMINO ACIDS

Isolation, Concentration, Separation, and Identification of Amino Acids in Potatoes by Ion Exchange and Paper Chromatography

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Previous studies (*4, 10*) showed that the color developed in chipped and fried potatoes was primarily the result of the reaction of amino acids with reducing sugars. Therefore, a study of the quality of potatoes for these purposes required not only the isolation and determination of reducing sugars, but also the separation and quantitative identification of the constituent amino acids.

Most procedures for investigating amino acids have employed vacuum evaporation for concentration, followed by one- or two-dimensional paper chromatography. The solvent systems normally used in the latter step (*2, 4, 8, 11*) contained phenol, collidine, or other noxious materials. A study was made of the use of ion exchange for isolating and concentrating the amino acid fraction in potato extracts. Several solvent systems were investigated for the paper chromatographic separation of the amino acids.

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Experimental

Apparatus. An ion exchange column containing Dowex 50-X8 cation exchange resin, 50- to 100-mesh, in the hydrogen form is prepared (*7, 9*) by placing 15 grams of the wet resin in an appropriate column; in this investigation a 50-ml. buret containing a small piece of glass fiber and several glass beads next to the stopcock is used. The column is backwashed with deionized water to remove air and to distribute the resin uniformly. All residual cations are removed by washing with 50 ml. of 2*N* hydrochloric acid, followed by deionized water until the effluent is neutral. The column is now ready for use. If numerous samples are to be analyzed, it is convenient to have as many as 20 columns set up to run concurrently.

Procedure. Preparation of Sample. Potatoes are extracted with 80% ethanol as described by Furuholmen *et al.* (*3*). The combined potato extract is protein-free.

Isolation and Concentration of Amino Acids. Two-hundred milliliter aliquots of potato extract are passed through the prepared ion exchange column at a maximum flow rate of 100 ml. per hour. The amino acids are retained on the resin, while the alcohol and other solutes are washed from the column with 150 ml. of distilled water.

The amino acids are eluted with 2*N* ammonium hydroxide. When this procedure is followed, the first 20 ml. of