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

The amino acids in alcohol extracts of potatoes are isolated and concentrated by means of a cation exchange resin. The amino acids are then separated by one-dimensional, ascending-flow paper chromatography using a new solvent system composed of methyl ethyl ketone–propionic acid–water–*tert*-butanol (75 : 25 : 30 : 20 v./v.). By means of three solvent passes, a separation of nine amino acids into distinct spots occurs within a period of 6 hours—a much shorter time than is required by other methods. The nine amino acids identified in potatoes are phenylalanine, methionine, tyrosine, alanine, lysine, glutamic acid, glutamine, aspartic acid, and asparagine. The R_f values of the amino acids in the potato extract agree to within 0.01 R_f unit of the values found for standard amino acid solutions.

effluent are discarded. The next 40 ml. are collected in a 50-ml. volumetric flask, 5 ml. of 95% ethanol are added, and the solution is made up to volume with distilled water. Since the effluent fraction containing the amino acids is a function of resin and solution properties, it is necessary to run a test sample, checking each 5 ml. of effluent with ninhydrin. In this manner, the desired fraction for collection of the amino acids is determined.

After elution of the amino acids the column is washed with distilled water to remove excess ammonium hydroxide. The resin is restored to the hydrogen form by the passage of 250 ml. of 2*N* hydrochloric acid.

Separation and Identification of Amino Acids in Potato Extract. An 8-inch square of Whatman No. 1 paper is spotted with suitable aliquots of samples for analysis and placed in a chamber equilibrated with the solvent: methyl ethyl ketone–propionic acid–water–*tert*-butanol (75:25:30:20 v./v.). The solvent is allowed to flow to within approximately 2 cm. of the upper edge of the sheet. The chromatogram is removed from the chamber, and the residual solvent is evaporated from the paper by means of forced air-drying in a hood for 30 minutes. This procedure is repeated for a total of three passages. It is recommended that the chromatograms be run at room temperature and away from sunlight. When the paper is thoroughly dried, the amino acids are developed with 0.5% ninhydrin in acetone by either the spray or dip method. The spots are identified by reference to chromatograms of standard amino acids.

Results and Discussion

Two methods of concentrating amino acids in potato extracts were tried. The first involved vacuum evaporation of the aqueous alcoholic extract solution. Two-hundred milliliter aliquots of potato extract were placed in 500-ml. round-bottomed flasks, which rotated in a 50° C. water bath, and evaporated under vacuum to about 3 ml. in volume. Residues were transferred quantitatively to 10-ml. volumetric flasks and diluted to volume with 70% ethanol. All prepared samples were then stored at 4° C. Vacuum evaporation produced a yellow-to-brown gummy residue which was soluble in 70% ethanol at room temperature but which formed a finely divided precipitate within a few days when stored at 4° C. This precipitate gave a positive ninhydrin test, indicating the presence of amino acids. As attempts to redissolve the precipitate and retain a stable solution were unsuccessful, this technique was discarded.

The second method, the one chosen for concentrating and isolating the amino acids in potato extracts, consisted of retaining the amino acids on a cation exchange resin, Dowex 50-X8, removing potentially interfering substances by washing with distilled water, and eluting amino acids with 2*N* ammonium hydroxide. This resulted in full recovery and maximum concentration of the amino acids. The amino acids could be easily located in the column by means of the heat evolved as the solvent front of ammonium hydroxide reached fresh resin. Collection of effluent was begun when the lowest portion of the resin became slightly warm to the touch. It was shown experimentally that the first 20 ml. of effluent contained no detectable quantities of amino acids and that the amino acids were completely eluted from the column in the next 35 ml. of effluent.

Model systems containing the amino acids listed in Table II, except for β-alanine and asparagine, were subjected to this ion exchange procedure. Paper chromatography of the ammonium hydroxide eluate showed that recovery of all amino acids was accomplished.

A reducing sugar analysis of the original extract and of the effluent collected when washing with distilled water showed complete recovery of the reducing sugars. Therefore, the use of ion exchange resins provided an effective method of separating amino acids from sugars, as well as an efficient concentration method.

Several solvent systems, including the one used by Clayton and Strong (*1*) of methyl ethyl ketone–propionic acid–water (75:25:30: v./v.), were investigated. The solvent system which was used for the separations described in this paper was similar to Clayton and Strong's except that inclusion of *tert*-butanol reduced the flow rate of the solvent and thus provided better separations. The addition of 20 parts of *tert*-butanol to 130 parts by volume of the original solvent also prevented streaking of the spots nearest the solvent front.

A variety of techniques, including one- and two-dimensional, ascending and descending flow of solvents on buffered and unbuffered chromatograms, were investigated. The one-dimensional, ascending flow method using Whatman No. 1 paper was selected as the most favorable technique when using the designated solvent system.

Multiple passage of the solvent system was evaluated also for increased separation of amino acids. Two and three passages of the solvent greatly improved the separation of amino acids in both standard solutions and potato extracts. Four solvent passages resulted in only slight additional separation, and five passages of solvent resulted in one of the spots reaching the solvent front.

Standard 0.01*M* solutions of amino acids, including alanine, asparagine, cystine, glutamic acid, glycine, leucine, lysine, methionine, proline, and tyrosine, were spotted both singly and in groups on Whatman No. 1 paper. The R_f values found for the amino acids for one solvent passage and for three solvent passages (calculated as for a single passage) are given in Table I. Table I also lists the R_f values given by Clayton and Strong (*1*), whose solvent did not contain *tert*-butanol. Triple passage resulted in complete separation of the amino acids and gave 10 well defined spots. The relative positions of the amino acids were not changed by the addition of *tert*-butanol. In addition, three passages of the solvent required only 6 hours, whereas Clayton and Strong's single passage on 46- × 52-cm. paper required 20 hours. Also, as shown in Table I, a slightly greater difference between the largest and smallest R_f values was obtained by triple passage of the designated solvent on

Table I. Effect of Multiple Passage of Solvent on R_f Values of Amino Acids

Amino Acid	R_f Value: Single passage	Triple passage	Clayton and Strong (1)
Leucine	0.32	0.82	0.81
Methionine	0.22	0.66	0.65
Tyrosine	0.17	0.59	0.57
Proline	0.12	...	0.51
Alanine	0.10	0.40	0.47
Glutamic acid	0.08	0.35	0.44
Glycine	0.07	0.29	0.39
Asparagine	0.05	0.19	0.30
Lysine	0.04	0.14	0.25
Cystine	0.02	0.07	0.14

Table II. Identification of Amino Acids in Potato Extracts by Comparing R_f Values with Standard Solutions

Amino Acids	R_f Values: Standards	Potato extract
Leucine	0.82	...
Isoleucine	0.79	...
Phenylalanine	0.76	0.76
Tryptophan	0.70	...
Methionine	0.66	0.67
Valine	0.63	...
Tyrosine	0.59	0.59
β-Alanine	0.49	...
Alanine	0.40	0.39
Glutamic acid	0.35	0.34
Glycine	0.29	...
Aspartic acid	0.26	0.25
Glutamine	0.21	0.22
Asparagine	0.19	0.20
Lysine	0.14	0.15
Cystine	0.07	...

8-inch paper than was reported by Clayton and Strong for a single passage of their solvent on the large sheets.

When 1- to 50-μl. volumes of potato extracts (concentrated to 50 ml. by the ion exchange method) were chromatographed, nine distinct spots were revealed. Two-microliter aliquots gave small, poorly defined spots, whereas 4- and 5-μl. volumes resulted in well defined spots of optimum size and intensity after development with ninhydrin. Volumes of 10 μl. or larger gave large spots with some streaking. Well defined spots were obtained with volumes of 2 to 4 μl. of the 0.01M standard amino acid solutions.

A tabulation of the R_f values for amino acids on chromatograms prepared from standard solutions and from concentrated potato extracts is given in Table II. In all cases, the R_f values of the amino acids in the potato extract agreed within 0.01 R_f unit of the standards. Asparagine, the identification of which might be dubious on the basis of R_f value alone, gave a brown color when treated with ninhydrin. Amino acids identified from potato samples included phenylalanine, methionine, tyrosine, alanine, lysine, glutamic acid, glutamine, aspartic acid, and asparagine. Other investigators (*5*, *6*) reported some amino acids in potatoes which were not found in this investigation. These discrepancies might be due to varietal differences or to cultural and environmental factors, particularly the latter. The Florida potatoes were produced during the winter and early spring months of the year in contrast to summer and early fall production in most other areas.

When ninhydrin in acetone was used for detection of the amino acids, no significant differences were observed between the spray and dip techniques, and no background color was developed. However, spraying the paper with ninhydrin in butanol resulted in a light yellow background.

The methods of isolation and concentration of amino acids in potato extracts and the separation and identification method described in this paper were applied to the analysis of amino acids in a large number of potato-tuber samples having different storage and environmental histories. A visual quantitative estimate of the amount of each amino acid was made by determining the lowest detectable amount of each amino acid and the intensity of the color corresponding to a spot containing 2 μl. of a 0.01M solution. Since all spots had an intensity between the two limiting concentrations, it was possible to estimate the amount of amino acid in each spot once the spot had been identified. Before it would be possible to predict the quantitative effect of amino acids on the browning reaction in the processing of potatoes, many more analyses and correlation studies would have to be made to evaluate the effects of storage and environment.

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COMPOSITION OF CITRUS OILS

Isolation, Identification, and Gas Chromatographic Estimation of Some Esters and Alcohols of Lemon Oil

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Saponification and acetylation values of essential oils are commonly used to estimate their ester and alcohol content. These values usually are expressed in terms of a major ester or alcohol constituent. One of the difficulties encountered in the use of saponification values when applied to lemon oil is that compounds such as coumarins and psoralens (*11*) may be hydrolyzed and calculated as esters. Another problem is that acetylation values do not account for the tertiary alcohols, which are the major alcohols present in lemon oil. Therefore, saponification and acetylation values are not always reliable for estimating the ester and alcohol content of essential oils.

Reports on the ester and alcohol content of lemon oil have been reviewed by Guenther (*3*), and more recently by Kefford (*4*). During the past few years, the composition of lemon oil has been investigated by a number of workers using gas chromatography (*1*, *5*, *6*, *8*). In most of these investigations, the oils were separated into hydrocarbon and oxygenated fractions before examination by gas chromatography.

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