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RECOVERY OF FREE AMINO COMPOUNDS FROM POTATO STARCH PROCESSING WATER BY USE OF ION EXCHANGE[1]

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The "protein water," discharged as one of the wastes from potato starch factories, contains all the water-soluble constituents of the potato. It typically is a dilute solution containing only about 1 per cent solids. Nitrogenous compounds constitute about 60 per cent of the total solids. Approximately two-thirds of the nitrogenous constituents are free amino compounds and one-third is protein. The non-nitrogenous constituents of the protein water are sugars, acids, inorganic salts and minor constituents including polyphenolic-type compounds.

If feasible methods can be devised for recovery of useful nitrogenous compounds from potato starch factory waste water, two benefits would thereby accrue: 1) the pollutional effect of the protein water would be reduced; 2) the economics of potato starch processing would be improved through the creation of salable by-products. In removing the nitrogenous substances from the potato processing water, one can consider the alternatives of preparing a concentrate containing several of the principal amino compounds and of isolating purified individual compounds. Certain of these free amino compounds, for example asparagine and glutamine, give some promise of finding medicinal applications. A concentrated mixture of the principal amino compounds, in addition to potential pharmaceutical uses, might also be of value as a nutritional supplement, food flavoring agent, or nutrient in industrial fermentations.

Another approach to the problem of utilization of potato starch factory soluble wastes is to evaporate under reduced pressure most of the water in the effluent to provide a soluble concentrate. Although this work is not reported in the present paper, we found it possible to make a fluid, stable concentrate containing as high as 60 per cent solids. It is apparently better, from the standpoints of stability and fluidity of the product, to remove the protein by heat coagulation before preparing the concentrate. Since such a concentrate contains most of the potato solubles, conversion of the plant effluent to a concentrate would greatly reduce the pollutional problem. We have information that a concentrate containing all the non-coagulable solubles of potato processing water should be useful as a nutrient in fermentations.

Our interest in this study lay in the free amino compounds of this processing water. The composition of potato extracts with respect to free amino compounds has been investigated by various workers. Using partition chromatography Dent, Stepka, and Steward (3) reported the presence of 24 amino compounds in the soluble, non-protein fraction of the potato. Thompson and Steward (10) presented quantitative data obtained by paper chromatographic technique. They reported the following percentages based on the percentage of nitrogen: glutamine, 46.9; asparagine, 23.6; arginine,

[1]Accepted for publication May 12, 1958.
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9.3; ammonia, 4.2; γ-amino butyric acid, 3.3; valine, 2.4; alanine, 1.7; glutamic acid, 1.4; threonine, 1.0; lysine, 1.0; phenylalanine, 1.0; aspartic acid, 0.9; leucines, 0.9; tyrosine, 0.8; serine, 0.7; methionine, 0.6; glycine, 0.4, and a trace of proline.

Procedure and Analytical Methods

Preliminary work was carried out on processing water received from Maine starch factories .It is necessary to use an additive, such as sodium benzoate, to preserve the processing water during shipment. In addition to the inconvenience of transporting a dilute solution a long distance, possible compositional changes present an uncertainty. Hence, it was decided to simulate processing water in the laboratory by preparing dilute potato extract. Washed potatoes were ground in a hammer mill; the slurry was treated with sulfur diozide, and basket-centrifuged to recover the juice. The centrifuge cake was then washed with water using one-third as much as the weight of potatoes ground, and the washings added to the straight juice.

The diluted juice was treated with acid or heat to coagulate .the protein. After the protein was filtered off, the liquor was diluted to 1 per cent total solids and run through Dowex 50[1,2] (H+ form) ion-exchange resin. The effluent was collected and set aside. The column was washed with water and the washings added to the effluent. The column was next eluted with ammoniacal solution.

All solutions on which data are reported here were analyzed by the following methods:

Total nitrogen was determined by the microkjeldahl method (5). The free ammonia content of the eluate was estimated, after appropriate dilution, by direct titration using the microkjeldahl titration technique. Total solids content was determined by heating to dryness in a forced-draft oven- at 80° C. followed by one hour in a vacuum oven at 80°. Ashing was carried out at 650° for five hours. The content of total polyphenols was determined using the method of Rosenblatt and Peluso (8). The latter method was modified, however, to the extent that chlorogenic acid was used instead of tannic acid as the standard for determining the amount of polyphenols present. This was done since chlorogenic acid is the major polyphenol in potatoes. Total sugars content was determined by the Java Sugar Experiment Station method (2).

The free amino compounds were studied qualitatively by the small scale, two dimensional paper chromatographic technique of Rockland and Underwood (7). Paper sheets ("S and S 589 Blue Ribbon") 5½ inches square were suspended in number 4 American Medical Museum jars by means of wooden dowels passed through two holes punched in the top of the paper. The solvents used were: (I)—tertiary butyl alcohol-water-formic acid (69.5-29.5-1) and (II)—phenol-water-ammonium hydroxide (73.5-20.5-1). After irrigation in the above solvents and drying the papers were dipped in 0.75 per cent ninhydrin solution in water-saturated N-butanol to detect the amino compounds.

[1]Mention of trade names in this paper does not imply that these products are recommended or endorsed by the U. S. Department of Agriculture over others not mentioned.

[2]The Dowex 50 resin used in all experiments was labeled, "Absorbent for Chromatography," and obtained from the Will Corp., Rochester 3, N. Y.

The above qualitative paper chromatographic technique was extended to yield a quantitative estimation of the amounts of aspartic acid, gultamic acid, asparagine, glutamine, and γ-aminobutyric acid plus valine (determined as valine.) Gamma-aminobutyric acid and valine were also determined separately by a one-dimensional technique using n-butanol-water-acetic acid (8-10-1). There are many references in the literature to quantitative paper chromatography of amino compounds. After some experimentation, we found the following procedure to give satisfactory results. The solution was applied to the paper with micropipettes. After irrigation in the appropriate solvents and drying, the color was developed by dipping the papers in 0.75 per cent ninhydrin in water-saturated n-butanol and drying for 2 hours at room temperature. The spots were cut out and put into test tubes, and the color extracted from the paper with 50 per cent aqueous n-propanol at room temperature. The intensity of the colored solution was read in a Beckman B Spectrophotometer at 570 mu for all the amino compounds except asparagine, which was read at 365 mu. Standard solutions were run daily to correct for any small variation that might occur in the standardized conditions.

Development of Optimum Conditions and Discussion of Results

Extraction.

In the preparation of simulated processing water, juice can be separated from the ground potato slurry by basket-centrifuging directly without any dilution. Using one lot of Maine Russet potatoes, an undiluted extract containing 4.6 per cent solids was obtained. This amounted to a yield of 2.8 per cent of soluble solids based on weight of fresh potato. On washing the centrifuge cake (without resuspension) the yield was increased as shown in table 1.

It is seen that 76 per cent of the soluble solids was obtained without any washing. The yield was increased to 89 per cent by washing with water equivalent to one-third the weight of potatoes ground and to 93 per cent by washing with twice this much water. Further washing increased the yield only slightly.

Table 1.—*Yields of potato solubles using various amounts of water to wash centrifuge cake.*

Ratio Wt. Wash Water *vs.* Wt. Potato Slurry	Yield Based on Wet Potato	Theoretical Yield
	Per cent	Per cent
0 : 3	2.80	76.0
1 : 3	3.28	88.9
2 : 3	3.44	93.2
3 : 3	3.49	94.5

*The theoretical yield is calculated by assuming that the amount of juice potentially available is equal to the weight of potato multiplied by its moisture content divided by 100. This volume of juice multiplied by the solids content (divided by 100) of the juice obtained by basket-centrifuging the straight slurry equals the theoretical yield of soluble solids.

Using the paper chromatographic procedure outlined above, a study was made of the amino compounds in protein water samples prepared from several different varieties of potatoes. The general pattern was the same for all the varieties. In all, about 20 spots were obtained on treating the paper with ninhydrin; however, all but three of the spots were very faint. Principally from data obtained with known compounds, it was ascertained that the three intense spots represent asparagine, glutamine, and a combination of γ-aminobutyric acid, valine, and a small amount of methionine. Using the solvent system n-butanol-water-acetic acid (8-10-1) γ-aminobutyric acid and valine were separated. Typically there is more γ-aminobutyric acid present than valine; however, both are present in considerable quantity. The methionine remained with the valine. Its presence was detected by treatment with platinic iodine reagent (1). The quantity of methionine is very small compared to γ-aminobutyric acid and to valine. It was found that the six amino compounds aspartic acid, glutamic acid, asparagine, glutamine, γ-amino-butyric acid and valine, constitute approximately 20 per cent of the total solids and 50 per cent of the total nitrogen of the protein water.

Protein Removal

Acid vs. Heat

Protein must be removed prior to ion-exchange treatment of potato juice to prevent its precipitation on the column and resultant clogging. The simplest methods of carrying out the precipitation are by heating to about 80° or acidification to pH of 3.0 or slightly below. The acid required to lower the pH of 1 per cent solids processing water to 3.0 is about 3 milliliters of concentrated HCl/1000 mls. liquor. Investigation of both the acid and heat treatment led to the conclusion that neither method resulted in any appreciable hydrolysis of the protein or conversion of the free amides (asparagine and glutamine) to the acids or the ring compounds. Using an extract of Pennsylvania Katahdin potatoes, believed to be typical of eastern potatoes, the acid treatment removed 11.3 per cent of the solids and the heat treatment, 24.2 per cent. The acid treatment removed 23.3 per cent of the total nitrogen and the heat treatment, 32.3 per cent. All data indicated that heat is the more complete precipitant. This is in agreement with the results of others (4, 9). Acidification at ordinary temperature precipitates only the difficultly soluble protein, leaving much of the globulin and albumin in solution whereas the heat treatment precipitates practically all the protein. However, although acid precipitation is not complete, it is sufficiently complete for preventing clogging of the ion-exchange column.

When the potatoes are treated with sulfur dioxide immediately on grinding, the deproteinized liquor subsequently obtained, either by acid or heat treatment, is light yellow and needs no decolorization. A disadvantage of the acid treatment is that a filter aid must be used to filter the precipitated material. As for subsequent behavior of the liquor on the ion-exchange column, there is no appreciable difference between the two methods of removing protein.

Amino Compounds in Non-protein Fraction.

The principal amino compounds of heat-deproteinized liquor were determined quantitatively by the small scale, two-dimensional paper chromatographic technique. Table 2 gives the data obtained with five varieties of potatoes from different growing areas.

It should be noted that with the five eastern-grown lots of potatoes, the relationships of the amounts of the amino compounds to one another within a lot were similar. The asparagine content was greater than the glutamine in all five cases. However, the California White Rose potatoes had more glutamine than asparagine and were much higher than the eastern potatoes in total amino compounds content. With the thought that perhaps some glutamine had been converted to the ring compound pyroglutamic acid (non-reactive with ninhydrin), the procedure of Rydon and Smith (9) was used in the attempt to detect the latter substance. No pyroglutamic acid was detected, though, in any of the samples.

TABLE 2.—*Amino compounds in deproteinized potato processing water (mg/100 mls. of 1 per cent solids liquor)*

	Maine Russet		Maine Kennebe	Pa. Katahdi	Del. Katahdin	Calif. White Rose
	(a)	(b)				
Aspartic acid	14	13	17	10	8	13
Glutamic acid	6	12	15	17	15	3
Asparagine	89	94	131	162	85	140
Glutamine	63	55	92	46	45	195
γ-Aminobutyric acid + Valine (as valine)	36	20	36	46	22	76
Total	208	194	291	281	175	427

*Separate lots.

ION EXCHANGE TREATMENTS

Charging the Column.

By passing deproteinized potato liquor through Dowex 50 (H^+ form) resin and eluting under proper conditions, it can be purified and concentrated with respect to the free amino compounds. A column 1-inch inner diameter containing 300 ml. of the wet commercial grade resin (123 g. dry weight) was used. This quantity of wet resin filled the column to a height of 23 inches.

All the amino compounds and inorganic cations were retained on the resin; the effluent containing the sugars, organic acids, and minor constituents including polyphenols was discarded. The resin was eluted with ammoniacal solution. This removed the amino compounds but left the inorganic cations on the column. The column could then be regenerated with dilute hydrochloric acid and the cycle repeated.

Our laboratory-simulated processing water, prepared by combining undiluted juice centrifuged from ground potatoes with washings from the centrifuge cake typically contained 3.7 per cent solids. To standardize conditions and enable the collection of data at the same concentration as

found in commercial practice, the laboratory-prepared deproteinized liquor was diluted to 1 per cent solids for use in all experiments reported. A few experiments conducted with potato liquor at 3-4 per cent solids concentration indicated that the results in absorption and elution were esentially the same as with 1 per cent solids. It would be much more economical, of course, to base a product recovery process on the more concentrated liquor if the potato starch industry can provide such an effluent by adjusting its operations.

The relationship between the flow rate of the liquor and the capacity of the column to absorb nitrogenous compounds was studied. A concentration of 15 mg. N/100 ml. effluent was taken as the threshold of nitrogen outgo, beyond which the capacity of the column would be considered exceeded. As would be expected, the capacity of the column was reduced as the flow rate was increased. Nitrogen-concentration values of the effluents are plotted against effluent volume data in figure 1. Taking 15 milligrams N/100 milligrams as the maximum allowable nitrogen in the effluent, the capacities of the column using flow rates of 2, 4, 5 and 6 liters/hour were respectively 6.5, 6.2, 5.9 and 4.2 liters of 1 per cent juice. These results were obtained with juice that had been deproteinized with acid. Heat-deproteinized juice gave essentially the same values.

The flow rate can be expressed in terms of contact time, *i.e.,* time in minutes that the liquor is in contact with the resin. Thus, the contact time is equal to the volume of liquor (in liters) required to wet the column of resin multiplied by 60 (minutes), divided by the flow rate in liters per hour. The contact times at flow rates of 2, 4, 5 and 6 liters/hour were 2.7, 1.35, 1.08 and 0.9 minutes respectively.

Eluting the Column

Here, two methods of approach presented themselves: 1) fractionation of the non-protein nitrogen group into the individual amino compounds and 2) stripping the amino compounds collectively. The first technique is necessarily complicated and would involve very dilute eluting reagents and a very slow flow rate. The amino compounds would thus be recovered in dilute solution, probably much more dilute than the original processing water. The second method, *i.e.,* stripping the amino compounds collectively from the column, enables the use of a minimum volume of the ammoniacal eluting agent. A concentrate solution of a mixture of amino compounds is thus obtained.

The alternative of fractionating the amino compounds was investigated by use of the procedure of Partridge (6), essentially without modification. The column of Dowex 50 resin (300 ml.) was loaded to one-third its capacity, that is, by passing through 2 liters of 1 per cent deproteinized liquor. A relatively slow flow rate of 1 liter/hour was used. The column was washed with 1 liter of water, then eluted with 0.15 normal ammonium hydroxide, NH_4OH). Sixty-nine fractions of 100 ml. each were collected. Fractions 1 to 21 contained no amino compounds. Fractions 22 to 61 gave a positive ninhydrin test. Small-scale, two dimensional chromatograms were run on selected fractions and from the information gained regarding the amino compounds content it was apparent that very little fractionation had been obtained. Aspartic acid came off alone in the early ninhydrin-active fractions; however, the middle fractions contained a mixture of

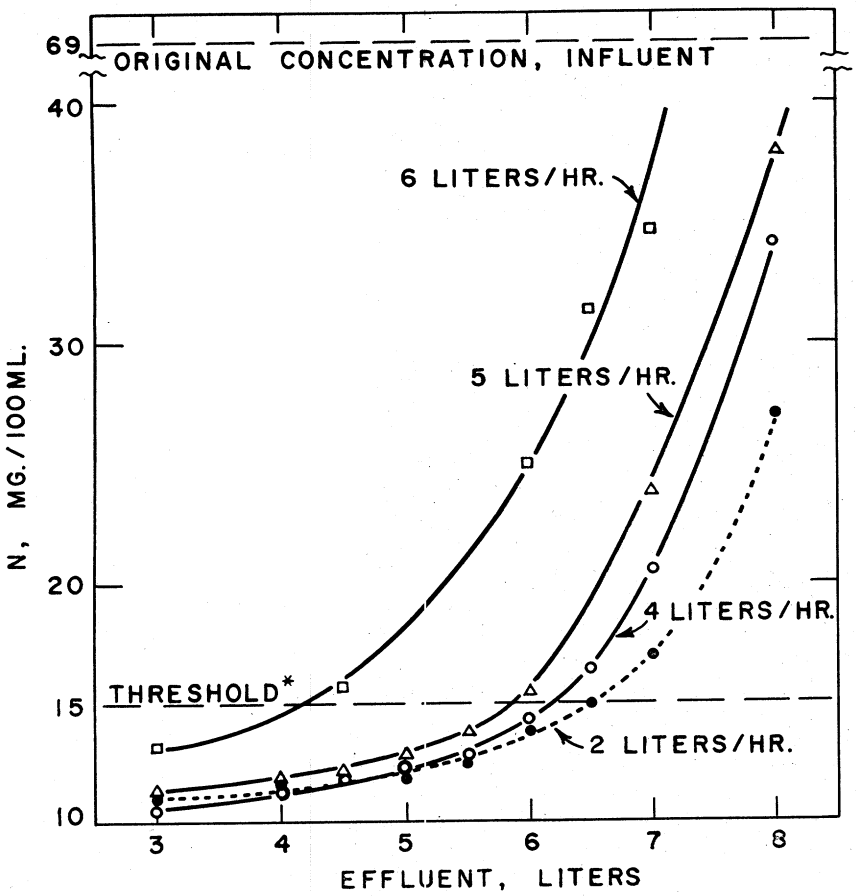

FIGURE 1.—Concentration of nitrogen in effluent from column at different rates of flow.

*This value taken as maximum allowable nitrogen concentration in effluent without considering the capacity of the column exceeded.

glutamic acid, asparagine and glutamine. The middle fractions were also rich in γ-aminobutyric acid and valine.

The basic amino compounds came off last, in fractions 43 to 61. The highest concentration of solids obtained in the eluate was 0.33 per cent, found in one of the middle fractions.

From the results of this preliminary fractionation work, it was decided to concentrate on the other alternative, that of eluting the amino compounds as quickly as possible. A series of experiments was carried out of which the concentration of NH^4OH (ammonium hydroxide) was varied from 0.6 to 2.5 normality (Table 3). The column was loaded to capacity, washed with water, and then eluted with the aqueous ammonia. A flow rate of 2 liters/hour was used in this set of experiments. Fractions

TABLE 3.—*Elution of amino compounds from resin columns, using various concentrations of NH_4OH.*

NH_4OH normality	0.6	1.0	1.5	2.0	2.5
Max. concen. in any fraction, per cent solids	4.8	8.5	10.7	14.3	14.6
Total vol. fractions containing min. of 1 per cent solids, ml.	700	500	450	350	350

of eluate containing 50 milliliters each were collected and the solids content of each fraction determined. All the fractions containing at least 1 per cent solids were combined and the others discarded.

The amounts of total solids and of amino compounds eluted from the column were substantially the same for all the concentrations of ammonia, and furthermore, the amounts of the individual amino compounds eluted remained essentially constant throughout the range of NH_4OH used. The elution data shown in table 3 indicated that 2 normal NH_4OH is the optimum concentration for eluting the column in that it elutes the amino compounds in minimum volume.

The rate at which the amino compounds are eluted from the column does not depend on the concentration of ammonia alone. The immediately preceding data on the effect of the normality of the ammonium solution on the concentration of the eluate were taken with the elution flow rate held constant at 2 liters/hour. Elution of loaded columns by use of 2 normal ammonium hydroxide was then carried out employing various flow rates. The volume of eluate required to remove substantially all of the amino compounds varied from 500 milliliters at 5 liters/hour to 350 milliliters at 2 liters/hour. No reduction in eluate volume was attained by eluting at a rate slower than 2 liters/hour. Hence, the 2 liters/hour flow rate is the optimum for obtaining the amino compounds in minimum volume of eluate.

CONCENTRATING AMINO COMPOUNDS BY METHODS DEVELOPED

Table 4 presents data on a typical experiment in concentrating amino compounds of potato liquor using optimum methods of charging and eluting the column. Six liters of acid-deproteinized liquor from Maine Russet potatoes was passed through the resin column at the rate of 5 liters/hour. The resin was washed with 1 liter of water and then eluted with ammonia.

Eluate fractions 6-12 inclusive (Table 4) were retained and the others containing less than 1 per cent solids each were discarded. The 350 milliliters composite of the seven more concentrated fractions contained 16.3 grams solids. This represented a recovery of 28 per cent of the solids passed through the column. It is seen that the original liquor contained 3.8 grams nitrogen and the eluate 2.9 grams providing a recovery of 76 per cent. The weight of nitrogen present in the effluent plus the washings, 0.77 grams, represented a loss of 20 per cent of that originally present. This loss of nitrogen through failure to be adsorbed on the column ap-

TABLE 4.—*Concentrating amino compounds by ion exchange in resin*-packed column.*

		Volume		Total solids			Nitrogen	
		Mls.		Per cent	Gms.		Per cent	Gms.
Deproteinized liquor passed		6000		0.97	58.4		0.064	3.84
Effluent plus wash water		7000		0.35	24.5		0.011	0.77
Retained on column					33.9			3.07
Eluate**—fractions	1	50		..				
	2	50		..				
	3	50		0.01				
	4	50		0.16				
	5	50		0.36				
	6	50		1.83	0.92			
	7	50		4.43	2.22			
	8	50		6.54	3.27			
	9	50	350	8.26	4.13	16.3	0.818	2.86
	10	50		7.69	3.84			
	11	50		2.61	1.30			
	12	50		1.28	0.64			
	13	50		0.49				
	14	50		0.40				
	15	50		0.21				

*Dowex-50, H+ form.
**Eluted with 2 normal NH_4OH at 21./hr.

parently is not connected with any conversion of glutamine to pyroglutamic acid, as indicated by the Smith-Rydon test (9). Only 4 per cent of the nitrogen introduced to the column was lost in discarding the dilute fractions. The basic amino acids are rather tightly held by the resin and were thus largely eluted in the last fractions in dilute form. These compounds include arginine, lysine, and histidine.

The factor 6.25 is believed to hold fairly well for converting nitrogen weight to amino compounds weight in the eluate. If the weight of nitrogen in the composite of fractions 6-12 (Table 4) is multiplied by 6.25, a somewhat larger figure but still a fair approximation to the weight of the total solids is obtained. This indicates that the solids content of these middle fractions is high in amino compounds.

Table 5 compares the composition of the deproteinized liquor with the eluate. The total solids content was increased 4.65-fold and the amino compounds content, 12.7-fold by the ion-exchange treatment. The eluate contained predominately amino compounds with very small amounts of sugars, inorganic salts, and minor constituents including polyphenols. The six amino compounds listed comprised 20 per cent of the total solids of the deproteinized liquor and 58 per cent of the total solids of the eluate.

RECOVERY OF INDIVIDUAL AMINO COMPOUNDS FROM THE ELUATE

Ion exchange methods described above can thus be made to yield the amino compounds of potatoes in relatively concentrated solution, essentially free of contamination by other types of substances. Besides the six amino

TABLE 5.—*Composition of deproteinized potato liquor and corresponding eluate.*

	Deproteinized liquor		Eluate*	
Volume ml.	6000		350	
Total solids, Per cent	0.97		465	
	Per cent		Per cent	
	W.B.	M.F.B.	W.B.	M.F.B.
Amino compounds (N x 6.25)	0.40	40.8	5.1*	
Ash	0.22	22.6	0.02	0.43
Sugars	0.31	32.0	0.10	2.15
Polyphenols	0.03	2.8	0.23	4.58
Individual amino compounds	0.01	1.3	0.31	6.6
Aspartic acid	0.01	1.2	0.15	3.1
Glutamic acid	0.09	9.7	1.34	28.8
Asparagine	0.06	5.7	0.59	12.8
Glutamine				
γ-aminobutyric acid plus valine	0.02	2.1		
		20.0		57.8

*Composite of fractions 6-12 of table 4.
**6.25 conversion factor results here in somewhat higher value for amino compounds than sotal soilds.

compounds listed in table 5, the eluate also contains other amino compounds including alanine, theonine, leucine, isoleucine, tryosine, serine, methionine, glycine, arginine, histidine and lysine.

Asparagine can be recovered in nearly 100 per cent yield as crystals containing 90 per cent asparagine by concentrating the eluate to 45 per cent solids and chilling to approximately 35°. (Asparagine can also be separated from a crude concentrate of potato solubles by similar treatment, but the yield is lower.) The main contaminant of these crystals is glutamine. Pure asparagine can be obtained by taking up the impure crystals in a minimum amount of hot water and allowing the solution to cool slightly, upon which asparagine crystallizes out and glutamine remains in solution.

After removal of the asparagine by chilling the concentrate eluate to 35° and filtering off the crystals, the remaining liquor is again cooled upon which impure glutamine is obtained. This second crop of crystals is about 90 per cent pure with respect to glutamine, but represents only a minor part of the total glutamine present,

After removing the asparagine and crystallizing out part of the glutamine, crude aspartic acid can be obtained. The concentrated eluate (40 to 50 per cent solids) is acidified to pH 3 and enough ethanol added to yield a solution containing 60 per cent ethanol. A crystalline mass then separates, containing about 80 per cent aspartic acid. The main contaminants are glutamic acid and glutamine. Aspartic acid can be recovered from the eluate in 60 per cent yield.

SUMMARY AND CONCLUSIONS

Ion exchange methods have been developed for concentrating the free amino compounds in dilute potato extract simulating starch factory

processing water. The solubles are extracted from ground, sulfited potatoes by centrifuging and washing the centrifuge cake with water equal to one-third the weight of potatoes ground. The combined juice and washings typically contain about 3.7 per cent total soilds. This extract was diluted to 1 per cent to conform to the concentration of commercial starch processing water. The protein was removed by heat coagulation to prevent clogging during subsequent passage of the liquor through an ion-exchange column.

The amino compounds were concentrated and separated from most of the other potato solubles by absorption on Dowex 50 resin (H+ form) followed by elution with 2 normal NH_4OH. The middle fractions of the eleuate were rich in asparagine, glutamine, aspartic acid, glutamic acid, γ-amino-butyric acid, and valine. Thus, in a typical experiment, 28 per cent of the total solids and 75 per cent of the amino compounds originally present in 6000 ml. of dilute liquor were recoverable in 350 milliliters of eluate combined from the middle fractions. The total solids content of this eluate was nearly five times and the amino compounds content, thirteen times the corresponding values of the original dilute liquor.

Amino compounds comprised most of the solids in the composite of middle fractions of the eluate. Half of the weight of amino compounds was present as the six previously-named compounds.

Nearly all of the asparagine, the most abundant amino compound present, can be easily recovered in 90 per cent-pure form. The composite of the middle fractions of the eluate is concentrated to 45 per cent solids or higher and chilled to about 35° C. to induce crystallization. Recrystallization results in nearly 100 per cent pure asparagine. Glutamine and aspartic acid were also recoverable by simple crystallization procedures

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