determinations or tests at some intermediate vapor velocity. All variations in operating conditions are listed.

An asterisk (*) in the column giving the number of theoretical plates means that at this point flooding conditions in the column were beginning.

The wire used in making the aluminum helices was No. 24 B. and S. gage; that used in the 4-mm. and 3.2-mm. stainless steel or nickel helices was No. 26 B. and S. gage. The wire used in the 2.4-mm. helices was No. 30 B. and S. gage.

In the tests on the open tube given in section 1A of the table, provision was made for the reflux liquid to descend along the wall of the column.

Conclusions

1. The helix-type packing material (*2*) is again found to be more suitable for fractional distillation columns than other types of packing material, both as regards efficiency and throughput.
2. For certain packing materials, particularly the wire helix type, the decrease in efficiency between columns about 2–3 cm. and 5 cm. in diameter is not as marked as might be expected. Good efficiencies are possible in 5-cm. diameter columns.
3. With fine wire helix type packing in 5-cm. diameter columns it is possible to obtain good throughputs and from forty to fifty theoretical plates in a 2.5-meter (100-inch) length of packing.
4. With certain packing materials it is possible to have a pressure drop per theoretical plate of from 0.2 to 1.0 mm. of mercury while still obtaining reasonable throughputs and efficiencies.
5. Certain packing materials may possess relatively high efficiencies but will permit only very low throughputs, and vice versa. In the wire helix type packing it is possible to obtain the combination of high efficiency and good throughput, with low pressure drop per theoretical plate.

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Inactive Inositol and Other Products from Starch Factory Steep Water

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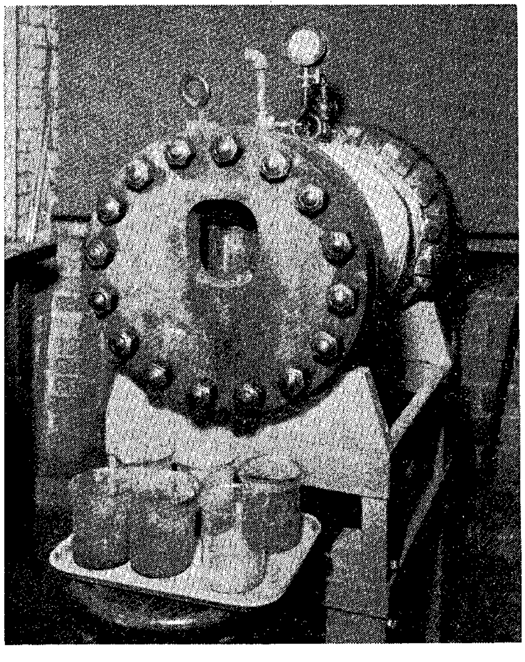


Small Autoclave, Open

STEEP water is obtained in the manufacture of cornstarch when the grain is soaked (steeped) in a warm dilute solution of sulfurous acid. Steep water contains the acid-soluble portion of the corn, and when it is heated some protein is precipitated. The addition of alkaline reagents, usually lime, precipitates phytin, a (calcium magnesium) salt of the phosphoric acid ester of inositol. Sugars, gums, other natural organic compounds, and inorganic substances remain in solution.

Originally the steep water, a highly putrescible liquid, was discharged into the nearest body of water. To prevent a nuisance, starch manufacturers concentrated the steep water and added it to stock feed. Later when wash water which contains a considerable quantity of organic matter was used as a source of steep water, the so-called bottling-up process resulted. This addition of steep water increases the protein and carbohydrate content of the feed so that these former wastes are valuable products.

Attempts have been made to find more profitable uses for steep water. Complex organic phosphoric acid compounds have been prepared by alkaline precipitation, and a number of patents have been granted. Recently some steep water has been used in the manufacture of yeast. The bulk of starch-factory steep water, however, continues to be used in stock feeds. An attempt has been made here to find other uses, and inositol and other substances have been prepared from the alkaline (calcium) precipitate.

Production of compounds from starch factory steep water by fractional precipitation, distillation, or use of common organic solvents was unsuccessful. Hydrolysis yielded inositol, formic acid, a mixture of amines, inorganic salts, and unidentified products. Glutamic acid was not identified in the mixture. Oxidation gave oxalic acid, unidentified crystals, and gums. Addition of lime gave a precipitate of phytin.

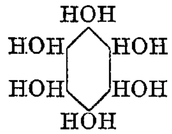
Hydrolysis of phytin with varying time, pressure, and concentration of acid was investigated. Higher concentration of acid did not increase the yield of inositol. Apparent adsorption of inositol on the insoluble sludges formed in its manufacture was overcome by boiling and agitating the hydrolyzed mixtures with steam.

Vigorous oxidation of inositol with concentrated nitric acid, bromine, and a trace of iodine gave oxalic acid as the only product. Mild oxidation with the same reagents gave a mixture of products, the most important being tetrahydroxyquinone and rhodizonic acid, which were removed as the sodium, potassium, or ammonium salts.

Historical

Scherer (*28*) in 1850 isolated inositol from Liebig's extract of meat and determined its empirical formula. It was first classed as a sugar because of the empirical formula, $C_6H_{12}O_6$, and the pronounced sweet taste, and was called "inosite" or "muscle sugar" because it was found in animal tissue.

Maquenne (*15*) showed in 1887 that it was a cyclic hexamethylene compound or cyclohexanehexol:



Inositol, free or as the phosphoric acid ester, is widely distributed in the plant and animal kingdoms, particularly during the growing stage (*18*, *27*). The natural compound is usually inactive. It crystallizes in the anhydrous form or with two molecules of water.

Wieland and Wishart (*32*) used purest hexahydroxybenzene and synthesized a product identical with natural inositol.

Inositol has been made from calcium phytate or phytin by hydrolysis with 10 to 60 per cent sulfuric acid. Prior to 1934 (*23*) the lowest acid concentration was reported by Moldavskii (*19*) who heated phytin in an autoclave at 150° to 160° C. for 5 or 6 hours with four parts of 10 per cent sulfuric acid. The highest acid concentration was reported by Contardi (*9*) who prepared inositol by mixing powdered phytin with 60 per cent sulfuric acid and heating in a porcelain beaker in an oil bath at 160° C. for 15 hours. Others (*4*, *5*, *22*, *28*) report the use of 30 to 40 per cent acid and temperatures of 150° to 170° C. under pressure. Recently hydrolysis without acid was reported by Otolski (*23*).

The decomposition of phytin with the formation of inositol was investigated by Palladin in 1895 (*24*), Winterstein in 1897 (*33*), Posternak in 1903 (*26*), and others. There is a difference of opinion concerning the reaction. Starkenstein (*30*) claimed that commercial phytin always contains free inositol and inorganic phosphates, and that merely drying at 100° C. causes nearly complete decomposition. Contardi (*8*), on the other hand, reported that he obtained only small quantities of inositol when he heated phytin in an autoclave with pure water for several hours at 200° C. Anderson (*4*, *5*) doubted whether phytin is easily decomposed. Several months of work had shown that it was relatively stable when pure and when no mineral acids were present, and that phytin could be boiled for hours in dilute sulfuric acid without suffering marked decomposition. In fact, it could be boiled for days with 30 per cent sulfuric acid without forming a determinable quantity of inositol.

Contrary to the opinions of Starkenstein and Anderson, this paper will show that inositol is not obtained by heating dry phytin at 140° C. Contrary to Contardi's findings, it is shown to be obtained by heating under pressure with water or with acid.

The writers hoped to make inositol cheaply and thus to stimulate research and to find commercial uses. The price of inositol has been $0.80 to $1.20 per gram, and research has been limited.

Possible Uses

The widespread distribution of inositol in nature indicates possible physiological importance (*6*) which further investigation, with larger quantities available, may explain.

Inositol, if available at a cheaper price, may be used as bacteriological media. In 1917 Weiss and Rice (*31*) investigated inositol fermentation, using 144 strains representing the paratyphoid enteriditis, typhoid, dysentery, and colon groups, and suggested its use for differentiating various strains of bacteria. Meillère (*17*) and Poe and Field (*25*) report favorably on inositol for bacteriological use.

Inositol can be oxidized with nitric acid, potassium permanganate or other oxidizing agents yielding a variety of products. Maquenne (*16*), Müller (*21*), and others have investigated these reactions. In 1930 Gelormini and Artz (*13*) gave a summary of previous work on the oxidation of inositol with nitric acid. They proved that hexahydroxybenzene is formed and obtained good yields of the salts of tetrahydroxyquinone, rhodizonic acid, and croconic acid.

A recent British patent (*7*) described a synthetic resin using inositol as a base. Inositol hexanitrate has been patented for use as a detonating agent (*10*).

Experimental Procedure

Apparatus. Two autoclaves were available for hydrolysis of the phytin. One was a horizontal steel cylinder (*12*), 30 cm. in diameter and 60 cm. long, in which sixteen 600-ml. beakers could be placed on a wire grating located below the center. Steam pressure to 14 kg. per sq. cm. was obtained by heating water below the frame with electric elements. The other autoclave was a vertical boiler 76 cm. in diameter and 94 cm. deep, in which twenty-one 7.5-liter (2-gallon) stone jars could be placed on three wire frames. A steam pressure of 6.3 kg. per sq. cm. (90 pounds per square inch) was obtained from the university steam line. The condensate was removed automatically through a trap.

Procedure. Both steep water and phytin were obtained from the A. E. Staley Manufacturing Company, of Penick and Ford, Ltd., and the Clinton Company. Pure compounds were not obtained from the steep water by the addition of any precipitating reagents. Fractional distillation, concentration in vacuum to varying densities, or extraction with common organic solvents did not yield pure compounds. Considerable inorganic material was present because the solid residue on evaporation had an ash content of 31.35 per cent. A study made at the University of Illinois (*1*) had given similar results.

HYDROLYSIS OF STEEP WATER. An unsuccessful attempt was made to obtain glutamic acid by hydrolyzing concentrated steep water, using the method developed by Bartow and Albrook (*2*, *3*) for obtaining glutamic acid from corn gluten. After removal of insoluble decomposition products by filtration and concentration of the filtrate, an excess of a concentrated alkaline reagent precipitated almost pure secondary phosphate salts. Impure formic acid was obtained from the hydrolyzate by distillation with sulfuric acid. A mixture of amines was obtained when the concentrated filtrates were made alkaline and distilled.

INOSITOL FROM STEEP WATER. Inositol was obtained by hydrolyzing steep water with varying concentrations of sulfuric acid at pressures from 10.5 to 12.7 kg. per sq. cm. It was identified as inositol by the reaction of Scherer (*29*) and by the formation of the hexacetate. The yields were very poor, usually a fraction of one per cent.

PHYTIN. After many attempts to obtain better yields of inositol directly from concentrated steep water and since phytin was the constituent of steep water from which inositol had been obtained, it was decided to precipitate phytin for use as starting material.

To determine whether heating dry phytin would yield inositol, as claimed by Starkenstein (*30*), 100-gram samples were heated in an electric oven for 96 hours at 140° C. The samples were treated with water, boiled, filtered, and washed. No inositol was found after the filtrates were concentrated to small volumes.

INOSITOL FROM PHYTIN. Inositol was prepared from phytin by hydrolysis, using different concentrations of acid, different pressures, and different times of heating. Most of the phytin was prepared from steep water at the laboratory. Some commercial phytin of much better quality was obtained from the Staley Manufacturing Company (marked SC in the tables).

TABLE I. YIELD OF INOSITOL WITH VARYING ACID CONCENTRATION, PRESSURE, AND TIME

Test No.	Wt. CaP[a]	H_2SO_4	H_2O	Pressure	Time	Sludge	Inositol	
	Grams	*Ml.*	*Ml.*	*Kg./sq. cm.*	*Hr.*	*Grams*	*Grams*	*%*
70	50	10	50	7.0	5	26	3.552	7.10
71	50	15	50	7.0	5	28	3.387	6.77
78	100	20	100	7.0	4	55	7.980	7.96
79	100(SC)	20	100	7.0	4	60	9.068	9.07
86	100(SC)	20	100	8.4	4	59	9.065	9.07
88	100(SC)	10	100	10.5	4	64	9.893	9.89
99	100(SC)	20	200	8.4	8	55.5	7.593	7.59
102	100(SC)	30	200	8.4	8	63.0	7.479	7.48
103	100(SC)	40	200	8.4	8	64.0	7.488	7.49

[a] CaP = phytin (calcium phytate).

In four tests of Table I (70, 71, 78, and 79) excess of acid was neutralized with barium hydroxide. In the other tests (86 and following) calcium hydroxide was used. Filtrates from the insoluble sludges were concentrated to the consistency of a thick sirup. Inorganic impurities which separated during the concentration were removed by filtration. Practically pure inositol was obtained from the concentrated filtrates by addition of glacial acetic acid and denatured alcohol. Recrystallization from water and then from 50 per cent alcohol gave very white, ash-free, feathery crystals, melting at 222° C. (uncorrected). Qualitative tests confirmed the absence of calcium, magnesium, phosphate, sulfate, and chloride.

The variable yields of inositol (Table I) did not appear to be caused by variation in acid concentration. They were not caused by incomplete hydrolysis since a second treatment of the sludge did not increase the yield. Variation in the moisture content of commercial phytin does not affect the yield, for the experiments show that the proportion of water to dry phytin in the mixture prepared for hydrolysis had no effect.

In another series of experiments with larger quantities of material (Table II), it was found that dilute acid could be used for the hydrolysis and that inositol could be made at lower steam pressures. Consequently in this series it was practical to use the larger autoclave which has an available steam pressure of not to exceed 6.3 kg. per sq. cm.

TABLE II. YIELD OF INOSITOL IN 5 HOURS WITH VARYING PRESSURE

Test No.	Wt. CaP	H_2SO_4	H_2O	Pressure	Dry Sludge	Inositol	
	Grams	*Ml.*	*Ml.*	*Kg./sq. cm.*	*Grams*	*Grams*	*%*
107	3250(SC)[a]	325[b]	3250	10.5	1970	255.5	7.69
117	200(SC)	25	300	5.5	..	16.4	8.20
118	200(SC)	48.1	300	5.5	..	18.5	9.25

[a] Sixteen 600-ml. beakers. [b] 15% solution.

TABLE III. YIELD OF INOSITOL WITH VARYING PRESSURE AND TIME

Test No.	Wt. CaP	Pressure	Time	Inositol	
	Kg.	*Kg./sq. cm.*	*Hr.*	*Weight*	*%*
119	16.521	8.8–10.5	6	1496 g.	9.06
120	2.027	5.3–5.6	11	198.5 g.	9.79
121	38.82	5.6–6.0	12	3.30 kg.	8.51
122	38.16	6.3	15	4.71 kg.	12.62

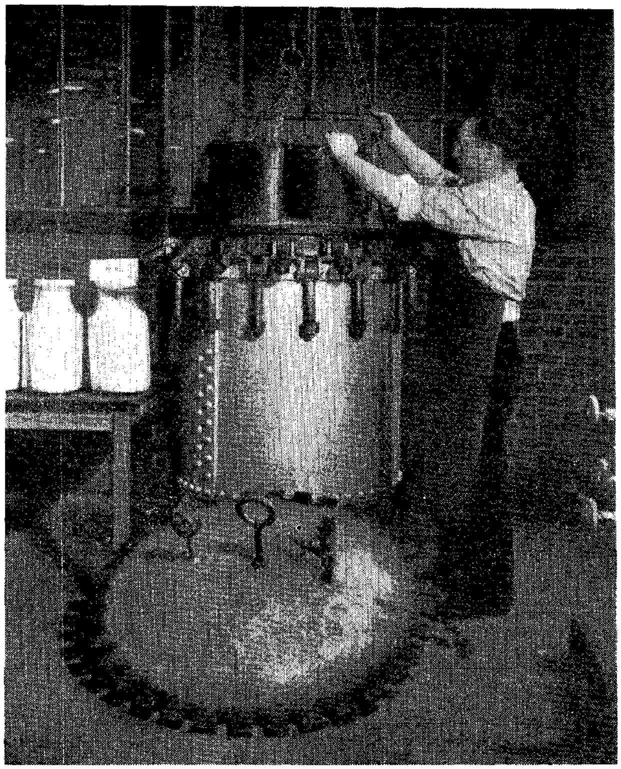
Without acid, phytin and water were mixed to the consistency of a thin paste and were heated under pressure in the autoclave; good yields were obtained (Table III). The best result gave 12.62 per cent inositol from the dry phytin.

LOSS BY ADSORPTION. Apparently adsorption of inositol on the insoluble sludges, formed in its manufacture, decreases the yield. Needham (*22*) confirmed this observation in his report that inositol tends to be held, perhaps in an adsorbed state, by a precipitate of lead sulfide formed in his method of purification. Two extractions with water at about 70° C. were required to remove it completely. He also noted that it is adsorbed by fuller's earth, though not by kieselguhr. The writers removed inositol from the lime sludge by extracting several times with hot water, heating, and agitating with steam.

Oxidation of Inositol

Inositol was oxidized with fuming nitric acid at a temperature below 60° C., as described by Gelormini and Artz (*13*). A 57.5-gram portion of oxidized product was obtained from 50 grams of inositol. When this product was treated with sodium bicarbonate, 38.4 grams of disodium tetrahydroxyquinone were obtained. The sodium and potassium salts of rhodizonic acid were also made from portions of the oxidized product; 50 grams of inositol yielded 13.4 grams of potassium rhodizonate.

Inositol was oxidized with concentrated nitric acid—one part of inositol to four parts of nitric acid (a saturated solution), bromine, and a few crystals of iodine. After warming to 45° C., the reaction was allowed to proceed without further heating; more bromine and iodine were added as they were consumed in the reaction. After 48 hours, crystals began to form in the liquid and continued to form as the reaction continued. They were identified as pure oxalic acid.



INTRODUCING A 65-LITER CONTAINER INTO THE LARGE AUTOCLAVE

Jars of inositol stand on the table at the left.

Complete reaction yielded 76.19 per cent of the theoretical amount of oxalic acid. No unoxidized inositol remained, and no trace of tetrahydroxyquinone or rhodizonic acid was found.

On the other hand, if the reaction was stopped before the oxalic acid stage was reached, by pouring the reaction mixture into a flat-bottomed dish and allowing it to dry, incomplete oxidation caused tetrahydroxyquinone and rhodizonic acid to be formed. The yield of disodium tetrahydroxyquinone was 41.9 per cent of the weight of inositol used.

At room temperature when no catalysts were used, lower yields of tetrahydroxyquinone and rhodizonic acid were obtained. Even on long standing no oxalic acid was formed.

Bromination of Inositol

Former workers (*14*, *20*) made brominated derivatives from the hexacetate and hydrobromic acid in sealed tubes. Attempts to brominate inositol directly were unsuccessful here. Inositol was refluxed with bromine, using various halogen carriers; although some reaction occurred, unchanged inositol remained principally. When red phosphorus was used, small amounts of the lower phosphate esters of inositol were formed.

The method of Datta and Chatterjee (*11*), where bromine and nitric acid are used, gave an oxidation reaction as mentioned previously.

Oxidation of Steep Water

When concentrated steep water (61 per cent dry solids) was treated with concentrated nitric acid, bromine, and a trace of iodine, a violent reaction occurred. The reaction was less violent with concentrated nitric acid alone but required cooling in a water bath to prevent excessive foaming. Crystals of oxalic acid separated from the mixture. The reaction was slow and after 12 days was not complete.

Conclusions

1. Production of compounds from starch factory steep water by fractional precipitation, distillation, or use of common organic solvents was unsuccessful.
2. The study and use of inositol has been limited by its previous high price and complicated method of preparation.
3. The method of preparation has been simplified by hydrolyzing a phytin and water mixture in an autoclave at 5.6 to 6.3 kg. per sq. cm. pressure for 5 or 6 hours. The best yield of inositol was between 12 and 13 per cent. The yield was not increased by the use of acid, higher pressure, or longer time.
4. Variations in inositol yields are caused by (*a*) purity of the phytin used and (*b*) adsorption on the insoluble sludge formed during the manufacture of inositol.
5. Oxidation of inositol, using concentrated nitric acid with catalysts, gave oxalic acid as a final product. Incomplete oxidation with the same reagents gave such intermediate products as tetrahydroxyquinone and rhodizonic acid.
6. Attempts to brominate inositol directly were unsuccessful.
7. Oxidation of steep water with concentrated nitric acid yielded a mixture of products among which oxalic acid was identified.

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