

[CONTRIBUTION FROM THE DIVISION OF HORTICULTURAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

## Isolation and Identification of the Organic Nitrogenous and Non-Nitrogenous Compounds Occurring in the Alaska Pea.

### I. The Presence of Citric Acid in the Alaska Pea

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From the work of Osborne and his associates<sup>1</sup> we know the proteins, and from that of Jodidi,<sup>2</sup> Boswell,<sup>3</sup> and Schulze,<sup>4</sup> we know the classes of compounds other than proteins which occur in the pea (*Pisum sativum*). The presence of citric acid in peas was reported by Ritthausen,<sup>5</sup> who has isolated the acid by a method *different* from the one used by the writer, namely, by extracting peas with acidified water, neutralizing the extract and precipitating the acid with lead acetate. He did not mention the *variety* of peas used by him. Yet different varieties differ in their chemical composition. Nor did he give the *quantity* of citric acid present in peas, all of which are given in this paper.

### Experimental Part

The peas employed were of the Alaska variety used chiefly by the canning industry. The seed, which was of size 2, was dried rapidly in a specially constructed forced-draft drying apparatus at the temperature of 60–65° to prevent enzymic action and ground to pass a 40-mesh sieve. The average percentage composition of the pea meal was as follows: dry matter, 92.90; ash, 3.88; fat (ether extract), 1.79; total nitrogen (Kjeldahl), 4.48; protein nitrogen (Stutzer method), 2.16; reducing sugars, 0.19; sucrose, 15.92; starch, 31.51—all calculated on the basis of the water-free meal.

One hundred gram portions of the meal were treated with 900 to 1000 cc. of distilled water at 55°, toluene being added to prevent bacterial action. The flasks with their contents were allowed to stand at that temperature for about four hours, being shaken occasionally. The mash was then filtered off with suction. The cake remaining on the filter was pressed out well and the juice thus obtained was added to the main extract. The slightly turbid filtrates were refiltered until clear, when they were evaporated to dryness *in vacuo*. Altogether 750 g. of the pea meal was worked up as described.

The dry residue obtained from the aqueous extract of the peas was now repeatedly boiled out with absolute alcohol under reflux, allowed to cool for some time and filtered. On standing the filtrates ordinarily became cloudy or gave precipitates which were filtered out and combined with the bulk of the substance found to be insoluble in absolute alcohol. The combined clear wine-red filtrates were then evaporated to dryness under reduced pressure, the dry residues again boiled out with absolute alcohol, filtered and the clear filtrates evaporated in a vacuum. The extraction with absolute alcohol and evaporation was repeated once more. The final residue obtained was easily soluble in water, fairly soluble in alcohol, and very difficultly soluble in ether. This residue was

(1) Osborne and co-workers, THIS JOURNAL, **13**, 583 (1896); **20**, 348, 410 (1898); *J. Biol. Chem.*, **3**, 213 (1907).

(2) Jodidi, *J. Agr. Research*, **43**, 811 (1931).

(3) Boswell, *Maryland Agr. Expt. Sta. Bull.*, 306 (1929).

(4) Schulze, *Landw. Vers.-Stat.*, **39**, 269 (1891); **46**, 23 (1895); *Z. physiol. Chem.*, **15**, 140 (1890–91).

(5) Ritthausen, *J. prakt. Chem.*, **29**, 357 (1884).

assumed to contain proline, which is the only amino acid that is soluble in absolute alcohol. With this in mind we prepared the copper salt by dissolving the bulk of the residue in water, boiling the solution with an excess of freshly prepared copper hydroxide for half an hour and evaporating the filtrate from the excess copper hydroxide to dryness in a vacuum. The copper salt was found to be readily soluble in water, fairly soluble in hot alcohol and apparently insoluble in ether. However, to our surprise the solution of the copper salt in water showed a pretty *green* color, while the copper salt of proline in aqueous solution is known to have a deep *blue* color. On further examination it was found that the aqueous solution of the residue showed a strongly acid reaction, while the solution of proline in water has a very slight acid reaction. It was now evident that the substance in hand is not proline. In order to establish the identity of the substance, the copper salt was dissolved in water, decomposed with hydrogen sulfide, and the filtrate from copper sulfide evaporated to dryness under diminished pressure. Twice recrystallized and dried for several hours in the oven the substance melted at 153°. Water-free citric acid melts at 153–154°. At this point it seemed of interest, from the standpoint of identification, to prepare the copper salt of citric acid. Several grams of Kahlbaum citric acid was dissolved in water and boiled with freshly precipitated copper hydroxide, the excess being filtered out. The filtrate showed a *green* color and the other properties observed on the copper salt of the isolated substance.

**The Calcium Salt.**—It was prepared by dissolving the isolated substance in water to which calcium chloride and ammonia was added and the whole boiled. The precipitate obtained was filtered off while boiling hot and washed with boiling hot water. The precipitate was then dried in the air for several days until its weight became constant. For the moisture estimation the air-dry substance was dried at 190°.

*Anal.* Calcd. for  $[\text{C}_6\text{H}_5\text{O}_7]_2\text{Ca}_3 + 4\text{H}_2\text{O}$ :  $\text{H}_2\text{O}$ , 12.63. Found:  $\text{H}_2\text{O}$ , 12.70 and 12.81.

For the calcium estimation the air-dry substance was first heated over the Bunsen burner and finally over the blast burner.

*Anal.* Calcd.:  $\text{CaO}$ , 29.45. Found: 29.39 and 29.56.

### Summary

By repeatedly extracting the evaporated aqueous extract of Alaska pea with absolute alcohol and by treating the alcoholic extracts in a definite way as outlined in this paper, citric acid was obtained. It was identified by its acid reaction, the melting point, the properties of the copper salt, and by the analysis of its calcium salt. The quantity of citric acid found was equal to 0.36% of the oven-dried peas.

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