[CONTRIBUTION FROM THE FOOD RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE ORGANIC ACIDS OF SPINACH, BROCCOLI AND LETTUCE[1]

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The value of acids in food metabolism depends upon whether the acid is easily metabolized, as are citric and malic, or difficultly metabolized, as oxalic or benzoic, since by the oxidation of the organic acid radical the alkaline mineral is left in the system and helps to maintain a proper alkaline reserve.

Much work has been done to determine the organic acids of fruits, but little seems to be on record as to their presence in the common vegetables, no doubt for the reason that the acids in fruits are free or partly free, whereas in vegetables they are, for the most part, present as salts.

The examination of certain green vegetables for oxalic acid prompted an extension of the work to include identification of other organic acids present.

Arbenz[2] and Esbach[3] have reported the presence of considerable amounts of oxalic acid in spinach. Arbenz found 0.29% and Esbach 0.32% of oxalic acid in the fresh material.

A more recent investigation by Alice E. Ryder[4] appeared while the present investigation was in progress. In three samples of spinach Ryder found 0.486, 0.652 and 0.692% of oxalic acid.

Examination of Spinach

The spinach used for the present investigation was purchased on the Washington market early in February, 1930.

Oxalic acid was determined by the method of Arbenz.[2] Twenty grams of dry spinach was extracted with 150 cc. of 15% boiling hydrochloric acid, the mixture was filtered, and the filtrate was evaporated to dryness, dissolved in 20 cc. of water and extracted with ether in a perforation outfit to complete extraction. The residue from the ether was dissolved in water, and ammonia was added until the solution was alkaline. It was then reacidified with acetic acid and precipitated hot with calcium chloride solution. The precipitate was redissolved in hydrochloric acid and reprecipitated as above until the crystals were pure calcium oxalate as determined by the microscope. The precipitate was ignited over the blast lamp and weighed as calcium oxide. By this method the dried spinach was found to contain 3.72% of anhydrous oxalic acid, equivalent to 0.31% on the basis of the fresh material (the spinach contained 8.3% dry matter).

For the separation of the other non-volatile acids of spinach, 500 g. of the dried spinach was extracted with boiling water, and the acids were precipitated from the extract with lead subacetate. The acids recovered from the lead precipitate were allowed to crystallize and filtered from the greater part of the oxalic acid. When esterified the sirupy filtrate afforded 6 g. of crude esters.

[1] Food Research Division Contribution No. 92.

[2] Arbenz, *Mitt. Lebensm. Hyg.*, **8**, 98 (1917).

[3] Esbach, *Bull. Gen. Ther. Med. Chir.*, **114**, 385 (1883).

[4] Ryder, *J. Home Econ.*, **22**, 309 (1930).

Two fractionations at 10 mm. yielded 0.7 g. boiling at 120°, 0.7 g. boiling at 120–140°, and 2.0 g. boiling at 140–160°. The lower fraction, on treatment with hydrazine hydrate, afforded an abundant precipitate of needles of oxalic acid hydrazide, melting at 235°. The intermediate fraction yielded *l*-malic hydrazide melting at 178–179°. The higher boiling fraction gave two hydrazides, one evidently *l*-malic hydrazide, crystallizing in nodules, and another crystallizing in clear rosets of prisms which melted at 107°, showing that it was citric hydrazide. It was further identified as citric hydrazide by optical crystallographic examination.[5]

Examination of Broccoli

The broccoli used, described as *Brassica Oleraceae*, var. Botrytis, or sprouting variety, was purchased on the Washington market. As no complete analysis of broccoli has been published, a thorough examination was made.

The buds, leaves and stems were examined separately. The methods of analysis used were those prescribed in "Methods of Analysis" of the A.O.A.C.

An investigation was also made of the organic acids of broccoli according to the ester distillation method.

The results of the analysis of broccoli are given in Table I.

TABLE I

ANALYSIS OF BROCCOLI, *Brassica Oleraceae*

	Buds		Leaves		Stems	
	Fresh, %	Dry, %	Fresh, %	Dry, %	Fresh, %	Dry %
Moisture	88.1		88.3		90.9	
Ether extract	0.74	6.19	0.84	7.17		
Crude fiber	1.42	11.91	1.11	9.45		
Protein	4.39	36.93	4.04	34.41		
Invert sugar		Trace				
Sucrose		None				
Starch	1.30	10.88				
Pentosans	0.91	7.61				
Ash	1.69	14.23	2.41	20.55		
Acid insol. ash	0.028	0.24	0.061	0.52		
P_2O_5	.17	1.39	.12	1.03		
CaO	.30	2.56	.38	3.27		
Ferric and aluminum oxides	.012	0.10	.026	0.22		
Mn_3O_4	.006	.051	.005	0.041		
MgO	.021	.18	.16	1.33		
K_2O	.30	2.51	.34	2.90		
Alkalinity of ash, cc. $N/10$ per 100 g.	93.4	785	144	1485		
Nitrates as KNO_3	0.09	0.76	0.12	1.05	0.25	2.74
Oxalic acid	0.02	0.16	0.02	0.15	0.01	0.11

Oxalic acid was determined by the method of Arbenz.[2]

For the investigation of the non-volatile acids twenty and one-half kilograms of broccoli was extracted with boiling water and the extract concentrated to two liters. After dilution with an equal volume of alcohol, the acids were precipitated with lead subacetate, and after being washed the lead salts were decomposed with sulfuric acid. After removal of the excess of sulfuric acid, the solution of organic acids was concentrated

[5] Optical examinations were made by G. L. Keenan of the Food, Drug and Insecticide Administration.

to small volume and thoroughly extracted with ether. The residue from the ether was partly crystalline and weighed slightly less than one gram. The crystalline acid, after recrystallization, did not have a sharp melting point, but succinic and oxalic acids were identified in it by optical crystallographic methods.

The acids unextracted by ether were esterified in the usual manner and afforded 26.5 g. of the ethyl esters of organic acids.

The mixed esters were fractionated three times at 10 mm. The following fractions were obtained

1	90–125°	0.9 g.	
2	125–135°	5.1 g.	α_D −10.5°
3	135–145°	0.4 g.	
4	145–155°	1.0 g.	
5	155–165°	1.6 g.	
6	165–170°	10.8 g.	

Residue and loss, 6.7 g.

The hydrazides of the acids were prepared from these fractions and purified. Fractions 1, 2, 3 and 4 yielded *l*-malic hydrazide, melting at 178–179°; fraction 5 was a mixture of ethyl malate and ethyl citrate; fraction 6 yielded citric hydrazide in its hydrated form melting at 104–106°.

The non-volatile acids of broccoli are therefore *l*-malic and citric acids with small amounts of oxalic and succinic acids.

Examination of Lettuce

Thirty-nine and seven-tenths kilos of lettuce, purchased on the Washington market, was dried, yielding 2630 g. of the dried vegetable.

Twenty grams examined by the Arbenz[2] method for oxalic acid yielded 0.164% of oxalic acid, equivalent to 0.01% on the basis of the undried lettuce.

Two and one-half kilos of the dried vegetable was extracted with boiling water and the extract was concentrated and mixed with an equal volume of alcohol. The lead salts were precipitated with lead subacetate, and the acids recovered from the lead precipitate were converted into the ethyl esters; 73.5 g. of crude ethyl esters was obtained. The ester mixture was fractionated three times at 10 mm. Fractions were obtained as follows

1	100–110°	1.0 g.	
2	127–135°	32.5 g.	α_D −10.6°
3	135–145°	0.9 g.	
4	145–155°	1.0 g.	
5	155–165°	1.3 g.	
6	165–170°	24.5 g.	

Residue 5.0 g.

The hydrazides were prepared from these fractions. Fraction 1 yielded *l*-malic hydrazide and a little levulinic hydrazide. Fraction 2 afforded *l*-malic hydrazide, melting at 178–179°, as did also fractions 3 and 4. Fraction 5 was a mixture. Fraction 6 yielded citric hydrazide, hydrated form, melting at 104–106° and identified by optical crystallographic examination.

The proper ester fractions, calculated to *l*-malic and citric acids, show that fresh lettuce contains approximately 0.065% of *l*-malic acid and 0.048% of citric acid.

Summary

Fresh spinach was found to contain 0.31% of oxalic acid. Citric acid and a small quantity of malic acid were separated by the ester distillation method.

Analysis of broccoli shows that the leaves and buds have materially the same composition and nutritive value. Both buds and leaves contain proteins somewhat in excess of that reported in spinach.[6]

The predominating organic acid in broccoli is citric acid. It also contains *l*-malic acid and small amounts of oxalic and succinic acids. The proportion of citric and malic acids is 3:2.

The organic acids of lettuce were found to be oxalic acid, 0.011%, *l*-malic acid, about 0.065%, and citric acid, about 0.048%.

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MIXED BENZOINS. IV. DETERMINATION OF THE STRUCTURE OF MIXED BENZOINS BY THE BECKMANN REACTION[1]

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The rigid determination of the structure of a number of mixed (unsymmetrically substituted) benzoins would allow important generalizations to be made as to the reactivity of various aromatic aldehydes, and would also contribute to the wider use of benzoins in synthetic work. Up to the present time a few mixed benzoins have been assigned structures, largely on the basis of their production (or the production of the isomer) by the Grignard reaction. Benzanisoin,[2a] benzfuroin[2b] and *p*-dimethylaminobenzoin[3] have been so determined. In addition, the structure of benzfuroin has been deduced by a Beckmann reaction,[4] that of *p*-dimethylaminobenzoin by an indirect method,[5] and that of benzanisoin by synthesis from the desoxy compound.[6] The structures of the three foregoing benzoins may therefore be taken as definitely settled, and the authors have taken these compounds as standards in this paper.

For determining the structure of mixed benzoins, the Grignard reaction, although probably trustworthy, is anything but convenient. Indirect

[6] Werner, "Die Pflanzenstoffe, **1911**, p. 180.

[1] Since submitting this paper, a note has appeared by Tiffeneau and Lévy, *Compt. rend.* **192**, 287 (1931), in which the structures of some mixed benzoins are determined by fission with potassium hydroxide. The results with benzfuroin, benzpiperoin and benzanisoin agree with those of the present authors. Hörbye, Ref. 11, has determined the structures of benzpiperoin and benzanisoin by an oxidation method.

[2a] McKenzie, Luis, Tiffeneau and Weill, *Bull. soc. chim.*, [4] **45**, 414 (1929).

[2b] Asahina and Terasaka, *J. Pharm. Soc. Japan*, **494**, 219 (1923).

[3] Jenkins, Bigelow and Buck, THIS JOURNAL, **52**, 5198 (1930).

[4] Werner and Detscheff, *Ber.*, **38**, 69 (1905).

[5] Jenkins, Buck and Bigelow, THIS JOURNAL, **52**, 4495 (1930).

[6] Meisenheimer and Jochelson, *Ann.*, **355**, 249 (1907).