scribed method, furnishes strong evidence as to the agricultural significance of the results thus obtained, but so far no one has been able to show a close agreement between crop yields and the lime requirements of soil as determined by laboratory methods.

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THE ANALYSIS OF VANILLA EXTRACT.

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HESS AND PRESCOTT, in papers published in this Journal,[1] have discussed the adulteration of vanilla extract and have described methods devised by them for the separation and determination of vanillin and coumarin, for the distinction of genuine from artificial extracts, and for the detection of artificial coloring-matter, all of which methods, with slight changes, are included among the "Provisional Methods for the Analysis of Foods", adopted by the Association of Official Agricultural Chemists.[2]

Preliminary to the examination of commercial extracts, under the Connecticut pure food law, we have subjected these methods to a critical investigation, the results of which, while substantiating in the main the accuracy of the methods, suggested certain modifications. The Hess and Prescott method for determination of vanillin and coumarin was found to be thoroughly reliable but susceptible of abridgment without diminishing its accuracy.

The methods as finally amended were employed in the analysis of five extracts made in the laboratory and sixty-two commercial extracts.[3]

METHODS OF ANALYSIS.

Determination of Vanillin and Coumarin. Modified Hess and Prescott Method.—The modified method differs from the original method in three details:

First. Two per cent. instead of 10 per cent. ammonia is used, and consequently a less concentrated ammonium chloride solution is obtained after neutralizing with hydrochloric acid, thus reducing the chance of carrying this salt into the extract.

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[1] Vol. **21**, 256, 721.

[2] Bulletin 65, U. S. Dept. Agr., Bureau of Chem., pp. 69-71.

[3] Conn. Agr. Expt. Sta., Rep. 1901, pp. 152-161.

Second. A greater bulk of ammonia is used, to diminish the error due to possible mechanical loss during shaking.

Third. The vanillin and coumarin are weighed after evaporation of the ether solutions, whereas in the original process they are not weighed until they have been redissolved in petroleum ether and again evaporated. This procedure not only materially shortens the process, but avoids the errors due to crawling of the solutions while redissolving and reevaporating. Any matter insoluble in the petroleum solvent is afterwards weighed with the dish and a correction introduced, but if care is exercised in the separation, this residue is insignificant. The evaporation of the petroleum extract is, however, necessary if melting-points are to be determined, but this evaporation can go on at leisure after obtaining the quantitative results.

A full description of the method follows:

Dealcoholize 25 grams of the extract in an evaporating dish upon a water-bath, at a temperature of about 80° C., adding water from time to time to retain the original volume. After removal of the alcohol, add normal lead acetate solution, drop by drop, until no more precipitate forms. Stir with a glass rod to facilitate flocculation of the precipitate, filter through a moistened filter, and wash three times with a few cubic centimeters of hot water. Cool the filtrate and extract with ether by shaking out in a separatory funnel. Use 15 to 20 cc. of ether at each separation, repeating the process three or four times, or until a few drops of the ether, evaporated upon a watch-glass, leave no residue. Place the combined ether extracts containing all of the vanillin and coumarin in a clean separatory funnel, and shake out four or five times with 2 per cent. ammonia, using 10 cc. for the first shaking, and 5 cc. for each subsequent shaking.

Set aside the combined ammoniacal solutions for the determination of vanillin.

Wash the ether solution into a weighed dish, and allow the ether to evaporate at the room temperature. Dry in a desiccator and weigh. Usually the dried residue is pure coumarin. Treat the residue with 5 to 10 cc. of cold petroleum ether, boiling between 30° and 40° C. and decant off the clear liquid into a beaker. Repeat the extraction with petroleum ether until a drop, evaporated on a watch-glass, leaves no residue. Dry the dish for a few moments in a water-oven, cool and weigh. Subtract the weight of the dish and the residue (if any) from the weight previously obtained after evaporation with ether, thus obtaining the weight of pure coumarin. Allow the petroleum ether to evaporate at the room temperature, and dry, if necessary, in a desiccator. The residue should be crystalline and have a melting-point of 67° C. This, with the characteristic odor of coumarin, is sufficient for its identification.

Slightly acidulate the ammoniacal solution reserved for vanillin with 10 per cent. hydrochloric acid. Cool and shake out in a separatory funnel with four portions of ether as described for ether extraction. Evaporate the ether at room temperature in a weighed platinum dish, dry over sulphuric acid, and weigh. Treat the residue with boiling ligroin (boiling-point, 80° to 85° C.), decanting into a dry beaker. Repeat the treatment until all vanillin is removed. Dry the dish with residue (if any) for a few moments at 100° C. and weigh. Subtract the weight from the weight previously obtained after evaporating the ether. The difference is the weight of vanillin. Evaporate the ligroin at room temperature and dry in a desiccator. The residue should be crystalline, have a melting-point of 80° to 81° C. and have the characteristic odor and crystalline form of vanillin.

Hess and Prescott,[1] as a test of their process, determined vanillin and coumarin in two 25-gram portions of vanilla extract U. S. P., to each of which had been added 0.5 gram of pure coumarin. The following are their results: Vanillin (I), 0.3081 gram; (II), 0.2997 gram.[2] Coumarin (I), 0.4910 gram: (II), 0.4820 gram. These figures illustrate the accuracy of the process for determination of coumarin, but throw no light on its value for determination of vanillin, as the amount present was unknown.

As a further test of the Hess and Prescott method, we have made determinations in 25-gram portions of a solution containing the same amount of sugar and alcohol as vanilla extract U. S. P., to which weighed quantities of pure vanillin and coumarin had been added, proceeding as follows:

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[1] This Journal, 21, 256.

[2] Owing probably to a typographical error, these amounts are about ten times what are usually present.

Method *A*. The original Hess and Prescott method, using ether in all extractions and 10 per cent. ammonia for separation of vanillin and coumarin.

Method *B*. Same as *A*, except that chloroform was substituted in all cases for ether.

Method *C*. Same as *A*, except that chloroform was used in the extraction of vanillin from the ammonia extract.

Method *D*. The modified method used by us, as already described.

In each case the separated vanillin and coumarin was weighed after the evaporation of the ether or chloroform (first evaporation), and only in a few instances was it reweighed after dissolving in the petroleum solvent and reevaporating (second evaporation). The results follow:

RESULTS OBTAINED BY VARIOUS MODIFICATIONS OF HESS AND PRESCOTT'S METHOD, ON MIXTURES CONTAINING KNOWN QUANTITIES OF VANILLIN AND COUMARIN.

	Vanillin.			Coumarin.		
Method.	Taken. Gram.	Found after first evaporation. Gram.	Found after second evaporation. Gram.	Taken. Gram.	Found after first evaporation. Gram.	Found after second evaporation. Gram.
A	0.0120	0.0101	0.0100	0.0170	0.0162	0.0165
B	0.0695	0.0723	0.0705	0.0975	0.0902	0.0900
C	0.0495	0.0490	.....	.....	.....	.....
C	0.0280	0.0261	.....	.....	.....	.....
C	0.0610	0.0630	0.0575	0.0855	0.0751	0.0740
C	0.0120	0.0136	0.0120	0.0170	0.0160	0.0162
C	0.0290	0.0280	.....	.....	.....	.....
C	0.0495	0.0455	.....	.....	.....	.....
D	0.0625	0.0636	0.0610	0.0875	0.0800	0.0786
D	0.0625	0.0620	.....	0.0875	0.0796	.....
D	0.0625	0.0621	0.0585	0.0875	0.0803	0.0795
D	0.0625	0.0602	.....	0.0125	0.0125	.....
D	0.0625	0.0630	.....	0.0125	0.0124	.....
D	0.0625	0.0615	.....	0.0125	0.0122	.....

From these figures it is evident that the process, both as originally described and as abbreviated by us, gives remarkably accurate results for vanillin and sufficiently accurate results for coumarin.

Where the amount of coumarin was large, there was an unaccountable deficiency of this substance, but for the amounts present in extracts, which, in our experience, has not exceeded 0.0600 gram per 25 grams of extract, the method is all that could be desired. The melting-points of the coumarin and vanillin in all cases were close to the theory.

Without doubt, this excellent method is in all points the best that has yet been devised.

Tests for Caramel, Coal-tar Dyes, etc.—Valuable indications of the nature of an extract are obtained in the process of determination of vanillin and coumarin. Pure extracts of vanilla beans give, with lead acetate, a bulky, more or less glutinous, brown-gray precipitate, and a yellow or straw-colored filtrate, whereas purely artificial extracts colored with caramel give a slight, dark brown precipitate and a dark brown filtrate. If both vanilla bean extract and caramel are present, the precipitate is more or less bulky and dark-colored, and the filtrate is more or less brown. The solution remaining after extraction of the vanillin and coumarin with ether, if dark-colored, should be tested both for caramel and coal-tar colors.

In our experience the most satisfactory test for caramel is to shake with fullers' earth as recommended by Crampton and Simons.[1] If the color is due to caramel and a grade of fullers' earth is used, which experience has proved suitable, the solution, after filtering, is yellow or colorless. This test does not positively identify the color, as some other brown substances may give similar reactions, but no such substance is liable to be present in vanilla extract.

Amthor's test[2] for caramel has not given in our hands decisive results, and Hess' test,[3] owing to the formation of caramel from the sugar of the extract during the heating with acid, has given reactions in extracts known to contain none whatever of this substance.

Coal-tar dyes may be tested for by Arata's method.[4]

Determination of Total Residue.—Introduce into a flat-bottomed aluminum dish 10 grams of ignited sand and a short stirring-rod,

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[1] This Journal, **21**, 355; **22**, 810.

[2] *Ztschr. anal. Chem.*, **24**, 30.

[3] This Journal, **21**, 721.

[4] *Ibid.*, **22**, 582.

and weigh. Add 5 grams of extract and heat on a boiling water-bath, with constant stirring, until the bulk of the water is removed. Dry in a water-oven until the loss sustained in one hour is less than 2 mg.

The "total residue" not only includes the total solids, but also glycerin which is often present in such amount that the residue appears moist after drying. Owing to the slow evaporation of this substance, absolutely constant weight can not be secured but the loss is so gradual that the above method is usually sufficiently accurate.

Determination of Alcohol.—Dilute 25 grams to 150 cc. and distil into a 100 cc. pycnometer. Determine the specific gravity of the distillate at 15.5° C. and calculate the percentage by weight.

Determination of Cane-Sugar. Detection of Glucose.—Dissolve 13.024 grams (one-half the normal weight) of the material in about 80 cc. of water. Add 3 cc. of basic lead acetate and 2 cc. of alumina cream, make the volume up to 100 cc. and filter through a dry filter. Determine the rotatory power of the solution in a 200 mm. tube. To 50 cc. of the filtrate referred to above, add 5 cc. concentrated chemically pure hydrochloric acid. After thorough mixing, place in a cold water-bath and heat quickly to 68° C. After standing at that temperature for ten minutes, cool quickly, filter from lead chloride, if necessary, and examine in a 220 mm. tube, first at the room temperature and finally at 86° C.

Estimate the percentage of cane-sugar by Clerget's formula. A considerable plus reading at 86° shows the presence of glucose.

Determination of Glycerin.—Determine in 5 grams of the extract as directed for sweet wines.[1]

ANALYSES OF GENUINE AND ARTIFICIAL VANILLA EXTRACTS.

Five extracts were made in the Station laboratory, from different grades of beans, after the U. S. P. formula.

An extract of tonka beans was also prepared in the laboratory in the same manner as the vanilla extracts, substituting, however, tonka beans for vanilla beans.

Analyses of these extracts and of fourteen representative commercial extracts, both pure and adulterated, are given in the table:

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[1] Borgmann: Anleitung zur Chem. Analyse des Weines, 2 Auf., p. 50. U. S. Dept. of Agr. Div. of Chem., Bull. 46, Revised ed., p. 63.

ANALYSIS OF VANILLA EXTRACTS.

Serial No.		Vanillin.	Coumarin.	Total residue.[1]	Cane-sugar.	Glycerin.	Solids other than cane-sugar.	Alcohol by weight.	Coloring.
4562	U. S. P. Mexican beans	0.13	0.00	22.60	19.90	0.00	2.70	37.96	natural
4561	U. S. P. Mexican beans (cut)	0.07	0.00	23.10	19.20	0.00	3.90	39.92	"
4504	U. S. P. South American beans	0.22	0.00	22.00	19.00	0.00	3.00	38.58	"
4505	U. S. P. Bourbon beans	0.14	0.00	23.13	20.40	0.00	2.73	38.32	"
4506	U. S. P. Tahiti beans	0.11	0.00	21.75	20.00	0.00	1.75	38.84	"
4509	U. S. P. Tonka beans	0.00	0.21	....	....	...	...	....	"
4193	Commercial extract (not found adult'd)	0.08	0.00	21.84	10.36	8.87	2.61	40.00	"
4132	Commercial extract (not found adult'd)	0.18	0.00	13.52	0.90	10.76	1.86	27.51	"
4137	Commercial extract (not found adult'd)	0.15	0.00	16.43[2]	4.54	4.54	7.35[2]	21.27	"
4180	Commercial extract (not found adult'd)	0.09	0.00	16.72	15.44	0.00	1.28	20.90	"
4052	Commercial extract (not found adult'd)	0.22	0.00	22.66	20.00	0.00	2.66	23.79	"
4129	Adulterated commercial extract	0.22	0.08	0.86	0.47	0.00	0.39	6.34	artificial
4152	Adulterated commercial extract	0.68	0.15	14.00	10.64	2.42	0.94	17.32	natural
4179	Adulterated commercial extract	0.02	0.10	14.34[3]	0.75	0.00	13.59[3]	14.15	"
4141	Adulterated commercial extract	0.15	0.04	15.15	12.28	0.00	2.87	5.28	artificial
4078	Adulterated commercial extract	0.14	9.05	12.94	0.60	9.74	2.60	41.42	natural
4053	Adulterated commercial extract	0.24	0.18	14.62	10.92	0.00	3.70	11.91	"
4056	Adulterated commercial extract	0.07	0.00	14.35	11.20	0.00	3.15	31.72	artificial
3959	Adulterated commercial extract	0.06	0.23	36.16[2]	12.96	0.00	23.20[2]	7.53	"
3972	Adulterated commercial extract	0.01	0.09	13.44	11.14	0.00	2.30	9.68	"
3725	Adulterated commercial extract	0.44	0.11	26.54	21.00	0.00	5.54	10.73	"
4042	Adulterated commercial extract	0.06	0.05	35.50	31.88	0.00	3.62	13.36	natural

[1] Includes glycerin.

[2] Contains glucose.

[3] Contains invert sugar.

From the analyses of the U. S. P. samples it is evident that the percentage of vanillin bears no relation to the value of the extract. Sample No. 4562, made from Mexican beans costing $11.00 per pound, contained less than Nos. 4504 and 4505 made respectively from South American and Bourbon beans costing less than $7.00 per pound and but little more than No. 4506 made from Tahiti beans costing but $1.50 per pound. The average percentage of alcohol was 38.72 and of cane-sugar, 19.70. The residue other than cane-sugar ranged from 1.75 to 3.90 per cent. None of these extracts contained the slightest trace of coumarin; on the other hand, the Tonka bean extract contained 0.21 per cent. of coumarin but no vanillin.

The commercial extracts classed as "not found adulterated" appeared to have been made from vanilla beans, without addition of other coloring or flavoring matter. In most of these, however, the percentages of alcohol and sugar were less than in extracts prepared according to the Pharmacopoeia, and in some of them considerable amounts of glycerin (maximum 10.76 per cent.) or glucose were present, but these differences bear little or no relation to the value of the product.

All of the U. S. P. and the pure commercial extracts gave with lead acetate a bulky precipitate of a light brown-gray color and a filtrate from this precipitate of a light yellow color.

The adulterated samples contained vanillin in amounts ranging from 0.01 to 0.68 per cent. and nearly all of them contained coumarin in amounts up to 0.23 per cent. The minimum percentage of alcohol was 5.28 and of cane-sugar was 0.47. Glycerin was found in several samples, glucose in one and invert sugar in four. Many of these extracts gave only a slight precipitate with lead acetate, the precipitate as well as the filtrate being dark brown in color. After shaking the filtrate with fullers' earth and again filtering, colorless solutions were obtained in nearly all such cases, indicating that the color was due to caramel. In one extract only was a coal-tar color detected.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
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