The amount of moisture in green alfalfa varies considerably. A sample cut during a rain contained 81 per cent moisture while in other samples the moisture content was as low as 60 per cent. The average of those samples dried in the laboratory was nearly 75 per cent. This of course is much greater than would be found in hay cut under ordinary conditions. The samples used for these tests contained about 64 per cent water, while a sample taken at the same time, but dried immediately, contained 74 per cent water, showing a loss of 10 per cent from handling and lying in the pile over night. Mowing, raking and hauling on a bright day would probably show a greater loss.

Assuming the moisture content of a hay to be 75 per cent when it reaches the drier, it would require the evaporation of 6000 pounds of water per ton of dry hay; $6000 \times 23.2/100 = 1390$ pounds of coal per ton of dry hay. At \$2.60 per ton, this would amount to \$1.81 per ton of hay for heat. To this should be added something like 25 cents per ton for interest and depreciation of plant and about \$1.00 per ton for chopping, power and labor, making a total cost of \$3.06 for drying one ton, dry weight, of hay.

Assuming the moisture content to be 64 per cent, as was found in the trials here shown, the production of one ton of dry hay would require the evaporation of but 3600 pounds of water, using 835 pounds of coal at a cost of \$1.09. Adding to this \$1.25 for interest, power, labor, etc., a total cost of \$2.34 per ton is shown. Although these cost figures are only approximations, they lead to some interesting conclusions.

As it was shown that the total loss in the value of hay due to field curing and handling amounts to from 20 per cent under favorable conditions to 50 per cent under adverse weather conditions, and as the calculated cost for artificial drying is from \$2.35 to \$3.00 per ton, producing only choice alfalfa of uniform grade, it is apparent that artificial drying may be conducted at a profit in districts where the drier can be situated near both field and source of fuel. The alfalfa meal industry has grown to considerable proportions in the last few years and the demand is for well-cured alfalfa of good color. Several alfalfa millers have expressed the opinion that a hay of such dryness could be ground at about half the cost of the average hay, taken from the stack.

Some millers subject their hay to a short, preliminary drying before grinding. A drier run in connection with an alfalfa-grinding plant would be an excellent combination.

This same principle of drying could doubtless be applied to other fodders and perhaps even to small fruits and other farm products which are occasionally damaged by unfavorable weather during the drying season. Among the other fodders to which drying might be applicable might be mentioned the pea vines from the canning factories and the common weed, sweet clover or yellow Mellilot. Pea vines are often made into ensilage but as such they have but a limited demand and the canner often has trouble disposing of the enormous quantity of vines which accumulate and become a nuisance. Pea-vine meal has been

used to a certain extent as an addition to alfalfa meal. Young sweet-clover is used as forage to a small extent in Europe but sun curing does not produce a good hay. Drying in an oven at 50°, however, gave a hay of good appearance and flavor. Samples of these plants examined gave analyses as follows:

	Protein Per cent	Diastatic activity	Water solubility Per cent
Pea vines.....	17.73	0.54	44.2
Sweet clover.....	18.85	0.03	32.7

The moisture contents of the two plants in the green state were 84.7 per cent and 83.2 per cent and this would probably be the principal drawback in curing them for feed.

SUMMARY

I—The diastatic activity of alfalfa is greater in the morning or after a period of darkness than after a period of exposure to light. There is much more diastase present in the plant during the warm, active, growth-producing summer than during the spring or fall. Young plants contain more diastase than older ones.

II—Drying at elevated temperatures in a humid atmosphere decreases the diastatic activity, even though the temperature is only 50°. Drying in a current of air with gradually increasing temperature, on the other hand, increases the activity markedly. Light and weathering in the field tend to destroy the diastase. Rain during curing is very detrimental.

III—Highly diastatic alfalfas generally show a greater solubility in water by auto digestion than samples low in diastase. However, the degree of solubility in water cannot be increased above a certain limit.

IV—The loss in digestible constituents during handling and curing in the field may vary from 20 per cent under favorable conditions to as much as 50 per cent under adverse weather conditions.

V—Curing by artificial heat, using the principle of counter-currents, gives a hay of better color, odor and flavor than can be produced by other means. The hay appears to retain many of the valuable properties of the green plant, which are ordinarily lost in curing.

VI—The cost of artificial drying is estimated to be less than the losses generally sustained in field curing and, therefore, it ought to be possible to conduct drying at a profit when the drier can be located near both field and source of fuel.

The writer desires to acknowledge his indebtedness to the late Robert Kennedy Duncan for helpful direction and valuable advice in connection with this work.

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THE ORIGIN OF VANILLIN IN SOILS—VANILLIN IN WHEAT AND IN THE WATER IN WHICH WHEAT SEEDLINGS HAVE GROWN

By M. X. SULLIVAN

Received August 19, 1914

Vanillin, the chief odorous principle of the vanilla pod, the fruit of *vanilla planifolia*, has been reported as present in a number of plants and plant products. The presence of vanillin in plants and its possible

passage into the medium of growth is of great interest since (1) vanillin has been found injurious to plant growth¹ and (2) it has been found in a number of soils.² As yet it has not been reported as formed by microorganisms.

Among the plants and plant material in which vanillin has been reported are asparagus sprouts, seeds of white lupine, ilex leaves, dahlia tubers, grain of oats, and roots of couch grass, potato, pine wood, peat, raw beet sugar, and in resins such as Asafoetida and Siam benzoin.³ It has been found also in orchid flowers exposed to sudden frosts⁴ and in the incrusting pigment of sugar cane on warming with dilute acids.⁵

Vanillin or a closely related mother substance, in large or small quantities, seems to be fairly wide-spread in the vegetable world. Accordingly, as a possible explanation of its occurrence in field soils, it became of interest to determine whether or not vanillin could be found in wheat as a type of field crop and in the water in which wheat had grown.

VANILLIN IN WHEAT—When wheat seedlings were extracted with hot alcohol (recently distilled) there was obtained on evaporating the alcohol extract a syrupy mass, part of which was soluble in ether. The ether extract was treated with a half saturated solution of sodium bisulfite in the usual way in testing for aldehydes. The bisulfite solution was freed from sulfite by means of dilute sulfuric acid and the excess of sulfur dioxide driven off by passing air through the liquid. After extraction with ether and evaporation of the ether solution a small amount of matter which smelled of vanillin was obtained. This gave the characteristic vanillin color reactions: blue with ferric chloride, green with ferrous sulfate and bromine water, violet with a mixture of hydrochloric and sulfuric acids and acetone water.

With the recognition that wheat contained vanillin, it became of interest to determine how much of the aldehyde was present. For the quantitative estimation recourse was had to the colorimetric method described by Folin and Denis.⁶

For the quantitative determination, 200 g. of wheat seeds were extracted with alcohol and the extract was then treated in the customary way for extracting aldehydes. The final ether extract from the solution freed from bisulfite was evaporated and taken up with warm water. This water solution was treated with equal parts of lead acetate and lead subacetate as long as a precipitate formed. The filtrate or an aliquot part of it was treated with 5 cc. of the phosphotungstic-phosphomolybdic reagent as described by Folin and Denis and after 5 minutes a saturated solution of sodium carbonate was added until the volume of the liquid was 50 or 100 cc. After standing 10 minutes, the solution was filtered and the blue color generated in the reactions compared in a Schreiner colorimeter with that produced by a similarly treated standard

solution of vanillin. The standard was of such a strength that 10 cc. diluted with the reagents to 100 cc. contained 1 mg. of vanillin or 10 parts vanillin per million. The stock solution of vanillin was made, as recommended by Harder,¹ by dissolving 2 g. of vanillin in 200 cc. of 95 per cent alcohol and diluting to 1 liter and then using 10 cc. of this solution to make 200 cc. of the standard solution as needed. Ten cc. of this standard were treated with 5 cc. of the phosphotungstic-phosphomolybdic reagent and then with sodium carbonate as outlined above.

Vanillin was similarly determined (1) in the ether-soluble part of a solution obtained by heating wheat seeds 4 hours on a steam bath with 5 per cent sulfuric acid; (2) in the alcohol extract of wheat bran; (3) in the alcohol extracts of the roots, tops, and seeds of wheat seedlings 5 to 10 days old; (4) in the seed water or the water bathing the seedlings for the first 24 hours; (5) in the root water or the water which bathed the roots for 4 to 10 days with a change of water every two days. The water solutions were concentrated under reduced pressure and extracted with ether. The quantitative results are given in the following table:

TABLE I—VANILLIN IN WHEAT AND IN THE WATER IN WHICH WHEAT SEEDLINGS HAD GROWN

MATERIAL	QUANTITY	VANILLIN Parts per million
Wheat seeds.....	200 and 500 g.	1-6 3
Wheat seeds digested with 5% H ₂ SO ₄	200 g.	5-14 11
Wheat bran.....	200 g.	3(a) 3
Roots of wheat seedlings.....	1500 seedlings	7-18(a) 13
Tops of wheat seedlings.....	1500 seedlings	13-16(a) 15
Seeds of wheat seedlings.....	1500 seedlings	6-8(a) 7
Seed water.....	4000 cc.	Trace-3
Root water.....	5000 cc.	Trace to 10(a)

(a) Based on weight of original seeds.

The effect of germination on the amount of vanillin in wheat may be seen more clearly in the following experiment where the vanillin in 1717 ungerminated seeds and in the same number of seeds germinated is compared:

TABLE II—VANILLIN IN UNGERMINATED SEEDS AND IN SEEDS, TOPS, AND ROOTS OF SEEDLINGS 5 DAYS OLD

	P. p. m.
Seed.....	3.6
Plant seed.....	7.7
Plant tops.....	15.2 (Based on weight of 1717 seeds or 58 g.)
Plant roots.....	13.0
WHOLE PLANT.....	35.9

The tables show that vanillin occurs free in ungerminated wheat to a slight degree, that it is increased in amount during the early growth of the plant and can pass from the plant to the medium of growth either directly or more probably as a result of cell sloughing and disintegration. Treatment of ground seeds with dilute sulfuric acid increases the amount of vanillin extractable. Accordingly, it would seem that a mother substance of vanillin occurs in wheat and yields vanillin on hydrolysis by acids or in the process of germination.

Coniferin is regarded by many as the mother substance of vanillin. Coniferin gives a blue color with phenol and hydrochloric acid especially in the presence of a little potassium chlorate, gives glyco-vanillin on treatment with potassium permanganate and chromium trioxide, yields vanillin on treatment with chromic

¹ THIS JOURNAL, 5 (1913), 619.

¹ Schreiner and Reed, *Bull.* 47, Bur. of Soils, U. S. Dept. Agric. (1907); Schreiner and Skinner, *Bull.* 77, Bur. of Soils, U. S. Dept. Agric. (1911).

² E. C. Shorey, *J. Agr. Res.*, 1 (1914), 357.

³ Abderhalden, *Biochemische Handlexikon*, 1 (1911), 837.

⁴ V. Lippmann, *Ber. d. chem. Ges.*, 45 (1912), 3431.

⁵ Langguth and Steuerwald, *Chem. Zentr.*, 1912, I, 83.

⁶ *J. Biol. Chem.*, 12 (1912), 239; THIS JOURNAL, 4 (1912), 670.

acid mixture, yields glucose and a resin by boiling with dilute sulfuric acid. From the roots of wheat seedlings there was obtained, by extraction with hot water and hot alcohol and precipitation from alcohol by means of ether, material which gave a greenish blue color with phenol and hydrochloric acid; a blue color with phenol, hydrochloric acid and a little potassium chlorate; a reducing substance on boiling with dilute sulfuric acid. The method of obtaining the material, its reaction with phenol, and the formation of reducing substances on boiling with acid would indicate the presence of coniferin in the wheat roots. Coniferin, it might be said in passing, has been found in the lignified tissue of a number of plants.

Vanillin we have found also in rotten oak wood, in pineapple pulp and parings and in the hot water extract of lawn grass, consisting of blue grass and a small amount of white clover. The amount of vanillin extractable from grass is considerably increased by heating with 5 per cent sulfuric acid for 4 hours on a steam bath. Undoubtedly it occurs to a greater or less degree in many plants either free or in a conjugated form, from which it may be liberated by oxidation and hydrolysis. Its presence in wood and various forms of vegetation would lead to the conclusion that the vanillin in soil has its origin in vegetable debris and to a minor degree also in direct excretion or cell sloughing by growing plants.

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AN EXACT AND EASY METHOD FOR PREPARING A NEUTRAL AMMONIUM CITRATE SOLUTION

By J. M. McCANDLESS

Received August 8, 1914

Since the writer made his official report as Referee on Phosphoric Acid, in 1908 to the Association of Official Agricultural Chemists, there have appeared a number of interesting articles on the subject matter of that report showing how the ammonium citrate solution could be rendered neutral both by physical and chemical methods. All of this work has confirmed the accuracy of the work done, and the conclusions reached by the writer in his original report. As this report was never published in any chemical journal, being printed only in the Proceedings of the A. O. A. C.,¹ and has therefore never been seen by many who are interested in the subject, the writer may be pardoned for reproducing here a part of that report.

PARTIAL REPRODUCTION OF REPORT OF REFEREE ON PHOSPHORIC ACID FOR 1908

"As the referee was to decide whether the various solutions of ammonium citrate sent him were neutral or not, and as scarcely any two chemists can agree upon the exact point of neutrality, whether from lack of sensitiveness of the indicators, or color-blindness on the part of the operators, he decided to make an analysis of each sample according to the method outlined in his last report to the Association, and be guided by those results in deciding upon neutrality.

METHOD OF ANALYSIS—"Twenty-five cc. of each solution were pipetted into a 250 cc. flask, diluted to mark, shaken, and 25 cc. of this solution were pipetted into a distillation flask. To the

solution in the flask, 40 cc. *N*/4 caustic soda solution were added, and the contents of the flask distilled into 20 cc. *N*/2 acid, continuing the distillation till the volume of the distillate measured from 65 to 70 cc. The ammonia in the distillate was then titrated by means of *N*/10 alkali and cochineal. The residue in the distillation flask was washed into an Erlenmeyer, excess of standard acid added, a few drops of phenolphthalein, and the excess acid estimated by *N*/10 alkali. From the result, the weight of citric acid originally combined with the ammonia was calculated. Calculating from the formula of the pure salt, $(\text{NH}_4)_2\text{C}_6\text{H}_5\text{O}_7$, that the ratio of ammonia to citric acid was 1:3.765, a basis of comparison was established. Results obtained are given in table below:

Number of analyst	Milligrams of citric acid in ammonia 25 cc. of in 25 cc. diluted solution = 2 1/2 cc. original	Milligrams of citric acid in ammonia 25 cc. of in 25 cc. diluted solution = 2 1/2 cc. original	Ratio of ammonia to citric acid	Ratio in neutral salt $(\text{NH}_4)_2\text{C}_6\text{H}_5\text{O}_7$	REACTION WITH CORALLIN
1	113.9	433.2	1:3.803	1:3.765	Neutral
2	109.3	412.8	1:3.775	1:3.765	Alkaline
3	104.9	424.96	1:4.051	1:3.765	Acid
4	113.7	433.9	1:3.816	1:3.765	Neutral
5	110.5	430.08	1:3.891	1:3.765	Slightly acid
6	111.5	436.48	1:3.915	1:3.765	Acid
7	108.7	421.1	1:3.874	1:3.765	Slightly acid
8	104.7	398.7	1:3.808	1:3.765	Neutral
9	102.8	430.7	1:4.189	1:3.765	Acid

"In the above table, all the solutions which showed materially more citric acid than 3.765 parts to 1 of ammonia, also showed a decidedly acid reaction to corallin.

"It appears that some chemists prepare their ammonium citrate solution by treating the citric acid with excess of ammonia, and then leave the hot solution to neutralize itself, or finally adjust by means of red and blue litmus paper, or corallin. No. 3 in the above table was prepared in this way. The heat of the mixture not only drove off sufficient ammonia to render it neutral, but *continued* and left it decidedly acid."

The writer has, only in the past few days, had his attention called to the article of Patten and Marti¹ where in referring to the solutions, analyses of which are given in the above table, they use the following language: "The three solutions *claimed* to be neutral by McCandless contained 44.3 g. NH_3 , and 168.76 g. $\text{C}_6\text{H}_5\text{O}_7$ per liter, the ratio being 1:3.809. The results obtained by us conclusively prove that such a solution would be slightly acid, which fact is further substantiated by the ratio of ammonia to anhydrous citric acid being too wide."

Patten and Marti had evidently failed to note the headings of Columns 5 and 6 in the above table, or they would not have attributed to the writer the "CLAIM" that solutions Nos. 1, 4 and 8 were really neutral, since the heading of Column 6 expressly stated that the words "neutral," "acid" and "alkaline" in the column below referred to the reactions with corallin, and the heading of Column 5 showed that the only ratio of ammonia to citric acid which the referee recognized as being neutral was that of 1 : 3.765.

The method given by Patten and Marti is an interesting and excellent method of preparing a neutral solution. The writer proposes the following exact and easy method of making a neutral solution of ammonium citrate which involves no analyses or distillations, but only a couple of titrations with solutions always ready in every laboratory:

¹ Bur. of Chem., U. S. Dept. of Agr., *Bull.* 132, 11.

¹ THIS JOURNAL, 5 (1913), 568.