ECKSTEIN

A Study of Caramel:
Its Normal Occurrence as a Color

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED A Study of Caramel: Its Normal Occurrence as a Color.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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A STUDY OF CARAMEL
ITS NORMAL OCCURRENCE AS A COLOR

INTRODUCTION

Caramel is a dark-brown substance obtained by heating a carbohydrate such as sucrose, dextrose, or maltose at a high temperature. In the process of caramelization no great color change is noted at temperatures as high as 150° C. Above this point, however, a light straw color is obtained. This gradually deepens and becomes almost black at 210° C. During the early stages of caramelization, a great deal of water is evolved. Later formaldehyde, benzaldehyde, acetone, and phenolic compounds are given off. The work of Zoller* seems to show that caramel is a compound of a definite composition, and that there is a difference between the caramels formed from the different sugars. The compound is quite soluble in water, and may be precipitated from aqueous solutions by the addition of strong alcohol. It is also precipitated by ammoniacal lead acetate, zinc chloride, and barium hydroxide. It reduces metals in alkaline solution, can be acetylated and benzoylated, and gives a dark-brown precipitate with phenyl hydrazine hydrochloride.

Caramel is used commercially as a color and as a flavor, and, since some of our foods are at times adulterated with this compound, its identification is of great importance. The following are some of the most important tests for caramel.

The Amthor test. 

Place 10 cc of the solution to be tested in a high

* Unpublished work of Beal and Zoller.
# Bureau of Chemistry—Bulletin No. 107.
narrow glass; add from 30 to 50 cc of paraldehyde, depending on the intensity of the coloring, and enough absolute alcohol to make the solutions mix. In the presence of caramel, a brownish-yellow to dark-brown precipitate will collect in the bottom of the flask. Decant the liquor, wash with absolute alcohol, dissolve in a small amount of hot water, and filter. The color of the solution will give some idea of the amount of caramel present. In order to identify the color, pour the solution into a freshly prepared solution of phenyl hydrazine (2 parts of phenyl hydrazine hydrochloride and 3 parts of fused sodium acetate and 20 parts of water). The presence of a considerable amount of caramel gives a dark-brown precipitate which is hastened in formation by heating. In case only a small amount is present, it takes hours to collect.

The Woodman-Newhall test for caramel coloring.*

Mix 15 cc of the solution to be tested with a 5 per cent solution of zinc chloride, add 2 cc of a 2 per cent potassium hydroxide solution, and filter. Wash the precipitate with hot water, dissolve in 15 cc of hot acetic acid, neutralize, and concentrate the filtered neutralized solution to one half its original volume. To one half add 3 parts of paraldehyde and enough alcohol to make the mixture homogeneous; to the other part add an equal volume of a mixture of 2 parts of phenyl hydrazine hydrochloride, 3 parts of fused sodium acetate, and 20 parts of water. After standing over night, both solutions will develop dark-brown precipitates if caramel is present.

The following modification has been suggested by Mr. Loomis* of the Seattle food and drug inspection laboratory.

* Bureau of Chemistry --- Bulletin No. 137 Page 69.
Mix 15 cc of the solution to be tested with 2 cc of a 5 per cent solution of zinc chloride; add 2 cc of a 2 per cent potassium hydroxide solution, and filter. Wash the precipitate with hot water, dissolve on the filter in 15 cc or less of hot acetic acid, nearly neutralize, concentrate to one half its original volume, filter if necessary, and divide into two parts. From this point, follow the Woodman-Newhall test in all its details.

IDENTIFICATION OF CARAMEL BY MEANS OF TANNIC ACID.*

Dissolve 1 gram of tannic acid in 30 cc of water and .75 gr. of sulphuric acid, and make up to a volume of 50 cc. For vanilla extract, add 5 cc of the above reagent to 5 cc of the extract, heat until the precipitate first formed is almost dissolved, and set aside for 17 hours. A light or dark-brown substance, according to the amount of caramel present, will separate out. For liquors evaporate the alcohol, take up with water, and proceed as above.

THE FURFURAL TEST FOR CARAMEL. #

This test is based on the distillation of the furfural in the caramel, and was especially used for the detection of caramel in beer and wine. The test is applied by neutralizing the free acids by the addition of magnesium carbonate, and distilling 100 cc until 75 cc have passed over. The distillate is then made up to 100 cc, and 20 cc are shaken with pure glacial acetic acid and 5 drops of analine. If caramel is present, a red coloration will be produced in 15 minutes. Distillation of 15 kinds of Italian beer and 21 samples of red wine showed that although traces of furfural were separated by direct distillation of the samples, the distillate

*Chemical Abstracts, 1911 739. # The Analyst 1912 37 18.
obtained when the liquid was neutralized with magnesium carbonate was quite free from furfural. On the other hand, when caramel was added to the neutralized samples the distillate invariably contained furfural.

THE BENZOYL CHLORIDE TEST FOR CARAMEL. #

The benzoyl chloride test, which was used in the identification of caramel coloring in straight whiskey and of which more shall be said in the experimental part of this work is made in the following manner. The sample to be tested is made slightly alkaline with sodium hydroxide, warmed, and shaken with benzoyl chloride until no further precipitation occurs. The solution is then filtered, the precipitate boiled first with 95% alcohol and then with amyl alcohol and the solution again filtered. If caramel is present, the residue from the alcoholic washings will be soluble in hot glacial acetic acid, and reprecipitated by the addition of water.

Of the five tests discussed, three seem to give quite satisfactory results. These three are the Amthor test, the Woodman-Newhall test, and the Benzoyl Chloride test. Schidrowitz * has thrown some doubt on the use of the Amthor test, because he has shown that spirits which were apparently uncolored gave a precipitate, whereas a plain spirit colored with caramel prepared by himself gave no reaction.

# Unpublished work of Beal and Zoller.
* Journal Soc. Chem. Ind. 21, 816.
THE COLORING OF STRAIGHT WHISKEY.

The question of caramel coloring in whiskey has been of such importance that some very extensive work has been done in this country. The work of C.A. Crampton and L.M. Tolman* in this connection was carried out in the following manner.

Thirty barrels of new spirits were set aside in as many different ware-houses and from as many different distilleries, and a quart sample from each barrel was taken for analysis. Once a year in a period of eight years, a quart sample was taken. These samples were all set aside in glass containers, and a complete analysis made at the end of eight years. The results of the analyses of the 248 samples indicated that in no case was there even a trace of caramel coloring. The caramel tests employed in this work were the Amthor and the Marsh tests. The former has already been described, the latter is made in the following manner: To 5 cc of whiskey add 10 cc of a reagent prepared by adding 3 cc of phosphoric acid and 3 cc of water to 100 cc of amyl alcohol. Shake vigorously for a few minutes and allow to settle. With a pure whiskey the lower layer will be perfectly colorless, but if caramel is present the layer will be colored.

From the work of Crampton and Tolman, it would seem that the presence of caramel coloring in straight whiskey is out of the question. The work of Schidrowitz, however, throws some doubt upon the Amthor test; and, when one considers the fact that the Marsh test was made upon a 5 cc portion of the liquor, the question as to the delicacy of this test arises. They say that when caramel was added to their whiskies that a positive test was obtained by

the Marsh test. They do not, however, state how much they added. The presence of a large amount of caramel might give a positive test, whereas a small amount might not. It was therefore thought that, if a comparison of the different qualitative tests were made on solutions of pure caramels of different concentrations, a choice could be made of one which would be delicate enough to show the presence of very minute quantities of this color.
EXPERIMENTAL

PART I

THE PREPARATION OF PURE CARAMEL.

As already mentioned, the caramel of any sugar can be prepared by heating the carbohydrate at a temperature of 210° C. Since it is necessary to keep the temperature as constant as possible, this heating was carried out by immersing the flask in an oil bath. The first caramel prepared in this manner was that of dextrose. In this preparation a great deal of frothing occurred, and quite a large amount of water insoluble caramel was obtained. The preparation was therefore repeated, and during this second process the sugar was constantly stirred. This time much less frothing occurred and a much larger yield of water soluble caramel was obtained. The sugar was, however, not completely caramelized, and so the final step in the isolation of the desired product was to separate it from the unchanged sugar. Two methods were available for this separation. One could either destroy the sugar by fermentation or separate it by dialysis. The method employed in this work was that of dialysis. Several methods have been used for dialysis, and among these are found the use of parchment paper and collodion bags. Both of these methods were used in this work. To study the rate and completion of dialysis, tests were made for reducing sugar both inside and outside of the parchment and collodion bags. From these tests the following data was obtained.

Dialysis through collodion bags.

Changes in the dialysate.

At the end of 8 hours. Heavy precipitate of cuprous oxide.
At the end of 21 hours.  Medium precipitate of cuprous oxide.
At the end of 50 hours.  Slight precipitate of cuprous oxide.
At the end of 120 hours.  Faint precipitate of cuprous oxide.

Changes in the caramel solution.
At the end of 8 hours.  Heavy precipitate of cuprous oxide.
At the end of 21 hours.  Heavy precipitate of cuprous oxide.
At the end of 50 hours.  Heavy precipitate of cuprous oxide.
At the end of 120 hours.  Some reduction of the Fehling solution.

Dialysis through parchment bags.
Changes in the dialysate.
At the end of 8 hours.  Medium precipitate of cuprous oxide.
At the end of 10 hours.  Medium precipitate of cuprous oxide.
At the end of 50 hours.  Medium precipitate of cuprous oxide.
At the end of 58 hours.  Medium precipitate of cuprous oxide.
At the end of 72 hours.  Faint precipitate of cuprous oxide.

Changes in the caramel solution.
At the end of 8 hours.  Heavy precipitate of cuprous oxide.
At the end of 10 hours.  Heavy precipitate of cuprous oxide.
At the end of 50 hours.  Heavy precipitate of cuprous oxide.
At the end of 58 hours.  Heavy precipitate of cuprous oxide.
At the end of 4 weeks.  Faint precipitate of cuprous oxide.

The color of the dialysate from the parchment bags was much lighter than that from the collodion bags. The amount of caramel obtained by using parchment was somewhat larger than that obtained in the other method of dialysis, but, since the former required so much more time than the latter, collodion bags were used in the purification of the caramel prepared after this preliminary work.
PART II

A STUDY OF THE QUALITATIVE TESTS FOR CARAMEL.

After a sufficient amount of the carameis of both dextrose and sucrose had been prepared, experiments were made to determine which of the qualitative tests was the most delicate. These tests were made on solutions whose concentrations were 1%, .1%, and .01%. The methods employed were the Amthor test, the Woodman-Newhall test, and the Benzoyl Chloride test.* The results of these experiments were as follows:

The Amthor test.
On 5 cc of a 1% dextrose caramel solution. A positive test.
On 5 cc of a .1% dextrose caramel solution. A positive test.
On 5 cc of a .01% dextrose caramel solution. A negative test.

The Woodman-Newhall test.
On 5 cc of a 1% dextrose caramel solution. A positive test.
On 5 cc of a .1% dextrose caramel solution. A positive test.
On 5 cc of a .01% dextrose caramel solution. A positive test.

The Benzoyl Chloride test.
On 5 cc of a 1% dextrose caramel solution. A positive test.
On 5 cc of a .1% dextrose caramel solution. A positive test.
On 5 cc of a .01% dextrose caramel solution. A negative test.

The above experiments were repeated on solutions of sucrose caramel of the same concentrations used above, and the results obtained were identical with those of the dextrose caramel. These results show that the Woodman-Newhall test is the delicate test for caramel, and for this reason a further study of the test was made.

* See pages 2 and 4 for the details of the tests.
On account of the reaction of caramel with phenyl hydrazine, it seems quite probable that the sugar group still exist in the caramel molecule and that the compound obtained by this reaction might be either the hydrazone or the osazone. With this in mind, a study was made of the compounds resulting from the reaction between phenyl hydrazine and the caramels of dextrose and sucrose. The same method employed in the preparation of the osazones of sugars was used in this case. The best yield was obtained by dissolving in water one gram of the caramel and in another portion of water a mixture of 2 grams of phenyl hydrazine hydrochloride, which had been purified by washing in alcohol and ether, and 3 grams of fused sodium acetate. After filtering both solutions, they were mixed, and added to 30 cc of water. The mixture was then placed in a bath of boiling water for 45 minutes, cooled, and filtered. The dark-brown compound obtained in this manner was found to be insoluble in water, completely soluble in hot glacial acetic acid, and partly soluble in hot 95% alcohol. On the addition of water to the acetic acid and alcoholic solutions reprecipitation occurred. The residue from the alcohol was light-brown in color, while that from the acid was dark cherry red. The product soluble in the acid was far in excess of that soluble in the alcohol.

Melting point of the alcohol soluble substance.

Melting point of the alcohol soluble compound from dextrose caramel 170-175 °C.

Melting point of the alcohol soluble compound from sucrose caramel 175-180 °C.

The compounds soluble in acetic acid did not melt when heated to 360 °C.

The above experiment was carried out on caramels prepared
at different times, and, in order to determine whether or not the compound soluble in acetic acid and insoluble in alcohol was constant with caramels of the same sugar prepared at different times, analyses of total nitrogen by means of the absolute method of Dumas were made. The results of these experiments are as follows:

Total nitrogen in dextrose product No.1 6.89% 7.07%
Total nitrogen in dextrose product No.2 6.62% 6.92%
Total nitrogen in sucrose product No.1 7.71%
Total nitrogen in sucrose product No.2 4.89% 4.67%

Since the above work shows that the compounds obtained with phenyl hydrazine are quite constant with dextrose caramels prepared at different times, the following work was done for the purpose of obtaining a modification of the Woodman-Newhall test.

The original test, which is described on page 2, was carried to completion, and the dark-brown precipitate washed first with hot 95% alcohol and then with hot glacial acetic acid. The alcoholic solution was light-yellow in color, while that of the acetic acid was dark cherry red. When water was added to both of these solutions, reprecipitation occurred. The product obtained by reprecipitating the acid solution did not melt when heated to a temperature of 360 C. These results were obtained with solutions of dextrose and sucrose caramels, and are sufficiently constant to be used as confirmatory tests in the Woodman-Newhall test.

Since it has been shown that the Woodman-Newhall test is the most delicate of the three important tests for caramel, and since the confirmatory test described in the preceding paragraph is desirable; the following method was used in the qualitative
estimation of caramel in the subsequent work of this research:

To 15 volumes of the solution to be tested add 2 volumes of a 5 per cent solution of zinc chloride, and 3 volumes of a 2 per cent solution of potassium hydroxide. Filter, wash the precipitate with hot water, and dissolve by allowing the fumes of boiling glacial acetic acid to condense on the filter paper. Neutralize, add an equal part of a mixture of 2 parts of phenyl hydrazine hydrochloride, 3 parts of fused sodium acetate, and 20 parts of water. Allow the mixture to stand over night, filter, wash the precipitate with boiling 95% alcohol, and dissolve the residue in boiling glacial acetic acid. The filtrate from the alcohol should be light yellow, while that from the acetic acid should be dark cherry red. To the latter add 5 volumes of water and a small amount of sodium chloride. A dark-brown precipitate which does not melt when heated to 360°C indicates the presence of caramel coloring.
PART III

THE QUALITATIVE DETERMINATION OF CARAMEL IN DISTILLED SPIRITS.

The work on the qualitative determination of caramel in distilled spirits was carried out because it has been shown that the paraldehyde test, which was so extensively used in the work of Crampton and Tolman, does not give accurate results, and because it is not as delicate as the Woodman-Newhall test.

Blank tests were first made on 50% alcohol, zinc chloride, sodium hydroxide, and acetic acid. In each case these tests gave only a faint turbidity with the Woodman-Newhall reagent. The color of straight whiskey comes from the char of the oak barrel in which it has been stored, and, since tannins are always present in oak wood, a blank test was made on a 1% tannin solution. In this case a faint yellow precipitate was obtained. The test was next applied to certain distilled liquors with the following results:

- Whiskey of unknown origin. Heavy brown precipitate.
- Straight whiskey. Medium brown precipitate.
- Blended whiskey. Heavy brown precipitate.

Since every one of the above whiskies gave tests which seemed to indicate the presence of caramel coloring, it was decided to make a further study of the whiskey color. Since this color comes from the char of the oak barrel, a stave of one of these barrels was obtained, charred, and extracted for 72 hours with a solution of a boiling sour mash. The extract was concentrated on the water bath and tested for with the Woodman-Newhall modification described on page 12. A dark-brown precipitate was obtained with the phenyl hydrazine. The solubilities of this compound
were identical with those mentioned in the modification. There was, however, not sufficient material for a melting point determination, and, since this was very desirable, it was decided to repeat this work using larger amounts of the alcohol and oak stave. The method of extraction was as follows:

One half of the oak stave was completely charred, ground up in a mortar, and placed in a 5 liter flask. Two liters of 50% alcohol were added, and the flask set aside for 4 months. At the end of this time, the extract was was concentrated to 750 cc and filtered.

300 cc of this extract were tested for caramel, and as in the preceding case a dark-brown precipitate with solubilities identical with those mentioned in the Woodman-Newhall modification was obtained. The melting point of the product soluble in acetic acid was 178°C. Its color was brownish-yellow.

In concluding this experimental work, a more detailed study of the coloring of a straight whiskey was made. A sample of 14 liters was taken from a barrel marked "Straight Whiskey", the liquor evaporated to 750 cc, made up to 1100 cc, and filtered. 200 cc of this concentrated solution were benzoylated, and the resulting brown product washed with amyl and ethyl alcohol. The residue was quite soluble in hot glacial acetic acid, but could be reprecipitated by the addition of water. According to the Benzoyl Chloride test described on page 4, caramel coloring is present in the straight whiskey. As a confirmatory test, the reprecipitated product was saponified with dilute alkali, and the dark-brown solution tested for caramel by means of the Woodman-Newhall modification. Although this test responded both in solubility and color reactions, there was not sufficient product for a melting point determination.
As a final test, 300 cc of the concentrated extract were treated with an excess of zinc chloride. The color, which was precipitated in this manner, was dissolved by means of hot glacial acetic acid, and the dark colored solution neutralized with dilute alkali. At this point a precipitate soluble in 95% alcohol was obtained. A small portion of the neutralized solution was treated with an excess of 95% alcohol, and tested for caramel. Since a positive test was obtained, the remainder of the solution was treated in the same way. The solubility and color reactions of the dark-brown precipitate obtained were identical with those obtained with solutions of pure caramel. Furthermore the product reprecipitated from acetic acid did not melt when heated to 360°C.
SUMMARY.

The method of preparing pure caramels as described by Beal and Zoller has been repeated with satisfactory results.

The methods for the identification of caramel have been studied in detail, and the benzoyl test and a modification of the Woodman-Newhall test found to be the most conclusive.

A combination of the two tests just mentioned gives the most conclusive results.

By these tests caramel has been detected in straight whiskey.

By these tests caramel like bodies have been detected in alcoholic extracts of charred oak wood, and the presence of caramel in straight whiskey attributed to the charred barrels in which the whiskey is aged.