Colorimetric Determination of Betaine in Glutamate Process End Liquor

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Existing methods for the determination of betaine in glutamate process end liquor were not sufficiently simple, reproducible, and accurate. This led to a systematic investigation of the colorimetric method, which is based upon measuring the color of the reineckate ion in 70% acetone at 525 mμ after the betaine as the reineckate has been separated from an acid solution of the sample. Optimum conditions for the reproducible precipitation of betaine reineckate have been established for a concentration range of 1 to 5 mg. of betaine, and techniques for washing and dissolving the precipitate and measuring the color have been clarified. Reproducible standard curves were obtained and after a preliminary carbon treatment it was possible to obtain reproducible and accurate results on the end liquor. The procedure provides a simple and accurate control method for the determination of betaine and should be applicable to other material from natural sources.

THE END LIQUOR FROM GLUTAMATE processes which use waste products from the sugar beet industry as a raw material contains a large amount of betaine (trimethylglycine). Because this end liquor is sold for cattle feed and there is interest in the nutritional value of betaine, a control method was needed for the determination of the betaine in this material.

It has been the practice to determine betaine by a procedure first described by Stanek (*5*), which is based upon the precipitation of betaine with potassium triiodide, followed by a titration with thiosulfate or a Kjeldahl nitrogen determination. More recently methods have been based upon the precipitation of betaine reineckate, first suggested as a gravimetric procedure by Strack and Schwaneberg (*6*).

Walker and Erlandsen (*7*) dissolved the betaine reineckate in aqueous acetone, removed the reineckate ion by precipitation with silver nitrate, and titrated the betaine nitrate with sodium hydroxide. The method was applied with satisfactory results to sugar beet diffusion juices. Results obtained on glutamate end liquor with this procedure were not entirely satisfactory. The principal objection was the difficulty of removing all traces of the inorganic acid from the betaine reineckate precipitate and the usual inherent problems that arise in titrating with dilute alkali.

Bandelin and Pankratz (*1*) and Parnöja (*4*) described colorimetric procedures that called for measuring the red color of the reineckate ion in the aqueous acetone solution. The first authors used the method for the determination of betaine in the presence of large amounts of choline. The choline was removed as the reineckate in alkaline solution prior to precipitating the betaine reineckate in acid solution. The latter author applied the method to deproteinized beet press juice.

A considerable amount of time was spent in an effort to develop a gravimetric procedure using the betaine reineckate precipitation. The results confirmed the conclusion of Walker and Erlandsen that it is impossible to isolate betaine reineckate quantitatively and at the same time analytically pure. However, the authors support the observation of Parnöja, that the same is not necessarily true for a colorimetric procedure. Because of the greater sensitivity of the colorimetric method, lower sample concentrations can be used. Thus, the coprecipitation of small amounts of other insoluble reineckates and the Reinecke salt can be avoided.

It was established that with a preliminary carbon treatment to remove the colored and colloidal substances, the colorimetric procedure could be applied to glutamate end liquor samples. The results obtained with the procedures described above were not reproducible. Therefore, a systematic investigation of the choice of conditions for the precipitation of betaine reineckate and subsequent operations for measuring the color of the reineckate ion was carried out. This paper describes the results of the investigation and the application of the method to glutamate process end liquors.

Materials and Apparatus

Process Samples. The process samples used in this investigation were either process end liquor from glutamate production at Ac'cent International, San Jose, Calif., or slightly diluted end liquor which is sold as cattle feed under the trade-mark MC-47.

Ammonium Reineckate Solution. The ammonium reineckate solution used for the precipitation of the betaine was prepared from ammonium reineckate monohydrate, Eastman No. 3806, by adding 100 ml. of water to 1.50 grams of the salt and adjusting to pH 1.0 with hydrochloric acid. The solution was stirred at room temperature for 45 minutes and filtered. This reagent must be prepared just prior to use, because it decomposes upon standing, which leads to lower values for betaine.

Acetone. Reagent grade acetone was used. It was diluted to 70% by volume with water.

Ether Wash Solution. Reagent grade ether free from alcohol was used. It was diluted with 1 ml. of water per 140 ml. of ether.

Carbon. Darco KB from the Darco Corp. was used.

Standard Betaine Solutions. The standard betaine solutions were prepared from recrystallized betaine hydrochloride.

Instrument. A Beckman Model DU spectrophotometer equipped with 1-cm. Corex cells was used in this investigation. Presumably, any spectrophotometer or a colorimeter equipped with a proper filter could be used.

Procedure

Preparation of Standard Curve. Pipet 5-ml. aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 mg. per ml. of standard betaine solutions into 30-ml. beakers. Place the beakers in a crushed ice bath and chill for 10 to 15 minutes. Add 5 ml. of the ammonium reineckate solution to each beaker dropwise from a buret with constant swirling, return the beakers to the crushed ice bath, and set the bath in a refrigerator for at least 3 hours. Remove the beakers one at a time from the refrigerator, suspend the crystals in the mother liquor by gentle swirling and filter the suspension with suction on fritted-glass crucibles of medium porosity. Rinse the beaker and the crystals with three 2-ml. portions of the ether wash solution and allow excess ether to evaporate from the crystals. Dissolve the betaine reineckate in the crucible with three 5-ml. portions of the 70% acetone solution, collect the filtrate in a 25-ml. volumetric flask, and make up to volume with 70% acetone. Transfer a portion of this solution to the absorption cell and measure the absorbance at 525 mμ, using 70% acetone as a reference solution. Correct the readings for a reagent blank, which has been carried through the same procedure, plot the corrected readings against milligrams of betaine, and draw the best straight line through the points.

Because of the slight solubility of the betaine reineckate in the mother liquor, the line does not pass through the origin but intersects the abscissa at a point between 0.1 and 0.2 mg. of betaine. The slope of the line shows an absorbance of 0.045 per mg. of betaine and the data are reproducible to ±0.06 mg. of betaine.

Process Samples. Dilute 10 grams of sample to exactly 100 ml. with water. Pipet a 5-ml. aliquot into a 50-ml. beaker, add 35 to 40 ml. of water, and adjust to pH 1 with hydrochloric acid. Add about 0.25 gram of carbon and heat nearly to boiling with occasional stirring. Filter the solution hot and wash the beaker and carbon cake with five 7-ml. portions of hot water. After cooling, adjust the filtrate and washings to pH 1.0 with hydrochloric acid, transfer to a 100-ml. volumetric flask, and make up to volume with water. Take a 5-ml. aliquot of this solution and continue the procedure as directed for preparation of the standard curve.

The percentage of betaine in the original sample is calculated by estimating the milligrams of betaine from the standard curve.

Experimental Results

Precipitation Time and Temperature. It was found that the times and temperatures recommended elsewhere for the complete precipitation of betaine reineckate were not adequate for conditions described in the above procedure. It is also probable that incomplete control of these variables accounted for some of the nonreproducibility of standard curves reported by Bandelin and Pankratz (*1*). Figure 1 illustrates that a 3-hour precipitation time is required for equilibrium to be established at about 0° C. on a sample precooled 2° C. before the addition of the ammonium reineckate precipitant. A duplicate sample that was precooled to 6° C. took 1 to 2 hours longer to reach equilibrium. Therefore, the temperature of the sample prior to precipitation must be considered before a time is established for the complete precipitation of betaine reineckate.

Chilling the sample to 2° C. before the addition of the ammonium reineckate and a standing time of 3 hours in a crushed ice bath were chosen as satisfactory conditions for obtaining reproducible results.

Table I. Stability of Betaine Reineckate Color

	Absorbance		
Sample, Mg.	1 to 2 hours after preparation	18 hours later	Loss, %
1	0.036	0.031	13.9
3	0.123	0.108	12.2
5	0.215	0.192	10.7
End liquor	0.112	0.100	10.7

Table II. Effect of Sample Size on Results from a Glutamate Process End Liquor

Sample Size, Ml.	Betaine, Wt. %
1	10.3
2	9.9
3	10.0
4	10.1
5	10.0

Table III. Recovery of Betaine Added to Glutamate Process End Liquor

	Betaine, Mg.			Recovery,
Sample	Present[a]	Added	Found	%
1	2.66	1.00	3.63	97
2	2.63	1.00	3.67	104
	2.63	1.00	3.69	106

[a] Colorimetric analysis.

Table IV. Analysis of Glutamate Process Samples

Sample	Betaine, Wt. %
1	9.9
	9.8
2	9.9
	10.2
3	9.8
	10.2
4	10.1
	9.9
5	10.4
	10.7

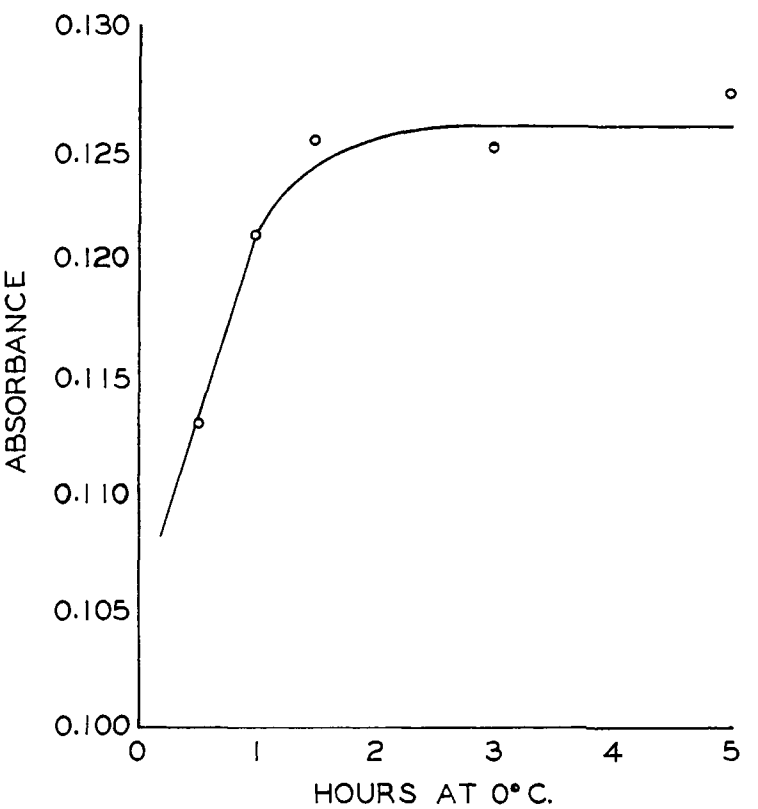

Figure 1. Time required for complete precipitation of betaine reineckate at 0° C

3 mg. of standard precooled to 2° C. before addition of ammonium reineckate

Ether Wash Solution. The addition of the small amount of water to the anhydrous reagent grade ether had no effect upon the results from synthetic betaine standards. This was not so when water was added to U.S.P. ether. Apparently the small amount of alcohol present in the U.S.P. ether combines with the water to dissolve some of the betaine reineckate. The wash solution of anhydrous ether with a small amount of water was chosen because it was somewhat easier to transfer the crystals and displace the mother liquor with this solution.

Stability of Betaine Reineckate Color. The data in Table I show that 10 to 14% of the betaine reineckate color in acetone is lost over an 18-hour period. Therefore, it is advisable to measure the absorbance of the samples within 2 hours after the betaine reineckate has been dissolved in 70% acetone.

Effect of Sample Size and Recovery of Added Betaine. Experiments on sample size and recovery were carried out on an end liquor sample (Tables II and III). The results illustrate that a linear relationship exists between the sample size and color being measured over the range of sample concentrations tested, and that satisfactory recovery of known added amounts of betaine can be obtained. Therefore, it can be concluded that other compounds present in the original material have very little if any effect upon the results. The absence of an insoluble reineckate precipitate in an alkaline solution of these samples and a negative test for amino acids on the precipitate by paper chromatography substantiate this conclusion.

Analysis of Process Samples. The results of duplicate analyses on several process samples are given in Table IV. These samples were analyzed on the same day by one analyst and a standard curve was prepared at the same time. Therefore, they illustrate the degree of precision that can be obtained by the method. It can be concluded that the method will give reliable and consistent results on the process samples tested.

Discussion

As the exact composition of the betaine reineckate precipitate is not known, the method is empirical. Therefore, it is necessary to prepare a standard curve under the same conditions used for unknowns.

The method is not specific, since many organic bases of high molecular weight are precipitated by Reinecke salt in acid solution (*2*, *3*, *8*). It is particularly applicable to samples containing a considerable amount of betaine, where interferences can be avoided by diluting. However, as shown in the work on mixtures of betaine and choline (*1*), many interferences can be avoided by a preliminary precipitation of reineckates insoluble in alkaline solution.

The method is especially suited for routine control, as it is simple and capable of yielding results with a fair degree of precision. With proper regard to standardization and interferences it should find considerable use as a method for betaine in other material from natural sources.

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FROZEN FOODS STORAGE EFFECTS

Formation of Alcohol, Acetaldehyde, and Acetoin in Frozen Broccoli Tissue

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The carboxylase activity of broccoli tissues was investigated to determine the role of pyruvic carboxylase in the production of volatile aldehydic and ketonic compounds which might serve as precursors of the off-flavors developing in underscalded frozen broccoli. Broccoli carboxylase was found to catalyze synthesis of acetoin and diacetyl chiefly from added pyruvate and to a much smaller extent from acetaldehyde, unlike pea and wheat germ carboxylase. Acetaldehyde inhibited broccoli carboxylase activity. In frozen broccoli, both inhibition by and restricted diffusion of acetaldehyde would favor production of acetoin. The concentration of acetaldehyde, acetoin, or diacetyl was not related to the organoleptically objectionable formation of off-flavors, whereas ethyl alcohol content was related to extent of off-flavor.

INVESTIGATIONS on the accumulation of acetaldehyde, ethyl alcohol, and related products in broccoli during freezing storage (*3*) were continued, with particular emphasis on pyruvic carboxylase activity. These investigations of the nature of the biochemical reactions which result in abnormal flavors in improperly scalded, frozen vegetables also led to a study of the behavior of pyruvic carboxylase in situ. The immature floral shoot of sprouting broccoli was used as the source of the enzyme system. Additional data on the accumulation of ethyl alcohol and acetoin are reported here and in more detail in the thesis of Buck (*2*).

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Materials and Methods

Commercially grown Italian green sprouting broccoli (whose structure is shown in Figure 1), packed in crates with ice, was obtained from a local wholesale distributor and stored at 1° C. The broccoli was obtained in several lots in order to ensure fresh tissues at the time of utilization. Broccoli shoots were