QUANTITATIVE DETERMINATION OF UREA, THIOUREA, AND CERTAIN OF THEIR SUBSTITUTION PRODUCTS BY SPECTROPHOTOMETRIC TECHNIQUES

by

RUSSELL CARL HOSENEY

B. S., Kansas State University, 1957

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1960

TABLE OF CONTENTS

INTRODUCTION

A new approach to studying protein quality by Finney (7, 8) is based on the ability of the wheat plant to take up, translocate, and synthesize into gluten protein the urea nitrogen sprayed on the leaves (9).

The root system of the wheat plant seems to do a fairly good job of regulating the uptake of many important elements such as sulfur that occurs in cystine and cysteine of gluten protein and is associated with such important dough properties as degree of extensibility and oxidation requirement. Finney (8) points out that foliar spraying possibly permits the uptake, translocation, and incorporation of important elements in the gluten protein complex in concentrations much higher than normally occur, and possibly permits incorporation of specific nitrogen groups in the protein molecule.

In previous foliar spraying experiments (8), sulfur and nitrogen in the form of thiourea, N,N'-diethylthiourea, and ethylenethiourea were applied to the leaves of the wheat plant prior to and/or at heading. Protein contents of the flours were increased by 2.5 to 4.5 per cent. Total sulfur contents of the flours were increased about 20 per cent above those for flours milled from wheats that were sprayed with ammonium nitrate and such nonsulfur-containing ureas as N,N'-dimethylurea, N,N-diethylurea, and urea.

The materially sub-normal physical and baking properties of some wheats compared to the normal and above normal properties of others suggested that certain ureas had been synthesized to varying degrees into gluten protein, thereby pointing to the need of a sensitive quantitative method for determining residual quantities, if any, of the ureas and thioureas in the wheats and flours involved. Residual amounts of certain ureas and thioureas possibly could account for the below as well as above normal properties of certain

wheats and flours. Accordingly, in the present study, two spectrophotometric methods for the quantitative determination of ureas have been investigated, one using p-dimethylaminobenzaldehyde, and the other diacetylmonoxime.

REVIEW OF LITERATURE

Reaction with p-Dimethylaminobenzaldehyde

The classical method for determining urea involves its hydrolysis with the enzyme urease followed by determining the ammonia produced by nesslerization or by the Kjeldahl procedure. This method is clearly inappropriate for the present study because of the extreme specificity of urease which attacks only urea.

A color reaction of urea with Ehrlich's aldehyde reagent, a solution of p-dimethylaminobenzaldehyde in hydrochloric acid, was first described by Barrenscheen (3). He found that the reaction used one mole of urea with one mole of p-dimethylaminobenzaldehyde, and that it was impossible to obtain higher condensation products. The bright yellowish-green product, formed by the direct addition of the acid to form a salt, was found to be relatively unstable. He found that urea, methylurea, and phenylurea gave the same color and similar crystallization characteristics.

Systematic investigation of other substituted ureas by Barrenscheen (3) showed that an unbound amino group was essential for the reaction. Barrenscheen found that thiourea reacted to give colored monoclinic needles that hydrolyzed very easily. He found that increasing the acid concentration led to almost a complete loss of color, and that the compound formed with sulfuric acid did not have as sharp a melting point as that with hydrochloric acid, but did not

hydrolyze as easily. Barrenscheen failed in his attempt to use the reaction for the quantitative determination of urea because the colored compounds readily were hydrolyzed.

The reaction appears to have been ignored until two Frenchmen, Pesez and Petit (16), found that hydrazine reacted with p-dimethylaminobenzaldehyde to give an azine of intense red-orange color which was directly proportional to the hydrazine concentration. The colored compound was soluble in mineral acids and was stable after reacting for 10 to 15 minutes. They also observed that urea reacted slightly.

Watt and Crisp (18) published a spectrophotometric method for the determination of hydrazine based on the observation by Pesez and Petit. A characteristic color was developed upon the addition of a solution of p-dimethylaminobenzaldehyde in ethyl alcohol and hydrochloric acid solution. In the course of their work on the determination of hydrazine in the presence of urea, they found that solutions containing urea and p-dimethylaminobenzaldehyde exhibited a broad transmittancy minimum at 420 mμ.

In a later publication Watt and Crisp (19) describe a spectrophotometric method for urea with p-dimethylaminobenzaldehyde. The color reagent, the same as that used for hydrazine, contained p-dimethylaminobenzaldehyde 2.000 g, 95 per cent ethyl alcohol 100.0 ml, and concentrated hydrochloric acid 10.0 ml. After adding ten milliliters of this reagent to appropriate urea aliquots, the mixture was diluted to a total volume of 25 ml with distilled water. The optimum urea concentration range of 50 to 240 ppm corresponds to 63 to 23 per cent transmittance, or 4.7 ppm per unit of transmittance.

Several workers have used this method successfully without major modification. Cline and Fink (5) used the reaction to locate urea spots on paper chromatograms. They found the reaction to be equimolar, thereby substantiating

the earlier work of Barrenscheen. Brown (4) adapted the reaction to determining blood urea. Jongen and Berkhaut (10) used the reaction to determine urea in feed and fertilizer. They used a water extract that was clarified and decolorized in one step by Carrez[1] solution and charcoal.

The preceding review of the literature suggested that the reaction between urea and p-dimethylaminobenzaldehyde might provide a method for the determination of urea, thiourea, and their monosubstituted derivatives. In the present study, however, it appeared advisable to investigate the possibility of increasing the sensitivity of the reaction by decreasing the range. It was evident that the reaction would not provide a method for the determination of disubstituted ureas and thioureas. Therefore a further search of the literature was undertaken.

Reaction with Diacetylmonoxime

Fearon (6) reported that a color was produced with urea by strongly acidifing with hydrochloric acid, adding a few drops of 3 per cent diacetylmonoxime, and boiling for 3 to 4 minutes. He obtained a positive reaction with urea, methylurea, butylurea, phenylurea, betanaphthurea, dimethylurea, allantoin, semicarbazide, citrulline, and all higher proteins examined. He failed to obtain a positive reaction with ammonium salts, hydrazine, carbamate, cyanate, acetamide, acetylurea, diphenylurea, guanidine, creatine, creatinine, glycocyamine, uroxanic acid, uric acid, indole, and all amino acids examined. On the basis of these tests he concluded that the reaction was positive with compounds

---

[1] A solution of 180 g lactic acid in 200 ml potassium hydroxide solution (sp. gr. 1.332) and 200 ml water; after boiling, and neutralization with acid or alkali, as required, it is mixed with a solution of 34.65 g cupric sulfate crystals in 250 ml water, and diluted to 1 liter.

containing the system RNHCONHR', where R was either hydrogen or a simple aliphatic radicle, and R' was not an acyl radicle. Fearon stated that the color was improved when a drop of 1 per cent potassium persulfate was added.

The method was modified by Barker (2) working with urea in blood and urine. His modification employed 0.25 ml of a 3 per cent solution of diacetylmonoxime, 4.0 ml of 50 per cent sulfuric acid, and 10 minutes in a boiling water bath, followed immediately by adding 0.25 ml of 1 per cent potassium persulfate. Spectrophotometer readings had to be taken exactly 15 minutes after addition of the persulfate.

Further work on the reaction was done by Koritz and Cohen (12). They found that sulfur-containing compounds inhibited color formation, but that the inhibition could be overcome by adding more persulfate.

Ormsby (15), in studies with the diacetylmonoxime reaction, found that direct sunlight replaced the oxidizing agent in the production of color, but that the color thereby produced faded rapidly. Direct sunlight also faded the color produced with potassium persulfate.

Wheatly (20) suggested the use of sodium diphenylamine-p-sulfonate, an aromatic amine, which gave an orange-red color instead of the characteristic yellow color. Archibald (1) used α-isonitrosopropiophenone, a compound closely related to diacetylmonoxime, to determine urea. This reagent was more specific for urea than diacetylmonoxime.

Natelson, Scott, and Beffa (14) found that potassium persulfate enhanced the reaction color about 10 to 15 per cent, but also caused fading to the extent that if the developing color was allowed to stand for more than one-half to one hour, appreciable decrease in color intensity occurred even in the absence of light. They showed that the persulfate destroyed the hydroxylamine that was formed. They also found that hydroxylamine decreased the color

produced in the reaction of diacetyl and urea, and described a method using diacetyl instead of diacetylmonoxime. Diacetyl produced the same color as diacetylmonoxime, but the reaction time for maximum color was shorter.

The above method with diacetyl was modified by LeMar and Bootzin (13) to one that was analytical instead of clinical.

A rather thorough study of the reaction of urea with diacetylmonoxime was undertaken by Rosenthal (17) who worked on a modification by Kawerau (11) of using arsenic acid to destroy the hydroxylamine as it was formed. Rosenthal found optimum concentrations of 3.8 N for hydrochloric acid, 0.08 N for arsenic acid, and 0.25 per cent for diacetylmonoxime. He found that a heating time of 30 minutes in a boiling water bath produced the greatest intensity. The final color was somewhat photolabile, fading at the rate of approximately 5 per cent per hour.

METHODS AND MATERIALS

All spectrophotometric readings in this study were taken on a Beckman DU spectrophotometer equipped with an a.c. power supply and a photomultiplier tube. A matched set of corex cells having a path length of 0.999 cm was used throughout the study. All readings were taken at 25° C by means of a thermoregulated water bath that was attached to the spectrophotometer. All readings were taken at a slit width of 0.01 mm, corresponding to a nominal band width of 0.26 $m\mu$ at 430 $m\mu$.

The ethylenethiourea used in the study was practical grade recrystallized twice from water. The t-butylurea and 1,3-dimethylurea were practical grade recrystallized twice from 95 per cent ethyl alcohol. The other ureas used in the study were reagent grade and included the following: urea, thiourea,

methylurea, allylurea, phenylurea, acetylurea, phenylthiourea, allylthiourea, acetylthiourea, 1,3-diethylthiourea, 1,3-diphenylurea, 1,3-diphenylthiourea, 1,1-diphenylurea, 1,1-diphenylthiourea, and 1,3-diethyldiphenylurea. All other chemicals used in the study were reagent grade and used as received, except for drying which was accomplished by heating at 70° C in a vacuum oven overnight.

The analysis of flour containing added quantities of ureas were performed on a composite flour. The flour sample was composed of several varieties grown at a number of locations in the southern and central regions of the United States.

EXPERIMENTAL RESULTS AND CONCLUSIONS

Analysis of Ureas Using p-Dimethylaminobenzaldehyde

Choice of Solvents. Before studing the reaction of p-dimethylaminobenzaldehyde with the various ureas, the data of Watt and Crisp was first verified. Several of the monosubstituted ureas gave a color similar to that given by urea. The thioureas gave no color in the water solution.

In working with the reaction it was noticed that p-dimethylaminobenzaldehyde was only slightly soluble in water. With the addition of a strong acid, however, it became very easily soluble, but an excess of strong acid depressed the color reaction between urea and p-dimethylaminobenzaldehyde. Accordingly, 95 per cent ethyl alcohol was selected as a solvent more acidic than water and one in which p-dimethylaminobenzaldehyde was more soluble. Samples were dissolved in 95 per cent ethyl alcohol and diluted to volume with alcohol. Since hydrochloric acid contains a significant amount of water, sulfuric acid was tried and found to be equal to, if not better than hydrochloric acid.

The change to 95 per cent ethyl alcohol as a solvent increased the sensitivity or change in concentration per unit of transmittance. In addition, a positive reaction was obtained with thiourea which did not react in water.

The success of 95 per cent ethyl alcohol as a solvent warranted an investigation of a number of other solvents. Among those tried were absolute ethyl alcohol, n-propyl alcohol, i-propyl alcohol, sec-butyl alcohol, i-butyl alcohol, n-butyl alcohol, t-butyl alcohol, and acetic acid.

Absolute ethyl alcohol and n-propyl alcohol offered little if any advantage over 95 per cent ethyl alcohol. Difficulty was encountered in obtaining a color with the butanols. Iso-propyl alcohol and acetic acid both gave a severalfold increase in color intensity. The reaction with an acetic acid solvent was unstable, probably due to the instability of the urea compounds in the acetic acid standard solutions. Therefore, with one exception, i-propyl alcohol was used as the solvent.

Color Solutions. Color solution (A) consisted of 2.000 g p-dimethylaminobenzaldehyde, 100.0 ml i-propyl alcohol, and 0.5 ml concentrated sulfuric acid. Ten ml per 25 ml total volume was used.

Color solution (B) consisted of 2.000 g p-dimethylaminobenzaldehyde, 100.0 ml i-propyl alcohol, and 1.0 ml concentrated sulfuric acid. Ten ml per 25 ml total volume was used.

Color solution (C) consisted of 2.000 g p-dimethylaminobenzaldehyde, 150.0 ml glacial acetic acid, and 0.6 ml concentrated sulfuric acid. Fifteen ml per 25 ml total volume was used.

General Method. To an appropriate aliquot of the urea or thiourea solution, add 10 or 15 ml, depending upon the color solution used, into a 25 ml volumetric flask, dilute to volume, and place in a water bath at 25° C for 15 minutes. The color is stable for at least one hour. Extreme care must be taken to

exclude water from the reaction. Thus, all samples must be dried and flasks or pipettes either dried or rinsed with i-propyl alcohol.

Certain Ureas that React with p-Dimethylaminobenzaldehyde

Urea. For urea, 10 ml of color solution (A) was used. The range of 1.0 to 8.0 ppm for urea corresponded to a transmittance range of 80 to 12 per cent, or 0.103 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.16 ppm. Maximum absorbance occurred at 425 mμ (Figure 1). The standard curve for urea is given in Figure 2.

Methylurea. For methylurea, 10 ml of color solution (A) was used. Maximum absorbance occurred at 430 mμ (Figure 1). The range of 0.5 to 3.0 ppm for methylurea corresponded to a transmittance range of 68 to 11 per cent, or 0.044 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.01 ppm. The standard curve for methylurea is given in Figure 2.

t-Butylurea. For t-butylurea 10 ml of color solution (A) was used. The maximum absorbance occurred at 430 mμ (Figure 1). The range of 0.5 to 2.5 ppm for t-butylurea corresponded to a transmittance range of 69 to 11 per cent, or 0.034 ppm per unit of transmittance. The standard deviation of a simple determination (30 replications) was 0.007 ppm. The standard curve for t-butylurea is given in Figure 2.

Allylurea. For allylurea 10 ml of color solution (A) was used. The maximum absorbance occurred at 435 mμ (Figure 1). The range of 0.5 to 4.0 ppm for allylurea corresponded to a transmittance range of 73 to 11 per cent, or 0.056 ppm per unit of transmittance. The standard deviation of a single determination (29 replications) was 0.12 ppm. The standard curve for allylurea is given in

Figure 2.

Phenylurea. For phenylurea 10 ml of color solution (A) was used. The maximum absorbance occurred at 440 mμ (Figure 1). The range of 0.5 to 4.0 ppm for phenylurea corresponded to a transmittance range of 72 to 13 per cent, or 0.059 ppm per unit transmittance. The standard deviation of a single determination (30 replications) was 0.14 ppm. The standard curve for phenylurea is given in Figure 2.

Thiourea. For thiourea 10 ml of color solution (B) was used. The maximum absorbance occurred at 450 mμ (Figure 1). The range of 5.0 to 25.0 ppm for thiourea corresponded to a transmittance range of 72 to 11 per cent, or 0.328 ppm per unit transmittance. The standard deviation of a single determination (27 replications) was 0.44 ppm. The standard curve for thiourea is given in Figure 2. The somewhat photolabile color produced by the reaction of thiourea and p-dimethylaminobenzaldehyde was eliminated by using low actinic glass volumetric flasks.

Phenylthiourea. For phenylthiourea 10 ml of color solution (B) was used. The maximum absorbance occurred at 445 mμ (Figure 1). The range of 7.0 to 30.0 ppm for phenylthiourea corresponded to a transmittance range of 65 to 14 per cent, or 0.451 ppm per unit transmittance. The standard deviation of a single determination (25 replications) was 0.72 ppm. The standard curve for phenylthiourea is given in Figure 2.

Allylthiourea. For allylthiourea 10 ml of color solution (A) was used. The maximum absorbance occurred at 445 mμ (Figure 1). The range of 5.0 to 25.0 ppm for allylthiourea corresponded to a transmittance range of 64 to 12 per cent, or 0.385 ppm per unit transmittance. The standard deviation of a single determination (20 replications) was 0.32 ppm. The standard curve for allylthiourea is given in Figure 2. The photolabile color produced with

allylthiourea and p-dimethylaminobenzaldehyde faded appreciably within 30 minutes. By employing low actinic glass volumetric flasks, color was stable for over one hour.

Ethylenethiourea (2-Imidazolidmethione). Ethylenethiourea presented a special case, because the color intensity produced in a straight i-propyl alcohol solvent was too low, and that produced in an acetic acid solvent was rather unstable. Therefore, a combination of the two solvents was used. Thus ethylenethiourea previously dissolved in i-propyl alcohol was added to 15.0 ml of color solution (C), followed by dilution with i-propyl alcohol to a total volume of 25 ml. Maximum absorbance occurred at 450 mμ (Figure 1). The range of 10 to 40 ppm for ethylenethiourea corresponded to a transmittance range of 66 to 12 per cent, or 0.555 ppm per unit of transmittance. The standard deviation of a single determination (25 replications) was 1.21 ppm. The standard curve for ethylenethiourea is given in Figure 2.

Analysis of Flours Containing Added Quantities of Certain Ureas. Analysis for each of the ureas was made after adding a known amount of the urea in question to a composite flour sample. Samples to be analyzed were dried in a vacuum oven overnight at 70° C. A 2.000 g sample containing 12.5 mg of an urea or thiourea compound was agitated in a reciprocating shaker for one hour with 100.0 ml i-propyl alcohol. The sample was then filtered through Whatman no. 4 filter paper. Thereafter, analysis were made on aliquots of the filtrate. Data for the analyses are found in Table 1.

Table 1. Analyses of flours containing added quantities of certain ureas (p-dimethylaminobenzaldehyde reaction).

Compound	Quantity		Average Quantity	
	Added mg	Found mg	Found mg	Recovery %
Urea	12.5	12.74	12.67	101.4
	12.5	12.74		
	12.5	12.53		
Methylurea	12.5	12.89	12.77	102.2
	12.5	12.89		
	12.5	12.54		
t-Butylurea	12.5	12.11	12.02	96.4
	12.5	12.04		
	12.5	11.90		
Allylurea	12.5	13.31	13.29	106.3
	12.5	13.58		
	12.5	12.98		
Phenylurea	12.5	12.46	12.55	100.4
	12.5	12.48		
	12.5	12.72		
Thiourea	12.5	13.13	13.32	106.5
	12.5	13.48		
	12.5	13.35		
Phenylthiourea	12.5	12.39	12.31	98.5
	12.5	12.22		
	12.5	12.32		
Allylthiourea	12.5	12.17	12.12	97.0
	12.5	12.18		
	12.5	12.02		
Ethylenethiourea	12.5	12.13	12.80	102.2
	12.5	13.49		
	12.5	12.77		

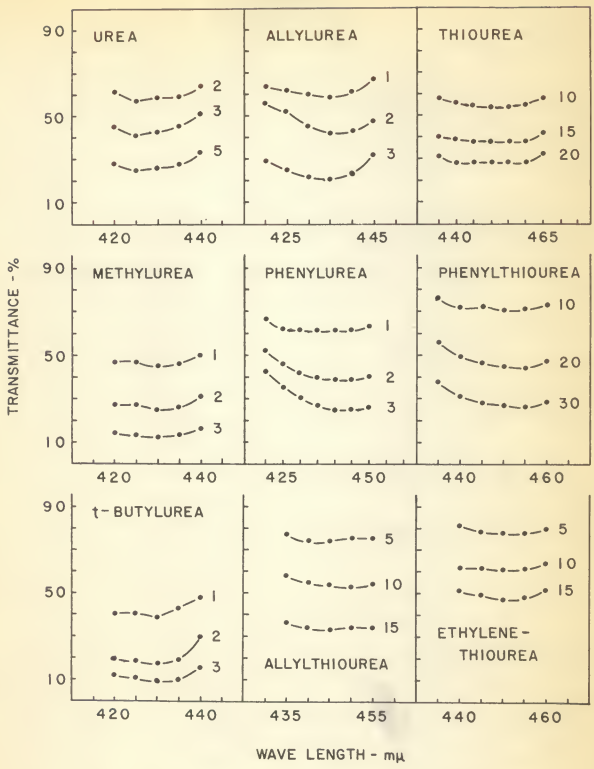


Fig. 1. Transmittance vs. wave length for nine ureas reacting with p-dimethylaminobenzaldehyde, each urea at three concentrations in ppm.

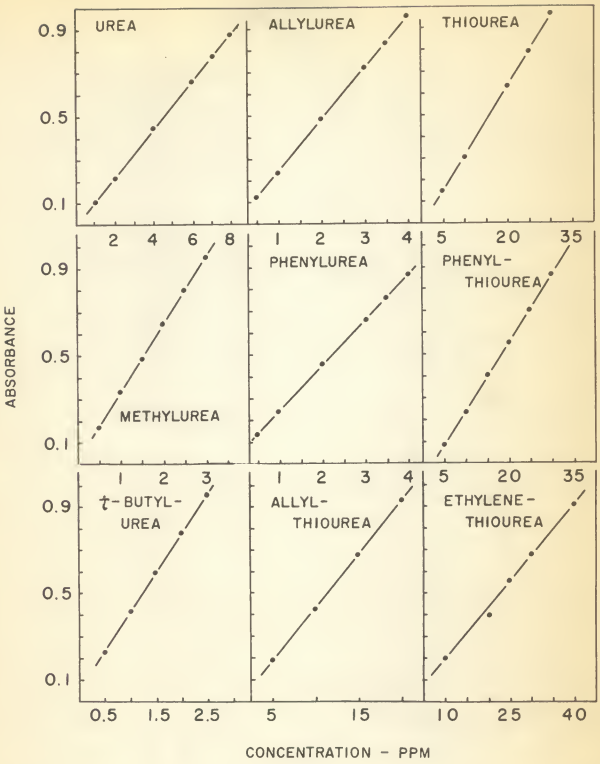
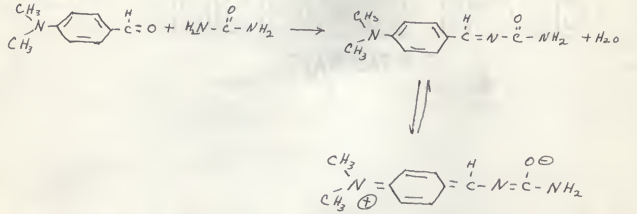


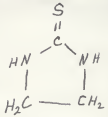
Fig. 2. Standard curves of concentration vs. absorbance at minimum transmittance for each of nine ureas reacting with p-dimethylamino-benzaldehyde.

p-Dimethylaminobenzaldehyde Reaction with Ureas

The mechanism of the reaction of urea with p-dimethylaminobenzaldehyde is well known.



Barrenscheen (3) stated that a compound must have an unbound amino group to react. The present study included six compounds, 1,3-dimethylurea, 1,3-diphenylurea, 1,3-diphenylthiourea, 1,3-diethyldiphenylurea and ethylenethiourea, that did not have an unbound amino group. Ethylenethiourea was found to be the only exception to the rule. Ethylenethiourea is a ring compound having the following structure:



Four compounds possessing an unbound amino group did not give the color reaction. They included 1,1-diphenylurea, 1,1-diphenylthiourea, acetylurea, and acetylthiourea. However, a color was noted with 1,1-diethylurea, the only disubstituted compound found to react. No method was established for 1,1-diethylurea because of the inability to obtain a sample of sufficient purity.

Although no attempt was made to apply the reaction with p-dimethylaminobenzaldehyde to samples other than wheat flour, there is no apparent reason why others, including feeds containing added ureas, should not work equally well, particularly if they can be dried and extracted with i-propyl alcohol. If the pigments in the feed interfere, Jongen and Berkhaut (10) described a method whereby the sample can be clarified and decolorized in one step.

Analysis of Ureas Using Diacetylmonoxime

Choice of Reagent. The study of the reaction between diacetylmonoxime with certain ureas was undertaken to see if it could be used to determine some of the disubstituted ureas and thioureas that did not react with p-dimethylaminobenzaldehyde. It appeared from a review of literature that the use of diacetyl as a reagent had the advantages of requiring no oxidizing agent and less heating time than for diacetylmonoxime. At the same time, the color produced was the same as that with diacetylmonoxime (14).

In preliminary experiments, no conditions were found whereby diacetyl gave a color of the same intensity as that with diacetylmonoxime. Diacetyl gave the same color and absorption curve, but only about one-tenth the color intensity as diacetylmonoxime. Apparently the more intense color with diacetylmonoxime can be attributed to a slow production of diacetyl and a continuous destruction of the generated hydroxylamine.

General Method. Diacetylmonoxime, 2.500 g, was dissolved in 100.0 ml of 5 per cent acetic acid. Concentrations of hydrochloric acid, arsenic acid, and diacetylmonoxime, together with heating time, varied with the compound under investigation. These details are specified as each individual compound is discussed. In general, 10 ml of acid mixture is added to each 25 X 200 mm reaction

tube, followed by 1.5 or 2.0 ml of diacetylmonoxime solution, and sufficient water to adjust the volume to 20-25 ml. Thereafter the tubes were immersed in a rapidly boiling water bath. After heating, the tubes were cooled in running water for 10 minutes, and the contents transferred to 25 ml volumetric flasks and diluted to volume with distilled water. Best results were obtained when readings were taken within one hour. Although no fading by light was encountered in this study, it appeared advisable to heat the tubes under subdued light.

Certain Ureas that React with Diacetylmonoxime

Urea. A suitable quantity of urea solution and 2.0 ml diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 60.0 ml concentrated hydrochloric acid, and 0.7354 g arsenic acid diluted to 100.0 ml (Figure 5). Heating time for maximum color production was found to be 40 minutes (Figure 4). Maximum absorbance occurred at 480 mμ (Figure 3). The useful range of 2.0 to 6.0 ppm corresponded to a transmittance range of 61 to 11 per cent, or 0.08 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.10 ppm. The standard curve for urea is given in Figure 6.

Acetylurea. A suitable quantity of acetylurea solution and 2.0 ml of diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 60.0 ml concentrated hydrochloric acid and 0.7354 g arsenic acid diluted to 100.0 ml (Figure 5). Maximum absorbance occurred at 485 mμ (Figure 3). Heating time for maximum color production was found to be 50 minutes (Figure 4). The useful range of 2.0 to 10.0 ppm corresponded to a transmittance range of 76 to 10 per cent, or 0.12 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.24 ppm. The standard

curve for acetylurea is given in Figure 6.

1,3-dimethylurea. A suitable quantity of 1,3-dimethylurea solution and 1.5 ml of diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 60.0 ml concentrated hydrochloric acid and 0.7354 g arsenic acid diluted to 100.0 ml (Figure 5). Maximum absorbance occurred at 550 mμ (Figure 3). Heating time for maximum color production was 20 minutes (Figure 4). The useful range of 10 to 30 ppm corresponded to a transmittance range of 63 to 19 per cent, or 0.45 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.75 ppm. The standard curve for 1,3-dimethylurea is given in Figure 6.

Thiourea. A suitable quantity of thiourea solution and 2.0 ml diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 60.0 ml concentrated hydrochloric acid and 2.941 g arsenic acid diluted to 100.0 ml (Figure 5). Maximum absorbance occurred at 480 mμ (Figure 3). Heating time for maximum color production was 45 minutes (Figure 4). The useful range of 4.0 to 16.0 ppm corresponded to a transmittance range of 75 to 13 per cent, or 0.21 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.43 ppm. The standard curve for thiourea is given in Figure 6.

Acetylthiourea. A suitable quantity of acetylthiourea solution and 2.0 ml diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 70.0 ml of concentrated hydrochloric acid and 0.7354 g arsenic acid diluted to 100.0 ml (Figure 5). Maximum absorbance occurred at 485 mμ (Figure 3). Heating time for maximum color production was 35 minutes (Figure 4). The useful range of 5.0 to 25.0 ppm corresponded to a transmittance range of 80 to 15 per cent, or 0.31 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 0.48 ppm. The standard curve for

acetylthiourea is given in Figure 6.

1,3-diethylthiourea. A suitable quantity of 1,3-diethylthiourea solution and 2.0 ml diacetylmonoxime solution were added to 10 ml of an acid mixture composed of 60.0 ml concentrated hydrochloric acid and 1.103 g arsenic acid diluted to 100.0 ml (Figure 5). Maximum absorbance occurred at 510 mμ (Figure 3). Heating time for maximum color production was 30 minutes (Figure 4). The useful range of 100 to 500 ppm corresponded to a transmittance range of 77 to 14 per cent, or 6.35 ppm per unit of transmittance. The standard deviation of a single determination (30 replications) was 14.18 ppm. The standard curve for 1,3-diethylthiourea is given in Figure 6.

Analysis of Flours Containing Added Quantities of Certain Ureas. Analyses were made after adding known amounts of a given urea or thiourea to a composite flour sample. A 2.000 g sample of flour containing the urea or thiourea under investigation was agitated in a reciprocating shaker for one hour with 100.0 ml water, followed by filtering through Whatman No. 4 filter paper. Analyses for specific ureas were performed on aliquots of the filtrates. Data for the analyses are given in Table 2.

Diacetylmonoxime Reaction with Ureas

The reaction of diacetylmonoxime with urea is not understood, and the reaction product is unknown. Natelson, Scott, and Beffa (14) have shown that the active reagent is diacetyl and not diacetylmonoxime. It is noteworthy to point out again that the present study shows that a greater color intensity was produced with diacetylmonoxime than with diacetyl. Being volatile, appreciable amounts of diacetyl may have been lost before having an opportunity to react. It appears that a slow production of diacetyl by the hydrolysis of the oxime

Table 2. Analyses of flours containing added quantities of certain ureas (diacetylmonoxime reaction).

Compound	Quantity Added mg	Quantity Found mg	Average Quantity Found mg	Recovery %
Urea	12.5	12.06	12.22	97.8
	12.5	12.35		
	12.5	12.25		
Acetylurea	12.5	12.04	12.34	98.7
	12.5	12.40		
	12.5	12.58		
1,3-Dimethylurea	12.5	12.45	12.36	98.9
	12.5	12.11		
	12.5	12.53		
Thiourea	12.5	12.22	11.85	94.8
	12.5	11.57		
	12.5	11.77		
Acetylthiourea	12.5	12.79	12.62	100.9
	12.5	12.17		
	12.5	12.89		
1,3-Diethylthiourea	25.0	25.8	24.5	98.0
	25.0	23.6		
	25.0	24.0		

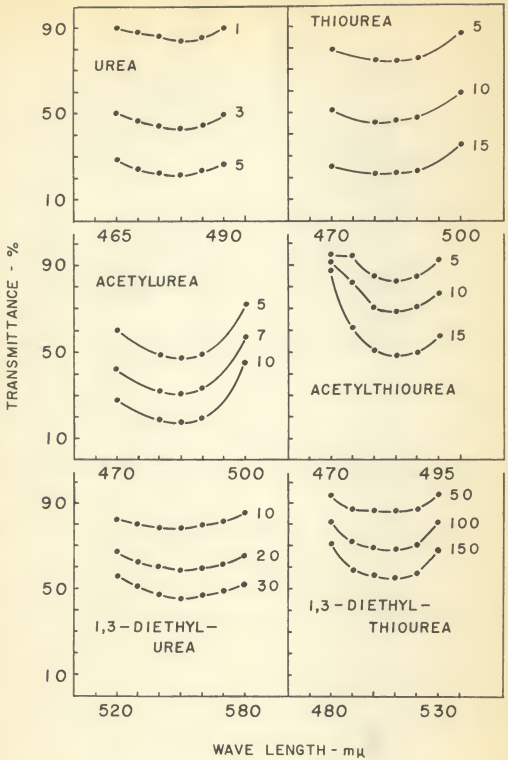


Fig. 3. Transmittance vs. wave length for six ureas reacting with diacetylmonoxime, each urea at three concentrations in ppm.

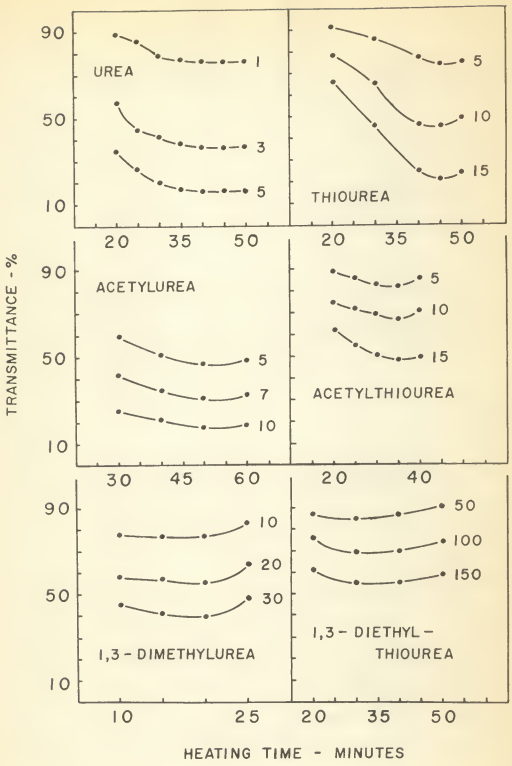


Fig. 4. Transmittance vs. heating time for six ureas reacting with diacetylmonoxime, each urea at three concentrations in ppm.

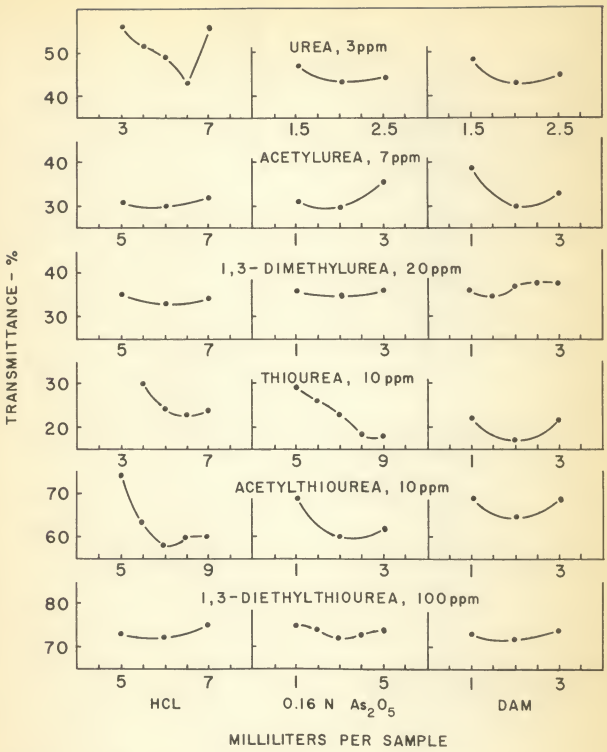


Fig. 5. Transmittance vs. three reagent concentrations for six ureas reacting with diacetylmonoxime, two reagents being held at optimum concentration while that of the third reagent was varied.

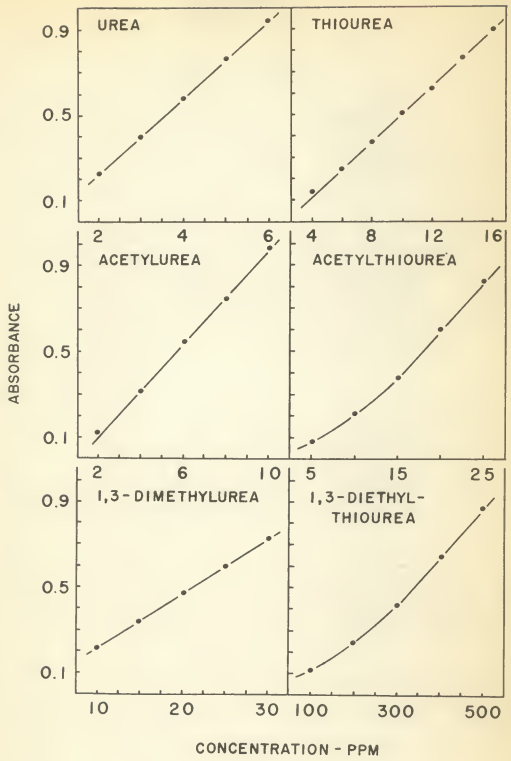


Fig. 6. Standard curves of concentration vs. absorbance at minimum transmittance for each of six ureas reacting with diacetylmonoxime.

permits a more favorable reaction. Fearon (6) stated that the reaction was positive for compounds containing the system RNHCONHR', where R is either hydrogen or simple aliphatic radicle, and R' is not an acyl radicle. In the present study and contrary to Fearon's statement, a positive reaction was obtained with acetylurea and acetylthiourea. No color was produced with 1,3-diphenylurea, 1,1-diphenylurea, ethylenethiourea, 1,3-diphenylthiourea, and 1,1-diphenylthiourea. A positive reaction was obtained with 1,1-diethylurea, but because of the difficulty in purifying the sample, a method was not worked out.

In view of the preceding discussion, it would appear that the reaction with diacetylmonoxime is positive for compounds containing the system RNHCO(S)NR'R'', where R is hydrogen or a simple aliphatic radicle and R' is hydrogen, a simple aliphatic radicle or a phenyl group, and R'' is not a phenyl group.

Although no effort was made to apply the methods to other types of samples, it appears that they would be applicable to a wide range of food products. It has been reported (6) that color is obtained with protein, however no color was noted with the water-soluble extracts of flour.

SUMMARY

Two methods have been modified and extended to determine urea, thiourea, and several of their substitution products. Both methods were modified to decrease the working range, and hence increase the sensitivity, two advantages not realized by other existing methods. The range of concentrations was lowered to values generally below 10 ppm for the ureas, and below 50 ppm for the thioureas.

The ureas reacting with p-dimethylaminobenzaldehyde gave a yellowish-green color having a broad transmittancy minimum from 425 to 445 mμ. The thioureas

gave the same color but their transmittance minimums were from 440 to 460 mμ.

An improved method using p-dimethylaminobenzaldehyde employed i-propyl alcohol solvent instead of water, and sulfuric in place of hydrochloric acid. When using i-propyl alcohol as a solvent, however, extreme care must be taken to exclude water from the reaction. As little as 0.25 per cent water in the color solution was found to decrease by 50 per cent the color produced with urea.

Methods are given for the determination of five ureas and four thioureas with p-dimethylaminobenzaldehyde. The five ureas include urea, methylurea, t-butylurea, allylurea, and phenylurea. The four thioureas include thiourea, allylthiourea, phenylthiourea, and ethylenethiourea. The determination of ethylenethiourea required the use of a mixture of i-propyl alcohol and acetic acid as a solvent.

In the reaction of diacetylmonoxime with the ureas, the concentrations of reagents were altered to increase the stability of the color, and extend the straight line portion of the concentration versus absorbance curves over a longer portion of the transmittance scale. This was done without decreasing the sensitivity of the method.

Methods employing diacetylmonoxime are given for the determination of three ureas and three thioureas. The three ureas include urea, acetylurea, and 1,3-dimethylurea. The three thioureas are thiourea, acetylthiourea, and 1,3-diethylthiourea. Since neither the p-dimethylaminobenzaldehyde nor the diacetylmonoxime method gives a color intensity that follows Beer's law, concentrations must be read from standard curves.

ACKNOWLEDGMENTS

The author is indebted to Professor Karl F. Finney for his supervision of the study and guidance in the preparation of this manuscript.

Grateful acknowledgment is also given to Assistant Professor Gerald D. Miller for his unending cooperation and his granting of laboratory and office space in which most of this work was done. The author also acknowledges the cooperation of all members of the Department of Flour and Feed Milling Industries staff.

The author is grateful to Patrick Latham Finney for his assistance in preparing the figures in this manuscript.

LITERATURE CITED

(1) Archibald, Reginald M.
Colorimetric determination of urea. J. Biol. Chem. 157:507-518. 1945.

(2) Barker, S. B.
The direct colorimetric determination of urea in blood and urine. J. Biol. Chem. 152:453-463. 1944.

(3) Barrenscheen, H. K.
A reaction of urea with p-dimethylaminobenzaldehyde. Biochem. Z. 140:426-434. 1923.

(4) Brown, Harold H.
Determination of blood urea with p-dimethylaminobenzaldehyde. Anal. Chem. 31:1844-1846. 1959.

(5) Cline, R. E., and R. M. Fink.
Color reaction between p-dimethylaminobenzaldehyde and urea or ureido acids. Anal. Chem. 28:47-52. 1956.

(6) Fearon, W. R.
The carbamide biacetyl reaction: a test for citrulline. Biochem. J. 33:902-907. 1939.

(7) Finney, K. F.
Results of spraying urea on wheat. Unnumbered Mimeograph Publication. Presented at Fertilizer Conference, K. S. U., Manhattan, Kansas. December 1950.

(8) _____.
Contributions of the Hard Winter Wheat Quality Laboratory to wheat quality research. Trans. Am. Assoc. of Cereal Chem. 12:127-142. 1945.

(9) _____, J. W. Meyer, F. W. Smith, and H. C. Fryer.
Effect of foliar spraying of Pawnee wheat with urea solution on yield, protein content, and protein quality. Agron. Jour. 49:341-347. 1957.

(10) Jongen, G. H., and H. W. Berkhaut.
Determination of urea in fertilizers and feeds. Chem. Weekblad 52: 909-910. 1956. Original not seen. Abstract in Chem. Abs. 51:5635. 1957.

(11) Kawerau, E.
Biochemical analysis with the Spekker absorptiometer. 1. Estimation of urea, citrulline, allantoin, and related carbamido compounds. Sci. Proc. Roy. Dublin Soc. 42:63-70. 1946. Original not seen. Abstract in Chem. Abs. 40:5085. 1946.

(12) Koritz, S. B., and P. P. Cohen.
Colorimetric determination of carbamylamino acids and related compounds. J. Biol. Chem. 209:145-150. 1954.

(13) LeMar, Ralph L., and David Bootzin.
Modification of the diacetyl determination of urea. Anal. Chem. 29: 1233-1234. 1957.

(14) Natelson, Samuel, Mary Lou Scott, and Charles Beffa.
A rapid method for the estimation of urea in biologic fluids, by means of the reaction between diacetyl and urea. Am. Jour. Clin. Pathol. 21:275-281. 1951.

(15) Ormsby, Andrew A.
A direct colorimetric method for the determination of urea in blood and urine. J. Biol. Chem. 146:595-604. 1942.

(16) Pesez, M., and A. Petit.
Investigation on and determination of hydrazine. Bull. soc. chim. (France) 122-123. 1947.

(17) Rosenthal, Harold L.
Determination of urea in blood and urine with diacetylmonoxime. Anal. Chem. 27:1980-1982. 1955.

(18) Watt, George W., and Joseph D. Crisp.
Spectrophotometric method for the determination of hydrazine. Anal. Chem. 24:2006-2008. 1952.

(19) _____, _____.
Spectrophotometric method for the determination of urea. Anal. Chem. 26:452-453. 1954.

(20) Wheatly, V. R.
An improved diacetyl reaction for the estimation of urea in blood. Biochem. J. 42:xli. 1948.

QUANTITATIVE DETERMINATION OF UREA, THIOUREA, AND CERTAIN OF THEIR SUBSTITUTION PRODUCTS BY SPECTROPHOTOMETRIC TECHNIQUES

by

RUSSELL CARL HOSENEY

B. S., Kansas State University, 1957

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1960

In previous foliar spraying experiments, sulfur and nitrogen in the form of thioureas, N,N'-diethylthiourea, and ethylenethiourea were applied to the leaves of the wheat plant prior to and/or at heading. Protein contents of the flours were increased by 2.5 to 4.5 per cent. Total sulfur contents of the flours were increased about 20 per cent above those for flours milled from wheats that were sprayed with ammonium nitrate and such nonsulfur-containing ureas as N,N'-dimethylurea, N,N-diethylurea, and urea.

The materially below normal physical and baking properties of some wheats compared to the normal and above normal properties of others suggested that certain ureas had been synthesized to varying degrees into gluten protein, thereby pointing to the need of a sensitive quantitative method for determining residual quantities, if any, of the ureas and thioureas in the wheats and flours involved. Residual amounts of certain ureas and thioureas possibly could account for the below as well as above normal properties of certain wheats and flours. Accordingly, in the present study, two spectrophotometric methods for the quantitative determination of ureas have been investigated, one using p-dimethylaminobenzaldehyde, and the other diacetylmonoxime.

The two methods have been modified and extended to determine urea, thiourea, and several of their substitution products. Both methods were modified to decrease the working range, and hence increase the sensitivity, two advantages not realized by other existing methods. The range of concentrations was lowered to values generally below 10 ppm for the ureas, and below 50 ppm for the thioureas.

The ureas reacting with p-dimethylaminobenzaldehyde gave a yellowish-green color having a broad transmittance minimum from 425 to 445 mμ. The thioureas gave the same color but their transmittance minimums were from 440 to 460 mμ.

An improved method using p-dimethylaminobenzaldehyde employed i-propyl alcohol solvent instead of water, and sulfuric in place of hydrochloric acid. When using i-propyl alcohol as a solvent, however, extreme care must be taken to exclude water from the reaction. As little as 0.25 per cent water in the color solution decreased by 50 per cent the color produced with urea.

Methods are given for the determination of five ureas and four thioureas with p-dimethylaminobenzaldehyde. The five ureas include urea, methylurea, t-butylurea, allylurea, and phenylurea. The four thioureas include thiourea, allylthiourea, phenylthiourea, and ethylenethiourea. The determination of ethylenethiourea required the use of a mixture of i-propyl alcohol and acetic acid as a solvent.

In the reaction of diacetylmonoxime with the ureas, the concentrations of reagents were altered to increase the stability of the color, and extend the straight line portion of the concentration versus absorbance curves over a longer portion of the transmittance scale. This was done without decreasing the sensitivity of the method.

Methods employing diacetylmonoxime are given for the determination of three ureas and three thioureas. The three ureas include urea, acetylurea, and 1,3-dimethylurea. The three thioureas are thiourea, acetylthiourea, and 1,3-diethylthiourea. Since neither the p-dimethylaminobenzaldehyde nor the diacetylmonoxime method gives a color intensity that follows Beer's law, concentrations of the compounds being measured must be read from standard curves.