Table II. Dates Found Marketable as Fresh Fruit, Products, and Culls[a] by Packinghouse Grading of Deglet Noor Dates Stored 0-5 Months Outdoors then Held 7-12 Months in Cold Storage

	Months in storage outdoors					
Dates marketable	0	1	2	3	4	5
Fresh fruit, %	89	85	53	29	5	25
Products, %	5	6	35	59	84	49
Culls, %	6	7	12	12	11	26

[a] Values represent the average of three bins of dates and are expressed in percent by weight.

Browning took place in the dates during storage, as shown by the regression line of soluble pigment measurements (Figure 2). Browning was more obvious visually than is indicated by the soluble pigment values. Dates held outdoors 5 months were very dark. We concluded from visual observations of all the treatments that dates should not be stored outdoors for more than 1 month if optimum color and quality of dates are to be maintained.

We also measured certain other changes that took place in the dates during storage. Those held zero months outdoors contained 10.9% fructose, 11.4% glucose, and 59.3% sucrose, on a dry weight basis, at the end of the test. Sucrose inverted to fructose and glucose during outdoor storage; at the end of the test, 1, 9, 11, 15, and 34% of the sucrose had inverted in dates stored outdoors for 1, 2, 3, 4, and 5 months, respectively. The average moisture content of the dates was 17.1 ± 2.1% before storage and 15.7 ± 0.4% after storage.

All the bins of dates were graded (Norman et al., 1976) in the packinghouse at the end of the test for determination of the quantities marketable as fresh fruit, products, and culls (Table II). Marketability was about the same for dates stored outdoors 0 and 1 month but decreased markedly for those stored outdoors 2 months. The amount of dates marketable as fresh fruit was lower for dates that had been stored outdoors 2 and 5 months than for dates not stored outdoors by 36 and 64 percentage points, respectively.

These results are preliminary in our study to determine if total aldehyde assay could be used for quality indexing. Further studies with adequate replication of different storage conditions and quality measurements are required to fully relate total aldehyde content to quality changes of dates. Individual carbonyl compounds need to be studied so that we can determine whether particular components increase or decrease with deterioration of dates.

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Received for review November 15, 1976. Accepted February 1, 1977. Mention of a proprietary product in this paper does not constitute a recommendation or an endorsement of the product by the U. S. Department of Agriculture.

An Enzymic Assay for Ammonia in Waste Matter

An enzymic method for ammonia analyses has been shown to compare favorably with the standard method, Nesslerization. Favorable comparison was found for accuracy, simplicity, and cost. The enzymic method was shown to be about three times as sensitive as Nesslerization and more directly applicable with physiological samples. Samples that contained considerable color negated the use of the Nessler procedure whereas this presented no problems for the enzymic method.

The application of the enzyme, glutamate dehydrogenase (GDH) (EC 1.4.1.2), to the analysis of ammonia levels in serum has been shown to be an extremely sensitive method (Mondzac et al., 1965). The exploitation of the fluorescence of nicotinamide adenine dinucleotide (NADH) makes this method the most sensitive of any method in use. The measurement of the radiation adsorption of NADH, while less sensitive, is more generally useful in most laboratories. Thus, we have chosen to compare the enzymic reaction by absorption spectroscopy with standard methods in the determination of ammonia levels in water samples and solutions containing animal wastes. This method is of interest to scientists concerned with pollution of water supplies and those interested in the use of animal

and human waste. The method is based on the reaction catalyzed by GDH as shown below:

$$NH_4^+ + \alpha\text{-ketoglutarate} + NADH \xrightarrow{GDH} \text{glutamate} + NAD^+ + H_2O$$

There has been interest in the ammonia content of fecal matter as well as other products which may be obtained from fecal matter (Murdock and Turner, 1975; Fu et al., 1974; Smith, 1973; Institute of Gas Technology, 1975). Thus a simple, sensitive, and low-cost analysis would be advantageous for these interests. The interest in this laboratory is in regard to the feasibility of obtaining ammonia as a salable product from animal wastes. The treatment of animal wastes at present allows most of the ammonia to be lost to the atmosphere. As a commercial product, this ammonia has been estimated to be worth billions of dollars per year (Murdock and Turner, 1975). Thus, to study the feasibility of releasing and recovering ammonia content called for a simple and versatile method of analysis.

The GDH reaction was compared with the Nessler reaction in sensitivity, precision, accuracy, and simplicity. The basis for comparison was analyses performed by both methods on solutions of known concentrations of ammonium chloride and on fecal matter extracts, both decolorized and untreated.

METHODS AND MATERIALS

Nicotinamide adenine dinucleotide, reduced (NADH), α-ketoglutarate (α-KG), adenosine diphosphate (ADP), glutamate dehydrogenase (GDH) (ammonium sulfate free), and ethylenediaminetetraacetic acid dipotassium salt (EDTA) were purchased from Calbiochem. Ammonium chloride (NH_4Cl) was purchased from J. T. Baker Analyzed Reagents. Activated charcoal (Darco G-60) was purchased from Sargent-Welch, and Celite Analytic Filter Aid was purchased from Johns-Manville.

All solutions except ammonia standards were prepared daily. NADH and GDH solutions were prepared daily to insure good activity, since they decrease with time, but were stable for 24 to 36 h under refrigeration. Ammonia-free doubly distilled water was used to prepare phosphate buffer which was used as the solvent for all solutions.

Preparations of bovine fecal samples prior to assay consisted of high-speed centrifugation (39 000g for 30 min) at a twofold dilution with 0.05 M, pH 7.8 potassium phosphate buffer. Upon completion of centrifugation, a second dilution of tenfold was performed using the same buffer as before to bring the fecal sample to a final dilution of 20-fold. The 20-fold diluted sample had a yellow-brown color but had no particulate matter.

The dehydrogenase ammonia assay requires three stock solutions and an ammonia standard. Two ammonia standards, 38.42 mg of NH_4/liter and 21.08 mg of NH_4/liter were prepared with NH_4Cl and ammonia-free doubly distilled water. The NADH solution was prepared to be approximately 3×10^{-3} M NADH. The substrate cocktail contained the following concentrations: 3×10^{-3} M α-KG, 1×10^{-4} M ADP, and 1×10^{-4} M EDTA. The GDH solution was diluted, as obtained from Calbiochem, to obtain 100–150 IU/mL. The ammonia standards were found to be stable for several months. ADP in the substrate cocktail provides high GDH activity during the assay (Frieden, 1959) while EDTA in the cocktail prevents heavy metal inhibition of GDH.

The dehydrogenase ammonia assay mixture was prepared in a 1-cm path quartz curvette of 3.3-mL capacity.

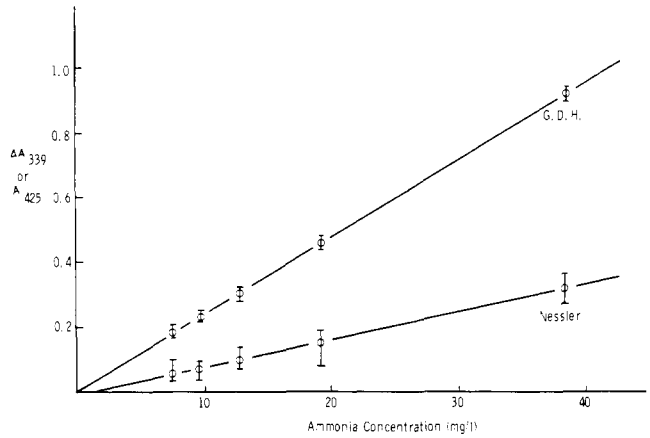

Figure 1. A comparison of sensitivity and precision between the enzymic and Nessler methods. Each graphical point consists of a minimum of five determinations on solutions of known ammonium concentration. The intermediate sample was used as a synthetic unknown to calculate the relative error for each procedure. ΔA_{339} and A_{425} represent the change in absorption at 339 nm (enzymic) and absorption at 425 nm (Nessler), respectively.

The total volume was 3.00 mL, consisting of 0.30 mL of the NADH solution, 2.40 mL of the substrate cocktail solution, 0.20 mL of the sample or ammonia standard solution, and 0.10 mL of the enzyme solution. The reference cuvette contained 0.10 mL of the enzyme solution and 2.9 mL of buffer.

The enzymic assay procedure consists of combining all solutions but the enzyme solution in the cuvette and recording the absorption due to NADH at 339 nm in a dual-beam spectrophotometer. The enzyme solution was added, allowed to react for 20 min, and the absorption due to NADH again recorded. The change in absorption at 339 nm was then related to the ammonia in the sample by comparison to the ΔA of an ammonia standard assay.

The standard methods for the decolorization and analysis of ammonia in wastewater are distillation and the Nessler ammonia analysis. Both procedures were performed as described in Standard Methods for the Examination of Water and Wastewater (1973).

An alternative method for decolorization of fecal samples by means of charcoal decolorization was also performed. The procedure for charcoal decolorization consisted of combining 20 mL of 20-fold diluted fecal sample with 0.50 g of charcoal. After 5 min the charcoal-treated sample was filtered through a pad consisting of filter paper and Celite Analytic Filter Aid. Complete decolorization was observed. Some standard solutions of ammonium chloride were treated in like manner.

RESULTS AND DISCUSSION

Comparison of the Enzymic and Nessler Methods on Known Concentrations of Ammonium Chloride. Five solutions were used and five individual determinations were made on each sample, with the exception that in some cases as many as eight separate determinations were performed. The results are shown in Figure 1. These data indicate the enzymic method performs well in comparison with the standard Nessler method. Upon careful analysis of these data, the enzymic method showed a slightly better performance in precision and accuracy. The mean standard deviations were ±1 to 5% or considerably less than the Nessler methods (see also Standard Methods for Examination of Water and Wastewater, 1973). The relative mean error for the enzymic method was <2%, whereas for the Nessler method a relative mean error of

Table I. Comparison of the Enzymic Method with the Nessler Method on Extracts of Fecal Matter and on Standard Solutions[a]

Untreated fecal extract	Charcoal filtered	Distilled		Standard solution of NH_4Cl	Charcoal filtered	Distilled
Enzymic values 295	276	157[c]	292[d]	20.63	14.50	20.32
Nessler values N.D.[b]	129	155[c]	298[d]	20.43	14.01	20.30

[a] The standard solution was used an an additional check for comparing the two methods in these experiments. The values for ammonia reported are averages of duplicate determinations. The standard ammonia solution used was 21.08 mg/L in ammonia. All values reported as mg/L. [b] The Nesslerization of colored extract results in an optically opaque solution, thus, undeterminable. [c] Distilled directly without the addition of surfactant. [d] Addition of Tween 20 prevented foaming and presumably this improved the quantitative transfer of NH_3.

10% was calculated from data obtained in this laboratory. The sensitivity of the two methods can be compared by the ratio of the extinction coefficients or the ratio of the absorption given on a known concentration of ammonium chloride. The molar extinction coefficients are 6.22×10^3 L mol^{-1} cm^{-1} for NADH and 2.14×10^3 L mol^{-1} cm^{-1} for the ammonium–mercury complex. This approximates a ratio of 3 to 1. Thus the enzymic method is three times more sensitive.

Comparison of the Enzymic Method and the Nessler Method with Solutions of Fecal Matter Extract. It has been noted recently by Elliott and Travis (1975) that ammonia assays on fecal matter show interference from low molecular weight alkylamines with the Nessler reagent. Assays for ammonia on fecal matter extracts performed in this laboratory have shown that the Nessler method requires decolorization by distillation prior to analysis. This additional step is not only time consuming but also can produce losses of ammonia as the results here show. This is substantiated by the results given in Standard Methods for the Examination of Water and Wastewater (1973). In order to avoid the distillation requirement, some laboratories resorted to the Kjeldahl method which is expensive in technician time and usually requires 2 to 3 days to complete an analysis.

The enzymic method was compared with the Nessler method on some decolorized extracts of fecal material, and the enzymic method was used to determine the ammonia content on extracts that were not decolorized. In addition, some extracts were decolorized by filtering with charcoal. Thus three comparisons can be made: (1) enzymic method vs. Nessler, (2) charcoal treatment vs. distillation, and (3) ammonia content before and after decolorization. Further, standard solutions of NH_4Cl were subjected to the same treatments for comparison. The results of these analyses are given in Table I.

It can be seen that: (1) the enzymic method was preferable from the fact that decolorization is not necessary, (2) ammonia may be lost by decolorization by either charcoal treatment or distillation, and (3) charcoal treatment causes interference with the Nessler method.

It appears from the data presented that the enzymic analyses compare well with direct Nesslerization of samples, but samples requiring decolorization would be better served by the enzymic method. The cost per analysis by the enzymic method was calculated to be <6¢/determination for chemicals. The Nessler analysis is only 2¢/determination. Thus the enzymic method costs 4¢ more per determination unless one encounters difficult samples such as the fecal extracts. Then the technical time required is greater for the Nessler method plus a possible sacrifice in accuracy. The enzymic method is specific, i.e., it has no interfering reactants. The assay procedure showed no interference from methylamine. There are some cautionary steps which need to be observed. One is the inhibition of the enzyme by heavy metals. This was found to be a problem in the fecal samples tested. The inclusion of EDTA was a satisfactory means of avoiding this inhibition. Also, one should be aware that the enzymic method is a kinetic measurement, and thus the reaction completion is of concern. This can be negated by making an ammonia standard which provides a ΔA approximating that of the test samples, i.e., $\pm 0.1 \Delta A$.

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Received for review November 14, 1975. Accepted January 14, 1977. This paper is published as MAES Journal Series No. 641.