

CHEMICAL ESTIMATION OF AVAILABLE
LYSINE IN DEHYDRATED DAIRY PRODUCTS

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The most commonly employed procedures for the chemical estimation of available lysine in dehydrated dairy products involve the di- or trinitrophenylation of the free epsilon amino groups followed by acid hydrolysis and separation and quantitation of the colored mono-derivative. Application of these techniques to pure milk proteins yields good results but difficulties are encountered with milk products whenever large amounts of the reducing sugar, lactose, are present. These procedures have been modified to permit the routine determination of available lysine in dehydrated milk and whey. A dye binding method employing the reactive dye remazol brilliant blue R and claiming no lactose interference has been applied to casein and whey powders. Accuracy of this method is dependent on the protein:dye ratio of the product under investigation. A new technique, quantified by measuring ^{19}F NMR after acetylation of the free epsilon amino groups of lysine by S-ethyl trifluorothioacetate, has been developed.

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INTRODUCTION

Since Magendie (1816) established the indispensability of organic nitrogen, the sole practical tool for over 100 years for the evaluation of protein by chemical means was the determination of total nitrogen. The discovery of amino acids as necessary dietary factors led to the classic work of Rose (1936) which established the definitive list of amino acids essential for the rat; this work opened the way for amino acid analysis to be used as a solid base for protein quality evaluation. Block and Mitchell (1946) in their review showed the usefulness of amino acid analysis in predicting the nutritive value of the protein in a particular food; agreement to a certain extent with biological values determined in vivo could be readily obtained. However, some products, particularly milk products which had been heat processed or stored under poor conditions, had lower values than were to be expected from their amino acid analyses.

At that time, there was already some evidence that these results were associated with the amino acid lysine (Harris and Mattill, 1940; Block et al., 1946). Lysine is the only essential amino acid that still has a free amino group (epsilon) when in condensed form within the peptide chain. Work with casein-glucose model systems and with milk powders appeared to show that these free amino groups would react with the aldehydic groups of reducing sugars even under relatively dry conditions to form compounds that had no nutritional value, but that could be hydrolyzed by strong acids to free lysine (Eldred and Rodney, 1946; Henry et al., 1948). Such behavior is explained by the Maillard reaction (Maillard, 1912), the chemistry of which was worked out by Hodge (1953). Carpenter and his associates (Carpenter et al., 1957; Lea et al., 1958) were the first to relate the structure of lysine to its nutritional availability and definitively demonstrated by chemical means that in certain circumstances not all of the lysine present in a protein is nutritionally available to an animal. Therefore, the total lysine content was not always a reliable guide to the true

quality of the protein in relation to lysine.

The idea of lysine being present but apparently nutritionally "unavailable" to the metabolic processes aroused considerable interest. Because of the extreme importance of lysine in the diet, attempts to measure its nutritional availability in all types of protein-containing foods have been made using biological evaluation studies (Gupta et al., 1958; Guthneck et al., 1953; Ousterhout et al., 1959; Schweigert and Guthneck, 1953; Kuiken et al., 1948; Kuiken and Trant, 1952), in vitro digestion methods (Mauron et al., 1955; Sheffner et al., 1956; Ford, 1964; Stott and Smith, 1966), and chemical analyses (Carpenter and Booth, 1973; Walz and Ford, 1973).

Reliable chemical methods for measuring available lysine are desirable because of the advantages of speed of analysis and suitability for routine assay. Several methods have been developed, all depending upon the freedom of the ϵ -amino group of lysine to react with the reagent of choice. The application of these methods for the determination of available lysine in dehydrated dairy products is the subject of this chapter.

1-FLUORO-2,4-DINITROBENZENE (FDNB) (CARPENTER PROCEDURE)

The first good chemical method for the determination of nutritionally available lysine was developed by Carpenter and his associates (Carpenter and Ellinger, 1955a, b; Bruno and Carpenter, 1957; Carpenter, 1958). Carpenter's original procedure, schematically outlined in Figure 1, was based on the work of Sanger (1945) who showed that when the products of the reaction of FDNB with amino acids are heated in hydrochloric acid, bright yellow dinitrophenylated amino acids are formed. Carpenter (1960) used a selective solvent extraction to separate ϵ -dinitrophenyl lysine (ϵ -DNP-lysine) from the reaction mixture and also used methyl chloroformate to separate α -DNP arginine from the ϵ -DNP-lysine derivative.

Schober and Prinz (1956) first applied the FDNB procedure to a variety of heat treated milks and milk powders dehydrated by

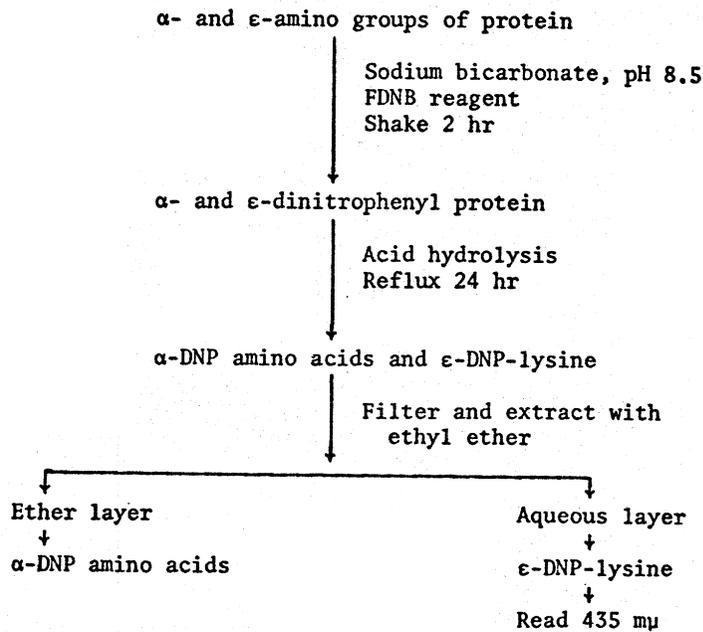


FIG. 1. FDNB technique for determination of available lysine. From Carpenter and Ellinger (1955a).

different means, and demonstrated a decrease in available lysine content with increasing heat treatment. However, 50-60% of the total solids of milk is composed of the reducing sugar, lactose. Handwerck et al. (1960) showed that 68% of the ϵ -DNP-lysine was destroyed during acid hydrolysis in the presence of lactose. They attributed this effect to the reduction of aromatic nitro groups to amino groups during the hydrolysis step; derivatives were formed which did not have the properties used to measure ϵ -DNP-lysine. The carbohydrates were less destructive if they were treated with FDNB prior to hydrolysis. Blom et al. (1967) also found less destruction due to carbohydrates if the refluxing was carried out in the presence of other aromatic nitro compounds such as dinitrophenol or FDNB itself; these compounds confer protection most likely by competing with the nitro groups of ϵ -DNP-lysine.

Carpenter's modified procedure (Carpenter, 1960) retains the

dinitrophenol and FDNB from the first step of the reaction in the refluxing mixture. Bujard et al. (1967) used the modified Carpenter procedure and other in vitro methods (Mauron and Mottu, 1958) to evaluate lysine content and lysine availability in processed milk. Mottu and Mauron (1967) then checked these results using the in vivo procedure. A summary of their data is shown in Table 1. It is readily apparent that use of the modified Carpenter procedure yielded values of available lysine in close agreement with those obtained by the growth assay method. These values, when compared to the total lysine value, clearly demonstrate the effect of heat applied during evaporation and dehydration procedures. Roller drying not only reduces the availability of lysine but also partially destroys it. Holm (1971) using his modification of the FDNB procedure confirmed the effect of the drying method used on the concentration of available lysine found.

TABLE 1
Lysine Content of Dairy Products^a

| Sample | Total lysine ^b | Available lysine ^c | Available lysine ^d |
|-------------------------------------|------------------------------|----------------------------------|----------------------------------|
| | g lysine/16 g nitrogen | | |
| Lyophilized whole milk | 8.3 | 8.4 | 8.4 |
| Evaporated milk | 7.6 | 5.5 | 6.1 |
| Spray dried milk | 8.0 | 8.2 | 8.1 |
| Roller dried milk (low heat) | 7.6 | 5.5 | 6.3 |
| Roller dried milk (conventional) | 7.1 | 4.6 | 5.9 |
| Roller dried milk (high heat) | 6.1 | 1.9 | 2.0 |

^aFrom Mottu and Mauron (1967).

^bChromatographic procedure (Moore and Stein, 1951).

^cChemical assay (Carpenter, 1960).

^dGrowth assay (Gupta et al., 1958).

Residual moisture in dried milks can also influence the available lysine content during storage. Erbersdobler (1970), using the FDNB procedure, found that stored skim milk powder showed a decrease in available lysine of up to 35%. The amount of lysine inactivated was dependent on the moisture content; with a moisture content of 4%, available lysine decreased 2%, but with a moisture content of 8%, decreases of 20-40% were observed. These results show that careful storage of properly dried milk powders is necessary to maintain the lysine in a nutritionally available form.

The modified FDNB procedure, although widely used, suffers from several disadvantages. Although it represents a considerable improvement over growth assays and enzymatic procedures, it still requires a considerable amount of time for multiple routine analyses. It also employs two particularly unpleasant chemicals: FDNB, which is not water-soluble and has a vesicant effect on human skin, and methylchloroformate which is a lachrymator.

In his excellent review Carpenter (with Booth, 1973) has thoroughly discussed factors affecting the analytical procedures for FDNB-reactive lysine; this paper should be consulted for further information.

2,4,6-TRINITROBENZENE SULFONIC ACID (TNBS)

TNBS reacts with the free amino groups in peptides and proteins to yield trinitrophenyl derivatives of the amino acids (Okuyama and Satake, 1960). Although the α -TNP derivatives are more sensitive to hydrolysis than the corresponding DNP derivatives, the TNP- ϵ -NH linkage is quite resistant to acid hydrolysis (Kotaki and Satake, 1964). Utilizing this principle, Kakade and Liener (1969) published a procedure for available lysine which claimed to be faster and more accurate than the FDNB procedure. A schematic of the TNBS procedure is shown in Figure 2.

The TNBS procedure requires only a 1-hr acid hydrolysis compared to an overnight hydrolysis of the FDNB procedure. The authors

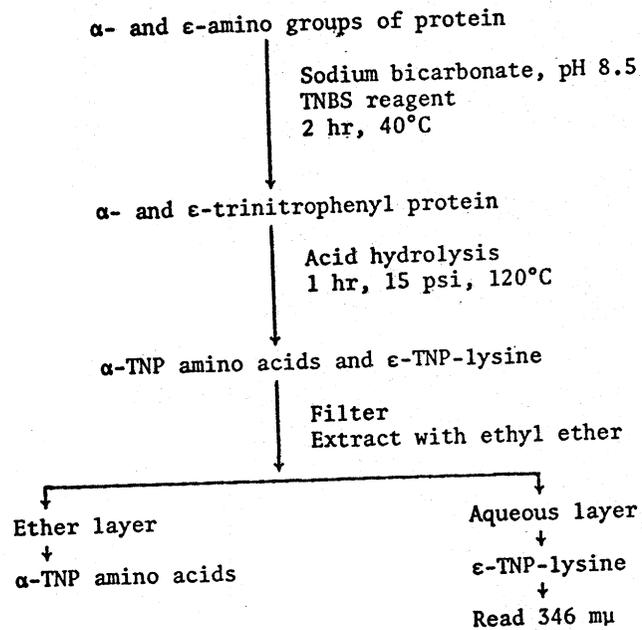


FIG. 2. TNBS technique for determination of available lysine. From Kakade and Liener (1969).

also claimed that the short hydrolysis time obviated the need for the correction factors encountered with the FDNB procedure when applied to foods rich in carbohydrate.

Holsinger et al. (1970a) were interested in determining the available lysine in dehydrated instant breakfasts, body builders, and diet drinks utilizing milk. As it was necessary to evaluate a large number of samples, they attempted to utilize the TNBS procedure. The results are shown in Table 2.

The data for available lysine shown in Table 2 are quite low when compared to skim milk. The vanilla drink and chocolate drink A both depended on the addition of milk for reconstitution, while chocolate drink B specified water. Because of the chocolate, all samples were dialyzed prior to analysis, which had the benefit of preventing any interference from lactose. When the TNBS procedure was applied to whole casein, however, an anomalously high value was

TABLE 2
Total and Available Lysine Present in Commercial Dairy Products^a

| Sample | Total lysine ^b | Available lysine ^c | % of total lysine available |
|--|---------------------------|-------------------------------|-----------------------------|
| | g/100 g dry sample | | |
| Skim milk | 6.39 | 5.79 | 90.6 |
| 2:1 Evaporated skim milk | 6.10 | 4.30 | 70.5 |
| Whole casein | 5.78 | 6.70 | ? |
| Vanilla drink (a body builder) | 6.50 | 4.30 | 66.2 |
| Chocolate drink A (diet) | 6.07 | 3.77 | 62.1 |
| Chocolate drink B (a body builder containing soy) | 4.92 | 3.12 | 63.4 |

^aFrom Holsinger et al. (1970a).

^bChromatographic procedure (Spackman et al., 1958).

^cTNBS procedure (Kakade and Liener, 1969).

obtained. This raised doubts about the method, so Holsinger et al. (1970b) examined a series of caseins.

The results of analysis of the various caseins, shown in Table 3, yielded anomalously high values of available lysine in those caseins containing amino sugars. Amino sugars reacted with TNBS to form colored derivatives which were not removed by ether extraction. It was concluded from these results that, although exact values of available lysine could not be determined by TNBS in milk products containing large amounts of glycoproteins, the procedure could still find application in the determination of relative values of lysine inactivation during processing of products of identical composition.

The economical salvage of cheese whey, an underutilized source of high grade animal protein and a serious pollution problem in some areas, has provided a challenge for considerable research. As shown in Table 4, the whey proteins represent an especially rich nutritive source of the essential amino acids, particularly lysine. As whey

TABLE 3
Lysine Content of Various Caseins^a

| Sample | Total lysine ^b | Available lysine ^c |
|----------------------------------|---------------------------|-------------------------------|
| | g/100 g dry sample | |
| Whole casein (acid precipitated) | 6.12 | 6.76 |
| "Nutritious" casein | 6.93 | 7.41 |
| α_{s1} -AA casein | 8.45 | 7.61 |
| α_{s1} -CC casein | 8.72 | 7.40 |
| β -Casein | 6.36 | 6.11 |
| κ -Casein A + B | 5.22 | 6.01 |
| N-acetyl neuraminic acid | -- | -- |
| D-Glucosamine | -- | 13.2 |
| D-Galactosamine | -- | 7.55 |

^aFrom Holsinger et al. (1970b).

^bChromatographic procedure (Spackman et al., 1958).

^cTNBS procedure (Kakade and Liener, 1969).

TABLE 4
Essential Amino Acid Content of
Whey Protein Compared to Whole Egg Protein^a

| Amino acid | Whey | Egg |
|--------------------|----------------------------|-----|
| | g amino acid/100 g protein | |
| Lysine | 9.2 | 6.4 |
| Methionine | 2.2 | 3.2 |
| Cystine + cysteine | 3.5 | 2.4 |
| Isoleucine | 6.4 | 6.6 |
| Leucine | 12.6 | 8.8 |
| Tryptophan | 2.2 | 1.7 |

^aFrom Orr and Watt (1957).

began to be utilized in a variety of dairy products, effects of processing and drying on the total and available lysine content of whey and whey protein concentrates became important. As whey contains low levels of glycoproteins, Posati et al. (1972) reasoned that application of the TNBS procedure would permit the evaluation of the available lysine content of a large number of samples in a short period of time.

As fluid cheese whey contains approximately 5% lactose and 1% protein and the TNBS procedure had already yielded anomalous results with proteins containing amino sugars, Posati and her associates (1972) investigated the effect of added lactose on pure ϵ -TNP-L-lysine. These results, shown in Figure 3, indicated that although some lactose could be tolerated, the addition of lactose in levels approaching the lysine:lactose ratio in whey resulted in severe destruction of the ϵ -TNP-lysine derivative.

Posati et al. (1972) next investigated the extent of ϵ -TNP-lysine derivative destruction in a model system containing all the whey proteins and increasing amounts of lactose. Their results are shown in Figure 4. The points were obtained by application of the TNBS procedure to solutions containing 0.5 mg whey proteins and increasing amounts of lactose. Additional data obtained in a similar

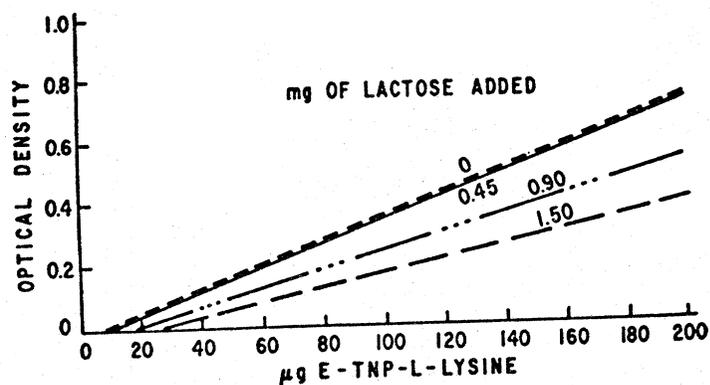


FIG. 3. Effect of added lactose on the concentration of ϵ -TNP-L-lysine. From Posati et al. (1972).

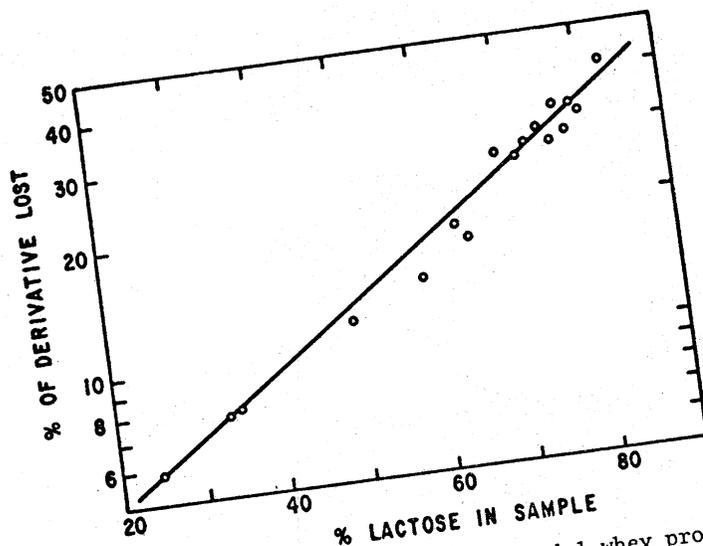


FIG. 4. Effect of added lactose on a model whey protein mixture containing 0.5 mg protein. Method of averages analysis of the data yields a straight line described by $\log y = 0.225 + 0.0167x$. From Posati et al. (1972).

fashion are shown in Figure 5. The observed scatter in the data points was attributed to variation in protein sample size of from 0.2 to 0.5 mg. Least squares analysis showed the data to represent a straight line described by the equation $\log y = -1.4936 + 0.9057 \log x$ where $y = \% \epsilon\text{-TNP-L-lysine derivative lost}$ and $x = \text{concentration of lactose in the sample}$. Posati et al. (1972) employed this equation to correct their data for lactose interference because it was useful over a range of sample sizes.

That protein sample size seriously influenced the available lysine found by TNBS is clearly shown in Figure 6. Therefore, Posati et al. (1972) recommended standardization of the sample size. In the analysis of protein-containing foods, Kakade and Liener (1969) recommended filtering the derivative-containing sample after digestion to remove insoluble particulate matter. Posati et al. (1972) found that this step also resulted in loss of derivative and devised a correction curve which permitted the use of a standard curve constructed with data from unfiltered solutions to be used to determine available lysine in solutions which required filtration.

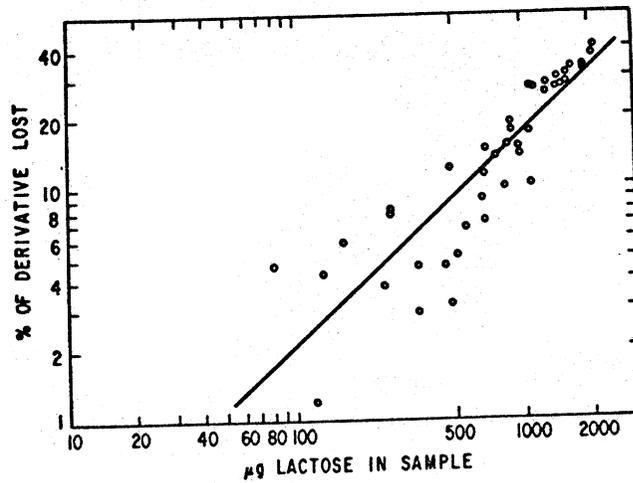


FIG. 5. Effect of added lactose on model whey protein mixtures of three different sample sizes. From Posati et al. (1972).

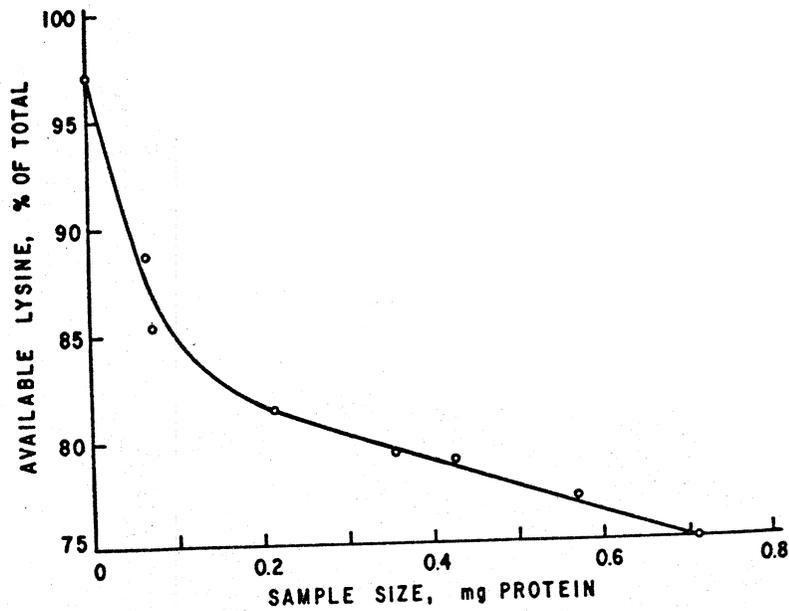


FIG. 6. Effect of sample size on available lysine content of bovine serum albumin. From Posati et al. (1972).

By employing their corrections for filtration and concentration of lactose in the product and standardizing the sample size, Posati et al. (1972) demonstrated concurrence with data procured by a more established method. Their results for dehydrated whey products are shown in Table 5. Although these results were still somewhat low, Posati et al. (1972) were satisfied that their corrected TNBS procedure could still yield valuable information about heat damage occurring in the processing of whey products when relative values are considered.

A possible explanation for these findings may rest on the reactivity of free lysine toward TNBS. There is some conflict in the literature regarding this point. Under the conditions of the reaction used, Okuyama and Satake (1960) prepared α,ϵ -di-TNP-lysine, 81% of which was recovered as ϵ -mono-TNP-lysine after acid hydrolysis. Ousterhout and Wood (1970) found, however, that free lysine was converted directly to mono-TNP-lysine. Hall and associates (1973) found that recoveries of free lysine and ϵ -TNP-lysine added as internal standards to protein suspensions which were then subjected to the TNBS reaction and acid hydrolysis were variable;

TABLE 5
Lysine Content of Whey Powders Determined by Different Methods^a

| Sample | Total lysine ^b | Available lysine ^c | Available lysine ^d |
|----------------------------|---------------------------|-------------------------------|-------------------------------|
| | g/100 g protein | | |
| Roller dried | 5.63 | 4.77 | 5.06 |
| Spray dried | 7.92 | 7.84 | 7.44 |
| Delactosed and spray dried | 6.94 | 6.18 | 6.31 |
| Lactalbumin | 9.79 | 8.75 | 9.24 |

^aFrom Posati et al. (1972).

^bChromatographic procedure (Spackman et al., 1958).

^cCorrected TNBS procedure.

^dFDNB procedure (Blom et al., 1967).

the authors could offer no explanation for the lack of consistency of their results.

Up to 30% of the total nitrogen present in cheese whey is in the form of free amino acids and soluble peptides. Mavropoulou and Kosikowski (1973) reported the lysine content of this fraction to be 10% for cottage cheese whey and 6.6% for cheddar whey. This means that an average of 20% of the total lysine present in whey is contributed by the nonprotein nitrogen fraction.

Because the concentration of lysine in the nonprotein nitrogen fraction represents a significant percentage of the total lysine present, its reactivity toward TNBS assumes great importance in the available lysine determination. Failure to react could lead to the low results observed by Posati et al. (1972).

From the results presented above, it is apparent that the TNBS procedure also requires many corrections and modifications to yield data in fair agreement with other methods. However, the TNBS procedure does have the advantage of being more suited to the routine assay of large numbers of samples if only relative values of available lysine are needed.

FDNB REAGENT: THE DETERMINATION OF AVAILABLE LYSINE BY DIFFERENCE

Because of the technical difficulties encountered in the complete recovery of ϵ -DNP- or ϵ -TNP-lysine after reaction of a food sample with FDNB or TNBS, an alternative approach has been to measure available lysine indirectly. The difference between the total lysine content of a sample measured after direct acid hydrolysis and the residual lysine content measured after acid hydrolysis of the sample after blockage of the free ϵ -amino groups by reaction with FDNB is assumed to be the available lysine of the sample. The main advantages of this procedure are that partial destruction of the derivative during acid hydrolysis does not cause difficulties and that lysine itself is extremely stable to destruction by acid. However, automatic ion-exchange chromatography equipment is necessary for ease in estimation of lysine concentration.

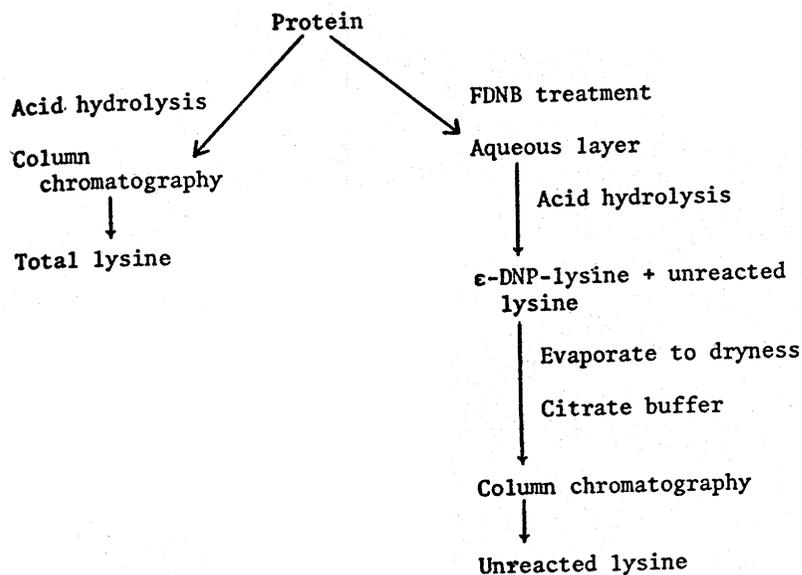


FIG. 7. Determination of available lysine by difference. Total lysine - unreacted lysine = available lysine. From Blom et al. (1967).

Rao et al. (1963) first used the difference technique as a check on the procedure they developed for determination of available lysine in oilseed meal proteins. Blom et al. (1967) also applied the technique for similar reasons. Roach et al. (1967) were the first to use the difference procedure as a working replacement for the Carpenter (1960) FDNB procedure. Ostrowski et al. (1970) modified the Roach et al. (1967) technique so as to make an automatic analyzer unnecessary and to allow the rapid routine determination of total and available lysine in foods.

Posati et al. (1974), using the difference technique described by Blom et al. (1967), measured total and available lysine in samples of spray dried and instantized nonfat milk. A schematic of the procedure is shown in Figure 7. Posati et al. (1974) were able to detect lysine which was blocked to reaction with FDNB by using a Beckman 120C amino acid analyzer in accordance with the method of Spackman et al. (1958). Posati (1973) found that a column employing

Beckman PA35 resin which was shorter than 5.5 cm did not give adequate separation of lysine from the acidics and any 2,4-dinitrophenol formed. The ϵ -DNP-lysine derivative eluted from the column with arginine under the elution conditions used. Kedenburg (1969), by altering the elution conditions, was able to separate ϵ -DNP-lysine from the arginine peak.

Theoretically, TNBS or other reagents which react with ϵ -NH₂ groups could be used. However, under her elution conditions, Posati (1973) found that ϵ -TNP-lysine did not separate from free lysine during the chromatographic step.

The difference procedure suffers from the major disadvantage that it is extremely time-consuming. However, in the opinion of the authors of this chapter, it offers the most accurate means presently available for the chemical determination of available lysine in dehydrated dairy products.

REMAZOL BRILLIANT BLUE R DYE

Ney and Wirotama (1970) have recently reported that the reactive dye, remazol brilliant blue R, whose structure is shown in Figure 8, when converted to the vinyl form by heating in strong alkali, will form a covalent bond with the free ϵ -amino group of the lysine residues and with the thiol group of cysteine. No reactions were observed with the hydroxyl groups of serine and tyrosine. The authors claimed that reaction with cysteine can be disregarded in the case of milk proteins.

Figure 9 shows the procedure for the determination of available lysine by the remazol brilliant blue R technique. Dyed protein is separated from excess dye by passage over Sephadex G-25 coarse. The excess dye in solution is then read at 280 m μ . The authors chose to work in the UV region because the extinction coefficient of the dye is double that in the visible region.

Although Ney and Wirotama (1970) obtained good results with casein, and in a later publication, Pruss and Ney (1972) demonstrated

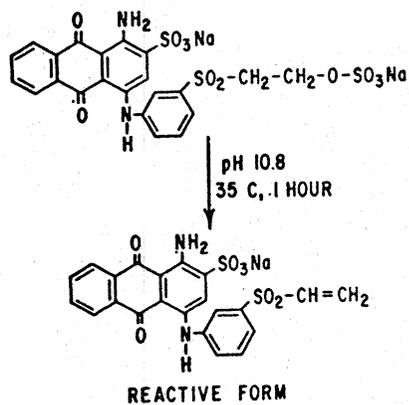


FIG. 8. Structure of the reactive dye remazol brilliant blue R. From Ney and Wirotama (1970).

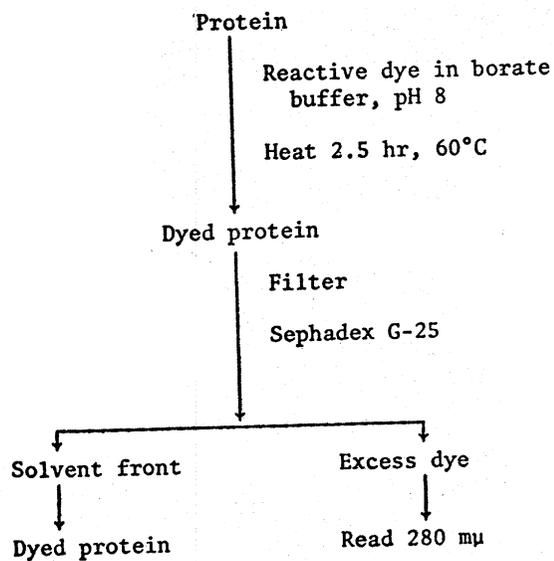


FIG. 9. Determination of available lysine with remazol brilliant blue R dye. From Ney and Wirotama (1970).

TABLE 6
Effect of Concentration of Whey Protein on Dye Binding

| Protein, mg | mg dye bound/mg protein |
|-------------|-------------------------|
| 19.5 | 3.54 |
| 41.8 | 2.40 |
| 80 | 1.73 |
| 117 | 1.55 |
| 165 | 1.41 |

^aFrom Pruss and Ney (1972).

no interference from lactose and other sugars, the method suffers from the disadvantage that the reaction is dependent on the quantity of protein present in the sample. Table 6 shows the effect of concentration of whey protein on dye binding. As the quantity of protein increases, the amount of dye bound decreases. Pruss and Ney (1972) have developed an extrapolation procedure to eliminate this problem.

Although quantitative determination of available lysine is difficult with this technique, it should find ready application in following lysine deactivation in the processing of dairy products of similar protein content.

S-ETHYL TRIFLUOROTHIOACETATE

A promising new technique for the determination of available lysine, thus far only applied to pure milk proteins, has been developed by Ramirez et al. (1975). Free ϵ -amino groups of lysine are trifluoroacetylated with the reagent S-ethyl trifluorothioacetate in dimethyl sulfoxide solution and the number of such groups is quantitatively determined using standard ^{19}F NMR techniques. The procedure employed is a modification of the Goldberger-Anfinsen procedure for trifluoroacetylating lysine in the protein chain (Goldberger and Anfinsen, 1962). Dimethyl sulfoxide was selected

as the reaction medium in order to improve the homogeneity of the system. For all proteins studied, the derivatized product was soluble in dimethyl sulfoxide.

After the solution became homogeneous, a period of several hours, a portion was added directly to an NMR sample tube and the ^{19}F spectrum obtained. Figure 10 shows a typical spectrum. The resonance at lowest field (A) corresponds to trifluoroacetic acid, a hydrolysis product of the thiol ester. The peak at intermediate field (B) corresponds to the trifluoroacetylated ϵ -amino group of lysine and the peak at highest field (C) to the trifluoroacetyl group of the thiol ester (unreacted reagent). Relative integrated intensities were calculated from the weights of the appropriately cut out peaks. The molar ratio of lysine to the original thiol ester is given by the ratio of the weight of the intermediate peak to the total weight of all peaks.

Table 7 shows a comparison of the lysine content determined with ^{19}F NMR to the compositional data for five pure proteins. The excellent agreement which can be observed suggests this as a new technique for the determination of available lysine in foods. The method offers a reasonably accurate, fast, and relatively simple procedure without the difficulty of variability of results from

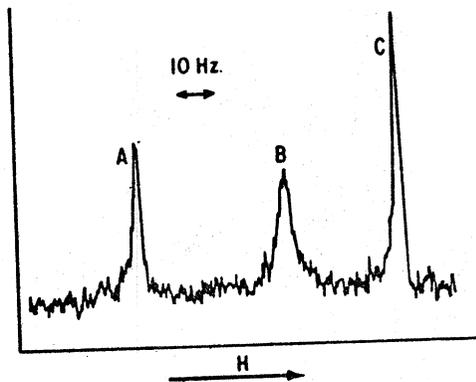


FIG. 10. ^{19}F NMR spectrum of the reaction products of a protein and S-ethyl trifluorothioacetate in dimethyl sulfoxide solution. From Ramirez et al. (1975).

TABLE 7
Comparison of Lysine Content Determined by
¹⁹F NMR with Compositional Data for Pure Proteins^a

| Protein | Lysine content g/100 g protein | |
|----------------------|-----------------------------------|--------------|
| | Composition ^b | Experimental |
| Bovine serum albumin | 12.8 | 12.5 ± 0.4 |
| β-Casein | 6.5 | 5.9 ± 0.1 |
| α-Lactalbumin | 11.5 | 11.7 ± 0.5 |
| β-Lactoglobulin | 11.7 | 11.5 ± 0.4 |
| Lysozyme | 5.7 | 5.0 ± 0.1 |

^aFrom Ramirez et al. (1975).

^bLiterature values.

varying amounts of protein present. In addition, the α-amino groups of the protein chains do not appear to be attacked in these experiments. The method does require access to a ¹⁹F NMR spectrometer. It is not known at this time whether the large amounts of lactose found in most dairy products would interfere with the accurate determination of available lysine using this method; research is continuing in this area.

CONCLUSIONS

By application of one of the chemical methods described, a good estimation of the amount of available lysine present in dehydrated dairy products can be made. The difference technique for available lysine determination employing FDNB, although time-consuming, probably yields results which come closest to the true biological value, when lysine is the limiting factor in the product under consideration. However, a single, simple chemical technique for the determination of available lysine applicable to all types of foods still remains to be developed.

NOTE

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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