



The cystine and methionine content of Montana range grass
by Earl W Turner

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University
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Abstract:

The leaf proteins of several Montana range grasses are extracted using sodium dodecyl sulfate. The advantages claimed for this method of extraction are simplicity, rapidity, and high yields of a final product containing as high as 80% pure protein. Cystine and methionine are determined on the leaf protein preparations using both chemical and micro biological assay methods. A comparison is made of the results obtained by the various assay methods. Methionine and cystine values obtained by both microbiological and chemical methods are found to be in good agreement and also consistent with results obtained by other methods. Methionine and cystine are determined on *Agropyron smithii* and *Agropyron cristatum* at two different stages of growth. The methionine and cystine contents of the younger grasses are considerably higher than of the more mature plants.

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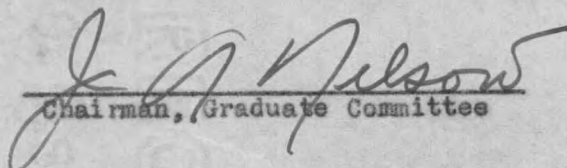
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PERMANENT RECORDS

I. ABSTRACT

The leaf proteins of several Montana range grasses are extracted using sodium dodecyl sulfate. The advantages claimed for this method of extraction are simplicity, rapidity, and high yields of a final product containing as high as 80% pure protein. Cystine and methionine are determined on the leaf protein preparations using both chemical and microbiological assay methods. A comparison is made of the results obtained by the various assay methods. Methionine and cystine values obtained by both microbiological and chemical methods are found to be in good agreement and also consistent with results obtained by other methods. Methionine and cystine are determined on Agropyron smithii and Agropyron cristatum at two different stages of growth. The methionine and cystine contents of the younger grasses are considerably higher than of the more mature plants.

II. INTRODUCTION

The presence of the sulfur containing amino acids, cystine and methionine, in plant proteins has been known for some time. Since these amino acids are quite easily altered by the action of ordinary hydrolytic reagents and probably by reagents employed as protein extractants, the determination of these acids in plant leaf proteins presents a special problem to the biological chemist. Cystine and methionine are essential to the growth of many organisms. In view of these facts, a more exacting study of methods of determining these amino acids in plant proteins is desired.

In the past, quantitative procedures for the determination of methionine and cystine have not been sufficiently accurate to afford more than approximate results. Although there are a number of chemical methods available for the estimation of methionine and cystine, many of these methods present serious difficulties which leave some doubt as to the reliability of the results obtained. Recently much work has been done in an effort to improve the older methods and to develop new methods of analyses which would give more consistent and accurate results. Of the more recently developed methods microbiological assaying offers interesting possibilities.

In view of these developments and the interesting problems that are presented, it was decided to investigate the desirability of the microbiological as well as the chemical methods of analysis. The leaf protein extracts from two common varieties of Montana range grass, Agropyron smithii and Agropyron cristatum, were selected for this study.

III. HISTORICAL RESUME

The Discovery of Methionine and Cystine:

Two sulfur derivatives have been found as constituents of the protein molecule. These are cystine and Mueller's thio acid (methionine). Cystine was isolated in 1810 by Wollaston and was found to be di, β -thio- α -amino propionic acid. Mörner, Osborne and Guest, Dakin, Osborne, Harris and others found that there was a probability of sulfur compounds in the protein molecule other than cystine. In 1922 Mueller isolated from casein a new sulfur containing amino acid, to which he gave the empirical formula $C_{11}H_{22}SN_2O_4$. Subsequent work on this amino acid by Mueller indicates that the amino acid has the empirical formula $C_5H_{11}SNO_2$ instead of $C_{11}H_{22}SN_2O_4$. In 1928 Barger and Coyne showed that Mueller's thio acid had the constitution α amino- γ -methyl thiol-n-butyric acid, $CH_3-S-CH_2-CH_2-\underset{\substack{| \\ NH_2}}{CH}-COOH$ and gave it the name methionine.

Protein Extraction Methods:

One of the most difficult problems in the study of leaf-proteins is the extraction and purification of the protein without subsequent loss of amino acid. The loss of amino acids occurs most readily when there is humin formation. Humin is formed when the hydrolysis is carried out in the presence of carbohydrates.

To determine the protein cystine content it is necessary to hydrolyze the protein with strong acid or with the appropriate proteolytic enzymes. As no method of preparing these proteins has hitherto been available, early workers found it necessary to submit the whole grass to acid hydrolysis or enzymic digestion. This means in effect that the proteins in the leaf material have been treated in the presence of from 3-6 times their own

