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BIOCHEMICAL AND BIOLOGICAL STUDIES ON SOME LEAF PROTEIN

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By

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B. Sc. (Biochemistry)

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THESIS

Submitted in partial fulfillment of the requirements for the Degree of

Master of Science in Biochemistry

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M.Sc.(Agricultural Biochemistry)

Faculty of Agriculture
Ain Shams University

1977



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Date : 26 / 2 / 1977

A C K N O W L E D G E M E N T

The author wishes to acknowledge the sincere help offered by Prof. Dr. S.I. El-Hinnawy, Professor of Agricultural Biochemistry Ain-Shams University, for the continuous help, careful guidance and criticism during the supervision of this work.

Thanks to Prof. Dr. A.S.Hamed Prof. of Biochemistry and Chairman of Soils Dept. Fac. of Agric., Zagazig Univ. for this kind help and assistance during the course of the present investigation.


Thanks also to Mr. G.M. Hamed Assist. Prof. of Biochemistry, Fac. of Agric., Ain Shams Univ. for his encouragement and valuable suggestions offered throughout this work.

Special thanks to Dr. Fikry El-Mahry, of the Institute of Nutrition, for his kind and continuous help throughout this work.

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I N T R O D U C T I O N

INTRODUCTION

The world food problem is one of greatest challenges of mankind to day, because of the continually widening gaps between the increase of world population and world food supplies. The major nutritive material in food are proteins which are the most limiting factor in the human and animal diets.

Several attempts had been tried to increase the food supplies as well as to improve the protein quality of foods.

Protein is, perhaps, the most important not only because it is required in small amounts for normal growth and development both physically and mentally as stated by Scrimshaw (1967) but also because it is quite essential for building up new tissues.

The joint of FAO/WHO Expert Group which met in (1971) studied carefully the data of the nitrogen balance for the human and they came to the conclusion that 0.57 g. and 0.52 g. of protein per day for each kilogram of body weight as the safe level of protein intake in terms of cow's milk or egg protein for are adult man or woman.

Food consumed by a great majority in developing countries, including Egypt, is usually deficient in proteins, both in quality and quantity, causing widespread malnutrition.

Therefore, efforts are currently directed to increase the world supply of protein for human consumption and improving its nutritive value. Plant sources of proteins are always more cheaper than the animal proteins, but they are usually of lower biological value, quality of cereal proteins can be improved through the addition of synthetic amino acids. Vegetable protein-rich food mixtures for preschool children such as Incaparena, Supramino and others were formulated to improve the quality of cereal proteins. Such products are now available on the local markets.

Introduction of new sources of proteins that will help as animal food and human food has received great deal of attention. Therefore the utilization of existing resources such as leaf-proteins as a source of food protein has expanded.

Moreover, attempts are undertaken to introduce unconventional protein sources such as the single cell proteins of algae, fungi, yeasts....etc.

During our lifetime, the higher plants will probably become one of the main sources of the nutrients needed by the world i.e. to supply proteins for man, yet the conversion and concentration of plant proteins into animal proteins by animals capable of doing this, cause a waste of 85-95% of the essential amino-acids present in the original plant products. So it is advisable to replace this by exploitation of higher plant proteins. It will, not only eliminate much of the 85-95% waste, but will allow the use of many plant species that, are not readily consumed by animal or man, leading to a marked increase in the productivity of our food.

However, more research on plant proteins is needed to determine the best form in which they may be incorporated into human diets, to find those crops suitable for the preparation of leaf-protein concentrate, and to determine production cost. In this connection, it should be mentioned that during the last 25 years, work has progressed so far at research laboratories, that they now

have machinery able to handle several tons of leaves a day, and extract most of the protein present in the leaves. The protein extracted is coagulated, filtered off and washed. The washed leaf protein has relatively little flavour, that could be masked by spice, curry and other flavouring agents. It can be prepared in manner to resemble the natural meats, to be introduced in meals for human consumption as suggested by Pirie (1963).

The work embodied in this investigation was to study the possibility of the use of some local leaves of plants grown in Egypt, for the preparation of leaf protein concentrates. The plants used in this work were Turnip green and sweet potato leaves which still did not find their way in any economical applications. The objectives of this study were :-

I. Selection of suitable method for the extraction of native proteins from the green leaves of sweet potato and Turnip green leaf plants.

II. Study of the Biological evaluation of the extracted leaf proteins through the determination of their net protein utilization (N.P.U.), protein efficiency ratio (P.E.R.) and net protein ratio (N.P.R.).

S E C T I O N I .
R E V I E W O F L I T E R A T U R E

REVIEW OF LITERATURE

I- Extraction of leaf protein:

As early as (1924), Chibnall et al obtained pure protein preparations from leaves using ether to plasmolise the leaves before their extraction with an object of improving the purity of the protein preparations.

In the same year, Osborne described also a method using 60% ethanol containing 0.3 % sodium hydroxide, for the extraction of proteins from the green leaves.

Davis (1926) dissolved the plant material in alkali and then reflocculated with acid. These processes successfully increased the purities and nitrogen contents of the isolated proteins.

The sequence of improvements of the leaf protein extractions were also tackled by Hoak (1927) who separated the protein from the leaves by simple grinding with water which was followed by heating the extractions to coagulate their proteins.

Miller and Chibnall (1932) in their numerous experiments to improve the preparation of protein from grasses found that the ether method previously mentioned by Chibnall et al (1924) was not so efficient. By substituting ether with ether-water mixtures they obtained better yields of leaf-proteins. The same authors in (1933) modified the ether-water method for the preparation of the protein from leaves and obtained excellent yields of protein from several grasses and forage crops.

Lugg (1939) extracted the proteins from plant leaves in various ways and showed that the use of mild alkaline buffers when macerating leaves with the object of dispersing the protein was the most suitable method. On the other hand, the difficulties of avoiding the loss of the proteins belonging to the granular units during the removal of cellwall material and other impurities were minimized.

Moreover, it had been shown that the addition of lipid solvent, such as alc hol and ether, to the mildly alkaline juice permitted most of the protein of the granule fraction to pass into solution.

From the results, Igg and Weller (1944) who described the extraction of the protein of reasonable purity from the leaves of four important species of pasture plants. The procedure involved the liberation of protein from the chloroplasts and possibly other granular protoplasmic bodies with slight alkalinity buffer i.e. pH 9.2 and in the presence of lipid solvents.

Crook (1946) reported a process which can extract 90 - 95 % of the nitrogenous materials from tobacco leaves. The steps in this process were : preliminary mincing and washing, grinding in a tripleroller mill, extraction with dilute sodium hydroxide at pH 8 several times.

With respect to the conditions that influence the protein extraction from leaves; Crook and Holden (1947) indicated that the nitrogen content and the dry matter percentage of the leaves showed great influence on the extraction of the leaf protein. Further more they found that the proteins extracted from leaves differed among the different plant species and the percentages of the extracted proteins ranged from 41-93 %.