BIOCHEMICAL STUDIES ON PLANT PROTEIN CONCENTRATES.

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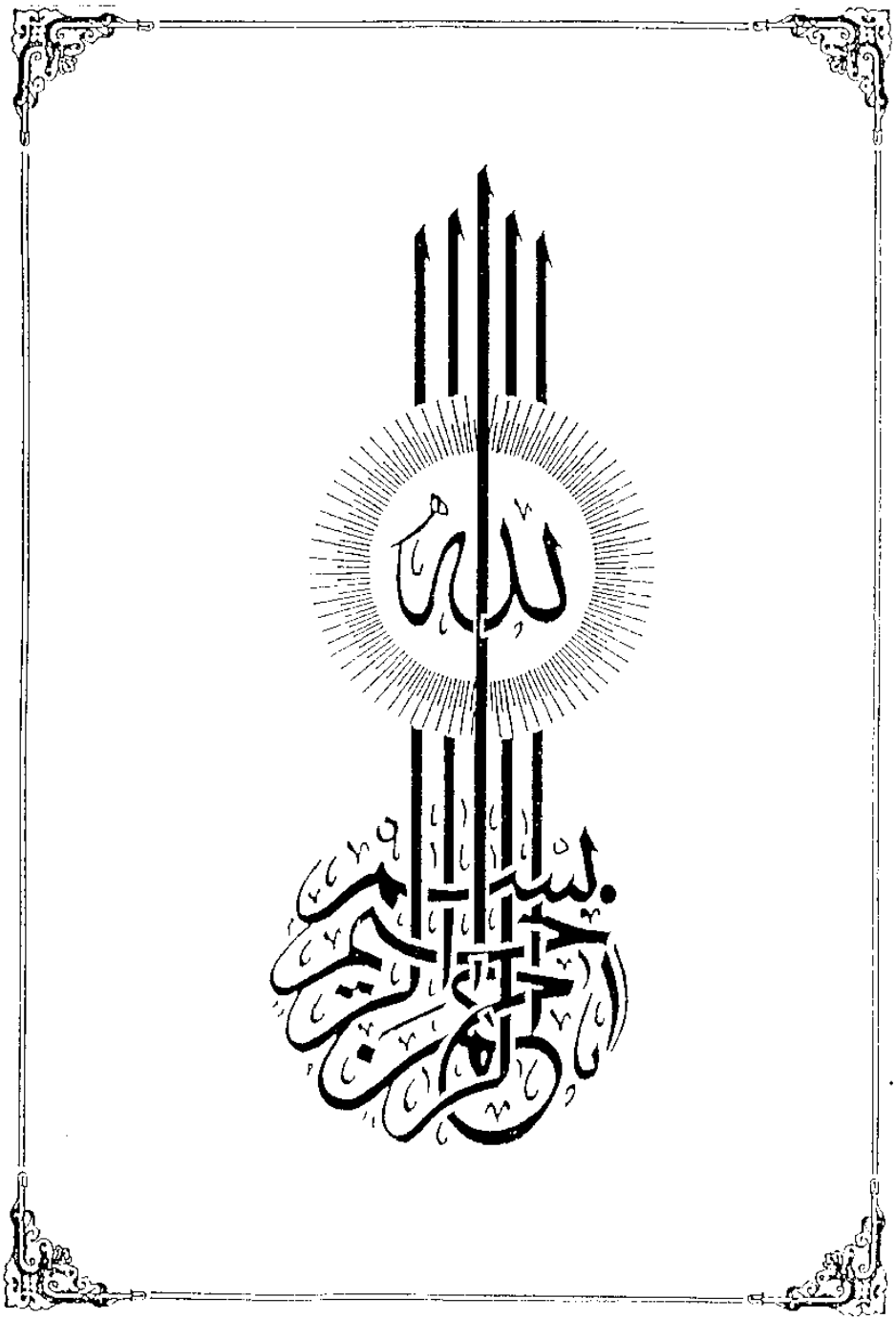

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1. INTRODUCTION

Shortage of world's protein production is one of the most serious problems presently facing the world.

During the last FAO Conference , Octobre 1983 the General Secretary of the United Nations announced that there are ten millions of children which yearly died in addition to 1000 millions of the world population which are under critical level of feeding. This finding suggested a black shadow on the future of human feeding all over the world.

For producing more protein the President Advisory Committee (1967) proposed ten methods included searching of the best genetic type of crops and animal producing meat in addition to these innovating source of proteins e.g. algea protein, leaf protein concentrate and single cell protein (Snyder, 1970).

However, green leaves represented the largest world's protein source. Furthermore, green leaf usually contains 90% of the synthesized protein in the plant in comparison to 5 - 10% only saved by seeds. But the most promising green source for leaf protein production are forages especially alfalfa plant or clover, (Saunder, et al, 1973).

The extraction of more than 10% of the clover leaves dry matter into two leaf protein fractions directily used for nutrition, one of them of highly percentage of digestibility, by the mechanical and chemical means, may markedly increase the protein productivity.

Several methods of leaf extraction were used started by (Roulle, 1773). The most common is this American method ProXan. The method used in this study proposed a connection between the production of leaf protein concentrates and a higher amount of protein from yeast using some leaf by-products for the yeast propagation. Seven to 10% of clover dry matter are still distributed however into another two by products, the chloroplastic or green leaf protein and residues. The first has already been suggested as a good ingredient for poultry feeding. The latter with its high amount of cellulosic content is suitable to be a good silage for reuminants.

The scheme of this research work consists of two main points, protein preparation and protein analysis. The first includes leaf protein concentrate production from green leaves with high percentages of cytoplasmic protein of highly biological value in addition to single cell protein production using the leaf serum by-products as a new yeast's media.

The second point is to study the biochemical properties and biological values of these new proteins in comparison with some of other common protein foodstuffs.

2. REVIEW OF LITERATURES

2.1 Protein Production :

2.1.1. Leaf Protein Extraction and Preparation :

Two hundreds and more than ten years ago, leaf protein was prepared, Roulle (1773) for instance, was one of the pioneers in this field. He mentioned that protein occurs in parts of plants other than seeds. He also used heat coagulation in order to separate protein from the plant juice into two fractions : a green one and pigment free one. Osborne and Wakeman (1920) obtained protein from the juice of macerated leaves. Chibnall et al (1933) were able to separate 80 - 90% of leaf nitrogen using an end runner mill with six separate lots of water. Also they obtained purified protein preparation from leaves with ether as anaesthetics to plasmolise leaf materials before extraction.

The main attempts to purify protein by those earlier investigators were dissolving in alkaline and refloculation with acid. However, Noak (1927) was able to obtain high protein extracts of 11 - 12% nitrogen using water and acid coagulation. A modified ether-water technique had also been used by Lugg (1939) who pointed out that the use of mild alkaline buffers while macrating leaves and the addition of lipoid solvents allowed the extraction of the most of leaf protein in a purer condition. Another procedure was out-lined by Grook (1946) in which 90 - 95% of the nitrogenous

compounds of leaf were extracted using a dilute sodium hydroxide solution at pH 8.

First large-scale extraction for leaf protein was carried out by Lugg and Weller (1944) they prepared protein samples containing 14 - 15% nitrogen from leaves of some species by mincing and squeezing them to obtain the protein extracts, from which the protein precipitated at a proper pH value. Whith an object to increase both yield and purity of protein, Guha (1961) suggested to crush leaves with 2% sodium carbonate, then centrifuged, and precipitated with HCL at pH 3.8. The proteins were then separated by centrifugation, drying and finally refind by several extractions with aceton. Byers (1961) minced leaf materials in a domestic mincer, the resultant pulp squeezed through a fine fibrous filter, after heating at 80°C, the preciptate is centrifuging off and washed repeatedly with distilled water until a clear supernatant.

Another large-scale producing for leaf protein concentrate (LPC) have been done by Morrison and Pirie (1961), fresh pulped leaf juice in this method is freed from starch grains fibers, then coagulated with steam, the coagulum protein is filtered off, washed with water at pH 4.0 and pressed into blocks while freeze dried. They noticed also that leaves juice has pH ranged from 5.5-6.2, and extraction is improved when pH 8 was used. The Western Regional Research Laboratory developed a process which has economically

sound and is used for largescale production. The procedure was mentioned by Saunder et al (1973) it has been designated the proxan process which divided into two phases: Phase (I) process consists of pressing the green juice from the alfalfa, coagulating the protein with heat, and separating the protein curd from the juice. The curd is then driet at standard dehydrating temperatures. The phase (II) process consists of flash-heating the raw juice, removing the chloroplastic protein centrifugally, and precipitating the cytoplasmic protein from the supernatant by heat, or lowering the pH.

Similarly, Free and Satterlee (1975) used a method in which leaf material were pressed with sodium bisulfate, the juice was centrifuged at 18000 x g for 20 min. The precipitate discarded and the supernatant "brown juice" was fractionated as follows: (a) Acid precipitated using concentrated HCL to final pH 3.5, storing for six hours at 4°C, then the precipitate of LPC is collected centrifugally at 15000 x g for 10 min. The supernatant fluid called "brown serum", (b) Heat precipitation whereby the brown juice heated at 80°C under continuous stiring and immediatly cooled, the mixture allowed to stand for 6 hours at 4°C and separated as previously described.

Alfalfa leaves have been pressed also according to Livingston et al (1980) using a twin screw press provided a whole green juice adjusted to pH 8.5 with concentrated NH_4OH then heated to 80-85°C by direct steam injection,

the whole coagulated green protein is collected after centrifugation.

More recently, Fantozzi and Sensidoni (1983) reported that protein extraction from tobacco leaves gives a smoking material with reduced toxicity, also tobacco leaf could be used as alternative to plants as a source of food protein. Tobacco plants were grinding with reducing agents pressed and centrifuged to separate fibers and juice. The latter was coagulated using citric acid at pH 3.5. The contrifugation then lyophilisation directly gives a whole green leaf protein concentrate.

2.1.2. Factors Influencing Leaf Protein Extraction and Preparation :

There is no general rules governing the preparation of extracts from leaves (Pirie, 1961). He stated also that mechanical damage is most obvious hazard to which an intercellular structure is exposed, but in many tissues the subcellular particles have considerable mechanical strength, the greatest hazard then is during grinding substances are exposed to an environment different from that surrounding then normally.

These factors however may be summarized as follows; Leaf species and stage of maturity (Boud, 1968; Chayen et al; 1961). The presence of mucilagenous materials (Nazir and Shah, 1966); Post harvest treatment (Huang

et al, 1970); pH and temperature of extraction (Lu and Kinsella, 1972; Poppe et al, 1970 and Evans, 1982).

From the point of view of freezing pretreatment, Wildam and Jagendorf (1952) showed in their experiment that if a high speed of centrifugation is not available that is possible to remove the particulate matter at lower speeds if the cell-free protoplasm is first frozen for 24 hours or longer and thawed before centrifugation, Whereas Pirie (1957) declared that they were unable to detect any changes in the solubility or in the physical behaviour of the cytoplasmic protein which had been frozen in neutral solutions as well as in case of extracted materials which can stored in the frozen state for several weeks and still return completely to solution after being thawed.

Also, the pH of extraction play a specific role, Betschart and Kinsella (1973) stated that the solubility of protein nitrogen of some leaves as a function of pH was most soluble at pHs 2.0 and 6.0 and above. Minimum solubility occuried between pH 3.2 and 3.7. Furthermore, Evans (1982) reported that dry matter and crude protein distribution in the fractions were influenced by the pH. At pH 4.0, the greatest amount of chloroplastic protein is extracted from costel bermuda grass while the least amount is extracted from white clover. The pH optima for extraction of the cytoplasmic proteins occurred at pHs 7 and 8 for the two plants, respectively. The amounts of cytoplasmic crude

protein extracted from both of two forages at their optimal pH were equivalent. The non protein nitrogen fractions increased in nitrogen content with increasing pH while crude protein in residue fractions decreased with the increasing of the pH for both of the two plants. He also showed that the dry matter distribution paralleled the crude protein distribution. Merodio et al (1983) tried, from another side, to make use of the characteristic of changing pH during the preparation of leaf protein concentrate in order to produce chlorophyll free protein, they estimated lowest chlorophyll percentage between 3.4 and 4.2 pHs while the whole results suggested that the pH which is the best for obtaining chlorophyll free protein were very sharp and ranged from 3.4 to 3.6 pHs, furthermore, low centrifugation i.e. 2000 x g is able to separate cytoplasmic leaf protein concentrate at this pH from chloroplastic one.

As noticed before, plant species as well as plant age play also a particular effect. The colour of final protein, for instance, depends upon the plant species from which the protein was made and varied from nearly white to pale brown. Different species have also different protein fiber ratios and the higher the ratio the greater the amount of protein that can potentially recovered (Edward and Hill, 1978). In another words, during processing the fiber material traps the protein and renders it unavailablbe for recovery (Lexander et al, 1970).

From the point of view of plant age, Morrison and Pirie (1961) pointed out that as the crop matures the percentage extraction diminishes, then only 15-20% of this leaf protein will be extractable even if water or alkaline are added. Byers (1961) claimed also that proteins were easily extractable from the younger leaf.

2.2 Yeast Propagation For Producing Single Cell Protein:

2.2.1. Single Cell Protein (SCP) Yeast Strains:

In this respect, Linder (1922) showed that the organisms which are commonly used is a strain of torula utilis which gives comparatively undiscriminating in its nutrition demands, and grows rapidly in good yield. Some of others were mentioned by Atkin (1944) included saccharomyces cerevisiae, candida arborea, c. tropicals and torulopsis utilis as the most specific microorganisms used in yeast food. After several experiments, White (1954) added the names of s. logos, s. lactis, Endomyces vernalis, T. candida, and Mycotorula lipolytise and others to the list of fodder yeast. But Peppler (1970) has found that among good species which are more suitable for the production of fodder yeast were s. carlbergensis, s. fragilis, c. tropicalis and c. utilis.

Lodder and Kreger van Rig (1952), ascribed s. cervisiae as the yeast of choice when molasses is used as raw material for the production propagation purposes, Prescott and Dunn