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EXTRACTION AND CHARACTERIZATION OF PROTEIN FROM *Acacia mellifera* SEED

Sharmeen Naaz[1], Dipika Bepary[2], Dolanchapa Sikdar[3], Riya Dasgupta[4]
[1,2]UG Student, [3,4]Assistant Professor
[1,2,3,4]Department of Food Technology, Guru Nanak Institute of Technology
[1,2.3,4]157/F, Nilgunj Road, Panihati, Sodepur, Kolkata-700114, India

Abstract*: Acacia mellifera* , commonly known as blackthorn, is a dense thorny shrub native to many semi-arid regions in Africa and western Asia. It is an underutilized legume which can be a potential source of plant protein. Research and Development of these seeds can cater to the world food shortage and further quality plant-based protein might be discovered. Seed Protein Isolates could be a favourable replacement of animal-based protein and are known to provide various beneficial activities like antimicrobial, anti-hypersensitive, antiviral and antioxidant. In the present study, proximate analysis of the Acacia mellifera seed was carried out with the aim to get a better understanding of the composition the blackthorn seeds and it was followed by estimation of soluble protein. The soluble seed protein has been separated in a solution using Microwave Treatment of defatted seed powder. The protein content in the seed samples ranged from 4.9% to 40.9% with varying duration of microwave treatment.

Keywords: *Acacia mellifera,* Acid-Alkali Extraction, Total Protein Proximate analysis, Soluble protein;

1. Introduction

Plant seed proteins are known to provide various beneficial activities like antimicrobial, anti-hypersensitive, antiviral and antioxidant. They are essential source of amino acids; act as a source of nutrition booster (Sonawane et. al. 2018). With the recent advancements and increasing consumer demands, visibly there is a shift occurring from traditional animal sources of protein to the plant-based protein sources, therefore, seed protein isolates can be a favourable replacement for animal-based protein sources. There is a worldwide interest in finding low-cost protein sources so far not exploited. Edible legumes play a major role in meeting widespread protein malnutrition (Bressani & Elias, 1974). Seeds of acacia plants (containing over 1,350 species) have considerable amount of protein (18.25% to 35.5%) and nutritionists have shown great interest in assessing the quality and functionality of proteins from these protein- rich plants. Seeds of acacia plants (containing over 1,350 species) have considerable amount of protein (18.25% to 35.5%) and nutritionists have shown great interest in assessing the quality and functionality of proteins from these protein-rich plants.

Seeds of Acacia plants, containing over 1350 species, have considerable amount of proteins and has significant commercial potential because of its high nutritional value (Adiamo et. al 2019). Theses seeds are commercially traded and have used traditionally for many purposes aiding to its tumor inhibitory activity (Jayatilake et al., 2003; Mujoo et al., 2001), and in vitro anticancer properties (Haridas et al., 2001; Li, Davis, Haridas, Gutterman, & Colombini, 2005). *Acacia mellifera,* commonly known as blackthorn, is a dense thorny shrub native to many semi-arid regions in Africa and western Asia. It is a very thorny shrub to small tree, 2-5 m tall. Crown rounded or flat and spreading, with branches that may reach down to the ground. Bark light to dark grey and longitudinally fissured, fissures generally darker. Branchlets flattened, 1.5-5 mm thick, glabrous; stipules deciduous, not spinescent; spines in pairs, 3-5 mm long, black, recurved and in pairs at nodes that are only 5-15(-30) mm apart and thus there are more thorns per unit length of branch than with other species (Abdelsalam et.al. 2020). A shallow rooted nitrogen fixing species, its protein rich leaves provide a useful browse. Clean burning, it valued as fuelwood and also for making charcoal, its wood being hard. It flowers prolifically and is a valuable honeybee plant, its specific epithet mellifera referring to producing honey (Cabi compendium et. al 2022). *Acacia mellifera* leaves and roots are used in traditional African ethnomedicine for the treatment of cold, malaria (A. Koch et. al. 2005), primary infection of syphilis, sterility, and bowel problems (C. Mutai et. al. 2004), including inflammation, diarrhoea, and bleeding (A. H. Fatima et. al. 2013). The published reports

of various biological activities of *Acacia mellifera* include its antimalarial (C. Mutai et. al. 2008) and antimicrobial (C. Mutai et. al. 2009) potentials. Phytochemical studies on *Acacia mellifera* extracts have indicated that the main components are alkaloids, saponins, flavonoids, tannins, and triterpenoids (S. Lalitha et. al. 2010, C. Mutai et. al. 2007,H. F. Abdel-Razik et. al.,2006). Acacia mellifera leaves also shows very promising hepatoprotective and cell-proliferative effects (Ahmed H. et. al., 2015). In this resent study, the analysis of ash content, moisture content, carbohydrate content, lipid content and Protein content of *Acacia mellifera* was done for characterizing the seed

2. Materials and Methods

2.1. Raw Materials:

Raw materials like sample seeds were obtained from Calcutta Seed Nursery, 155, B. B. Ganguly Street, Kolkata – 700012. All the other chemicals used were of laboratory standard taken from the Food Analysis and Quality Control Laboratory in Guru Nanak Institute of Technology, Sodepur, Kolkata.

2.2. Methods

2.2.1. Preparation of The Plant Material-

The method of seed preparation for protein extraction was followed as per the method stated by Babiker et. al. 2019 with slight modifications. Blackthorn seeds (*Acacia mellifera*) were acquired from the Calcutta Seed Nursery in Sealdah, West Bengal. They were manually cleansed to remove any foreign substances or contaminants such as debris, plant pieces, or damaged seeds. The shells (seed coats) were frequently peeled from the seed kernel. The stones were removed using sieves. The cleaned seeds were placed in a beaker, and 100gm of them were weighed for grinding. After grinding the seeds, they are sieved again to obtain fine seed powder. The known weight of the sample was then used to investigate the subsequent experiment.

2.2.2. Extraction of Seed Protein by acid alkali Treatment

It has been identified that acid –alkali mediated treatment for the preparation and extraction of protein from the seed as a simplest and promising technology throughout the globe. Based on this fact, the method of acid –alkali mediated treatment was conducted with slight modification as stated by Zhang, et.al. 2018. In this experiment, water served as a solvent for microwave-based protein extraction. Microwave treatment was performed at five different time intervals in order to conduct a comparative evaluation of the extracted soluble protein. As a result, the extraction duration was set to 0 minutes, 5 minutes, 10 minutes, 15 minutes, and 30 minutes for five batches of samples, at a power of 320 Watts. Centrifugation was done to remove the grounded seed debris from the water, the rpm was initially set to 10,000 for 5 minutes. The supernatant was then removed from the seed precipitates and subjected to pH adjustment to coagulate the protein in solution. To coagulate the protein molecules, the isoelectric point of all test samples was adjusted to around 5.9-6 pH by adding 1N HCl and 1N NaOH drop-wise while continuously swirling the sample solutions. The entire arrangement was kept warm in a water bath at 60-65 °C.

Following the second centrifugation, the protein precipitates were removed from the tube by mixing with whey protein dropwise.

2.2.3. Extraction of Tannin:

Tannin present in the seed coat is regarded as the anti-nutrient component while value adding seed protein. In this context, the removal of tannin from the seed coat has been considered as inevitable part of the research work, considering this fact, a method of solvent immersion was adopted to extract as well as remove the tannin. To extract tannins from defatted grounded seeds, 30 ml of acetone and 70 ml of distilled water were mixed and the sample seeds were left overnight by adding it into the solution. The processing temperature was kept reasonably low (room temperature to 60 °C).

2.2.4. Analytical Procedures

2.2.4.1. Proximate Analysis of Seed

Proximate Analysis such as determination of moisture, ash, crude fat, crude protein and fibre was conducted for seed as per the AOAC method, 1990. The proximate analysis of seed provides better understanding about the composition of seed for further value addition.

2.2.4.2. Protein Quantification by Folin-Lowry Method:

The experimental procedure was conducted with the preparation a fresh Bovin Serum Albumin (BSA) stock solution of 1000 micrograms per ml, followed by preparing Reagents A of 2% Na2CO3 in 0.1% NaOH and Reagent B of 0.1% CuSO4 in 1% Na-K Tartarate in 100ml Distilled water for Reagent C (Folin Lowry Reagent) of 50ml reagent A and 1ml reagent B. The Folin Ciocalteau reagent should be in a 1:1 ratio. Taking six test tubes, label them B (blank), 0, 1, 2, 3, 4, and 5. BLANK is made up of 1ml of distilled water and 4ml of Reagent C, and the other samples are made up of 1ml of sample and 4ml of Reagent C. All six test tubes are incubated at room temperature in the dark for 10 minutes. After incubating the test tubes, add 0.4ml of Folin Ciocalteau reagent to the solution and incubate for another 15 minutes at room temperature in the dark. In this experiment, T1 is diluted tenfold. After incubation, the absorbency was measured with a spectrophotometer at 660 nm.

3. Results and Discussions

3.1. Proximate Analysis of Seed

Table 1 shows moisture, fibre, carbohydrate, ash, and oil content present in seed sample. The fibre content observed in the sample was 6.5%, carbohydrate content found to be 37.8%, ash 4.6%, moisture 6.2%, and oil 7.4%.

table 1: proximate analysis *acacia mellifera* of seeds

Ash	4.6%
Moisture	6.2%
Fibre	6.5%
Carbohydrate	37.8%
Fat	7.4%

3.2. Yield of Protein extracted from Seed

The seeds of *Acacia mellifera* were investigated for protein contents in different time parameters. The seed samples were treated for time duration ranging from 0 minutes to 30 minutes in the microwave to observe the extraction of protein. The variation observed in the extracted protein (Figure 1) for 0 minute was 4.97%, 5 minutes- 10.02%, 10 minutes- 23.98%, 15 minutes- 35.62%, and for 30 minutes, it was found to be maximum- 40.95%. This data is found to be in close comparison to the results observed by (Adiamo et. al 2019).

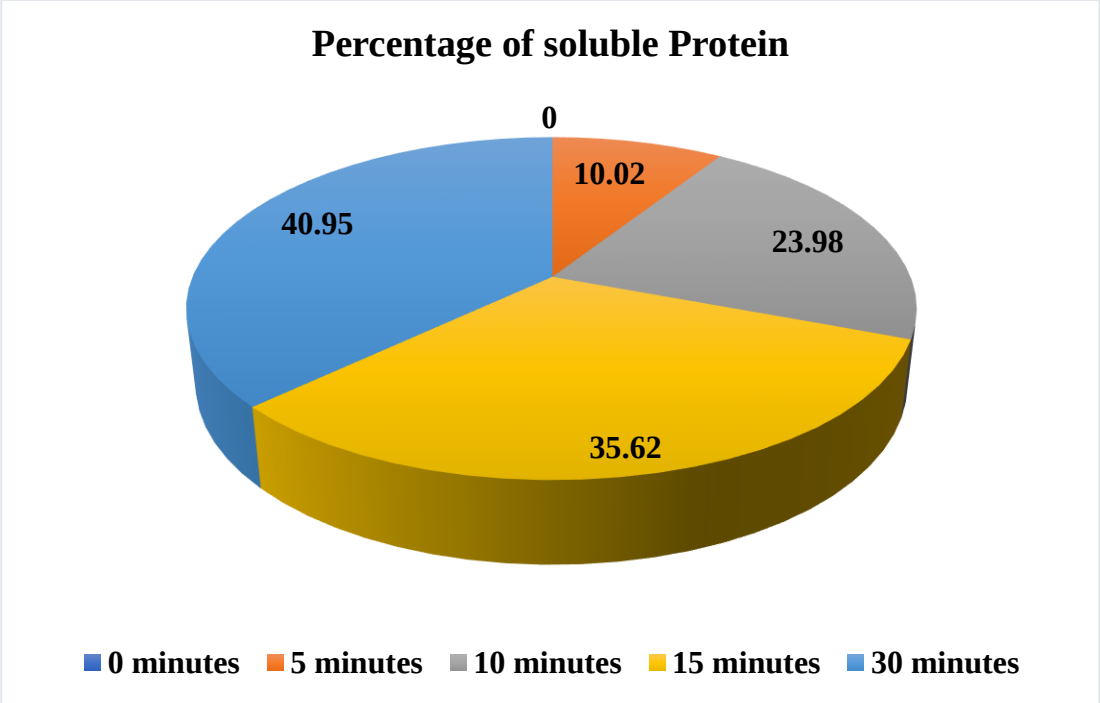

figure 1: protein extraction at different time intervals

4. Conclusion

The current results provide useful information regarding underutilised legumes for future research to meet consumers' increasing protein demand. In this study, the seeds of a leguminous species, called Acacia mellifera, belonging to the Acacia genera, were analysed for protein content, which was found to be approximately 40.95% when microwave treatment was done at different time intervals, with attractive proximate analyses as ash (4.6%), moisture (6.2%), crude fibre (6.5%), carbohydrate (37.8%), and crude lipid (7.4%). Investigations into the presence of various anti-organoleptic, proteinase inhibitors, antinutritional, or toxic substances, as well as the development of appropriate technologies to eliminate them, are required to improve the nutritional quality for the use of other species in the same genera.

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