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Akshay S Chandran
Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Corresponding Author:
Akshay S Chandran
Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

A review on extraction of protein from mahua seed (*Madhuca longifolia*) using different extraction method

Akshay S Chandran

Abstract
The genus Madhuca, a versatile plant belonging to the Sapotaceae family, includes species such as *Madhuca longifolia, Madhuca longifolia*, and *Madhuca butyracea*. Among these, *Madhuca longifolia*, commonly known as Mahua, is a large deciduous tree found both in the wild and cultivated throughout India. The flowers and seeds of this tree have significant economic value, particularly for rural forest dwellers, and serve as a source of livelihood. Mahua seeds are an abundant oilseed crop in southern India, with annual production of around 0.5 million tonnes and yields ranging from 20 to 200 kg per tree. The seeds have a high vegetable fat content compared to other tropical fruits. Mahua seed oil extraction is commonly carried out using commercial screw press expellers due to the high oil content of the seeds. The residual seed cake, after oil extraction, contains approximately 30% protein and can be detoxified to serve as a good source of protein for food and feed products. Both alkaline extraction and ultrasound extraction are valuable techniques with unique benefits for protein extraction, offering opportunities for the modification and enhancement of proteins for various applications. Alkaline extraction involves the use of alkaline conditions to extract proteins from plant and animal sources while modifying their structure and function. Ultrasound extraction, on the other hand, is a safe, non-intrusive, and environmentally friendly method. It utilizes mechanical waves with a frequency higher than the audible range for humans.

Keywords: Mahua, uses, protein extraction

1. Introduction
The genus Madhuca, which belongs to the Sapotaceae family, is a versatile plant with the most common species being *Madhuca longifolia, Madhuca longifolia*, and *Madhuca butyracea.* Mahua is a large, shady deciduous tree that grows wild and cultivated all over India. This tree's flowers and seeds provide a great source of revenue and livelihood, particularly for forest resider in rural India. Mahua is a valuable crop because of the industrial applications of its flower, fruit, and seed. This is a forest plant that grows in India's dry climatic conditions. It is categorized as a minimal waste plant since its parts are used for different purposes (Nayak & Sahoo, 2021; Ramadan, Abdelrazek, *et al*., 2016) [11, 10]. Mahua flower, leaves, bark, seed oil, seed protein, seed cake, and other parts of the mahua plant have a wide range of applications. The high reducing sugar and nutrient content of Mahua flowers is well known. The plants' flowers are edible. The corolla, also known as mahua flowers, is a sugar-rich flower that also contains a significant number of vitamins and minerals. (Bisht *et al*., 2018) [12]. Mahua fruits can be consumed either raw or cooked. The fruit pulp can be used to make sugar, while the dry husk can be used to ferment alcoholic beverages. Oil can be found in abundance in seeds. Saponin, an alkaloid glucoside, is found in the leaves of the Mahua tree. In the seeds, sapogenin and other basic acids have been discovered (Bisht *et al*., 2018) [12].
Mahua is valued for its seeds. In the southern part of India, mahua seed is the most abundant oilseed crop. Mahua seed production in India is around 0.5 million tonnes per year, with seed yields ranging from 20 to 200 kg per tree (Pradhan *et al*., 2020) [13]. Green fleshy fruits 6 with three to four ellipsoidal-shaped seeds are produced by its flowers. Compared to seeds of other tropical fruits, mahua seeds have potentially high vegetable fat content. Mahua seeds are harvested during periods of high rainfall. In India, Mahua has about 18 lakh metric tons of oil production capacity annually. Fresh form of mahua seed oil has a light-yellow color with an unpleasant taste and odor at room temperature. Because the seeds of mahua may contain 50 to 61% oil, there is a good chance that oil can be extracted from them using commercial screw press expellers. The deoiled seed cake of mahua contains around 30% protein (Ramadan, Mohdaly, *et al*., 2016) [2].

Defatting of Mahua seed increased the level of protein, saponin and tannin. Treatment with isopropanol could lower the levels of saponins. For food and feed products, mahua seed flour after it is detoxified is found to be a good source of protein (Ramadan, Mohdaly, *et al*., 2016) [2].

The Mahua oil cake (MOC) is a by-product obtained during the extraction of oil from Mahua plant seeds, comprising approximately 60-70% of the raw seed. Despite its abundance and rich nutritional and mineral composition, MOC remains underutilized. Currently, it is primarily used as feed for ruminants and fish, as well as low-grade fertilizers and adsorbents. However, considering its high protein content (>19%), we recognized the potential for extracting protein from MOC and exploring its physicochemical properties and structure for various applications. In the process of separating proteins from a biological source, it is crucial to ensure the overall feasibility of the extraction method and maintain the native structure of the protein molecule (Biswal *et al*., 2020) [24].

2. Composition of Mahua seeds and seed cake

The composition of mahua seeds includes different constituents such as oil, protein, fiber, carbohydrates, ash, saponins, and tannins. The seeds consist of 50-61% oil, 16.9% protein, 3.2% fiber, 22% carbohydrates, 3.4% ash, 2.5% saponins, and 0.5% tannins. The oil content is the highest among all components and is three times greater than the protein content. After extracting the oil, the remaining seed cake called deoiled seed cake comprises 30% protein, 1% oil, 8.6% fiber, 42.8% carbohydrates, 6% ash, 9.8% saponins, and 1% tannins (Ramadan *et al*., 2016). The protein, saponin, and tannin concentrations of mahua seeds increased after the removal of fat.

Table 1: Uses of different parts of mahua

Part	Use	Reference
Fruit	Fruits are astringent and used in chronic tonsillitis and pharyngitis	(Sinha *et al*., 2017) [3]
Seed	Source of oil, low-grade fertilizer, bio-pesticide	(Ramadan *et al*., 2016) [2]
Flower	As coolant, aphrodisiac, galactagogue, expectorant and carminative, making vinegar, Preparation of bakery and confectionary goods	(Gupta *et al*., 2012) [1]
Root	Root paste used to expel intestinal worms	(Tomar, 2009) [4]

3. Use of Mahua as a food

Despite being a highly versatile species with a rich nutritional profile and widespread availability in rural areas, the consumption of these flowers as a food source is not very popular among the local population. Raw, cooked or fried, only a small quantity of the flowers are observed to be consumed in different parts of the country.

Sugar syrup

The dry flowers of the Mahua tree can be used to make sugar syrup, which can then be utilized as a sweetening ingredient in various food items (Sinha *et al*. 2017) [3]

Jam, Jelly, marmalade, pickle

According to (Patel and Naik 2010) [19] unripe fruits that are fully matured are used to make jam with the addition of citric acid. The pulp is converted into marmalade or syrup and used as a food ingredient. Jelly is also made from the pulp either alone or mixed with guava to modify its astringent taste. The pulp is also pickled, while the majority of the flowers are utilized for producing distilled liquors.

Fermented products

Kumari *et al*. (2016) [22] discovered that the flowers of the mahua plant are used to produce alcoholic beverages by fermenting the material. In North-West India, the local population collects and dries the flowers for the preparation of "mahua daaru," which contains approximately 20-40% alcohol. The flowers are mixed with water and left to ferment, during which Ammonium chloride (Navshar) and jaggery are added. To create a strong, hot flavor, black pepper is sometimes included. Following fermentation, the mixture is transferred to a traditional container with a distillation setup.

4. Protein Extraction Methods

4.1 Alkaline extraction of protein

Alkaline-based methods are straightforward and versatile techniques commonly employed to restore and modify protein structure, thereby affecting its function. The utilization of alkaline conditions serves two purposes: extracting proteins from plant and animal sources and modifying the functional properties of the proteins (Momen *et al*., 2021) [7]. The addition of alkali enhances the yield of protein extraction through two mechanisms: (1) breaking down the surrounding matrix where the proteins are located, and (2) increasing the solubility of the protein molecules. Proteins tend to carry a higher charge when they are further away from their isoelectric point (Typically within a pH range of 4.5-5.0). This elevated charge promotes their solubility in water-based solutions. However, high pH levels can also induce alterations primarily in the tertiary and quaternary structure of proteins, as well as their overall composition. Additionally, changes may occur during the subsequent acidic precipitation step that is often performed. These structural and/or compositional modifications, including isomerization, crosslinking, and degradation, can have significant implications in terms of technological functionality and nutritional aspects (Deleu *et al*., 2019) [5].

4.2 Ultrasound extraction of protein

Ultrasound (US) has become increasingly recognized as a highly advantageous and cutting-edge method due to its safety, non-intrusive nature, absence of ionizing radiation, effectiveness, and environmentally friendly qualities, allowing treated products to maintain a clean label status [19]. US technologies operate by producing mechanical waves with a frequency exceeding the audible range for humans (20 Hz to 20 kHz). These waves cause the particles within the medium they pass through to move back and forth, creating a pattern of compression and rarefaction (Suchintita Das *et al*., 2022) [9]. Over the past decade, there has been significant interest in utilizing ultrasound for extraction purposes. Ultrasound-assisted extraction offers numerous benefits, such as enhanced solvent penetration into cellular material, improved mass

transfer, and more effective release of the extract through the disruption of cell walls (Ly *et al*., 2018) [6]. The efficiency of protein extraction and the improvement in extraction yields are influenced by various operational factors, including sonic energy density, sonication duration, substrate-to-slurry ratio, agitation, and more. Ultrasonication is also utilized for altering the physical, structural, and functional characteristics of protein-based ingredients, in addition to concurrent extraction and modifications. The application of sonication leads to notable alterations in protein properties such as size reduction, rheology, electrical conductivity, and zeta (ζ) potential (Rahman & Lamsal, 2021) [8].

5. Health benefits of Mahua seed

5.1 Antihyperglycemic activity

In a study conducted by (Khan *et al*. 2011) [18] it was found that the ethanolic extract derived from mahua seeds could reduce the plasma glucose levels in normal albino rats in a dose-dependent manner. This extract produced a hypoglycemic effect by stimulating the release of insulin from the β-cells or by increasing the uptake of glucose from plasma.

5.2 Anti-inflammatory activity

(Gaikwad *et al*., 2009) [23] conducted a study to evaluate the anti-inflammatory activity of the ethanol extract and saponin mixture of mahua seeds. The study used acute, sub-acute, and chronic models of inflammation in rats, including carrageenan-induced inflammation, formaldehyde-induced inflammation, and cotton pellet granuloma. The results showed that both the ethanol extract and saponin mixture had a significant anti-inflammatory effect, particularly in the acute and sub-acute models. The extracts were also found to be more effective than the reference drug diclofenac sodium in the sub-acute inflammation model. The study concluded that mahua saponins have significant anti-inflammatory activity in cotton pellet granuloma.

5.3 Antiulcer activity

The potential anti-ulcer activity of the crude alkaloid extract and ethanolic extract obtained from the seeds of mahua was investigated. The results of the study revealed that the ethanolic extract exhibited a significant protective effect against pylorus ligation-induced gastric ulcers. In particular, the administration of the ethanolic extract at a dose of 10 mg/kg resulted in a significant reduction in ulcer formation. Other bioactive compounds that were isolated and characterized from the plant include n-hexacosanol, as well as the β-glucoside and free form of β-sitosterol. In addition, the nut-shell of the plant was found to contain quercetin, hydro quercetin, and the β-glucoside of β-sitosterol (Saha *et al*. 2010) [2].

6. Conclusion

Mahua is a significant tree in India, providing a valuable source of revenue and livelihood, particularly for rural communities. It is considered a minimal waste plant as its flowers, leaves, bark, seed oil, seed protein, seed cake, and other parts are utilized for different purposes. The seeds are abundant in oil, making them a prominent oilseed crop in southern India. Mahua seed oil extraction is feasible using commercial screw press expellers due to the high oil content. The deoiled seed cake contains a significant amount of protein, which can be further increased through defatting. Protein extraction methods play a crucial role in obtaining proteins from various sources and modifying their structure and functionality for diverse applications. Both alkaline extraction and ultrasound extraction have their advantages and considerations. The choice of extraction method depends on the specific requirements of the protein and the intended application. Factors such as pH, sonic energy density, sonication duration, and agitation play crucial roles in optimizing extraction efficiency and protein properties. Understanding and optimizing protein extraction methods is essential for various industries, including food, pharmaceuticals, and biotechnology, as proteins are vital components for a range of products. Further research and development in protein extraction techniques will contribute to the advancement of protein-based products, improving their functionality, nutritional value, and overall sustainability.

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