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Effect of various processing treatments on *Macrotyloma uniflorum* (Horse gram)

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Abstract
Horse gram (*Macrotyloma uniflorum*) also known as poor man's pulse is an underutilized pulse. It is a latent pulse grain having great nutritional worth and the capability to acclimate in harsh environment. The pulse is rich in protein, have low glycemic index and a great source of fiber. The main objective of this review is to study the effect of different processing on nutritional composition, physical properties, functional properties, vitamin analysis, cooking properties and anti-nutritional factors in Horse gram pulse. Different types of diseases can be treated these different studies showed that horse gram has potential to be utilized in product formulation in bakery and confectionary.

Keywords: Horse gram, cooking, anti-nutritional, functional, fermentation, germination

Introduction
Legumes are regarded as one of the world's most crucial sources of food in underdeveloped nations. At the moment, there is a lot of buzz about using neglected legumes for human consumption (Abhirami *et al.*, 2018). Legumes play a significant role in maintaining soil fertility, making them an essential component of sustainable agriculture. In many developing countries, these are a primary source of dietary nutrients for many people, especially vegetarians. Horse gram is an underutilized legume that is grown as a pulse crop in India and has been found to have great nutritional value (Pal *et al.*, 2015) [18]. Horse gram, also known as Kulth, Kulthi-Kalai, horse gram (English), and kulattha, was previously known as *Dolichos biflorus*. It is considered a poor man's food because it is an inexpensive source of proteins for human consumption and cattle. It can be consumed in the form of whole meal, dhal, splits, and sprouts (Handa *et al.*, 2017) [8]. Admitting that there is defined genetic information on horse gram, it has been considered as a future pulse due to the high therapeutic properties. Because of its tolerance or salinity, drought, and heavy metals, horse gram species have a variety of medicinal properties, including antimicrobial and antioxidant activity, as well as being effective in the dissolution of kidney stones (Trivedi *et al.*, 2015) [27].
Horse gram is a good source of protein, carbohydrate, fiber, and micronutrients as a food. In some parts of India, this is also used as cattle feed (Sahoo and Mohanty 2020) [23]. It is primarily grown in India, Africa, Australia, Burma, Malaysia, and Sri Lanka as an underexploited food crop. Despite the high quality of nutritional supplements, there is widespread prejudice in the population against the use of Macrotyloma uniflorum. Global population growth has raised food demand, prompting food and agricultural researchers to hunt for underutilized low-cost agricultural produce as a substitute for traditional human diets. Horse gram (*Macrotyloma uniflorum*) is a valuable but underutilized legume grown mostly in India's southern states, such as Karnataka and Andhra Pradesh. The plants can be grown in low-fertile soil and in bad weather conditions. Horse gram was once thought to be a medicinal legume having healing powers in ancient Ayurveda. Recent research has discovered the nutritional makeup of horse gram. Horse gram is a good source of protein, glucose, vital amino acids, and energy (Banerjee *et al.*, 2022) [1].
The reason for this is that Macrotyloma uniflorum has long been regarded as a low-quality food. Despite this prejudice, Macrotyloma uniflorum (horse gram) is India's fifth most widely grown pulse (Ramachandraiah *et al.*, 2019). It lacks methionine and tryptophan like other legumes, although it is high in calcium, iron, and vitamins such as thiamin, riboflavin, niacin, and L-ascorbic acid. It's important to keep in mind that the higher dietary fiber content may have positive effects on the intestine and colon.

Despite the availability of essential nutrients, horse gram is underutilized, which could be due to anti-nutritional factors found within the gram (Ojha *et al.,* 2020) [15]. Horse gram is a low-fat legume and is high in protein, dietary fiber, micronutrients, and phytochemicals.

Due to the presence of certain phytochemicals with antinutrient effects that limit the nutritive value of these legumes, the use of horse gram flour as an ingredient in composite flours and functional food is limited (Bhokre *et al.,* 2015) [3, 4]. Because of components like tannin, trypsin inhibitor, and phytic acid, which interfere with the bioavailability of supplements present in horse gram, the most extreme use of horse gram is missing (Pagar *et al.,* 2021) [16-17]. Horse gram has a high iron content, but the phytates, tannins, and oxalic acid it contains reduce the iron's availability. Horse gram is a good source of protein and appears to be a calcium source as well. However, the amount of oxalic acid in horse gram is high, which combines with calcium and iron to form an insoluble salt, rendering the calcium and iron unavailable for absorption (Pal *et al.* 2015) [18].

Even so, significant progress has been made in reducing antinutrient factors and improving the nutritive value and functional properties of legumes through dehulling, germination, fermentation, dehydration, soaking, and partial hydrolysis of the proteolytic enzyme (Vandarkuzhali1 and Sangeetha 2016). Even so, a large number of references exist in the traditional system of medicine of horse gram demonstrating its medical benefits such as anti-diabetic, anti-calcifying, anti-hypercholesterolemic, analgesics, anti-oxidant, and larvicidal properties, making it critical to compare the nutrient compositions of the underutilized food legume crop horse gram with other well-known legumes (Sumeet and Rahul 2020). It lacks methionine and tryptophan like other legumes, although it is high in calcium, iron, and vitamins such as thiamin, riboflavin, niacin, and L-ascorbic acid. It's important to keep in mind that the higher dietary fiber content may have positive effects on the intestine and colon.

Despite the availability of essential nutrients, horse gram is underutilized, which could be due to anti-nutritional factors found within the gram (Ojha *et al.,* 2020) [15]. Due to the presence of certain phytochemicals with antinutrient effects that limit the nutritive value of these legumes, their use as ingredients in composite flours and functional foods is limited. Soaking, boiling, germination, and fermentation are common processing methods used to reduce the content of these undesirable components, resulting in improved acceptability and nutritional quality, as well as optimal use of these legumes as human food (Sreerama *et al.,* 2011). In this study, we will evaluate the impact of different processing methods on the Physical, Functional, and cooking properties and Anti-nutritional, Anti-oxidant factors of Horse gram.

Review of Literature

The current research focuses on the chemical composition of raw and processed Macrotyloma uniflorum (horse gram) seeds. To compile the following systematic version, several reviews on the nutrients, antioxidants, enzymes, and antinutrients in horse gram seeds, as well as the effects of various processing treatments, were gathered.

Table 1: Classification and nomenclature of Macrotyloma uniflorum

Kingdom	**Plantae**
Phylum	Tracheophyte
Class	Magnolyopsida
Order	Fabaies
Family	Fabaceae
Genus	Macrotyloma
Species	*M. uniflorum*
Chromosome no.	20, 22, 24

Its taxonomic classification was clarified by Ranasinghe and Edifier in 2017. Horse gram is classified as a member of the Kingdom Plantae. It belongs to the phylum Tracheobionta. Magnoliopsida is the class. It belongs to the Dicotyledon family. Fabales is the order, and Fabaceae is the family of pea plants. *Mycrotyloma uniflorum* is the species that belongs to the Macrotyloma genus.

Plant features

The second most important crop group after cereals is Food legumes. It has been a staple food of a well-balanced human diet, as well as the second most valuable plant source for human and animal nutrition. Although a few conventional legumes dominate the production and market chains in developing countries and play a critical role in eradicating protein malnutrition, some underutilize indigenous legumes, such as horse gram which is termed *Macrotyloma uniflorum* in scientific nomenclature. It plays a significant role in the nutritional security of rural, tribal, and underprivileged populations (Bhartiya *et al.,* 2015) [2].

Horse gram is a potential food crop belonging to the Fabaceae family with highly nutritious and medicinal qualities as well as greater climate-resilient to acclimate to adverse climatic conditions. It is among the most crucial untapped food leguminous plants, grown everywhere across the globe, which includes milder climates and sub-tropical areas such as East and Northeast Africa, Asian countries such as India, China, and the Philippines, Bhutan, Pakistan, Sri Lanka, and Queensland, Australia. Horse gram is a minimal pulse crop grown in southern Asia, primarily from India to Myanmar, as well as feedstuffs and organic manure crop across many tropical countries, notably in Australia and Southeast Asia (Bhartiya *et al*., 2015) [2]. In dry regions of India, Australia, Sri Lanka, and Burma also it is consumed as a vegetable in India, where it is recognized as the poor man's pulse crop (Sriram *et al.,* 2011).

Nutritional Composition

Horse gram is a pulse crop grown in India, accounting for about 0.33 percent of the overall food grain production. According to findings on the nutritional content of horse gram, it is a great source of protein which is approximately 25%, 60% carbs, essential amino acids, energy, and has a low lipid content of 0.58%, Fe, and content (Bravo *et al.,* 1999).

Horse gram is an elevated source of fiber in reduced-carbohydrate food. In addition to other homoeostatic and therapeutic functions in human nutrients, have positive effects on the intestine and colon (Ramteke *et al.*, 2016).

Instead of being an excellent source of proteins and minerals, horse gram is not widely consumed by human beings due to its poor cooking aspect. Moreover, it is a nutritious outstanding minor staple food that is grown as a pulse crop and ingested as sprouts in many regions of our country. This legume has recently been recognized in the United States as future human food. The Fe content of the crop is generally high, however, the existence of trypsin inhibitors, phytates, haemagglutinins, and polyphenols lessens its usage (Guna Shree and Govindarajulu 2016). Horse gram is a low-cost protein source that is high in minerals like calcium, phosphorus, and iron, as well as vitamins like carotenes, thiamine, riboflavin, niacin, and L-ascorbic acid. In Andhra Pradesh, horse gramme water is used to treat jaundice. It is well-known for its therapeutic properties, as various parts of the plants are used to treat heart illness, asthma, bronchitis, urine discharges, and kidney stones (Ramteke *et al.*, 2016).

Horse gram is high in protein (17.9%–25.3%), carbs (51.9–60.9%), essential amino acids, calories, low fat content (0.58–2.06%), Mo, P, Fe, and vitamins such as carotene, thiamine, riboflavin, niacin, and vitamin C (Saroj and Manoj 2015). Aishwarya and Kasturiba in 2018 found moisture content, ash, protein, carbohydrates, fat and crude fiber 7.4%, 3.88%, 24.51, 63.67, 0.58% and 4.41% respectively. While performing isolation and characterization of starch from horse gram (Chavan *et al.*, 2010) found that moisture content and ash percentage are 12% and 0.08% respectively.

Like many other legumes large variation in nutrition is found while studying about horse gram. According to Marimuthu and Krishnamoorthi in 2013 the moisture content 6.72%, ash 2.24%, fat1.25%, carbohydrates 58.32% and proteins 22.12% was found. Pagar *et al*., 2021 [16-17] found that carbohydrates, moisture content, fats, protein and ash content was 58.86%, 10.3%, 1.36%, 21.06% and 3.47% respectively. Per 100g of dry matter, horse gram seed contains 57.2 percent carbohydrate, 22% protein, 5.3 percent dietary fiber, 0.50 percent fat, calcium (287 mg), phosphorous (311 mg), iron (6.77 mg), calories (321 Kcal), and vitamins like thiamine (0.4 mg), riboflavin (0.2 mg), and niacin (1.5 mg) (Bhartiya *et al.*, 2015) [2].

Sreerama *et al*., 2007 studied mean proximate analysis was calculated as % of dry matter. They found the moisture content, ash, fat, protein and carbohydrates as 8.83%, 2.72%, 1.25%, 22.50%, 64.70% respectively. Another study was carried out in 2019 by Shilpa and Sangita which shows that the moisture content, ash, fat, protein and carbohydrates are 6.65%, 3.45%, 1.8%, 21.28% and 61.42% respectively. Summet and Rahul in 2020 conducted the study on proximate analysis of horse gram which states that ash, fats, protein and carbohydrates are 2.24%, 1.9%, 28.8% and 58.32% respectively.

Table 2: Proximate content of Horse gram in different studies

Fiber	Carbohydrates	Fats	Protein	Ash	Moisture	References
-	60%	0.58%	25%	-	-	Bravo *et al*., 1999
4.41%	51.9%-60.9%	0.58%-2.06%	17.9%-25.3%	-	-	Saroj and Manoj 2015
-	63.67%	0.58%	24.51%	3.88%	7.4%	Aishwarya and Kasturiba 2018
-	-	-		0.08%	12%	Chavan *et al*., 2010
-	58.32%	1.25%	22.12%	2.24%	6.72%	Marimuthu and Krishhnamoorthi 2013
-	58.86%	1.36%	21.66	3.47%	10.3%	Pagar *et al*., 2021 [16-17]
5.3%	57.2%	0.58%	22^%	-	-	Bhartiya *et al*., 2015 [2]
-	64.70%	1.25%	22.50%	2.72%	8.83%	Sreerama *et al*., 2007
-	61.42%	1.8%	21.28%	3.45%	6.65%	Shilpa and Sangeeeta 2019
-	58.32%	1.9%	28.8%	2.24%	-	Sumeet and Rahul 2010

Processing prospective

Women in developing countries have traditionally processed food legumes at home as part of their meal preparation. The nutritional value of the food, as well as the type and quantity of processing losses, will be influenced by a number of factors. The genetic makeup of the plant, the land in which it is cultivated, fertilizer treatment, weather conditions during harvest, packing, storage conditions, and processing techniques are all aspects to consider. The nutritional value of food is also influenced by how it is stored and treated after processing.

Effect of Germination

Sadawarte *et al.*, 2018 has been studied the effect of germination in which they found that after germination, the total phenols were decreased. In horse gram malt, the total phenol concentration was determined to be 1.06. After two days of germination, the total phenolic content declined dramatically, but subsequently increased as the germination duration increased to four days. The quality attributes of horse gram were severely impacted by sprouting. Moisture, lipid, and ash levels were observed to be decreased after germination, whereas protein and carbs enhanced. As a result, it's possible to conclude that the germination process aids in the production of nutritionally dense malt. Phytonutrients such as alkaloids, flavonoids, phenol, and tannins, as well as their anti-nutritional effects, were shown to be reduced after sprouting.

Study has been conducted by Handa *et al.*, 2017 [8] and observed that the TSS, moisture, titratable acidity, ascorbic acid, and total sugars all increased as germination time increased, whereas total protein, starch, total phenols, antioxidant activity, tannins, and oxalate all decreased. Water absorption capacity and solubility index of horse gram flour were considerably altered by improved germination, however the effects on oil absorption capacity, swelling power, foaming capacity, and bulk density were non-significant.

Pagar *et al*., 2021 [16, 17] discovered the effect of germination on horse gram and concluded that in which they found that Soaking, germination, and drying of horse gramme results in changes in the horse gram's characteristics. With soaking, germination, and dehydration, the level of tannin and phytic acid in horse gramme decreased by 24.77 percent and 39.66 percent, respectively. After soaking, germination, and drying,

the calcium and iron content of horse gramme was reduced by 31.68 percent and 20.68 percent, respectively. According to the properties, the treatment with 6 hours of soaking, 72 hours of germination, and 70 degrees of drying was the best, with the lowest anti-nutritional factors and the highest flour functional qualities due to the soaking and germination. As a result, it can be stated that 6 hours of soaking, 72 hours of germination, and 70 degrees Celsius drying are the best conditions for making snack sticks. The maximum acceptance was achieved with a commercial product (Snack sticks) containing 10% GHF.

Effect of roasting

The influence of roasting on properties of horse gram was carried out by Sawant *et al*., 2015. The moisture content ranges from 9.22 to 3.90, according to the research (% w.b.). It was initially higher, but as the roasting time increases, it reduces because free water evaporates first. The moisture content was highest in the treatment where it was roasted for 5 minutes (9.22%) and lowest in the treatment where it was roasted for 10 minutes with sand (1:2) that is (3.90%). The 1000 grain weight was found to be between 28.01gm and 31.82gm. It was initially -higher. The water in the sample evaporates as the roasting time increases. As a result, the grain loses moisture and weight drops. The treatment in which horse gram was roasted for 5 minutes had the highest value of 31.82gm, while the treatment in which horse gram was roasted for 10 minutes with sand had the lowest value of 28.01gm.

Ojha *et al*., 2020 [15] had studied effects of roasting and soaking in which they studied that soaking and roasting, polyphenols dropped by 19.5% and 28.3%, respectively, whereas flavonoids dropped by 33.3 percent and 48.7%. The antioxidant activity (AOA) of whole horse gram was discovered to be 52.682.24 DPPH percent inhibition, which dropped after soaking (28.77%) but increased with roasting (29.13%), germination (51.61%), and fermentation (51.92%).

Kadam *et al*., 2009 studied the nutritional composition, processing and utilization of horse gram and moth bean. The purpose of their research was to consolidate the data available in the literature on these two undervalued legumes in order to improve their use as food for people. They found that cooking of legumes should be done before consumption. Except cooking different processing like roasting and puffing of legumes could be done. Legumes after roasting are used in a variety of snacking recipes that are popular among the impoverished. Roasting did not lead to a significant reduction in different amino acids, according to studies conducted by Vijaylaksmi and Venkatrao. When compared to the values indicated by cooked horse gram, roasting improved the rats' growth rate, PER, and digestibility.

Effect of Fermentation

Fermentation process increase the nutritional values of horse gram legume. Similarly, the drying process will affect both nutrients and functional properties of legumes. Also, the flour quality and shelf life of products will increase with these processing methods. Therefore, fermentation and dehydration methods will improve nutritional utilization but also upsurge its application in remedial benefits for health conditions. Sarvani *et al*., 2020 studied the effect of processing on fermentation on different functional properties. They have found that the fermented processed flours had lower phytate levels than non-fermented flours. Germinated flour (0.44 mg / g) had the largest reduction in fermented flours, followed by cooking (0.51 mg / g) and soaking (0.62 mg / g), compared to non-fermented flours (0.56, 0.65, 0.71 mg / g for germinated, cooked, and soaked, respectively. Ojha *et al*., 2020 [15] found that when it came to reducing anti-nutrients, fermentation as well as germination were found to be more efficient than roasting and soaking (more than 50%).

Effect of Soaking

Handa *et al*., 2017 [8] studied the effects of soaking on physicochemical and functional attributes of horse gram. The results showed that as the soaking period lengthened, water absorption, oil absorption, solubility index, swelling power, and foaming capacity increased, while bulk density decreased. Horse gram flour increased water absorption capacity could be due to changes in total protein structure as well as the loosening of starch polymer structures as soaking time increases. Moisture, titratable acidity, ascorbic acid, and total protein content increased as soaking time increased, whereas TSS, starch, total sugars, total phenols, antioxidant activity, tannins, and oxalate content decreased when compared to control.

The results of the study show that soaking time has a significant impact on most of the product's engineering properties, as well as a section of the angle of repose and coefficient of static friction recorded after 10 minutes of soaking, indicating that length, width, and thickness are among the most important factors in the design of cleaning, sorting, and grading equipment for both cowpea seed varieties. In addition, rubber's high static coefficient of friction makes it ideal for use as a chute or chamber surface when biomaterial separation is critical (Ogunnigbo *et al*., 2018).

Nutraceutical benefits of Horse gram

- The health-promoting benefits of phytochemicals, nutraceuticals, and/or functional foods are most likely the result of a complex mix of biochemical and cellular interactions that work together to improve an individual's overall health (Saroj and Manoj 2015).
- The tremendous global growth of the nutraceuticals and functional food industries is due to the clinical success of nutraceutical products and rising health consciousness. Phenolics, flavonoids, alkaloids, carotenoids, prebiotics, phytosterols, tannins, fatty acids, terpenoids, saponins, and soluble and insoluble dietary fibers are the key chemical components known as potential health promoters (Saroj and Manoj 2015).

Horse gram leaves are also consumed as a vegetable in some regions of India, and the leaves include significant health-promoting properties including such anthocyanins, which seem to be powerful antioxidants that serve as free radical scavengers and have been demonstrated to be anti-inflammatory. Nontoxic compounds from horse gram aerial parts support its traditional medicinal usage (Bhartiya *et al*., 2015) [2].

Conclusion

Since prehistoric days, legume seeds have played a vital role in human nutrition. Grain legumes, also known as pulses in India, play an imperative role in human diet. They are not only crucial and cost-effective protein sources for vegetarian diets in developing countries, but they are also virtuous

sources of energy, B-complex vitamins, and minerals. In terms of amino acid balance, they are a good complement to cereals. The pulse horse gram, which is undervalued, has positive effects on human health. After reviewing various studies, it is clear that it is high in both nutrients and antinutrients. Horse gram is an underutilised legume crop that is high in protein (22%), as well as other important nutrients. It comprises a lot of lysine, which is an essential amino acid. Ca, Fe, P, and Mo are all copious in horse gram, as are vitamins like carotenes, thiamine, riboflavin, niacin, and L-ascorbic acid. Germinated and blanched horse gram seeds can be used as a salad ingredient with other vegetables, including green leafy vegetables and fruits, even though horse gram has the potential to treat a variety of diseases, according to Ayurveda. Horse gram seeds, in real sense, revealed that it contains numerous biomolecules with anti-urolithiatic qualities. Aside from that, horse gram has substantial medicinal value. As according Ayurvedic wisdom, daily intake of horse gram portions for one month will dissolve kidney stones. Finally, the horse gram should be considered an exploited source of nutraceutical and food industry due to its plentiful health benefits.

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