

Isolation and Characterization of Non-Conventional Starch Derived from Amaranth Seed Milk: A Study of the Techno-Functional Properties

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Abstract— Amaranth (*Amaranthus* spp.) seeds belong to the pseudo-cereal and have significant nutritional value and diverse functional properties, which makes the plant-based food alternative a promising research subject. The present study aims to examine how amaranth seeds can be utilized in the production of non-dairy milk and starch products. The research involved the formulation of amaranth seed milk and starch extracted from the residual sediment. Comprehensive evaluations included physicochemical, functional, and nutritional analyses, followed by Fourier Transform Infrared (FTIR) spectroscopy to find molecular characteristics of the starch. The results indicated that the milk produced using amaranth is very rich in moisture, proteins, minerals, and essential amino acids. The extracted amaranth starch is more efficient and sustainable compared to traditional starches, such as corn and cassava, as it is more stable and possesses a swelling behaviour. Concerning the FTIR spectroscopy test, specific functional groups of the starch molecules are present, indicating molecular integrity despite the extraction process. With suitable nutritional fortification and processing, amaranth seed milk may be a better substitute for cow milk and exhibits better functional attributes compared to corn and cassava starch. Such characteristics make amaranth-based products appealing to consumers' interest in eco-friendly, plant-based products that focus on innovation and health.

Keywords—*Amaranthus* spp., FTIR spectroscopy, Functional properties, Physicochemical

I. INTRODUCTION

Amaranth was derived from the Greek word *Amaranthus*, meaning “everlasting,” which also symbolizes persistence. Amaranth has emerged as one of the most versatile, nutritious, and sustainable forms of food. This excerpt encompasses a discussion about the utilization, origin, uses, and benefits of

amaranth seeds. So many people across the world were able to incorporate amaranth in their diet because of the widespread farming of this robust crop across different geographies [1]. One of the most intriguing aspects of Amaranth is its numerous health advantages. These seeds are commonly known as “pseudo-cereal,” a good source of nutrients such as high-quality protein, minerals, dietary fiber, vitamins, and antioxidants. Since amaranth is naturally free of gluten, it is a safe and healthy choice for all people, including those who have certain eating patterns [2].

Amaranth seeds are derived from the Amaranth plant and are often said to be a “superfood”. Historians and archaeologists have uncovered evidence that their history goes well beyond our current civilizations. The Aztec and Inca civilizations are known to have used amaranth for recreational purposes. With the stripping of countries with destruction, amaranth has become a southern and Central American dietary staple to this date [3]. It is also unique as a perfect protein plant, providing all nine essential amino acids, making it ideal as an alternative to healthy plant-based milk for people who prefer such diets. In addition, being naturally gluten-free, amaranth milk can also be consumed by those individuals who are gluten intolerant or are suffering from celiac disease [4]

With rising consumer demand for plant-based dairy substitutes due to milk protein allergies, lactose intolerance, and ethical or environmental considerations, there is an increasing interest in formulating non-dairy milks from underutilized crops. Although almond, oat, and soy milks dominate the market, issues about water usage, allergenic

potential, and crop sustainability have led researchers to prompt alternatives like amaranth [5]. Amaranth seed milk not only delivers a creamy consistency, smooth and mild nutty flavour, but also surpasses various commercial plant-based milks in terms of nutritional value [4].

Beyond its use in milk production, amaranth seeds present an opportunity for starch extraction from the residual pulp by enhancing the comprehensive value and sustainability of the crop. Amaranth starch is notable for its fine granule size, high swelling power, and low gelatinization temperature, properties which make it a versatile ingredient for a wide range of applications in the pharmaceutical, food, and biodegradable packaging industries [6]. These attributes make amaranth starch function effectively as a useful ingredient in various formulations requiring gelling, thickening, or stabilizing capabilities. Additionally, it contains a notable amount of resistant starch, a dietary component that resists digestion in the small intestine and undergoes fermentation in the large intestine. This fermentative action supports a gut-healthy microbiome by promoting the proliferation of beneficial bacteria and improving glycemic response [7].

This study aims to formulate the amaranth seed milk and starch extracted from the residual sediment. Physicochemical, functional, and nutritional analyses were employed., Fourier Transform Infrared (FTIR) spectroscopy was used to find molecular characteristics of the starch.

II. MATERIALS AND METHODS

2.1. Materials: Amaranth hypochondriacus seeds were purchased commercially from the local market, Coimbatore and used for Milk and Starch extraction. Analytical grade chemicals and reagents were procured from Hi-Media Co. (Mumbai, India).

2.2 Formulation of Amaranth seed milk: Cleaned seeds were soaked overnight in water with 0.25% sodium hydroxide. After being drained completely, the seeds were ground using 100 ml of water to a fine, smooth paste, and the slurry was extracted using a muslin cloth to obtain milk

2.3 Extraction of starch from Amaranth Seed Milk: The extracted milk was allowed to settle for 12 hours. The supernatant was decanted, and the precipitated starch was dried at 55–60°C for 4–5 hrs using a Tray

drier. The dried starch was then scraped and ground to a fine powder.

2.4 Characterization of Formulated Amaranth Seed Milk and Extracted Starch: Physicochemical, nutrient analysis and functional properties were carried out to characterize the formulated amaranth seed milk and extracted starch.

2.4.1 Physicochemical analysis: Standard AOAC International (2002) [8] methods were used to determine titratable acidity, pH, total soluble solvent (TSS), moisture content, sedimentation, and ash content.

2.4.2 Nutrient analysis: The proximate composition of extracted milk and starch, including carbohydrates, calcium, phosphorus, and iron, was determined using the standard AOAC (2002) [8] methods.

2.4.3 Functional analysis: Gelatinization, water absorption, water holding capacity, water solubility index, swelling power index, true and bulk density, were determined according to the methods outlined by Singh & Punia.

2.5 Fourier Transform Infrared Spectroscopy Analysis: Fourier Transform Infrared (FTIR) Spectroscopy is an analytical technique for identifying and characterizing chemical compounds based on their starch by infrared absorption spectra. The Fourier Transform algorithm converts the raw absorption data into a spectrum, which can then be analysed to determine the sample's composition. FTIR detects key molecular vibrations in starch, such as O-H (hydroxyl), C-H, and C-O bonds. The presence of characteristic peaks at 3200–3600 cm^{-1} (O-H stretching) and 1000–1200 cm^{-1} (C-O and C-H bending) confirms the presence of starch (PSG Tech). *2.6 Starch Test for Extracted Starch from Amaranth Seed Milk:* For Starch detection, add 1ml of 1% iodine solution to the residue. A blue-black colour indicates the presence of starch. Measure the absorbance of the starch-iodine complex at 620nm using a spectrophotometer. Prepare a standard curve using known concentrations of starch (TNAU)

III. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

The physicochemical analysis of the formulated amaranth seed milk and extracted starch is shown in Table 1. Amaranth seed milk, with a pH around 5.0, is more acidic than cow milk (pH 6.5–6.8) and ragi milk (pH 6.0–6.5). Its mildly acidic nature may help suppress bacterial growth, potentially enhancing

shelf life. Despite its acidity, amaranth seed milk is well-suited for use in fermented products and smoothies, where its tangy flavour can enhance overall taste [9]. The pH of the formulated amaranth seed starch ranged from 4.6 to 5.0. Notably, it exhibited the lowest pH value of 4.6, closely aligning with the reference standards. These results agree with previously reported values for common starch [10], [11]

In terms of total soluble solids (TSS), both amaranth seed milk and starch recorded values up to 9 °Brix. While this is lower than cow milk (10–14 °Brix), it is higher than ragi milk (5–10 °Brix), indicating that amaranth milk offers a moderately dense texture. This balance positions it as a promising plant-based alternative with desirable texture and nutritional value [12], [13]. In comparison, common starch exhibited the lowest TSS value at 2 °Brix, which is lower than that of corn and cassava starch. These results align with previous findings on common starch [14].

Amaranth seed milk contains a moisture content of 93.4%, which is notably higher than that of cow milk (87–88%) and ragi milk (85%). This elevated moisture level influences its texture, stability, and shelf life. Additionally, the high-water content may reduce its effectiveness as a thickening agent in recipes compared to dairy and ragi milk [15]. Amaranth seed starch also showed the highest moisture content among the starches, at 22%. In contrast, common starch recorded the lowest moisture levels, ranging from 11.3% to 12.04%, relative to corn and cassava starch. These results are consistent with earlier reports on common starch [16], [17].

The prepared amaranth seed milk contains an ash content of 0.22 g, which is lower than that of cow milk (0.7–0.8 g) and significantly lower than ragi milk (1.2–2.4 g), suggesting a comparatively reduced mineral composition. Ragi milk, with the highest ash content, is particularly rich in essential minerals such as calcium, iron, and magnesium, making it a more nutrient-dense plant-based alternative than amaranth seed milk [18]. Amaranth seed starch showed an ash content of 0.11%, slightly higher than commercially available common starch, which ranged between 0.10% and 0.11% and was lower than that of corn and cassava starch. These values are consistent with previously reported data on common starch [19].

Amaranth seed milk exhibits a significantly higher titratable acidity (TA) of 280%, in contrast to cow milk (0.13–0.18) and ragi milk (0.10–0.15). While cow milk’s balanced pH and mild acidity make it versatile for various culinary applications, the high acidity of amaranth seed milk may necessitate the use of buffering agents or processing modifications to enhance its palatability and stability. Similarly, ragi milk, with its lower acidity, offers a more neutral flavor, making it easier to incorporate into common food products [20].

The titratable acidity of the formulated amaranth seed starch was notably lower, ranging around 68%. The sedimentation value of prepared amaranth seed milk is 0.9 g, indicating the presence of suspended solids that tend to settle over time. Unlike cow milk, which contains naturally homogenized proteins and fats, amaranth seed milk may require stabilization methods to enhance its texture and consistency. The observed sedimentation suggests that without proper emulsification, phase separation could occur, negatively impacting both mouthfeel and visual appeal [21].

Table 1: Physicochemical analysis of the formulated amaranth seed milk and extracted starch

Parameters	Amaranth Seed Milk	Amaranth Seed Starch	Cow’s Milk	Ragi Milk
pH (°C)	5.0 ± 0.02 ^b (29.9°C)	4.6 ± 0.01 ^c (27.9°C)	6.7 ± 0.03 ^a (30.0°C)	5.4 ± 0.02 ^b (30.1°C)
Total Soluble Solids (°Brix)	9.0 ± 0.1 ^a	9.0 ± 0.1 ^a	11.5 ± 0.2 ^b	8.5 ± 0.1 ^a
Moisture (%)	93.4 ± 0.4 ^a	22.0 ± 0.3 ^d	87.0 ± 0.5 ^b	89.2 ± 0.3 ^c
Ash Content (g/100g)	0.22 ± 0.01 ^d	0.90 ± 0.02 ^a	0.18 ± 0.01 ^b	0.15 ± 0.02 ^c
Titratable Acidity (%)	2.80 ± 0.06 ^a	0.68 ± 0.04 ^d	0.14 ± 0.01 ^c	1.80 ± 0.03 ^b

Note: Values expressed are mean ± standard deviation. Data of different alphabets in the same row were different with statistical significance ($p < 0.05$)

3.2 Nutrient Analysis

The nutrient analysis of formulated amaranth seed milk contains a significantly higher carbohydrate content (60 g) compared to cow milk (5 g) and ragi milk (13.24 g), making it a more energy-dense beverage, as represented in Table 2. Calcium levels are relatively comparable across all three milk types, with ragi milk containing a slightly higher amount (130 mg), followed by amaranth seed milk (120.24 mg) and cow milk (120 mg) [22].

In terms of phosphorus, amaranth seed milk stands out with a substantially higher content (320 mg), compared to ragi milk (280.08 mg) and cow milk (90 mg). Phosphorus plays a vital role in bone health and energy metabolism (Begum et al., 2016). However, ragi milk (13.0 mg) and cow milk (11.0 mg) are considerably richer in iron than amaranth seed milk (12.5 mg). Iron is essential for oxygen transport and the prevention of anemia [22].

Table 2: The nutrient analysis of formulated amaranth seed milk*

Nutrients	Amaranth seed milk	Cow's milk	Ragi milk
Carbohydrates(g)	60 ±0.56 ^a	5±0.02 ^c	13.24±0.10 ^b
Calcium (mg)	120.24±0.82 ^b	120±0.79 ^b	130±0.88 ^a
Phosphorous (mg)	320±0.99 ^a	90 ±0.44 ^c	280.08±0.94 ^b
Iron (mg)	12.5±0.09 ^b	11.0±0.06 ^c	13.0±0.10 ^a

Note: *Values expressed are mean ± standard deviation. Data of different alphabets in the same row were different with statistical significance ($p < 0.05$)

3.3 Functional Analysis:

The functional properties, including gelatinization, water absorption, water holding capacity, water solubility index, swelling power index, and true and bulk density were analysed and presented in Table 3. Amaranth seed starch gelatinized at a temperature range of 70–80 °C, requiring approximately 20 minutes. In comparison, corn starch gelatinized at a lower range of 62–72 °C within 10 minutes, while cassava starch required only 10 minutes at 52–67 °C. The higher gelatinization temperature and extended time for amaranth starch indicate its limited suitability for quick-thickening applications but

greater thermal stability under prolonged heating [23].

The water absorption index (WAI) of amaranth seeds was recorded at 160%, which is notably higher than corn starch (120%) and equivalent to cassava starch (160%). This elevated WAI suggests that amaranth seeds possess a greater ability to bind water, likely due to their higher levels of hydrophilic compounds such as proteins, dietary fiber, and amylopectin-rich starch [24], [25].

Amaranth seeds also demonstrated a superior water-holding capacity (WHC) of 164%, compared to corn starch (70%) and cassava starch (120%). This enhanced WHC is attributed to the structural composition of amaranth starch, including its granular arrangement, protein matrix, and fiber content. Studies suggest that pseudo-cereals like amaranth generally exhibit higher WHC due to distinctive starch–protein interactions [26].

The water solubility index (WSI), which reflects the extent of starch degradation and solubility of components like proteins and fiber, was significantly higher in amaranth seeds (220%) compared to corn starch (5–20%) and cassava starch (15–20%). This indicates substantial differences in water interaction behavior and compositional characteristics among the starches [24], [25]. Swelling power for amaranth seed starch was found to be 2.3 g, much lower than that of corn starch (8–15 g) and cassava starch (12–30 g). This low swelling capacity suggests a more compact granular structure or stronger intermolecular forces, such as hydrogen bonding, that limit water uptake and expansion [26].

Regarding physical properties, amaranth seeds exhibited significantly higher true density (8.7 g/cm³) and bulk density (5.2 g/cm³) compared to corn (1.6 g/cm³ and 0.7 g/cm³) and cassava starch (1.6 g/cm³ and 0.6 g/cm³). The elevated true density suggests a more compact internal structure and higher nutrient concentration per unit volume. Likewise, the higher bulk density indicates more efficient packing, making amaranth starch more space-saving during storage and transport [27].

Table 3: Functional Properties of the formulated Amaranth Seed Starch

Starch	Gelatinization	Water absorption index	Water holding capacity	Water solubility index	Swelling power	True & bulk density
Amaranth seed starch	Takes 20mins at 70-80°C	160% ^a	164% ^a	220% ^a	2.3g ^c	8.7 g ^a & 5.2g ^a
Corn starch	Takes 10mins at 62-72°C	120% ^b	70% ^c	5-20% ^c	8-15g ^b	1.6 g/c m ^{3b} & 0.7 g/c m ^{3b}
Cassava starch	Takes 10mins at 52-67°C	160% ^a	120% ^b	15-20% ^b	12-15g ^a	1.6 g/c m ³ & 0.6 g/c m

Note: Values expressed are mean ±standard deviation. Data from different alphabets in the same column differed statistically significantly ($p < 0.05$).

3.4 FTIR Analysis for Extracted Amaranth Seed Starch

The FTIR spectrum of amaranth seed starch was analyzed to identify the functional groups present, based on the characteristic absorption peaks observed. The results, as illustrated in the provided Fig. 1, reveal the following distinct peaks and corresponding functional group assignments.

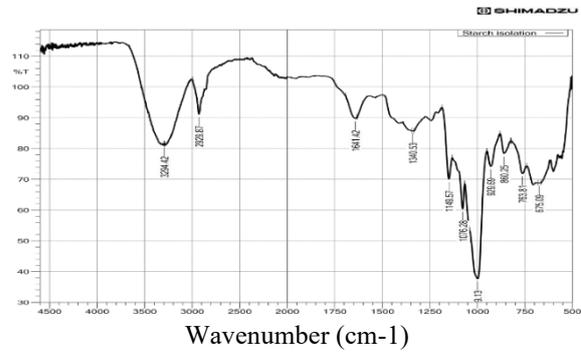


Fig. 1 – FTIR spectra of Amaranth Seed Starch

FTIR spectral analysis of amaranth seed starch reveals key functional groups that influence its behavior in food applications, as depicted in Table 4. The broad absorption band at 3294.42 cm⁻¹ corresponds to O–H stretching, indicating strong water-binding capacity, which supports effective gelatinization ((C₆H₁₀O₅)_n + H₂O → Gelatinized Starch). The peak at 2929.87 cm⁻¹ (C–H stretching) contributes to starch molecular stability. A peak at 1641.42 cm⁻¹ (C=O stretching) is associated with retrogradation, impacting shelf life. Peaks at 1149.57 cm⁻¹ and 1076.28 cm⁻¹ (C–O–C stretching) are linked to digestibility. Additionally, cyclic ether vibrations observed between 929.69–675.09 cm⁻¹ suggest a rigid molecular structure. These functional features make amaranth starch well-suited for roles as a thickener, gelling agent, stabilizer, and fat replacer in various food products.

Table 4: Band assignment of Amaranth Seed Starch

S. No	Functional groups	Wave number literature	Amaranth Seed Starch
1	O-H Stretching vibration	3500-3000	3294.42
2	C-H Stretching	3000-2500	2929.87
3	C-O Bending associated with the OH group	1750-1500	1641.42
4	C-H Symmetric bonding	1500-1250	1340.53
5	C-O-C Asymmetric stretching	1250-1000	1149.57 & 1076.28
6	C-O-C Ring vibration of COH	1000-750	929.69, 860.25, 763.81

7	C-O-C Ring vibration of COH	750-500	675.09
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3.5 Starch Analysis for Extracted Amaranth Seed Starch

Starch extraction from amaranth seeds yielded a content of 38.98%, which is considerably lower than that of conventional sources such as corn starch (70–72%) and cassava starch (80–85%). Despite its lower yield, amaranth starch offers distinct physicochemical properties. Its small granule size is advantageous for industrial applications requiring fine starch, including pharmaceuticals and cosmetics. Additionally, its high digestibility makes it suitable for functional foods and gluten-free diets. The starch also exhibits unique gelatinization and pasting behaviors, differing from those of traditional starches, which may offer functional benefits in food processing applications.

IV. CONCLUSION

The study concludes that amaranth seed milk and starch are economically viable and cost-effective alternatives with minimal environmental impact compared to traditional dairy farming. Amaranth cultivation is resource-efficient, and its derived products show strong potential for large-scale production. With appropriate nutritional enhancement and processing improvements, amaranth seed milk could serve as a superior alternative to cow's milk. Additionally, amaranth starch demonstrates higher functional properties when compared to corn and cassava starch. These qualities position amaranth-based products as attractive options for health-conscious and environmentally aware consumers seeking plant-based innovations.

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