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Effect of different heat moisture treatment modifications on Faba Bean (*Vicia faba*) Starch: A Comparative Study

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Abstract
Heat-moisture treatment (HMT) is a promising physical modification technique used to enhance starch properties without chemical agents, making it suitable for clean-label and functional food applications. This study comparatively evaluated the impact of three HMT methods—conventional heating (CHFBS), microwave-assisted (MHFBS), and autoclave-based (AHFBS)—on the physicochemical, structural, and thermal properties of starch isolated from faba bean (*Vicia faba* L.). Native starch (NFBS) was extracted and characterized alongside modified samples. Parameters such as amylose content, water absorption capacity, oil absorption capacity, swelling power, solubility, light transmittance, structural patterns (FTIR), crystalline characteristics (XRD), and granular morphology (SEM) were investigated. Results indicated that amylose content decreased significantly in modified starches, particularly AHFBS by 16.25%, while water and oil absorption capacities increased by 151.98% and 184.32% respectively. Native starch exhibited higher solubility, 14.06% at 90°C and swelling power, 15.36% at 90°C, while modified starches showed improved functional stability with lower retrogradation tendencies. FTIR spectra confirmed structural stability of starch molecules, while SEM revealed surface roughness and fissures in modified starches compared to smooth native granules. XRD analysis showed a typical C-type crystalline pattern in all samples, with a slight reduction in relative crystallinity after HMT, particularly in AHFBS, indicating partial disruption of double-helical order and rearrangement of amylose-amylopectin regions. Overall, autoclave-based HMT imparted the most pronounced functional improvements. These findings demonstrate the potential of eco-friendly HMT processes to tailor faba bean starch functionality for diverse food and industrial applications, paving the way for broader utilization of this underutilized legume starch in sustainable product formulations.

Keywords: Faba bean, starch, heat-moisture treatment, hydrothermal modification, physicochemical properties, functional properties

Introduction

Starch is a vital biopolymer with wide applications in food and non-food industries due to its thickening, gelling, and stabilizing properties (Punia *et al.*, 2019) [1]. However, the limitations of native starch such as poor solubility, low stability, and susceptibility to retrogradation necessitate modification for industrial use. Conventional chemical modifications, though effective, raise safety and environmental concerns. In contrast, physical modifications such as heat-moisture treatment (HMT) provide safe, cost-effective, and eco-friendly alternatives for starch enhancement (BeMiller *et al.*, 2015; Piecyk *et al.*, 2021) [15, 2].

Faba bean (*Vicia faba* L.), also known as broad bean, is an ancient pulse crop valued for its protein- and starch-rich seeds (Etemadi *et al.*, 2019) [10]. Its starch constitutes nearly 40-48% of the dry seed matter, making it a promising ingredient for food applications. Despite its potential, faba bean remains an underutilized crop in India and globally, partly due to limited research on its starch functionality (Etemadi *et al.*, 2019) [10]. Exploring modification strategies for faba bean starch can add value to this orphan crop, improve its industrial utility, and promote dietary diversification.

HMT involves subjecting starch to elevated temperatures (100-120°C) at restricted moisture (<30%) for a defined period, causing structural rearrangements in amorphous and crystalline domains without gelatinization. These modifications influence amylose-lipid interactions,

crystalline order, swelling capacity, solubility, and thermal stability. Comparative studies of different HMT methods conventional heating, microwave-assisted, and autoclaving are scarce for faba bean starch (Alfauomy *et al*., 2017) [11]. Understanding their differential impact will help optimize starch properties for food systems requiring stability, reduced retrogradation, and enhanced functional performance (Zhao *et al*., 2018) [17].
This study aimed to:

- Isolate starch from faba bean seeds;
- Modify starch using three different HMT methods;
- Evaluate changes in morphological, physicochemical, structural, and functional properties.

By providing comparative insights, this study contributes to the valorisation of faba bean starch and its incorporation into value-added food formulations.

Materials and Methods

Materials

Commercially available faba beans were purchased from the local market in Delhi, India. All chemicals utilized were analytic-grade to ensure purity and reproducibility.

Starch Isolation

Starch was isolated using the method given by Wani *et al*., 2016 [3]. Faba bean seeds were washed repeatedly, soaked in 0.16% sodium hydrogen sulphite solution for 24 hours, drained, ground to slurry, and sieved progressively through mesh sizes (0.250-0.045 mm). The filtrate was allowed to settle, supernatant discarded, and the starch layer resuspended and centrifuged at 3000 rpm for 10 minutes. The upper non-white layer was scraped off, and the collected starch was oven-dried at 40°C for 12 hours. The dried starch was then powdered and stored for further analysis.

Starch Modification Methods

- **Conventional Heat-Moisture Treatment**

Starch moisture was adjusted to 25%, equilibrated overnight, then heated in an oven at 100-110°C for 1 hour. The product was dried at 45°C for 12 hours, ground, sieved, and stored.

- **Microwave Heat-Moisture Treatment**

Moisture adjusted starch was subjected to microwave heating at 180W for 5 minutes, dried as above, then ground and sieved.

- **Autoclave Heat-Moisture Treatment**

After moisture adjustment, starch was equilibrated 24 hours at 4°C and then autoclaved at 100-110°C for 1 hour, followed by drying, grinding, and sieving.

Amylose Content

Amylose Content was determined spectrophotometry by iodine binding and absorbance was measured at 625 nm (Atrous *et al*., 2017) [4].

Water and Oil Absorption

For water and oil absorption 1g starch dispersed in 10ml water and oil seperately, centrifuged, and sediment weighed. The water absorption of starch was determined by the amount of water or oil hold by starch per gram of sample (Mathobo *et al*., 2021; Sangokunle *et al*., 2020) [6, 5].

$$WAC\% = \frac{weight\ of\ water/\ oil\ absorbed}{weight\ of\ starch\ sample} X 100$$

Solubility and Swelling Power

1g starch in 99ml water was heated at 60-90 °C for 1 hour, cooled and centrifuged. Solubility and swelling power determined gravimetrically (Leach *et al*., (1959; Desam *et al*., 2018) [7, 16].

$$\text{Solubility (\%)} = \frac{Weight\ of\ soluble\ starch\ (g)}{weight\ of\ sample\ (g)} x 100$$

$$\text{Swelling Power (\%)} = \frac{weight\ of\ sediment\ paste\ (g)}{weight\ of\ sample\ (g)(db) \times (100 - \text{solubility})} x\ 100$$

Scanning Electron Microscopy (SEM)

Starch samples were sprayed on a metal plate previously covered with double-sided adhesive tape and shadowed under vacuum with gold-palladium for Scanning Electron Microscopy. An accelerating potential of 5.0kV was used during microscopy (Punia *et al*., 2019; Chen *et al*., 2021) [1, 8]. Scanning electron micrographs were taken for each sample at different magnifications by scanning electron microscope (JSM-6100, Jeol, USA) at Department of Biotechnology, GJU, Hisar, Haryana.

X-ray Diffraction (XRD)

X-ray diffraction was used to examine the presence and characteristics of starch granules' crystalline structure, and it provides the evidence of an ordered structure (Chen *et al*., 2021; Kim *et al*., 2020) [8, 9].

FT-IR Spectroscopy

The FT-IR spectra were obtained using FT-IR. The spectra were recorded in transmission mode from 4,000 to 450 cm^{-1} (mid-infrared region). The sample was diluted with KBr (1:100, w/w) before acquisition and the background value from pure KBr was acquired before the sample was scanned (Amir *et al*., 2013; Warren *et al*., 2016; Fazio *et al*., 2020) [12, 18, 19].

Transmittance

Light transmittance (%) was measured as described by Piecyk *et al*., 2021 [2]. An aqueous suspension (2%) of starch was heated in a water bath at 90°C for 1h with constant stirring. The suspension was cooled and held for 1 h at 30°C. The sample was then stored for 7 days at 4°C, during which time the transmittance was determined every 24 h by measuring the absorbance at 640 nm (Systronic spectrophotometer 106) (Achille *et al*., 2007; Zhang *et al*., 2020) [13, 14].
Statistical analysis of the results was conducted using Minitab statistical software version 14 (Minitab Inc, State College, PA, USA). The data reported in all the tables are an average of triplicate observations and were subjected to one-way analysis of variance (ANOVA).

Results

Starch sample obtained from different treatments were identifies as NFBS (native faba bean starch), MHFBS (microwave HMT faba bean starch), CHFBS (conventional

HMT faba bean starch), and AHFBS (autoclave HMT faba bean starch).

Amylose content

Amylose content decreased from 23.92% in NFBS to 16.25% in AHFBS.

Water absorption capacity

Water absorption capacity increased significantly from 111.40% in NFBS to 151.98% in AHFBS.

Oil absorption capacity

Oil absorption capacity also improved, with highest in AHFBS (184.32%). Effect of different HMT on Amylose content, Water absorption capacity, and Oil absorption capacity faba bean starches is shown in table 1.

Table 1: Physicochemical properties of native and HMT modified faba bean starch.

Sample	Amylose content (%)	Water absorption (%)	Oil absorption (%)
NFBS	23.92± 0.79	111.40± 1.02	159.22± 1.20
MHFBS	17.46± 0.59	136.96± 1.21	178.82± 1.42
AHFBS	16.25± 0.53	151.98± 1.38	184.32± 1.49
CHFBS	21.20± 0.71	123.32± 1.92	171.06± 1.43

Solubility

Solubility increased with increase in temperature from 60-90°C but remained lower in all HMT starches compared to native starch. Effect of temperature on solubility on different HMT starches is shown in Table 2.

Table 2: Solubility of native and HMT modified faba bean starch.

Sample	60°C	70°C	80°C	90°C
NFBS	1.65± 0.05	7.1± 0.29	12.47± 0.24	14.06± 0.42
MHFBS	1.45± 0.03	6.95± 0.26	10.8± 0.25	13.9± 0.46
CHFBS	1.55± 0.07	4.6± 0.12	9.8± 0.24	13.27± 0.40
AHFBS	0.87± 0.02	4.37± 0.23	7.8± 0.32	10.42± 0.38

Swelling Power

NFBS showed the highest swelling power (15.36% at 90 °C), while AHFBS showed the lowest (9.84%) as shown in Table 3.

Table 3: Swelling power of native and HMT modified faba bean starch.

Sample	60°C	70°C	80°C	90°C
NFBS	2.94± 0.13	7.41± 0.31	10.95± 0.39	15.36± 0.68
MHFBS	3.36± 0.15	7.40± 0.29	9.47± 0.35	11.34± 0.45
CHFBS	3.14± 0.12	6.65± 0.25	8.48± 0.34	11.71± 0.46
AHFBS	3.05± 0.10	6.63± 0.22	7.50± 0.32	9.84± 0.35

Scanning Electron Microscopy (SEM)

SEM images showed NFBS granules as smooth and oval, whereas HMT starches exhibited surface roughness, fissures, and partial deformation (Figure 1). Mean particle size ranged from 17.7 to 21.4 µm for the four faba bean starches given in Table 4. These values are within the range of mean diameters (11 to 33 µm) reported for other legume starches.

Table 4: Granular Dimension of native and HMT modified faba bean starch.

Sample	Length (µm)	Breadth (µm)
NFBS	16.4-24.8	10.8-14.8
CHFBS	16-25.6	13.2-20
MHFBS	16.8-21.2	12.2-15.6
AHFBS	12.8-25.2	10.8-17.6

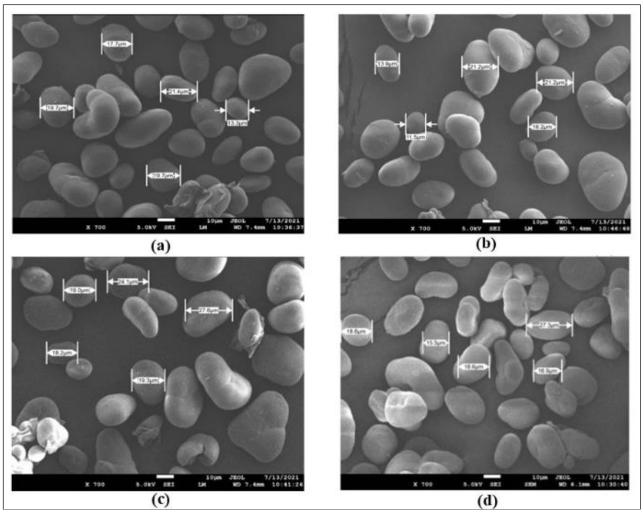

Fig 1: SEM of native and HMT modified starches Morphological properties of native and HMT modified faba bean starches, (a) NFBS: native faba bean starch; (b) CHFBS: conventional HMT faba bean starch, (c) MHFBS: microwave HMT faba bean starch (d) AHFBS: autoclave HMT faba bean starch.

X-ray Diffraction Analysis

XRD analysis showed that all faba bean starch samples exhibited a typical C-type crystalline pattern with characteristic peaks around 2θ = 15°, 17°, and 23°, confirming a mixture of A- and B-type polymorphs (Figure 2). The relative crystallinity varied slightly among samples, attributed to differences in crystal size, orientation, and molecular interactions within the starch granules.

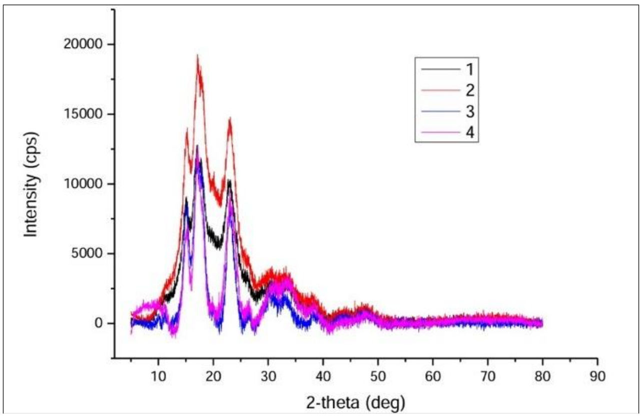

Fig 2: X-ray diffraction patterns of Faba bean starch; 1 (CHFBS), 2 (MHFBS), 3 (NFBS) and 4 (AHFBS).

FTIR (Fourier transform infrared spectroscopy) Analysis

The deconvoluted FTIR spectra of the faba starches are shown in Figure 3. The FTIR spectra of all these starches showed that modification did not impart any significant change in their spectrum pattern. The absorbance near 1000 cm-1 from the deconvoluted FTIR spectra have been associated with ordered (organized) and amorphous (less organized) starch structures, respectively.

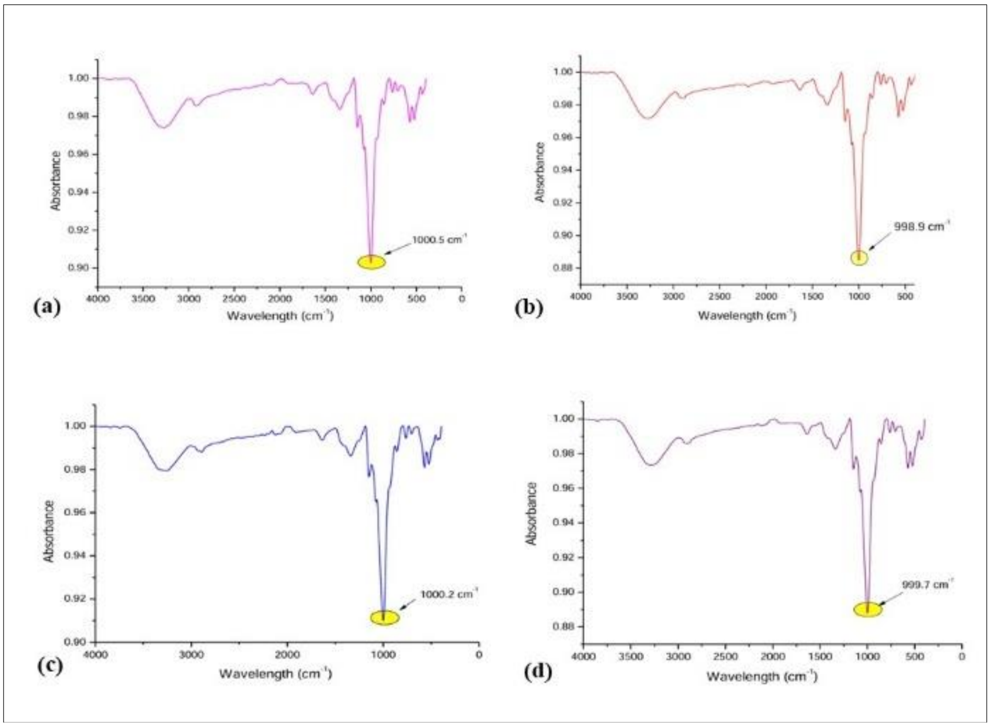

Fig 3: Spectra of NFBS and modified starches showed typical starch bands, with no major differences, indicating structural stability post-HMT (a) NFBS (native faba bean starch), (b) MHFBS (microwave HMT faba bean starch), (c) CHFBS (conventional HMT faba bean starch), and (d) AHFBS (autoclave HMT faba bean starch).

Light Transmittance

NFBS showed higher transmittance (20.8% initially), but modified starch AHFBS exhibited lower reduction rates (3%), suggesting improved retrogradation resistance.

Table 5: Light transmittance (%) of faba bean starch in different HMT treatment.

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	Light transmittance (%)						
NFBS	20.8± 0.89	8.4± 0.40	5.8± 0.25	4.6± 0.20	4.2± 0.22	3.8± 0.18	3.5± 0.13
CHFBS	15.8± 0.62	8.2± 0.36	8.2± 0.36	5.8± 0.24	4.3± 0.21	3.7± 0.16	3.5± 0.14
MHFBS	16.0± 0.60	8.1± 0.39	4.3± 0.23	3.5± 0.16	3.4± 0.14	3.3± 0.15	3.1± 0.12
AHFBS	12.9± 0.43	6.9± 0.24	5.4± 0.20	4.7± 0.24	4.1± 0.20	3.6± 0.17	3.0± 0.15

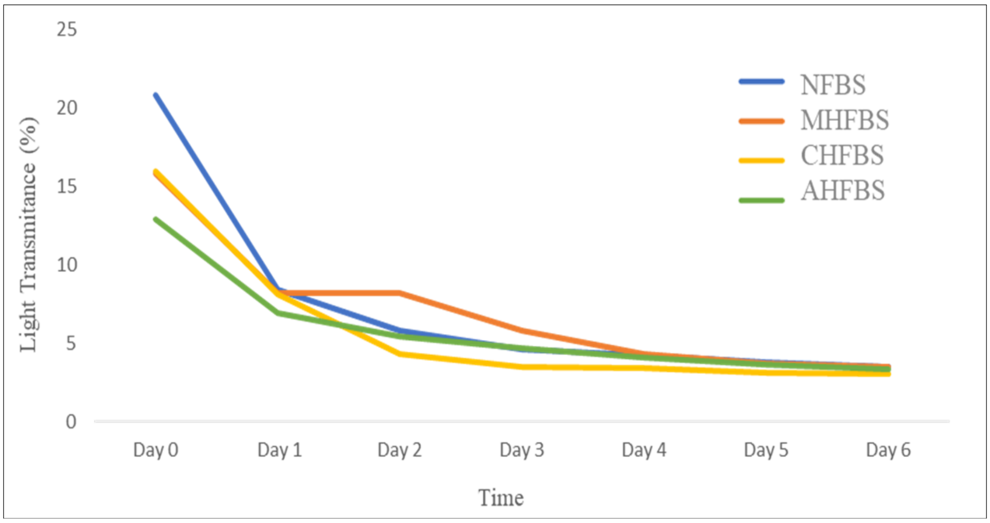

Fig 4: Light transmittance (%) of native and HMT modified starches; NFBS (native faba bean starch), MHFBS (microwave HMT faba bean starch), CHFBS (conventional HMT faba bean starch), and AHFBS (autoclave HMT faba bean starch).

Discussion

HMT significantly altered faba bean starch properties, consistent with findings on other legumes. Reduction in amylose content was attributed to reorganization of starch chains and formation of amylose-lipid complexes. The observed increase in water and oil absorption capacities suggests enhanced hydrophilic and lipophilic binding sites post-treatment, making HMT starch useful in formulations requiring moisture retention and emulsification.

Decreased swelling power and solubility in modified starches indicate stronger internal bonding and reduced granule disruption. Improved retrogradation resistance, as evidenced by higher light transmittance stability, is advantageous for refrigerated food storage. FTIR confirmed preservation of molecular integrity, XRD analysis showed a typical C-type crystalline pattern in all samples, with a slight reduction in relative crystallinity after HMT, particularly in AHFBS, while SEM highlighted granule surface disruptions, which likely contributed to altered hydration and functional behaviour.

Among treatments, autoclave-based HMT imparted the most pronounced improvements, likely due to combined effects of pressure and heat. These modifications broaden the functional versatility of faba bean starch in bakery, dairy, and convenience foods.

Conclusion

Physical modifications, particularly conventional, microwave, and autoclave-based heat-moisture treatments, significantly modulate the properties of faba bean starch. These treatments result in reduced amylose content, increased water and oil absorption capacity, decreased swelling power and solubility, raised gelatinization temperatures, and altered granular morphology. The absence of chemical changes, as confirmed by FTIR, makes these techniques especially attractive for food-grade and industrial starch applications.

The study demonstrates that autoclave-based HMT generally produces starches with the highest water and oil absorption, lowest amylose content, and most pronounced granule alteration. These modified starches have potential utility as viscofiers, emulsifiers, and gelling agents across a variety of food systems. Further research into the digestibility, pasting behavior, and potential health benefits of these modified starches will help optimize their use in health-promoting and functional food products.

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Conflict of Interest

The authors declare no conflicts of interest.

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