

Methodology

The methodology of the study entitled “Starch Characterization, Functional Properties, Prebiotic Potential of Unripe Banana Flours and Development of Ready-to-Eat and Ready-to-Cook Products” is presented in the following sections.

- 3.1. Phase 1: Exploring Consumers’ Knowledge, Attitude, and Practice of Ready-to-Eat and Ready-to-Cook Products**
 - 3.1.1. Selection of Survey participants
 - 3.1.2. Conduct of Survey
- 3.2. Phase 2: Identification and Authentication of Banana Cultivars and Preparation of Unripe Banana Flours of *Musa paradisiaca*, Peyan (PUBF) and Monthan (MUBF)**
 - 3.2.1. Selection of Banana Cultivars
 - 3.2.2. Procurement and Authentication of Unripe Bananas
 - 3.2.3. Assessment of Physical Characteristics and Ripening Stage
 - 3.2.4. Processing and Yield of Unripe Banana Flours (UBF)- PUBF and MUBF
- 3.3. Phase 3: Determination of Starch Morphology, Characteristics, and Functional Properties of Unripe Banana Flours- PUBF and MUBF**
 - 3.3.1. Analysis of Starch Morphology and Characteristics-- SEM Imaging, XRD, and FTIR Spectra Analysis
 - 3.3.2. Determination of Total Starch, Resistant Starch, Amylose, Amylopectin
 - 3.3.3. Evaluation of Thermal Properties
 - 3.3.4. Determination of Physicochemical Properties
 - 3.3.5. Determination of Flour Hydration and Gelling Properties
- 3.4. Phase 4: Estimation of Nutrient Composition, Phytonutrients, Antimicrobial Activity, Antioxidant Potential, Prebiotic Potential, and Predicted GI of Unripe Banana Flours- PUBF and MUBF**
 - 3.4.1. Estimation of Macronutrients and Micronutrients
 - 3.4.2. Estimation of Phytonutrients
 - 3.4.3. Assessment of Antimicrobial Activity and Antioxidant Assays

3.4.4. Determination of Prebiotic Potential

3.4.5. Determination of Predicted GI (eGI)

3.5. Phase 5: Development and Evaluation of Ready-to-Eat and Ready-to-Cook

Products Incorporated with Unripe Banana Flours- PUBF and MUBF

3.5.1. Product Concept

3.5.2. Trials and Standardization of the Developed RTE and RTC Products

3.5.3. Sensory Evaluation and Acceptability of RTE and RTC Products

3.5.4. Determination of Physicochemical Properties and Nutrient Composition of RTE and RTC Products Incorporated with 30% UBFs

3.5.5. Shelf-life Analysis of the Developed Products

3.6. Phase 6: Assessment of Prebiotic Potential, Predicted GI, and Purchase Intent of

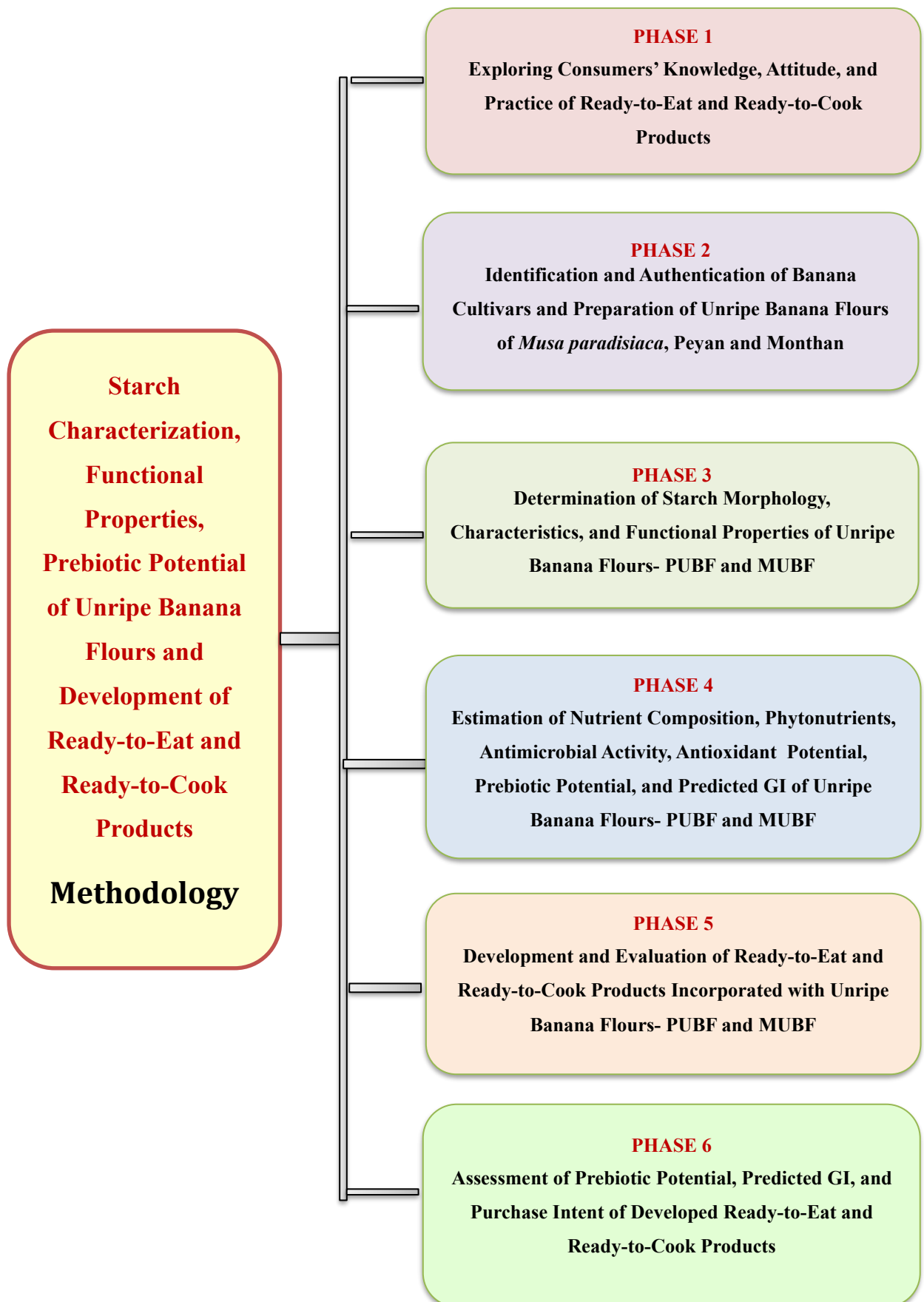
Developed Ready-to-Eat and Ready-to-Cook Products

3.6.1. Determination of Prebiotic Index of PUBF Cookies and Noodles

3.6.2. Determination of eGI of PUBF Cookies and Noodles

3.6.3. Purchase Intent of PUBF Cookies and Noodles

3.7. Statistical Analysis



The study on “**Starch Characterization, Functional Properties, Prebiotic Potential of Unripe Banana Flours and Development of Ready-to-Eat and Ready-to-Cook Products**” was carried out with the ethical clearance certificate issued by the Universal Ethics Committee, Chennai. (Appendix I)

3.1. Phase 1: Exploring Consumers’ Knowledge, Attitude, and Practice of Ready-to-Eat (RTE) and Ready-to-Cook (RTC) Foods

The transition from traditional cooking to processed and ready-to-eat foods is evident in the modern day due to lifestyle changes. RTE and RTC foods with health concepts are the future of consumption trends, and developing these foods with functional properties will bring beneficial changes.

To assess the knowledge, attitudes, and practices regarding ready-to-eat (RTE) and ready-to-cook (RTC) foods, a baseline survey was conducted to gain insight into the awareness, beliefs, and consumption behaviour of these foods from the general population.

Information from participants can be elicited through online surveys or questionnaire-based studies shared via digital communication channels, including electronic mail or web-based survey tools (Singh & Sagar, 2021). The online survey method was chosen to collect details on KAP due to the then-prevailing COVID-19 outbreak. Akintunde et al. 2021 stated that most studies had online data collection preferred over in-person interviews due to the travel bans and distancing protocols during the COVID-19 crisis.

3.1.1. Selection of Survey Participants

A Convenience sampling method was adopted to select 500 respondents aged 20 to 50 years, as it includes young adults, middle-aged, and older adults. As adults, they may exhibit varying interest in functional RTE and RTC foods and make independent dietary and purchasing decisions, so they were selected with the following admittance and exclusion guidelines.

Inclusion Criteria:

- Respondents 20-50 years old
- Both male and female
- Access to the internet and a valid email address

Exclusion Criteria:

- Respondents with limited digital literacy

3.1.2. Conduct of Survey

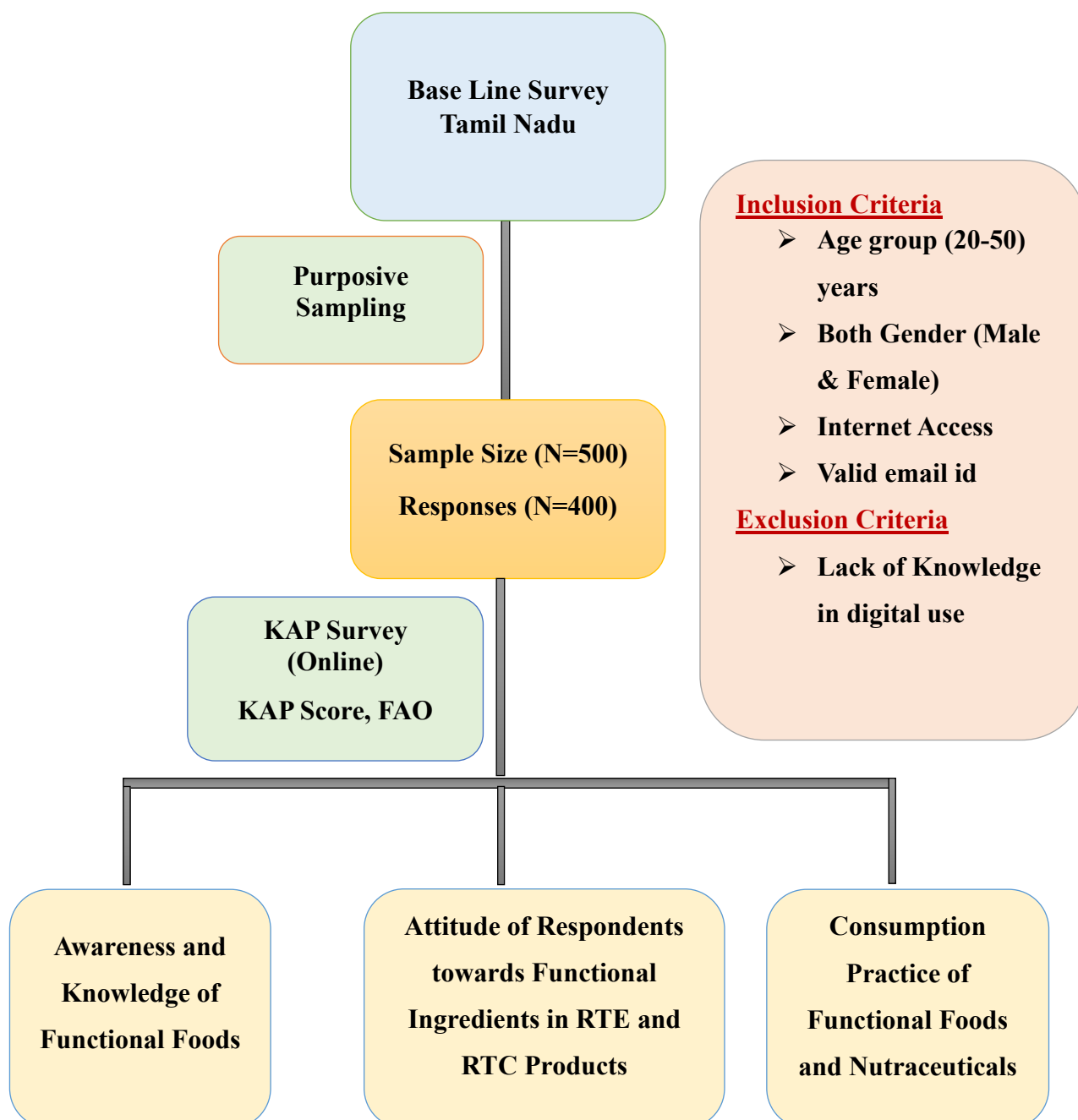
A structured questionnaire encompassing demographic details, awareness of health-promoting foods, perception of bioactive components in RTE and RTC foods, and dietary patterns related to health-focused RTE/RTC foods was created using Google Forms.

The coherence of the questions was assessed through Cronbach's Alpha test, a reliability metric. With an obtained α value of 0.7, the internal consistency was deemed reliably satisfactory. A pilot test was undertaken with 15 selected participants representing the respondents to identify any ambiguities in the questionnaire. Based on the feedback, minor revisions were implemented to improve clarity and effectiveness. The questionnaire was mailed to all 500 participants, and the response rate was 80% (400 respondents). (Appendix II).

The FAO's KAP Manual (2014) was followed to calculate the KAP score. Two closed-ended questions were used to calculate the knowledge score, and seven questions with a 3-point Likert scale were used to calculate the attitude score. Respondents reported their consumption of health-promoting foods and bioactive supplements to assess practice, and the total consumption score was normalized. The mean scores were then calculated on a 3-point scale. (Macías & Glasauer, 2014)

PHASE 1

Exploring Consumers' Knowledge, Attitude, and Practice of RTE and RTC Products



3.2. Phase 2: Identification and Authentication of Banana Cultivars and Preparation of Unripe Banana Flours of *Musa paradisiaca*, Peyan (ABB), and Monthan (ABB)

3.2.1. Selection of Banana Cultivars

The results of the online survey showed that 52% of respondents preferred RTE and RTC foods with reduced calorie content and enriched dietary fiber; therefore, an attempt was made to identify high-fiber functional ingredients to explore further.

The survey results further showed that only 29% of respondents considered unripe bananas, sweet potatoes, and yam as functional food, driving home the fact that unripe banana health benefits have to be explored and made aware among the population.

According to European Food Safety Authority (EFSA) guidelines (2010), “A food item is classified as fiber-rich when it provides a minimum of 1.5 g of fiber per 100 kcal and is designated as high-fiber when it delivers 3 g of fiber per 100 kcal”. Thus, unripe bananas and unripe banana products may qualify as fiber-rich foods (Falcomer et al., 2019) by high levels of resistant starch, along with indigestible carbohydrates such as cellulose, hemicelluloses, and lignin, which account for 60-70% of their composition (Powthong et al., 2020)

Because unripe bananas are excellent sources of both resistant starch and dietary fiber, they were chosen as the primary functional ingredient for incorporation into RTE and RTC foods, in line with consumer preferences. The rationale for selecting *Musa paradisiaca* is listed below

- Bananas are available throughout the season
- Versatile use
- Nutrient dense
- Rich in resistant starch
- Gut friendly
- Cost effective

The banana (*Musa* sp.) ranks among the most extensively grown tropical fruits globally, with over 1,000 varieties in production (FAO, 2023). Bananas are highly valued for their succulent taste, nutritional benefits, and year-round availability. Approximately 70–80% of the raw banana's dry matter consists of starch, with the majority being RS. This

resistant starch remains undigested in the intestinal tract and can help lower insulin responses and postprandial glucose levels (Fu et al., 2022).

India has numerous banana cultivars, each with distinct and unique culinary purposes. The extensive variability in composition and utility is swayed by factors such as cultivar types, planting methods, climate conditions, and nutrient availability. (Maseko et al., 2023). The enormous diversity of banana cultivars emphasizes the importance of selecting specific varieties for further exploration in this study. Hence, *Musa paradisiaca*, Peyan (ABB), and Monthan (ABB) cultivars were chosen for this research based on the following considerations.

- Indigenous to Southern India
- Widespread availability
- Underutilized and unexploited
- Limited research

These cultivars from Southern India, despite their widespread availability, significant cultural relevance, and traditional practices affirming curative and health-promoting qualities (Ajijolakewu et al., 2021), remain underutilized, supporting further research. This research seeks to investigate the nutritional and bioactive advantages of the chosen *Musa cultivars*, Peyan and Monthan, as well as their potential uses in food product development.

The taxonomy of *Musa paradisiaca* is shown in Table IV

Table IV
Taxonomy of *Musa paradisiaca*

Taxonomic Rank	Scientific Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Musaceae
Genus	<i>Musa</i>
Species	<i>Musa paradisiaca</i>

Adapted from Oyeyinka and Afolayan (2020)

3.2.2. Procurement and Authentication of Unripe Bananas-- *Musa paradisiaca*, Peyan and Monthan

Peyan (ABB) and Monthan (ABB) cultivars of *Musa paradisiaca* were sourced from a wholesale fruit market in Chennai, Tamil Nadu. Their identity was authenticated (Form No. PCOG002-ACF) by the Department of Pharmacognosy at the Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, GOI), Chennai. (Appendix III).

3.2.3. Assessment of Physical Characteristics and Ripening Stage

The physical characteristics of banana cultivars, particularly fruit weight, pulp, and peel weight, play a crucial role in determining overall yield. Dadzie and Orchard's (1997) guidelines were followed to assess the morphological attributes of unripe banana fruits. A total of twelve fruits were chosen for measurement. The length of each fruit was recorded from the distal end along the outer curve, while its maximum circumference at the midpoint was measured using a measuring tape. The total fruit weight, along with the separate weights of the pulp and peel, was determined using an electronic weighing balance (Ravi and Mustaffa, 2013).

According to Horie et al. (2020), to prepare unripe banana flour, the fruits should be gathered at approximately 75% maturity (stage 1, fully green) and handled in less than a day. Within bananas belonging to the same genomic group and growth stage, physiological ripeness does not necessarily develop simultaneously. Flour from less mature fruits can have a mild astringency attributed to its tannin content. Thus, choosing the appropriate ripening stage is important.

The fruit ripening stages were assessed using the Von Loesecke (1950) scale, which classifies the maturation process into seven stages based on peel color (Figure 10).

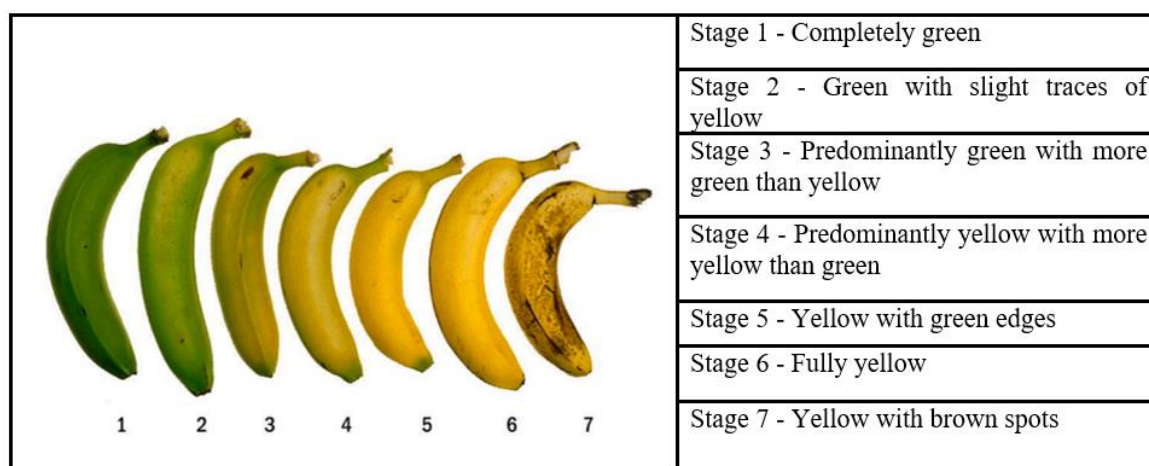


Figure 10: The Seven Ripening Stages of Banana (© Don Edwards, UC Davis, Postharvest Technology Center, California, USA). Adapted from Maseko et al. 2023

To confirm the optimal ripening stage of the bananas, the protocol outlined by Campuzano et al. (2018) focusing on the assessment of Total Soluble Solids (TSS) was used. Banana pulp (30 g) was homogenized with 90 mL of distilled water for two minutes, and the TSS was recorded using an Atago refractometer (scale range of 0 to 30 °Brix). The reference brix value for stage 1-4 bananas is presented in Table V.

Table V

°Brix value for Stages 1-4 Bananas

Ripening stage	Brix Value
Stage 1 (completely green)	1.2 - 2.1 °Brix
Stage 2 (green with yellow traces)	2.2 to 6.9 °Brix
Stage 3 (predominantly green with some yellow)	7.0 and 13.2 °Brix
Stage 4 (mostly yellow with some green)	13.3 to 18.0 °Brix

Adapted from Campuzano et al. (2018)

3.2.4 Processing and Yield of Unripe Banana Flours(UBF)- PUBF and MUBF

Figure 11 summarizes the steps in unripe banana flour preparation.

The protocol outlined by Kumar et al. (2019) was adapted for preparing unripe banana flour and calculating flour yield. Bananas in stage 1 were rinsed under running tap water, peeled using a sharp steel knife to minimize bruising damage, and then sliced into one cm thick pieces. The sliced samples were immersed in a 0.3 g/100 mL citric acid

solution to impede enzymatic browning. Subsequently, the slices were dehydrated in a tray dryer at 50°C for 48 hours until they reached a brittle texture (break or shatter easily when subjected to stress, without significant deformation or bending). The dried slices were powdered in a mixer (120s) and passed through a 60-mesh sieve. The Peyan and Monthan unripe banana flour (PUBF and MUBF) were stored in appropriately labelled Ziploc bags at ambient conditions.

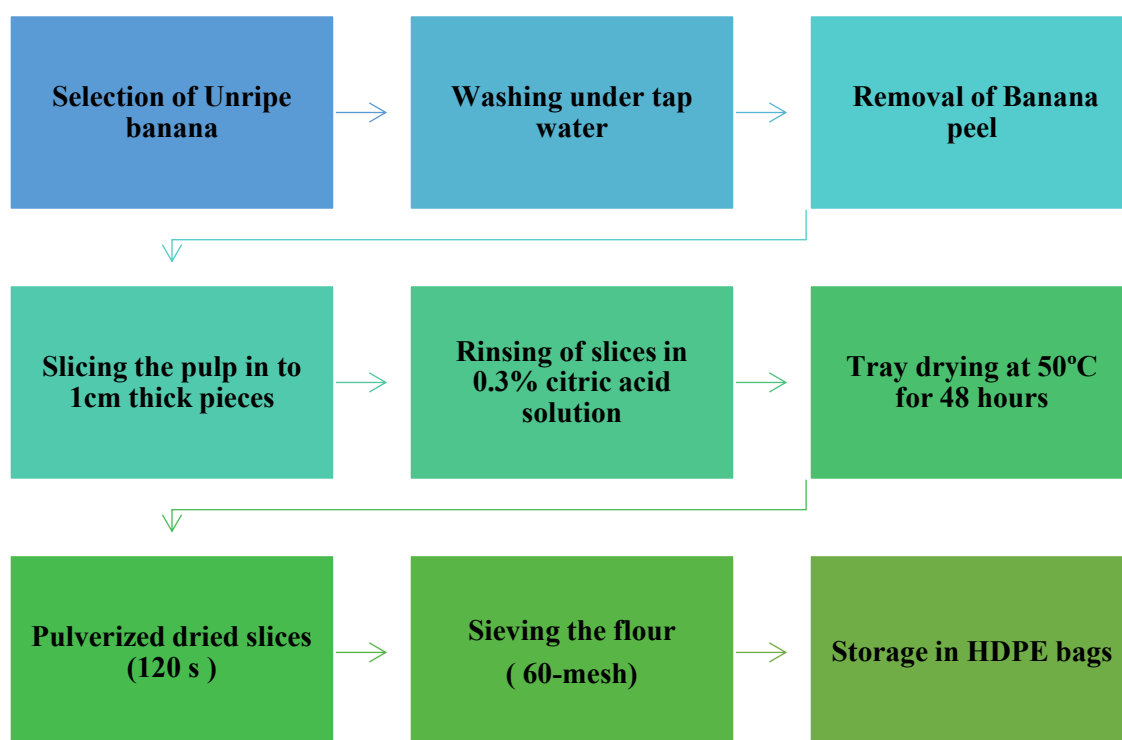


Figure 11: Steps in Unripe Banana Flour Preparation
(Kumar et al., 2019)

The flour yield was standardized with three trials and calculated with the following equation.

$$\text{UBF yield (\%)} = \frac{\text{Fresh weight of the pulp} - \text{Moisture content of the pulp}}{\text{Fresh weight of the pulp}} \times 100$$

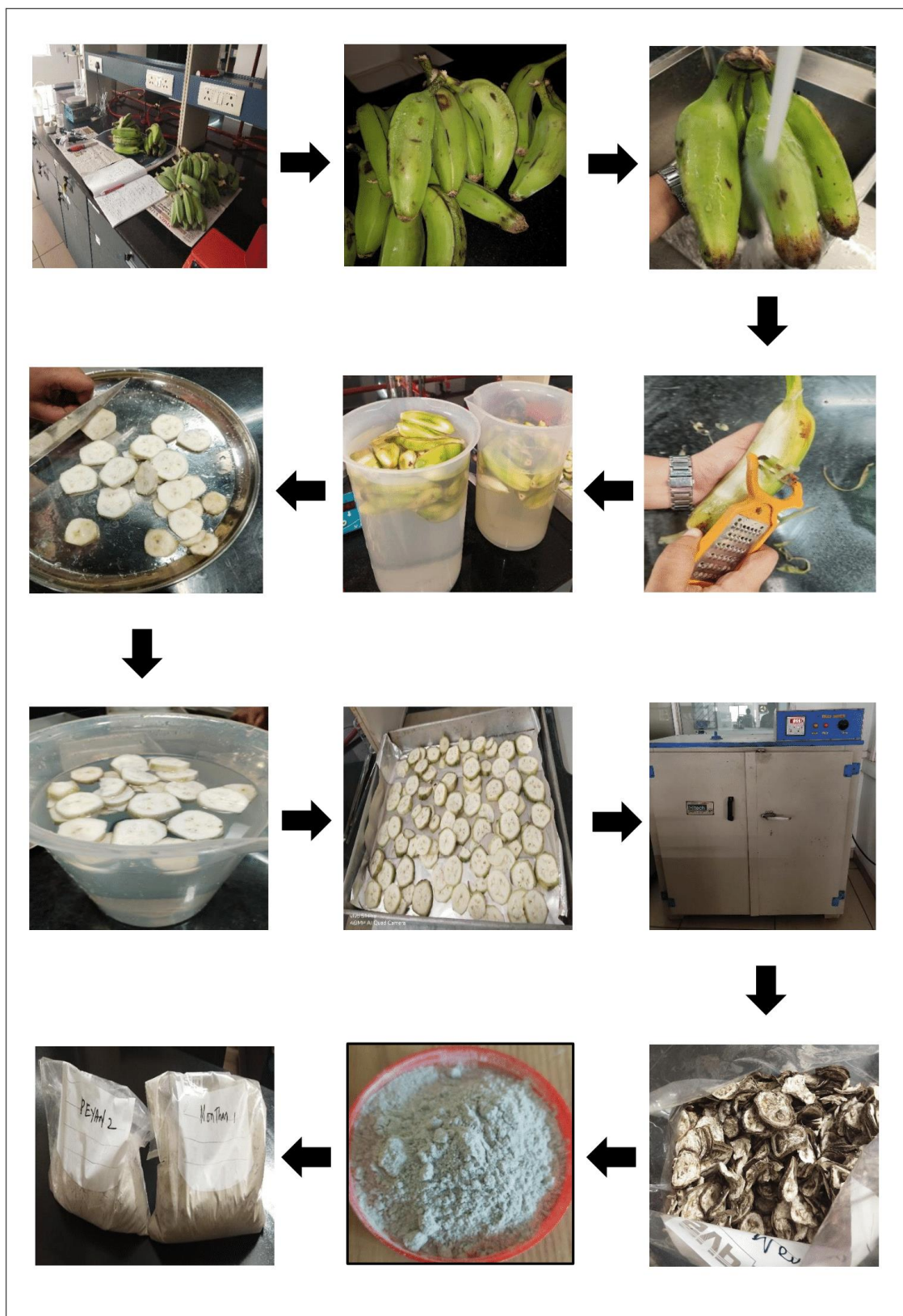
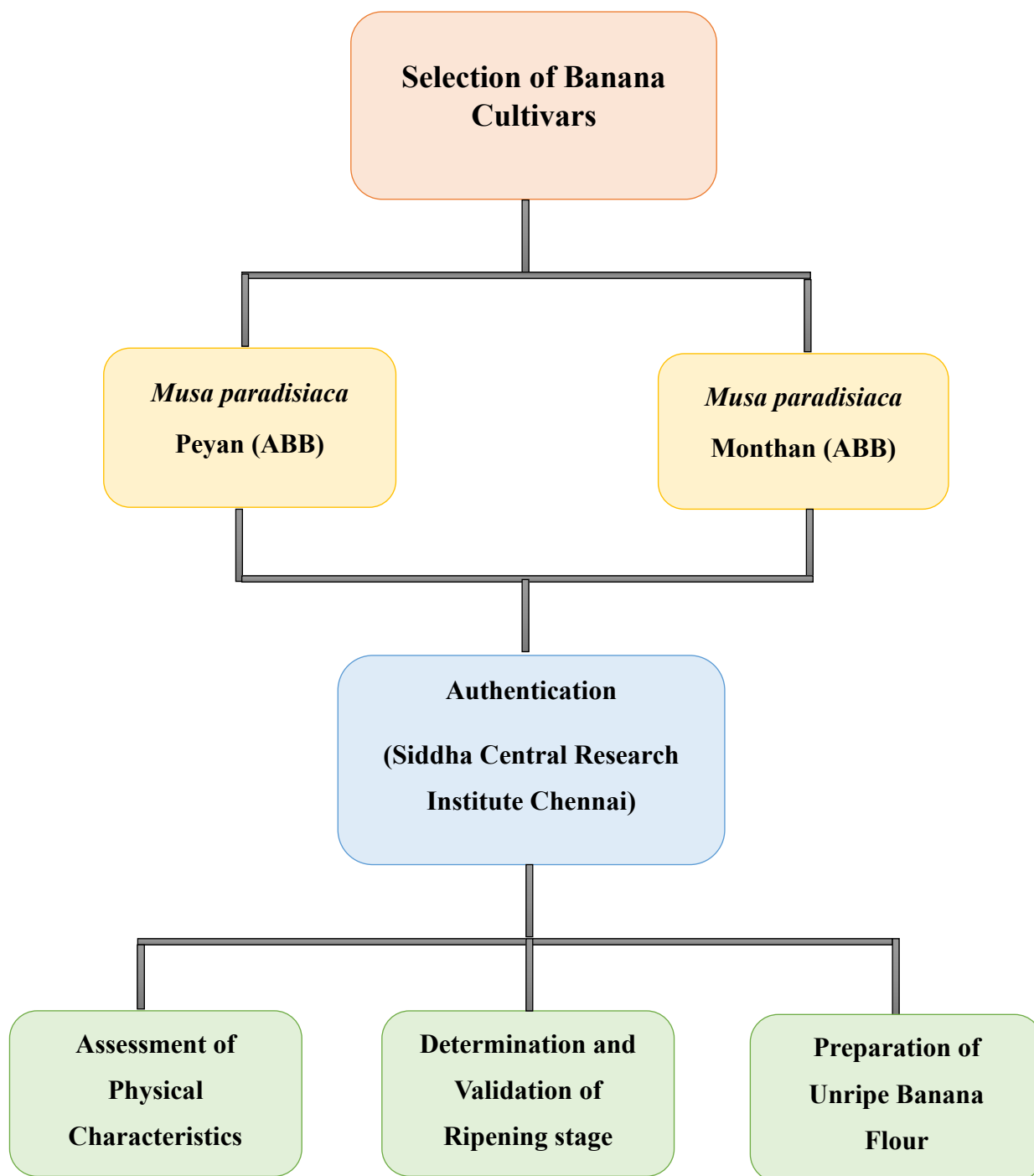


Plate 1: Steps in Unripe Banana Flour Preparation

PHASE 2

Identification and Authentication of Banana Cultivars and Preparation of Unripe Banana Flours (UBF) of *Musa paradisiaca*, Peyan (ABB) and Monthan (ABB)



3.3. Phase 3: Determination of Starch Morphology, Characteristics, and Functional Properties of Unripe Banana Flours- PUBF and MUBF

3.3.1. Analysis of Starch Morphology and Characteristics-- SEM Imaging, XRD, and FTIR Spectra Analysis

According to Jiang et al. (2015), banana cultivars differ in their digestibility, physicochemical properties, and starch structure. The starch composition, size, shape of the starch granules, crystallinity, and molecular weight of the starch influence the digestibility of UBF (Bi et al., 2017). Hence, an understanding of the starch morphology and characteristics is essential to explore the possible usage of unripe banana flour.

The starch granule characteristics, like their size, shape, presence of other compounds, and structural integrity, can be studied using Scanning Electron Microscopy (SEM) (Anyasi et al., 2016). The surface morphology of the unripe banana flours (PUBF and MUBF) was examined with an FEI-Quanta FEG 200F scanning electron microscope with a resolution of granule sizes from 5 to 100 μm . UBF were affixed to dual-sided adhesive strips, coated with a layer of gold plating, and examined (Kumar et al., 2019).

The FTIR spectra facilitate identifying various characteristic functional groups in unripe banana flours. FT-IR spectrophotometer (SHIMADZU IRAffinity-1S, Miracle 10 ZnSe Prism Plate) was used, and the range studied was 600-3600 cm^{-1} , and the UBF samples were mixed with potassium bromide (KBr) before testing (Kumar et al., 2021).

Starch can manifest in three distinct crystallinity patterns- A, B, and C and the crystallinity pattern determines the starch digestibility (Martens et al., 2018). The XRD technique was employed to inspect the crystallinity of the unripe banana flour. X-Pert Pro PANalytical instrument with a Cu $K\alpha$ source, operating at a voltage of 45 kV and an electricity supply of 30 mA was used for the study. The setting for the scanning was 10° to 80° (2 θ), step size of 0.0170 [°2Th.] and scan step time of 5.7150 S. OriginPro 2023b Software was used to calculate the relative crystallinity (%), and the following equation was used as outlined by Campuzano et al. (2018).

$$RC(\%) = \frac{\text{Crystalline peaks}}{\text{Crystalline peaks} + \text{Amorphous peak}} \times 100$$

3.3.2. Determination of Total Starch, Resistant Starch, Amylose, Amylopectin

Bananas in their mature green stage contain approximately 70–80% starch (Marta et al., 2022) and an appreciable quantity of resistant starch (49.80 to 65.56%) (Paramasivam et al., 2021). For the commercialization and development of innovative food products utilizing unripe banana flour, it is important to know the starch composition and the functional characteristics. Hence, the starch compositional analysis was carried out as listed in Table VI, and the detailed protocols are provided in Appendix IV

Table VI
Determination of Starch Composition

Starch Characterization	Method	Reference
Total Starch	Enzymatic method	Goñi et al., 1997
Resistant and Digestible Starch	AOAC 2002.02	AOAC
Amylose	Method of Mccready et al. (1950)	Outlined by Sadasivam and Manickam, 2008
Amylopectin	Total starch - Amylose = Amylopectin	Sadasivam and Manickam, 2008

3.3.3. Evaluation of Thermal Properties- DSC (Differential Scanning Calorimeter) Analysis

The pasting behaviour of unripe banana flour is essential for optimizing the use of UBFs in food product development, as they directly impact the functional properties (Padhi and Dwivedi, 2022). In a DSC apparatus (NETZSCH DSC 204 F1 PHOENIX), a UBF sample (2.8 and 3 mg on dwb) was directly placed into an aluminium pan, and 7 μ l of double-distilled water was added to the sample. The pan was sealed and allowed to reach equilibrium for one hour. The sample was heated at a rate of 10.0 K/min with a temperature range of 20°C to 200°C. An empty aluminium pan with a pierced lid was used as a reference without a sample. NETZSCH Proteus software was used to get the thermograms and derive the values for onset temperature (T_o), peak temperature (T_p), final temperature (T_c), gelatinization temperature range (ΔT), and enthalpy (ΔH). (Kumar et al., 2021a).

3.3.4. Determination of Physicochemical Properties

The analysis of physicochemical properties—bulk density, tapped density, Carr index, Hausner ratio, oil absorption capacity, foaming capacity, and emulsion activity and stability—was carried out using the Wani et al. (2015) method.

3.3.4.1. Density and Flowability Parameters

Bulk density is the mass of flour particles divided by their total volume, including particle, pore, and void spaces. Differences in starch content may lead to variations in the bulk density of foods, with higher starch levels resulting in increased bulk density (Awuchi et al., 2019). 50 g of UBF was added to a 100 mL capacity graduated measuring cylinder and gently compacted until a stable volume was reached. The bulk density results were reported as g/mL. To determine the tapped density, the cylinder was gently tapped with a glass rod while resting on a soft, stable surface until the volume remained constant, and the resultant tapped volume was divided by the sample mass.

The flowability of powders is commonly evaluated using parameters such as the Carr Index / Carr Compressibility Index and the Hausner ratio. Interpretations of the flow characteristics were made compared to standard charts presented in Appendix 4 as outlined by Alam et al., 2023

$$\text{Carr index \%} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

3.3.4.2. Foaming Capacity

Foaming capacity refers to the capacity of proteins to migrate to the surface layer and develop a flexible elastic membrane that prevents air bubbles from coalescing. Foaming ability is due to proteins reducing surface tension, preventing air bubble agglomeration, and stabilizing the foam (Devisetti et al., 2014). A sample of UBF weighing 1 gram was mixed with 50 mL of double-distilled water in a measuring cylinder. The mixture was then agitated with vigour for 5 minutes to produce foam.

The foam volume 30 seconds after whipping was expressed as foam capacity using the formula given below.

$$\text{Foaming capacity \%} = \frac{\text{Volume of foam after whipping} - \text{Volume of foam before whipping}}{\text{Volume of foam before whipping}} \times 100$$

3.3.4.3. Emulsion Activity and Stability

Emulsion capacity can be defined as the potential of a protein to enhance the dispersion of immiscible dissolvents. The potentiality of the emulsion system of foods to withstand and resist the changes in their physicochemical properties over a period is known as emulsion stability (Hu et al., 2017). In a graduated centrifuge tube, a one-gram sample, 10 mL of distilled water, and 10 mL of refined sunflower oil were added and centrifuged at $2000 \times g$ for 5 minutes. To analyze emulsion stability, the emulsion formed was heated at a temperature of 80°C for 30 minutes in a water bath. and then cooled for 15 minutes under running water and centrifuged again at $2000 \times g$ for 15 minutes.

$$\text{Emulsion Activity \%} = \frac{\text{Height of the emulsified layer}}{\text{Total height of the mixture}} \times 100$$

3.3.4.4. Oil Absorption Capacity

OAC of the flour influences the texture, flavor retention, and mouthfeel of a food product and can be defined as the interaction of fat with the hydrophobic region of proteins (Badjona et al., 2023). To the UBF sample (1g), 10 mL sunflower oil was added and left at room temperature for 30 minutes. It was then centrifuged for 30 minutes at $2000 \times g$. OAC was calculated as the weight of sediment after draining oil divided by the sample weight.

3.3.5. Determination of Flour Hydration and Gelling Properties

The interaction between amylopectin and amylose and the granular structure of the starch affect hydration. Flour hydration is a key functional property as it influences the consistency, bulk, and performance of products, particularly in baking. Gelation properties determine the texture, structure, and stability, making them valuable for various food applications (Iwe et al., 2016).

3.3.5.1. Flour Hydration Properties

To 0.2 g of the unripe banana flour sample, 20 mL of distilled water was added and heated at various temperatures (50, 60, 70, 80, and 90°C) for 15 minutes in a shaking water bath. The starch paste was cooled to room temperature and centrifuged at $3000 \times g$ for 10 minutes. The supernatant was decanted into a Petri plate, and its solid content was determined by drying it overnight at 110°C. The sediment was weighed, and the dry solid content was calculated (Cornejo and Rosell, 2015).

Water solubility index (WSI), water absorption capacity (WAC), water holding capacity (WHC), and swelling power (SP) were calculated using the respective equations (Table VII). For determining the swelling volume, 0.5 g (dry weight basis) of UBF was combined with 30 mL of distilled water and left at room temperature for 24 hours (Buckman et al., 2017).

Table VII
Flour Hydration Properties Equations

Hydration Properties	Equation
Water Solubility Index (%)	$WSI = \frac{\text{Weight of dry supernatant}}{\text{Weight of sample}} \times 100$
Water Absorption Capacity(g/g)	$WAC = \frac{\text{Weight of wet sediment}}{\text{Weight of sample} - \text{Weight of dry supernatant}}$
Water Absorption Index (g/g)	$WAI = \frac{\text{Weight of wet sediment}}{\text{Weight of sample}}$
Water Holding Capacity (g/g)	$WHC = \frac{\text{Weight of wet sediment} - \text{Weight of dry sediment}}{\text{Weight of sample}}$
Swelling Power(g/g)	$SP = \frac{\text{Weight of wet residue}}{\text{Weight of dry residue}}$
Swelling Volume (ml/g)	$SV = \frac{\text{Total volume of swollen sample}}{\text{Weight of sample}}$

3.3.5.2. Flour Gelling Properties

3.3.5.2.1. Least Gelation Concentration (LGC)

The LGC was estimated by Chandra and Samsher's (2013) method, where the prepared UBF samples, ranging from 2% to 30% (w/v) in 5 mL of distilled water, were heated in a water bath at 90°C for one hour. After cooling under running water and keeping them at 10 ± 2°C for 2 hours, the LGC was assessed by inspecting any emulsion droplets separated and fallen out to the top in inverted test tubes. The findings were categorized as no (-), complete (+), or partial (±) gelation.

3.3.5.2.2. Light Transmittance

Starch gel clarity directly affects the brightness and opacity of food products (Khawas and Deka, 2016). An aqueous 1% UBF suspension was prepared and heated in a shaking hot water bath (90°C) for 30 minutes. The suspension was then kept at ambient temperature (30 ± 2°C) for one hour and stored at 4°C for 72 hours. At 24-hour intervals, absorbance was recorded at 640 nm using a UV spectrophotometer, against a water blank (Wani et al., 2010).

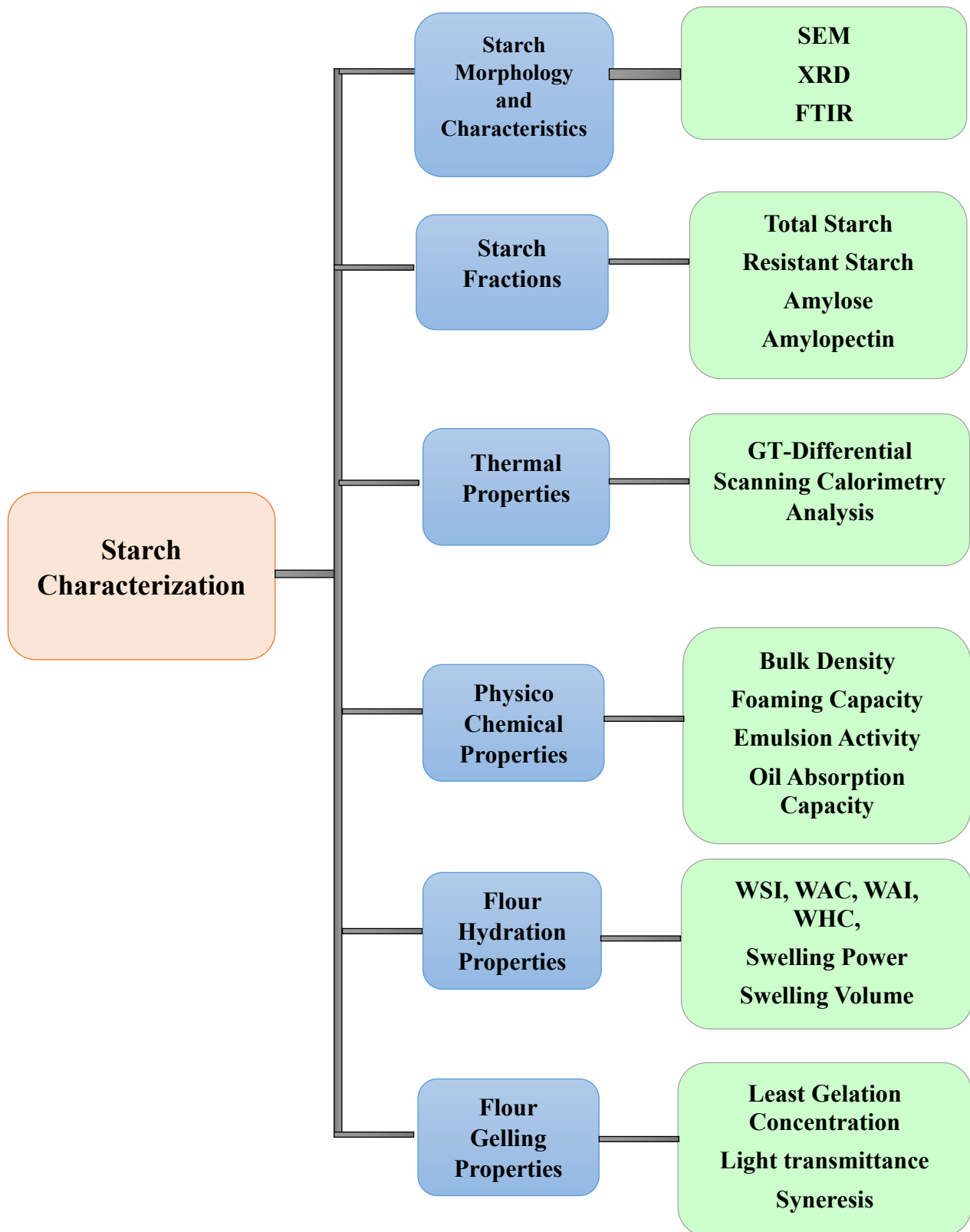
3.3.5.2.3. Syneresis

Starch retrogradation at low temperatures is important for foods that require freeze-thaw stability (Wang et al., 2015). Separate test tubes of UBF samples (2% w/w dry weight basis) were thermally treated (90°C) for 30 minutes in a shaking water bath, and later the suspensions were kept at 4°C for 1-5 days. Each day, the expelled water was weighed after centrifugation at 3000 × g for 10 minutes (Wani et al., 2010).

$$\text{Syneresis (\%)} = \frac{\text{Weight of water released}}{\text{Weight of gel}} \times 100$$

PHASE 3

Determination of Starch Morphology, Characteristics, and Functional Properties



3.4. Phase 4: Estimation of Nutrient Composition, Phytonutrients, Antimicrobial Activity, Antioxidant Potential, Prebiotic Potential, and Predicted GI of Unripe Banana Flours

3.4.1. Estimation of Macro and Micronutrients

Moisture, ash, TSS, and pH, the macro and micronutrient composition of the unripe banana flours were determined following the standard methods listed in Tables VIII, IX, and X on a dry weight basis, and the detailed protocols are presented in Appendix V.

Table VIII
Methods of Physicochemical Analysis

Parameters analyzed	Method and Reference
Moisture	925.10: AOAC, 2005
Ash	923.03: AOAC, 2005
Titrateable acidity and pH	942.15 and 981.12: AOAC, 2005
Total Soluble Solids (TSS)	Utilizing a portable Atago Refractometer (Salvador et al., 2007)

Table IX
Methods of Macro Nutrient Analysis

Parameters analyzed	Method and Reference
Protein	Kjeldahl method (conversion factor Nx6.25) 978.04: AOAC, 2005
Fat	960.39: AOAC, 2005
Total Carbohydrate	Anthrone method outlined by Sadasivam and Manickam (2008).
Total Sugars	Phenol-sulfuric acid assay outlined by Sadasivam and Manickam (2008).
Reducing Sugars	Nelson-Somogyi method outlined by Sadasivam and Manickam (2008).
Total dietary fibre	Sigma TDF 100A kit based on AOAC 16 th edition method
Crude fibre	Sadasivam and Manickam (2008).
Cellulose	Sadasivam and Manickam (2008).

Table X
Methods of Micro Nutrient Analysis

Parameters analysed	Method and Reference
Vitamin B6	EN 15652:2009 -HPLC method -CEN2009
Vitamin C	Silva et al., 1999
Calcium	Ranganna, 1997
Phosphorus	Ranganna, 1997
Iron	Ranganna, 1997
Potassium	AOAC Official Method 2011.14

3.4.2. Estimation of Phytonutrients

The increasing emphasis on natural products in recent years, driven by society's interest in a health-conscious diet, highlights the significant potential of phytonutrients in healthcare and clinical therapy due to their advantageous effects (Jain et al., 2021). Phytonutrients are bioactive compounds that are beneficial to human health. These compounds can improve metabolism, act as antioxidants, reduce, and prevent metabolic diseases (Kan et al., 2022). Limited research on the bioactive compounds in the selected cultivars necessitated this profiling.

3.4.2.1. Preparation of the UBF Extract

To one gram of UBF, 25 mL methanol and water v/v in a 20:5 ratio were added and agitated in an orbital shaker (100×g) for 8 hours at 37°C. Following this, it was centrifuged (4000×g) for 10 minutes, and the supernatant was used for analyzing phytonutrients, antimicrobial activity, and antioxidant assays (Savlak et al., 2016).

3.4.2.2. Qualitative Screening

Preliminary qualitative screening for phytochemicals -Alkaloids, Anthocyanin, Anthraquinone, Cardiac glycosides, Coumarins, Flavonoids, Glycosides, Lignin, Phenolic compounds, Quinones, Saponin, Sterols, Tannin, Terpenoids, Volatile oils was carried out using standard protocols (Sorescu et al., 2018, Kancherla et al., 2019, Shaikh & Patil, 2020)

3.4.2.3. Quantitative Analysis of Phytonutrients

Dietary phenolic compounds demonstrate antioxidant, anti-allergic, anti-inflammatory, antiviral, and anti-cancer effects and also support cardiovascular health. (Rahman et al., 2021). Based on the results of qualitative tests, total tannins, total polyphenols, flavonoids, and phytic acid were determined using the methods outlined in Table XI. The detailed protocols are presented in Appendix VI.

Table XI
Methods of Phytonutrient Analysis

Phyto nutrient	Method	Reference
Total phenolic content	Folin-Ciocaltaeu assay Standard -Gallic acid	(Singleton & Slinkard, 1977)
Total tannins	Folin-Ciocaltaeu method Standard -Tannic acid	Sadasivam and Manickam (2008)
Flavonoids	Aluminium chloride colorimetric method Standard- Quercetin	Alothman et al., (2009).
Phytic acid	Standard- Sodium phytate	Davies and Reid (1979)

3.4.3. Assessment of Antimicrobial Activity and Antioxidant Assays

3.4.3.1. Antimicrobial Activity

The exploration of compounds with strong antimicrobial properties has emerged as a key area of research, focusing on minimizing the threat of infectious diseases caused by pathogenic microflora to human beings. Secondary metabolites, including phenolics, flavonoids, alkaloids, saponins, and terpenoids, serve as key components of a plant's defense system while also exhibiting significant therapeutic potential for various diseases. Plant extracts have garnered considerable attention for their role in combating emerging antibacterial resistance (Riaz et al., 2023)

Well diffusion assay was selected to evaluate the bacteriostatic activity of the methanolic extracts of UBF Peyan and Monthan on *E. coli* (MTCC 452) and *Staphylococcus aureus* (MTCC 096) detailed protocol is presented in Appendix VI.

3.4.3.2. Antioxidant Assays

Antioxidants have gained attention due to their potential role in preventing various degenerative diseases, and different methods of assessment of antioxidant capacity have been extensively studied *in vitro* (Da Silva Mendonça et al., 2022). Antioxidant compounds in plant-based food matrices function through multiple mechanisms and it is widely accepted to employ more than one method for their evaluation. Accordingly, the antioxidant potential of UBF was determined by the methods presented in Table XII, and the detailed protocols are given in Appendix VI.

Table XII
Methods of Antioxidant Analysis

Antioxidant Assay	Reference
DPPH- Free Radical Scavenging Activity Assay	Carciochi et al., (2014)
ABTS -Free Radical Scavenging Activity Assay	Re et al., (1999).
Ferric Reducing Antioxidant (FRAP) Assay	Benzie and Strain (1996)

3.4.4. Determination of Prebiotic Index

Prebiotics are indigestible polysaccharides that act as a substrate for intestinal microbiota. Resisting gastric acid and digestive enzymes, they reach the intestine intact and undergo bacterial fermentation. They promote the growth of beneficial microflora and offer significant health benefits (Davani-Davari et al., 2019). In animal research studies, unripe banana flour has promoted gut health and various health benefits (Baek et al., 2023).

The high resistant starch content (44%) and other indigestible carbohydrates, such as dietary fiber (13 g/100 g) and cellulose, identified in phases two and three, have led to the study on the prebiotic potential of unripe banana flour. Numerous investigations explored the efficacy of *Lactobacillus* sp. concerning specific prebiotics. However, the optimal prebiotic carbohydrates for selectively promoting the growth of different strains remain uncertain (Figueroa-González et al., 2019).

The common gut bacteria *Lactobacillus acidophilus* (ATCC314), *Bifidobacterium longum* (ATCC BAA 999), and *Lactobacillus paracasei* (ATCC BAA 2839), were shortlisted for the study to test the growth promotion by unripe banana flour. Each strain was suspended in sterile saline, and the turbidity of the suspension was visually compared

to the 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL) to achieve a uniform inoculum density. This standardization ensured that all strains were in comparable cell numbers, minimizing variability and allowing reproducible and reliable quantification of bacterial growth in response to unripe banana flour.

1% W/V of the study samples (Peyan flour and Monthan flour) and inulin were incorporated into the MRS broth, and 1% bacterial inoculum was added and incubated at 37° C anaerobically. Inulin is well recognized as a prebiotic and it was used as the reference standard. MRS media with 1% dextrose was taken as the control carbohydrate.

3.4.4.1. Determination of pH, Optical Density, and Colony Count

The prebiotic potential of unripe banana flours was assessed by studying the colony count, change in pH, and optical density (OD) over 24-96 hours to understand the growth of the selected probiotics. The readings were recorded every 24 hours. This duration allows the assessment of the initial adaptation phase of the bacteria to the substrate and the subsequent growth phases, to understand the pattern of utilization of specific prebiotics. At each period of 24, 48, 72, and 96 hours, five millilitres of broth was taken for analysis. The pH of the broth was determined, and the OD was recorded at 600 nm with a UV spectrophotometer. One mL aliquot of broth from study samples was serially diluted to get concentrations ranging from 10^{-1} to 10^{-12} . One millilitre of these diluted samples was used for colony enumeration using the pour plate method. Following incubation at 37°C, the colonies were quantified and reported as colony-forming units (CFU/mL) (Powthong et al., 2020). The broth was centrifuged at 10,000 rpm for 10 min, and the supernatant was used to assess its inhibitory effect on *E. coli*.

3.4.4.2. Calculation of Prebiotic Index

According to Huebner et al. (2007), prebiotic activity is defined as “The ability of a given substrate to support the growth of an organism relative to growth on a non-prebiotic substrate, such as glucose. The prebiotic index (PI or I_{preb}) provides a quantitative value for comparing different prebiotic carbohydrates.” When the ratio determined by the equation below surpasses a value of one, it signifies that the microorganism's growth is favoured by the studied prebiotic in contrast to the control carbohydrate (Palframan et al., 2003).

$$I_{preb} = \frac{\text{CFU of probiotics in prebiotic carbohydrate}}{\text{CFU of probiotics in control carbohydrate}}$$

3.4.4.3. Inhibition of *E. Coli* by Fermentation Supernatant

Probiotics support gastrointestinal health by inhibiting the growth of harmful pathogens through antimicrobial secondary metabolites produced during the fermentation of undigested carbohydrates (Latif et al., 2023).

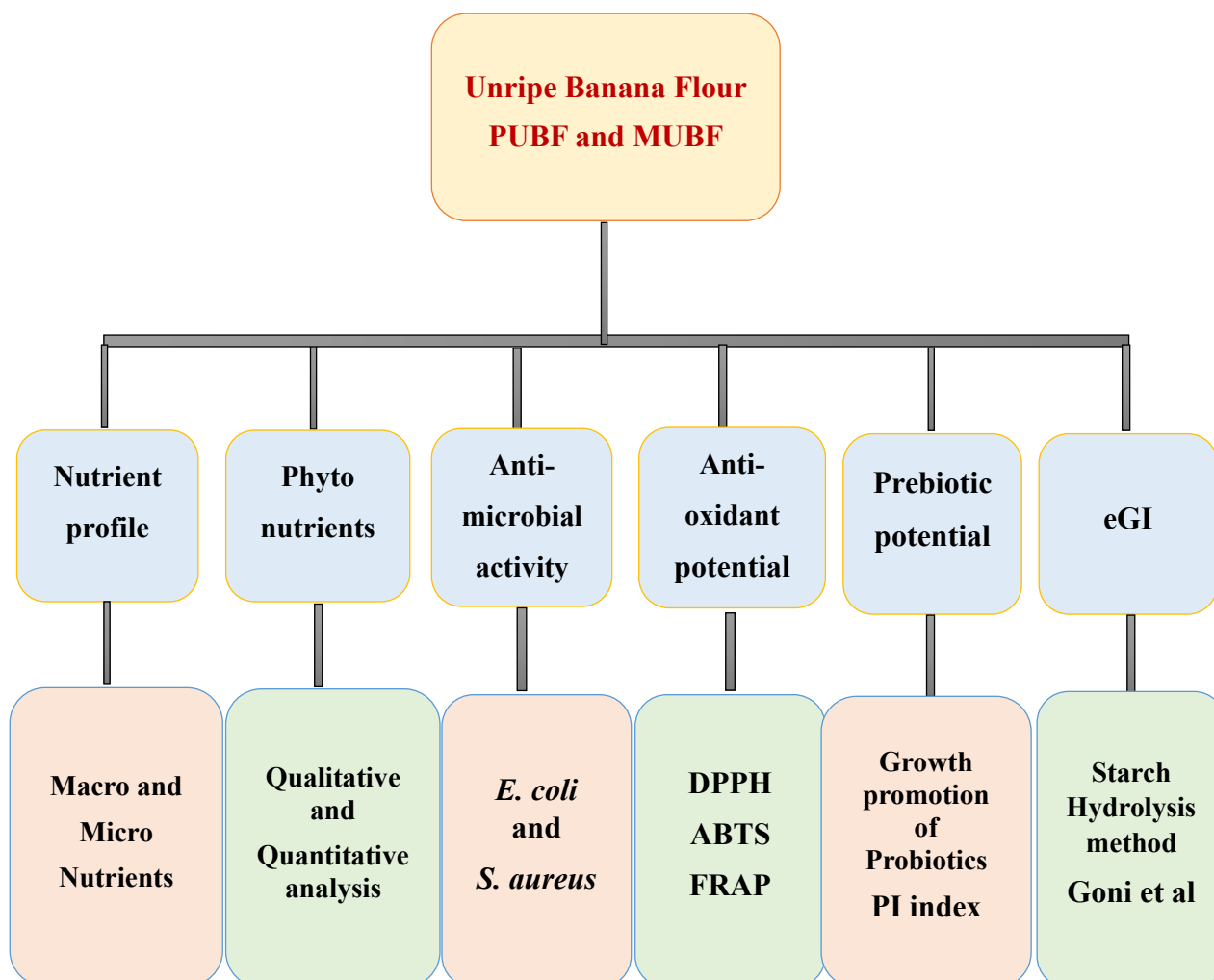
The antimicrobial activity of the fermentation supernatant at 72 h was determined by the agar well diffusion method (Balouiri et al., 2016). Mueller Hinton Agar plates were prepared and inoculated with 200 microliters of a 0.5 MacFarland standard of *E. coli* MTCC 452 in 20 ml of media. Wells of 5 mm diameter were created in the agar, and 100 microliters of each fermentation supernatant were loaded into the wells and incubated at 37°C for 24 hours, after which the inhibition zones were gauged to evaluate the antimicrobial effect.

3.4.5. Determination of eGI

Starch is a primary energy component in our diet, and its digestion plays a significant role in regulating blood sugar levels (Yang et al., 2022). According to Gourineni et al. (2019), consumer demand for foods containing carbohydrates that promote sustained glucose release and a lower glycemic index (GI) is steadily increasing. The GI serves as a key indicator of carbohydrate quality. Incorporating slowly digestible carbohydrates presents a viable approach to enhancing carbohydrate quality while addressing consumer preferences. *In vitro*, starch digestibility assays simulate the physiological conditions of *in vivo* digestion and serve as valuable tools for studying starch changes, interactions, and digestibility. The *in vitro* GI assay is simple and rapid, predicting the GI value by simulating the enzymatic digestion of various food products rather than using human subjects (Sulung et al., 2023).

In vitro GI was estimated by Goñi et al. (1997) method. The hydrolysis index (HI) represents the proportion of glucose released from a food sample compared to a standard reference, such as white bread, over 180 minutes. It is determined by calculating the ratio of the area under the hydrolysis curve of the test sample to that of the reference sample. The bread was used as reference food, and eGI was determined for PUBF and MUBF. The detailed protocol is presented in Appendix VII

Phase 4: Estimation of Nutrient Composition, Phytonutrients, Antimicrobial Activity, Antioxidant Potential, Prebiotic Potential, and Predicted GI of Unripe Banana Flours



3.5. Phase 5: Development and Evaluation of Ready-to-Eat and Ready-to-Cook Products Incorporated with Unripe Banana Flours- PUBF and MUBF

Fresh green bananas are typically not consumed by people due to the usual firmness and pronounced astringency, which is attributed to soluble phenolic compounds such as tannins (Sarawong et al., 2014). Unripe banana flour has the potential to partially or entirely replace other flour in novel product development (Cândido et al., 2023).

The investigated physicochemical properties of UBF, its rich nutrient and phytonutrient profile, and the prebiotic potential of unripe banana flours (PUBF and MUBF) have sparked interest in incorporating them as a functional ingredient for product development.

3.5.1. Product Concept

In line to develop functional RTE and RTC foods, the present study focused on incorporating unripe banana flour as the functional food ingredient in food products that could offer the following benefits

- Nutritional and bio-actives enrichment
- High in resistant starch and fiber content
- Possessing antioxidant and prebiotic potential

RTE and RTC foods were chosen as the platform for functional food delivery, supported by several key considerations listed below

- Convenient and accessible means to deliver functional ingredients
- Catering to diverse tastes and preferences
- Broader consumer base

Insights from the consumer survey conducted in Phase 1 also emphasize consumer interest in incorporating functional ingredients into RTC and RTE products. Further high fiber noodles made with wheat and millet, high-fiber biscuits, and cookies were consumed by most of the respondents (48 %).

Two product categories, baked foods and extruded products, were selected for developing RTE and RTC foods. A total of five products, namely jeera (cumin) cookies,

muffins, and baked choco cereals (RTE category), and noodles and pasta (RTC category). were chosen for the study.

The rationale behind choosing baked foods to incorporate UBF and develop functional RTE and RTC foods is based on the following considerations

- High acceptability among younger consumers
- Long shelf life
- Easy to fortify
- Convenient and portable
- Potential for nutritional enhancement
- Growing demand for healthier snack options

Jeera Cookies: Cookies are appealing and favourable to all age groups, including children, teenagers, and the elderly (Park et al., 2015). They are recognized for their extended shelf life, convenient consumption, and diverse ingredients, textures, and flavours. Traditionally, cookies are made from three fundamental ingredients: flour, sugar, and fat. They can be modified to meet specific dietary requirements by incorporating components rich in dietary fibers, antioxidants, and prebiotics, with low or no gluten, thus providing additional health advantages (Rao et al., 2018). Incorporating unripe banana flour into jeera cookies at varying proportions can provide a healthy option for individuals seeking low-glycemic, high-fiber snacks.

Muffins: According to Rabail et al. (2022), ready-to-eat bakery products, such as muffins, are widely enjoyed by consumers of all ages worldwide. The composition of muffins comprises of wheat flour, sugar, fat, and egg, and there is a possibility for modifications in caloric values, physical attributes, and physicochemical properties during processing (Belorio& Gómez, 2020). Adding UBF to muffins could enhance the nutritional profile by increasing fiber and resistant starch content.

Baked Choco Cereal: As consumers are inclined towards convenience and healthful eating, exploring the incorporation of unripe banana flour in baked chocolate cereal becomes essential, for its nutritional benefits. The baked choco cereal formulation with the incorporation of PUBF and MUBF can enhance its healthful attributes and have consumer appeal.

Extruded RTC products, noodles, and pasta were selected for developing functional food variants based on the following rationale.

- Blending of ingredients uniformly during extrusion
- Suitability for incorporation of functional ingredients
- Easy and quick preparation
- Potential for nutritional enrichment
- Popular across diverse age groups
- Growing demand for health-conscious products

These products are ideal for incorporating bioactive compounds such as resistant starch and antioxidant phenolics, which can significantly enhance their nutritional value and meet consumer demand for healthier food options.

Noodles: Noodles have gained global popularity, and their appeal has expanded, making them an ideal candidate for nutritional enhancement. Resistant starch has been linked to improved digestive health and a lower glycemic response, making it particularly desirable for health-conscious consumers (Raungrusmee et al., 2020). Typically made from wheat flour, noodles offer a promising opportunity for nutritional improvement by substituting part of the wheat flour with alternative flours such as unripe banana flour, which can significantly increase resistant starch content and offer additional health benefits.

Pasta: Pasta is another globally consumed product, widely recognized for its ease of preparation, extended shelf life when dried, and affordability. The nutritional value of pasta is approximately 70–76% carbohydrates, 10–14% protein, 1.8% fats, and 2.9% dietary fiber, along with trace amounts of vitamins and minerals (Sissons, 2022). However, conventional pasta contains limited resistant starch, dietary fiber, and antioxidant capacity, making it a strong candidate for nutritional enhancement (Ovando-Martinez et al., 2008). Over the past two decades, researchers have explored ways to improve the nutritional profile of pasta by incorporating non-traditional ingredients in response to rising consumer interest in functional foods (Sissons, 2022).

3.5.2. Trials and Standardization of the developed RTC and RTE Products

A standardized recipe has been tested and consistently produces the same product under identical conditions. Standardized recipes provide inventory control, foster

creativity, ensure simplicity, and maintain consistency (Naumov, 2023). The RTE recipes were prepared using the standard recipe making from the cookbooks, and the RTC products were adapted from research papers (Vernaza et al., 2011; Ovando-Martinez et al., 2008).

All five products were developed by replacing wheat flour with PUBF and MUBF in variations of 15%, 30%, and 50% levels, and the products were tried out and standardized. These products were treated as the test products. Control products were developed with 100% wheat flour alone for all five products for comparison purposes. The codes for referencing the RTE and RTC products are presented in Table XIII.

Table XIII
RTE and RTC Products Coding

% UBF Incorporation/ Products		Wheat Flour	Peyan (PUBF)			Monthan (MUBF)		
		0%	15% (1)	30% (2)	50% (3)	15% (1)	30% (1)	50% (3)
RTE	Jeera cookies	CWFC	PUBF 1C	PUBF 2C	PUBF 3C	MUBF 1C	MUBF 2C	MUBF 3C
	Muffin	CWFM	PUBF 1M	PUBF 2M	PUBF 3M	MUBF 1M	MUBF 2M	MUBF 3M
	Baked Choco Cereal	CWFB	PUBF 1B	PUBF 2B	PUBF 3B	MUBF 1B	MUBF 2B	MUBF 3B
RTC	Noodles	CWFN	PUBF 1N	PUBF 2N	PUBF 3N	MUBF 1N	MUBF 2N	MUBF 3N
	Pasta	CWFP	PUBF 1P	PUBF 2P	PUBF 3P	MUBF 1P	MUBF 2P	MUBF 3P

3.5.3 Sensory Evaluation and Acceptability of RTE and RTC Products

Sensory analysis is about the appraisal of a product's quality via panellists' senses to determine the acceptance or rejection of the product. It has been a supporting methodology for the technological and microbiological assessments, for judging food quality (Ruíz-Capillas & Herrero, 2021).

Nine-point hedonic scale is commonly employed in sensory evaluation studies to measure the degree of liking or preference for a specific product or attribute. It allows panellists to express their sentiments on a scale, ranging from "extremely dislike" to "neither like nor dislike" to "extremely like" (Lim, 2011). The scale is presented with the following response options (Figure 12 and Appendix VIII)

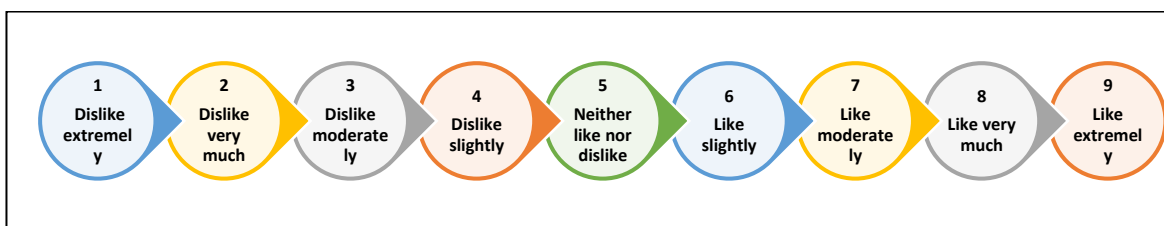


Figure 12: 9 -Point Hedonic Scale



Plate 2: Sensory Analysis by Panel Members

The sensory acceptability study was carried out for the 21 products (jeera cookies, muffins, baked choco cereals with PUBF and MUBF, and three controls) formulated in the RTE category and 14 products (noodles and pasta with PUBF and MUBF and two controls) formulated in the RTC category. Fifteen semi-trained panel members evaluated the sensory attributes of the products. Using the nine-point Hedonic scale provided, panellists selected the number on the scale that best reflects their perception of the product or attribute. The average of the responses was calculated for all the attributes and overall acceptability.

3.5.4. Determination of Physicochemical properties and Nutrient composition of RTE and RTC Products incorporated with 30% PUBF and MUBF

Nutrient profiling helps to identify the composition of nutrients and the existence of interacting elements. It also offers insights into the bioavailability and overall contribution to a balanced diet, and is essential to estimate nutrient intake (Mondal et al., 2023). Hence, nutrient profiling and characterization of the RTE and RTC foods with good sensory acceptability were carried out.

The findings from the sensory analysis indicated the following.

- Product formulations containing 15% and 30% PUBF and MUBF varieties were more favourably accepted and are comparable to the control samples.
- 50% UBF formulations had a lower score for aftertaste and overall acceptability.
- Consequently, considering the higher percentage of UBF incorporation feasible, 30% UBF formulations comprising 15 products were selected for further nutrient profiling.

Figure 13 presents the product formulations selected for further analysis. Tables XIV and XV outline the methods of analysis of physical and cooking characteristics and nutrition compositional analysis. The detailed protocols are presented in Appendices V and IX.

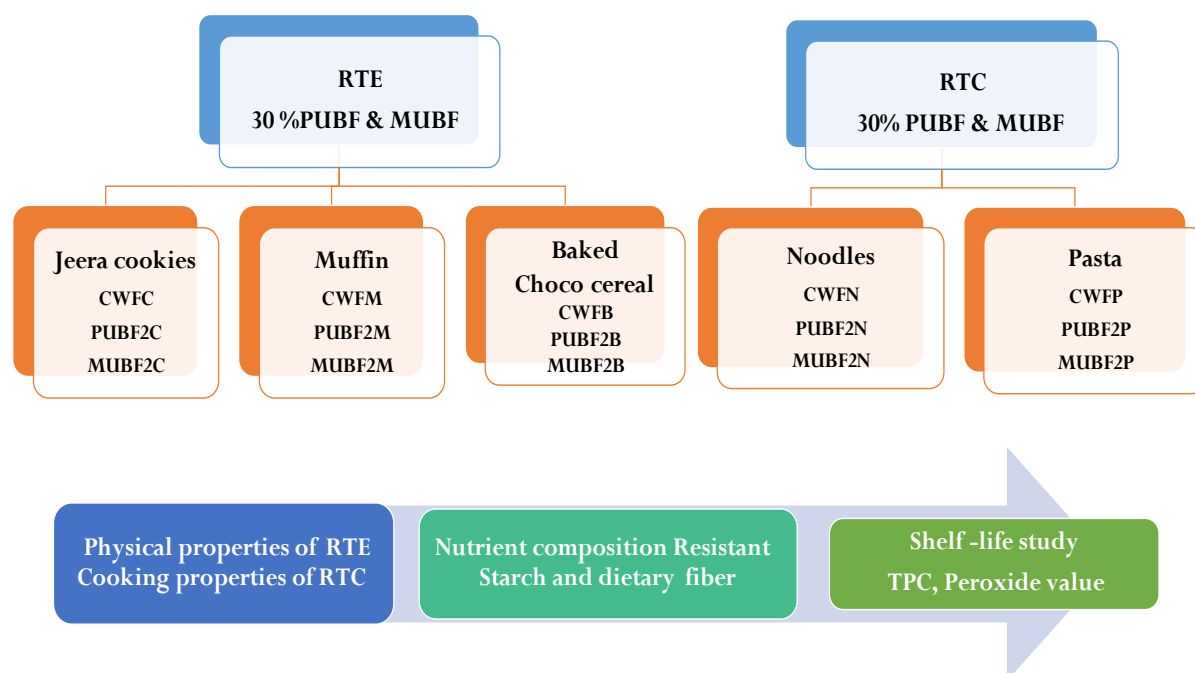


Figure 13: Product Formulations for Nutrient Profiling and Characterization

Table XIV
Physical and Cooking Characteristics of the Developed RTE and RTC Products

Foods	Characteristics Analysed
Cookies	Weight, Diameter, Thickness, Spread ratio, Volume, Bulk Density Ahmed et al., 2018
Muffin	Weight, Height, Diameter, Volume, Bulk Density (AACC, 2000, Guan et al., 2023)
Noodles and pasta	Cooking Time- Yadav et al. (2014)
	Cooked Weight - Galvez & Resurreccion (1992).

Table XV
Nutrient Composition of the Developed RTE and RTC Products

Parameters analysed	Method and Reference
Moisture	925.10: AOAC, 2005
Total mineral	923.03: AOAC, 2005
Protein	Kjeldahl method (conversion factor Nx6.25) 978.04: AOAC, 2005
Fat	960.39: AOAC, 2005
Total Carbohydrate	Anthrone method outlined by Sadasivam and Manickam (2008)
Total Sugars	Phenol-sulfuric acid assay outlined by Sadasivam and Manickam (2008)
Total dietary fibre	Sigma TDF 100A kit based on AOAC 16 th edition (1997)
Resistant starch	AOAC 2002.02

In addition, resistant starch was determined for uncooked and cooked noodles and pasta to understand the degradation of resistant starch in the moist cooking process. The protocols are presented in Appendix IV

3.5.5. Shelf-life Analysis of the developed RTE and RTC Products

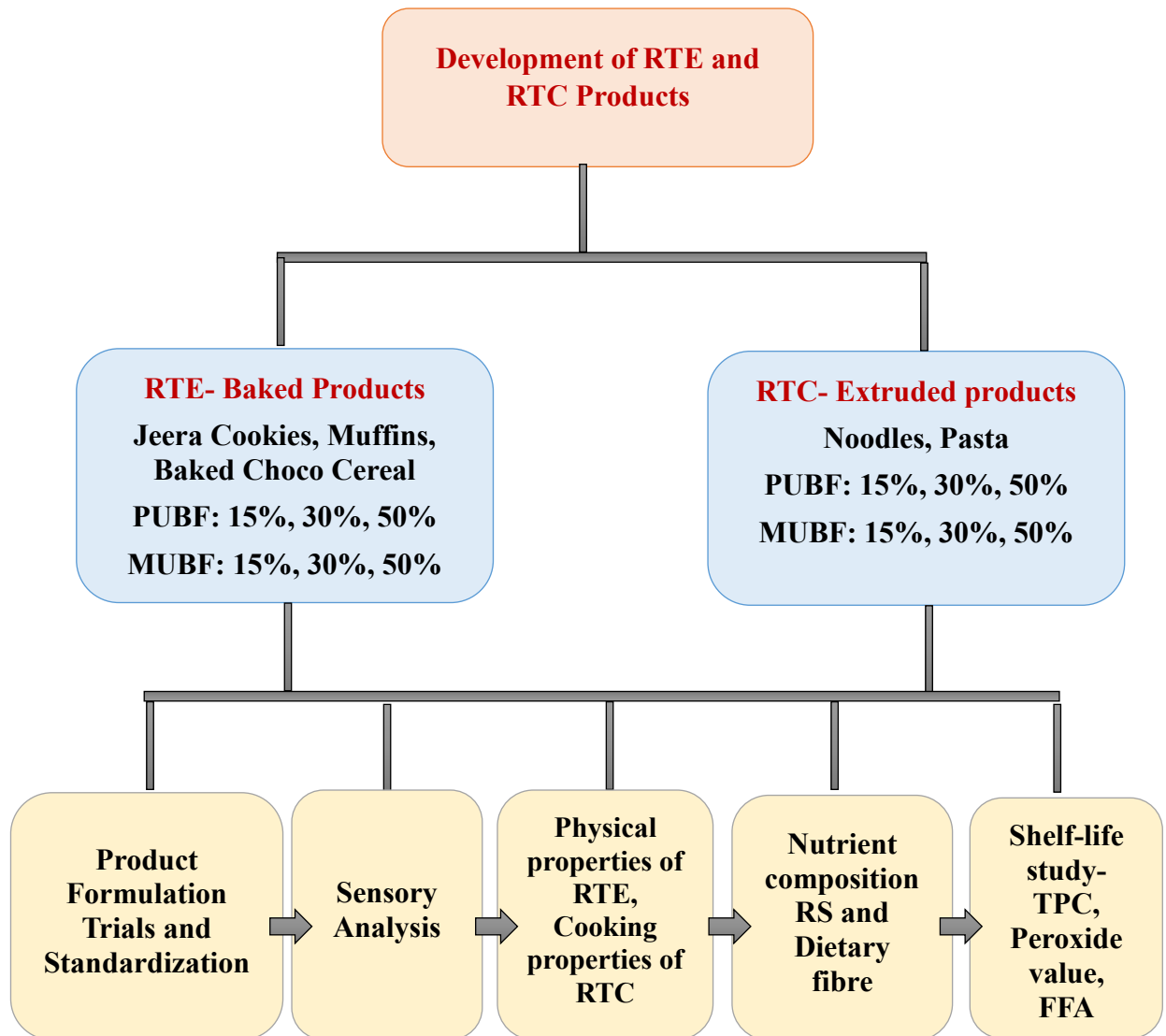
The Institute of Food Science and Technology (IFST) has outlined considerations for assessing shelf-life: (a) safety; (b) sensory, chemical, physical, and microbiological attributes; and (c) adherence to nutritional label declarations (Clodoveo et al., 2021). The shelf-life analysis of the products was carried out by analyzing the microbial standards, peroxide value, and free fatty acid by real-time stability tests. For the shelf-life study of ready-to-cook (RTC) products, 100 g products were placed in polyethylene zipper bags and kept at ambient conditions for 90 days. Periodic sampling was performed at 0, 30, 60, and 90 days. For ready-to-eat (RTE) products, the shelf-life study was conducted for 5 days, with samples drawn at 0, 2, 3, and 5 days (Table XVI). Peroxide value was assessed according to the AOAC-965.33 method, FFA by the method outlined by Kaur et al. (2023a), and the Total plate count was determined according to the FSSAI manual 2016. (Appendix X).

Table XVI
Shelf-life Study for the RTE and RTC Products

RTC / RTE Products	Shelf-life study period	Frequency of Analysis
Noodles	3 months	0, 30, 60, and 90 days
Pasta	3 months	0, 30, 60, and 90 days
Jeera cookies	5 days	0, 2, 3, 5 days
Muffin	5 days	0, 2, 3, 5 days
Baked Choco Cereal	3 months	0, 30, 60, and 90 days

PHASE 5

Development and Evaluation of Ready-to-Eat and Ready-to-Cook Products
Incorporated with Unripe Banana Flours



3.6 Phase 6: Assessment of Prebiotic Potential, Predicted GI, and Purchase Intent of Developed Ready-to-Eat and Ready-to-Cook Products

Product formulations with 30% PUBF in Jeera cookies (ready-to-eat) and Noodles (ready-to-cook) were shortlisted for further studies based on the selection criteria, which included the inclusion of one product from each category (RTE and RTC), the absence of added sugar in the formulations, and well-accepted sensory scores obtained in Phase 5. These products exhibited a substantial presence of resistant starch and dietary fiber, as estimated in Phase 5. Based on these factors, the shortlisted formulations were further evaluated for their prebiotic potential, predicted glycemic index (GI), and consumer purchase intent.

3.6.1. Determination of Prebiotic Index of PUBF Cookies and Noodles

The prebiotic potential of the products was determined along with their wheat control parts. The potential of these products to enhance the growth of selected probiotics, prebiotic index calculation, and inhibition of pathogenic *E. coli* by the fermentation supernatant was carried out based on the methods described in phase 4.

3.6.2. Determination of Predicted GI of PUBF Cookies and Noodles

In vitro GI was estimated by the Goñi et al. (1997) method. The bread was used as reference food, and eGI was determined for 30 % PUBF cookies and 30% PUBF noodles and compared to their wheat control. The detailed protocol is presented in Appendix VII

3.6.3 Purchase Intent of PUBF Cookies and Noodles

A consumer acceptability survey was conducted with 50 women participants to assess the overall liking and intention to purchase 30% PUBF-incorporated cookies and noodles based on the sensory attributes of the products. The participants were provided with the coded sample to taste and provide their purchase intention. The purchase intent scale used by Mora et al. (2022) was adapted for the study. The 5-point scale with its description is presented in Table XVII and Appendix XI.

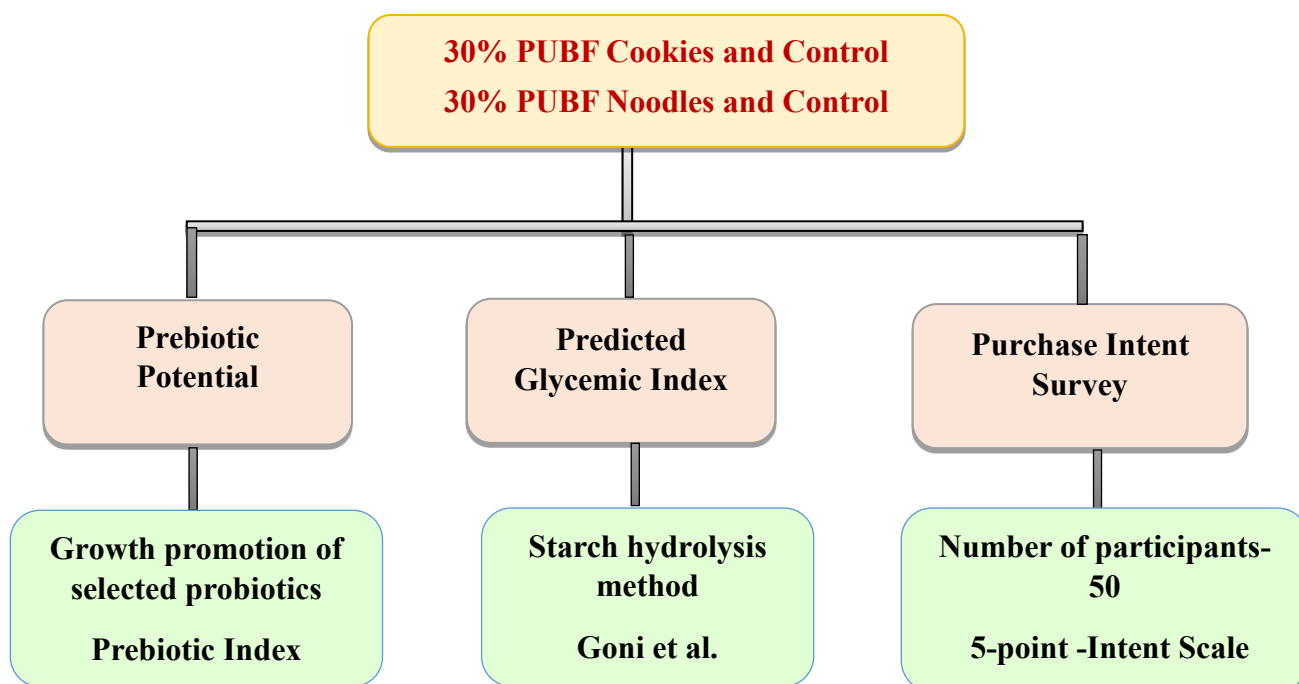
Table XVII
Purchase Intent Scale

Point Scale	Description of the Scale
1	Definitely would not purchase
2	Probably would not purchase
3	May or may not purchase
4	Probably would purchase
5	Definitely would purchase



Plate 3: Purchase Intent Survey participants tasting the RTC Noodles and RTE Cookies

**Phase 6: Assessment of Prebiotic Potential, Predicted GI, and Purchase Intent of
Developed RTE and RTC Products**



3.7. Statistical Analysis

The data analysis was conducted using SPSS version 14.0. In Phase 1, descriptive statistics, including the mean, standard deviation, and frequency distributions, were used to summarize the data. The chi-squared test was applied to examine the relationship between categorical variables in the data. Spearman's rank correlation was used to assess the strength and direction of relationships between ordinal or ranked variables.

For the experimental analysis, the results were presented as mean values with standard deviation. Two-tailed independent t-tests were applied to compare the cultivars Peyan and Monthan. For sensory analysis and nutrient profiling of the RTE and RTC products developed, one-way ANOVA was used, followed by the Duncan Multiple Range Test (DMRT). Pearson correlation coefficient tables were presented to assess the relationships between phytonutrients and antioxidant potential, dietary carbohydrate components, resistant starch (RS), and dietary fiber with respect to glycemic index (GI) and prebiotic index.