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Formulation and evaluation of buttermilk enriched with encapsulated banana pseudostem extract

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Nutritional value of banana pseudostem extract. Encapsulation of banana pseudostem extract with sodium alginate. Analysis of bioactive properties of encapsulated extract. Estimation of nutritional value of banana pseudostem extract.

ABSTRACT
Banana pseudostem is a potentially rich source of phytochemicals, which include phenolics and flavonoids which possess functional properties such as antioxidant and anti-diabetic activities. However, these bioactive compounds are less stable within the food matrix and their extract may impart undesirable sensory attributes. Encapsulation of the extract presents a viable solution to these shortcomings. This study aimed to encapsulate thee banana pseudostem extract from the *Palayamkodan* variety using alginate-based wall material. Additionally, the research involved developing an enriched beverage incorporating the encapsulated extract. Developing an enriched beverage with the encapsulated pseudostem extract offers consumers a convenient and nutritious option. The encapsulation process was performed using high-speed homogenizer with sodium alginate as the wall material. The ethanolic extract of banana pseudostem and its encapsulated form exhibited a total phenolic content of 10.62±0.21 mg GAE/g of extract and 2.97±0.09 mg GAE/100 mL, respectively. Both the extract and its encapsulated form demonstrated significant antioxidant activity, as measured by DPPH radical scavenging assay. The *in vitro* anti-diabetic activity was determined by α-amylase inhibition assay revealing that both the extract, and the encapsulated form exhibited strong activity. The encapsulated extract was incorporated into spiced buttermilk for developing the enriched beverage. A storage study of the enriched butter milk, packaged in bottles and stored under refrigerated conditions (4±2 °C) indicated that the product remains acceptable for over 10 days, maintaining favourable scores across various sensory attributes. From the current investigation, it can be concluded that developing enriched buttermilk with the encapsulated banana pseudostem extract is feasible, providing good antioxidant and anti-diabetic activity.

Introduction

The market for functional foods has been rapidly growing in recent years as a result of the rise in lifestyle diseases and growing public awareness on the importance of maintaining a healthy lifestyle. Functional foods, in their broadest sense, comprise of prepared foods, altered foods, dietary supplements, and fortified goods such as baked goods, cereals, confections, dairy-based foods, fruit- and vegetable-based foods, meat-based foods, and beverages. Among these, beverages are the most popular category of functional foods. They are also significantly less expensive to produce, easier to manage within the the cold chain, and are readily accepted by consumers (Corbo et al., 2014). A number of investigations are focusing on utilizing bioactive compunds from agricultural by-products as ingredients of functional foods. This approach will eventually reduce the cost of raw materials and provide additional income to farmers (Gumisiriza et al., 2017). One such underutilized agricultural by-product is the banana pseudostem, which is a goldmine for many bioactive compounds with positive health benefits (Kumar and Reddy, 2015). In a study conducted by Deng et al. (2020), a wide variety of metabolites (373 in total), categorized into 10 primary recognized types based on their structural characteristics, were reported. These included flavonoids (95), lipids (66), phenolic acids (62), amino acids and their derivatives (44), alkaloids (30), nucleotides and their derivatives (23), organic acids (20), lignans and coumarins (14), tannins (14), and terpenes (5). In another study, the phenolic profile of banana pseudostem revealed the presence of gallic acid, protocatechuic acid, tannic acid, *p*-hydroxy benzoic acid, catechin, epicatechin, gentisic acid, vanillic acid, caffeic acid, syringic acid, *p*–coumaric acid, trans-ferulic acid, trans-cinnamic acid and kaempferol, as reported by Gayathry and John (2023). Bananas (Order: *Zingriberales*; Family: *Musaceae*; Genus: *Musa*) are the second most widely cultivated fruit after citrus fruits (Kumar and Reddy, 2015). They are predominantly grown in the Asian subcontinent, Africa and South America, with a total production of 124.97 million tonnes. India is the largest producer and consumer of bananas, yielding approximately 33.06 million tonnes (FAOSTAT, 2021). The pseudostem is the part of the banana plant composed of tightly overlapping leaf sheaths surrounding a fragile core. After harvesting the fruit, the pseudostem is often discarded and incinerated. Conventionally, there have been several constraints to utilising it; such as limited acceptance due to discolouration and taste. Nevertheless, pseudostem is widely recognized in traditional medicines for its properties against hyperglycaemia, obesity control and detoxification.

Numerous studies have explored the creation of beverages using banana pseudostem juice. These studies include the addition of banana pseudostem juice to papaya juice by Bornare and Sumaiya (2015) and the utilization of pseudostem as a novel substrate for producing ethanol and probiotic beverages by Shriniketan et al. (2023). Researchers have explored and established the presence of various bioactive compounds in banana pseudostem (Ramírez-Bolaños et al., 2021; Ramu et al., 2017). Alkaloids, flavonoids, phenolic acids, tannins, terpenes, lignans and coumarins were the primary bioactive types found in the banana pseudostem according to a study conducted by Deng et al. (2020). However, the application of these potential bioactives in food and other industrial sectorsis limited by factors such as the instability of these compounds and their negative impact on the sensory and nutritional properties of food (Risch, 1995; Shahidi and Han, 1993). It is well established that encapsulating such bioactive components can prevent destructive interactions that may lead to a loss of bioactivity. Therefore, protecting phenolic compounds by subsequent encapsulation after extraction would be a more effective method for preserving the structural integrity of polyphenols until their application in the food and nutraceutical industries (Nwabor et al., 2020; Rajapaksha and Shimizu, 2020; Saikia et al*.,* 2015).

Encapsulation is a technique that involves enclosing an active agent within a carrier material. It serves as a valuable method for enhancing the transport of bioactive compounds and living cells into food (Nedovic et al., 2011). The primary applications of encapsulation include the controlled release of compounds over an extended period, the protection of components from unwanted exposure both inside and outside the body, the enhancement of the stability of active pharmaceutical ingredients, the combination of two such ingredients in a formulation, and the masking of unpleasant flavours and odours (Desai and Park, 2005). Various techniques have been used for the encapsulation of bioactive components which include spray drying, spray chilling or spray cooling, extrusion coating, fluidized bed coating, liposome entrapment, coacervation, inclusion complexation, centrifugal extrusion, and rotational suspension separation. Utilizing instruments that can produce high-intensity forces to split dispersed phase droplets and generate nanoscale droplets is the basis of the high-energy approach, which may be scaled for industrial usage.

These forces can be produced by a variety of instruments, including evaporators, ultrasonic generators, high-shear homogenizers, high-pressure homogenizers, and high-pressure microfluidizers (Galvão et al., 2018). The current study has used high-speed homogenizer for developing such a force for the encapsulation. Many materials can be employed to encapsulate or cover various types of solids, liquids, or gases each with distinct properties. The ultimate product's functionality, possible coating material restrictions, encapsulate concentration, release type, stability requirements, and cost constraints are the most crucial factors to consider when choosing an encapsulating material (Wandrey et al., 2010). The sodium salt of alginates has been widely used to encapsulate cells, medicinal ingredients, and essential oils. Due to its unique colloidal propertiessuch as thickening, stabilizing, suspending, film-forming, gel-producing, and emulsion-stabilizing, alginate iscapable of creating biopolymer films or coating components (Khanvilkar et al., 2016; Rhim, 2004; Kester and Fennema, 1986). The wall material utilized in this study for encapsulating bioactive compounds was sodium alginate (SA) and the process employed was high-speed homogenization (HSH).

The present study was thus undertaken to extract bioactive compounds from the pseudostem extract of the *Palayankodan* variety of banana *(Musa × paradisiaca Mysore AAB group)* and to develop an encapsulated formulation for incorporation into a beverage. Additionally, the study aimed to evaluate the functional properties of this formulation, particularlyits antioxidant and anti-diabetic activities.

Materials and methods

Materials

Banana pseudostem *(Musa paradisiaca)* of *Palayamkodan* variety was obtained from a local farm near Thiruvankulam, Kochi, Kerala, India (9.9435°N 76.3739°E, 11m above sea level).

Methods

Sample Preparation

The collected raw materials were manually cut into small cubes (approximately 2×2×2cm) using a knife. The cut cubes were then treated with a 0.2% citric acid solution for 30 minutes to inhibit browning (Wickramarachchi and Ranamukhaarachchi, 2005). The pre-treated samples were subsequently dried in a tray dryerr at a temperature of 45 °C for 24 hours (Padam et al., 2014). The dried material was then ground into a powder using a mixer grinder (Preethi Grinder, India) at medium speed, stored in zip pouches and kept at -32 °C for further analysis.

Extraction of phytochemicals

A 10 g sample of dried powder was combined with 150 mL of 60% ethanol. The mixture was then subjected to ultrasonication at room temperature for 20 minutes. Following initial filtration, the remaining residue was re-extracted with 50 mL of 60% ethanol for an additional 10 minutes at room temperature, and the resulting filtrates were combined. The ethanol solvent from the extract was evaporated using a rotary vacuum evaporator (IKA RV10, Germany) and the concentrated extract was stored in a screw-capped bottle at -20 °C.

Estimation of total phenolic content

A modified method based on the procedure described by Singleton and Rossi (1965) was employed to determine the total phenolic content of the banana pseudostem extract and the encapsulated extracts of various ratios. 0.5 mL of Folin-Ciocalteu's phenol reagent was added to a test tube containing 0.5 mL of the sample. Subsequently, 1 mL of saturated sodium carbonate was added, followed by the addition of 10 mL of distilled water. The mixture was vortexed thoroughly and then incubated at room temperature for 45 minutes. Centrifugation was performed at 4000 rpm for 5 minutes. The supernatant was collected to measure the absorbance at a wavelength of 725 nm. A standard curve prepared with gallic acid was used to calculate the total phenolic content in each encapsulated extract, with the results expressed as milligrams of gallic acid equivalents (mg GAE) per gram of encapsulated extract.

Estimation of total flavonoid content

Total flavonoid content was determined by the aluminium chloride colorimetric assay described by Zhishen et al. (1999). The absorbance of the mixture was read at 510 nm. Quercetin was used as the standard and the results were expressed as mg QE/g of sample.

Antioxidant potential by Ferric reducing antioxidant power (FRAP) assay

The antioxidant potential of the extract was determined by FRAP (Ferric reducing antioxidant power) according to the process described by Yen and Chen (1995). The solutions were mixed properly and the absorbance was read at a wavelength of 700 nm. The standard used was Trolox.

Anti-oxidant potential by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The scavenging activity of both the extract and the encapsulated extract of different ratios was determined using DPPH scavenging inhibition assay according to the procedure described by Ko et al. (1998). To 1mL of the sample, an equal volume of ethanol was added, followed by the addition of 0.25 mL 1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl). The mixture was then incubated in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm. The percentage inhibition was calculated using Formula 1. Formula 1:

$$\%\ Inhibition\ of\ DPPH\ free\ radical = \frac{A_0 - A_1}{A_0} \quad (1)$$

where, A_0 – absorbance of control,

A_1 – absorbance of sample.

Alpha amylase inhibition assay

The anti-diabetic activity of both the extract and the encapsulated extract was assesse during the chromogenic method described by Banerjee et al. (2017) with slight modifications. A volume of 100 µL

of the sample was added to a test tube, followed by the addition of 200 µl of α-amylase enzyme (0.275 mg/mL). The mixturewas then incubated at 37 °C for 20 minutes. After incubation, 100 µL of 1% starch was added, and the mixture was allowed to stand at 37 °C for an additional 10 minutes. Subsequently 200 µL of dinitrosalicylic acid (DNSA) was added, and thenmixture was boiled for 5 minutes. The solution was then diluted with distilled water by adding 2.2 mL of distilled water. The absorbance was measured at 540 nm.

The percentage inhibition of the alpha amylase enzyme was calculated using the Formula 2.

Formula 2:

$$\%\ Inhibition\ of\ alpha\ amylase\ activity = \frac{[C_{blank} - \langle S - |S_{blank}\rangle] \times 100}{C_{blank}} \quad (2)$$

where, C_{blank} – absorbance of control blank,

S – absorbance of sample,

S_{blank} – absorbance of sample blank.

Encapsulation of phytochemicals

The encapsulation of the extract using the wall material sodium alginate was performed according to the procedure described by Purwanti et al. (2018), with modifications. The wall material used for the encapsulation was sodium alginate with a concentration of 1.5% (w/w).

Three different ratios of extract to wall material were prepared: 1:3, 1:2, 1:1 (v/v) with a total volume of 50 mL. The process employed for the encapsulation was high-speed homogenization (T18 Ultra-Turrax®, IKA, Germany) at 5000 rpm for 10 minutes, conducted in an ice-water mixture to prevent execessive heat. The prepared solutions were stored in the refrigerator for further analysis and processing.

Retention efficiency

The retention efficiency was calculated according to Piacentini (2016) with slight modifications. The efficiency was calculated as shown in Formula 3. Formula 3:

$$Retention\ efficiency = \frac{W\mathrm{p}}{W\mathrm{f}} \times 100 \quad (3)$$

Where, Wp – phenolic content in the encapsulated extract,

Wf – phenolic content in the feed.

Zeta potential

The zeta potential of the encapsulated extract was determined using a Zetasizer Nano (Malvern Panalytical Ltd., Malvern, UK). A 3 mL sample was taken for analysis. The zeta potential of the nanoparticle was measured by detecting its mobility in the applied electric field at a temperature of 25 °C and an angle of 15°.

Poly dispersity index (PDI) and particle size

The poly dispersity index and particle size were determined by dynamic light scattering analysis using a Zetasizer Nano series (Malvern Panalytical Ltd., Malvern, UK).

Development of enriched beverage

Spiced buttermilk incorporated with encapsulated pseudostem extract was prepared as described. The ingredients used for the formulation included desi buttermilk and spices, specifically fresh ginger, bird's eye chilli, curry leaves, and salt (Table 1). The encapsulated extracts in the ratio (v/v) 1:2 and 1:3 (exract: sodium alginate) were selected for the development of enriched beverages.

The selection of the appropriate ratio was based on the sensory attributes. These extracts were incorporated into the spiced buttermilk in the following ratios (v/v) 1:1, 1:2, 1:3 and 3:1 (encapsulated extract: spiced buttermilk).

Table 1. Ingredients and its quantity taken for preparing spiced buttermilk.

Ingredients	Quantity
Ginger	5 g
Bird's eye chilly	3 g
Curry leaves	0.5 g
Desi Buttermilk	400 mL
Salt	As required

Storage study of enriched buttermilk

A shelf-life study of enriched buttermilk for the selected ratio was carried over a period of 10 days. The buttermilk was packed in polyethylene terephthalate (PET) bottles and stored under refrigerated conditions.

Each bottle contained 100 mL of enriched buttermilk and the samples were stored in duplicates along with a control.

Analyses were performed at two days interval sspecifically on the 0, 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} days. The storage parameters evaluated included sensory evaluation, sedimentation, viscosity, acidity, total phenolic content, and antioxidant potential.

Sensory evaluation

Sensory evaluation was carried out by a panel of 10 members by using a 9-point hedonic scale score sheet.

Sedimentation

The sedimentation of samples was determined by measuring the height of the sediment formed from the base of bottle, expressed in centimetres. Analysis was performed at two day interval.

Viscosity

The viscosity of the enriched buttermilk was analyzed using a Brookfield DVE viscometer (DVEEELVTJO, USA). The spindle used was 61. Readings were taken at two different RPM settings: 60 and 100, and the results were expressed in centipoise.

Acidity

The titratable acidity of the beverage was determined through titration with 0.1 N NaOH.

A 5 g sample was taken in a beaker, followed by the addition of an equal amount of distilled water. The mixture was then titrated with NaOH using phenolphthalein as the indicator.

The percentage acidity of the beverage was calculated using Formula 4. Formula 4:

$$Acidity(\%) = \frac{9 \times A \times N}{W} \quad (4)$$

where, A – titre value,
N – normality of NaOH,
W – weight of the sample.

Statistical analysis

The statistical analysis of the data was conducted using the statistical package WASP 2.0 (Web Agri Stat Package 2.0). All analyses were performed in triplicate and the values representing the mean were subjected to univariate and multiple-way analysis to find out the significance. For each tests, statistical significance was expressed at the $p<0.05$ level.

Results and discussion

This study involved the phytochemical evaluation of banana pseudostem extract and its encapsulated form. Additionally, an enriched beverage was developed using the extract and a storage study was conducted. The research focused on incorporating the encapsulated extract into the beverage in its liquid phase. Ethanol was selected for the extraction of bioactive compounds from the banana pseudostem, as it is considered to have no harmful effects on humans in contrast to methanol which has been identified by Sumathy et al. (2011) as the most effective solvent for extracting bioactive compounds from banana stem.

Phytochemical evaluation of the extract

Total phenols and flavonoids

The phenolic content of the ethanolic extract from banana pseudostem of the *Palayamkodan* variety was found to be 10.62±0.21 mg GAE/g of extract (Table 2). The value is comparable to that reported by Loganayaki et al. (2010) which was 12±0.1 mg GAE/g of extract. However, the total phenolic content of banana peel extract has been reported to be higher than that of the banana pseudostem (16.83±0.39 mg GAE/g of extract) (Yusof et al., 2023). Additionally the amount of phenolics extracted can also be adversely affected by the solvent mixture used during ther extraction process (Sidhu and Zafar, 2018).

Table 2. Yield, phytochemical and bioactive evaluation of banana pseudostem extract.

Phytochemical andbioactiveproperties of pseudostem extract				
Yield (g/100 g dried pseudostem)	**Phenolic Content (mg GAE/g extract)**	**Flavonoid Content (mg QE/g extract)**	**Antioxidant activity-DPPH Assay (%inhibition/0.5 g extract)**	**Antidiabetic Activity IC_{50} (mg extract/mL)**
15.5±1.3	10.62±0.21	238.51±3.57	53.66±0.38	0.19±0.02

Values are expressed as Mean ± S.D (n=3)

The flavanoid content obtained in this study is relatively high compared to the value reported by Ravindran et al. (2021) for banana inflorescence (3.29 mg QE/g). This comparison suggests that banana pseudostem is more advantageous in terms of flavonoid content, as flavonoids have been shown to

effectively lower the risk of cardiovascular diseases by reducing LDL oxidation and preventing other degenerative conditions.

Anti-diabetic activity of the pseudostem extract

In order to reduce the rate of glucose release into the bloodstream, it is essential to inhibit the carbohydrate-associated enzyme α-amylase. Inhibiting this enzyme can help slow the absorption of glucose during digestion (Marikkar et al., 2016). Numerous studies have been published on the anti-diabetic efficacy of traditional medicinal plants. Research has demonstrated that plants like *Gynura procumbens, Ficus deltoidea, Phyllanthus niruri*, etc., are effective against diabetes. Furthermore, it has been shown that pseudostem extracts contain significant levels of phenolics and flavonoids, which may contribute to the notable inhibitory effect of these extracts on α -amylase.

The samples were diluted to various concentrations and the IC_{50} values for both the standard (acarbose) and the samples were estimated. The IC_{50} value for the banana pseudostem extract was found to be 0.19±0.02mg/mL. The inhibitory effect of the extract was lower than that of the standard (IC_{50}– 0.039±0.01mg/mL); clearly indicating that the extract possesses anti-diabetic activity, althoughit is less potent than the standard acarbose. The results are consistent with those obtained by Ramu et al. (2022) regarding banana pseudostem extract.

Encapsulation of bioactives in banana pseudostem extract

The optimization of the encapsulation ratio was conducted based on both preliminary phytochemical evaluations and the sensory characteristics of the beverage containing the encapsulated extract. Moreover, the quantity of wall material required to encapsulate the bioactive compounds present in the extract was taken into account.

Table 3 shows the phenolic content and antioxidant activity of both the feed and encapsulated extract in different ratios of extract to wall material. The results indicated that the 1:1 and 1:2 (extract to wall material) ratios exhibited the highest phenolic content and antioxidant activities. These selected ratios provided an adequate amount of wall material to effectively encapsulate the bioactive compounds in the extract. In contrast, the 3:1 ratio contained an insufficient wall material, which may not have been adequate to entrap all the core materials.

Table 3. Total phenolic content (mg GAE/100 mL), inhibition % of feed and encapsulated extract of different ratios (extract: wall material).

Sample	Ratio	Total phenolic content (mg GAE/100 mL)	Inhibition-DPPH (%/mL of extract)	FRAP Assay (mg TE/100 mL)
Feed	1:3	1.97±0.13^{c}		
	1:2	2.93±0.09^{b}		
	1:1	3.52±0.08^{a}		
Encapsulated extract	1:3	1.55±0.08^{f}	14.53±2.2^{g}	7.62±0.23^{j}
	1:2	2.22±0.05^{e}	32.09±1.48^{h}	14.19±0.18^{k}
	1:1	2.97±0.09^{d}	38.92±4.14^{i}	21.18±0.51^{l}

Values are expressed as Mean ± S.D (n=3). Means in columns with similar superscripts (a-l) are not significantly different (p≥0.05), based on ANOVA

Retention efficiency of encapsulated extract

Retention efficiency was calculated by comparing the retention of bioactive compounds in the encapsulated extract after high-speed homogenization with the amount present in the feed solution (Table 4). The extract with a wall material in a 1:1 ratio demonstrated the highest total phenolic content (TPC) retention among the various ratios tested. In terms of total flavonoid compound (TFC) retention, the 1:3 ratio showed the highest value. Conversely, in both cases, 1:2 ratio exhibited the lowest retention value.

Table 4. Effect of different ratios on TPC and TFC retentions.

Encapsulated ratio (extract:wall material)	TPC Retention (%)	TFC Retention (%)
1:3	78.68[b]	88.44[a]
1:2	75.76[c]	76.45[c]
1:1	84.37[a]	83.43[b]

Values in columns with similar superscripts (a-c) are not significantly different (p≥0.05), based on ANOVA

Zeta potential of encapsulated extract

Zeta potential is a crucial parametre that indicates the charge and stability of a system against coalescence and aggregation (Sezgin-Bayindiret al., 2015). A zeta potential of ±30 mV or greater is generally considered as stable. High absolute values create repulsive forces between particles, which can enhance the physical stability of multiphase systems. Absolute values between 5 and 20 mV provide short-term stability, while values below 5 mV lead to rapid aggregation (Wang et al., 2016; Honary and Zahir, 2013). The formulation exhibited weak electrostatic stability, with net charges approaching zero (Table 5). The low surface charge might be the reason to the reduced zeta potential, adversely affectings the stability of the system.

Table 5. Zeta potential, PDI & particle size of encapsulated extract.

Ratio	Zeta potential (mV)	Polydispersity index	Particle size (µm)
1:1	-8.4±0.056	0.711±0.003	3.53±0.07

Polydispersity index and particle size

The polydispersity index (PDI) measures the uniformity and homogeneity of the produced particles. PDI values below 0.3 indicate a narrow particle size distribution, suggesting a homogeneous system (Tamjidi et al., 2013). Consequently, the PDI value of the sample indicates that the encapsulated system is less homogeneous (Table 5). Particle size is a critical physical characteristic that directly influences the release and absorption ofen capsulated bioactive substances (Bhushani et al., 2017; Rajam and Anandharamakrishnan, 2015). According to Desai et al. (1997), nanoparticles possess a superior intracellular retention capacity compared to microparticles, owing to their enhanced mobility and small size. In this context, the particle remains within the micrometer range.

Phytochemical evaluation of encapsulated extract

Total phenolic content

TPC was determined for all ratios of extract to wall material attempted for the encapsulation of the extract. The total phenolic content of both the feed (prior to encapsulation process) and the

encapsulated extract of different ratios are shown in Table 3. The values for both the feed and the encapsulated extract across different ratios were found to be similar, indicating a satisfactory encapsulation efficiency. However, the lower value for the encapsulated extract compared to the feed, as shown in Table 3, may result from polyphenols being more tightly bound due to interactions with the coating material used (Šaponjac et al., 2016). A similar trend was observed in previous studies conducted by Arriola et al. (2019) and Stojanovic et al. (2012). Statistical analysis of the TPC of both the feed and the encapsulated extract reveals a significant difference (p≥0.05) among the different ratios.

Total flavonoid content

In contrast to the TPC content comparison between the feed and extract, a significant difference can be observed between the TFC of the feed and the encapsulated extract, as shown in Table 4. The TFC of the encapsulated extract for each ratio was found to be lower than the TFC of the feed as,shown in Table 6.

Table 6. Total flavonoid content (mg QE/100 mL) of feed and encapsulated extract of different ratios.

Sample	Ratio	Total flavonoid content (mg QE/100mL)
Feed	1:3	63.62±1.36^{c}
	1:2	88.59±.29^{b}
	1:1	107.41±2.63^{a}
Encapsulated extract	1:3	56.27±2.83^{e}
	1:2	67.73±10.11^{e}
	1:1	89.62±4.66^{d}

Values are expressed as Mean ± S.D (n=3). Means in columns with similar superscripts (a-e) are not significantly different (p≥0.05), based on ANOVA.

According to the results obtained by Essifi et al. (2021), alginate microparticles prepared using the ionotropic gelling method represent a promising approach for encapsulating and controlling the release of water-soluble phenolic and flavonoid compounds such as gallic acid, thereby expanding their application in functional formulations.

Antioxidant activity: DPPH$^{\cdot}$ scavenging inhibition assay & FRAP method

The percentage of inhibition exhibited by the encapsulated extract of different ratios is shown in Table 3. Encapsulated extract at a ratio 1:1 exhibited the highest DPPH$^{\cdot}$ inhibition activity, measuring 38.92±4.14 % and the lowest inhibition was observed at a 1:3 ratio, which recorded 14.527±2.2 %. The percentage inhibition increased with the amount of extract as anticipated. Statistical analysis revealed significant difference in the percentage inhibition between the feed and the encapsulated (p≥0.05). Additionally, the percentage inhibition of the encapsulated extract of different ratios was found to be lower than that of the feed of same ratios, as detailed in Table 3.

In the study conducted by Arriola et al. (2016), the TPC and antioxidant activity levels, assessed through ABTS$^{\cdot}$ scavenging and FRAP assays,demonstrated a significantly strong linear correlation (r = 0.99 and 0.97, p<0.001, respectively). This indicates that phenolic compounds are important contributors to the antioxidant activity of stevia leaves. In the encapsulated extract, the phenolic compounds may be trapped within the wall material, which accounts for the lower concentration of phenolic compounds in the encapsulated extract compared to the feed. Consequently, the antioxidant activity of the encapsulated extract was found to be lower than that of the feed.

The antioxidant properties of the final carriers may vary depending on the wall material used. The antioxidant activity of dietary items that are encapsulated will be boosted, because wall materials can

participate in antioxidant processes depending on their capacity to donate an electron or hydrogen (Maqsoudlou et al., 2020). Mahmoud et al. (2018) utilized chitosan and sodium alginate to nano-encapsulate antioxidants extracted from the skins of Egyptian prickly pears. According to their results, depending on the extract concentration and wall material, DPPH radical scavenging and reducing power activity enhanced after the nanoencapsulation procedure. In the case of antioxidant capacity determined by the FRAP method, the values increased with a higher extract-to-encapsulating ratio, as expected, which are shown in Table 5. The enhanced antioxidant activity can be attributed to the increased TPC in the encapsulated extract.

Anti-diabetic activity of the encapsulated extract

Encapsulated extract of banana pseudostem was tested for its ability to inhibit the α-amylase enzyme. The encapsulated extract of 1:1 ratio and a a concentration of 1 mg/mL demonstrated an inhibition of 27.22±1.52%.

A study by Haładyn et al.(2021) established that the types of polysaccharide coatings and their mixtures would affect the ability of chokeberry extracts to inhibit the enzymes alpha-amylase and alpha-glucosidase. Their results indicated that the microspheres composed entirely of sodium alginate had the most significant impact on alpha-amylase inhibition, measuring 113.04 mg/mL. Our findings align with this conclusion. Furthermore, in an ethanolic extract of *Peltophorum pterocarpum*, Manaharan et al. (2012) demonstrated a strong correlation between anti-diabetic and antioxidant properties. Similarly in the present study, the anti-oxidant and antidiabetic activities could be correlated.

Development of enriched buttermilk

Spiced buttermilk incorporated with encapsulated pseudostem extract was prepared as described in the previous section. The ratios of encapsulated extract to spiced buttermilk developed in this study were 1:3, 1:2, 1:1, and 3:1. The optimization of the amounts of encapsulated extract and spiced buttermilk was based on sensory characteristics. The 1:1 ratio of extract to wall material and the 1:2 ratio of encapsulated extract to spiced buttermilk were selected based on various sensory parameters (data not shown). Numerous investigations have been conducted to create beverages using banana pseudostem juice. However, this study incorporates a concentrated extract that has undergone encapsulation, which protects the bioactive components from potential degradation processes that may occur during the storage of the beverage.

Storage study of the selected enriched buttermilk

Sensory evaluation

Sensory evaluation of enriched buttermilk was conducted by a panel of 10 members. Both the control (spiced buttermilk) and the enriched spiced buttermilk were presented for comparison during the sensory evaluation. The enriched buttermilk was well recieved by the sensory panel members throughout the shelf-life study. All sensory attributes, except for the taste, remained unchanged during the storage. The taste of the beverage improved in terms of astringency over time, even though the levels of bioactive compounds remained constant. Overall, the product's acceptability was rated positively throughout the study, as indicated by the scores shown in Table7.

Table 7. Sensory evaluation of enriched buttermilk during storage study with the control.

Days	Sensory Attributes											
	Colour		Appearance		Consistency		Aroma		Taste		Overall acceptability	
	*Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample	Control	Sample
0	8.00±0.00^{b}	8.00±0.67^{a}	7.80±0.52^{a}	7.70±0.48^{bc}	7.80±0.65^{b}	7.80±0.79^{ab}	8.00±0.00^{a}	8.10±0.57^{a}	8.20±0.37^{a}	7.10±0.88^{a}	8.10±0.88^{a}	7.40±0.84^{a}
2	8.20±0.57^{a}	7.7±0.48^{c}	7.70±0.67^{b}	7.60±0.70^{c}	8.00±0.00^{a}	7.50±0.71^{d}	8.00±0.00^{a}	7.5±0.71^{c}	8.10±0.58^{b}	6.80±1.52^{b}	8.00±0.56^{a}	7.00±1.1^{b}
4	8.00±0.00^{b}	8.00±0.00^{a}	7.50±0.72^{d}	7.90±0.52^{a}	7.80±0.50^{b}	8.00±0.00^{a}	8.00±0.00^{a}	7.80±0.54^{b}	8.00±0.72^{c}	6.90±0.70^{b}	8.20±0.68^{a}	7.40±0.68^{a}
6	8.00±0.00^{b}	7.90±0.82^{b}	7.80±0.52^{a}	7.80±0.79^{ab}	7.60±0.32^{c}	7.60±0.70^{c}	7.50±0.60^{d}	7.70±0.48^{b}	8.10±0.58^{b}	7.20±0.63^{a}	8.00±0.00^{ab}	7.40±0.52^{a}
8	8.00±0.00^{b}	7.70±0.42^{c}	7.70±0.48^{b}	7.70±0.48^{bc}	7.80±0.55^{b}	7.70±0.48^{bc}	7.80±0.36^{bc}	7.20±0.42^{d}	8.00±0.00^{c}	6.90±0.74^{b}	7.80±0.92^{bc}	7.00±0.67^{b}
10	8.00±0.00^{b}	7.70±0.48^{c}	7.60±0.65^{c}	7.70±0.48^{c}	7.80±0.66^{b}	7.70±0.48^{bc}	7.70±0.62^{c}	7.20±0.42^{d}	8.10±0.62^{b}	6.90±0.74^{b}	7.60±0.83^{c}	7.00±0.65^{b}

Values are expressed as Mean ± S.D (n=10). Means in columns with similar superscripts are not significantly different (p≥0.05), based on ANOVA. *Control: Spiced buttermilk; Sample: Enriched spiced buttermilk.

Sedimentation

The sedimentation of the sample was found to be higher than that of the control on the 0^{th} day itself, as the enriched buttermilk contains a greater liquid component (encapsulated extract) compared to the control. The curdling that occurred during the addition of the encapsulated extract to the buttermilk may have further promoted sedimentation despite efforts to minimize curdling through magnetic stirring. The sedimentation throughout the shelf-life study period has shown similar values as illustrated in the Figure 1(a). Statistical analysis indicated that the sedimentation of the beverage at various intervals during the shelf-life study period showed no significant differences ($p \geq 0.05$).

Viscosity

The viscosity of the beverage was measured using a Brookfield viscometer at a speed of 100 rpm. The viscosity obtained at 100 rpm exhibited a consistent trend. During the initial days, the viscosity remained stable; however, it began to increase as illustrated in the Figure 1(b). Nevertheless, the increment shown in the viscosity was minimal. According to a study conducted by Farahani et al. (2023), alginate and gellan improved the specimen's ability to bind water, thereby slowing the flow rate and forming a more robust network of fluid gels. This may explain the slight increment in the viscosity of the beverage, as it contained sodium alginate, which has been used as the wall material for encapsulating bioactive compounds.

Statistical analysis shows that the viscosity of the beverage on different intervals throughout the shelf-life study period had shown a significant difference ($p \geq 0.05$).

Acidity

As the purchased buttermilk was from a local market and prepared using traditional method, the control sample exhibited a higher level of acidity. The acidity of the enriched buttermilk remained stable, as the values were consistent, as shown in Figure 1(a). The enriched buttermilk was stored under refrigerated conditions, which helped maintain a stable acidity level throughout the shelf-life study.

Statistical analysis shows that the acidity of the beverage at various intervals during the shelf-life study period showed no significant differences ($p \geq 0.05$).

Total phenolic content

The total phenolic content of the beverage was determined using the same procedure employed for the encapsulated extract. In addition, the sample taken from the beverage was first deproteinized according to the method described by Kuhnen et al. (2014) as proteins may interfere with the evaluation of total phenolic content.The values obtained during the shelf-life study were found to be similar as illustrated in Figure 1(b). The trend of TPC throughout the study can be interpreted as stable although a very slight variation is evident as shown in Figure 1(b). This indicates that the phenolic content present in the enriched buttermilk didnot undergo degradation. In the study conducted by Muhammad et al. (2019), the inclusion of colloidal cinnamon nanoparticles enhanced the antioxidant activity and total phenolic content of the chocolate beverages made with both types of cocoa powder.

Antioxidant activity: $DPPH^{\cdot}$ scavenging inhibition assay

The supernatant obtained after deproteinization was used to assess the antioxidant activity of the beverage.

Zokti et al. (2016) investigated the impact of spray-dried green tea catechin extract microparticles on the antioxidant activity of mango beverages. They found that the encapsulated catechin components were more stable in the supplemented mango drinks than in the non-encapsulated catechin powder. Similarly, the enriched buttermilk demonstrated potential antioxidant activity throughout the shelf-life study period, as depicted in Figure 1(a).

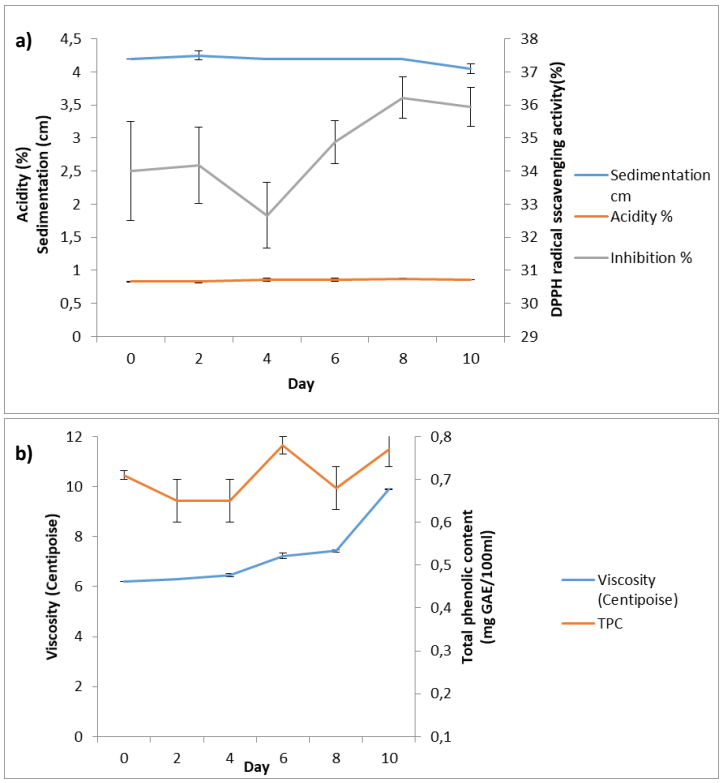

Figure 1. a) line graph showing trends of acidity, sedimentation, and inhibition (DPPH) during storage study, b) line graph showing trends of viscosity and TPC during storage study.

A strong correlation was found between the phenolic content and the antioxidant activity in the study done by Arriola et al. (2019) which is relevant to this context. The TPC which remained stable, supports the antioxidant activity observed in the enriched buttermilk during the shelf-life study.

Conclusions

In the present study, attempts were carried out to encapsulate the bioactive compounds present in the banana pseudostem extract of the *Palayankodan* cultivar (*Musa × paradisiaca Mysore AAB group*). The study also evaluated the phytochemical and functional properties of both the extract and its encapsulated form. Additionally, an enriched beverage was developed, and its shelf-life was assessed. The ethanolic extract of the pseudostem was found to be an excellent source of polyphenols. Results indicated that the pseudostem extract exhibited significant antioxidant as well as anti-diabetic activities. In order to protect the bioactive components in the banana pseudostem extract from various degradation reactions, encapsulation was carried out using sodium alginate as the wall material through high-speed homogenization, with ratios standardized at 1:1 and 1:2. The phenolic as well as flavonoid contents, were found to be higher in the feed than in the encapsulated extract, suggesting that the bioactive components may be trapped within the wall material. The antioxidant assays of the

standardized encapsulated extracts demonstrated inhibition percentages of 32.09±1.48% and 38.92±4.14% per mL. A concentration of 1mg/mL of the 1:1 ratio encapsulated extract showed an inhibiton percentage of 33.16±0.23%. The ethanolic extract from the banana pseudostem displayed a high astringent taste, rendering it unacceptable to consumers. This undesirable astringent taste was masked by the incorporation in spiced buttermilk, resulting in an enriched beverage. The beverage was prepared in different ratios of the encapsulated extract and spiced buttermilk. Optimization was done based on sensory characteristics and phytochemical evaluation, resulting in the selection of the ratio 1:2 (encapsulated extract: spiced buttermilk). Shelf-life study of the selected beverage was conducted over a period of 10 days under refrigerated conditions. The shelf-life study indicated that the product was acceptable throughout the study period.

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