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Improving Nutritional and Physicochemical Characteristics of Yoghurt with Arrowroot (*Maranta arundinacea*) Powder Incorporation

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ABSTRACT: This study aimed to develop standardized parameters for yoghurt fortified with arrowroot powder and assess its chemical, microbiological, sensory characteristics, and shelf life. The yoghurt sample incorporated with arrowroot powder demonstrated comparable sensory properties to the control yoghurt, with improvements in flavor, body and texture, and color and appearance. The addition of arrowroot powder increased the crude fiber content of the yoghurt, potentially acting as a prebiotic substrate for the probiotics. The yoghurt exhibited slightly lower titrable acidity and higher water-holding capacity compared to the control, indicating improved texture and quality. Throughout the storage period, the arrowroot-incorporated yoghurt maintained its sensory acceptability and water holding capacity than the control and the product was found to keep well for 10 days.

Keywords: Yoghurt, Arrowroot powder, Prebiotics, Shelf life.

INTRODUCTION

The global functional foods market is experiencing a rapid surge in popularity, driven by consumers who are increasingly seeking food products that offer both taste and health advantages (Karabagias *et al.*, 2018). These functional foods have garnered considerable interest for their potential to prevent various health issues, enhance physiological functions, and offer superior nutritional value (Huang *et al.*, 2022; Qasim *et al.*, 2021). The nutritional and therapeutic functionalities of dairy formulations supplemented with functional ingredients like probiotics and prebiotics are leading to a growing acceptance of these products worldwide (Balthazar 2019). Yoghurt, a popular fermented milk product, is produced by combining pasteurized milk with specific lactic acid bacteria such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* in controlled conditions (Rashwan *et al*., 2022; Wijesekara *et al*., 2022). It is a highly nutritious food that provides essential micro and macronutrients, contributing to better gut health, daily energy intake, and enhancing protein and lipid digestibility. The beneficial effects of yoghurt can be attributed to the metabolic activities of the starter cultures, which can be further enhanced through co-culturing with probiotics (Kennas, 2020).

Probiotics are live microbial preparations that are added to food and have been shown to provide various health advantages in humans, such as enhancing digestion and promoting intestinal hygiene (Fuller, 1991). In 2014, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill *et al.*, 2014). *Lactobacilli* and *Bifidobacteria* are the most common probiotic strains found in over 90% of probiotic products, making them widely available to health-conscious consumers (Sah *et al*., 2016). Probiotics offer a wide range of advantageous effects on human health, including managing diarrhea, preventing certain types of cancer, alleviating symptoms of irritable bowel syndrome, reducing cholesterol levels, and treating inflammatory bowel diseases like ulcerative colitis and Crohn's disease (Kumar *et al*., 2010; Guandalini *et al.*, 2011; Moayyedi *et al*., 2008; Ooi *et al*., 2010; Sheil *et al*., 2007). Furthermore, several recent studies have demonstrated that adding prebiotic ingredients to probiotic yoghurt can enhance the survival of these microorganisms. Prebiotics, such as fructooligosaccharides and inulin, are the components that induce specific changes in the composition and/or activity of the gastrointestinal microbiota, primarily *Lactobacillus* spp. and *Bifidobacterium* spp. resulting in beneficial effects on host well-being and health (Abesinghe *et al*., 2012; Pendyala *et al*., 2012; Hemarajata and Versalovic 2013).

Arrowroot (*Maranta arundinacea*) is an herbaceous plant with rhizomes that are known for their high content of fructooligosaccharides, which have the potential to exhibit prebiotic properties (Abesinghe *et al*., 2012). The starch extracted from arrowroot is recognized for its easy digestibility and remarkable gelling properties making it suitable for addition to yoghurt (Reddy 2015; Kay 1973). It is valued for its ability to enhance the texture and consistency of food products. Furthermore, arrowroot has been explored for potential therapeutic purposes due to its beneficial properties (Tarique *et al*., 2021). Hence, Arrowroot starch, when added to yoghurt, not only serves as a prebiotic source, but also acts as a natural stabilizer and texturizing agent, enhancing the microstructure, color, and texture of the final product (Buchilina 2021; Mohamed Ahmed *et al*., 2021; Mohammadi-Gouraji *et al*., 2019).

The primary objective of the present research is to establish the standardized parameters for probiotic yoghurt fortified with arrowroot powder. Moreover, the study aims to examine the chemical and microbiological characteristics of the resulting product, and assess its shelf life to ensure its quality and safety throughout its storage period.

MATERIAL AND METHODS

The current research was conducted in the Dairy microbiology laboratory, College of Dairy Science and Technology Pookode, Wayanad. Toned, Homogenized Milk (Milma), arrowroot powder, and sugar for the study were procured from the local market.

Preparation of cultures. Probiotic cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidium* used in this study were procured from Varghese Kurien Institute of Dairy and Food Technology, Thrissur, Kerala, and yoghurt culture was obtained from Department of Dairy Microbiology, College of Dairy Science and Technology, Wayanad, Kerala. The Cultures were activated in 10% skimmed milk suspension, incubated at 37-38°C for 6 hrs.

Preparation of yoghurt. Three replicate trials were conducted in the manufacturing of yoghurt. There were three treatments of probiotic yoghurt with 0.5%, 1.5%, and 2.5% arrowroot powder. Based on the sensory trials with 9point hedonic scale, yoghurt added with 1.5% arrowroot powder was selected for the study. Homogenized, toned milk was heated to 45°C and added with arrowroot powder followed by sugar addition @ 8% to enhance its sweetness. The yoghurt mixture was heated at 90°C for 5 minutes. After cooling to 42°C, milk was inoculated with an active culture combination comprising yoghurt starter culture (ST and LB at the ratio of 1:1) @ 1% and probiotic culture @1% v/v of final milk volume. The inoculated mix was dispensed in 80ml polyethylene cups and incubated for 4 hours at 42° C. Then the samples were stored at 4 °C and withdrawn on the 0^{th}, 5^{th}, 10^{th}, and 15^{th} day of storage for quality evaluation.

Analysis of yoghurt.

Sensory evaluation. Sensory evaluation of the samples were carried out by a panel of 5 semi-trained judges. The parameters of the study were the color and appearance, body and texture, flavor, acidity, and overall acceptability. The sensory evaluation was carried out based on a 9-point Hedonic scale in which a score of 1 represented 'dislike extremely' and a score of 9 represented 'like extremely'. The samples for analysis were presented before the judges after suitable marking. The judges were provided with a room with good lighting and appropriate facilities. The optimum product selection from all the treatments was done based on the sensory scores obtained.

Proximate composition analysis. Probiotic yoghurt, incorporated with arrowroot powder was subjected to proximate analysis. The samples' total solids and fat content were determined as per IS: 1479 (part-II), 1961.The fat content was determined by using the standard Gerber method as per IS: 1224 (part-I), 1977, and the acidity was estimated according to IS: 1479, (part–I), 1960.

Water holding capacity. The water-holding capacity of yoghurt was determined according to the procedure reported by Guzmán-González *et al*. (1999). A sample of approximately 20g of yoghurt (Y) was centrifuged at 1250 x g for 10 minutes at 4 °C. The expelled whey (W) was subsequently removed and weighed.

The water holding capacity (WHC) % was calculated as,

$$WHC(\%) = \frac{Y - W}{Y} \times 100$$

Where, Y= Weight of Yoghurt; W= weight of expelled whey

Crude fiber. The determination of crude fiber was conducted following the method outlined by Mbaeyi-Nwaoha *et al*. (2017). It involved the use of standard sulfuric acid and sodium hydroxide for digestion. 2g sample was hydrolyzed in a beaker with 299 mL of 1.25% sulfuric acid and boiled for 30 minutes. The resulting mixture was filtered under a vacuum, and the residue was washed three times with hot distilled water. Subsequently, it was boiled again for 30 minutes with 200 mL of 1.25% sodium hydroxide and filtered once more. The digested sample was washed with hydrochloric acid to neutralize the sodium hydroxide, followed by three additional washes with hot distilled water. The residue was then transferred to a crucible and dried in an oven at 100°C for 2 hours. After cooling in a desiccator, the sample was weighed. The crucible with the sample was incinerated at 500°C for 5 hours until all carbonaceous matter was completely burnt. Finally, the crucible containing the resulting ash was cooled in the desiccator and weighed.

The calculation of the crude fiber percentage was performed using the following formula:

$$\text{Crude Fibre (on moisture basis) \% by mass} = \frac{100(M_1 - M_2)}{W} \times 100$$

Where, M_1 = Mass (g) of the digested sample and crucible before ash; M_2 = Mass (g) of crucible and ash; W = Mass (g) of sample use

Microbial analysis. Throughout the storage period, the coliform count, and yeast and mold count were periodically assessed. The enumeration was done using the pour plate technique using the guidelines provided in IS: 1224, 1981.

RESULTS AND DISCUSSION

Sensory analysis. The sensory properties of the product were comparable to the control yoghurt in terms of color and appearance and could be attributed to the similar visual appeal of both products. There were slight improvements in flavor, body and texture, and color and appearance for the product, but these differences were not significant. The high overall acceptability score of 9 for both yoghurt types indicated that the panelists found the taste, texture, and visual appeal of the products to be satisfying and enjoyable.

Arrowroot starch, with its neutral taste and thickening properties, enhances the body and texture of yoghurt. Its white appearance and lack of odor make it versatile for improving overall acceptability (Malinis and Pacardo 2012; Nogueira *et al*., 2021). Combining arrowroot starch with probiotics creates yoghurt that matches or surpasses the sensory qualities of natural yoghurt. Probiotics add nutritional value and potential health benefits, while arrowroot starch enhances texture. This synergy produces a satisfying yoghurt experience, showcasing the potential for enhanced sensory appeal in yoghurt products.

Physico-chemical analysis. Arrowroot tuber has been found to contain a significant amount of dietary fiber. The starch derived from arrowroot flour consists of 8.7% insoluble dietary fiber and 5.0% soluble dietary fiber, indicating its potential as a prebiotic ingredient (Harmayani *et al.,* 2011). Moreover, recent studies have suggested that arrowroot flour possesses prebiotic properties. Also, the crude fibre content of arrowroot powder is found to be 3.96% (Chit, 2016). Comparing the results from Table 2, it is evident that the control yoghurt lacked fiber, whereas the product was enriched with crude fiber sourced from arrowroot. This fiber content in arrowroot effectively transferred to the yoghurt, potentially acting as a prebiotic substrate for the probiotics present in the yoghurt. These findings highlight the potential of arrowroot as a dietary fiber source and its ability to enhance the fiber content of yoghurt, thereby providing additional health benefits and supporting the growth of beneficial gut bacteria.

The titratable acidity of the product was measured at 0.65±0.003, while the control yoghurt exhibited a slightly higher titrable acidity of 0.71±0.003. This observation aligns with the findings of Abesinghe *et al.* (2012), who reported that probiotic yoghurts supplemented with arrowroot carbohydrates tend to have higher pH levels and lower titratable acidity.

In the present study, the product demonstrated a slightly higher WHC (89.06±0.01) compared to the control sample (88.25±0.12). This can be attributed to the unique properties of arrowroot starch, which is commonly used as a thickener in various food products like puddings, sauces, cookies, and baked goods (Otegbayo, 2014; Rodrigues *et al*., 2018). The high digestibility of arrowroot starch, along with its ability to form a stable gel and provide structure to starch binders, contributes to its effectiveness as a water-binding ingredient (Kumalasari *et al*., 2012). These characteristics make arrowroot starch a desirable choice for improving water retention and texture in yoghurt formulations, ultimately enhancing the overall quality of the product.

Shelf life analysis.

Changes in sensory score on refrigerated storage (7±1°C). On the initial day of analysis (0^{th} day), arrowroot incorporated yoghurt and the control sample secureda commendable score of 9 for all sensory attributes, with the former achieving a higher score. As the evaluation progressed to the 5th day, the product exhibited slight improvements in scores offlavor, body and texture, color and appearance, and overall acceptability compared to the control sample. However, the overall acceptability of the product decreased in comparison to the initial day. Despite this, the panelists still regarded the product as acceptable, rating it an 8 for all sensory attributes. Upon reaching the 10^{th} day of analysis, it was observed that the product exhibited slightly better flavor, body, and texture, and overall acceptability when compared to the control sample. However, there was a slight decrease in the overall acceptability of the product in comparison to the evaluation conducted on the 5th day. During the 15^{th} day assessment, significant variations were noted in the flavor, body, and texture of the product. However, the color and appearance, as well as the overall acceptability, remained consistent compared to the sensory analysis conducted on the 10th day. Overall, despite the insignificant difference, the product consistently obtained higher sensory scores compared to the control yoghurt throughout the entire storage period.

The consistently higher sensory scores obtained by the sample yoghurt compared to the control can be attributed to the potential improvements in sensory quality of the product. Value addition through the inclusion of beneficial components should not have a negative impact on the sensory and nutritional quality of the conventional product (Bourn and Prescott 2002). The product should either demonstrate superior sensory quality or maintain the same quality as the original conventional product. he higher sensory scores obtained by the arrowroot-incorporated yoghurt in terms of flavor indicate that arrowroot powder, being a tasteless and odorless substance, does not have a detrimental impact on the taste of yoghurt (Kumalasari *et al*., 2012).

Arrowroot starch, with its gelling and thickening properties, can be used as a stabilizer alternative in food. It contains around 40% amylose (Sandoval Gordillo *et al*., 2014), which undergoes gelatinization, increasing viscosity and forming a gel during storage. This gel formation, attributed to the aggregation of gelatinized starch molecules, creates a rigid network that holds water and provides strength (Tabilo-Munizaga *et al*., 2005; De Vries, 2009). The product consistently achieved the highest scores for body and texture, indicating that the inclusion of arrowroot starch helped reduce syneresis and contribute to a firm texture throughout the storage period.

During storage, both the control and product exhibited a significant decrease in color and appearance values, suggesting that chemical reactions occurring in yoghurt contribute to a decrease in lightness (white color), resulting in reduced acceptance (Jańczuk *et al*., 2023). Similarly, as the storage period progressed, all sensory attributes such as flavor, color and appearance, and body and texture were affected, leading to a decline in the overall acceptability of the yoghurt.

Changes in physicochemical properties on refrigerated storage (7±1°C).

Titratable acidity. The titratable acidity of the arrowroot-incorporated yoghurt and the control sample were analyzed over a period of 15 days (Fig. 2). On the 0th day, the product had a lower titratable acidity compared to the control, indicating a slightly lower acidity level in the product yoghurt. As the storage period progressed, significant changes were observed. On the 5th day, the titratable acidity increased for both the product and control yoghurts, with the former having a slightly higher acidity level. On the 10^{th} day, a similar trend of acidity increase was noticed, with the product displaying higher levels of acidity. Finally, on the 15^{th} day, the titratable acidity was similar for both the product and control yoghurts, but the control yoghurt exhibited a higher acidity level.

The increase in acidity in the control yoghurt was attributed to the action of starter cultures, which converted lactose into lactic acid. In contrast, the product showed a slower yet steady increase in acidity. During storage, the starch in yoghurt might have undergone breakdown into smaller molecules, which were subsequently metabolized by the yoghurt bacteria, leading to the production of acids (Menzel *et al*., 2014; Saccaro *et al*., 2009). The development of acidity is important for yoghurt production as it affects syneresis, body, and texture, with the arrowroot-incorporated yoghurt displaying reduced characteristics. Similar results have been reported by Singh and Byar (2009) as well as MwizERwA *et al*. (2017) in yoghurt incorporated with starch.

Water holding capacity (WHC). The water-holding capacity (WHC) of both the product and the control sample was evaluated during a 15-day period (Fig. 2). On the 0th day, the sample had a WHC of 89±0.01, slightly higher than the control at 88±0.01. As the storage progressed, both samples experienced a gradual decrease in their WHC. On the 5^{th} day, the product exhibited a WHC of 88±0.01, compared to 87±0.01 for the control. By the 10th day, the WHC of the product had slightly increased to 91.43±0.72, surpassing the control at 90±0.02. Finally, on the 15th day, the product displayed a WHC of 95.2±0.15, while the control remained at 91±0.12.

The results suggest that the arrowroot-incorporated yoghurt consistently maintained a slightly higher WHC throughout the storage period compared to the control, indicating improved moisture retention in the sample yoghurt.

Water holding capacity (WHC) refers to the ability of a substance to absorb and retain water (Ramasamy, 2014). In the case of yoghurt, WHC tends to decrease over time due to factors such as increased acidity and syneresis (Sakandar *et al*., 2014). However, in this study, the product higher WHC compared to the control. This finding is consistent with the research conducted by Shaheryar *et al*. (2023), which also reported the highest WHC in yoghurt supplemented with starch. The enhanced water retention in the starch-incorporated yoghurt can be attributed to the swelling power of starch, which enables it to effectively hold water (Kaur *et al*., 2011).

Shelf life studies based on Microbial Analysis. The microbial analysis conducted during the shelf life study is expressed in $\log_{10}$CFU/gin Table 4. The analysis on 0^{th} day indicated that no coliform, yeast or molds were present in both product and the control. The same results were observed on the 5^{th} day. However, on the 10^{th} day of storage, the yeast and mold count of the product was found to be higher for the product than for control but maintained to stay within the standard limits. Despite the absence of coliforms in both product and control, the yeast and mold count surpassed the acceptable limit on the 15^{th} day. Hence the product was rejected on 15^{th} day, highlighting the shelf life limit of the product as the 10^{th} day.

Table 1: Sensory analysis of sample and control yoghurt.

Attributes	Sample	Control	F value
Flavor	9.09±0.03	9.02±0.02	0.32^{ns}
Body & Texture	9.01 ± 0.01	9.0±0.01	0.37^{ns}
Colour& appearance	9.06±0.03	9.03±0.03	2.5^{ns}
Over all acceptability	9.04±0.01	9.03±0.02	0.09^{ns}

Figures are mean ± standard error of three replications, ns-non significant (p>0.01)

Table 2: Physico-Chemical Analysis of sample and control yoghurt.

Parameters	Sample	Control	F value
Fibre	3.23±0.1	0.00±0.00	482.2**
Total solids	20.00±0.05	18.93±0.03	271.4**
Titrable Acidity (% Lactic acid)	0.65 ±0.003	0.71±0.003	11.52*
WHC	89.06±0.01	88.25±0.12	39.7**

Figures are mean ± standard error of three replications, ns-non significant (p>0.01)

Table 3: Changes in sensory scores during refrigerated storage (7±1°C).

Attributes	Sample	Days of Storage			
		0	**5**	**10**	**15**
Flavor	Sample	9.09 ± 0.03^{a}	8.54±0.02^{ab}	8.20±0.06^{ab}	7.59±0.2^{b}
	Control	9.02 ± 0.03^{a}	8.53±0.02^{ab}	8.12±0.06^{ab}	7.5±0.2^{b}
	Z value	0.13^{ns}	0.128^{ns}	0.96^{ns}	0.02^{ns}
Color and appearance	Sample	9.06 ± 0.03^{a}	8.54±0.02^{ab}	7.8±0.05^{b}	7.89±0.05^{b}
	Control	9.02 ± 0.01^{a}	8.53±0.02^{ab}	7.71±0.01^{b}	7.87±0.01^{b}
	Z value	2.5^{ns}	0.128^{ns}	0.016^{ns}	0.016^{ns}
Body and Texture	Sample	9.11 ± 0.01^{a}	8.91±0.01^{ab}	8.59±0.05^{b}	7.9±0.2^{b}
	Control	8.66 ± 0.03^{a}	8.22±0.01^{a}	7.87±0.01^{b}	7.2±0.2^{b}
	Z value	1.65**	24.7**	0.015^{ns}	0.012^{ns}
Overall Acceptability	Sample	9.04 ± 0.01^{a}	8.53±0.01^{ab}	7.97±0.01^{b}	7.54±0.02^{b}
	Control	8.96±0.03^{a}	8.46±0.02^{ab}	7.92±0.01^{b}	7.51±0.05^{b}
	Z value	1.2^{ns}	5.25^{ns}	4.97^{ns}	1.81^{ns}

Figures are mean ± standard error of three replications, **-Significant at one per cent level ($p<0.01$), a-b - Means with different superscript vary significantly within a column

Table 4: Changes in microbial quality during refrigerated storage (7±1°C).

Days	Change in yeast and mould count (log10cfu/g)			
	0	**5**	**10**	**15**
Sample	Nil	Nil	2	2.60
Control	Nil	Nil	2	2.47
	CHANGE IN COLIFORM COUNT (log10cfu/g)			
Sample	Nil	Nil	Nil	Nil
Control	Nil	Nil	Nil	Nil

Fig. 1. Arrow root incorporated probiotic yoghurt.

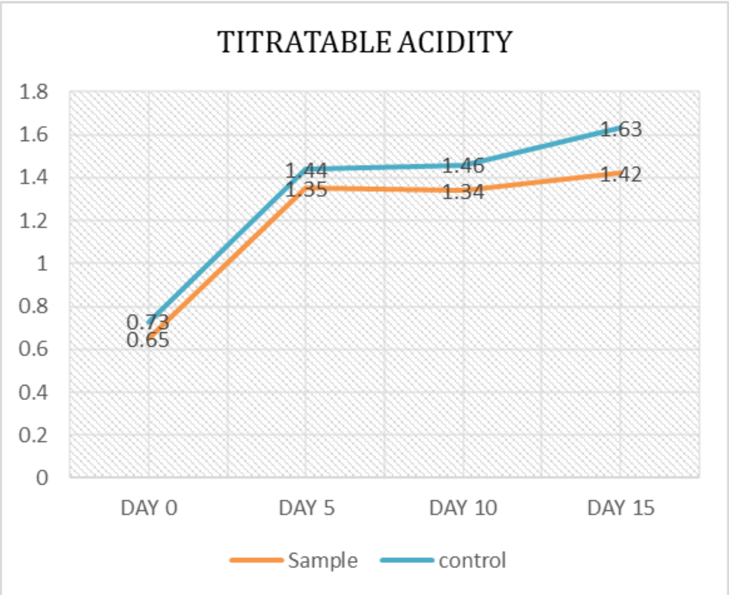

Fig. 2. Changes in Titratable acidity during storage (7±1°C).

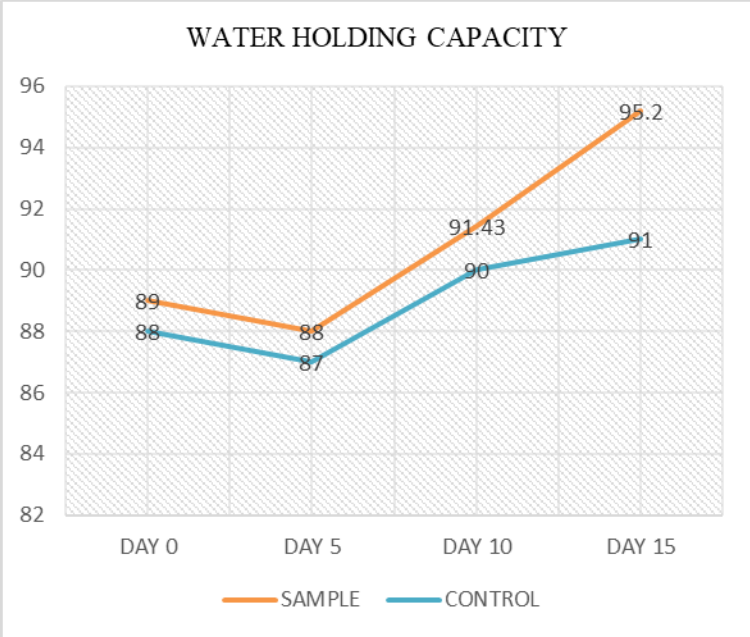

Fig. 3. Changes in Water holding capacity during storage (7±1°C).

On the initial days of storage period, there were no yeast and mold detected in either the product or control. However, as the storage progressed, the counts increased beyond the permissible limits set by FSSAI (2022), which specify a maximum of 100 per gram. In contrast, throughout the entire storage duration, both the control and product consistently showed the absence of coliforms, indicating that the product was prepared following hygienic standards. An increased presence of yeast and mold was detected in the arrowroot powder incorporated yoghurt after 15 days, potentially due to the presence of additional nutrients like soluble fibre and starch.

CONCLUSIONS

In conclusion, the addition of arrowroot powder to probiotic yoghurt resulted in a product with enhanced sensory properties, including improved flavor, body and texture, and overall acceptability compared to the control yoghurt. Arrowroot starch acted as a natural stabilizer and texturizing agent, contributing to the microstructure, color, and texture of the final product. Moreover, arrowroot powder enriched the yoghurt with dietary fiber, potentially acting as a prebiotic substrate for the probiotics that may be present, thus offering additional health benefits. The arrowroot-incorporated yoghurt also exhibited a slightly higher water-holding capacity and comparable titratable acidity to the control yoghurt. Throughout the storage period, the arrowroot-incorporated yoghurt maintained its sensory quality, with consistently higher scores compared to the control. Thus, arrowroot can be considered as a valuable ingredient for incorporation in production of functional yoghurt, offering both enhanced taste and additional nutritional values.

FUTURE SCOPE

Future prospects of this work can include the critical aspect of enumerating and validating the probiotic count in arrowroot-enhanced probiotic yoghurt. Accurate enumeration ensures that the product's desired level of probiotics is present, providing the intended health benefits to consumers. Conducting thorough consumer acceptance studies and market research will provide valuable insights into consumer preferences and enable the development of targeted marketing strategies. Overall, this research not only leads to innovation in the dairy industry but also presents opportunities for the creation of healthier and more diverse probiotic yoghurt products that cater to consumer demands.

Author contributions. All authors contributed substantially to the successful completion of the work. Archana S - Investigation, Data collection and Draft preparation; Muhsina A- Investigation and data collection; Archana Chandran - Conceptualisation, supervision, review and editing; Rahila MP, Grace Thachil, Ankitha Anto - Review and editing.

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