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Effect of α-D-glucopyranosyl-[β-D-fructo-furanosyl] (n-1)-D-fructose-furanosides and Sodium Alginate coated Caesalpinia Bonducella Seed Coat Extract in Developed Yogurt

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Abstract
Encapsulation technology is an emerging technology to enhance bioavailability, hydrophilicity and endurability of the food product. The objective of this study was to evaluate the effects of adding Caesalpinia bonducella seed coat aqueous extract in both encapsulated and non-encapsulated forms to the herbal yoghurt. For extraction, ultrasonication was performed, followed by encapsulation with a spray drier. Spray-drying was done (at optimal outlet- 80°C inlet- and 160°C temperatures) to encapsulate the aqueous seed coat extracts. Sodium alginate and α-D-glucopyranosyl-[β-D-fructo-furanosyl] (n-1)-D-fructo-furanosides were cross-linked in a 1:2 ratio as the coating substance. The pH, Titrable acidity, Synersis, culture viability, antioxidant activity, antiradical activity, total phenolic content, texture and sensory evaluations were done for the optimization. With increasing amounts of both encapsulated and non-encapsulated extract, the total polyphenolic contents, and antiradical activity increased proportionately. When different herbal yoghurt samples were contrasted, it was found that the one with 2% encapsulated powder (3% fat) had the best sensory profile and texture. In comparison, the herbal yoghurt sample with 2% aqueous extract (3% fat) had the highest phenolic content 25.08 mg GA eq./g and Antioxidant percentages in terms of DPPH, ABTS, and SOSA-37.79%, 31.43%, and 38.02%, respectively. The Caesalpinia bonducella seed coat extract in capsule form can be useful as a natural food component for making herbal yoghurt.
Keywords: Herbal Yoghurt, Caesalpinia bonducella, Antioxidant, Extraction, Encapsulation

INTRODUCTION

Recently, meals with biologically active ingredients have caught the attention of scientists. Functional foods, which provide physiological or metabolic health advantages in addition to their fundamental nutritional value, are increasingly popular around the world (Granato et al., 2020; Alongi & Anese, 2021). These days consumers better understand the link between eating habits and nutritional status. As a result, people seek food products with natural ingredients rather than artificial chemical additives (Saraiva et al., 2020). The urgent need to increase food production has resulted from the need to feed a growing global population. This increased output must be more affordable while maintaining high standards of quality. A food must be kept for the longest possible duration in terms of taste, appearance, texture, and microbiological safety (Giller et al., 2021; Díaz et al., 2023). As compared to the original ingredients, food quality in terms of nutrition and biochemistry may change during the fermentation process. Comparing fermented foods to plain foods, customers can benefit from the antioxidants, peptide synthesis, organoleptic and probiotic qualities, and antibacterial activity that these foods possess. Additionally, it reduces pollution and anti-nutrient levels. Based on the production method, storage circumstances, and durability, different microbial communities can be found in different amounts and different quality in fermented foods (Sharma, et al., 2020). One of the most popular and widely regarded probiotic products made from fermented milk is yogurt. It is recognized as a very beneficial food product because of the symbiotic link between the cultures of Lactobacillus bulgaricus and Streptococcus thermophiles. Yogurt is used to provide probiotics and prebiotics to the consumer and is primarily regarded as a health product. However, it appears that there is a dearth of studies containing important data on global regulatory frameworks, the findings of therapeutic trials, and methodological strategies for counting multiprobiotic bacteria in Yoghurt (Nyanzi, et al., 2021). This association claims to provide a variety of health advantages along with improvements in nutritional value, such as a reduction in fat content, hiding the textural effects of the addition of proteins, polysaccharides, or probiotic microorganisms to provide specific nutrition for the microbiota of gut. It also has a large customer base to support it (Vecchio et al., 2016). Depending on the ingredients used and how they affect the product's texture, water-holding capacity, and other sensory qualities, the functional and nutritional qualities will vary. Due to its excellent taste, low cost, and all-natural ingredients in a single, readily accessible product, yogurt has a high level of public interest. Yogurt is a dairy product produced by fermenting milk with lactic acid bacteria. Numerous studies show that adding these ingredients raises the quantity of antioxidant, polyphenols, flavonoids, and antibacterial properties of the yogurt (Kang et al., 2018; Lim, 2018). In addition to promoting gut health, Yoghurt is a useful treatment for gastrointestinal disorders such as lactose intolerance, constipation, bowel illness, and diarrhea. Yogurt eating has also been linked to a lower incidence of vaginal yeast infection in diabetic women by regulating pH and stifling Candida overgrowth (Ghosh 2019). Yogurt has been found to have both therapeutic and preventative effects on several conditions, including cancer, infections, gastrointestinal problems, asthma, and antibiotic-associated diarrhea (Nazir et al., 2018). Numerous phytoactive chemicals with therapeutic characteristics are present in the herbal extract, but their direct addition to the food product may alter its texture, flavor, and other attributes. To sustain the activity of these actives, encapsulation methods are used for entrapment (Kong, et al., 2022). To stabilize, safeguard, and preserve foods, active chemicals are added as entrapped particles (Talón et al., 2019; Tavares et al., 2021). Using an encapsulating technique through entrapment and preservation, competent transportation of bioactive chemicals is carried out. In the food industry, several uses for extracts containing bioactive substances with beneficial nutritional, practical, and therapeutic qualities have emerged. Functional yoghurts have been claimed to contain micronutrients such as iron, zinc, iodine, and vitamin A in addition to vitamin D, acacia fiber, sweet pumpkin, MACA (Lepidium meyenis) hot water extract, black garlic extract, red ginseng extract, and spice oleoresins (Kang et al., 2018; Lim, 2018; Hong et al., 2021). The purpose of this study is to develop herbal yogurt incorporated with optimized encapsulated C. bonducella seed coat extract powder (ECBSCP) and C. bonducella seed coat extract (CBSCE). In this study, the encapsulation technology works as a taste reducer for seed coat extract, and texture enhancer, and increased the availability of the phytoactives and supplementary nutritive possessions of the herbal extracts. Sensory analysis and texture analysis have been used to assess the acceptability and quality of herbal yogurt enriched with varying concentrations of optimal ECBSCP and CBSCE. The developed herbal yoghurt was further used to examine the impact of extract additives on the pH, Titrable acidity, synersis, culture viability, antioxidant and antiradical activities, nutritional value, Texture and and sensory evaluations of the herbal yoghurt.

MATERIALS AND METHODS

Materials

From the neighbouring BHU market in Varanasi, Uttar Pradesh, the two fat percentages milk was collected 1.5% and 3.0% (F1 and F2). Sodium alginate and α-D-glucopyranosyl-[β-D-fructo-furanosyl] (n-1)-D-fructo-furanosides were combined in a 1:2 ratio and spray dried to form capsules with the Caesalpinia bonducella seed extract that had been extracted in aqueous phase using ultrasonication. The study used only analyticalgrade chemicals and reagents, all of which were obtained from Sigma Chemicals Co. in St. Louis, Missouri, in the United States. The herbal yoghurt was made by using both fat percentage milk with optimised ECBSCP and CBSCE. The two ratios of ECBSCP and CBSCE were made to develop 10 samples of herbal yoghurt, including the control (3 replicates of each treatment). Control with1.5% fat level (F1C), 1% ECBSCP (F1T1), 2% ECBSCP (F1T2), 1% CBSCE (F1T3), and 2% CBSCE (F2T4) and 3.0% fat level (F2C), 1% ECBSCP (F2T1), 2% ECBSCP (F2T2), 1% CBSCE (F2T3), and 2% CBSCE (F2T4). The manufacture of yoghurt was carried out in the manner depicted in Figure 1.

2.2 pH, Titrable acidity and Synersis

Prior to the analysis, the pH of all the samples were analyzed with the calibrated pH meter that was done with standarized pH buffer solutions of 4.0, 7.0, and 9.0 (Fisher Scientific). A computerized titratable acidity titrator was used to measure the titratable acidity of all the homogenized herbal yoghurt samples. The homogenizarion of the samples was done by taking 9 gram of herbal yoghurt in 40 mL of water (848 Titrino plus, Switzerland). TA was determined by constantly swirling while titrating with 0.1 N NaOH. To express titratable acidity, lactic acid was utilised. According to the process outlined by Aprodu et al., the syneresis (%) of yoghurt samples was measured by centrifugation (2012).

2.3 Culture viability

Samples were examined before and after 30 Days of storage at 4°C in every set of the herbal yoghurt. Using water sterilized with peptone (0.1%), herbal yoghurt samples were serially diluted. Following a 48h aerobic incubation at 37°C, S. thermophiles was counted on M17 agar plates. After being grown anaerobically for 72h at 45°C in a jar with anaerobic gas packets, L. bulgaricus was counted on MRS at pH 5.2 with acetic acid.

2.4 Antioxidant evaluations and Total phenolic content

The Nishino et al. (2000) method was used to measure the antiradical activity against DPPH with only minor changes to sample preparation and reaction volume. With a few minor adjustments, the radical scavenging activity in terms of ABTS was analyzed (Re et al., 1999)). With just minor modifications, the Liu et al. (1997) method was used to analyse the superoxide anion scavenging activity (SOSA). The Folin-Ciocalteu reagent and gallic acid was used as a standard, the total phenolic components of the extracts were analyzed by applying the method of Druckerei, 2002.

Textural analysis

The texture profile analyser (TA-XT micro-system Plus, U.K.) was used to examine the textural properties including hardness, adhesion, springiness, cohesion, and chewiness. An extension bar and the disc back extrusion probe of (A/BE) were used in conjunction with a 5 kg load cell. The height and width of the tests were 30 mm with back extrusion containers (50 mm). The probe applied a compressive force to the product (herbal yoghurt) up to a distance of 20 mm on either side. Pre-test and test speeds as well as trigger force and evaluation times for the texture analyzer are all set to 3 mm/sec, 0.5 mm/sec and post-test, respectively. During texture analysis, roughly 30 g of herbal yoghurt were utilized.

2.6 Sensory evaluations

Different sensory aspects of the yoghurt, such as colour and appearance, flavour, body and texture, mouthfeel, and overall acceptability, were examined. In order to conduct the study, experimental samples and control samples were distributed to a panel of 12 semi-trained adjudicators (aged 25 to 65) from the Department of Dairy Science and Food Technology, IAS, Banaras Hindu University, Varanasi. A nine-point hedonic rating scale, as described by Amerine et al., (1965) was used to conduct sensory evaluation at temperatures between 25 and 30°C.

2.7 Statistical analysis

Utilizing statistical designs, the data collected during the current inquiry was appropriately examined. Preliminary study findings are presented as an average and standard error (Mishra et al., 2015). The C.D., SE(m), as well as the SE(d) were analyzed to compare the means. One-way analysis of variance (ANOVA) was performed to assess for statistical significance.

RESULTS AND DISCUSSION

3.1 pH, Titrable acidity and Synersis

By measuring pH and TA (% of lactic acid), the fermentation characteristics of both herbal yoghurt samples i.e. ECBSCP and CBSCE have been identified. Encapsulated seed coat extract powder supplementation had a minor impact on the initial pH, TA, and synersis, which varied from 4.77-2.90, 0.93 to 0.96, and 21.29-38.28, respectively (Table 1). The fermentation characteristics of herbal yoghurt samples were analysed by measuring pH and titrable acidity (TA). Fermentation ceased at pH 2.90 after 30 days, with a significant reduction in fermentation time to 6 hours for all herbal yoghurts supplemented with encapsulated seed coat extracts compared to 12 hours for plain yogurt, serving as the negative control. All supplemented herbal yoghurts exhibited a more rapid pH decrease and TA increase than plain yogurt, indicating enhanced metabolic activity of yogurt bacteria due to the presence of herbs (Wajs et al., 2023; Joung et al., 2016).

Recent studies have shown that supplementation with plant extracts can enhance the metabolic activities of yoghurt starter cultures, leading to shorter fermentation times (Dabija et al., 2018; Joung et al., 2016; Jung et al., 2016). Moreover, the viability of S. thermophilus and L. delbrueckii subsp. bulgaricus during fermentation was significantly improved in all yogurt samples supplemented with plant extracts, with counts increasing by approximately 2.95 log units and 1.14 log units, respectively, from the initial fermentation stage. At the end of fermentation, viable starter cultures exceeded 8.5 Log CFU/mL for S. thermophiles and 5.8 Log CFU/mL for L. delbrueckii subsp. bulgaricus, with significantly higher counts in supplemented yogurts compared to plain yogurt (Amirdivani and Baba, 2011). Syneresis, a critical factor influencing consumer acceptance, varied significantly with supplementation and type of plant extracts. Plain yogurt exhibited greater syneresis than supplemented yogurts, with the best water holding capacity observed in NN yogurts after 28 days of storage at 62.90%. This finding aligns with previous research indicating that the pH decreased while the TA level increased due to acid production in red ginseng extract- and ginseng extract-supplemented yogurt (Jang et al., 2018; Jung et al., 2016). The viscosity of yogurt supplemented with plant extracts tended to be slightly lower than that of plain yogurt, with significant decreases observed in certain concentrations. This is consistent with previous studies suggesting that viscosity is related to the aggregation of casein micelles (Joyce et al., 2017). Furthermore, carthamin yellow from safflower has been reported to decrease blood viscosity parameters in animal studies (Li et al., 2009). supplementation with plant extracts in yoghurt manufacturing has the potential to shorten fermentation time, increase the viability of starter cultures, and improve the physiological characteristics of yogurt samples such as pH, titrable acidity, and syneresis. Further research is warranted to explore the specific mechanisms underlying these effects and optimize the use of plant extracts in yogurt production.

3.2 Culture viability

The culture viability of herbal yoghurt samples supplemented with encapsulated and non-encapsulated seed coat extracts was analysed over a period 30-day, focusing on pH, titrable acidity (TA), and the viability of S. thermophilus and L. bulgaricus. The pH of all herbal yoghurt samples was decreased slowly up to 30 days. Herbal yoghurts supplemented with encapsulated seed coat extracts generally exhibited lower pH values compared to herbal yoghurt samples supplemented with non-encapsulated seed coat extract and plain yoghurt samples. Conversely, TA tended to increase initially and then remained stable until day 30. These results suggest that the addition of encapsulated and non-encapsulated seed coat extracts supported the production of lactic acid by yoghurt starter cultures (Table 2).

The viability of S. thermophilus and L. bulgaricus was significantly influenced by the addition of encapsulated and non-encapsulated seed coat extracts, the type of seed coat extract, and the storage period. S. thermophilus counts tended to increase until day 30 of storage, while the counts of L. bulgaricus showed a significant increase from day 5 through day 30, in all supplemented yoghurts samples. This indicates that S. thermophilus remained viable throughout the storage period. Additionally, yoghurts supplemented with encapsulated and non-encapsulated seed coat extracts showed significantly higher counts of L. bulgaricus compared to plain yogurt throughout the storage period, suggesting improved viability. Further research is needed to elucidate the specific mechanisms underlying these effects and to optimize the use of herbal extracts in yoghurt manufacturing. Similar studies show that the NN yoghurt (yoghurt supplemented with Nelumbo nucifera leaf extract) exhibited the highest counts of L. bulgaricus on day 14. This increase in viability could be attributed to the presence of prebiotics or sodium acetate in plant extracts, as suggested by previous research (Joung et al., 2016; Michael et al., 2010). Joung et al. (2016) and Robinson (2002) reported that S. thermophilus counts in all yoghurts exceeded 8.77 Log CFU/mL on day 28, with no significant difference between yoghurt samples.

3.3 Antioxidant and phenolic contents

The antioxidant and phenolic content of herbal yoghurt samples containing ECBSCP and CBSCE @1% and 2% have displayed in table 3. The herbal yoghurt sample (@3% fat) with 2% CBSCE showed higher antioxidant and phenolic levels as compare to the other samples. Both herbal yoghurts (1.5% and 3.0% fat percentages) with CBSCE had higher levels of total phenolic content and antioxidant activity than the herbal yoghurt with ECBSCP. The high temperature of the spray dryer used to create the powder of the encapsulated extract may be the cause of this; normally, temperatures of more than 60°C diminish the activity of the bioactive components found in herbal extracts. The findings were compared to related study in that the testing of the functional symbiotic yoghurt made from plant wastes (carrot pomace extract), Lactobacillus plantarum, and beads made from Alyssum homolocarpum seed gum (AHSG) and sodium alginate (SA) revealed that the Yoghurt's total phenolic content and DPPH radical scavenging activity were in the range of 16.13-48.30 g GAE/ml (Sharifi et al., 2023). In another study it was determined that both the tulsi yoghurt and the beet-root extract yoghurt samples were superior in terms of the biochemical properties of cow milk curd (Ghosh, 2019). Tavakoli et al. (2018) reported that the yoghurt samples that contain free and encapsulated olive leaf extract had significantly higher antioxidant activity than the control yoghurt. The outcomes were contrasted with those of a related study in which the levels of aronia juice were increased relative to the control, increasing the total polyphenol and flavonoid contents (Nguyen and Hwang 2016).

3.4 Texture profile analysis of Yoghurt

The texture profile analysis (TPA) presented in Table 4 evaluates the textural characteristics of herbal yogurt formulations containing different concentrations of encapsulated C. bonducella seed coat extract (ECBSCP) and non-encapsulated C. bonducella seed coat extract (CBSCE) across two levels of fat content. The data indicates that the firmness of herbal yogurt samples decreases with increasing concentrations of both encapsulated extract and fat levels. This trend suggests that higher concentrations of encapsulated extract and fat lead to softer textures in the yogurt. Notably, the firmness of 3% fat herbal yogurt containing 1% and 2% ECBSCP is significantly lower than that of 1.5% fat herbal yogurt with the same concentrations of ECBSCP. This disparity in firmness underscores the influence of both fat content and encapsulated extract concentration on the texture of the yogurt. Additionally, the firmness of herbal yogurt with CBSCE is observed to be higher compared to that of herbal yogurt with ECBSCP. This difference in firmness could be attributed to the weakening of the protein network, resulting in a reduction in firmness due to the presence of CBSCE. Moreover, other texture properties such as cohesiveness, springiness, gumminess, and chewiness also show alterations after the addition of both encapsulated and free extract. These changes are likely due to the reduced potency of protein-protein and protein-lipid interactions caused by the presence of the extract. The TPA data highlights the complex relationship between encapsulated extract concentrations, fat content, and their effects on the textural attributes of herbal yogurt. Understanding these relationships can aid in optimizing yogurt formulations to achieve desired texture profiles and enhance consumer satisfaction.

These findings align with previous research indicating that supplementation of plant extracts in yoghurt formulations can enhance viscosity and texture, possibly due to improved water holding capacity. For instance, many researchers demonstrated better viscosity and texture in plant extract-supplemented yoghurts compared to plain yogurt (Delikanli-Kiyak and Ozcan, 2023; Ahmad et al., 2022; Joung et al., 2016). Furthermore, a study done by Li et al. (2019) and Yi et al. (2010) have shown that the addition of α-D-glucopyranosyl-[β-D-fructo-furanosyl] (n-1)-D-fructo-furanosides and its hydrolysates at different concentrations can increase the textural properties of yoghurt.

3.5 Sensorial evaluations of Yoghurt

The sensory evaluation data presented in Figure 1a&b provides insights into the sensory attributes of herbal yogurt formulations containing different percentages of encapsulated C. bonducella seed coat powder (ECBSCP) and C. bonducella seed coat extract (CBSCE), along with their effects on various sensory parameters such as color and appearance, flavour, body and texture, mouthfeel, and overall acceptability. The results indicate that herbal yogurt formulations containing 1% and 2% ECBSCP and CBSCE significantly influence sensory parameters compared to control samples. Specifically, these formulations exhibit statistically significant effects on color and appearance, flavour, body and texture, mouthfeel, and overall acceptability.
Color and appearance scores for both 1.5% and 3.0% fat control yogurt samples are comparatively lower (7.58±0.09 and 7.69±0.40, respectively) compared to samples containing free and encapsulated extracts. Additionally, the scores increase with higher concentrations of both encapsulated and non-encapsulated seed extract, suggesting an improvement in visual appeal with increased extract concentration.
Flavour scores for yogurt with 2% extract at both fat levels are significantly lower (5.83±0.79 and 5.43±0.96, respectively) compared to other samples, including the control. However, yogurt with encapsulated extract shows significantly higher flavour scores than yogurt containing free extract. Furthermore, yogurt with 3% fat exhibits higher flavour scores compared to samples with 1.5% fat content.
Body and texture scores for control yogurt samples with 1.5% fat are slightly lower compared to samples with 3.0% fat. The addition of extract at various percentages significantly decreases body and texture scores, with yogurt samples containing 2% encapsulated powder showing higher scores compared to samples containing 1% encapsulated extract at both fat levels.
Mouthfeel scores for yogurt containing free extract at both fat levels are lower than those for encapsulated extract. Specifically, yogurt samples with 2% encapsulated powder at 3.0% fat level demonstrate higher mouthfeel scores.
Overall acceptability is highest for yogurt with 2% encapsulated extract at 3.0% fat, indicating that encapsulation helps enhance the overall sensory appeal of yogurt by minimizing the bitterness of the seed extract. In summary, encapsulation strategies effectively improve the sensory properties of herbal yogurt by minimizing undesirable attributes such as flavour, color, and mouthfeel associated with the extract, ultimately enhancing overall consumer acceptance.
The findings are consistent with previous studies like Nguyen and Hwang (2016) found that yogurt containing 2% Aronia juice had higher taste compared to samples with 1% and 3% Aronia juice, while Tavakoli et al. (2018) reported that yogurt containing olive leaf extract in the form of nanoliposomes had the highest acceptability compared to yogurt with free extract.

CONCLUSION

The antioxidant and total phenolic contents of the herbal yoghurt sample (both fat percentage) containing 2% non-encapsulated seed coat extract (CBSCE) were highest but the sensory acceptance was very less as compared to others. The consumer acceptance score of the 3.0% fat herbal yoghurt with a 2% encapsulated seed coat extract (ECBSCE) was best among all in terms of taste and texture. All the herbal yoghurt samples with 3.0% fat wore better responses as compared to the 1.5% fat yoghurt. Employment of encapsulated C. bonducella seed extract in yoghurts enhanced their textural characteristics. Sensorial evaluation of herbal yoghurts contains encapsulated seed coat extract had an enhanced body, appearance, and smoother texture than yoghurts with non-encapsulated seed coat extract. Although the antioxidant activity and phenolic contents of yoghurt containing non-encapsulated extract are higher. The sensorial evaluations of the 3% fat yoghurt with 2% encapsulated seed coat extract was best among all. Consequently, the present study was dedicated to the incorporation of the encapsulated C. bonducella seed coat extract with remedial properties to facade the bitter taste and enhance the immovability of the herbal extract in yoghurt system.

Conflict of interest
There is no conflict of interest among authors
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