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RESEARCH

Effect of Spray- and Freeze-Dried Microcapsules Containing Probiotics and γ-Aminobutyric Acid on Nutritional, Physicochemical, Textural, Pasting, Rheological, and Microstructural Characteristics of Composite Dough

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Abstract

This study aims to develop probiotics and γ-aminobutyric acid (GABA)-enriched composite dough based on Bengal gram, finger millet, oat flakes, groundnuts, date paste, and honey with the incorporation of spray- and freeze-dried microcapsules containing probiotics and GABA and their techno-functional properties were evaluated. The nutritional and mineral profiles of dough samples did not vary significantly ($p > 0.05$) after adding probiotic-GABA co-encapsulated powders. The enrichment of probiotics and GABA decreased the bulk density while increasing the water absorption and solubility indices with a higher lightness and whiteness index of the composite mix. The dough samples showed a significantly higher probiotics count (> 8.0 log CFU/g) and GABA content (56.5–62.4 mg/g). The FTIR peaks at 1500–1700 cm^{-1} related to amide I and amide II bond vibrations with lower intensities for probiotics-GABA-enriched composite dough indicated deformation in the secondary structure in doughs' protein network with the addition of encapsulated powder. The addition of freeze-dried powder resulted in a discontinuous structure and uneven distribution of protein particles, with large angular voids in dough samples. The inclusion of spray-dried probiotic-GABA powder resulted in better dough properties in terms of improved textural (decrease in adhesiveness, cohesiveness, chewiness, and increase in springiness) and pasting properties (decrease in setback viscosity) and exhibited higher viscoelasticity compared to the freeze-dried powder added samples.

Keywords Composite dough · Probiotics · γ-Aminobutyric acid · Spray drying · Freeze drying · Quality attributes

Introduction

The awareness of consuming healthy and nutritious food products promoted the demand for the development of functional foods (Kumar et al., 2016). The composite flour mix is generally formulated by combining different grain flours of cereals, pseudo-cereals, and legumes to provide balanced nutrients in the daily diet, and this process is more convenient for enriching proteins, minerals, energy, fiber, and vitamins in the formulated product than the foods prepared from a specific grain flour (Hasmadi et al., 2020). Probiotics are living organisms, when subjected in adequate quantity (10^6–10^7 CFU/g or mL of end product), confer health benefits to the host (FAO/WHO, 2006). The probiotics-enriched food products account for 60% of the functional food market, making it the largest growing sector (Misra et al., 2021). Probiotics-enriched products such as beverages, cheese, yogurt, ice cream, mousse, and chocolate soufflé have been developed, but few research literature are available on bakery applications (De Prisco et al., 2017; dos Santos et al., 2019; Frakolaki et al., 2021; Gao et al., 2019; Khorshidi et al., 2021; Malmo et al., 2013; Martins et al., 2018; Misra et al., 2022; Pourjafar et al., 2020; Rodrigues et al., 2012). The growing interest in functional foods imparting physiological benefits has pivoted on supplementing bioactives such

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as antioxidants, vitamins, essential oil, prebiotics, and other natural extracts (Chawda et al., 2017). γ-Aminobutyric acid (GABA), a nutraceutical compound, is a non-proteinogenic amino acid and is also found in natural foods in minute quantities (< 700 nmol/g dry weight), rendering insufficient daily requirement of GABA through intake of raw foods (Dhakal et al., 2012; Oh et al., 2003). GABA-enriched food products easily deliver GABA to the human body. The GABA at a level of 0.3 to 300 mg/kg was found to be effective for reducing systolic blood pressure, whereas the daily oral administration of GABA at 10 and 26.4 mg could be effective for hypertensive patients and preventing neurological disorders, respectively (Kimura et al., 2002; Okada et al., 2000). It may improve gut health and other physiological functions related to the glycemic index by activating the GABA receptors (Hyland & Cryan, 2010). Hence, incorporation of GABA in food products has garnered considerable attention for enrichment in food products (Gramazio et al., 2020).

Co-encapsulation of probiotics and bioactive compounds such as black bean extract, anthocyanin pigments, and apple skin polyphenols by spray drying, freeze drying, and co-extrusion process with their incorporation into food products has been a recent focus to enhance the functional properties of various food products such as cheese, yogurt, milk drink, and strawberry nectar and to protect the probiotics from the adverse conditions during processing and transit through acidic gastrointestinal conditions (Liu et al., 2015; Morsy et al., 2022; Sharma et al., 2022; Shinde et al., 2014; Vasile et al., 2020). Similarly, the objective of this study emphasizing on functional foods enriched with probiotics and bioactive compounds such as GABA via co-encapsulation using spray- and freeze-drying techniques can provide a synergistic effect (Misra et al., 2021). The viability loss of bacteria is attributed to acidic gastric conditions, thermal stress during heating, interaction with the complex food matrices, and environmental stress during storage. GABA was found to be degraded by 88% at 121 °C temperature and pH 8.0 by increasing the treatment time from 15 to 20 min, thus making it a challenging task for its direct incorporation into food formulations (Misra et al., 2022). Encapsulating probiotics, prebiotics, and bioactive compounds such as GABA as a single entity were capable of enhancing the viable cell number, functionality of probiotics, and stability of GABA in gastrointestinal conditions (Apiwattanasiri et al., 2022; Pandey et al., 2021). The application of co-encapsulated probiotics-GABA with their bioactive effects is a novel approach in the functional foods domain. Thus, it would be beneficial if a portion of food with multi-functional activities is developed. Spray drying is a low-cost and commercially viable co-encapsulation technology for the encapsulation of probiotics and provides flexibility in producing good-quality products (Obradović et al., 2022). Freeze drying is a convenient process for enhancing the viability of probiotics due to the low-temperature application and provides a better result than spray drying for the encapsulation of heat-sensitive bioactives (Jouki et al., 2021). However, the time-consuming nature and high running cost limit its commercial application for the encapsulation of probiotics and bioactives.

Probiotic and GABA-enriched foods currently on the market are mainly based on dairy products like yogurt and cheese, so there is also a wide scope for the enrichment of GABA in bakery food products with a suitable selection of co-encapsulation technology along with the encapsulating materials in order to achieve a desired probiotic count in the end product after the intense thermal treatment during the baking process (Arepally et al., 2022). The dough preparation is the first and most important stage in producing multigrain bakery products, which involves intricate physical and biological transformations and plays a crucial role in the quality of the final product (Sciarini et al., 2012). Due to complex baking processing conditions, research on the addition of co-encapsulated probiotics and bioactives to bakery products is still meagre. The possibilities of applying free or encapsulated probiotics to the bakery food matrix are (i) directly added during dough preparation, (ii) onto the surface of the product, and (iii) in creams used for filling the product (Frakolaki et al., 2021). Reid et al. (2007) added freeze-dried probiotic probiotic *L. rhamnosus* cells directly to the dough for the preparation of probiotic biscuits and observed that the encapsulation using whey protein concentrate resulted in 77% viable cells, i.e., 10^7 log CFU/g in dough samples and after baking at 280 °C for 5 min, the final count in the biscuit was found to be 4.5×10^5 CFU/g. The authors concluded that the viability of probiotics in the final product after baking depended on the thermostability of the microcapsules and types of wall matrix used for encapsulation.

Food products with co-encapsulated probiotics and GABA powder can be a promising alternative approach to the natural enrichment of GABA by other processes, such as fermentation or natural synthesis of GABA by probiotic bacteria (Nakamura et al., 2009). The interaction of probiotics and GABA co-encapsulated in exopolysaccharides such as dextran, inulin, and maltodextrin on the dough characteristics have not been reported yet, which may affect the quality of probiotic-GABA-enriched bakery products. Therefore, this study aimed to evaluate the nutritional, physicochemical, pasting, textural, rheological, and microstructural characteristics of composite dough after incorporating spray and freeze-dried co-encapsulated probiotics-GABA microcapsules.

Materials and Methods

Materials

The probiotic strain *Lactococcus lactis* SKL 13 (LL) was procured from the Indian Institute of Technology Delhi (IITD), Delhi, India. Commercially available Bengal gram, finger millet, oat flakes, groundnuts, date paste, and honey were purchased from Technology Market, IIT Kharagpur, West Bengal, India. Food-grade maltodextrin D 20, inulin, and dextran were procured from M/s. Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India. All the reagents and chemicals used in the experiment were of analytical grade.

Preparation of Culture

The stock culture was stored in DeMan, Rogosa, and Sharpe (MRS) Broth at − 80 °C with 15% (v/v) glycerol as a cryopreservative and used as inoculum in further study. The fresh culture was prepared by adding 1% (v/v) inoculum into MRS Broth and kept overnight at 37 °C before use. The strain was grown in the MRS medium until the early stationary phase was achieved, followed by centrifugation at 5000 g for 20 min at 4 °C. After thorough washing with sterile NaCl solution (0.85%, w/v), the harvested cell pellets (2.2 g,̃ 9.2 log CFU/mL) were used for the microencapsulation process on the same day.

Co-Encapsulation by Spray Drying

The probiotic bacteria was microencapsulated according to the method of Pandey and Mishra (2021). The feed solution was prepared by the addition of core material (cell concentrate: 2.2 g/100 mL + GABA: 4.5 g/100 mL) and wall materials (maltodextrin, dextran, and inulin: 8.41, 4.59, and 0.40 g/100 mL, respectively) with a 20.1% (w/v) solid content. The feed solution was kept under magnetic agitation at room temperature and fed into the spray chamber at a feed rate of 2.5 mL min^{-1}. The wall materials were added with sterilized water, followed by a mixing of the core materials (GABA and freshly harvested probiotic cells) properly. The feed solution was then fed into the spray dryer ('Spray Mate,' Jay Instruments & Systems Pvt Ltd., Mumbai, India) at an air inlet temperature of 110 ± 2 °C and the outlet temperature 50 ± 5 °C. The collected spray-dried powder was stored in sterile glass vials and used for further study.

Co-Encapsulation by Freeze Drying

The same solution of core and wall materials prepared for spray drying was freeze-dried in a Lyodel freeze dryer (Model: LYO0555, Make: Delvac, India) at − 40 °C, 1 mbar pressure, and the produced microcapsules were packed in air-tight container for further analysis.

Preparation of Probiotic and GABA-Enriched Composite Dough

The composite dough was formulated based on the preliminary experiments that were conducted using the information from the literature (Bampi et al., 2016; Bhakha et al., 2019; Pereira et al., 2019) and popular knowledge of the ingredients. The dry ingredients were selected as Bengal gram (65 g), finger millet (40 g), oat flakes (30 g), groundnuts (20 g); date paste (50 g), and honey (15 g) were used as binder materials in order to enrich protein and mineral content in the final product. The dry ingredients were individually roasted in the oven under different conditions as finger millet at 110 °C for 30 min, Bengal gram at 180 °C for 10 min, groundnuts at 160 °C for 10 min, and oat flakes at 160 °C for 20 min (Thakur et al., 2022). The grains, after roasting, were cooled, and the grains (finger millet and Bengal gram) were then milled in the abrasive mill (M/s. Annapurna domestic flour mill, Pune, India) for 5–7 min. The flour samples were sieved using a sieve shaker (M/s. Retsch GmbH, Germany) for 15 min, and flours under the sieve size of 250 µm were mixed properly for 1 min. The eight medium-sized dates were finely chopped, incorporated into 100 mL of water and heated at 80–85 °C for 10 min until 85°Brix was achieved. The date paste and honey were added to the dry ingredient mixture, which acted as a binder for the development of the dough. During the mixing of dry ingredients and binder, the spray or freeze-dried co-encapsulated powder (1%, w/w) was incorporated into the dough samples during the mixing of ingredients in order to achieve a probiotic count above 8.0 log CFU/g. All these ingredients were blended properly using a bakery mixture for 15 min to ensure homogeneity. The developed dough was kept in zip-lock plastic covers for resting for 20–30 min at 4 °C and then was allowed 10 min to equilibrate at room temperature prior to further analysis.

Proximate and Mineral Analysis of Composite Dough

Moisture, protein, fat, ash, crude fibre, and total carbohydrate content (by difference) of the dough samples were analyzed by standard AOAC (2005) methods. Mineral contents were evaluated using an atomic absorption spectrometer (AAnalyst 700 Perkin-Elmer). The energy value (kcal/100 g) was calculated as protein × 4.00 + %carbohydrate × 4.00 + %fat × 9.00 (Sade, 2009).

Probiotic Viability

The probiotic count was determined by taking 5 g dough samples and rehydrating in 95 g of (0.85% w/v) sterile saline solution (Rajam et al., 2015). The solution was homogenized aseptically for 10 min, and the samples were kept in an incubator at 37 °C with an agitation speed of 100 rpm for 20 min for the complete release of cells from microcapsules. Cells were diluted in 0.85% NaCl solution, and cell viability was calculated using the standard plate count method and expressed in log CFU/g.

Quantification of GABA

The GABA in the composite dough samples was analyzed as per Kitaoka and Nakano (1969) with some modifications. Briefly, 5 g of dough and 45 mL of phosphate buffer solution (0.1 M, 7 pH) were mixed and then homogenized for 10 min in a high-speed homogenizer (IKA Turrax, Germany). After homogenization, the sample was centrifuged, and the supernatant was collected in which 0.2 mL borate buffer (0.2 M) and 1 mL phenol (6%) were added. The GABA standard solutions were prepared according to the method followed by Karladee and Suriyong (2012). Afterwards, 8% NaOCl (0.4 mL) was added, followed by vortex for 1 min with 5 min settling time. The solution was ultimately heated in a water bath at 100 °C for 10 min before cooling. Using 2-mL ethanol as a blank, optical density was evaluated at 63 0-nm wavelength by a spectrophotometer (Shimadzu UV–VIS, India). The GABA content in the sample was calculated by comparing the absorbance and standard curve.

Physicochemical Properties

Water Absorption Index (WAI) and Water Solubility Index (WSI)

The WAI and WSI of the composite mix were evaluated by the method adopted by Misra et al. (2023). Briefly, the composite mix (2 g) was suspended in 20-mL distilled water in 50-mL centrifuge tube and mixed for 10 min and centrifuged at 3000 g in REMI R8-C laboratory centrifuge for 20 min. After that, the supernatants were carefully removed. The WAI and WSI values were calculated as given in Eqs. (1) and (2):

$$WAI\left(\frac{g}{g}\right) = \frac{\text{Weight of sediment after centifugation}}{\text{Weight of composite mix}} \quad (1)$$

$$WSI(\%) = \frac{\text{Weight of dried supernatant}}{\text{Weight of composite mix}} x100 \quad (2)$$

Bulk density (BD)

The BD was estimated with the method suggested by Mandliya et al. (2022) and recorded in triplicate (expressed as g/cc).

Color

The color parameters (L^*, a^*, and b^*) of dough samples were calculated using a colorimeter (CM-5, Konica Minolta, Japan). The whiteness index (WI) and chroma values were also observed directly from the instrument. Measurements were performed at 3 different surfaces (left, right, and bottom) for each sample of the dough. The WI and chroma of dough samples can also be calculated as Eqs. (3) and (4):

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (3)$$

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

Pasting Properties

The pasting properties of composite dough samples were investigated by a Rheometer (MCR 52, Anton Paar, Austria) with the method of Dalbhagat and Mishra (2021). The dough samples were sieved below 300-µm particle size before the analysis. Approximately 10% (w/v) dough suspension was made in the canister and loaded into the instrument. The temperature program was set as incubation at 50 °C for 1 min, heating to 95 °C at a rate of 12 °C /min, holding for 2.5 min at 95 °C, cooling to 50 °C at 12 °C/min, and holding for 2 min at 50 °C. The pasting curve of each sample was used to determine peak viscosity (PV), pasting temperature (PT), trough viscosity (TV), setback viscosity (SV), and final viscosity (FV).

Textural Profile Analysis (TPA) of Composite Dough

The TPA of the dough samples was examined using the texture analyzer (CT3, Brookfield Engineering Labs, USA) by deforming the dough balls (10 mm height × 20 mm diameter, weight of approximately 5 g) in two cycles with a 25-mm diameter cylindrical probe (TA25), and the methodology was followed as described by Tanislav et al. (2022). The criteria, including hardness, adhesiveness, springiness, gumminess, cohesiveness, and chewiness, were noted (at 5 replications) after the dough samples were compressed to 75% with pre-test and test speeds at 1.0 mm/s and setting trigger load at 0.10 N. The dough samples were prepared and stored at 4 °C in an air tight container for 10 min before analysis.

Rheological Properties of Composite Dough

The strain and frequency sweep tests of the prepared dough samples were conducted using a Rheometer (MCR 52, Anton Paar, Austria) equipped with a temperature-regulated Peltier plate–plate system (P-PTD 200/Air) with a 25-mm diameter parallel plate geometry. A quantity of around 3 g of dough was supplied on the lower plate, and after that, the upper plate was descended with a distance between the plates of 1 mm. Dough surplus was eliminated, samples were left to rest for 10 min to enable dough relaxation, and silicone oil was supplemented on the outside of the upper plate geometry to prevent sample drying during the experiment (Precup et al., 2022). Before analysis, dough samples were tested for the limits of the linear viscoelastic region (LVR) based on the strain sweep determination, in which the strain was increased from 0.01 to 1% at a constant oscillation frequency of 5 rad/s. The oscillatory measurements were performed at a maximum strain of 0.7%, which was found to be in the LVR where dough samples have a linear relationship between stress and strain. Frequency sweeps were performed from 0.1 to 100 rad/s at 0.7% strain, and the parameters such as storage modulus (G'), loss modulus (G''), and loss factor (tan δ) were recorded at measuring temperature of 25 ± 0.1 °C. For each type of dough, the test was done in triplicate.

FTIR Analysis

The dough samples were lyophilized, ground, and sieved. The powder samples (2 mg) were mixed with anhydrous KBr and hydraulically compressed into disk-shaped pellets (Yang et al., 2022). The sample discs were kept in the light path which enabled the passing of IR light through them and IR spectra was recorded. FTIR spectroscopy was performed with an FTIR spectrophotometer (NICOLET 6700, Thermo Fisher Scientific, Waltham, MA, USA) with transmittance mode, recorded in 400 to 4000 cm^{-1} wavenumber range at a resolution of 4 cm^{-1} and a 128 scan frequency to obtain spectral information for each sample.

Morphology

The dough samples were characterized for the surface morphology and microstructure analysis through scanning electron microscopy (ZEISS EVO 60, Göttingen, Germany) at a voltage of 5 kV. The samples were defatted using hexane in soxhlet apparatus and finally freeze-dried (Bhatt et al., 2021). The samples were mounted on studs and were coated with a layer of gold palladium (360 Å thick) under a vacuum followed by scanning electron microscopy.

Data Analysis

All the studies were carried out in replicate, and the results were depicted as mean ± standard deviation. The data were analyzed using SPSS (SPSS Statistics 22.0, IBM, USA) for analysis of variance (ANOVA), and the mean values were compared using Tukey's honestly significant difference (HSD) test at a significance level of 5%.

Results and Discussion

Nutritional Properties and Mineral Profile of Composite Dough

The moisture content of the dough samples varied from 34.61 to 36.89% and was non-significantly ($p > 0.05$) affected by the addition of probiotics-GABA co-encapsulated powder (Table 1). The freeze-dried co-encapsulated powder-added dough exhibited a comparatively higher moisture content than the control, which might be due to the elevated residual moisture content of freeze-dried microcapsules (4.32%) compared to spray-dried microparticles (4.15%). Similarly, de Marins et al. (2022) found that the uncooked burger's moisture content remains unaffected by the addition of spray-dried microparticles containing probiotics *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* spp. *lactis* Bb-12, and *Lactobacillus acidophilus* La-5. A similar trend was observed for other proximate compositions of dough samples after incorporating spray- and freeze-dried probiotics-GABA microcapsules. The mean values of protein, fat, ash, dietary fiber, carbohydrate, and calorific value of dough ranged from 11.93 to 12.21%, 7.30 to 7.85%, 1.94 to 1.96%, 5.40 to 7.07%, 41.38 to 42.50%, and 283.83 to 284.10 kcal/100 g, respectively, with no significant differences between the formulations (Table 1). The results are in accordance with Silva et al. (2022), in which the proximate values of peanut butter were not significantly affected by the application of co-encapsulated *Bifidobacterium animalis* and guarana peel extract at the proportion of 1.5% (w/w) in Arabic gum matrix using freeze drying technique. It was also observed that both the probioticated and non-probioticated composite dough were also rich in minerals, for example, iron, calcium, sodium, magnesium, zinc, and manganese (Table 2).

Table 1 Effect of spray- and freeze-dried probiotic-GABA powder on the nutritional properties of composite dough

Components (g/100 g)	Control composite dough	Composite dough with SD	Composite dough with FD
Moisture	36.16 ± 1.51^{a}	34.61 ± 2.21^{a}	36.89 ± 0.27^{a}
Protein	12.21 ± 0.75^{a}	13.00 ± 0.12^{a}	11.93 ± 0.11^{a}
Fat	7.30 ± 0.21^{a}	7.82 ± 0.46^{a}	7.85 ± 0.35^{a}
Carbohydrate	42.40 ± 2.45^{a}	42.50 ± 2.54^{a}	41.38 ± 0.02^{a}
Crude fibre	7.07 ± 0.54^{a}	5.77 ± 0.28^{ab}	5.40 ± 0.11^{b}
Ash	1.94 ± 0.02^{a}	2.09 ± 0.01^{b}	1.96 ± 0.06^{ab}
Energy (kcal/100 g)	284.10 ± 4.94^{a}	292.30 ± 6.50^{a}	283.83 ± 2.58^{a}

SD spray-dried powder, *FD* freeze-dried powder

Data represented as mean ± standard deviation of triplicates with different letters in the superscript between the columns are significantly different ($p < 0.05$)

Table 2 Effect of spray- and freeze-dried probiotic-GABA powder on the mineral profile of composite dough

Minerals (mg/100 g)	Control composite dough	Composite dough with SD	Composite dough with FD
Iron	12.81 ± 2.57[a]	6.53 ± 0.06[b]	7.68 ± 0.63[b]
Calcium	83.03 ± 5.45[a]	81.67 ± 7.85[a]	77.33 ± 5.86[a]
Sodium	69.96 ± 6.87[a]	55.27 ± 8.14[a]	65.17 ± 7.39[a]
Zinc	6.31 ± 0.59[a]	6.61 ± 1.20[a]	6.69 ± 0.76[a]
Magnesium	134.70 ± 1.30[a]	136.23 ± 5.01[a]	132.37 ± 3.11[a]
Manganese	5.40 ± 0.27[a]	5.51 ± 0.13[a]	5.64 ± 0.10[a]

SD spray-dried powder, *FD* freezedried powder

Data represented as mean ± standard de-viation of triplicates with different letters in the superscript between the columns are significantly different ($p < 0.05$)

Probiotic Viability in the Composite Dough

The composite dough added with freeze-dried co-encapsulated powder revealed a significantly ($p < 0.05$) high probiotic count of 8.6 log CFU/g compared to spray-dried powder added dough sample (8.2 log CFU/g) as presented in Table 3 since the freeze-dried microcapsules exhibited higher probiotic encapsulation efficiency (95.08%) compared to the spray-dried powder (93.12%). Moumita et al. (2018) incorporated lyophilized and spray-dried microcapsules containing probiotics *E. faecium* in sattu and observed a higher viability of probiotics (8.67 log CFU/g) in sattu added with freeze-dried powder compared to spray-dried powder added formulation (8.18 log CFU/g).

GABA Content in the Composite Dough

The freeze-dried co-encapsulated powder incorporated in dough samples exhibited a GABA content of 62.4 mg/g, which was significantly higher ($p < 0.05$) than the samples added with spray-dried microcapsules (56.5 mg/g) as shown in Table 3, since the freeze drying resulted in a higher GABA yield (90.04%) compared to the spray drying (83.46%). The addition of both spray- and freeze-dried co-encapsulated powder resulted in sufficient quantity of GABA in the dough, which will be effective for preventing hypertension and neurological disorders (Kimura et al., 2002; Okada et al., 2000). This is the first attempt to develop a composite dough added with co-encapsulated probiotics and GABA powder, which is a promising alternative approach to the natural enrichment of GABA in food products by other processes such as fermentation or natural synthesis of GABA by probiotic bacteria that has been adopted by different researchers. Phuapaiboon et al. (2013) found an increased concentration of GABA from 1.46 to 2.33 mg/100 g of yogurt supplemented with immobilized *Lactococcus lactis* within Jerusalem artichoke powder during storage due to the fermentation process. Thus, microencapsulation is an alternative way for a higher enrichment of GABA in food formulations as compared to the method of natural synthesis by fermentation.

Table 3 Effect of addition of spray- and freeze-dried powder on the viability of probiotics and GABA content of composite dough

Composite mix	Probiotic count (log CFU/g)	GABA content (mg/100 g)
Control	-	-
SD	8.20 ± 0.1[a]	56.50 ± 1.32[a]
FD	8.60 ± 0.07[b]	62.40 ± 2.79[b]

SD spray-dried powder, *FD* freeze-dried powder

Data represented as mean ± standard deviation of triplicates with different letters in the superscript between the rows are significantly different ($p < 0.05$)

Physicochemical Properties of Composite Dough

Water Absorption Index (WAI)

The WAI is the volume that starch granules occupy after being suspended in water. In this study, it was observed that the WAI of the composite dough did not vary significantly ($p > 0.05$), but the value increased slightly from 1.28 (for control sample) to 1.30 and 1.31 g/g after the incorporation of microencapsulated freeze- and spray-dried powders, respectively (Table 4). The WAI of dough was slightly increased, when microencapsulated powder were added in a larger quantity due to the presence of maltodextrin and exopolysaccharides such as dextran and inulin in the encapsulating matrix (Afyounizadeh Esfahani & Goli, 2021). Seitter et al. (2020) also observed an increased water absorption of wheat dough to 62.5% with the supplementation of 1% crude exopolysaccharides (levan) of *Lactobacillus sanfranciscensis* strains compared to the control sample (59.8%). Ahmed et al. (2010) produced encapsulated sweet potato flours rich in anthocyanins by spray drying using various levels of ascorbic acid (AA) and maltodextrin (MD) as wall materials. The authors observed that encapsulated flour samples with AA (10 g kg^{-1}) and MD (30 g kg^{-1}) showed a higher WAI than non-encapsulated flours.

Water Solubility Index (WSI)

The WSI of the dough can be related to the presence of soluble fractions that can leach out from the starch granule in excess water. An alike trend of WSI was detected in the composite dough. Although no significant difference ($p > 0.05$) in the WSI values were found in composite dough after the addition of co-encapsulated powder, a slight increased WSI value of probiotic and GABA-enriched dough was found

Table 4 Effect of probiotic and GABA containing spray- and freeze-dried powder on the physicochemical properties of composite dough

Properties	Control composite mix	Composite mix with SD	Composite mix with FD
Bulk density (g/cc)	0.55 ± 0.01^{a}	0.42 ± 0.02^{b}	0.54 ± 0.02^{a}
Water absorption index (g/g)	1.28 ± 0.01^{a}	1.31 ± 0.02^{a}	1.30 ± 0.01^{a}
Water solubility index (%)	19.34 ± 0.16^{a}	20.09 ± 0.66^{a}	19.59 ± 0.12^{a}
L^*	50.54 ± 0.71^{a}	50.77 ± 0.37^{a}	53.26 ± 0.38^{b}
a^*	9.25 ± 0.19^{ab}	9.50 ± 0.12^{a}	9.12 ± 0.10^{b}
b^*	24.26 ± 0.31^{a}	25.20 ± 0.43^{b}	25.51 ± 0.06^{b}
Whiteness index (WI)	44.14 ± 0.66^{a}	43.89 ± 0.37^{a}	45.97 ± 0.35^{b}
Chroma (C^*)	25.96 ± 0.34^{a}	26.93 ± 0.43^{b}	27.09 ± 0.07^{b}

SD spray-dried powder, *FD* freeze-dried powder

Data represented as mean ± standard deviation of triplicates with different letters in the superscript between the columns are significantly different ($p < 0.05$)

indicating that the addition of microencapsulated powder enhanced the proportion of water-soluble solids (Table 4). The addition of probiotic-GABA co-microcapsules disturbed the protein network, thus resulting in a discontinuous protein matrix that permits more solids to leach into the water (Rajam et al., 2015).

Bulk Density

The quantity of air trapped in the mixture is indicated by the density of the product. The BD of composite dough varied from 0.42 to 0.55 g/cc, with lower values for co-encapsulated probiotics-GABA powder added composite dough compared to non-probioticated dough samples (Table 4). The lowering of the density of dough might be attributed to the higher water-holding capacity of exopolysaccharides present in the co-encapsulation matrix (Afyounizadeh Esfahani & Goli, 2021). The air bubbles entrapped into the batter during mixing can easily rise to the surface and are lost to the atmosphere during baking, as a result there may be collapse of the structure of the bakery products such as cake prepared from the low density dough (Turabi et al., 2008). Umashankar et al. (2016) detected the lowest density (0.89 g/cc) of whole wheat flour-based batter treated with additives (Guar gum, carboxy methyl cellulose, and gum Arabic), indicating lighter batter owing to air incorporation. The composite mix with freeze-dried microcapsules showed a higher bulk density than that of dough with spray-dried powder. The different density of microcapsules produced by various drying techniques might be influenced by their shape, surface morphology, and flowability. The rough surface and lower flowability of freeze-dried powder might be linked to the higher bulk density of composite mix (Caglar et al., 2021). The increased BD of dough is suitable for the preparation and packaging of nutritious foods, whereas lower BD is advantageous for the formulation of complementary foods (Kumar et al., 2016).

Color Attributes

The color parameters were observed for the control, spray-, and freeze-dried co-encapsulated powder added composite dough samples. The L^* value (lightness value), WI, and C^* value increased from 50.54 to 53.26, 44.14 to 45.97, and 25.96 to 27.09, respectively, and were significantly affected ($p < 0.05$) by the addition of freeze-dried microencapsulated powder having higher L^* value of 90.12 compared to spray-dried powder (L^* value of 86.58) (Table 4). The slight increase in the L^* and WI of composite dough with the inclusion of the microencapsulated powder can be associated with the reflectivity of the wall materials of the microcapsules. The increased C^* value for probiotics-GABA-enriched dough samples is desirable as the higher C^* values are correlated with greater vividness, which affects the purchase intention of the product (Salueña et al., 2019). Michalska and Lech (2018) also observed that the L^* values of beverage powder prepared from apple juice increased with the enrichment of maltodextrin. No significant difference in a^* values was found between control (9.25) and freeze-dried powder added dough samples (9.12), but increased significantly ($p < 0.05$) to 9.50 with the addition of spray-dried probiotic-GABA co-encapsulated powder. There was a significant increasing trend in b^* values from 24.26 to 25.51 of the composite dough sample with the incorporation of the whitish-yellow color of spray- and freeze-dried co-encapsulated probiotics-GABA powder in the formulation ($p < 0.05$) (Table 4). de Marins et al. (2022) also found an increase in L^*, a^*, b^*, and C^* values in the raw burger after the incorporation of spray-dried microparticles containing probiotics *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* spp. *lactis* Bb-12, and *Lactobacillus acidophilus* La-5. Similarly, Jouki et al. (2021) observed a non-significant variation in L^* values between the yogurt powder with and without the addition of freeze-dried *L. plantarum* microcapsules, whereas a decrease in a^* and an increase in b^* values were reported after the enrichment with cryoprotected probiotic microcapsules.

Pasting Properties

It was observed that the probiotic-GABA-enriched composite dough showed a significant ($p < 0.05$) reduction in the pasting temperature from 79.95 to 78.90 °C (Table 5), which implied more free starch as the substrate for the enzyme resulting in earlier gelatinization during the baking period. The addition of co-encapsulated powder significantly reduced ($p < 0.05$) the peak viscosity (PV) values from 143.40 to 135.95 cP, with the least value for spray-dried probiotic-GABA added composite dough (Table 5). Similarly, the final viscosity (FV) of composite dough significantly decreased ($p < 0.05$) from 232.60 to 218.60 cP after the enrichment of probiotic-GABA powder compared to the control sample showing a reduced ability of starch to form viscous paste due to the interaction of polysaccharides (Table 5). Corresponding declination in setback viscosity (SV) of probioticated dough samples from 92.55 to 86.20 cP showed the inhibition capacity of spray and freeze-dried powder to reassociate the amylose chains resulting from the effect of the exopolysaccharides on solubilized starch, giving these co-encapsulated powders the potential to be used as the anti-staling agents (Li et al., 2019). Since the spray- and freeze-dried probiotics-GABA microcapsules do not gelatinize under the conditions of the experiment, the pasting properties of the composite dough are generally attributed to the roasting of dry ingredients used in the formulation, not to the spray- and freeze-dried microparticles. Thus, the dried microcapsules act as a neutral filler and dilute the starch, thereby lowering the viscosity of the composite dough. Ashwar et al. (2021) also observed a decrease in peak, trough, final, and setback viscosities of rice flour blended with encapsulated *Lactobacillus casei* microcapsules at a level of up to 20%.

Textural Properties of Composite Dough

The textural properties affect the physical and organoleptic properties of foods. Hardness represents the food's resistance against the applied compressive stresses. The addition of spray- and freeze-dried microencapsulated powder maintained the hardness of the dough in the range of 24.58 to 24.99 N with a non-significant variation ($p > 0.05$) with the control sample (26.70 N) (Fig. 1). Moradi et al. (2020) observed that the elasticity of dough was associated with the fermentation process and higher WAI, which contributed to the decrease in hardness and was suitable for the manufacturing of bakery products. Springiness is linked with the elasticity and freshness of foods, and the higher value in dough samples incorporated with the spray-dried microencapsulated powder is desirable. However, no significant differences were found in the springiness varying between 0.80 and 1.02 mm regardless of the types of co-encapsulated powders added (Fig. 1). The adhesiveness of composite dough also significantly decreased ($p < 0.05$) from 0.97 to 0.47 mJ after adding microencapsulated powder compared to the control dough sample (Fig. 1). The higher adhesiveness of materials creates stickiness in the mouth that negatively affects the texture and taste of foods (Saberi et al., 2018). Similarly, the spray-dried powder-added composite dough exhibited a significant ($p < 0.05$) reduction in chewiness than the control. Chewiness is the energy required to chew a product into a swallowed state, and the probiotics-GABA incorporated dough samples showed lower chewiness (16.03–19.10 mJ), demonstrating better tasting properties (Peng et al., 2017) (Fig. 1). An analogous decreasing trend was also detected for the cohesiveness of probiotic-GABA enriched composite dough, leading to a softer dough. The addition of spray- and freeze-dried co-encapsulated powder significantly reduced ($p < 0.05$) the gumminess of dough samples from 24.82 to 15.96 N (Fig. 1). These findings might be intriguing since stickiness is a prevalent issue, particularly in the baking and confectionery sectors, where it can result in serious processing issues because the dough sticks to the handling equipment. Pérez-Chabela et al. (2013) encapsulated probiotic strain *Lactobacillus plantarum* with Acacia gum by spray drying followed by inoculation in cooked meat batters and observed a significant decrease in cohesiveness with no changes in the hardness and springiness of samples. Lopes et al. (2021) also found similar results in which goat Ricotta cheese with microencapsulated *Lactobacillus acidophilus* with alginate and chitosan did not

Table 5 Effect of probiotic and GABA containing spray- and freeze-dried powder on the pasting properties of composite dough

Composite mix	Pasting temperature (°C)	Peak viscosity (cP)	Final viscosity (cP)	Setback Viscosity (cP)
Control	79.95 ± 0.07^{a}	143.40 ± 3.39^{a}	232.60 ± 1.41^{a}	92.55 ± 1.48^{a}
SD	78.90 ± 0.11^{b}	135.95 ± 1.63^{b}	218.60 ± 2.83^{b}	86.20 ± 1.41^{b}
FD	79.25 ± 0.14^{c}	138.80 ± 1.13^{ab}	222.90 ± 2.40^{b}	87.50 ± 2.97^{ab}

SD spray-dried powder, *FD* freeze-dried powder

Data represented as mean ± standard deviation of triplicates with different letters in the superscript between the rows are significantly different ($p < 0.05$)

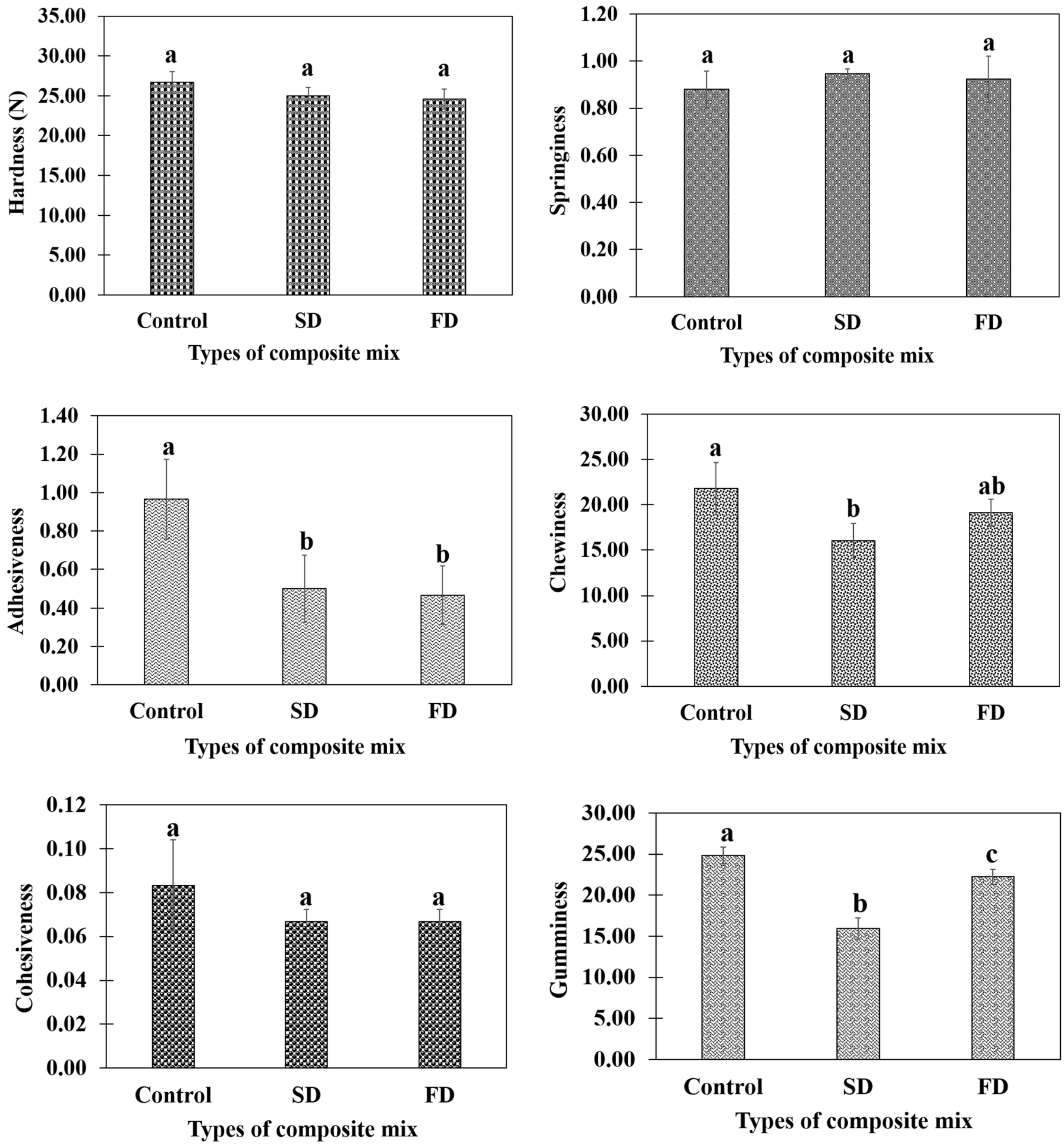

Fig. 1 Effect of the addition of spray- and freeze-dried (SD and FD) co-encapsulated probiotics-GABA microcapsules on textural properties of composite dough

affect the hardness but reduced adhesiveness and gumminess of the product, which was desirable from the technological point of view. Mishra and Mishra (2013) observed that soy yogurt added with a binary mixture of probiotics *L. bulgaricus–S. thermophilus* showed a better texture to the product with lower springiness and adhesiveness, higher gumminess, and average cohesiveness.

Rheological Properties

Viscoelasticity determines the final use quality of dough. The storage and loss modulus (G' and G'') represents the elastic and viscous characteristics of a material, while the loss factor (tan δ) is the ratio of G'' and G' of the viscoelastic deformation behavior. The results reported in Fig. 2a and b

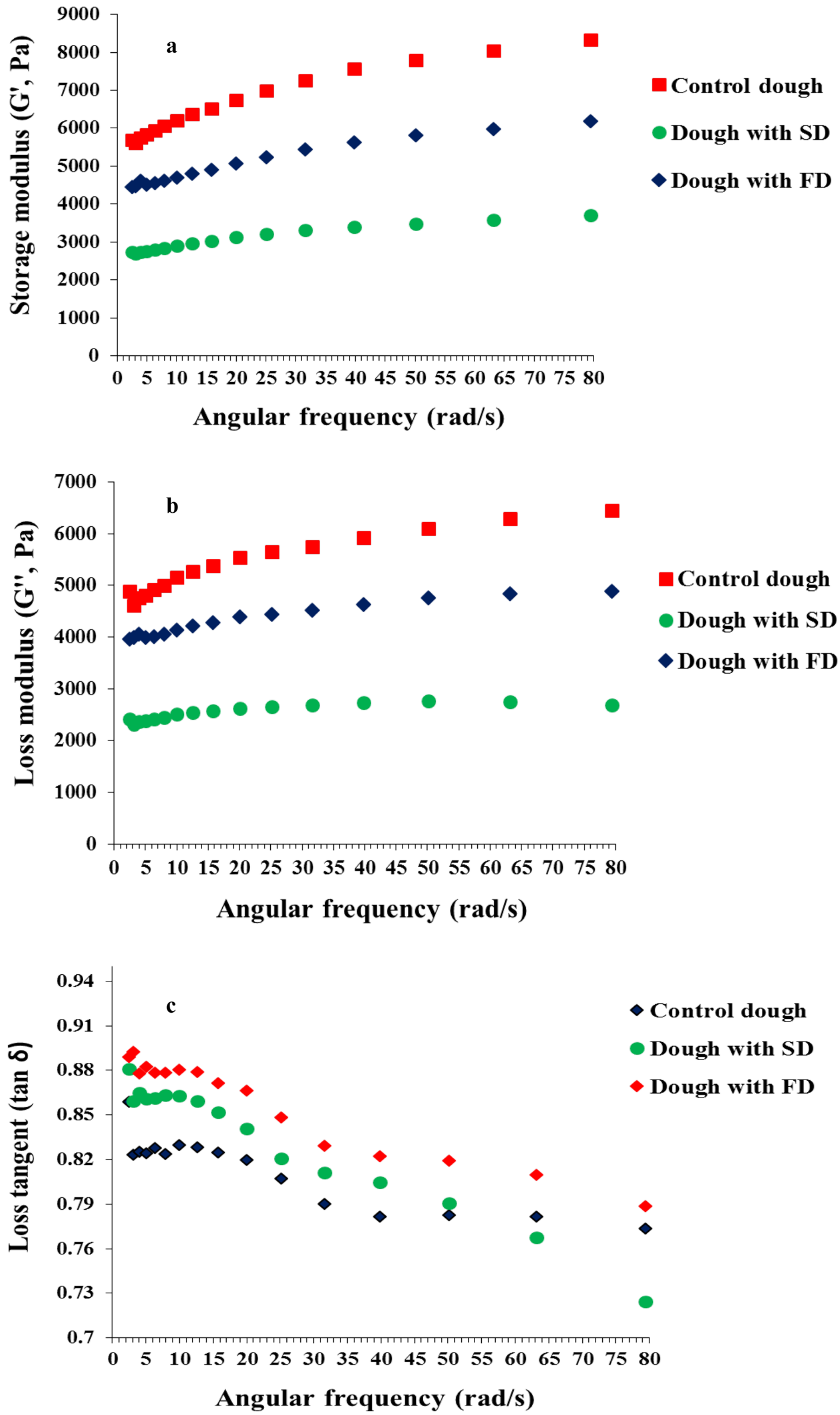

Fig. 2 Effect of addition of spray- and freeze-dried (SD & FD) co-encapsulated probiotics-GABA microcapsules on rheological properties: **a** storage modulus (G'), **b** loss modulus (G''), and **c** loss tangent (tan δ) of composite dough

presented that G' and G'' increased with angular frequency, indicating a good gel system. The G' value is higher than G'' value indicating highly structured materials. The incorporation of spray- and freeze-dried probiotic-GABA powder reduced the G' and G'' values; thus, the viscoelasticity of probiotic dough samples was also decreased (Fig. 3a and b). This was due to weak elasticity and viscous behavior that may be caused by high internal structure content or the resultant polysaccharide interfering with the formation of 3-D protein structure (Xu et al., 2022). The tan δ was lesser than 1 for all the dough samples signifying a solid elastic-like behavior (Fig. 2c). The decreasing tan δ with increasing shear frequency showed an increase in the elastic component and depletion in the viscous component (Zhao et al., 2013).

FTIR Analysis

Infrared spectroscopy helps in understanding the similarities or differences in the structure and presence or absence of functional groups (Gull et al., 2016). The composite dough incorporated with spray- and freeze-dried co-encapsulated powder did not show any remarkable variations in FTIR spectra with differing intensities. The GABA spectrum was found at 1591.8 cm^{-1}, explaining NH group vibration, and 991.23 cm^{-1} represented distinctive C–C stretching of GABA for both the spray- and freeze-dried powder added composite dough samples (Pandey & Mishra, 2021) (Fig. 3). The higher intensities of characteristic peaks of free bacterial cells and GABA at a particular wave number found in the dough with freeze-dried powder showed their presence in higher quantity than the spray-dried microcapsules added to dough samples. The spectra at 2850–3000 for C–H and 2900–3400 cm^{-1} correspond to O–H stretching (Fig. 3). The peaks at 1500–1700 cm^{-1} are related to protein molecules and indicate Amide I and Amide II bond vibrations with lower intensities for probiotics-GABA-enriched composite dough compared to control dough, which indicated deformation in the secondary structure in doughs' protein network with the addition of encapsulated powder (Cao et al., 2020; Fetouhi et al., 2019). The stronger peak intensity at around 2800–3010 cm^{-1} and 1710–1770 cm^{-1} for the dough samples was related to the lipid regions due to the presence of roasted groundnuts in the dough matrix (Kotsiou et al., 2022) and attributed to the symmetric and asymmetric vibration of the aliphatic CH_2 functional group stretching (Fig. 4) (Fanari et al., 2022). The increased peaks at 2922 and 2854 cm^{-1} for control and freeze-dried powder added dough samples showed a higher rate of oxidation reactions as compared to the dough with spray-dried microcapsules (Kotsiou et al., 2022). The main mechanism for lipid oxidation is autoxidation, an autocatalytic process initiated by formation of radicals in unsaturated lipids due to thermal processing (e.g., roasting) followed by oxygen attack (Giménez et al., 2011). It can be anticipated that the porous flaked structure of the freeze-dried microcapsules (Fig. 4b1 and b2) when added to the dough, it might initiate the oxidation process

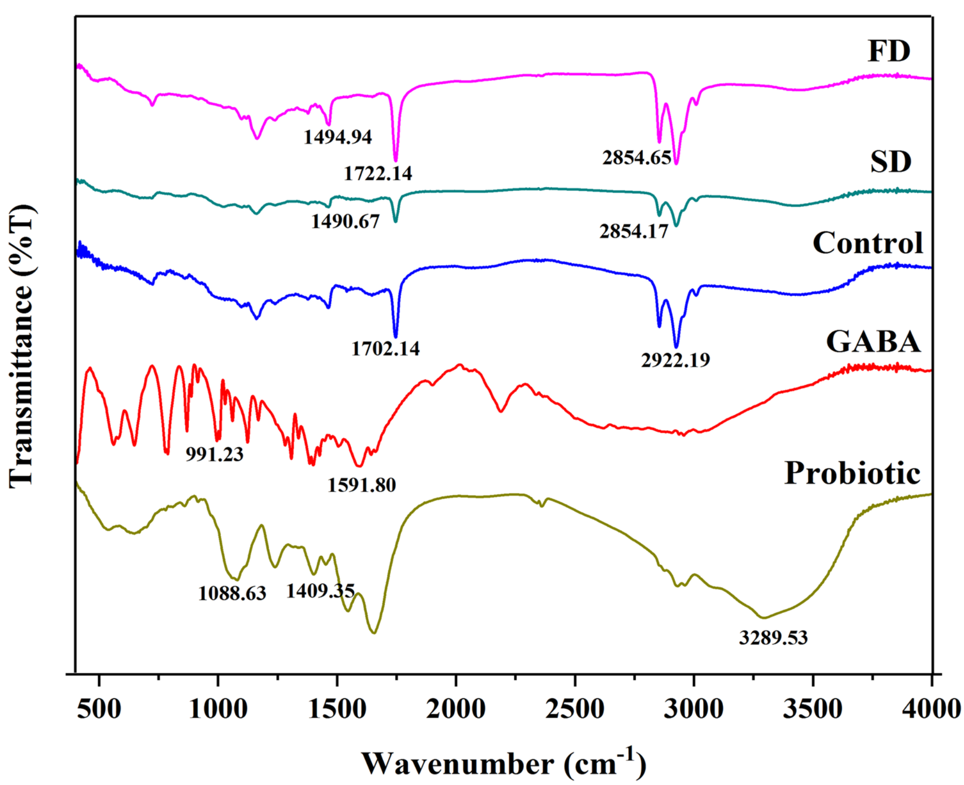

Fig. 3 Fourier transform infrared (FTIR) spectra of composite dough without and with the addition of spray- and freeze-dried probiotics-GABA microcapsules. Notations: SD: spray-dried powder; FD: freeze-dried powder

interacting with the lipids of the roasted groundnut in the dough matrix. In contrast, spray-dried microcapsules were found to be closed intact spherical structures (Fig. 4a1 and a2) thus limiting the oxidation reaction to some extent inside the dough samples.

Microstructure Characteristics

The control dough exhibited an intact, uniform, dense structure with the enmeshing of starch granules in the protein matrix (Fig. 4a). As shown in Fig. 4b and c, the incorporation of spray- and freeze-dried co-encapsulated powder showed the discontinuous structure, protein particle disruption, and aggregation of the protein, along with the large angular voids in the freeze probiotic-GABA-enriched dough samples. It was also observed that the addition of microencapsulated powder resulted in structural deformation, which was in accordance with the results of textural and rheological properties. The breakdown in the internal structure of dough added with microencapsulated powder was also reflected from the reduced values of cohesiveness and hardness of the matrix compared to the control sample (Fig. 4). The more structural deformation in the dough with freeze-dried powder might be attributed to the larger voids, which reduced the elasticity of dough samples and, consequently, caused loss of shape (Fig. 4c). The main reason behind this phenomenon is associated with the breakdown of macromolecules like proteins and fats, which produces protein-free cross-linked aggregates because of weaker protein interactions and little contraction of the gel network during formation, indirectly explaining the dough sample's weaker elastic and viscous behavior (Li et al., 2022).

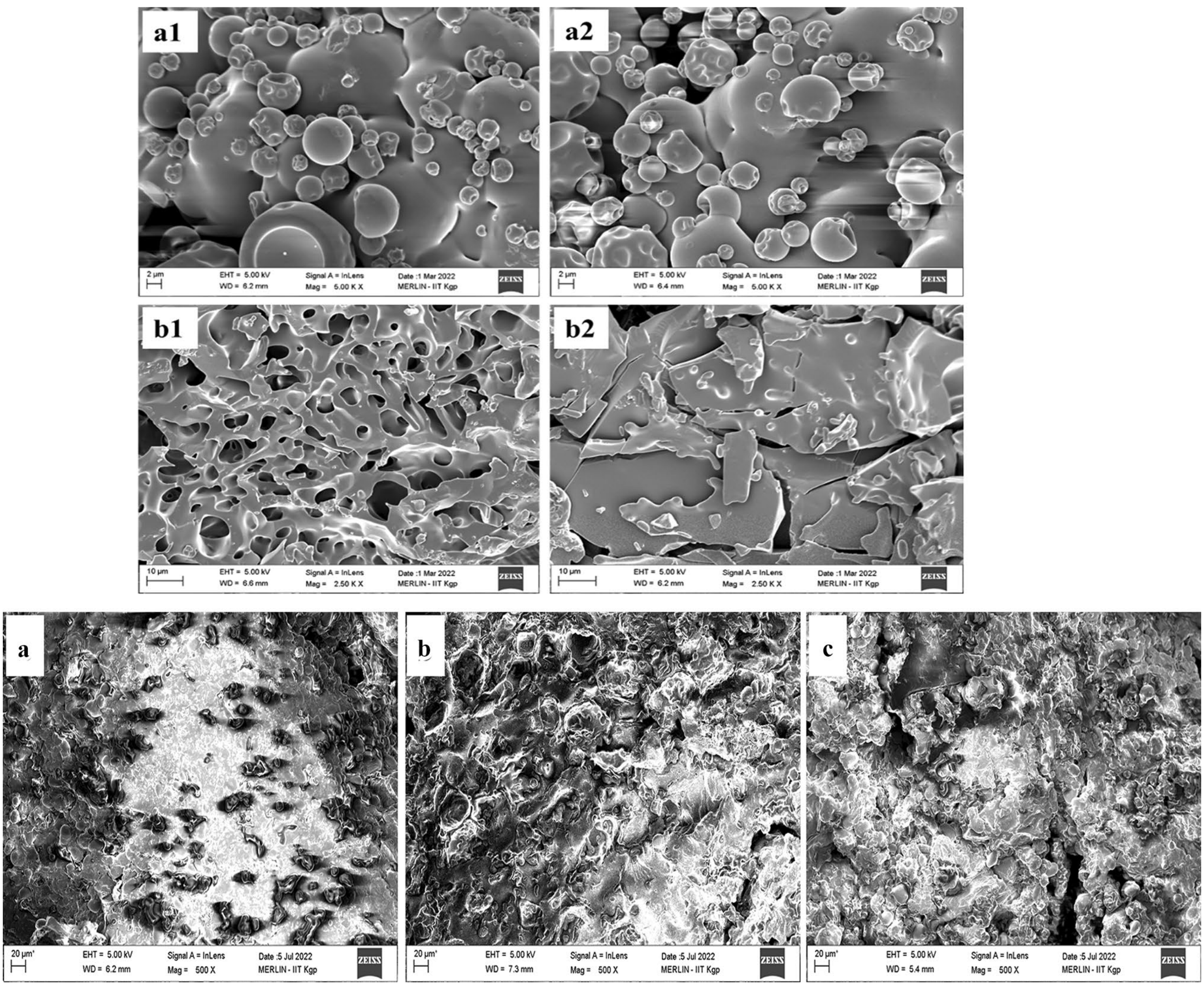

Fig. 4 Scanning electron microscope (SEM) of **a1**, **a2** spray-dried microcapsules (5Kx magnification) and **b1**, **b2** freeze-dried microcapsules (2.5Kx magnification); and **a** dough without and with the addition of **b** spray- and **c** freeze-dried probiotics-GABA microcapsules (500 × magnification)

Conclusion

In this study, spray- and freeze-dried co-microcapsules containing probiotics and GABA were incorporated into multigrain dough samples. The outcomes of this study revealed that the supplementation of co-encapsulated powder could be an effective approach for the enrichment of probiotics and bioactive compounds, such as GABA, for the development of functional dough without a notable change in the nutritional and physicochemical properties. The addition of microencapsulated powder resulted in structural deformation of the dough matrix, which also resulted in decreasing the viscoelasticity of samples. It was concluded that the incorporation of probiotics and GABA into dough via spray drying showed better dough properties in terms of improved textural and pasting qualities by exhibiting lower values of adhesiveness, cohesiveness, chewiness, setback viscosity, and higher value of springiness, as well as showing comparably higher viscoelasticity over the freeze-dried powder added samples, thus making it suitable for preparation of good quality bakery products.

Author Contribution Sourav Misra: collection of literature, methodology, conceptualization, writing the original draft, and data curation. Shubham Mandliya: methodology, data curation, and editing the original draft. Chandrakant Genu Dalbhagat: data analysis, figure editing, and editing the original draft. Pooja Pandey: conceptualization and methodology. Chirasmita Panigrahi: figure editing and data curation. Hari Niwas Mishra: project administration, conceptualization, and supervision.

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Data Availability No research data will be shared.

Declarations

Conflicts of Interest The authors declare no competing interests.

References

Afyounizadeh Esfahani, D., & Goli, M. (2021). The effect of Stevioside-Isomalt, whey protein concentrate, and Bacillus coagulans on the physicochemical and sensory properties of Iranian probiotic Masghati sweet. *Journal of Food Processing and Preservation*, e16028.

Ahmed, M., Akter, M. S., Lee, J. C., & Eun, J. B. (2010). Encapsulation by spray drying of bioactive components, physicochemical and morphological properties from purple sweet potato. *LWT-Food Science and Technology, 43*(9), 1307–1312.

AOAC. (2005). *Official methods of analysis* (16th ed.). Washington, DC: Association of Official Analytical Chemists.

Apiwattanasiri, P., Charoen, R., Rittisak, S., Phattayakorn, K., Jantrasee, S., & Savedboworn, W. (2022). Co-encapsulation efficiency of silk sericin-alginate-prebiotics and the effectiveness of silk sericin coating layer on the survival of probiotic Lactobacillus casei. *Food Bioscience, 46*, 101576.

Arepally, D., Reddy, R. S., Goswami, T. K., & Coorey, R. (2022). A review on probiotic microencapsulation and recent advances of their application in bakery products. *Food and Bioprocess Technology*, 1–23.

Ashwar, B. A., Gani, A., Gani, A., Ahmad, M., & Shah, A. (2021). Encapsulating probiotics in novel resistant starch wall material for production of rice flour extrudates. *LWT, 140*, 110839.

Bampi, G. B., Backes, G. T., Cansian, R. L., de Matos, F. E., Ansolin, I. M. A., Poleto, B. C., ... & Favaro-Trindade, C. S. (2016). Spray chilling microencapsulation of Lactobacillus acidophilus and Bifidobacterium animalis subsp. lactis and its use in the preparation of savory probiotic cereal bars. *Food and Bioprocess Technology*, *9*(8), 1422–1428.

Bhakha, T., Ramasawmy, B., Toorabally, Z., & Neetoo, H. (2019). Development, characterization and shelf-life testing of a novel pulse-based snack bar. *AIMS Agriculture and Food, 4*(3), 756–777.

Bhatt, S., Kumari, N., Abhishek, V., & Gupta, M. (2021). Elucidating the role of amaranth flour in formulation of gluten free black rice muffins and its premix: Nutritional, physico-chemical and textural characteristics. *Journal of Food Measurement and Characterization, 15*, 675–685.

Caglar, N., Ermis, E., & Durak, M. Z. (2021). Spray-dried and freeze-dried sourdough powders: Properties and evaluation of their use in breadmaking. *Journal of Food Engineering, 292*, 110355.

Cao, Y., Yang, Z., Zhang, H., Guo, P., Dong, S., & Li, H. (2020). Influence of potato pulp on gluten network structure in wheat dough and steamed bread. *Cereal Chemistry, 97*(2), 226–234.

Chawda, P. J., Shi, J., Xue, S., & Young Quek, S. (2017). Co-encapsulation of bioactives for food applications. *Food Quality and Safety, 1*(4), 302–309.

Dalbhagat, C. G., & Mishra, H. N. (2021). Drying modeling, cooking characteristics, pasting properties, and crystallinity of fortified rice kernels. *Journal of Food Processing and Preservation, 45*(6), e15579.

de Marins, A. R., de Campos, T. A. F., Batista, A. F. P., Correa, V. G., Peralta, R. M., Mikcha, J. M. G., ... & Feihrmann, A. C. (2022). Effect of the addition of encapsulated Lactiplantibacillus plantarum Lp-115, Bifidobacterium animalis spp. lactis Bb-12, and Lactobacillus acidophilus La-5 to cooked burger. *LWT*, *155*, 112946.

De Prisco, A., van Valenberg, H. J., Fogliano, V., & Mauriello, G. (2017). Microencapsulated starter culture during yoghurt manufacturing, effect on technological features. *Food and Bioprocess Technology, 10*(10), 1767–1777.

Dhakal, R., Bajpai, V. K., & Baek, K. H. (2012). Production of GABA (γ-aminobutyric acid) by microorganisms: A review. *Brazilian Journal of Microbiology, 43*, 1230–1241.

dos Santos, D. X., Casazza, A. A., Aliakbarian, B., Bedani, R., Saad, S. M. I., & Perego, P. (2019). Improved probiotic survival to in vitro gastrointestinal stress in a mousse containing Lactobacillus acidophilus La-5 microencapsulated with inulin by spray drying. *Lwt, 99*, 404–410.

Fanari, F., Carboni, G., Desogus, F., Grosso, M., & Wilhelm, M. (2022). A chemometric approach to assess the rheological properties of durum wheat dough by indirect FTIR measurements. *Food and Bioprocess Technology, 15*(5), 1040–1054.

FAO/WHO. (2006). *Probiotics in food Health and nutritional properties and guidelines for evaluation: Report of a Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*.

Fetouhi, A., Benatallah, L., Nawrocka, A., Szymańska-Chargot, M., Bouasla, A., Tomczyńska-Mleko, M., ... & Sujak, A. (2019). Investigation of viscoelastic behaviour of rice-field bean gluten-free dough using the biophysical characterization of proteins and starch: A FT-IR study. *Journal of food science and technology*, *56*, 1316–1327.

Frakolaki, G., Giannou, V., Kekos, D., & Tzia, C. (2021a). A review of the microencapsulation techniques for the incorporation of probiotic bacteria in functional foods. *Critical Reviews in Food Science and Nutrition, 61*(9), 1515–1536.

Gao, Y., Hamid, N., Gutierrez-Maddox, N., Kantono, K., & Kitundu, E. (2019). Development of a probiotic beverage using breadfruit flour as a substrate. *Foods, 8*(6), 214.

Giménez, B., Gómez-Guillén, M. C., Pérez-Mateos, M., Montero, P., & Márquez-Ruiz, G. (2011). Evaluation of lipid oxidation in horse mackerel patties covered with borage-containing film during frozen storage. *Food Chemistry, 124*(4), 1393–1403.

Gramazio, P., Takayama, M., & Ezura, H. (2020). Challenges and prospects of new plant breeding techniques for GABA improvement in crops: Tomato as an example. *Frontiers in Plant Science, 11*, 577980.

Gull, A., Prasad, K., & Kumar, P. (2016). Evaluation of functional, antinutritional, pasting and microstructural properties of Millet flours. *Journal of Food Measurement and Characterization, 10*, 96–102.

Hasmadi, M., Noorfarahzilah, M., Noraidah, H., Zainol, M. K., & Jahurul, M. H. A. (2020). Functional properties of composite flour: A review. *Food Research, 4*(6), 1820–1831.

Hyland, N. P., & Cryan, J. F. (2010). A gut feeling about GABA: Focus on GABAB receptors. *Frontiers in Pharmacology, 1*, 124.

Jouki, M., Khazaei, N., Rezaei, F., & Taghavian-Saeid, R. (2021). Production of synbiotic freeze-dried yoghurt powder using microencapsulation and cryopreservation of L. plantarum in alginate-skim milk microcapsules. *International Dairy Journal*, *122*, 105133.

Karladee, D., & Suriyong, S. (2012). γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination. *Science Asia, 38*(1), 13–17.

Khorshidi, M., Heshmati, A., Taheri, M., Karami, M., & Mahjub, R. (2021). Effect of whey protein‐and xanthan‐based coating on the viability of microencapsulated Lactobacillus acidophilus and physiochemical, textural, and sensorial properties of yogurt. *Food Science & Nutrition*.

Kimura, M., Hayakawa, K., & Sansawa, H. (2002). Involvement of γ-aminobutyric acid (GABA) B receptors in the hypotensive effect of systemically administered GABA in spontaneously hypertensive rats. *Japanese Journal of Pharmacology, 89*(4), 388–394.

Kitaoka, S., & Nakano, Y. (1969). Colorimetric determination of ω-amino acids. *The Journal of Biochemistry, 66*(1), 87–94.

Kotsiou, K., Sacharidis, D. D., Matsakidou, A., Biliaderis, C. G., & Lazaridou, A. (2022). Physicochemical and functional aspects of composite wheat-roasted chickpea flours in relation to dough rheology, bread quality and staling phenomena. *Food Hydrocolloids, 124*, 107322.

Kumar, K. A., Sharma, G. K., Khan, M. A., & Semwal, A. D. (2016). A study on functional, pasting and micro-structural characteristics of multigrain mixes for biscuits. *Journal of Food Measurement and Characterization, 10*(2), 274–282.

Li, J., Zhu, Y., Yadav, M. P., & Li, J. (2019). Effect of various hydrocolloids on the physical and fermentation properties of dough. *Food Chemistry, 271*, 165–173.

Li, Y., Zhao, F., Li, C., Ban, X., Gu, Z., & Li, Z. (2022). Fine structures of added maltodextrin impact stability of frozen bread dough system. *Carbohydrate Polymers, 298*, 120028.

Liu, M., Wang, Y., Jiang, L., Xia, Q., Qiu, Y., Fan, L., ... & Zhao, L. (2015). Effect of γ‐aminobutyric acid on the physicochemical, rheological and sensory properties of yoghurt. *International Journal of Dairy Technology*, *68*(4), 503–510.

Lopes, L. A. A., Pimentel, T. C., Carvalho, R. D. S. F., Madruga, M. S., de Sousa Galvão, M., Bezerra, T. K. A., ... & Stamford, T. C. M. (2021). Spreadable goat Ricotta cheese added with Lactobacillus acidophilus La-05: Can microencapsulation improve the probiotic survival and the quality parameters?. *Food Chemistry*, *346*, 128769.

Malmo, C., La Storia, A., & Mauriello, G. (2013). Microencapsulation of Lactobacillus reuteri DSM 17938 cells coated in alginate beads with chitosan by spray drying to use as a probiotic cell in a chocolate soufflé. *Food and Bioprocess Technology, 6*(3), 795–805.

Mandliya, S., Vishwakarma, S., & Mishra, H. N. (2022). Modeling of vacuum drying of pressed mycelium (*Pleurotus eryngii*) and its microstructure and physicochemical properties. *Journal of Food Process Engineering*, e14124.

Martins, I. B. A., Deliza, R., dos Santos, K. M. O., Walter, E. H. M., Martins, J. M., & Rosenthal, A. (2018). Viability of probiotics in goat cheese during storage and under simulated gastrointestinal conditions. *Food and Bioprocess Technology, 11*(4), 853–863.

Michalska, A., & Lech, K. (2018). The effect of carrier quantity and drying method on the physical properties of apple juice powders. *Beverages, 4*(1), 2.

Mishra, S., & Mishra, H. N. (2013). Effect of synbiotic interaction of fructooligosaccharide and probiotics on the acidification profile, textural and rheological characteristics of fermented soy milk. *Food and Bioprocess Technology, 6*(11), 3166–3176.

Misra, S., Pandey, P., & Mishra, H. N. (2021). Novel approaches for co-encapsulation of probiotic bacteria with bioactive compounds, their health benefits and functional food product development: A review. *Trends in Food Science & Technology, 109*, 340–351.

Misra, S., Pandey, P., Dalbhagat, C. G., & Mishra, H. N. (2022). Emerging technologies and coating materials for improved probiotication in food products: A review. *Food and Bioprocess Technology*, 1–42.

Misra, S., Pandey, P., Panigrahi, C., & Mishra, H. N. (2023). Evaluation of potentiality of erythritol on improving the physicochemical, functional, and pasting properties, along with the storability of multigrain flour using chemometric approach. *Journal of Stored Products Research, 101*, 102088.

Moradi, M., Bolandi, M., Karimi, M., Nahidi, F., & Baghaei, H. (2020). Improvement of gluten-free taftoon bread properties during storage by the incorporation of potato powder (Satrina V.), guar gum, sodium caseinate and transglutaminase into the matrix. *Journal of Food Measurement and Characterization*, *14*(4), 2282–2288.

Morsy, M. K., Morsy, O. M., Abdelmonem, M. A., & Elsabagh, R. (2022). Anthocyanin-colored microencapsulation effects on survival rate of Lactobacillus rhamnosus GG, color stability, and sensory parameters in strawberry nectar model. *Food and Bioprocess Technology, 15*(2), 352–367.

Moumita, S., Das, B., Hasan, U., & Jayabalan, R. (2018). Effect of long-term storage on viability and acceptability of lyophilized and spray-dried synbiotic microcapsules in dry functional food formulations. *LWT, 96*, 127–132.

Nakamura, H., Takishima, T., Kometani, T., & Yokogoshi, H. (2009). Psychological stress-reducing effect of chocolate enriched with γ-aminobutyric acid (GABA) in humans: Assessment of stress using heart rate variability and salivary chromogranin A. *International Journal of Food Sciences and Nutrition, 60*(sup5), 106–113.

Obradović, N., Volić, M., Nedović, V., Rakin, M., & Bugarski, B. (2022). Microencapsulation of probiotic starter culture in protein–carbohydrate carriers using spray and freeze-drying processes: Implementation in whey-based beverages. *Journal of Food Engineering, 321*, 110948.

Oh, S. H., Moon, Y. J., & Oh, C. H. (2003). γ-Aminobutyric acid (GABA) content of selected uncooked foods. *Preventive Nutrition and Food Science, 8*(1), 75–78.

Okada, T., Sugishita, T., Murakami, T., Murai, H., Saikusa, T., Horino, T., ... & Takahashi, T. (2000). Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. *Nippon Shokuhin Kagaku Kogaku Kaishi= Journal of the Japanese Society for Food Science and Technology*, *47*(8), 596–603.

Pandey, P., & Mishra, H. N. (2021). Co-microencapsulation of γ-aminobutyric acid (GABA) and probiotic bacteria in thermostable and biocompatible exopolysaccharides matrix. *LWT, 136*, 110293.
Pandey, P., Mettu, S., Mishra, H. N., Ashokkumar, M., & Martin, G. J. (2021). Multilayer co-encapsulation of probiotics and γ-amino butyric acid (GABA) using ultrasound for functional food applications. *LWT, 146*, 111432.
Peng, B., Li, Y., Ding, S., & Yang, J. (2017). Characterization of textural, rheological, thermal, microstructural, and water mobility in wheat flour dough and bread affected by trehalose. *Food Chemistry, 233*, 369–377.
Pereira, J. O., Soares, J., Monteiro, M. J., Amaro, A., Gomes, A., & Pintado, M. (2019). Cereal bars functionalized through Bifidobacterium animalis subsp. lactis BB-12 and inulin incorporated in edible coatings of whey protein isolate or alginate. *Food & Function*, *10*(10), 6892–6902.
Pérez-Chabela, M. L., Lara-Labastida, R., Rodriguez-Huezo, E., & Totosaus, A. (2013). Effect of spray drying encapsulation of thermotolerant lactic acid bacteria on meat batters properties. *Food and Bioprocess Technology, 6*(6), 1505–1515.
Phuapaiboon, P., Leenanon, B., & Levin, R. E. (2013). Effect of Lactococcus lactis immobilized within pineapple and yam bean segments, and jerusalem artichoke powder on its viability and quality of yogurt. *Food and Bioprocess Technology, 6*(10), 2751–2762.
Pourjafar, H., Noori, N., Gandomi, H., Basti, A. A., & Ansari, F. (2020). Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage. *Biotechnology Reports, 25*, e00432.
Precup, G., Teleky, B. E., Ranga, F., & Vodnar, D. C. (2022). Assessment of physicochemical and rheological properties of xylo-oligosaccharides and glucose-enriched doughs fermented with BB-12. *Biology, 11*(4), 553.
Rajam, R., Kumar, S. B., Prabhasankar, P., & Anandharamakrishnan, C. (2015). Microencapsulation of Lactobacillus plantarum MTCC 5422 in fructooligosaccharide and whey protein wall systems and its impact on noodle quality. *Journal of Food Science and Technology, 52*(7), 4029–4041.
Reid, A. A., Champagne, C. P., Gardner, N., Fustier, P., & Vuillemard, J. C. (2007). Survival in food systems of *Lactobacillus rhamnosus* R011 microentrapped in whey protein gel particles. *Journal of Food Science, 72*(1), M031–M037.
Rodrigues, D., Sousa, S., Gomes, A. M., Pintado, M. M., Silva, J. P., Costa, P., ... & Freitas, A. C. (2012). Storage stability of Lactobacillus paracasei as free cells or encapsulated in alginate-based microcapsules in low pH fruit juices. *Food and Bioprocess Technology*, *5*(7), 2748–2757.
Saberi, F., Naderi, M., & Naeli, M. H. (2018). Production of biochewing gum based on saqqez as the biopolymer: Its biodegradability and textural properties. *Journal of Polymers and the Environment, 26*, 3889–3901.
Sade, F. O. (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (Pennisetum glaucum). *Journal of Food Technology, 7*(3), 92–97.
Salueña, B. H., Gamasa, C. S., Rubial, J. M. D., & Odriozola, C. A. (2019). CIELAB color paths during meat shelf life. *Meat Science, 157*, 107889.
Sciarini, L. S., Ribotta, P. D., León, A. E., & Pérez, G. T. (2012). Incorporation of several additives into gluten free breads: Effect on dough properties and bread quality. *Journal of Food Engineering, 111*(4), 590–597.
Seitter, M., Fleig, M., Schmidt, H., & Hertel, C. (2020). Effect of exopolysaccharides produced by Lactobacillus sanfranciscensis on the processing properties of wheat doughs. *European Food Research and Technology, 246*(3), 461–469.
Sharma, R., Rashidinejad, A., & Jafari, S. M. (2022). Application of spray dried encapsulated probiotics in functional food formulations. *Food and Bioprocess Technology*, 1–20.
Shinde, T., Sun-Waterhouse, D., & Brooks, J. (2014). Co-extrusion encapsulation of probiotic Lactobacillus acidophilus alone or together with apple skin polyphenols: An aqueous and value-added delivery system using alginate. *Food and Bioprocess Technology, 7*(6), 1581–1596.
Silva, M. P., Farsoni, E. G., Gobato, C. F., Thomazini, M., & Favaro-Trindade, C. S. (2022). Simultaneous encapsulation of probiotic and guaraná peel extract for development of functional peanut butter. *Food Control, 138*, 109050.
Tanislav, A. E., Pușcaș, A., Păucean, A., Mureșan, A. E., Semeniuc, C. A., Mureșan, V., & Mudura, E. (2022). Evaluation of structural behavior in the process dynamics of oleogel-based tender dough products. *Gels, 8*(5), 317.
Thakur, A., Pandey, P., Dalbhagat, C. G., & Mishra, H. N. (2022). Development of grain-based carbonated beverage premix using maize (Zea mays), Bengal gram (Cicer arietinum), and finger millet (Eleusine coracana). *Journal of Food Science and Technology, 59*(4), 1637–1648.
Turabi, E., Sumnu, G., & Sahin, S. (2008). Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. *Food Hydrocolloids, 22*(2), 305–312.
Umashankar, K., Rajiv, J., & Prabhasankar, P. (2016). Development of hypoimmunogenic muffins: Batter rheology, quality characteristics, microstructure and immunochemical validation. *Journal of Food Science and Technology, 53*(1), 531–540.
Vasile, M. A., Milea, Ș. A., Enachi, E., Barbu, V., Cîrciumaru, A., Bahrim, G. E., ... & Stănciuc, N. (2020). Functional enhancement of bioactives from black beans and lactic acid bacteria into an innovative food ingredient by comicroencapsulation. *Food and Bioprocess Technology*, *13*(6), 978–987.
Xu, X., Cui, H., Yuan, Z., Xu, J., Li, J., Liu, J., ... & Zhu, D. (2022). Effects of different combinations of probiotics on rheology, microstructure, and moisture distribution of soy materials-based yogurt. *Journal of Food Science*.
Yang, L., Wang, S., Zhang, H., Du, C., Li, S., & Yang, J. (2022). Effects of black soybean powder particle size on the characteristics of mixed powder and wheat flour dough. *LWT, 167*, 113834.
Zhao, Q., Kuang, W., Long, Z., Fang, M., Liu, D., Yang, B., & Zhao, M. (2013). Effect of sorbitan monostearate on the physical characteristics and whipping properties of whipped cream. *Food Chemistry, 141*(3), 1834–1840.