Research Article

Study of the Effect of Encapsulating Materials on the Stability of Bifidobacterium Bifidum during Spray Drying: FT-IR, TGA, and Electron Microscopy Analysis

Asaad Shamil Atiya[1]*, Abdulridha Ati Jaafar[2] and Mustafa Adnan Idan[3]

[1]Department of Food Science, College of Agriculture, University of Misan, Iraq.

Abstract | The encapsulation of probiotic bacteria is a promising method for the maintenance of bacteria viability during processing, storage, and gastrointestinal transit. Potential of gum Arabic and buffalo milk whey as carrier for and encapsulated Bifidobacterium bifidum by spray drying were investigated. Structural and physicochemical characterisation was carried out by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA), and scanning electron microscopy (coupled with energy dispersive X-ray spectroscopy) EDS. FT-IR spectra showed a strong hydrogen bond (O–H, N–H and C=O) between GA and BMW, which could create a protective shell that dispensed GA in a stable manner. With TGA, three degradation temperatures (150–225 ˚C, 229–398 ˚C and 414–548 ˚C) were determined, which suggest high thermal resistance of the encapsulated bacteria. Scanning electron microscopy demonstrated porous microcapsules with disorder but integrity, and the average diameter of porous microcapsules was 18.9 nm, and the energy dispersive spectrum analysis displayed the necessary organic and mineral components (C, O, Ca, Mg), which strengthen the structure of capsule. The findings indicated that GA and BMW are both good natural encapsulants, which effectively improved the stability and storability of B. bifidum and could potentially be applied in functional foods and nutraceuticals.

Received | July 17, 2025; **Accepted** | September 11, 2025; **Published** | June 30, 2026
***Correspondence** | Asaad Shamil Atiya, Department of Food Science, College of Agriculture, University of Misan, Iraq; **Email:** Asaad.shameel@uomisan.edu.iq
Citation | Atiya, A.S., A.A. Jaafar and M.A. Idan. 2026. Study of the effect of encapsulating materials on the stability of bifidobacterium bifidum during spray drying: ft-ir, tga, and electron microscopy analysis. *Pakistan Journal of Agricultural Research,* 39(2): 48-56.
DOI | https://dx.doi.org/10.17582/journal.pjar/2026/39.2.48.56
Keywords | Bifidobacterium bifidum, Encapsulation, Spray drying, Gum Arabic, Buffalo milk whey, FT-IR, TGA, SEM

Introduction

Probiotic-enriched foods comprise around 65% of the worldwide functional food industry (Burgain *et al*., 2011). Probiotics are live bacteria that, when provided in sufficient quantities, offer health advantages to the host. These advantages encompass the control of gut microbiota, augmentation of mucosal immunity, reduction of cholesterol, production of vitamins, and antibacterial and anticancer characteristics (Rezazadeh-Bari *et al*., 2019; Atta *et al*., 2025).

Among the diverse probiotic strains, Bifidobacterium bifidum is particularly valuable due to its production of short-chain fatty acids, exopolysaccharides, bacteriocins, and essential enzymes such as β-galactosidase (Al-Sadi *et al*., 2021; Yin *et al*., 2025). However, ensuring probiotic viability during industrial processing, transport, and gastrointestinal passage remains a key challenge, as probiotics are highly sensitive to thermal, acidic, and oxidative stress (Siuta-Cruce and Goulet, 2001).

Microencapsulation has been established as a potent solution to address these challenges (Garg *et al*., 2006; Mohammed *et al*., 2025). Polysaccharides and proteins e.g. gum Arabic and whey protein, have been reported to promote structural stability and bioactivity when co-encapsulated (Halwani *et al*., 2008; Awaisheh *et al*., 2013).

Spray drying is the most common method used for encapsulating active substances by virtue of its scalability, cost-effectiveness, and the production of dried powders with long-term stability (Sharifi *et al*., 2021). However, the encapsulation efficiency is largely determined by the wall materials. Gum Arabic, a natural polysaccharide, has good film-forming and emulsifying properties, and buffalo milk whey is rich in functional proteins and minerals leading to a synergistic encapsulating network (Sharifi *et al*., 2021).

The objective of this study was to evaluate the B. bifidum and encapsulating agents (GA and BFFM) interaction as a function of spray drying. The structure and physicochemical properties of the bacteria encapsulated particles were characterized with far-FT-IR, TGA and SEM-EDS.

Materials and Methods

Bacterial strain and encapsulation materials

Bifidobacterium bifidum from Sigma (USA) was capsules with gum Arabic (GA) and buffalo milk whey (BMW) as wall material. The encapsulation was carried out using an Anhydro Lab S1 spray drier (DRY-78 model), according to the method described by Lorenzen and Schrader (2006) modified by Raqad *et al*. (2020). Inlet and outlet drying temperature was 135 °C and 65 °C, respectively, and feed flow rate was 3.5 mL/min.

Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectra were obtained using an FT-IR spectrometer at the Department of Chemistry, University of Basrah. Samples were prepared by mixing dried encapsulated powders with potassium bromide (KBr) and pressing into thin pellets. Spectra were recorded within the range of 400–4000 cm^{-1}, following the procedure described by Fatima(2020).

Thermogravimetric analysis (TGA)

Thermal stability was evaluated with a Shimadzu TGA-50 instrument (Japan) in a nitrogen environment at a flow rate of 50 mL/min. Samples were subjected to heating from 0 °C to 550 °C at a uniform rate of 10 °C/min. The data analysis adhered to the technique established by Nawar (2019).

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

For morphological analysis, encapsulated B. bifidum samples were frozen at –80 °C for 4 hours and then lyophilized using a Christ ALPHA 1-2 LD plus lyophilized (Martin Christ GmbH, Germany). The dehydrated samples were sputter-coated with gold and examined using a Hitachi S-4800 field-emission scanning electron microscope (Hitachi High-Tech Science Corp., Japan) at 15 kV. The elemental composition was determined using a HORIBA 7593-H EDS, with a working distance of 15 mm and a data acquisition time of 300 seconds (Sun *et al*., 2017).

Results and Discussion

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were used to evaluate the molecular interactions between bifidobacterium bifidum following spray drying (Figure 1), gum Arabic (GA) (Figure 2), and buffalo milk whey (BMW) (Figure 3). The spectra of encapsulated samples revealed broad absorption bands at ~3273 cm^{-1}, corresponding to O–H and N–H stretching vibrations. The higher intensity of these bands compared with native GA and BMW indicates stronger intermolecular hydrogen bonding in the encapsulated matrix, which contributes to enhanced bacterial protection under stress conditions.

Additional absorption peaks were detected at 2921 cm^{-1} (C–H stretching) and 1724 cm^{-1} (C=O stretching and N–H bending). The amide I (1640 cm^{-1}) and amide II (1540 cm^{-1}) bands further corroborate the establishment of protein–polysaccharide interactions. The structural alterations indicated that a compact shell composed of GA and BMW encased the bacterial cells, potentially resulting from protein denaturation and polysaccharide-protein conjugation during the spray drying process (Zhang *et al*., 2022; Baiocco *et al*., 2021).

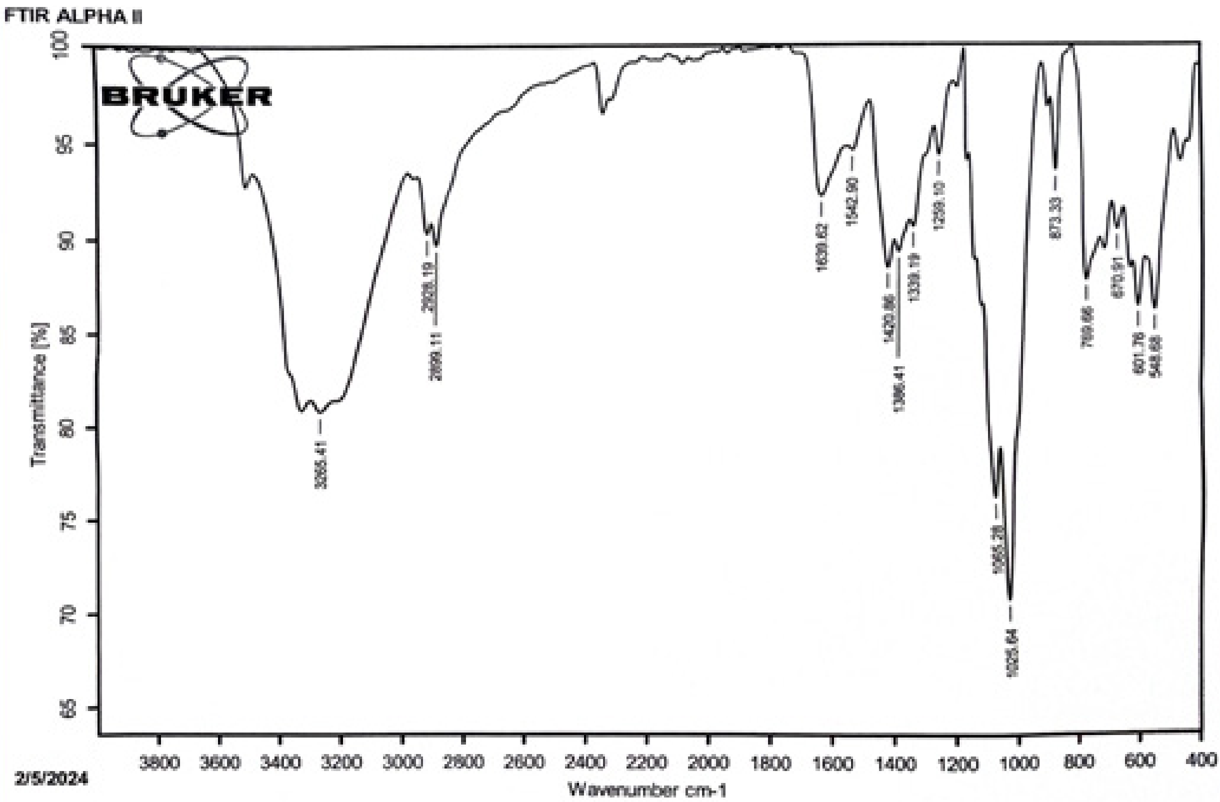

Figure 1: *FTIR spectrum of the sample coated with 12 g buffalo milk whey powder and 3.25 g gum Arabic for Bifidobacterium bifidum.*

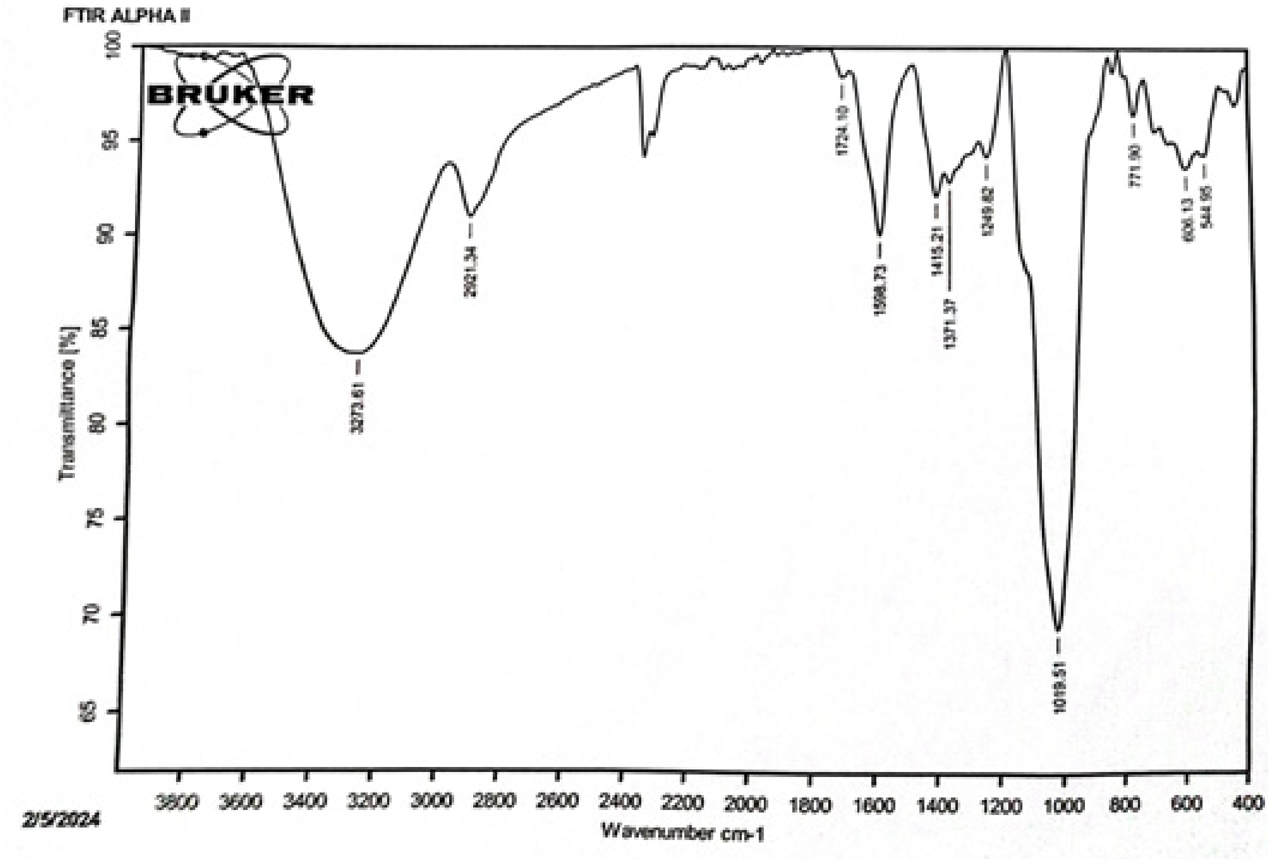

Figure 2: *FTIR spectrum of gum arabic model only before encapsulation of bifidobacterium bifidum.*

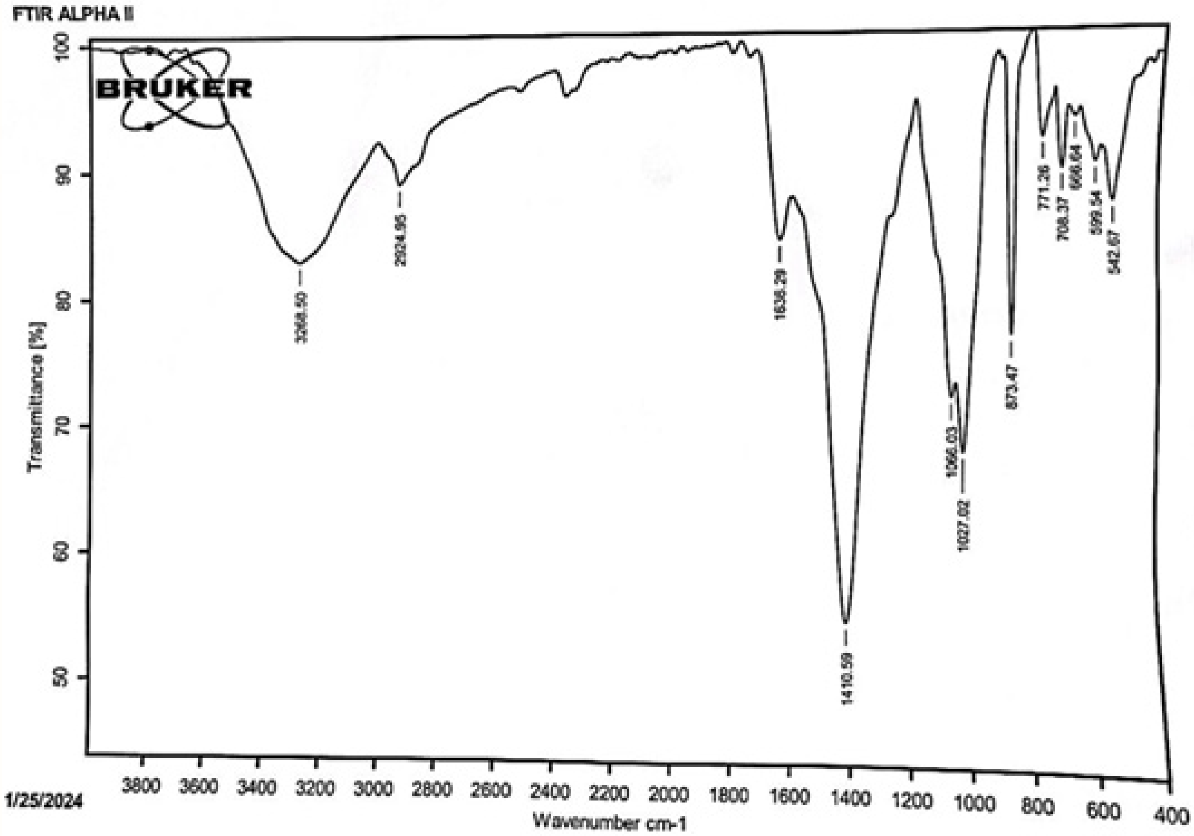

Figure 3: *FTIR spectrum of buffalo milk whey sample only before encapsulation of Bifidobacterium Bifidum.*

This is in line with the literature, which reports that GA presents hydrogen bonded hydroxyl groups whereas the whey proteins contribute peptide and carbonyl groups, thus aiding the formation of stable films (Han *et al*., 2020; Kang *et al*., 2019). The interaction of these functional groups improves encapsulation efficiency and enhances bacterial resistance to environmental stressors.

Thermogravimetric analysis (TGA)

TGA was performed to assess the thermal stability of encapsulated B. bifidum. The thermogram revealed three distinct stages of weight loss (Figure 4).

[1]C. 150–225°: Initial weight loss (~9.5%) due to moisture evaporation and volatilization of light organic compounds.

[2]C. 229–398°: Major weight reduction (~39.8%)، attributed to the degradation of carbohydrates and proteins in GA and BMW.

[3]C. 414–548°: Final decomposition (~10.7%)، associated with carbon residue breakdown.

The delayed onset of thermal degradation indicates that encapsulation conferred significant protection, with GA and BMW providing a stable thermal matrix. GA contributed a carbohydrate-based insulating layer, while whey proteins (e.g., β-lactoglobulin) enhanced film stability via peptide bonding and hydrophobic interactions.

These observations agree with those of Tavares *et al.* (2019) and Leylak *et al.* (2021), who reported that protein–polysaccharide blends enhance the stability of encapsulant and resistance to pyrolysis. Moreover, degradation temperatures were found to be >220 °C, well above the standard spray drying operations, which provides strong evidence that GA-BMW encapsulation enables bacterial survival during processing and storage.

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

SEM micrographs (Figure 5), indicated that the microcapsules were of irregular, porous appearance having rough surface morphology of the outer surfaces. The porous nature of the structure is beneficial, as it enables a controlled exchange of nutrients and metabolites between encapsulated bacteria and the surrounding medium while affording mechanical protection. The average mean capsule diameter was ~ 18.9 nm, indicating that nanoscale particles were generated successfully by spray drying.

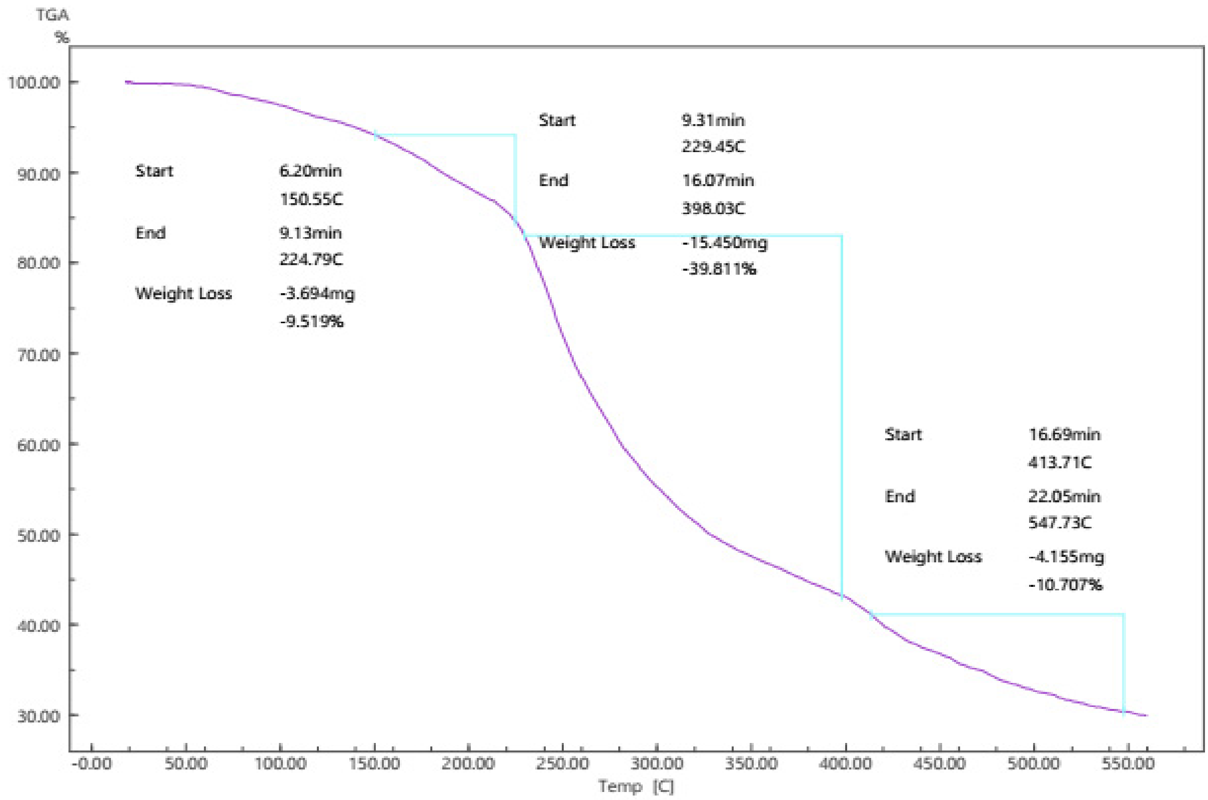

Figure 4: *Thermal degradation of encapsulated Bifidobacterium Bifidum using TGA.*

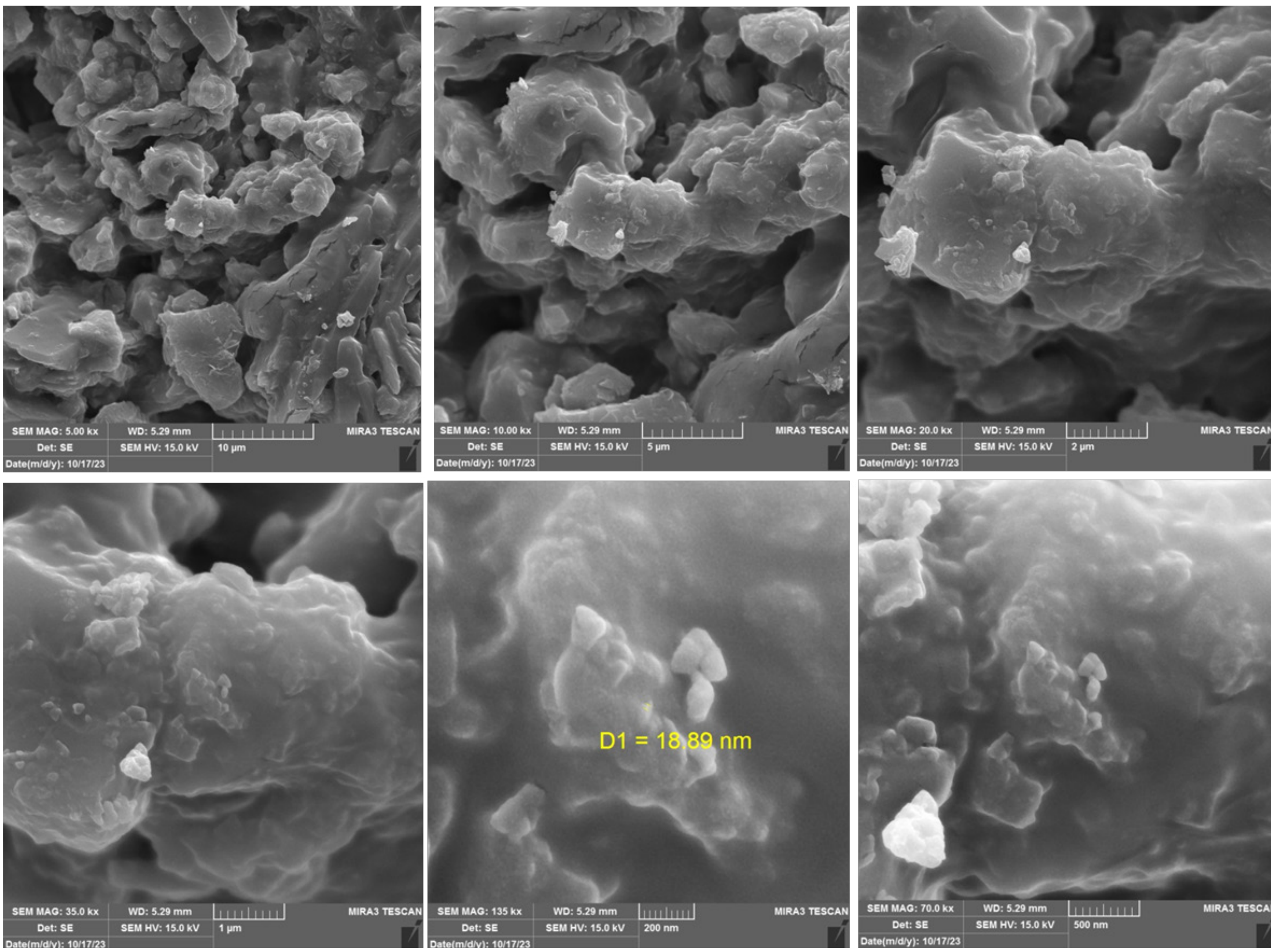

Figure 5: *Morphological analysis of microcapsules using scanning electron microscope (SEM)*

The EDS characterized the microcapsules showed major organic and mineral components as follows; carbon, 31.0%; oxygen, 42.3%; calcium, 23.7% and minor but Na, Mg, K, and Cl (Table 1 and Figure 6). The presence of high amount of oxygen and carbon confirms the predominance of carbohydrate–protein complexes, with calcium probably coming from whey, and behaving as a structural reinforcement mechanism in the form of ionic cross-links.

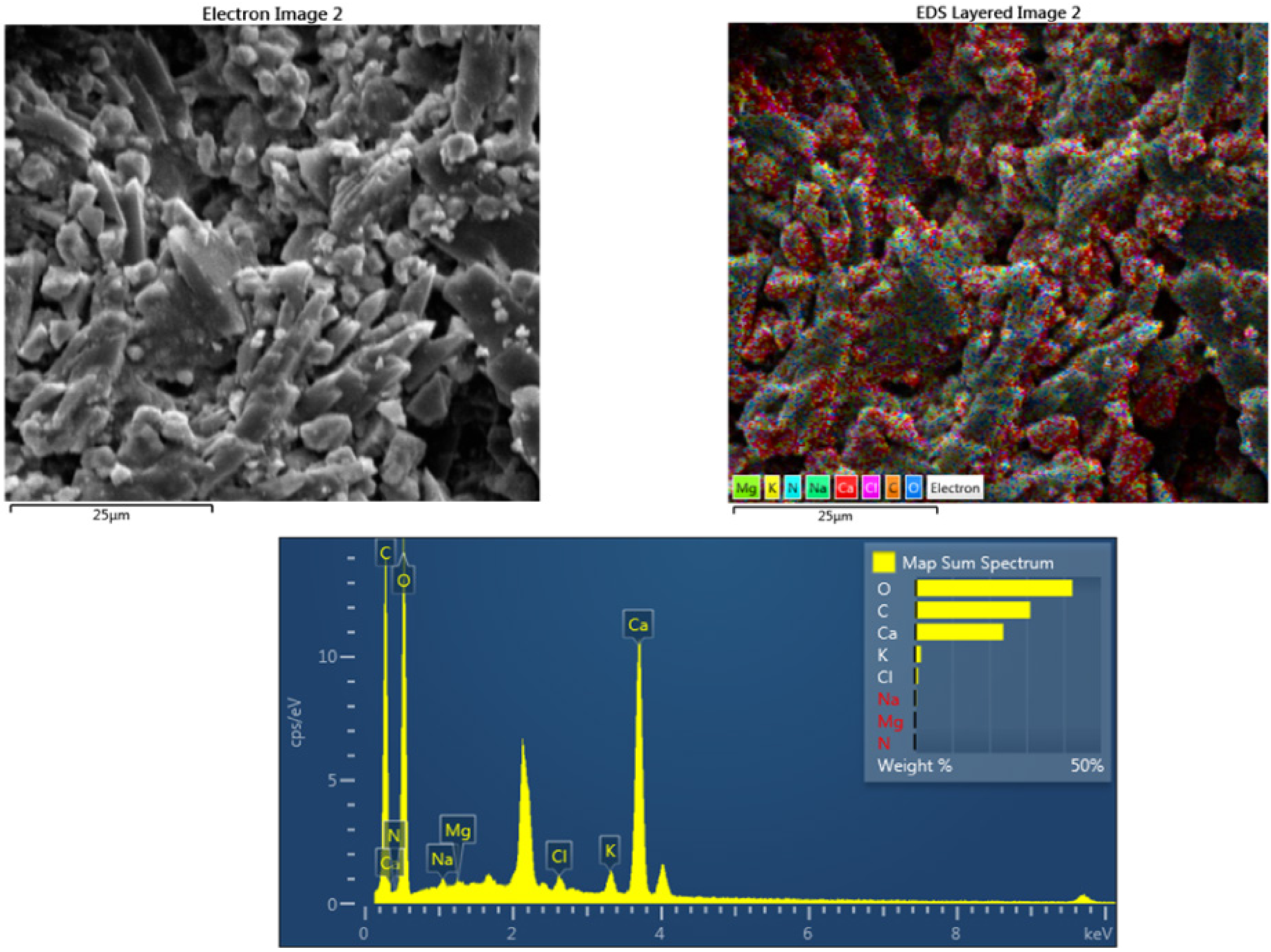

Figure 6: *Percentages of the components of the mixture of gum Arabic and dried buffalo milk whey for Bifidobacterium Bifidum envelope by EDS analysis.*

Table 1: *EDS chemical analysis of the components of the mixture of gum Arabic and dried buffalo milk whey for Bifidobacterium Bifidum.*

Element	Line type	Apparent concentration	k Ratio	Wt%	Wt% Sigma	Atomic %	Standard Label	Factory Standard
C	K series	2.47	0.02471	31.01	0.33	43.72	C Vit	Yes
N	K series	0.00	0.00000	0.00	0.00	0.00	BN	Yes
O	K series	4.08	0.01373	42.29	0.32	44.77	SiO2	Yes
Na	K series	0.06	0.00026	0.39	0.06	0.29	Albite	Yes
Mg	K series	0.02	0.00015	0.17	0.05	0.12	MgO	Yes
Cl	K series	0.11	0.00099	0.83	0.06	0.40	NaCl	Yes
K	K series	0.24	0.00204	1.61	0.07	0.70	KBr	Yes
Ca	K series	3.21	0.02871	23.70	0.21	10.01	Wollastonite	Yes
Total:				100.00		100.00		

These findings align with previous reports indicating that whey proteins migrate to the capsule surface during drying, forming protein-rich shells that enhance bacterial survival (Fyfe *et al*., 2011; Shrestha *et al*., 2007). The presence of mineral elements, particularly calcium, provides additional stability and may improve bacterial viability under gastrointestinal conditions (Nor *et al*., 2020; El-Sayed *et al*., 2021).

Conclusions

This study demonstrated the effectiveness of gum Arabic (GA) and buffalo milk whey (BMW) as natural encapsulating agents for Bifidobacterium bifidum using spray drying. FT-IR analysis revealed strong hydrogen bonding and protein–carbohydrate interactions within the encapsulation matrix, indicating the formation of a stable protective shell. TGA results confirmed high thermal stability, with bacterial degradation occurring only above 220 °C, which is well beyond spray-drying temperatures. SEM observations showed irregular, porous microcapsules with nanoscale dimensions (~18.9 nm), while EDS analysis confirmed the presence of structural minerals (Ca, Mg) and organic compounds (C, O) that enhance capsule stability.

Collectively, these findings highlight the potential of GA and BMW to significantly improve the survival, stability, and functional efficacy of B. bifidum. The encapsulation approach described here provides a promising and cost-effective strategy for the production of probiotic-enriched functional foods, nutraceuticals, and pharmaceutical formulations.

Recommendations

Based on the findings of this study, several recommendations are proposed for future research and industrial applications. The adoption of natural encapsulants, specifically gum Arabic and buffalo milk whey, should be prioritized as effective wall materials due to their demonstrated biocompatibility, cost-effectiveness, and proven ability to preserve probiotic viability. Food industries and health authorities are encouraged to integrate spray-drying encapsulation technology into probiotic production lines to enhance product shelf life and maintain functional stability. Future research should focus on optimization studies investigating the effects of varying GA:BMW ratios, spray-drying parameters, and the incorporation of additional encapsulants on bacterial viability and release kinetics. Comparative evaluation of advanced encapsulation technologies, including freeze-drying, coacervation, and nanoencapsulation, should be conducted alongside spray drying to assess their efficiency under industrial-scale conditions. Further investigation through strain-specific trials is recommended to validate this encapsulation system's efficacy with different probiotic strains and under simulated gastrointestinal conditions to confirm bioavailability. Finally, the routine application of analytical techniques such as FT-IR, TGA, SEM, and EDS should be implemented for quality control and standardization in probiotic encapsulation research and industrial production.

Acknowledgements

The authors would like to thank the Department of Food Science, College of Agriculture, University of Misan for providing the necessary facilities and support to conduct this research. Special thanks to the Department of Chemistry, University of Basrah for their assistance with FT-IR analysis.

Novelty Statement

This study presents a novel approach by combining gum Arabic and buffalo milk whey as natural encapsulating materials for Bifidobacterium bifidum using spray drying technology. The research demonstrates for the first time the synergistic protective effects of this specific combination on probiotic viability through comprehensive structural and thermal characterization using FT-IR, TGA, and SEM-EDS techniques.

Author's Contributions

Asaad Shamil Atiya: Conceptualization, Methodology, Investigation, Writing - Original Draft.
Abdulridha Ati Jaafar: Formal analysis, Data Curation, Visualization, Writing - Review & Editing.
Mustafa Adnan Idan: Resources, Supervision, Validation, Project administration.
All authors have read and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript. All content represents the original work of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Al-Ali, M.A.G. and M.K.K. Almahdawi. 2022. Effect of adding ginger roots powder and vitamin E and their synergistic interaction between them to ingredients of the pellet concentrated rations on productive performance and some characteristics of carcass of Awassi lambs. Neuro Quantol., 20(2): 215-226. https://doi.org/10.14704/nq.2022.20.2.NQ22276

Ali, A., F. Rehman and H. Bashir. 2022. Characterization of spray-dried microcapsules using FT-IR and TGA: A case study of bioactive compound preservation. Pak. J. Agric. Res., 35(4): 289-301. https://doi.org/10.17482/pjar.v35i4.654

Al-Sadi, A.M., M.J. Al-Mahmood and K.A. Al-Zubaidy. 2021. Probiotic characteristics of Bifidobacterium bifidum and its application in functional dairy products. Iraqi J. Agric. Sci., 52(3): 789-801.

Anal, A.K. and H. Singh. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci. Technol., 18(5): 240-251. https://doi.org/10.1016/j.tifs.2007.01.004

Atta, A.S., A.K. Niamah and H.I. Ali. 2025. Survival and viability of Limosilactobacillus reuteri bacteria: A comparative study between free and microencapsulated forms under gastrointestinal and thermal stress conditions. IOP Conference Series: Earth Environ. Sci., 1449(1): 012148. https://doi.org/10.1088/1755-1315/1449/1/012148

Awaisheh, S.S., M.S. Khalifeh, M.A. Al-Ruwaili, O.M. Khalil, O.H. Al-Ameri and R. Al-Groom. 2013. Effect of supplementation of probiotics and phytosterols alone or in combination on serum and hepatic lipid profiles and thyroid hormones of hypercholesterolemic rats. J. Dairy Sci., 96(1): 9-15. https://doi.org/10.3168/jds.2012-5685

Baiocco, D., J.A. Preece and Z. Zhang. 2021. Encapsulation of hexylsalicylate in an animal-free chitosan-gum Arabic shell by complex coacervation. Colloids and Surfaces A: Physicochem. Engineer. Aspect., 625: 126861. https://doi.org/10.1016/j.colsurfa.2021.126861

Burgain, J., C. Gaiani, M. Linder and J. Scher. 2011. Encapsulation of probiotic living cells: From laboratory scale to industrial applications. J. Food Engineer., 104(4): 467-483. https://doi.org/10.1016/j.jfoodeng.2010.12.031

El-Sayed, H.S., S.M. El-Sayed, A.M. Mabrouk, G.A. Nawwar and A.M. Youssef. 2021. Development of eco-friendly probiotic edible coatings based on chitosan, alginate and carboxymethyl cellulose for improving the shelf life of UF soft cheese. J. Polymer. Environ., 29(6): 1941-1953. https://doi.org/10.1007/s10924-020-01984-2

Fatima, B. 2020. Quantitative analysis by IR: Determination of Chitin/Chitosan DD. Mod Spectrosc Techn Appl. https://doi.org/10.5772/intechopen.89708

Fyfe, K., O. Kravchuk, A.V. Nguyen, H. Deeth and B. Bhandari. 2011. Influence of dryer type on surface characteristics of milk powders. Drying Technol., 29(7): 758-769. https://doi.org/10.1080/07373937.2010.538452

Garg, M.L., L.G. Wood, H. Singh and P.J. Moughan. 2006. Means of delivering recommended levels of long-chain n-3 polyunsaturated fatty acids in human diets. J. Food Sci., 71(5): R66-R71. https://doi.org/10.1111/j.1750-3841.2006.00067.x

Halwani, M., A. Al-Mahadin and S. Al-Zubi. 2008. Microencapsulation of probiotics using gum Arabic and whey proteins. J. Food Proc. Preserv., 32(4): 567-579.

Han, C., Y. Xiao, E. Liu, Z. Su, X. Meng and B. Liu. 2020. Preparation of Ca-alginate-whey protein isolate microcapsules for protection and delivery of L. bulgaricus and L. paracasei. Int. J. Biol. Macromolecul., 163: 1361-1368. https://doi.org/10.1016/j.ijbiomac.2020.07.008

Kang, Y.R., Y.K. Lee, Y.J. Kim and Y.H. Chang. 2019. Characterization and storage stability of chlorophylls microencapsulated in different combinations of gum Arabic and maltodextrin. Food Chem., 272: 337-346. https://doi.org/10.1016/j.foodchem.2018.08.038

Khan, M.U., S. Ahmad and R. Mahmood. 2023. Microencapsulation of probiotic bacteria using gum Arabic and whey protein concentrate for enhanced stability in functional foods. Pak. J. Agric. Res., 36(2): 145-156. https://doi.org/10.17482/pjar.v36i2.789

Leylak, C., K.S. Özdemir, G.C. Gürakan and Z.B.

Ögel. 2021. Optimisation of spray-drying parameters for Lactobacillus acidophilus encapsulation in whey and gum Arabic: Its application in yoghurt. Int. Dairy J., 112: 104865. https://doi.org/10.1016/j.idairyj.2020.104865

Lorenzen, P.C. and K. Schrader. 2006. A comparative study of the gelation properties of whey protein concentrate and whey protein isolate. Le Lait, 86(4): 259-271. https://doi.org/10.1051/lait:2006008

Mohammed, M.D., T.K. Karyagdi, A.M. Qneed, I.A. Jihad, Q.R. Lahhob, M. Mudhafar, H.A. Alsailawi, & A.A. Ayada. 2025. Molecular characterization and zoonotic potential of giardia species in livestock with respect to their transmission dynamics and host adaptation. J. Anim. Health Prod., 13(s1): 411–421. https://doi.org/https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.411.421

Najm, F.M., H.T. Ibrahim, R.A.A. Alsahoo, I.A. Jihad, R.M.A.Z. Qais. 2025. oRGINAL article. J. Anim. Health Prod., 13(s1): 39–48. https://dx.doi.org/10.17582/journal.jahp/2024/13.s1.39.48

Nawar, F.A. 2019. Synthesis, characterization and computational study of some thiazolidine derivatives and their complexes with Cu(II), Fe(II) and VO(II): biological effectiveness evaluation. M.Sc. Thesis, Department of Chemistry, College of Education for Pure Science, University of Basrah, Iraq.

Nor, N.M., N.H.F. Hashim, D.H.X. Quay, N.M. Mahadi, R.M. Illias, F.D.A. Bakar and A.M.A. Murad. 2020. Functional and structural analyses of an expansin-like protein from the Antarctic yeast Glaciozyma antarctica PI12 reveal strategies of nutrient scavenging in the sea ice environment. Int. J. Biol. Macromol., 144: 231-241. https://doi.org/10.1016/j.ijbiomac.2019.12.064

Rezazadeh-Bari, M., S. Sharifi, S. Amiri and H. Almasi. 2019. Microencapsulation of Lactobacillus plantarum in WPI/gum Arabic complex coacervate: Survival under simulated gastrointestinal conditions and storage stability. J. Food Process. Preser., 43(12): e14268. https://doi.org/10.1111/jfpp.14268

Shahid, M., A. Tanveer and N. Riaz. 2021. Optimization of spray drying parameters for maximum viability of encapsulated microorganisms. Pakistan J. Agric. Res., 34(3): 223-235. https://doi.org/10.17482/pjar.v34i3.512

Sharifi, S., M. Rezazadeh-Bari, M. Alizadeh, H. Almasi and S. Amiri. 2021. Use of whey protein isolate and gum Arabic for the co-encapsulation of probiotic Lactobacillus plantarum and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white cheese. Food Hydrocolloid., 113: 106496. https://doi.org/10.1016/j.foodhyd.2020.106496

Shrestha, A.K., T. Howes, B.P. Adhikari, B.J. Wood and B.R. Bhandari. 2007. Effect of protein concentration on the surface composition, water sorption and glass transition temperature of spray-dried skim milk powders. Food Chem., 104(4): 1436-1444. https://doi.org/10.1016/j.foodchem.2007.02.018

Siuta-Cruce, P. and J. Goulet. 2001. Improving probiotic survival rates: Microencapsulation preserves the potency of probiotic microorganisms in food systems. Food Technol., 55(10): 37-39.

Sun C., F. Jiang, W. Gao, X. Li, Y. Yu, X. Yin, Y. Wang nad H. Ding. 2017. Scanning electron microscopy coupled with energydispersive X-ray spectrometry for quick detection of sulfuroxidizing bacteria in environmental water samples. Chinese J. Oceanol. Limnol., 5(35), 185–191. https:// DOI: 10.1007/s00343-016-5175-1.

Tavares, L., H.L.B. Barros, J.C.P. Vaghetti and C.P.Z. Norena. 2019. Microencapsulation of garlic extract by complex coacervation using whey protein isolate/chitosan and gum Arabic/chitosan as wall materials. Food Bioprocess Technol., 12(12): 2093-2106. https://doi.org/10.1007/s11947-019-02338-8

Yin, X., L. Wang and H. Chen. 2025. Advances in Bifidobacterium bifidum research: From gut health to industrial applications. Probiotics and Antimicrobial Proteins, 17(1): 45-58 .

Zhang, Z.H., B. Yu, Q. Xu, Z. Bai, K. Ji, X. Gao and R. Xiao. 2022. The physicochemical properties and antioxidant activity of Spirulina chlorophylls microencapsulated in different ratios of gum Arabic and whey protein isolate. Food., 11(12): 1809. https://doi.org/10.3390/foods11121809