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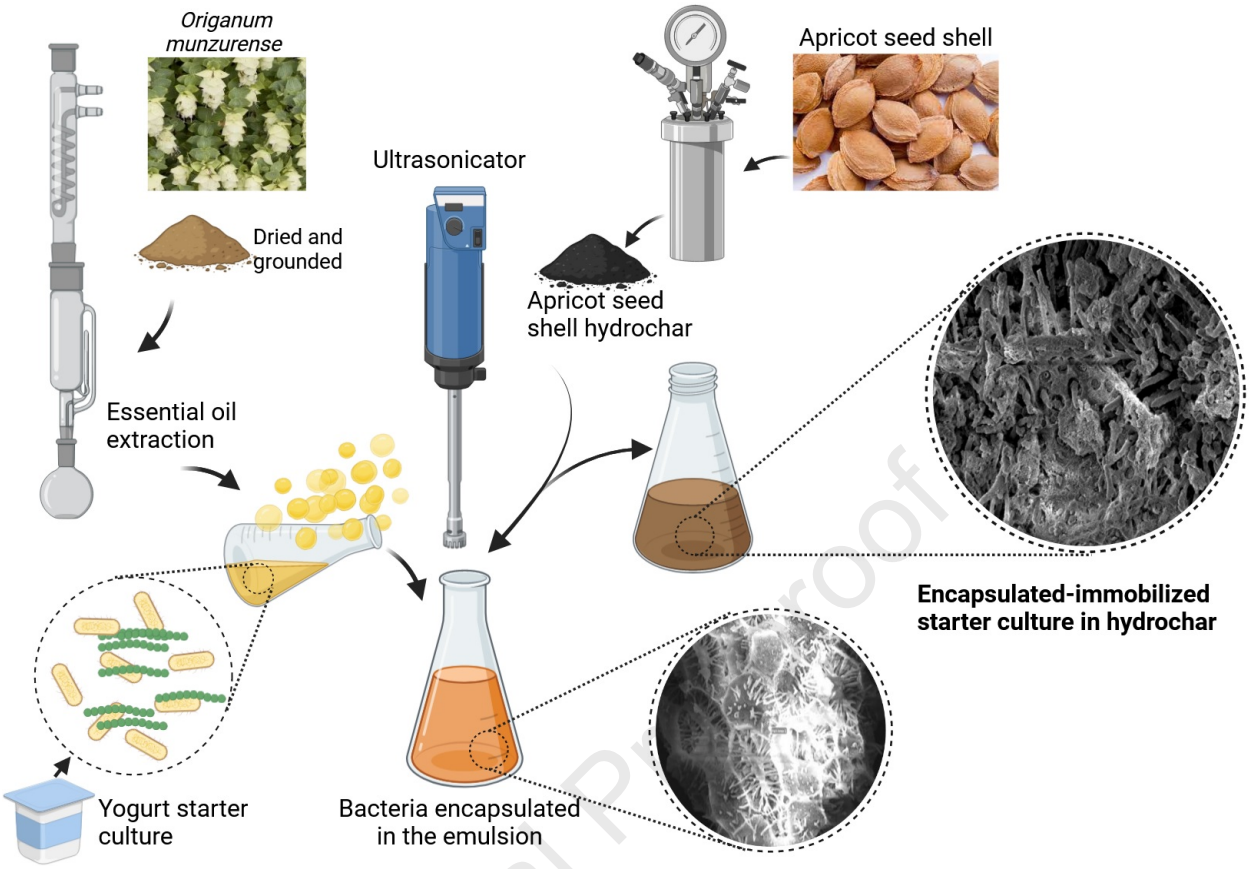


Immobilization of Yoghurt Starter Culture Encapsulated with *Origanum munzurense* Kit Tan & Sorger Essential Oil Emulsion into Apricot Seed Shell Hydrochar

Elif Cundul [1], Elif Ayşe Erdogan Eliuz [2,*] , Erdal Yabalak [3,4, **]

[1] Mersin University, Department of Chemistry, Faculty of Science, Mersin, Turkey
[2] Mersin University, Department of Seafood Processing Technology, Faculty of Fisheries, Mersin, Turkey
[3]Mersin University, Technical Science Vocational School, Department of Chemistry and Chemical Processing Technologies, Mersin, Turkey
[4]Mersin University, Department of Nanotechnology and Advanced Materials, Mersin, Turkey

Corresponding Authors: *eliferdogan81@gmail.com; yabalakerdal@gmail.com**

Abstract

The use of encapsulated starter cultures has become more important in recent years due to their improved survival and viability under adverse environmental conditions. In this study, apricot seed shell hydrochar and essential oil (EO) emulsion of *Origanum munzurenze* (*O. munzurenze*) was used in the microencapsulation and immobilization of starter culture (SC: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains). Bacteria included in EO emulsions of *O. munzurense* were immobilized on hydrochar layers using a lyophilizer. Physicochemical analyzes and surface analyzes of the final products were examined using FTIR, SEM, and EDS. The viability of encapsulated and/or immobilized SC bacteria was analysed using the colony counting method. As a result of the study, the encapsulated SCs were embedded in the layered and porous structures of the hydrochar (El-SC: immobilised SC in microemulsion into hydrochar). In EDS analysis, C, N, O, P, Na, S, and K elements were detected in encapsulated-immobilized SC (El-SC). The viability of free SC insignificantly differed ($6.5*10^7$) from the bacteria within oil emulsions ($6.6*^{107}$) within 5 h. In contrast, the colony number of El-SC was higher than free SC within 5 hours ($p \leq 0.05$). The number of live cells in El-SC at pH 2.0 was reduced as $\sim 10^1$ and $\sim 10^2$ times, respectively at the 5 h. As a result, starter culture bacteria can survive both in the emulsion and the hydrochar layers.

Keywords: Starter culture, *O. munzurenze*, Apricot seed hydrochar, Emulsion, Immobilization

1. Introduction

Hydrochar is the final product in which organic natural wastes are converted into valuable coal products known as hydrochar using the hydrothermal carbonization method (Islam et al., 2021). Hydrochar is produced from many biological materials such as kitchen waste, food waste and plants (Yabalak and Erdogan Eliuz, 2022). Biotechnologically, hydrochar is widely used as a soil conditioner in mostly degraded and polluted soils. Its porous and layered structure is thought to act as a micro-environment for microorganisms. Therefore, it can be argued that it supports the development of soil microflora (Thunshirn et al., 2021). Hydrochars are widely used in industrial applications such as soil amendment, stabilizers and carbon sequestration due to their advantages such as cation exchange capacity and efficient surface area (Yabalak and Erdogan Eliuz, 2022). Hydrochar production takes place in a water medium (subcritical water medium) under high temperature (373 - 647 K) pressure conditions. A higher yield of hydrochar can be achieved in the subcritical water medium compared to other conventional methods such as torrefaction, pyrolysis or gasification (Saleh et al., 2021; Yabalak and Elneccar, 2021).

There is a limited number of studies on the use of biochar or hydrochar as food additives or food. A small number of studies paved the way for biochar to be used in animal food formation. Although the use of biochar as a feed additive for animals entering the human food chain has been removed from the Food and Drug Administration's list of approved additives in the United States, the California Department of Food and Agriculture has permitted the use of biochar in animal feed. The use of biochar in animal feed is accepted in many countries, including Europe, Australia, Canada and Japan (Schmidt et al., 2019). After consumption and processing as food, apricot peels are thrown away as garbage or burned. A rare number of studies have been conducted on the micromorphology, phases and pore structure of the shell structure as hydrochar (Kabakcı et al., 2019; Zhang et al., 2022).

Essential oils obtained from *Origanum* species have some therapeutic effects, especially choleretic and antimicrobial effects, due to their various chemical and aromatic properties. Some of them are used in agriculture, pharmaceutical, and cosmetics industries,

for flavouring foodstuffs, making perfume and the soap industry (Leyva-López et al., 2017; Knez Hrnčič et al., 2020). *Origanum* species, which are about 50 species in the world, mostly spread in the Mediterranean region and the Balkans. They are perennial herbaceous or semi-shrub plants with more than one erect stem, and their flowers are clustered or clustered at the stem ends (Marrelli et al., 2018). *O. munzurense* is one of the most traditionally used endemic species found in Turkey (Güner et al., 2000; Özhatay et al., 2011). Due to its aromatic properties, essential oils and phenolic substances, it is widely used as tea, as a food additive and in traditional medicine (Yabalak et al., 2020).

Encapsulation is a technology that protects the active ingredient by covering solid, liquid and gaseous materials and keeping them in capsules, allowing them to be released under certain conditions and speeds (Pegg and Shahidi, 2017). Thus, it is aimed to protect and process the immobilized active material. Microencapsulation has taken place in different fields such as pharmaceutical, nutraceutical, pharmaceutical, food, paper, and cosmetics sectors. In the food industry, microcapsules in solid form are used as food additives and supplements (Nesterenkoa et al., 2013). Among the microencapsulation techniques, nanoemulsion increases the controlled release of essential oils and stabilizes their biological activity (Ghadetaj et al., 2018; Pirozzi, et al., 2020; Hou et al., 2021)). As a nanoencapsulation technique, the nanoemulsion method is a kinetically fixed encapsulation of the core material in two immiscible liquids. It has advantages of use in fields such as medicine, chemistry and cosmetics, especially in food (Solans et al., 2005; Karimirad et al., 2018; Kour et al., 2022).

Various carrier materials and preparation techniques are being investigated for the encapsulation of probiotics. Food-grade polymers such as alginate, chitosan, pectin, carrageenan, whey, gelatin, and lipids have been extensively studied to immobilize bacteria (Anal and Singh, 2007). Extrusion and emulsion techniques are widely used, but problems such as low mechanical stability and lifelessness have been observed in these techniques (Li et al., 2016). Encapsulation has been reported to be one of the best approaches to achieve the symbiotic effect of probiotic bacteria and enzyme-resistant starch (Fuentes-Zaragoza et al., 2011). Several approaches are being explored to increase the viability of probiotic bacteria in commercial and tested products, including the selection of acid and bile-resistant strains, the use of oxygen-tight containers, stress adaptation, and microencapsulation (Shah, 2000). Studies in which lactic acid bacteria are coated with essential oil emulsions are not common in literature. Hou et al. succeeded in trapping bacteria in nanoemulsion using sesame oil (Hou et al., 2003). In a study conducted by Tzen et al., (1998) the successful encapsulation of

probiotics in sesame oil emulsions showed that probiotics were randomly distributed in the oil matrix and remained viable. In this study, yoghurt culture was included in *O. munzurenze* essential oil emulsions.

Many beneficial bacteria such as lactic acid bacteria, which can be found as probiotics in these foods, support the protection of human health (Kavitake et al., 2018). Probiotic lactic acid bacteria strains are preferred due to their ability to survive in the gastrointestinal tract and colonize the intestinal tract. One of the most important properties of probiotics is that they protect against pathogens in the host's intestinal tract. In addition to all these, probiotics can be widely used in many fields such as pharmaceuticals in pharmacology (Ranadheera et al., 2019). The most common and valuable fermented milk product is yoghurt. Yoghurt is a probiotic fermented dairy product and has highly digestible proteins. Therefore, yoghurt bacteria are very important for human nutrition. The antimicrobial effect of yoghurt bacteria on pathogens also increases the importance of yoghurt probiotics (Yerlikaya et al., 2021).

This study aimed to embed immobilized bacteria in hydrochar cavities, which is a strong absorbent with a porous structure. Here, the immobilization of bacteria into microemulsion has been studied and *O. munzurense* essential oil was used as an important encapsulation material in bacterial stability. The presence of essential oils will also enrich the material in fatty acids and increase its nutritional value. In summary, this study involves the entrapment of starter culture in *O. munzurense* essential oil emulsion and subsequent immobilization into apricot hydrochar pores. The viability of the final products, in other words, SC bacteria in encapsulated and/or immobilized form ((EI-SC), was analysed using the colony counting method. Physicochemical analyses and surface analyses of encapsulated-immobilized bacteria were examined using FTIR, SEM and EDS analyses.

2. Material and methods

2.1. Materials and Apparatus

Stainless steel reactor (fixed bed high-pressure reactor, domestically made), Sieve shaker (SCoratory type), Ultrasonic homogenizer (Baandelin Sonoplus, Typ: UW3200, Probe: KE 76), Millipore Milli-Q Advantage A10 pure water device (Darmstadt, Germany), FT-IR (Jasco 6700), SEM (ZEISS SUPRA 55), Eliza spectrophotometer (Thermo Scientific, MULTISKAN, Finland), Autoclave (Core OT 40L), incubator (Core EN 055), Precision balance (Citizon, CX220, Jadever), Sterilizer (Nuve FN 500), Shaker (IKA®C-MAG HS7),

Syringe filters 0.45 μm (Agilent, Santa Clara, CA, USA) were used. Man, Rogosa, and Sharpe (MRS) agar (Darmstadt, Germany) were used for bacterial growth.

2.2. Microemulsion of *O. munzurense* essential oil

O. munzurense plant was collected from Tunceli-Hozat/Turkey region and identified by Prof. Dr. Ayşe Everest (2017). The samples were dried in a cool room away from sunlight and are stored in the research herbarium of Mersin University Department of Biology. *O. munzurense* essential oil was extracted using a Clevenger-type apparatus for 4 hours. The essential oil was transferred from the assembly to a 10 mL tube. The remaining oil on the tube was removed with a pipette. Sodium sulfate was added to the tube from which the oil was taken and centrifuged (Yabalak et al., 2021). In the study, the essential oil (100 μL) was slowly added to distilled water (1 mL) in and Tween 80 (up to 30 % v/v) of the oil and emulsified for 3 minutes at a mixing speed of 10000 rpm to obtain an emulsion. Next, the first emulsion was nano-emulsified with a probe sonicator operating at 20 kHz and 200 W for 15 minutes (Hemmatkhah et al., 2020).

2.3. Hydrochar synthesis from apricot seed

Apricot was purchased from a local market in Mersin. Hydrochar synthesis was carried out similarly to the method reported by Yabalak and Erdogan Eliuz as described below (Yabalak and Erdogan Eliuz, 2022). A certain amount of biomass was taken into the high-pressure and temperature-resistant stainless steel reactor and pure water was added to it. By closing the reactor, its internal pressure was increased to 100 bar with nitrogen gas and its temperature to 240 °C. At the end of the 1 h, the reactor was cooled and the internal pressure was reduced to atmospheric conditions, and the hydrochar formed was dried in an oven set at 97 °C. Then, the hydrochar size was reduced below 100 μm with the help of an automatic sieve. The resulting hydrochar was sieved and stored in dark, capped bottles for further use.

2.4. Preparing of starter culture

Yoghurt starter culture (SC) (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains) were purchased from BüyüYo (DANEM milk and dairy products food industry company, Isparta) in Türkiye. The viability of the cultures was checked by sowing on MRS agar. Ready-made cultures were grown anaerobically at 37 °C for 48 h in 10 mL of MRS broth (without ammonium citrate or sodium acetate). SC suspensions were spread onto the surfaces of modified MRS agar plates and were incubated for 48 h at 37 °C in anaerobic conditions. The glass tubes (MRS broth) and petri dishes (MRS Agar) which are inoculated bacteria were placed in the jar with candles. The candle was burned and the lid tightly closed, thus initiating anaerobiosis (Haldar et al., 2017; Gezginc et al., 2015; Uzunsoy et al., 2023).

2.5. Encapsulation of SC with *O. munzurenze* EO Emulsion

Firstly, a microemulsion solution of *O. munzurenze* EO was prepared by combining 100 µL of tween 80 and 100 µL of *O. munzurense* and mixed with an ultrasonic stirrer. To coat the starter culture with the EO: The microemulsified EO was mixed with 100 µL of SC solution, which was previously adjusted to McFarland 0.5, and all samples were mixed with the ultrasonic stirrer for 10 minutes. Microemulsions without SC were used as controls (0 % v/v). The final samples (Encapsulated starter culture: EnSC) were stored in a cabinet (+4 ºC) for experiments and all experiments were done in 3 replicates (Hemmatkhah et al., 2020; El-Sayed and El-Sayed, 2021).

2.6. Immobilization of EnSC on apricot seed hydrochar surface

Before using the hydrochar, they were sterilized with UV in a laminar cabinet for 15 minutes. Then, certain amounts (0.01 g) of the hydrochar were added to the polystyrene tubes containing the encapsulated bacteria with the microemulsion. It was mixed with a vortex until the hydrochar had absorbed all the emulsion. The finalized samples were remixed with an ultrasonic stirrer and dried with the aid of a lyophilizer and stored for experiments. Before lyophilizer treatment, samples were stored at -80 ºC for one day and then dried in a

lyophilizer for 24 hours. The final sample (Immobilized EnSC: ImEnSC) was then stored at +4 ºC (Kınacı et al., 2023).

2.7. Comparative characterization analysis of immobilized/encapsulated SC

After drying of ImEnSC, the preparations were analyzed using the SEM microscope, FTIR, and EDS according to Kınacı et al. (Kınacı et al., 2023).

2.7.1. SEM Analysis of Immobilized/Encapsulated SC

ASH Apricot seed hydrochar, EnSC Encapsulated starter culture (SC in microemulsion), ASH*SC Immobilized starter culture (SC immobilised to hydrochar) and El-SC encapsulated-immobilized SC (immobilise of SC in microemulsion to hydrochar) were dried at a 'critical point' in liquid CO_2 under 60-mbar of pressure before microscopic imaging and made platin-covered by spraying (EMITECH K850), Quorum 150 R ES). Then, the preparations were analyzed using the SEM instrument. The best images were selected from a large number of micro-scale images.

2.7.2. FTIR Analysis of Immobilized/Encapsulated SC

FT-IR spectra of ASH and EI-SC were analysed using FT-IR in ATR mode and the spectral measurement range was 4000–500 cm^{-1}. Bioactive compounds such as acyl groups, aromatic and phenyl in ASH, and EI-SC were determined according to the FT-IR spectra (Yabalak and Erdogan Eliuz, 2022).

2.7.3. EDS analysis of immobilized/encapsulated SC

The elemental composition of ASH, EnSC, ASH*SC, and EI-SC were evaluated by energy-dispersive spectroscopy. This analysis was performed to identify the C, N, O, Na, P, and K composition found on ASH, EnSC, ASH*SC, and EI-SC. Analysis was performed by sending a scanning electron beam onto the sample.

2.8. Comparative viability control in immobilized/encapsulated SC

SC cell counts were calculated by counting colonies on MRS agar plates and expressed as colony-forming units per mL (CFU/mL). For the experiment, 0.1 g of the SC, EnSC, and EI-SC preparations were taken and suspended in 9.9 mL of sodium citrate solution (0.06 mol/L), mixed for complete homogenization and serially diluted in ¼ strength Ringer's solution. Survival of cells in SC, EnSC, and EI-SC was determined by spreading on MRS agar and colony monitoring after 72 hours of incubation at 37 °C under anaerobic conditions (Hou et al., 2003).

To mimic high acid stomach conditions (gastrointestinal conditional), the pH of the MRS agar medium is adjusted to 2.0 or 3.5, and cells in the nanoemulsion are planted in this medium and incubated at 37 °C. Colony counts as logarithmically and percent reduction (P: Eq.1) are performed at the end of 24-48 hours (Vinderola et al., 1999; Dimitrellou et al., 2019).

$$\text{P, Reduction \%} = \frac{(A-B)\ X\ 100}{A} \qquad \text{Eq. 1}$$

where A and B indicate the starter population and the last population counted after treatment, respectively. P indicates Percent (%) reduction.

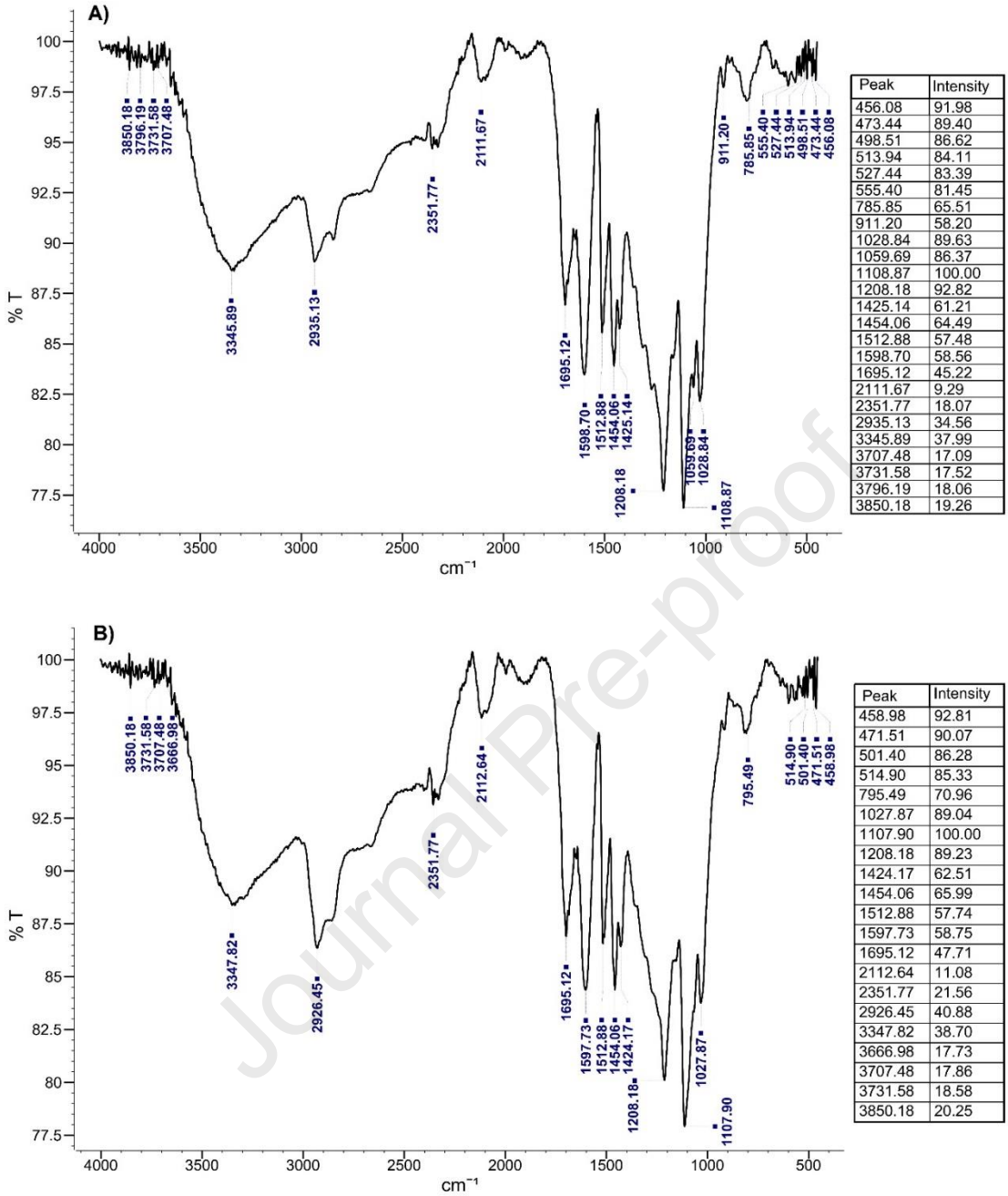
2.9. Statistical analyses

Statistical analyses of all experiments were performed by One-way ANOVA with post-hoc Tukey HSD Test (p<0.05).

3. Results and Discussion

3.1. FT-IR, SEM, and EDS Analysis of immobilized EnSC on the hydrochar surface

The efficiency of the EnSC in *O. munzurense* incorporated apricot seed hydrochar was evaluated by FT-IR and SEM analyses. As can be observed, bands of similar numbers were exhibited in ASH (Fig. 1, A), while in EI-SC (Fig. 1, B).



Peak	Intensity
456.08	91.98
473.44	89.40
498.51	86.62
513.94	84.11
527.44	83.39
555.40	81.45
785.85	65.51
911.20	58.20
1028.84	89.63
1059.69	86.37
1108.87	100.00
1208.18	92.82
1425.14	61.21
1454.06	64.49
1512.88	57.48
1598.70	58.56
1695.12	45.22
2111.67	9.29
2351.77	18.07
2935.13	34.56
3345.89	37.99
3707.48	17.09
3731.58	17.52
3796.19	18.06
3850.18	19.26

Peak	Intensity
458.98	92.81
471.51	90.07
501.40	86.28
514.90	85.33
795.49	70.96
1027.87	89.04
1107.90	100.00
1208.18	89.23
1424.17	62.51
1454.06	65.99
1512.88	57.74
1597.73	58.75
1695.12	47.71
2112.64	11.08
2351.77	21.56
2926.45	40.88
3347.82	38.70
3666.98	17.73
3707.48	17.86
3731.58	18.58
3850.18	20.25

Fig. 1. FT-IR spectra of ASH(A) and EI-SC (B)

Fig. 1 represents the comparative FTIR fingerprint of ASH and EI-SC. The fingerprint in the region 400-3850 cm^{-1} was generally similar for samples, the main differences were observed in peak intensities, the EI-SC have slightly higher intensities of peaks from 1695.12 to 2926.45 cm^{-1}. These regions were identified as C-H stretching at 2935.13 cm^{-1} (34 %) and 2926.45 cm^{-1} (40 %), in ASH and EI-SC, respectively. Similarly, O=C=O stretching at 2351.77 cm^{-1} (18 %) and 2351.77 cm^{-1} (21 %); N=C=S stretching at 2111.67 cm^{-1} (9%) and 2112.64 cm^{-1} (11 %); C-H bending aromatic compound at 1695.12 cm^{-1} (15 %) and 1695.12 cm^{-1} (47 %) were detected in ASh and EI-SC, respectively. Dziuba et al. (2007) analysed

lactic acid bacteria and found mean intensity values of 4.3 and 5.1 for *Streptococcus* and *Lactobacillus* species between 1500 and 3100 cm^{-1}, respectively. This may overlap with the increased density in the EnInSC sample. *O. munzurenze* essential oil, which is in microemulsion, may be dispersed in the hydrochar, possibly as peaks smaller than below 1000 cm^{-1}. It is known that there are compounds in the C-H bending structure whose essential oil microemulsions have very weak peaks. In a study, thymol nanoemulsion did not show any noticeable peak in the FTIR spectrum (Kumari et al., 2018). The bands below 1000 cm^{-1} in ASH and EnlmSC were attributed to isoprenoids (C–O) and many bands of deformation of C–H were present.

In both samples, intensities, and bands very close to each other were determined. O-H stretching (intermolecular bonded) at 4000-3000 cm^{-1} (3850.18, 3796.19, 3731.58, 3707.48, 3345.89 cm^{-1} in ASH; 3850.18, 3731.58, 3707.48, 3666.98, 3347.82 cm^{-1} in EnlmSC), CN at 1598.70 cm^{-1} in ASH and 1597.73 cm^{-1} in EnlmSC; C-N stretching at 1512.88 cm^{-1} in ASH and 1512.88 cm^{-1} in EnlmSC; bending alkane methyl group at 1454.06 cm^{-1} in ASH and 1454.06 cm^{-1} in EnlmSC; acid or ester at 1208.18 cm^{-1} were detected in both samples. Amino acid or tertiary alcohol (O–H and N–H) at 1108.87 and 1107.90 cm^{-1} in ASH and EI-SC, respectively, and C-O stretching primary alcohol at 1059.69 cm^{-1} were detected in ASH (Thummajitsakul et al., 2020; Erdogan Eliuz and Yabalak, 2022).

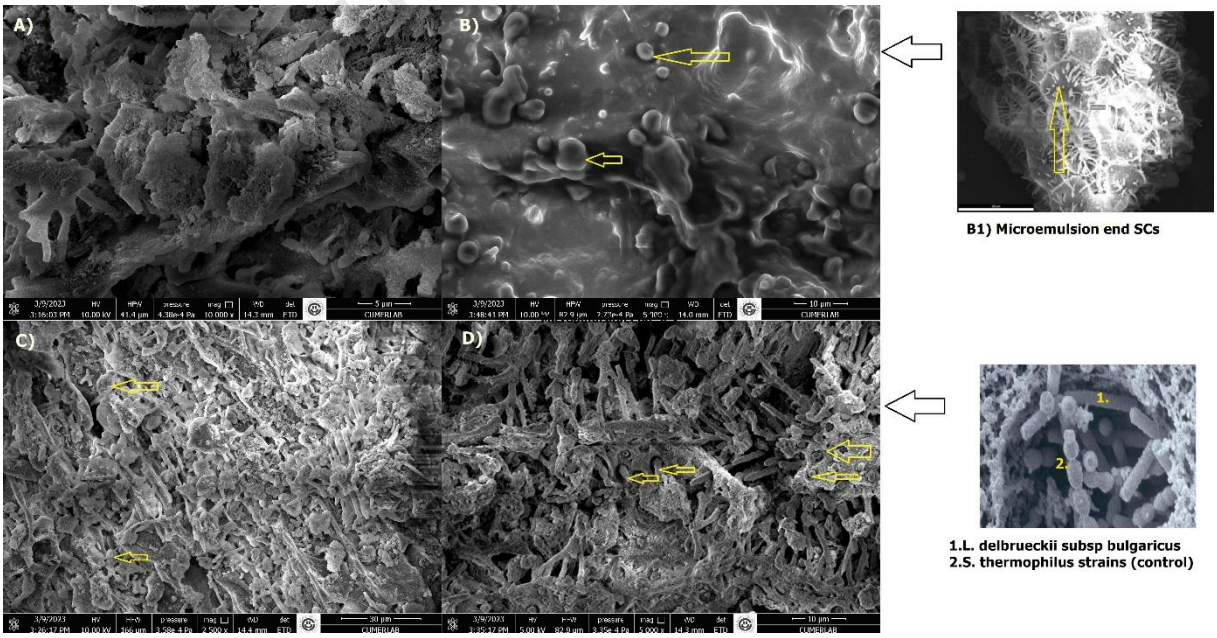


Fig. 2. SEM images of ASH (A), EnSC (B), ASH*SC (C), and EI-SC (D) with different magnification ratios.

SEM images of ASH, EnSC, ASH*SC, and EI-SC with different magnification ratios are shown in Fig. 2. It was observed that the shape of the ASH sample is irregular in size, layered, and disorganized (A). The SCs appeared (yellow arrow) to be intertwined with the *O. munzurense* EO microemulsion as a closed and amorphous structure (B). Similarly, Perumalsamy et al. (2022) observed the topographic structure of the nanoemulsion obtained from *Origanum vulgare* essential oil as spherical structures with agglomeration. Occasional bacterial breaks in the microemulsion structure were also observed, which may be due to the partial inhibition effect of the nanoemulsion on bacteria. The antimicrobial effect of *Origanum* species has been previously reported (Bhargava et al., 2015). SCs settled in the layered and porous structures of the hydrochar and appeared in the form of individual cells in some places and clusters in others (C). SCs suspended in the microemulsion, on the other hand, retained their cellular forms by clinging to the structure of the hydrochar (D). The layered structure of biochar, which is mostly used in wastewater studies, is also an important adsorbent because it creates physical advantages such as nutrient retention and collection between layers (Yang et al., 2020; He et al., 2022). In general, biochars are known to absorb bioactive compounds well, as they are a strong absorbent. For this reason, there may have been physicochemical processes between the bioactive agent and the hydrochar, which interpenetrated and thus increased the cohesion (Komnitsas and Zaharaki 2016).

As a result of the elemental composition analysis, the composition percentages of the ASH (A), EnSC (B), ASH*SC (C), and EI-SC (D) were defined. When the EDS analyses of ASH, EnSC, ASH*SC, and EI-SC were examined, many different element types were found. The surface area and the number of components it contains are very important in determining the application potential of hydrochar.

Table 1. Weight (W %) and Atomic (A %) rates of ASH, EnSC, ASH*SC, and EI-SC

Elements	**Samples**							
	ASH		**EnSC**		**ASH*SC**		**EI-SC**	
	W %	A %	W %	A %	W %	A %	W %	A %
C	60.16	66.32	62.15	68.16	60.73	66.86	56.79	64.62
N	6.07	5.74	5.74	5.4	5.74	5.12	7.67	7.48
O	33.77	27.95	32.11	26.44	33.53	27.72	28.83	24.62
Na							2.67	1.59
P							1.76	0.78
S							1.44	0.61
K							0.84	0.29
Total	100							

In the elemental analysis, C, N and O were determined in similar proportions in all samples. The total C values of ASH, EnSC, ASH*SC and EI-SC were found to be 60.16, 62.15, 60.73 and 56.79 %, respectively. The N value in all samples varied between 5 and 7 %. The highest O value was found in ASH at 33.77 %, while the lowest O value was observed in EI-SC at 24.62 %. Na, P, S, and K elements determined the hydrochar, starter culture bacteria and emulsion were together. This indicates that there are some biochemical processes in the EI-SC complex.

In literature, elemental analysis of biochar and hydrochars is carried out using many spectroscopic methods. Elemental analysis of biochar and hydrochars is carried out using many spectroscopic methods. For example, C, N, O, H, N, S could be detected from biochars obtained from *Brassica napus*, *Picea glauca*, *Triticum aestivum* plants (Nzediegwu et al., 2021). Among these elements, only C, N, and O were detected in ASH in this study. In addition, A high O content (about 20 %) was found in hydrochar derived from orange peels (Espro et al., 2021). With this report, oxygen value was found more than 33.77 % in ASH. As a mechanism, the high oxygen content has been associated with the surface retention of many residual oxygenated groups at the low temperature of hydrothermal carbonization (Espro et al., 2021). The presence of strong peaks of C (62.15 %), N(5.74 %), O (32.1 %) in EnSC, and C (60.73 %), N(5.74 %), O (33.53 %) in ASH*SC bacteria and hydrocarbon structures of essential oils are supported. *O. munzurenze* essential oil content was analyzed by the GC-MS method in our previous study. Accordingly, the main components of the essential oil 4-

hydroxy-3-methylbenzaldehyde (44.84 %), thymol (14.59 %), carvacrol (6.42 %) and *p*-cymene (4.32 %) were detected (Yabalak et al., 2020). These compounds consist of hydrocarbon chains containing C and H (Eslahi et al., 2017).

3.2. Survival rate of immobilized EnSC on the ASH surface

The survival rate of encapsulated/immobilized and free SC stored at 10 °C is shown in Table 2. The free cells dropped from 1.5×10^8 to 6.5×10^7 cfu/mL, whereas the viable cell count of microencapsulated SCs dropped from 1.3×10^8 to 6.6×10^7 cfu/mL over a storage period of 5 h. The encapsulated-immobilized SC cells dropped from 1.1×10^8 to 7.2×10^6 cfu/mL in the same conditions. The viability of free SC was insignificantly different (6.5×10^7) than bacteria within oil emulsions (6.6×10^7) within 5 h. In contrast, the colony number of encapsulated/immobilized SC was higher than free SC within 5 h ($p<0.05$). In the calculation made based on the starter population, the highest percentage of inhibitions (99.9 and 98.9 %) was seen at the 5th hour and in pH 2 and 3.5 pH environments, respectively.

Table 2: Viability (CFU/mL) and reduction (%) of encapsulated SCs (EnSC), encapsulated-immobilized SCs (EI-SC) and free SC during storage at 10 ˚C.

	Free SC				**EnSC**				**EI-SC**			
hr	**CFU/mL**		**%**		**CFU/mL**		**%**		**CFU/mL**		**%**	
0	1.5×10^8		$0^a\pm0$		1.3×10^8		$13.3^a\pm0.3$		1.1×10^8		$26.6^a\pm0.1$	
1	1.3×10^8		$13.3^b\pm1.1$		7.7×10^7		$48.6^b\pm2.4$		1.1×10^8		$26.6^a\pm2.8$	
3	6.6×10^7		$56^c\pm2.3$		7.1×10^7		$52.6^b\pm2.6$		5.9×10^7		$60.6^b\pm5.9$	
5	6.5×10^7		$56.6^c\pm2.2$		6.6×10^7		$56^b\pm3.7$		7.2×10^6		$95.2^b\pm5.1$	
	Incubation time (1 and 5 h)											
pH	**1 h**		**5 h**		**1 h**		**5 h**		**1 h**		**5 h**	
	CFU/mL	**%**	**CFU/mL**	**%**	**CFU/mL**	**%**	**CFU/mL**	**%**	**CFU/mL**	**%**	**CFU/mL**	**%**
2.0	1.3×10^8	13.3 ±0.1	1.1×10^5	99.9 ±2.8	7.8×10^7	48 ±0.3	7.7×10^6	94.8 ±2.2	1.3×10^8	13.3 ±0.3	1.9×10^6	98.7 ±0.3
3.5	1.5×10^8	0±0	1.9×10^7	87.3 ±2.4	7.9×10^7	47.3 ±0.3	1.6×10^6	98.9 ±4.1	8×10^7	46.6 ±0.1	6.9×10^7	54 ±0.3

Starter population*: 1.5×10^8 Statistical differences are indicated by a different column in each line for Percent inhibition.

Encapsulated-immobilized SC and free SC were separately exposed to *in vitro* simulated gastrointestinal environment conditions including high-acid to evaluate the potential durability of bacteria in acidic conditions. The number of live cells of encapsulated and encapsulated-immobilized SCs at pH 2.0 was reduced as ~10^1 and ~10^2 times, respectively at the 5 h. The live cell number of free SC was significantly reduced (~10^3) at the end of the 5 h at pH 2.0, compared with that at pH 3.5. Similarly, the reduction was also seen in encapsulated (~10^1) and encapsulated-immobilized (~10^2) SCs, but not more than free SCs.

The viability of encapsulated SC was 1.3×10^8 and 6.6×10^7, while the viability of encapsulated-immobilized SC was 1.1×10^8 and 7.2×10^6, at the 1 and 5 h, respectively.

In our study, although a decrease was observed in both free bacteria and immobilized bacteria, overall bacterial viability continued stably. This is because the essential oil emulsion or hydrocarbon does not have a permanent inhibitory effect. Hou et al., (2013) showed that bacteria in an essential oil emulsion reproduce over time. They also confirmed the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* stored at 4 °C for 16 days increased significantly from 0.023 % to 5.45 % after sesame oil emulsion encapsulation (Hou et al., 2013). In contrast, another study reported that lactic acid bacteria count was significantly affected after both essential oil/pectin coating and storage treatments (Gedikoglu 2022). Similarly, essential oil/hydrochar coating reduces bacterial viability to some extent but does not inhibit it completely. Possibly this may be related to how tolerant the bacteria are to the compounds present in the emulsion or to pH. It is clear that the presence of volatile compounds and microbial-based enzymatic activity will determine the viability of bacteria (Fang et al., 2018; Xiong et al. (2020). Therefore, the inhibition may have occurred from a symbiotic interaction of the microemulsified essential oil alone or together with the hydrochar. Hydrochar has some antimicrobial properties (Yabalak and Erdogan Eliuz 2022). The antimicrobial effect of Origanum essential oil and its emulsion forms has been known for a long time (Yabalak et al., 2020; Zeybek et al., 2023). SCs immobilized to the hydrochar structure were found to have a very high potential to survive at the end of incubation. This showed that SCs use hydrochar as a host and benefit from its micronutrients.

4. Conclusion

In this study, *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus* strains were encapsulated with *O. munzurenze* EO microemulsion. The resulting complex was immobilized on apricot seed hydrochar. In the elemental analysis of the final products (EI-SC); C, N, O, P, Na, S, and K elements were detected. It was determined that EI-SC could survive for 5 h under normal conditions and in an acidic environment. These findings show that the yoghurt starter culture maintains its viability in emulsions and its stability is preserved in hydrochar pores. Considering the bioavailability of the starter culture, the viability of bacteria settling in hydrochar layers by binding to the microemulsion may be an advantage in areas such as food coating and preservation of probiotics. Possible food shortages in the future and difficult agricultural conditions due to climate change necessitate sustainable

development. Some bacteria can be cultured continuously and hydrochars that can be produced from waste. Therefore, the ability to utilize both important materials are important resource for sustainable waste evaluation. The resulting complex can currently be used as a feed additive or for nutrient enrichment of the soil. In the future, new ideas may emerge in many areas such as food coating and drug development. There is a need for more detailed studies on the development of applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Highlights

Origanum munzurenze essential oil was microemulsified and combined with yoghurt bacteria.

The colony count method was used to determine that most of the bacteria lived in the emulsion.

The starter culture incorporated into the emulsion was then immobilized in the hydrochar layers.

Immobilized bacteria continued to survive even at the 5. h of incubation.

The study showed that the nanoemulsion and hydrochar did not inhibit the starter culture.

Hydrochar rich in fatty acids and probiotics have been developed.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: