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Research Article

Investigation of bioencapsulation possibilities of pithaya fruit extract with *Saccharomyces cerevisiae* yeast cells

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Abstract: Bioencapsulation is a technique that has been frequently studied in recent years, with the use of different yeast cells in encapsulation. The low cost, environmentally friendly, and non-toxic nature of yeast cells has enabled them to find applications as encapsulation agents in addition to their well-known uses in the food and fermentation industry. In this study, lyophilized powder extracts from pithaya fruits were encapsulated with plasmolyzed and non-plasmolyzed *Saccharomyces cerevisiae* yeast cells and bioactive tests, SEM, and FTIR analyses were performed. In addition, different concentrations of ethanol: water solvent media were tested for encapsulation. DPPH inhibition values of the capsules were found between 5.08% and 25.53% and the highest value was found in the encapsulation method performed with non-plasmolyzed cells in 80:20% ethanol medium. Similarly, ABTS inhibition values were determined as 1.91-6.13µg Trolox eq/g, and the highest value was found in the encapsulation method performed in 80:20% ethanol medium with non-plasmolyzed cells. Shrinkage and deformations in plasmolyzed cells were observed in SEM images and clearer images of capsules were obtained in non-plasmolyzed yeast cells. In the FTIR analysis of the fruit extract, the sharp peak obtained at approximately 2950 cm^{-1} indicated the presence of galacturonic acid and this band intensity decreased in encapsulated samples. Our study showed that yeast cells can be used in encapsulation for the preservation of low-stability components such as betacyanin and betanin in pithaya and more successful results were obtained in non-plasmolyzed yeast cells.

1. INTRODUCTION

Bioactives are substances with physiological or pharmacological actions that can improve human health, and they are derived from either plant or animal sources. Since bioactive substances exhibit a certain degree of instability when exposed to environmental conditions, encapsulation is seen to be a useful technology for preventing bioactive ingredients in food products from being exposed to outside influences and controlling their release in specific conditions (Qazi *et al.* 2025). Bioactive particles, ingredients, or products, which usually include a core material of interest coated by a secondary layer, can be developed through

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encapsulation processes. Encapsulation can provide controlled release of the core material, protection against non-specific chemical interactions, ease of handling and transport, and simple separation from matrices to the entire composite particle, depending on the coatings' qualities (Ayyaril *et al.* 2023). In food industries, there are various encapsulating materials such as proteins, lipids, starches, phospholipids, waxes, and some microorganisms such as yeast cells. Encapsulation in yeast cells is an inexpensive, biocompatible, and biodegradable option. It has long been known that yeast cells are capable of absorbing large amounts of hydrophobic or hydrophilic substances, and that these cells can be modified to create yeast-based capsules (Günal-Köroğlu *et al.* 2024). In contrast to many coating materials, yeast cells are natural, GRAS (generally recognized as safe), and a great source of proteins, minerals, nucleic acids, and B vitamins (Kurek *et al.* 2023). In many studies, it has been discovered that depleting the cell content using plasmolysis, autolysis, and enzymatic hydrolysis processes, can increase encapsulation efficiency (Ashkezary *et al.* 2024).
Pithaya (*Hylocereus* spp.) belongs to *Cactaceae* family and cultivated in tropical and subtropical regions. Because of its ease of handling, high productivity, and the income for producers made the pithaya popular and more common in recent years (de Lima Costa *et al.* 2025). High betalains and polyphenol content of pithaya gives it its antioxidant properties (Vieira *et al.* 2024). The bioactive and antioxidant characteristics of betalains as natural pigments are strongly connected. Approximately seventy-five compounds from two different types of betalains have been identified structurally and it is known that betacyanins and betaxanthins, two forms of betalains, are produced with condensation of betalamic acid (Fathordoobady *et al.* 2021).

Both betalains and phenolic compounds require methods that can increase their stability because they become unstable when subjected to environmental and processing elements such temperature variations, water activity, humidity, light, oxygen, and enzymes (Vieira *et al.* 2024). Micro- and nanoscale pigment encapsulation will provide broad and powerful platforms for the emergence of a new phase in the manufacturing of innovative and nutritious foods (Ghosh *et al.*, 2022). According to Khan (2016), the persistence of betalains in food products is increased when they are encapsulated in complex matrices.

In the literature, there are different reported encapsulation methods of pithaya (Utpott *et al.* 2020; Vargas-Campos *et al.* 2018; Xue *et al.* 2025). In this study, owing to the high efficiency, ease of handling and simple process of *S. cerevisiae* for encapsulation of bioactive compounds, it was aimed to investigate the possibilities of encapsulation of pithaya fruit with *S. cerevisiae* cells using different pre-treatments and different suspension media. To the best of our knowledge, this is the first study to evaluate the possibility of application using yeast cells in the encapsulation of pithaya fruit to form a natural food colorant.

2. MATERIAL and METHODS

2.1. Material

In the study, the yeast of *Saccharomyces cerevisiae* was purchased as Baker's yeast (Pakmaya Co. Düzce, Türkiye) from a local market in Kayseri (Türkiye). Pithaya fruits were procured from a local seller in Antalya, Türkiye.

2.1.1. *Pretreatments of yeast cells*

The plasmolyzed and non-plasmolyzed yeast cells were used after two different pre-treatments. For the production of non-plasmolyzed yeast cells, yeast was suspended with phosphate buffer, washed, and centrifuged at 4100 *g* for 10 min, and yeast cells were separated from the medium. For plasmolyzed yeast cell production, yeast cells were centrifuged in 10% NaCl solution at 180 rpm and 55 ºC in a shaking water bath at 4 ºC and 4100 *g* for 10 min after 48 h, and yeast cells were collected. The collected yeasts were washed with distilled water and then dried and ground by freeze drying method using a lyophilizer device (Christ Alpha 1–2 LDplus/1.4 mbar at −46 °C) (Karaman, 2020).

2.1.2. *Preparation of fruit bioactives*

Pithaya fruits were peeled, and the seeds were removed. Then the flesh part of the fruits was crushed with a fruit crusher. Then the samples were freeze dried (Christ Alpha 1–2 LDplus/1.4 mbar at −46 °C) and powdered by a coffee grinder (Bosch, Germany).

2.1.3. *Encapsulation process*

A 2.06 g of powdered pithaya fruit was extracted with 50 mL EtOH: water mixture (80 EtOH:20 water and 20 EtOH:80 water), and the supernatant was collected by centrifugation (Shi *et al.* 2008). Then, 30 mL of the extract was mixed with 5 g of yeast and 25 mL of distilled water for two hours to complete the encapsulation process. At the end of the time, the yeast cell microcapsules were separated from the medium by centrifugation at 4100 *g* for 10 min and then the pellets were collected, dried with a lyophilizer, and ground with a coffee grinder (Bosch, Germany). The powder samples as encapsules were used for further analysis.

2.2. Methods

2.2.1. *Betanin analysis*

For extraction, MeOH (100 mL) was used to dissolve pure sample extract powder (0.1 g). After weighing a 1 mL aliquot in a 10 mL volumetric flask, a 2% solution of HCl and MeOH (equally) was used to complete the volume (10 mL). After the sample was filtered into HPLC amber vials with a 0.45 µm nylon syringe, HPLC equipped with a UV detector (Shimadzu LC 2030C, Japan) at 538 nm and a Supelcosil LC-18 column was used to analyse the sample in triplicate. The pressure was set at 107 bar, acetonitrile (70%) and 30% ammonium acetate buffer in water (0.7 mM) as mobile phase in the isocratic phase, flow rate of 0.4 mL/min, and injection volume of 10 µL. The column was kept at a constant temperature of 35°C (Rotich *et al.* 2022).

2.2.2. *Encapsulation efficiency*

Encapsulation efficiency (EE) of the yeast microcapsules was calculated by using the following equations (Paramera *et al.*, 2011):

$$EE(\%) = (CE/CT) * 100 \quad (1)$$

CE is the weight of encapsulated betanin, CT is the initial betanin content.

2.2.3. *Color analysis*

Color measurements of the encapsules were performed by a colorimeter (PCE-CSM 5 PCE Instruments, Germany). Before each test, the instrument was calibrated with both the black and white reflective plate. Measurements were performed in the CIELab color space and carried out with 5 replications (Çetin & Sağlam 2023).

2.2.4. *Bioactive properties of yeast cell microcapsules*

2.2.4.1. Extraction of the samples. In order to determine the bioactive properties of the yeast microcapsules, the extraction process was first performed from the yeast microcapsules. For this purpose, 50 mg microcapsule sample was mixed with 15 ml of 80% ethanol solution containing 0.1% metaphosphoric acid, vortexed for 1 min, and then kept in ultrasonic bath for 10 min. Then the samples were centrifuged at 7500 *g* and 4 ºC for 5 min, and the supernatant was used for bioactivity analysis.

2.2.4.2. DPPH radical scavenging activity. The method by He *et al.* (2011) for determining DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was used. To do this, 3900 µL of DPPH methanolic solution (0.2 mM) was combined with 100 µL of extract at varying doses (diluted as 1/2, 1/5, and 1/10 v/v), and the tubes were vortexed. The samples were then left in a dark, room temperature incubator for half an hour. A spectrophotometer was used to record the absorbances at 517 nm. As a result, % inhibition values of the DPPH radical scavenging activity of yeast cell microcapsules were determined.

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

2.2.4.3. ABTS^{+} radical scavenging activity. The Gong *et al.* (2012) approach was utilized to ascertain the ability to scavenge free radicals ($ABTS^{\cdot+}$) of yeast cell microcapsules. Initially, the radical material was prepared by dissolving 96 mg of $ABTS^{\cdot+}$ in 2.45 mmol/L of potassium persulfate and keeping it for 16 hours at ambient temperature and darkness. Following the production of the stock solution, phosphate buffer solution (pH 7.4) was added to dilute it until an absorbance value of 0.7 ± 0.05 at 734 nm was measured. After mixing 20 µL of diluted samples (1/2) with 2 mL of $ABTS^{\cdot+}$ solution, the final samples were allowed to sit at room temperature for 6 minutes. After that, the absorbance values of the samples were measured at 734 nm by a spectrophotometer and the $ABTS^{\cdot+}$ radical scavenging activity of the samples was calculated as µg Trolox eq/g.

2.2.4.4. Ferric reducing antioxidant power. According to the method by (Rajurkar & Hande 2011), in a ratio of 10:1:1, 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ were combined to create the ferric reducing antioxidant power (FRAP) reagent. 100 µL of the sample and 3900 µL of freshly made working FRAP reagent were carefully mixed. After 30 minutes at 37 ºC, the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form, forming a strong blue color complex. The absorbance at 593 nm was measured against a reagent blank. All determinations were performed in triplicates. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mg of ascorbic acid equivalent per kg of sample.

2.2.5. *Morphological and conformational properties*

2.2.5.1 SEM analysis. Scanning electron microscopy and surface morphology of the samples were performed using a scanning electron microscope (SEM LEO 440 Stereoscan) equipped with EDX and WDX 600i X-Ray spectrometer.

2.2.5.2. FT-IR analysis. The FT-IR spectra of the samples were measured with a Perkin Elmer Spectrum 400 (Perkin Elmer Instruments, USA) FTIR spectrometer and the measurement was performed in the wave number range of 450-4000 cm^{-1}.

2.2.6. *Statistical analysis*

All data were subjected to statistical analysis using SAS statistical software (Version 9.0.1) ANOVA was used to determine the differences among the samples and Duncan test was applied to see the differences between the samples. Significance level was set as 0.05.

3. FINDINGS

Table 1 presents the encapsulation efficiency values of the produced yeast cell microencapsules. The highest encapsulation efficiency value was calculated for the S2 coded sample (nonplasmolyzed cell, 80:20 water: ethanol) while the lowest one was detected for the S3 coded sample (plasmolyzed cell 80:20 ethanol: water) (p<0.05). No statistically significant difference was found between samples coded S1 and S4 (p>0.05). Among pigment studies encapsulated by *S. cerevisiae*, Kurek *et al.* (2023) reported the encapsulation efficiency of microencapsulated anthocyanins by *S. cerevisiae* as 15.61%. In another study, the encapsulation efficiency of *Hibiscus sabdariffa* anthocyanin was about 27% for non-plasmolyzed baker yeast cells as similarly to our study (Nguyen *et al.* 2018). Dong *et al.* (2020) reported an information related to water success in the present study that a large concentration of ethanol could remove the permeability of cell membranes. Among the produced encapsules, nonplasmolyzed cells were more successful than plasmolyzed ones in terms of betanin entrapment. The pre-treatment process called plasmolysis is performed to remove the cell content by using inorganic salts or organic solvents. In the meantime, a few water-soluble cell components are also eliminated to free up more space for the core material to be loaded (Günal-Köroğlu *et al.* 2024).

Table 1. Encapsulation efficiency of the yeast cell microcapsules.

Samples	Encapsulation efficiency (%)
S1	4.91^{b}±0.33
S2	23.31^{a}±1.56
S3	2.29^{c}±0.15
S4	5.58^{b}±0.37

(S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water)

Plasmolysis process may alter the encapsulation in different ways. For example, it did not affect the curcumin encapsulation efficiency (Paramera *et al.* 2011), while it decreased the encapsulation efficiency of fisetin (de Camara *et al.* 2016). Similar to the mentioned results, Karaman (2021) encapsulation efficiency of gallic acid encapsules using nonplasmolyzed yeast cells was determined to be significantly higher than that of the plasmolyzed ones. And also, Cerit (2025) determined that the amount of lycopene in the non-plasmolyzed cells was 111.18 µg/g yeast while it was determined as 14.67 µg/g in the plasmolyzed capsules. Paramera *et al.* (2011) stated that it might be related to the adhesion of curcumin to the cytoplasmic cell membrane or hydrogen bonds between the constituents and cell wall. Figure 1 shows the images of produced encapsules, and it is clearly seen that the colour of the samples differed from each other.



Figure 1. Images of produced encapsules (S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water)

Figure 2 also represents of antioxidant properties of produced encapsules with different methods namely DPPH, ABTS and FRAP. %Inhibiton values for DPPH antiradical activity were observed in the range 5.08-25.50% and no statistically significant difference was found between samples coded S1-S3 (p>0.05). ABTS values changed between 1.92-6.13 µg Trolox equivalent/g and FRAP values was in between 845-2818 mg AAE/kg. Both FRAP and ABTS values, no statistically significant difference was found between samples coded S1- S3 and S2-S4 coded samples (p>0.05). According to activity tests, the values were determined in non-plasmolyzed capsules as higher (encoded S2 and S4) because the compounds responsible for antioxidant activity were better preserved. Using nonplasmolyzed yeast cells provided higher antioxidant activity compared to plasmolyzed ones because of higher entrapment of pithaya active compounds. Plasmolyzed cells, which retained less pithaya extract was the reason of lower antioxidant activity values. Similar trend was also observed in Karaman *et al.* (2021) for the antioxidant activity results for plasmolyzed gallic acid encapsules. In the study of Gümüşay

et al. (2025), it was stated that due to the high hydrophilic character of the phenolic components in the chokeberry, non-plasmolyzed yeast cell showed higher encapsulation efficiency compared to plasmolyzed yeast as similar to our results. The presence of organelles and intercellular cytoplasm in non-plasmolyzed yeast has been proposed as a factor in the binding of phenolic compounds (Young *et al.* 2017).

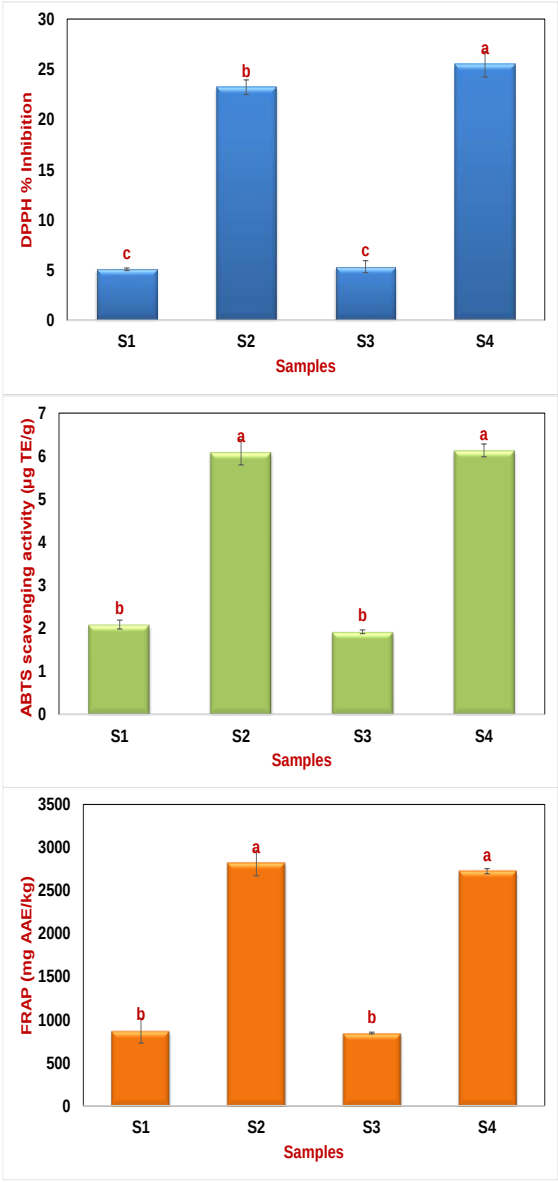


Figure 2. Bioactive properties of produced encapsules (S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water)

Table 2 shows the color properties of the produced encapsules. L^* value of pithaya samples was measured as 6.70 while the encapsulation process caused an increment the L^* values as expected. The highest L value was detected in S3 coded sample while the lowest one was measured in S2 coded sample. The color is defined in terms of luminosity (L), red versus green (a) and yellow versus blue (b). b^* value of pithaya powder was determined as -1.65 while the closest value was observed in S2 coded sample. A ΔE value in the range of 0–0.5 implies an imperceptible difference in color between two samples, 0.5 to 1.5 a slight difference, 1.5 to 3.0 a just noticeable difference, 3.0 to 6.0 a remarkable difference, 6.0 to 12.0 an extremely remarkable difference, and above 12.0 a color of a different shade (Coronel-Aguilera and Martín-González, 2015). Regarding to that information, the lowest ΔE difference was observed in S2 coded sample confirming the highest encapsulation efficiency.

Table 2. Color properties of produced encapsules (S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water).

Samples	L^*	a^*	b^*	ΔE
S1	26.61^{c}±0.79	23.38^{b}±0.33	-0.32^{e}±0.04	62.38^{c}±0.49
S2	15.52^{d}±0.61	18.49^{d}±0.28	-1.18^{d}±0.06	70.18^{b}±0.43
S3	56.54^{a}±0.08	19.89^{c}±0.06	4.92^{a}±0.01	36.47^{e}±0.01
S4	51.82^{b}±0.81	23.05^{b}±0.10	2.95^{b}±0.01	42.29^{d}± 0.44
Pithaya	6.69^{e}±0.29	26.43^{a}±0.17	-1.65^{c}±0.10	81.21^{a}±0.35

To determine functional groups and describe the connection between the yeast coating material and pithaya components, FT-IR analysis was performed. Prominent peaks were consistently seen for O–H stretching (3260 cm^{-1}) and C–H stretching (2925 cm^{-1}) in all encapsules (Figure 3) as similar to different studies (Widianto & Puangpraphant 2024; de Carvalho Alves *et al.* 2024). The absorption peak at approximately 1743 cm^{-1} is due to the stretching vibration of the pectin carbonyl group. These peaks were observed in strongly pithaya and weakly in S1 and S3 samples, while in S2 and S4 coded samples it had disappeared as a result of intermolecular interactions between the core and wall materials. The peak at about 2930 cm-1 is also due to the basic structure of betacyanin and can be attributed to the CH bond stretching peak (Raj & Dash 2022). The intensity of these peaks decreased in encapsules, and the peaks shifted to other wavenumbers. The absorption bands at wavelengths 800-1200 cm^{-1} are typical fingerprint regions of pectin polymers.

The surface morphologies of encapsules were evaluated using SEM. The nonplasmolyzed samples encoded S2 and S4 exhibited a comparatively smoother surface and oval-shaped cells, which are characteristic of yeast cells. However, in samples encoded S1 and S3 plasmolysis process caused deformation in yeast cells (Figure 4).

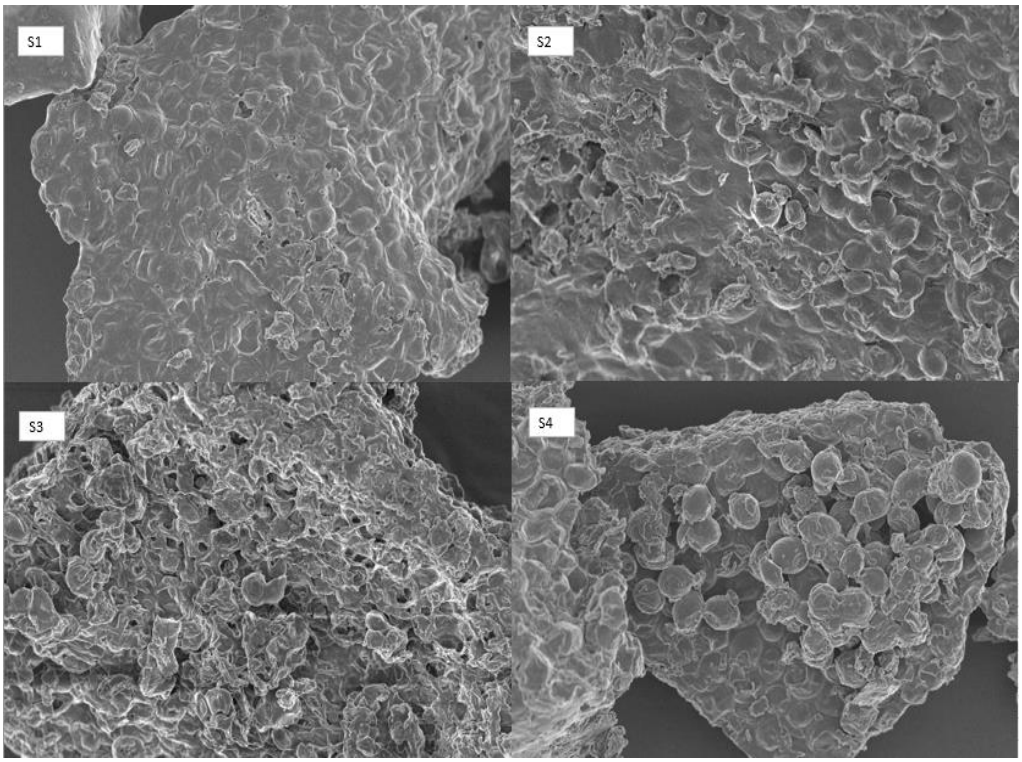


Figure 4. SEM images of produced encapsules (S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water).

Similar to our results, Fu *et al.* (2022) reported that yeast cells seemed smaller and shrunken, possibly as a result of cytoplasmic leakage brought on by the NaOH pre-treatment. Additionally, it was reported that, freeze-drying process of yeasts caused a high level of cell aggregation (Dadkhodazade *et al.* 2018; Kurek *et al.* 2023). It is possible that pithaya extract interacts with yeast cells since the yeast surface morphology greatly improved and maintained its unbroken form after encapsulation in S2 and S4 coded samples. Besides, the average dimensions of yeast capsules were determined as approximately 2 µm.

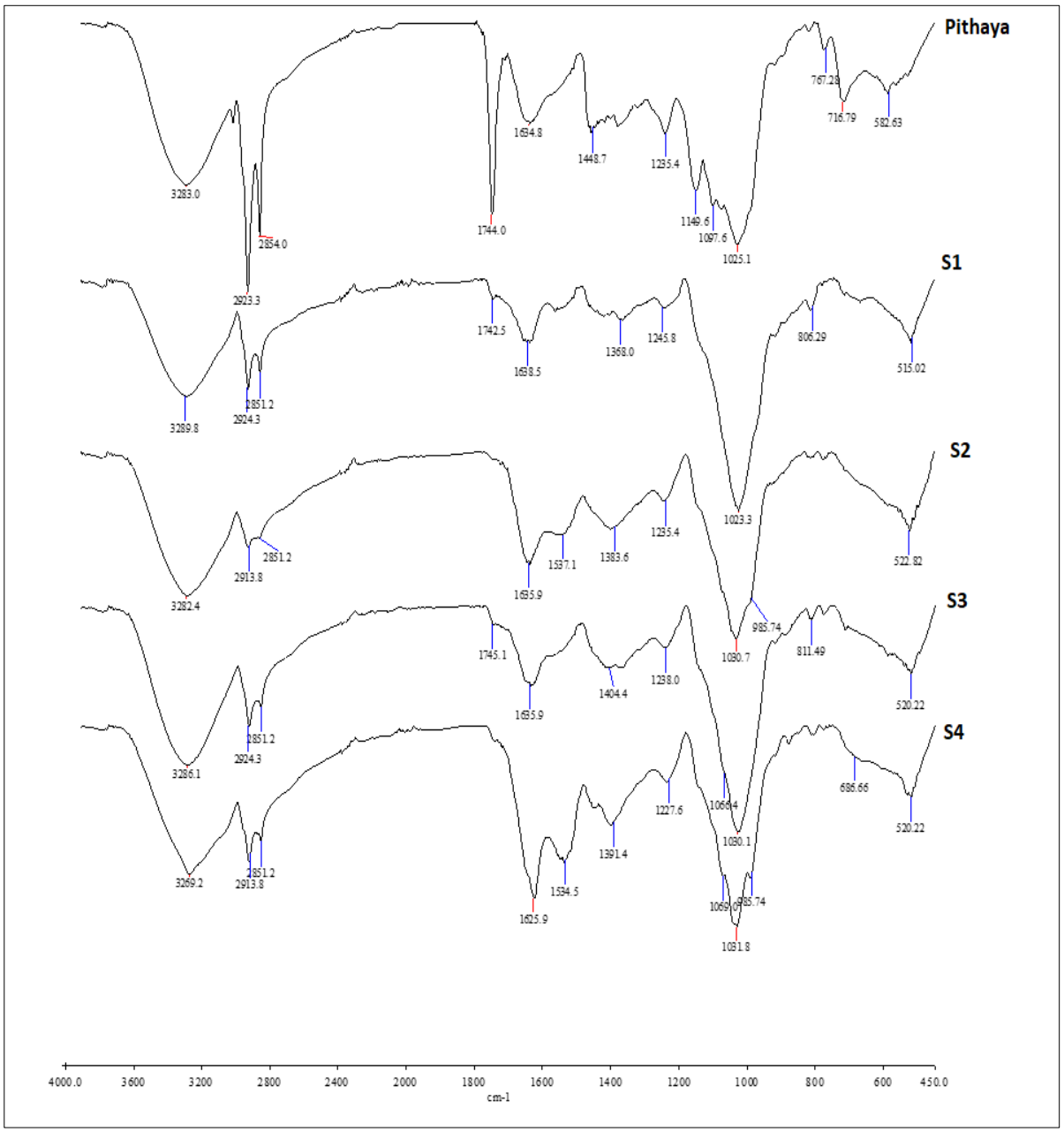


Figure 3. FTIR spectra of pithaya and produced encapsules (S1: plasmolyzed cell 80:20 water:ethanol, S2: nonplasmolyzed cell 80:20 water:ethanol, S3: plasmolyzed cell 80:20 ethanol:water, S4: nonplasmolyzed cell 80:20 ethanol:water)

4. CONCLUSION

Pithaya fruit extract was encapsulated in both plasmolyzed and nonplasmolyzed yeast cells. The results showed that nonplasmolyzed yeast cells were more successful than plasmolyzed ones in terms of encapsulation efficiency and bioactive properties. Besides, the solvent used in the encapsulation process of pithaya affected the encapsulation efficiency and water-based mixture was more effective. It could be concluded that yeast cells might be used with different

polymers together and to achieve higher efficiency values for future studies. And also, different pre-treatments such as ultrasound and high-pressure homogenization for yeasts could be evaluated for higher entrapment of pithaya pigments.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Kevser Karaman: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft. **Şeyda Nur Türkay Rifaioğlu**: Methodology and Validation.

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