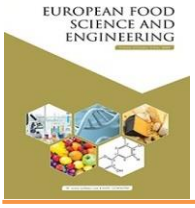


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# Encapsulation and characterization of catechin and epicatechin microcapsules using yeast cell biocarriers

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ABSTRACT

In this study, catechin and epicatechin compounds were encapsulated using *Saccharomyces cerevisiae* yeast cell and fabricated yeast cell microcarriers were subjected to bioactive analysis, conformational and morphological characterization. Encapsulation efficiency values of the yeast cell microcarriers were 24.74 and 31.87% for the epicatechin and catechin loaded yeast cell microcapsules, respectively. Total flavonoid content of the epicatechin and catechin loaded yeast cell microcapsules were determined to be 79.67 and 61.86 mg CE/g while the antiradical activity of the samples was in the range of 4.16-39.24%. FT-IR and XRD spectrum revealed that the catechin and epicatechin were encapsulated by the yeasts cell successfully.

## 1. Introduction

Catechins are a group of polyphenolic compounds belonging to the flavonoid class, found in high concentrations in various plant-based fruits, vegetables, and beverages. Although catechins are not essential for human nutrition, they help to improve human health by preventing various diseases. Catechins are classified under flavanols. Fruits such as grapes, apples, pears, cherries, and green tea are the main sources of catechins (Kondo et al., 2002). Many research articles have established that in vitro studies of catechins showed protection against degenerative diseases and a strong inverse relation between the intake of catechins and risk of CHD mortality has been reported (Stein et al., 1999; Wollny et al., 1999). It has been reported that catechins appear to have greater anti-bacterial activity against Gram positive than Gram negative bacteria (Ikigai et al., 1993). Gadkari & Balaraman (2015) reported that the catechins occur in the structure of plants and show phenolic structure and they can be consumed as nutraceutical for improving the human health. But it was also reported that the catechins had low stability against light and temperature (Munin & Edwards-Levy, 2011). Volf et al. (2014) stated that the catechins do not show long term stability and for these reasons, these groups of phenolic substance are not very popular in pharmaceutical sector. In the food industry, encapsulation process is commonly applied because the bioactive compounds encapsulated are popular because of their improved chemical stability, better handling structure and controlled targeted release (Castel, Rubiolo & Carrara, 2018; Fernandes et al., 2016).

Recently, *Saccharomyces cerevisiae* yeast cells have been used to encapsulate active ingredients and at the same time sensitive compounds. Yeast cells do not show health risk and they have low cost and widely used as a food ingredient (Czaja et al., 2015). They have a phospholipid membrane and can therefore act as a liposome and they can easily encapsulate the hydrophobic and hydrophilic compounds (Paramera et al., 2011). Czerniak et al. (2015) and Kavosi et al. (2018) showed that yeast cells are effective encapsulation agents for menhaden fish oil and purslane seed oil, respectively. Paramera et al. (2011) used yeast cells as an effective microcarrier for curcumin and reported the optimal conditions to produce curcumin-loaded microcapsules.

The main aim of the present research was to investigate the possibility of encapsulation of catechin (C) and epicatechin (EC) compounds by using *Saccharomyces cerevisiae* yeast cell and to characterize the bioactive, conformational, and morphological properties of loaded yeast cell microcarriers.

## 2. Materials and methods

### 2.1. Materials

Catechin and epicatechin were purchased from Sigma Aldrich (Sigma, Germany). Baker's yeast was provided from a local market in Kayseri, Turkiye.

### 2.2. Methods

*Encapsulation process using yeast cells*

For the encapsulation of catechin and epicatechin,

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*Saccharomyces cerevisiae* yeast cells were used. The encapsulation process using yeast cells was performed according to the method reported by Karaman (2021). For this aim, a certain amount of yeast cells (750 mg) and a certain amount of catechin or epicatechin (250 mg) were homogeneously mixed in aqueous media in a beaker. The samples were subjected to shake on a shaker (180 rpm) at 40 ºC for 12 h to conduct the encapsulation process. At the end, the yeast cell microcapsules were centrifuged and washed using distilled water and then freeze dried using a lyophilizer, ground with a grinder and prepared for analysis. The samples were stored at -18 ºC until analysis.

*Determination of encapsulation efficiency*

Encapsulation efficiency (EE) of microcapsule samples was calculated by using of the following equations (Paramera et al., 2011).

$$EE(\%) = \left(\frac{CE}{CT}\right) * 100 \quad (1)$$

CE is the weight of encapsulated catechin or epicatechin, CT is the initial catechin or epicatechin and CM is the weight of total yeast cell microcapsules.

*Bioactive characterization of produced microcapsules*

*Extraction of microcapsules*

The extraction process was performed to determine the bioactive properties of yeast microcapsules. For this aim, 50 mg sample was mixed with 5 mL of water and vortexed for 1 min and then kept in ultrasonic bath for 10 min and the extraction process was completed. The supernatant was then centrifuged at 7500 g for 5 min at +4 ºC and the supernatant was used for the bioactivity test.

*Total Flavonoid Content*

Total flavonoid content of the yeast microcapsules was determined according to the method described by Zhishen et al. (1999). One milliliter of extract was transferred into the tube containing 4 mL of distilled water. After that, 0.3 mL of $NaNO_2$ (5 % in water), and after 5 min, 0.3 mL of $AlCl_3$ (10 % in water) was added. After 6 min incubation, 2 mL of 1 M NaOH solution was placed in the tubes, and finally, the volume of tubes was adjusted to 10 mL with distilled water. The absorbance of sample was measured at 510 nm (Shimadzu UV-vis 1800, Japan). The results were expressed as milligram catechin equivalent /g sample.

*DPPH radical scavenging activity*

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity of the samples was determined according to Faller & Fialho (2009). For this aim, 100 µL of the extract was mixed with 3900 µL of DPPH radical solution (0.1 mM), and the samples were vortexed and incubated for 30 min at room temperature (25 ºC) and in the dark conditions. At the end of the incubation period, the absorbance values of the samples were recorded by UV-vis spectrophotometer at 517 nm wavelength and the DPPH radical scavenging activity of the samples was calculated as % inhibition by substituting the measured absorbance values in the following equation.

$$\%\ Inhibition = [(A_C - A_S)/A_C]x100 \quad (2)$$

where $A_c$ and $A_s$ refer the absorbance of control and sample, repectively.

*Determination of morphological and conformational properties of microcapsules*

*SEM analysis*

The surface morphology of the samples was analyzed by scanning electron microscopy using a scanning electron microscope (SEM LEO 440 Stereoscan) equipped with EDX and WDX 600i X-Ray spectrometer.

*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 wavenumber range of 450-4000 $cm^{-1}$.

*X-RD analysis*

X-ray diffraction analysis of the powdered samples was determined by X-ray powder diffractometer (Bruker AXS D8 Advance Model). The sample measurement range was performed in the standard measurement mode in the angle range of 10-90º in 2θ.

### 2.3. Statistical analysis

To show the differences between the samples, the statistical software was used for the statistical analysis of the samples (SAS Institute, 2000). For the comparative analyses at the significance level of 0.05, Duncan's multiple range tests were used.

## 3. Results and Discussion

### 3.1. Encapsulation Efficiency

Figure 1 shows the encapsulation efficiency (EE) of catechin and epicatechin loaded yeast cell microcapsules. As can be seen from the figure, EE of the samples ranged between 24.74-31.87% and epicatechin loaded yeast cell (ECLYC) microcapsules was determined to be significantly higher than that of the catechin loaded yeast cell (CLYC) microcapsules ($P<0.05$). Eugène et al. (2022) compared the catechin and epicatechin molecules in terms of reactivity and they reported that the reactivity of epicatechin was higher than that of the catechin but, the stability of epicatechin was lower than the stability of catechin. They indicated that their hydroxyl groups correspond to their most receptive sites. Karaman (2021) encapsulated the gallic acid by using yeast cells and EE values of the samples ranged between 2.59-37.91%. In another study, curcumin was encapsuled by yeast cells and EE of the samples were reported to be 31.8 and 30.6% for the microcapsules produced by plasmolyzed and non-plasmolyzed yeast cells, respectively (Paramera et al., 2011).

### 3.2. Bioactive properties of the yeast cell microcapsules

To determine the bioactive properties of CLYC and ECLYC microcapsules, total flavonoid content and DPPH radical scavenging performance were determined, and the results were tabulated in Table 1. As is seen from the table, the highest total flavonoid content was determined for the ECLYC (79.67 mg CE/g) while the CLYC and empty yeast cell (EYC) contained 61.86 mg CE/g 14.13 mg CE/g total flavonoid, respectively.

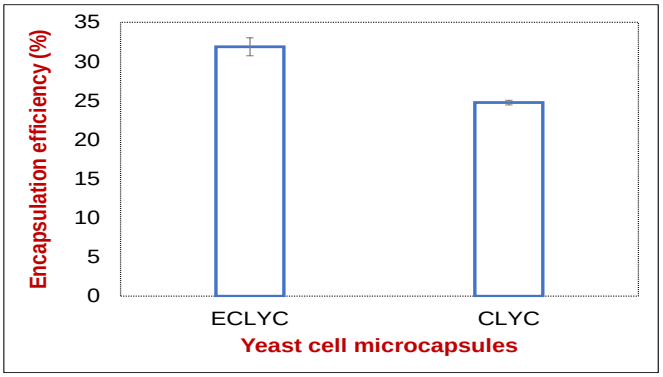


**Figure 1.** Encapsulation efficiency of the loaded yeast microcapsules. CLYC: Catechin loaded yeast cell, ECLYC: Epicatechin loaded yeast cell

DPPH radical scavenging activity of the samples ranged between 4.16-39.24%, and the highest antiradical activity was for the ECLYC while the lowest activity was observed in empty yeast cell as expected. Also, the differences among the samples in terms of total flavonoid and antiradical activity were found to be statistically significant ($P<0.05$). Karaman (2020) reported that the yeast cells loaded with carvacrol showed significant DPPH radical scavenging activity. In another research, it was reported that the thymoquinone, gallic acid and resveratrol loaded yeast cells performed good radical scavenging activity (Karaman, 2020; Karaman, 2021, Shi et al., 2008).

**Table 1.** Bioactive properties of the yeast cell microcapsules

| Samples | Total flavonoid content (mg CE/g) | Antiradical activity (%) |
|---|---|---|
| **EYC** | 14.13+1.56$^{c}$ | 4.16+0.82$^{c}$ |
| **CLYC** | 61.86+0.72$^{b}$ | 29.05+1.66$^{b}$ |
| **ECLYC** | 79.67+2.85$^{a}$ | 39.24+1.87$^{a}$ |

EYC: Empty yeast cell, CLYC: Catechin loaded yeast cell, ECLYC: Epicatechin loaded yeast cell

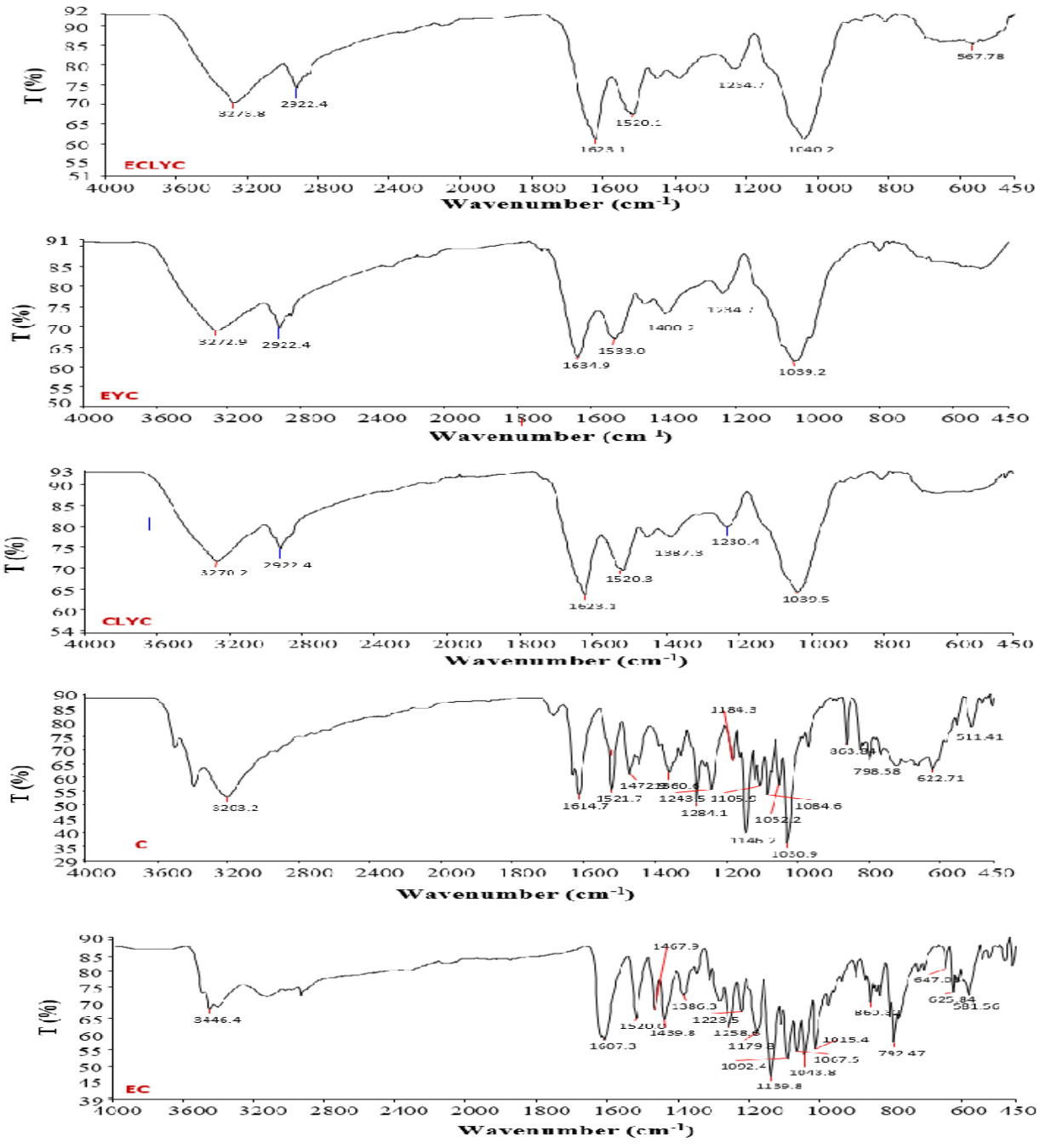


**Figure 2.** FT-IR spectrum of the samples. EYC: Empty yeast cell, CLYC: Catechin loaded yeast cell, ECLYC: Epicatechin loaded yeast cell, C: Catechin, EC: Epicatechin

### 3.3 Conformational and morphological properties of yeast cell microcapsules

**Figure 2** illustrates the FT-IR spectra of both catechin and epicatechin loaded and unloaded yeast cells in addition the FT-IR spectra of catechin and epicatechin standard compounds. As is clearly seen from the spectrum, catechin and epicatechin showed characteristic IR bands showing the phenolic structure. Also, from the bands, there are clear differences between the bands of catechin and epicatechin at 800-450 $cm^{-1}$. Some significant bands were not observed for the ECLYC and CLYC samples due to the encapsulation process and it shows that these compounds interacted with the yeast cells and diffused into the cell. Similar findings were also reported by Karaman (2021), Paramera et al. (2011) and Shi et al. (2008) for gallic acid, curcumin and chlorogenic acid, respectively.

Figure 3 shows the XRD spectra of both catechin and epicatechin loaded and empty yeast cells in addition catechin and epicatechin compounds. There is a clear difference between the spectra of phenolic compounds and yeast cells microcapsules. Catechin and epicatechin showed the crystalline structure giving the sharp peaks but both loaded and empty yeast cells had amorphous structure giving more broader peaks. Similar observation was reported by Karaman (2021) for the gallic acid which is a popular phenolic substance and yeast cells loaded with gallic acid.

Figure 4 illustrates the morphological images recorded by scanning electron microscopy of the loaded and empty yeast cells. As is seen, characteristic spherical structure was monitored for the loaded and empty yeast cells. Also, catechin (C) and epicatechin (EC) compounds showing crystalline structure (Figure 3) were clearly observed by SEM. When compared to loaded and empty yeast cell images, added catechin and epicatechin which are not encapsulated and adsorbed on the surface of the yeast cells were also monitored clearly (Figure 4). Similar spherical structure for the yeast cell used for the encapsulation of curcumin (Paramera et al., 2011) and gallic acid (Karaman, 2021).

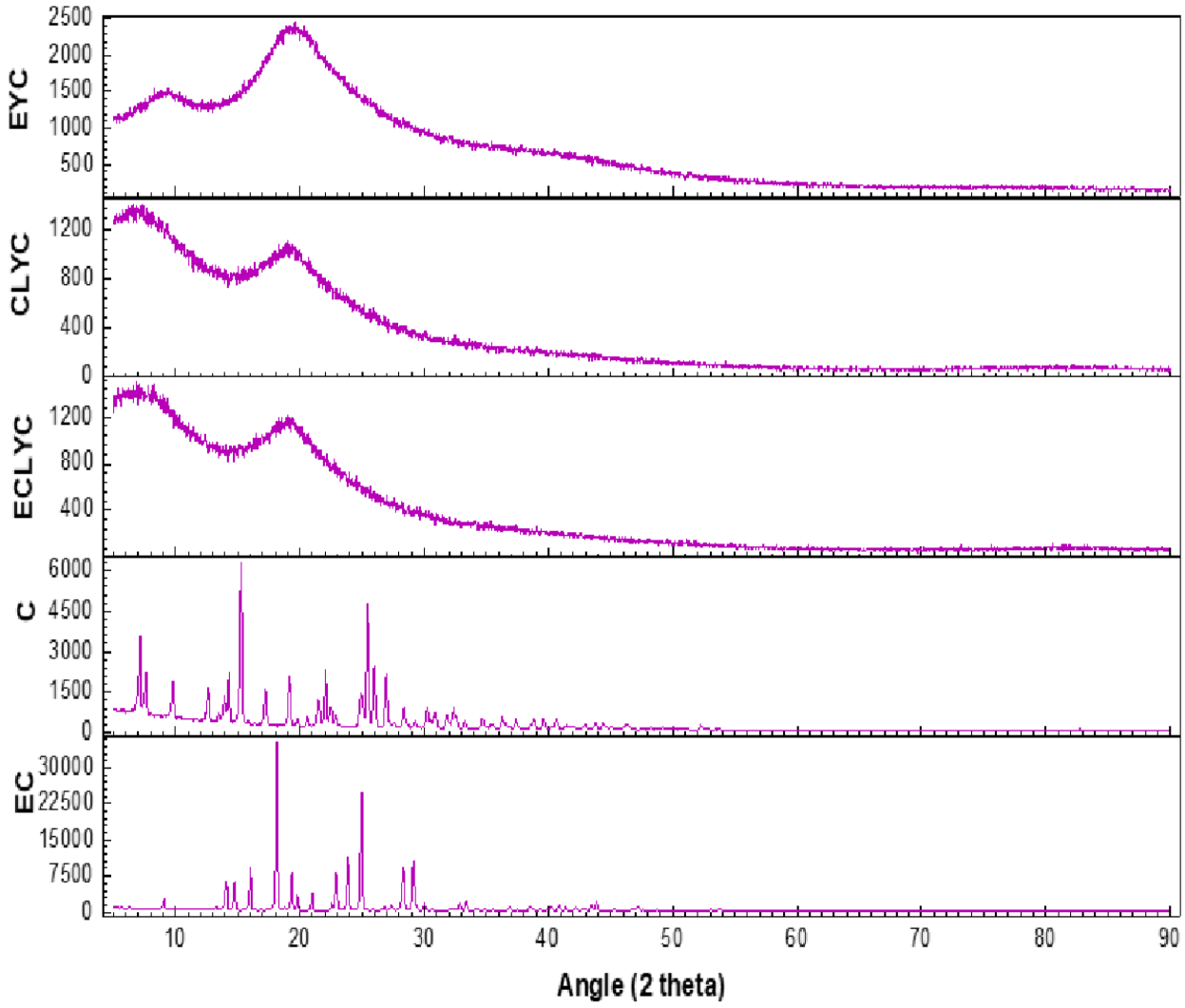


**Figure 3.** XRD spectrum of the samples. EYC: Empty yeast cell, CLYC: Catechin loaded yeast cell, ECLYC: Epicatechin loaded yeast cell, C: Catechin, EC: Epicatechin

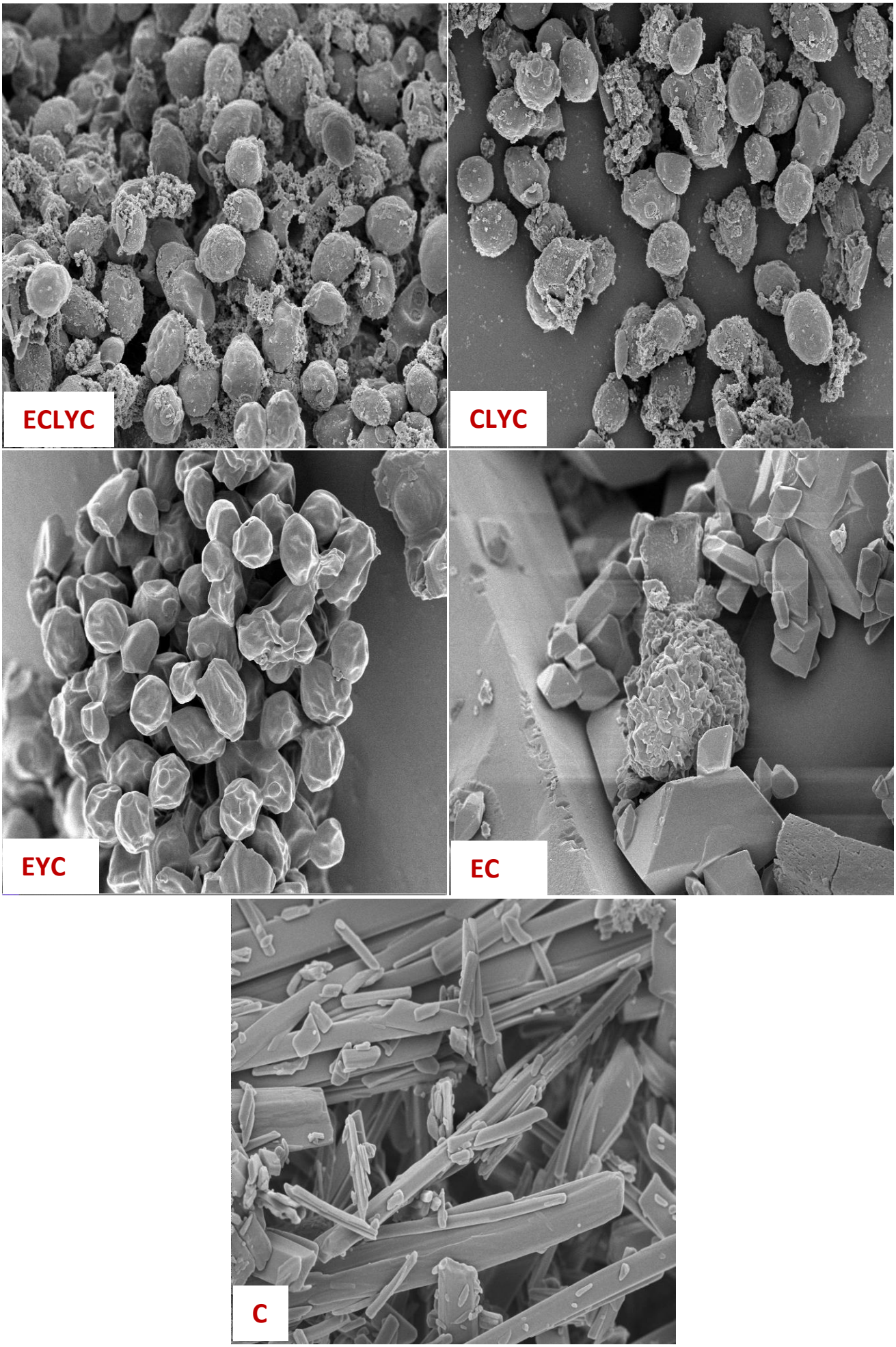


**Figure 4** SEM images of the samples. EYC: Empty yeast cell, CLYC: Catechin loaded yeast cell, ECLYC: Epicatechin loaded yeast cell, C: Catechin, EC: Epicatechin

## 4. Conclusions

This research shows that catechin and epicatechin could be encapsulated by using plasmolyzed yeast cells with quite high encapsulation efficiency. Encapsulation of the related phenolic substances was confirmed by Fourier transform infrared spectroscopy and scanning electron microscopy in addition to the bioactive performance of the loaded yeast cells compared to unloaded yeast cells. For further study, stability of catechins loaded yeast cell microcapsules at different media (in vitro and in situ) could be determined. It could be concluded that the catechin and epicatechin yeast cell microcapsules could find possible use in the food and pharmaceutical industries.

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