Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *86* (4) 367–380 (2021)
JSCS–5427
Original scientific paper

Encapsulation of peach waste extract in *Saccharomyces cerevisiae* cells

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(Received 1 December 2020, revised 2 January, accepted 4 January 2020)

Abstract: As a secondary industrial product, peach waste (PW) presents an ecological problem, but is potentially a rich source of natural antioxidants. A potential and novel way to improve the phytochemical stability of waste rich in phytochemicals is encapsulation in yeast cells that possess good structural characteristics. In the present study, PW extract was encapsulated in non-plasmolyzed, plasmolyzed and living *Saccharomyces cerevisiae* cells using the freeze-drying method. HPLC analysis revealed that β-carotene is the most abundant carotenoid, while epicatechin and catechin are the most abundant phenolics in PW. The highest encapsulation efficiency of carotenoids (86.59 %), as well as phenolics (66.98 %), was obtained with freeze-dried non-plasmolyzed yeast cells used as carriers. Although plasmolysis can cause some changes in the structure and properties of yeast cells, it did not enhance the encapsulation efficiency of present phytochemicals. Successful encapsulation of PW extract in yeast cells was confirmed by FTIR spectroscopy and SEM imaging. The obtained results present the encapsulation of sensitive compounds in yeast cells by freeze-drying as an excellent method for preserving valuable compounds and their potential use in the food and pharmaceutical industries.

Keywords: phenolics; carotenoids; yeast; phytochemical stability; epicatechin; catechin.

INTRODUCTION

In fields of food, agriculture, and drug ingredients, many substances are useful for human health (antioxidants, essential oils, vitamins, *etc*.) and have technological or organoleptic functionalities (enzymes, aroma, natural colorant, *etc*.). Some of them are very sensitive to adverse environmental conditions, such

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https://doi.org/10.2298/JSC201201001C

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as moisture, temperature, oxygen, or undesirable effects of light, or display negative properties of their targets, such as biocides or drugs.[1,2] One of the essential ways for preserving these substances is encapsulation in which small active compounds are surrounded by another material, thereby producing particles with diameters of a few nm to a few mm. The encapsulated substance may be called the core material, the active agent, fill or internal phase, while the surrounding material is called the coat, carrier, external phase or wall material.[3–5] Encapsulation prolongs the shelf life of some products under constantly changing storage conditions, stabilizes the active materials since the wall material acts as a physical barrier for oxygen or other molecules, preventing deleterious reactions, and enable the material to be released in a controlled way in product applications. Among the different techniques of encapsulation, freeze-drying is widely used in the food industry as one of the especially effective methods for bioactive compounds susceptible to degradation at high temperatures.[1,4,5]

The stability and release properties of encapsulates are dependent on the composition of the wall material and hence, various carriers have been used: sugars, cyclodextrins, maltodextrins, modified starches, gums, proteins, and liposomes.[5] The structure of the yeast cell wall made it an excellent and novel encapsulating material for the food industry,[1,4,6–8] which has many advantages over other encapsulation carriers: natural, food-grade, low cost, health benefits, thermostability and slow-release.[3,4,9] *Saccharomyces cerevisiae* cell wall is approximately 70–100 nm thick and is composed of *β*-glucans, a mannoprotein layer, and a small amount of chitin. Due to their phospholipid membranes, yeast cells can behave as liposomes and have been used for the encapsulation of both hydrophobic and hydrophilic molecules, such as essential oils,[10] resveratrol,[11] curcumin,[1] flavor compounds,[8] berberine,[4] chlorogenic acid[6] and enzymes.[7]

Peaches are a popular summer fruit, the increased consumption of which lowers the risk of chronic diseases, such as cardiovascular diseases, cancer, diabetes, *etc*. In the last decades, peach production is about 21 million tones around the world with an annual growth rate of more than 3 %. Peach cultivars rich in carotenoids and phenolics have higher antioxidant activities and represent excellent sources of natural antioxidants.[12] Phenolics in peach include hydroxycinnamic acids, such as caffeic, *p*-coumaric, ferulic, and chlorogenic acids, flavonols, such as quercetin glycosides (quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside), flavanols, such as catechin, epicatechin, procyanidin B1, and anthocyanins, such as cyanidin-3-glucoside and cyanidin-3-rutinoside.[13,14] The carotenoid profile of peach includes *β*-carotene, *β*-cryptoxanthin, lutein, and zeaxanthin.[15]

By-products of plant food processing represent a major disposal problem for the industry concerned, even though they present promising sources of compounds that may be used for their high nutritional and excellent technological

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properties.[16,17] Recovery of antioxidants from by-products of food processing plants has gained importance since the replacement of synthetic antioxidants with natural ones has benefits due to health implications and solubilities in food systems.[18] Peaches are composed of approximately 7–9 % of peel tissue and that the exocarp is a concentrated source of nutrients. It can be assumed that over 20 % of the total phytochemical concentration of a peach is lost if the peel is discarded and not consumed.[12] Peach juice by-product contains 37 % of dietary fiber and 0.5–8 % of total polyphenols, mainly kaempferol and quercetin 3-*O*-rutinoside.[19]

This study aimed at evaluating the feasibility of *Saccharomyces cerevisiae* cells as an encapsulation carrier for phytochemicals present in peach waste extract. Simple solvent extraction of peach waste and freeze-drying of the formulated encapsulation solutions was performed, and their potential to entrap and protect the present carotenoids and phenolics were evaluated. The distribution of peach waste extract in yeast cells was studied by Fourier transform infrared analysis and scanning electron microscopy.

EXPERIMENTAL

Preparation of Saccharomyces cerevisiae *cells*

The reference culture of *Saccharomyces cerevisiae* ATCC 9763 was grown on Sabouraud Maltose Agar slants (SMA, Himedia, Mumbai, India) for 48 h at 30 °C. After incubation, the cells were suspended in 5 mL phosphate buffer (pH 6.8), which was used as the inoculum for the second subculturing. The second subculturing was realized in Fernbach flasks with 250 mL Sabouraud Maltose broth (Himedia, Mumbai, India). The flasks were agitated at 180 rpm (Jeio Tech Lab, Korea) for 48 h at 30 °C.

From the yeast suspension, non-plasmolyzed (C1) and plasmolyzed (C2) yeast cells were prepared according to Paramera *et al*.[1] with some modifications. Briefly, before freeze-drying (Martin Christ, Apha 2-4 LSC, Germany), the samples were frozen in a deep freezer (Snijders Labs, Tilburg, The Netherlands). For yeast cell plasmolysis, a 10 % NaCl solution was used. For centrifugation, a Rotina 380R centrifuge (Hettich, Germany) was used. Alive yeast cells (C3) were prepared as the other two samples but without plasmolyzing and freezing.

Extraction of peach waste

Freeze-dried peach waste (PW), obtained using a Alpha 2-4 LSC Martin Christ freeze--drying apparatus (Osterode, Germany), was extracted three times using an acetone:ethanol mixture (36:64 volume ratio) at a solid to solvent ratio 1:20 w/v, with the same volume of solvents. The extraction was performed using a Unimax 1010 laboratory shaker (Heidolph Instruments GmbH, Kelheim, Germany) at 300 rpm, under light protection, at room temperature, for 10 min. The three obtained extracts were filtered (Whatman paper No. 1), combined, and stored in dark bottles at –20 °C until further analysis.

Encapsulation of peach waste extract in S. cerevisiae *cells*

Encapsulation by the freeze-drying technique was conducted following the method described by Šeregelj *et al*.[20] with modifications. Each yeast sample (7 g) was dissolved in 10 mL of water at 60 °C and kept under stirring until the temperature reached 30 °C. Separately, 50 mL of PW extract was combined with sunflower oil (1.5 mL), concentrated under reduced pressure on a rotary evaporator set at 40 °C to remove the organic solvent, and immediately

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mixed with the previously prepared carrier solution. The mixtures were homogenized at 11000 rpm for 3 min at room temperature and frozen in a deep freezer. All samples were freeze-dried at –40 °C for 48 h. The obtained encapsulates were stored at –20 °C until further use.

Determination of yeast cell number

The total count of living yeast cells was determined by the plate counting method using SMA as the medium. In C1, the cell number was determined after subcultivation but before freeze-drying. In C2, the cell number was determined after subcultivation and plasmolysis but before freeze-drying. In C3, the cell number was determined after subcultivation. The encapsulation number was determined as E1 (encapsulated peach waste extract in non-plasmolyzed yeast cells), E2 (plasmolyzed freeze-dried yeast cells) as well as E3 (in alive yeast cells). The cells numbers are expressed as log CFU mL^{-1} of suspension and log CF mg^{-1} of encapsulate.

Determination of carotenoids content

Total carotenoid content (*T*Car), expressed as mg β-carotene per 100 g of dry PW or mg *β*-carotene per 100 g of encapsulate, was analyzed spectrophotometrically by the method of Nagata & Yamashita.[21] For the HPLC analysis of carotenoids, a solvent gradient was performed by varying the proportion of solvent A (water:methanol 1:4 v/v) to solvent B (acetone:methanol 1:1 volume ratio) at a flow rate of 1 mL min^{-1} with the following gradient profile: 25 % B 0–3 min; 75 % B 3–6 min; 90 % B 6–10 min; 100 % B 10–18 min; 50 % B 18–25 min; 25 % B 25–32 min. The column temperature was 25 °C and chromatograms were plotted at 450 and 475 nm. The carotenoids were dissolved in hexane and identified by matching the retention time and its spectral characteristics against those of standards.

Determination of phenolics contents

Total phenolic content (*T*Ph), expressed as mg gallic acid equivalents GAE per 100 g of dry PW or mg GAE per 100g of encapsulate, was determined spectrophotometrically by the Folin–Ciocalteau method adapted to microscale. For HPLC analysis of phenolic compounds, chromatograms were recorded using different wavelengths for individual phenolic compounds: 280 nm for hydroxybenzoic acids, 320 nm for hydroxycinnamic acids, and 360 nm for flavonoids following the method of Tumbas Šaponjac *et al.*[22]

Antioxidant activity of peach waste extract and encapsulates

The antioxidant activity of the PW extract and the obtained encapsulates was expressed as μmol Trolox equivalent, *TE* per 100 g of dry PW and μmol *TE* per 100 g of encapsulate, respectively. Three different methods were utilized: the 2,2-diphenyl-1-picrylhydrazyl method (SA) described by Girones-Vilaplana *et al.*,[23] reducing power (RP) by Oyaizu,[21] and *β*-carotene bleaching assay (BCB) by Al-Saikhan *et al.*[24] The SA and RP were performed on 70 % methanol extracts, while BCB was realized on hexane extracts.

Encapsulation efficiency

Determination of carotenoids encapsulation efficiency (*EE*Car, %) was based on the calculation of the concentration of carotenoids detected in the encapsulates (CC) over the initial concentration of carotenoids added to make the encapsulates (IC). The *T*Car concentrations were determined spectrophotometrically by the method described above. Thus, the *EE*Car was determined using Eq. (1):[25]

$$EE\text{Car} = 100(w(\text{CC})/w(\text{IC})) \quad (1)$$

Determination of phenolics encapsulation efficiency (*EE*Ph, %) was performed by the Tumbas Šaponjac *et al.* method.[25] The concentration of the surface phenolics (*SP*) and total

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phenolics (*TP*) in the PW encapsulates were determined by the Folin–Ciocalteu method described above. The *EE*Ph was determined using Eq. (2):

$$EE\text{Ph} = 100(w(\text{TP})–w(\text{SP}))/w(\text{TP}) \quad (2)$$

Characterisation of the encapsulates

In order to confirm the encapsulation of peach waste extract in yeast cells, FT-IR analysis was performed among carriers and as well as the obtained encapsulates and PW. The FTIR spectra of the samples were recorded on a Thermo Nicolet Nexus 670 FTIR spectrophotometer using the transmission technique in KBr pellets. For all spectra, 32 scans were recorded and averaged with a resolution of 4 cm^{-1} for each spectrum. A DTGS detector was employed in the IR measurements.

Morphological analysis of the surface of the samples was performed using a JEOL JSM--6390LV scanning electron microscope (JEOL Ltd., Tokyo, Japan). Prior to the analysis, (acceleration voltage 15 kV, beam current 20 nA, spot size 1 mm), the samples were sputter--coated with gold during 100 s under 30 mA ion current on BALTEC SCD 005 sputter coater (Capovani Brothers Inc., Scotia, NY).

Statistical analysis

All experiments were run in triplicate. The results presented are means ± standard deviation (±*SD*, $n = 3$). Statistical analyses were realized using Origin 8.0 SRO software package and Microsoft Office Excel 2010 software. Significant differences were calculated by ANOVA ($p < 0.05$).

RESULTS AND DISCUSSION

A potential and novel way to improve the phytochemical stability of waste rich in phytochemicals is encapsulation in yeast cells that possess good structure characteristics. In this study, the number of yeast cells was determined during the above-mentioned treatments to show how these treatments affect changes in the number of yeast cells. The results of the number of yeast cells in C 1–3 and appropriate encapsulates are given in Table I. According to the obtained results for the cells number, C1 and C3 after subcultivation are similar. In C2, after plasmolysis, the cell number was reduced by about 6 log units, which means that the NaCl concentration for plasmolysis was insufficient for total reduction. According to the gained number in encapsulates after freeze-drying (Table I), showed that these processes significantly inhibit the growth of cells.

TABLE I. Number of *Saccharomyces cerevisiae* cells; data present the mean value of three replicates ±*SD*; values sharing the same letter (a, b and c) in the same row are not significantly different at the 0.05 level according to Tukey's HSD test

Sample number	Number of yeast cells	
	Carriers (C) (log (CFU ml^{-1}))	Encapsulates (E) (log (CFU mg^{-1}))
1	$9.62±0.04^c$	$2.69±0.01^a$
2	$3.69±0.11^a$	$< 4^c$
3	$9.30±0.08^b$	$3.84±0.01^b$

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Namely, the freeze-drying process was lethal for almost all cells in E2. In E1, the number was reduced for 7 log units, and in E3 for 6 log units. Plasmolysis can potentially increase the available encapsulation intercellular space and increase encapsulation efficiency. However, the loss in viability does not have a significant effect on yeast loading because encapsulation is mostly driven by passive diffusion of the substance throughout the cell envelope. On the other hand, proteins, polysaccharides, and other cellular components can make retention and interaction of the active ingredient more difficult.[2,9]

The phytochemical profile (carotenoids and phenolics) determined by spectrophotometric and HPLC analyses are presented in Table II. The data from the HPLC analysis revealed the presence of β-carotene as predominant carotenoid and *β*-cryptoxanthin.

The obtained results of the phytochemical content of peach waste extract and encapsulates (Table II) are in accordance with spectrophotometrical data, where E1 exhibited a significantly higher *T*Car ($p < 0.05$) compared with E2 and E3. Giuffrida *et al.*[26] found three free carotenoids (zeaxanthin, *β*-carotene, and *β*--cryptoxanthin) in the peach extract, juice, and jam, whereby *β*-carotene was the most abundant in all samples. The carotenoid profile reported by Dalla Valle *et al.*[15] included *β*-carotene, *β*-cryptoxanthin, lutein, zeaxanthin, and lesser amounts of additional related compounds.

The phenolics profile determined by HPLC analysis includes the presence of four phenolic acids (*p*-coumaric, *p*-hydroxybenzoic, caffeic, and chlorogenic acid), and four flavonoids (catechin, epicatechin, rutin, and quercetin). Epicatechin and catechin are the most abundant compounds, the quantities of which significantly differ among the samples. These findings could be explained by the difference in the applied encapsulation carriers based on yeast pretreatment, as well as their different capacity for bonding various compounds. Similar phenolics and flavonoids profiles were also reported by Stojanovic *et al.*[27] who studied peach pulp and peel extracts. They reported that the peach pulp contains mainly chlorogenic, neochlorogenic, and *p*-coumaric acids, whereas the peel contains chlorogenic, neochlorogenic and *p*-coumaric acids together with several flavanol glycosides in huge amounts. Furthermore, Andreotti, Ravaglia *et al.*[23] detected chlorogenic acid, catechin, epicatechin, rutin, and cyanidin-3-glucoside as the main phenolic compounds of ripened peach fruits. Generally, the present HPLC results showed the presence of the same groups of phenolic compound as in the available literature.

The antioxidant activity of carotenoids is based on the scavenging peroxyl radicals and quenching of single oxygen ($^1O_2$), while phenolics act as reducing agents, free radical scavengers, and quenchers of $^1O_2$ formation.[29] There is no single chemical assay that can accurately evaluate the contribution of the hydrophobic and hydrophilic compounds to the total antioxidant activity of the plants.

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Therefore, several assays were performed, *i.e.*, SA and RP with hydrophilic fractions, and BCB with hydrophobic fractions (Fig. 1). The SA and RP tests (Fig. 1) showed significantly higher values for E1 and E3, which could be correlated with the phenolic content of the mentioned encapsulates.

TABLE II. Total and individual contents of carotenoids and phenolics; *: non-plasmolyzed; **: plasmolyzed freeze-dried; ***: alive. Data present the mean value of three replicates±*SD*; values sharing the same letter (a, b, and c) in the same row are not significantly different at the 0.05 level according to the Tukey's HSD test

Compound	Unit	Sample			
		PW extract	Yeast cells		
			E1*	E2**	E3***
Spectrophotometrical analysis					
*T*Car	mg *β*-carotene (100 g^{-1})	1.9±0.3^{d}	1.4±0.1^{c}	0.7±0.0^{b}	0.2±0.1^{a}
*T*Ph	mg GAE (100 g^{-1})	641.6±13.9^{d}	571.4±39.8^{c}	146.8±17.1^{a}	386.5±4.6^{b}
HPLC analysis					
Epicatechin	mg (100 g^{-1})	12.9±2.6^{b}	303.2±2.1^{c}	24.0±0.1^{a}	351.6±1.3^{d}
Catechin		493.3±6.1^{d}	8.5±0.3^{a}	35.7±0.3^{b}	230.9±2.7^{c}
Caffeic acid		3.0±0.1^{c}	0.1±0.0^{a}	0.1±0.0^{a}	0.4±0.0^{b}
p-Coumaric acid		3.0±0.2^{d}	0.3±0.0^{b}	0.1±0.0^{a}	1.2±0.0^{c}
Chlorogenic acid		13.4±0.5^{d}	1.1±0.0^{b}	0.4±0.1^{a}	4.6±0.0^{c}
p-Hydroxybenzoic acid		30.4±1.8^{d}	2.0±0.0^{b}	1.0±0.0^{a}	6.8±0.1^{c}
Rutin		0.3±0.1^{c}	0.2±0.1^{b}	0.1±0.0^{a}	0.1±0.0^{a}
Quercetin		0.2±0.0^{b}	0.1±0.0^{a}	0.2±0.0^{b}	0.1±0.0^{a}
Total phenolics		556.6±14.3^{c}	315.5±2.4^{b}	61.6±4.5^{a}	595.7±12.3^{d}
β-Cryptoxanthin		0.2±0.0^{c}	0.1±0.0^{b}	0.1±0.0^{b}	0.0±0.0^{a}
β-Carotene		1.3±0.2^{c}	1.5±0.3^{c}	0.1±0.0^{a}	0.1±0.0^{a}
Total carotenoids		1.5±0.2^{c}	1.6±0.9^{c}	0.2±0.0^{a}	0.1±0.0^{a}

On the other hand, the BCB assay showed a strong relationship between carotenoids content and their potential to neutralize the linoleate free radicals and other free radicals formed in the *β*-carotene–linoleic acid system.

Phytochemicals derived from plants have numerous health benefits, and the therapeutic potential to cope with oxidative stress caused diseases when consumed at a proper level. These natural antioxidants, especially carotenoids and phenolics, exhibit a wide range of biological effects due to their action as free radical scavengers and inhibitors of lipid peroxidation. Generally, the antioxidant capacities of the samples were strongly dependent on the employed solvent, due to the different antioxidant potential of the compounds with different polarities.[26]

As presented in Table III, *Saccharomyces cerevisiae* yeast cells have been investigated for the encapsulation of hydrophobic (carotenoids) and hydrophilic compounds (phenolics).

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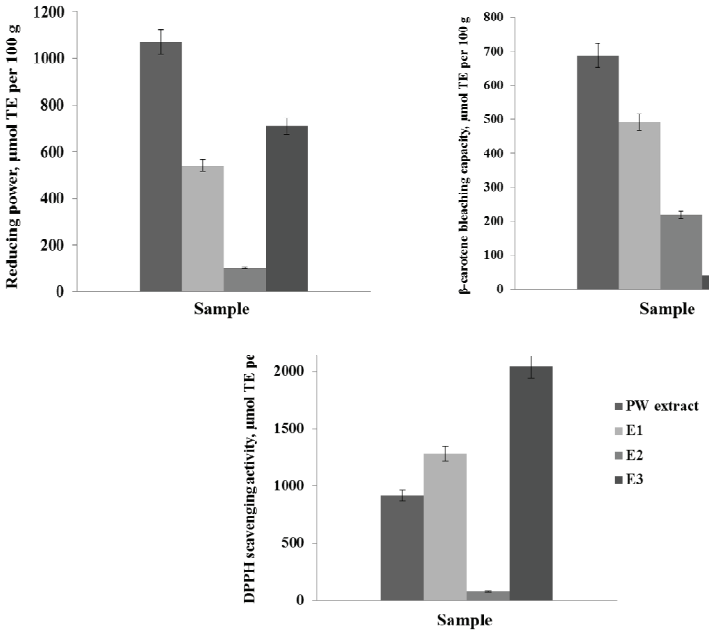

Fig. 1. Antioxidant activity of peach waste extract and encapsulates.

TABLE III. The encapsulation efficiency (%) of carotenoids and phenolics; the data present the mean value of three replicates±*SD*; Values sharing the same letter (a, b, and c) in the same row are not significantly different at the 0.05 level according to the Tukey's HSD test

Yeast sample	Component	
	Carotenoids (*EE*Car)	Phenols (*EE*Ph)
Non-plasmolyzed (E1)	86.59±4.61^{c}	66.98±0.39^{c}
Plasmolyzed freeze-dried (E2)	63.17±6.72^{b}	24.59±4.01^{a}
Alive (E3)	26.97±5.10^{a}	46.82±4.83^{b}

As presented in Table III, the *EE*Car value ranged from 26.97 to 86.59 %. The highest *EE*Car value (86.59 %) was obtained with freeze-dried non-plasmolyzed yeast suspension (E1). The same trend was observed for *EE*Ph, where the application of non-plasmolyzed yeast suspension as the carrier exhibited the highest efficiency (66.98 %). As previously mentioned, the increasing intracellular space by the loss of cytoplasmic materials could be effective concerning the encapsulation efficiency. For this purpose, plasmolysis with a NaCl solution is commonly used as a pretreatment for the reduction of the intracellular contents of cells. Some studies reported a greater increase in the encapsulation efficiency after yeast cell pretreatment. Thus, Shi *et al.*[6] showed a two-fold higher encapsulation efficiency of chlorogenic acid in plasmolyzed yeast cells. Dadkhodazade *et al.*[30] reported the highest *EE* value (76.10 %) obtained with spray-dried plasmolysed yeast cell encapsulates. Kavosi[31] *et al.* revealed increasing purslane oil

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encapsulation efficiency from 52.96 to 60.27 % on increasing the intracellular space. However, Paramera *et al.*[1] observed that cell plasmolysis had no significant increase on the encapsulation efficiency of curcumin. According to their explanation, curcumin was entrapped in the yeast cell by its adhesion to the cytoplasmic cell membranes or by interaction with the constituents of the cell walls *via* hydrogen bonds. Therefore, the removal of water-soluble components of the cytoplasm had no significant effect on curcumin uptake. Bishop *et al.*[10] also showed effective encapsulation of essential oils in *Saccharomyces cerevisiae* without prior plasmolysis. Differences among the results could be explained by the permeation of both hydrophobic and hydrophilic molecules, as well as the physicochemical properties of the cell wall.[2] The encapsulation efficiency also depends strongly on the purity of the target molecules.[6] Variations in the results could also be caused by the molecular size of the permeating compounds, diversity of treatments, and encapsulation protocols.

The FT-IR spectra of non-plasmolyzed, plasmolyzed freeze-dried yeast cells, alive yeast cells (C1–C3) and appropriate encapsulates (E1–E3) with PW extract, as well as the initial PW extract, are shown in Fig. 2.

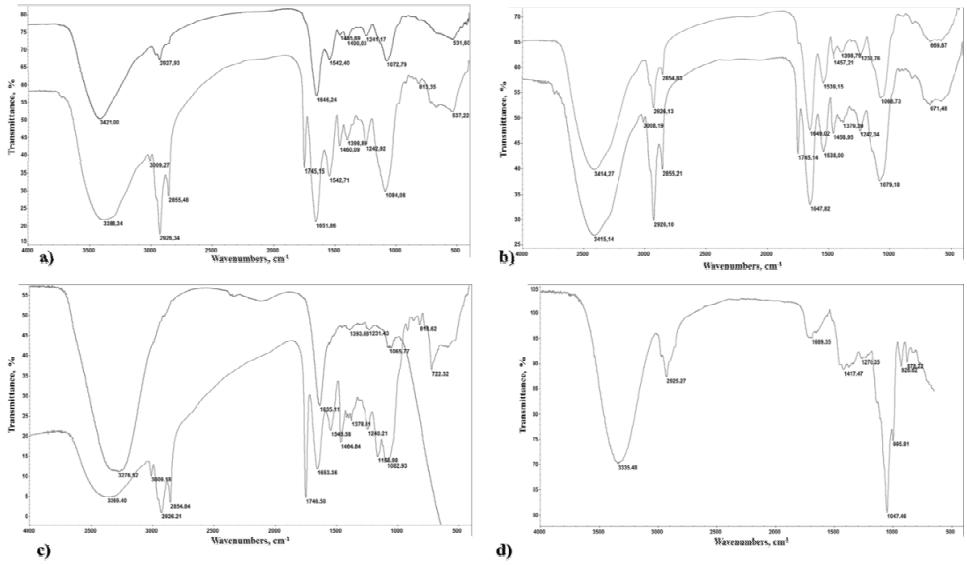

Fig. 2. FT-IR spectra of samples: a) non-plasmolyzed, b) plasmolyzed freeze-dried yeast cells, c) alive yeast cells (C1–C3) and appropriate encapsulates (E1–E3) with PW extract; d) initial PW extract.

Plasmolysis is the phenomenon in which intracellular water is drawn out of the cell and because of the internal water loss, the cell membrane contracts from the cell wall. This phenomenon occurs by modifying the structure of the cell wall

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and particularly of the membrane that affects the lipids, proteins, nucleic acids, and carbohydrates of the whole yeast cell, cell wall, and membrane.[1] Due to these changes, some differences between the spectra of non-plasmolyzed, alive, and plasmolyzed yeast cells appeared (Fig. 2a–c). Changes in the region 3000––2800 cm^{-1} indicate modifications of the length of the lipid chains and the structure of the yeast membrane. Changes in the peak shapes in the region 1800–1500 cm^{-1} indicate partial protein degradation mainly involving the yeast cell wall. Peaks within this region are associated with amide I and II signals of the protein, carbonyl stretching modes of the pectic polysaccharides, and the O–H bending mode of water.[32] Changes in peak shapes in the band region 1500–1390 cm^{-1} indicate degradation of one part of the yeast proteins mainly involving the cell membrane and the cell wall, while changes in peak shapes in the region 1100––700 cm^{-1} indicate degradation of the cell wall of the polysaccharides (glucans and mannans). The results showed that plasmolysis disorganized the yeast plasma membrane and increased its fluidity. Furthermore, the yeast cell wall became thinner since the mannoproteins were partially degraded along with β-1,4- and β-1,6-glucans.

From the FT-IR spectra of the PW extract (Fig. 2d) some peaks that belong to carbohydrates (region 1100–700 cm^{-1}) are visible (for example, peaks 1047, 995, 926, 877 cm^{-1}). These peaks did not exist in the spectra of yeast cells, while in the encapsulates,, these peaks disappeared or their shape is changed (Figs. 2a––c). This indicates to the fact that the main bands of the PW extract weakened when they interacted with yeast cell components and that extract molecules are rather located inside the yeast cells. This was confirmed by the fact that the FT--IR spectra of the encapsulates are similar to the spectra of the carriers (Figs. 2a––c). In all three encapsulates, it could be noted that the position of the carbonyl peak was at a high wavenumber value (1745 cm^{-1}). Since the position of the carbonyl group in the proteins (more bonded, less free) is around 1650 cm^{-1}, it could be assumed that this carbonyl group is freer in vibration, *i.e.*, protein degradation has occurred maybe because of interaction with the extract. The results are in accordance with those of Shi *et al.*[6] and Paramera *et al.*[1], who encapsulated chlorogenic acid and resveratrol, as well as curcumin in yeast cells, respectively.

From the results, it could be confirmed that peach PW was successfully incorporated into the yeast cells by intermolecular interactions and their integrity was preserved after the freeze-drying process. This means the different yeasts used as carriers are compatible materials for PW extract encapsulation according to the FT-IR analysis.

The SEM images of non-plasmolyzed, plasmolyzed freeze-dried yeast cells (C1–C2) and corresponding encapsulates (E1–E2), as well as encapsulates in alive yeast cells (E3), are shown in Fig. 3.

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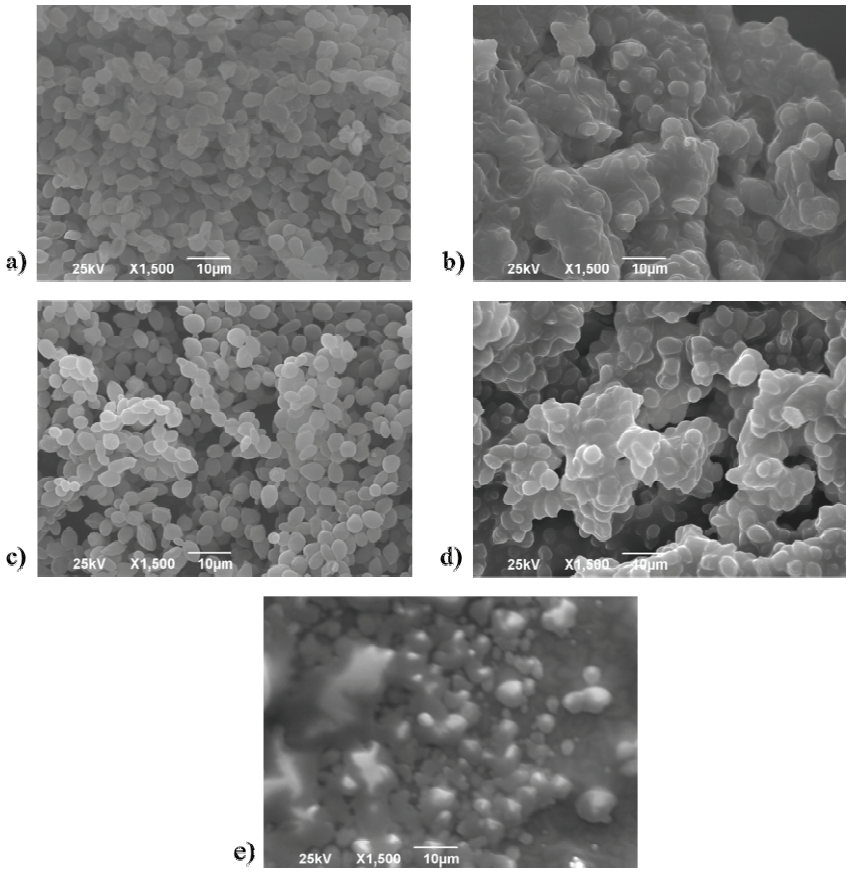

Fig. 3. SEM images of samples: a) non-plasmolyzed and c) plasmolyzed freeze-dried yeast cells (C1–C2), and b, d) appropriate encapsulates (E1–E2), as well as e) encapsulates in alive yeast cells (E3).

It could be seen from Fig 3a–c that the PW extract was successfully encapsulated in yeast cells, both non-plasmolyzed, plasmolyzed, and alive. Additionally, there were no differences in the morphological properties of the cell wall of the yeast cells and PW extract loaded yeast cells, *i.e.*, the surface morphology was intact and with no burst on the surface. This explains the absence of difference in non-plasmolyzed and plasmolyzed yeast cells (Figs. 3a and c) because the plasma membranes are often considered to be the primary site of osmotic injury.[28] The results are in accordance with those of Salari *et al.*[4] who concluded that the encapsulation process did not affect the organization of the cell wall.

CONCLUSIONS

This study shows that peach waste could be encapsulated in non-plasmolyzed, plasmolyzed and living *Saccharomyces cerevisiae* yeast cells. The best

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encapsulation efficiency was achieved with freeze-dried, non-plasmolyzed yeast cells, However, Fourier transform infrared spectroscopy and scanning electronic microscopy confirmed encapsulation was also obtained for plasmolyzed, and living cells. Overall, it could be concluded that peach waste, a rich source of valuable bioactive compounds (phenolics and *β*-carotenes), encapsulated in yeast cells as carriers could find possible use in the food and pharmaceutical industries. Struggling with the disposal of organic fruit waste around the world and providing recovery of health–improving substances that have great potential for utilization and high functional properties for the food industry, this study explains carotenoids and phenolics encapsulation in yeast cells which could be a promising technique for the stability of peach waste extract for all production levels.

Acknowledgment. The financial support of the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Contract No. 451–03–68/2020–14/200134) is gratefully acknowledged.

И З В О Д

ИНКАПСУЛАЦИЈА СПОРЕДНОГ ПРОИЗВОДА ПРЕРАДЕ БРЕСКВЕ У ЋЕЛИЈЕ КВАСЦА *Saccharomyces cerevisiae*

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Секундарни производ прераде бресквe (PW) представља еколошки проблем, али и потенцијални извор природних антиоксиданата. Један од иновативних начина да се повећа стабилност фитохемикалија из споредних производа јесте инкапсулација у ћелије квасца, који поседује добре структурне карактеристике. У овом раду, PW екстракт је инкапсулиран методом лиофилизације у неплазмолизираним, плазмолизираним и живим ћелијама *Saccharomyces cerevisiae*. HPLC анализом је утврђено да је β-каротен најдоминантнији каротеноид, док су епикатехин и катехин најдоминантнији феноли у PW екстракту. Највећа ефикасност инкапсулације каротеноида (86,59 %), као и фенола (66,98 %) је постигнута приликом инкапсулације PW екстракта у лиофилизиране, неплазмолизиране ћелија квасца. Иако плазмолиза може изазвати промене у структури и особинама ћелије квасца, овај поступак није утицао на повећање ефикасности инкапсулације присутних фитохемикалија. Успешна инкапсулација PW екстракта у ћелије квасца потврђена је и FT-IR спектроскопијом и SEM микроскопијом. На основу резултата овог рада може се закључити да је примењена метода инкапсулације осетљивих једињења у ћелије квасца адекватна основа за стабилизацију вредних компоненти и њихову потенцијалну употребу у прехрамбеној и фармацеутској индустрији.

(Примљено 1. децембра 2020, ревидирано 2. јануара, прихваћено 4. јануара 2021)

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