Research Article

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Saccharomyces cerevisiae as a delivery system of *Zataria multiflora* Boiss. essential oil as a natural preservative for food applications:

Encapsulation of Iranian *Zataria multiflora* Boiss. essential oil

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Abstract

BACKGROUND: The following study is an evaluation of the encapsulation, stability and release profile of Iranian *Zateria multiflora* boiss essential oil (ZEO) in *Saccharomyces cerevisiae* yeast cells. Encapsulation was performed with different essential oil / yeast weight ratios at different temperatures. The encapsulation efficiency and stability of the loaded yeasts and the release profiles of carvacrol and thymol (as the main active ingredients of ZEO) were also investigated.

RESULT: The encapsulation efficiencies of carvacrol and thymol at a ZEO / yeast weight ratio of 1.25 were 30.9% ± 0.01% and 44.5% ± 0.02%, respectively. Loaded yeast cells were stable during the 4-week storage period. Both carvacrol and thymol showed substantial releases of around 60% during the first hour and around 70% during the second hour at two different water temperatures, followed by steady release.

CONCLUSION: *Zateria multiflora* boiss essential oil can be encapsulated effectively in *S. cerevisiae* yeast cells, refrigerated without degradation, and released efficiently. *Zateria multiflora* boiss essential oil encapsulated into *S. cerevisiae* yeast may be used as a potential preservative for the food and drug industry.
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Keywords: Encapsulation; *Zateria multiflora*; *Saccharomyces cerevisiae*; essential oil; natural preservative; yeast cell

INTRODUCTION

Many methods are used nowadays for the preservation of food products including aseptic handling and packaging, mechanical removal of microorganisms by washing or filtration, physical or chemical sanitization, and finally environmental control. This can also be performed by adding chemical preservatives with inhibitory or bactericidal / fungicidal activity.[1] However, consumers consider the use of chemical preservatives as a drawback.[2] In recent years, natural antimicrobial compounds have attracted increasing attention due to consumer awareness of the food quality and safety aspects.[1]

Natural antimicrobials are an evolving technology in the food industry to increase shelf life as well as the safety of food. Essential oils (EOs) are the most abundant plant extracts that are tested for antimicrobial activity in foods. These are aromatic compounds, consisting of more than 60 ingredients, which are usually extracted by steam distillation.[3]

Essential oils have been shown to be effective *in vitro* against a wide range of foodborne pathogenic organisms such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio* species. They generally have a greater effect against gram-positive than gram-negative microorganisms, and can also be active against fungi.[3, 4]

Zataria multiflora Boiss. is an aromatic medicinal plant that belongs to the Labiatae family and grows in Iran, Pakistan, and

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Afghanistan. It has been traditionally used as a flavoring and preservative agent in food and drinks.[5] Carvacrol (2-methyl-5-(1-methylethyl)phenol) and thymol (5-methyl-2-(1-methylethyl)phenol) are isomers of monoterpenic phenol and the main active constituents of *Z. multiflora* essential oil (ZEO).[6, 7] These naturally lipophilic compounds damage the bacterial cell membrane considerably and result in permeability change and in the release of the cellular contents.[8]

Concerns about the negative effects of antimicrobial compounds on the physical and /or chemical stability of food, and the degradation of activity of antimicrobial compounds, make it necessary to use high enough concentrations of antimicrobial agents to inhibit microbial proliferation but at the same time make minimal alterations in quality of the food product.[1] The fat composition of foods influences the effectiveness of EOs.[3] A high concentration of plant extracts is limited due to their organoleptic properties.[2] Concentration optimization of plant extracts as preservatives in food products is therefore of the utmost importance. The organoleptic and stability problems can be overcome by encapsulation instead of using these compounds directly in food products.[2]

Microencapsulation is defined as enveloping a substance (usually active agents) within another one, in capsules smaller than one to several hundred microns in size.[9] Encapsulation can increase the concentration of antimicrobial agents in aqueous phase or liquid-solid interfaces where microorganisms are located.[1]

Yeast cells are attractive encapsulants as they are a 'generally recognized as safe' (GRAS) material. Yeast cells can be used for the encapsulation of both hydrophobic and hydrophilic active food ingredients, as well as pharmaceutical agents and probiotics for preserving or masking taste, and targeting drug delivery. Whole cells or only the cell wall of *Saccharomyces cerevisiae* have been used for encapsulation.[10-19] Other yeast cells such as *Saccharomyces bayanus*, *Candida utilis, Kluyveromyces fragilis*, *Torulopsis lipofera, Endomyces vernalis*, and oleaginous yeast have also been used for this purpose. In addition, encapsulation in yeast cells is not only simple, low cost, but can also be done without any additives.[10]

The objective of this study was the encapsulation and characterization of Iranian ZEO in yeast cells for food preservation applications.

MATERIALS AND METHODS

Materials

Lyophilized *S. cerevisiae* (PTCC 5269) was purchased from the Industrial and Scientific Research Organization of Iran. Iranian *Zataria multiflora* essential oil was purchased from Research Center of Chemical Industry of Iran, Karaj. Carvacrol and thymol standard solutions were purchased from Merck, USA. Deionized distilled water was purchased from Samen Pharmaceutical Company, Mashhad, Iran. Culture media (yeast extract, bacteriological peptone, glucose) were purchased from Himedia, India.

Methods

Cultivation of S. cerevisiae

The culture of yeast cell was done according to the method described by Ciamponi *et al*. (2012) with some modifications. Two hundred milliliters of yeast extract peptone dextrose (YPD) (yeast extract 1% w/v, bacteriological peptone 2% w/v, and glucose 2% w/v) was inoculated with 1.5 mL of 10^8 colony forming unit (CFU)/mL yeast suspension (in normal saline) and incubated at 37 °C at 100 rpm in a shaking incubator for 11 h.

Yeast cells at the logarithmic phase of proliferation were harvested by centrifugation (3000 ×g/10 min), followed by washing three times in phosphate-buffered saline (PBS) and freeze drying.[20, 21]

ZEO Analysis

Quadruplicates of ZEO (10 mg) were diluted in 100 mL acetonitrile (ACN):H_2O (80:20). Triplicates of each sample was injected into the high-performance liquid chromatography (HPLC) C18 column, Agilent 1260 System (Agilent Technologies, Germany, Frankfurt). The mobile phase was ACN:H_2O (50:50); flow-rate of 1.0 mL/min; and the detector: UV-visible at 275 nm.

The essential oil was also analyzed by gas chromatography–mass spectrometry (GC/MS) using a Varian Saturn 3 apparatus (Varian, Ontario, Canada) under the following operating conditions: capillary column, 50 m × 0.25 mm i.d., 0.25 μm film thickness (Chrompack, Germany, Lörrach), helium (He) carrier gas, 1 mL/min flow rate, oven temperature of 60–240 °C, with rates of 3 °C per minute, split injection injector mode, electron impact (EI) ionization mode, at 70 eV, 280 °C interface temperature, and 40–300 U scan range.[22]

ZEO Encapsulation in yeast cells

The encapsulation was done according to the method described by Bishop *et al*. (1998) with some modifications. Various ratios of ZEO/yeast cell (0.5 (0.5/1), 0.75 (1.5/2), 1 (1/1), and 1.25 (2/1.6) w/w) were mixed with distilled water (10 mL), in an Erlenmeyer flask, and incubated in a shaker incubator at 100 rpm, 40 °C for 48 h. Then the cells were centrifuged at 3000×g for 10 min. The supernatant was removed and yeast cells were washed three times with PBS and then freeze-dried for 48 h. The encapsulation process was repeated for the ratio with the best encapsulation result at two different temperatures (30 °C and 50 °C) and the encapsulation efficiency (%EE) was estimated.[23, 24]

Determination of encapsulation efficiency

The encapsulation efficiency was determined according to a method described by Paramera *et al*. (2011). Equation (1) was used to determine %EE:

$$\%EE = \frac{\text{concentration of encapsulated essential oil}}{\text{initial concentration of essential oil}} \times 100 \qquad (1)$$

Yeast cell micro-morphology

The structure of the loaded and unloaded yeast cells was studied using transmission electron microscopy (TEM). Samples were fixed first in 2.5% glutaraldehyde (v/v) (2-4 h – 4 °C) and then in 1% osmium tetroxide(v/v) for 2 h at ambient temperature. The fixed cells were embedded in acrylic resin for 2–24 h at ambient temperature, and stained with 1% (w/v) uranyl acetate and lead citrate (0.004 w/v) after sectioning with an ultramicrotome (75 nm section). The microsections were stained with toluidine blue (1% w/v, ambient temperature, 1 s) and photographed with light microscope. Some samples were also analyzed using TEM (Leo 912 AB, Germany, Waldbronn).[23]

Fourier-Transform Infrared Spectroscopy (FTIR) analysis

Fourier transform infrared spectra was acquired on a Thermo Nicolet, AVATAR 370, FTIR spectrometer using the KBr disk

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Table 1. Composition of Zataria multiflora essential oil (ZEO)

	RT	Area pct	Library/ID
1	11.04	1.68	IR-alpha-pinene
2	13.66	0.10	.alpha.-phellandrene
3	14.11	1.12	(+)-4-carene
4	14.43	5.23	p-cymene
5	15.71	4.44	a-terpinen
6	19.96	0.11	Borneol
7	20.37	0.39	Terpinen-4-ol
8	20.98	0.21	Alpha terpineol
9	24.50	0.20	Bornyl acetate
10	25.60	25.49	Thymol
11	26.07	51.55	Carvacrol
12	31.91	0.60	Valencen
13	39.23	0.32	Caryophyllene oxide

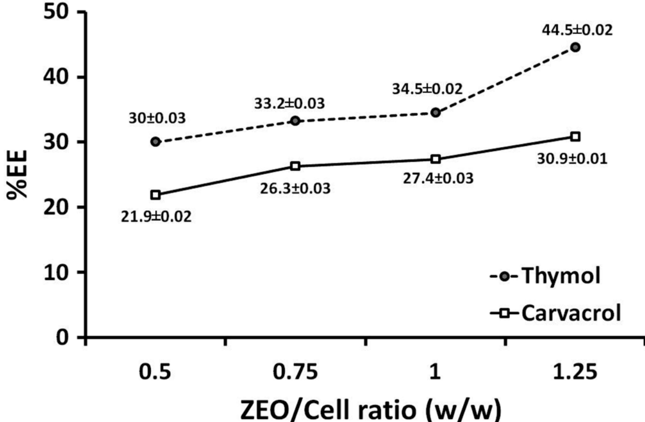

Figure 1. Encapsulation efficiency of carvacrol and thymol for different ZEO / cell ratios (0.5, 0.75, 1, and 1.25 (w/w)).

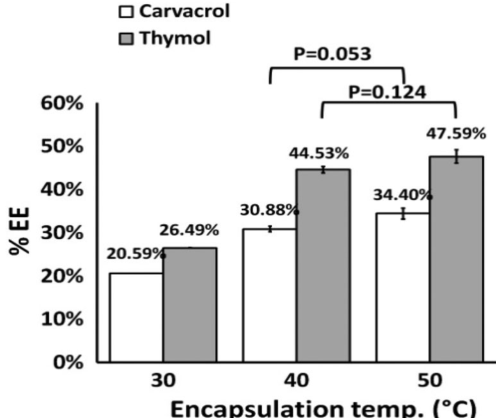

Figure 2. %EE of carvacrol and thymol in samples with 1.25 ZEO / yeast ratio at different temperatures.

method. The FTIR spectra of individual unloaded and loaded yeasts were assessed by mixing samples with potassium bromide (70 mg), compressed into a pellet by applying 1 ton/unit. The FTIR spectrum of ZEO alone was assessed by loading it on a KBr pellet. Spectral scanning was done in the 4000–400 cm^{-1} wave range at a resolution of 2 cm^{-1}.[21, 25]

Stability of loaded yeasts

The stability of the encapsulated yeast powder was determined by quantifying carvacrol and thymol in yeast cells at different time intervals (0, 1, 2, 3, and 4 weeks) after encapsulation through HPLC analysis (see above). Twenty milligrams of loaded yeasts was suspended in 2 mL distilled water and 8 mL ethanol was added and then sonicated for 15 s. The cell suspension was then centrifuged (3000×g, 10 min) and the supernatant was filtered through a 0.45 μm filter.[26]

Release profile of carvacrol and thymol

The *in vitro* release of carvacrol and thymol was assessed according to a method adapted from Shi *et al.* (2008)[25] with some modifications. Briefly, 2 mg of loaded yeast cells was suspended with 1 mL distilled water. The suspension was incubated in two different temperatures, 10 °C and room temperature, in a shaker incubator (100 rpm). The samples were centrifuged at predetermined intervals (0.5, 1, 2, 4, 6, 24, 48, 72, and 168 h). The supernatant was collected and replaced with 1 mL of fresh medium at each time interval. The supernatant was filtered using 0.45 μm membrane and analyzed through HPLC with the same method described in the previous section. All experiments were repeated three times.

Statistical analysis

Statistical analysis was carried out using SPSS ver. 21 (IL, USA). The stability of loaded yeast cells during the 4-week storage period was compared using the Friedman test. The **%**EE of carvacrol and thymol at different temperatures was compared by one-way ANOVA. Comparison of groups (different temperatures) for %EE was performed using the Dunnett T3 post hoc test. Repeated measures analysis of variance (ANOVA) followed by the Dunnett T3 post hoc tests were used to compare the release profiles of carvacrol and thymol in loaded yeasts at two different temperatures. A result of $P < 0.05$ was defined as statistically significant.

RESULTS

High-performance liquid chromatography (HPLC) analysis showed that carvacrol and thymol constituted 51.75% ± 1.14 (w/v) and 25.65% ± 1.4 (w/v) of total ZEO, respectively. The

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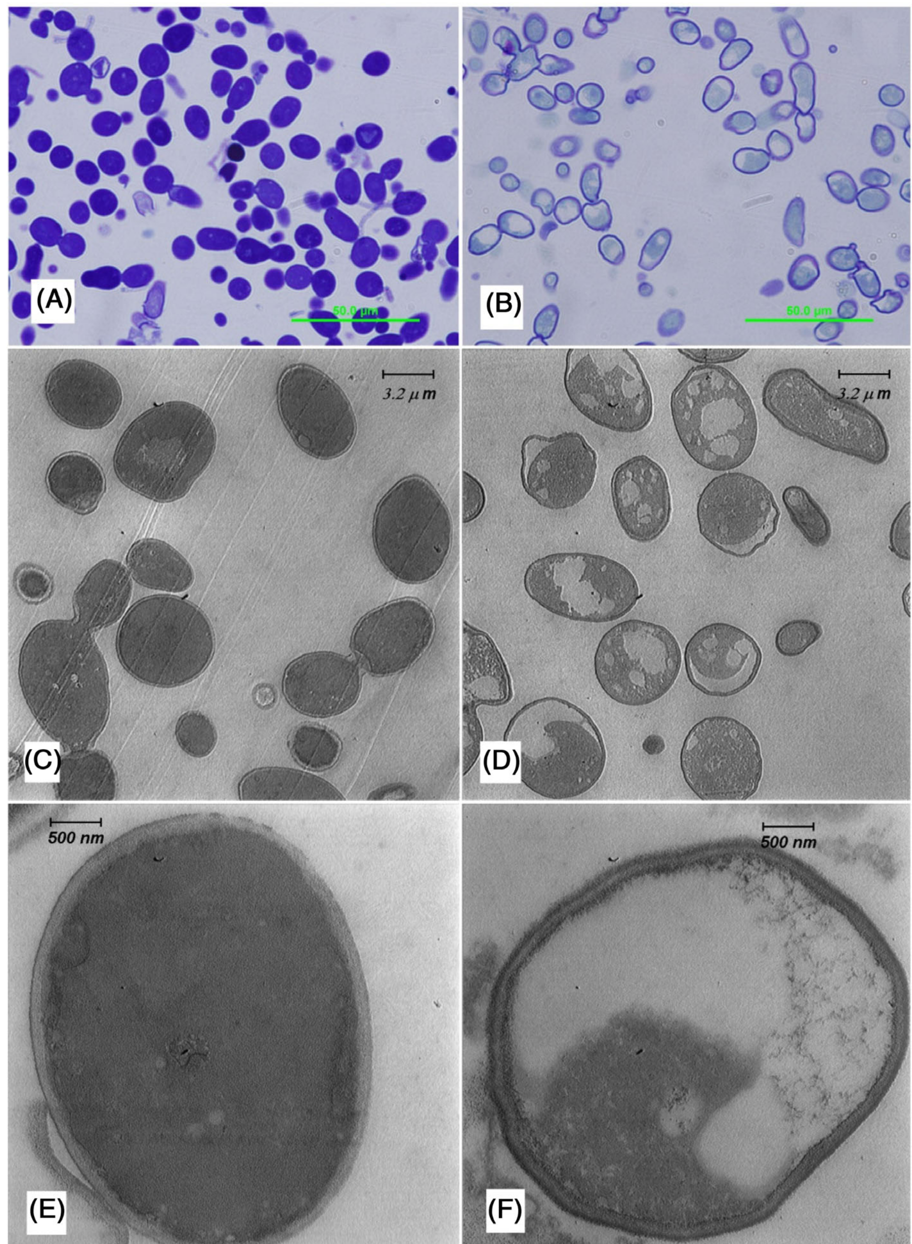

Figure 3. Light microscopy images of semi-thin sections (1000 nm) of (a) non-loaded and (b) loaded yeast cells, and TEM images of (c, e) non-loaded and (d, f) loaded yeast cells.

retention time peaks of carvacrol and thymol were observed at 12.95 and 14.08 min, respectively.

The limits of detection (LoDs) and quantization (LoQs) of carvacrol and thymol were 0.07 and 0.03 ppm, and 0.1 and 0.05 ppm, respectively.

Composition determination results of ZEO by GC/MS are shown in Table 1.

According to GC/MS data, carvacrol (51.55% ± 1.3 (w/v)) and thymol (25.49% ± 1.5 (w/v)) were the major components of ZEO. Good substantial similarities were seen between the GC/MS and HPLC analysis techniques.

The two major components of ZEO, carvacrol and thymol, were used for determination of %EE. Fig. 1 shows the %EE of carvacrol and thymol loaded into freeze-dried yeasts with four different weight ratios of ZEO to yeast cells.

According to our results, the carvacrol and thymol content (%) were in the range of 21.87–30.88% and 30–44.53%, respectively. In other words, the more the ZEO/cells weight ratio, the higher the %EE obtained in the observed range. As the ZEO/cells weight ratio of 1.25 resulted in the highest loading efficiency, this ratio was selected for further experiments. These samples were encapsulated at three different temperatures (30, 40, and 50 °C) and their %EE were determined (Fig. 2).

Increasing the temperature from 30 to 40 °C resulted in a significant increase in the loading efficiency of both carvacrol and thymol ($P < 0.005$); however, no significant increase in %EE was seen between encapsulation at 40 and 50 °C ($P = 0.053$ and $P = 0.124$ for carvacrol and thymol, respectively).

The microscopic morphology of yeast cells was examined. Stained toluidine blue unloaded yeasts appeared as separate,

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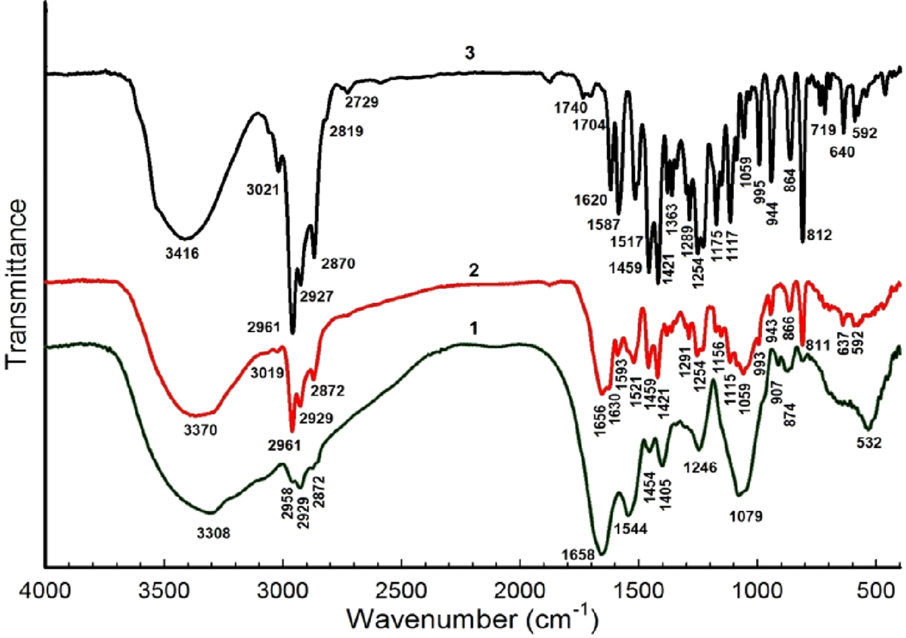

Figure 4. FTIR spectra of (1) unloaded yeast cells, (2) loaded yeast cell, and (3) essential oil.

Table 2. FT-IR peak analysis of unloaded and EO-loaded yeasts as well as EO alone

	Wave number (cm^{-1})	Functional group
EO	3416	Phenolic OH of carvacrol and thymol
	3020, 2961, 2927, 2870, 2819, and 2729	aromatic and vinylic C-H and asymmetrical and symmetrical CH_3 and CH_2
	1740, 1704	C=O (carbonyl) groups
	1620, 1587, 1517, 1459, and 1421	Non-conjugated C=C and benzene ring skeleton of terpinene, cymene, carvacrol and thymol, CH_3
	1289 and 1254	Phenolic C–O
	1254, 1175, and 1059	Methoxy C–O and C-O-C
	995, 944, 864, 812, and 719	Aromatic C–H
unloaded yeast cells	3700–3000	OH and NH of nucleic acids, proteins, and carbohydrates of the whole yeast cell, cell wall, and membrane
	2958, 2929, and 2872	Asymmetrical and symmetrical CH_3 and CH_2 of nucleic acids, proteins, and lipid chains
	1658 and 1544	Protein amide I and amide II
	1454 and 1405	Asymmetric and symmetric bending modes of CH_3
	1246	C-O or asymmetrical vibrations of PO_2^-,which are mainly derived from DNA and RNA
	1079, 907, and 874	Glucans and mannans
loaded yeast	3370	Phenolic OH of carvacrol and thymol
	Slight shift (2–3 cm^{-1}) at 3021–2819	C-H of essential oil
	1656 and 1593	Protein amide I and II
	Some changes in the 1110-1000 cm^{-1}region	
	Disappearance of the 1087	

entirely dark blue stained round or ovoid particles in light microscopy. Loaded yeasts had dark blue cell walls and pale blue cytoplasms with white spots. In other words there were the clear whitish regions within the cell cytoplasms that absorbed no dye (Fig. 3(a),(b)).

The unloaded yeasts appeared as homogeneously dark globules in TEM images, while the loaded cells clearly showed lipid droplets with various sizes (Fig. 3(c)–(f)).

Figure 4 illustrates the FTIR spectra of unloaded and loaded yeast cells as well as ZEO alone.

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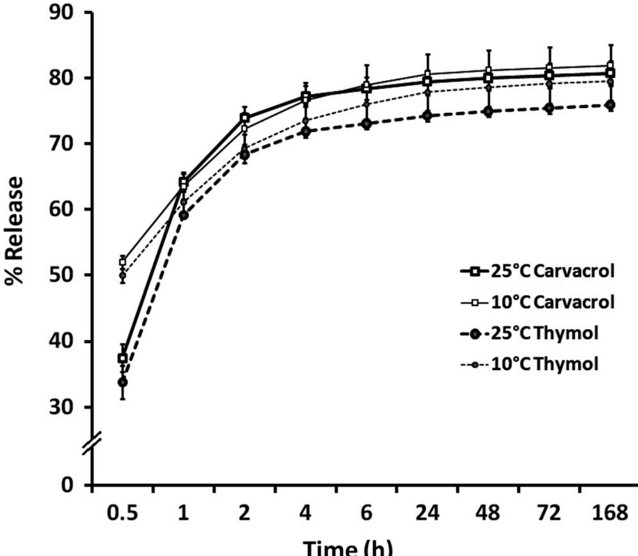

Figure 5. Release pattern of carvacrol and thymol from loaded yeast cells at room temperature and 10 °C over the period of 1 week.

The peak analyses of FTIR spectra are summarized in Table 2. The FTIR spectrum of the ZEO shows a broad absorption band in the region of 3416–3100 cm^{-1}(O-H stretching), 3021 cm^{-1} (Aromatic C-H stretching), 2961–2870 cm^{-1} (C-H stretching), 1620 and 1587 cm^{-1} (C=C stretching), 1363 cm^{-1}($C(CH3)_2$bending) and 1254 cm^{-1}(C-O stretching). The characteristic absorption bands of yeast were observed at 3400–2500 cm^{-1} (N-H and O-H stretching), 2958–2872 cm^{-1} (C-H stretching of CH3), 1658 cm^{-1} (amide-I, C=O stretching), 1544 cm^{-1} (amide-II, N-H bending and C-N stretching) and also 1079 cm^{-1}(C-O-C stretching of glycosidic bonds in polysaccharides). Further, the yeast cell encapsulated ZEO shows the characteristic peaks at 3500–2600 cm^{-1} (O-H stretching, N-H stretching), 3019 cm^{-1} (Aromatic C-H stretching), 2961–2872 cm^{-1} (C-H stretching), 1656 and 1593 cm^{-1} (C=O stretching of amide I, II group), 1630 and 1521 cm^{-1} (C=C stretching) and 1052 cm^{-1} (C-O stretching). Comparing these spectra, it can be concluded that when the ZEO was added to a yeast cell, an absorption band at 3019 cm^{-1}, attributed to aromatic C-H stretching vibration, was shown and there is no absence of any functional peaks in all the spectra. Thus, it revealed that ZEO was successfully encapsulated in the yeast cell.

The influence of storage conditions on loaded yeast cells, stored at 4 °C for up to 4 weeks, is shown in Table 3. Our results show that microcapsules were stable during the 4-week storage period, and no significant decrease was seen in the EO content in the cells during those periods.

The *in vitro* release profiles of carvacrol and thymol from loaded yeast cells prepared with 1.25 ZEO/yeast cells weight ratio at 40 ° C are shown in Fig. 5.

An initial burst was followed by sustained carvacrol and thymol release. Both carvacrol and thymol release processes showed significant differences between 10 and 25 °C ($P < 0.001$), but the average release of carvacrol and thymol during 1 week did not show any significant differences between the two temperatures ($P = 0.082$ and $P = 0.088$, respectively). Over a period of 168 h (1 week) carvacrol was released up to 81.94% and 80.79% at 10 °C and 25 °C, respectively. Similar results were obtained for thymol. The release of thymol over the period of 1 week was up to 79.53% and 75.92% at 10 °C and 25 °C, respectively.

Table 3. Stability of loaded lyophilized cells at 1, 2, 3, and 4 weeks after loading

Time	0	First week	Second week	Third week	Fourth week
Carvacrol content (ppm) ± SD	473.52 ± 17.26	470.85 ± 9.07	471.68 ± 13.92	474.03 ± 15.43	472.89 ± 11.56
Thymol content (ppm) ± SD	336.90 ± 16.75	334.61 ± 12.78	335.54 ± 12.58	337.10 ± 7.63	336.02 ± 13.63

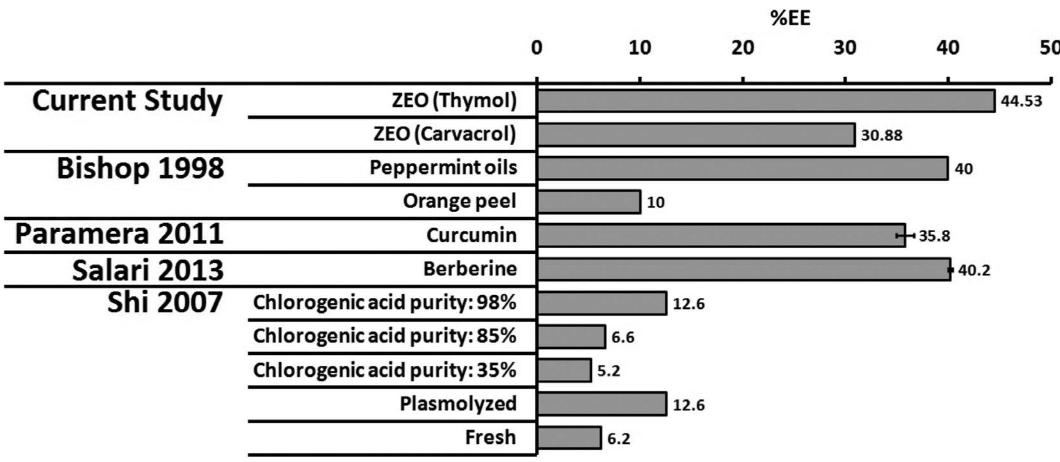

Figure 6. Comparison between the encapsulation efficiency in the current study with those reported previously by Bishop,[23] Paramera,[24] Salari,[31] and Shi.[21]

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DISCUSSION

In this research, we encapsulated ZEO in yeast cells as a delivery system for application in food preservation. Our results showed that ZEO was successfully encapsulated in *S. cerevisiae*.

The limited incubator temperature for loading ZEO in and its release from the yeast cells appears to have been a technical limitation in our study.

To the best of our knowledge there has been no previous report on the encapsulation of ZEO in yeast cells. Comparing the encapsulation efficiency of *S. cerevisiae* showed that the %EE for thymol in the current study was higher than other encapsulated active ingredients reported in previous studies, and the %EE for carvacrol was within the range of other ingredients (Fig. 6).

The encapsulation kinetics is mainly dependent on the lipid solubility of the ingredients in the cell wall, which is inversely related to the partition coefficient (log P).[20]

Carvacrol (log P: 3.52) and thymol (log P: 3.28) have relatively strong hydrophobic characteristics. They are structural isomers and each one has one hydroxyl group on its phenolic ring, at different positions. Their hydrophilic properties increase in relation to the hydroxyl group, which could be helpful for dissolving in the microbial cell membrane.[27] The hydroxyl or other polar functional groups promote their transport through the polar head groups of the membrane phospholipids. Afterwards, hydrophobic interactions, hydrogen bonds, and van der Waals attractive forces between the active compounds and the yeast cell components (organelles within the cytoplasm or cytoplasmic membrane) act synergistically to preserve the ingredient within the cell.[10] However, the hydrogen bonds formed between NH_2, OH, and COOH in the polar head groups of lipids and the OH group of active ingredients at the lipid–water interface are helpful in attracting and stabilizing the penetrating substances.[25]

The molecular weight of ZEO components is another important factor in yeast-cell penetration. Small polar and nonpolar molecules with molecular weights up to 760 Da can diffuse freely from the cell wall.[28] The low molecular weight of both carvacrol and thymol (150.22 g mol^{-1}) allows them to penetrate easily into the yeast cells.[29]

The cell membrane permeability is highly dependent on its fluidity, which is expressed by the phase transition temperature (30–37 °C), the temperature at which the membrane transits from the gel to the more fluid liquid crystalline phase.[10] The phase transition of the membrane at higher temperatures significantly increases the rate of permeation of oil into the yeast cells.[23]

The encapsulation is therefore strongly affected by the encapsulation temperature. Encapsulation is usually performed at temperatures in the range of 20–60 °C. However, temperatures around 40 °C (above the membrane phase transition temperature) allow penetration and increased permeation of the compound into the cell.[23] The yeast-cell plasmatic membrane phospholipids are in the liquid–crystalline state above 35 °C, resulting in enhanced diffusion of molecules inside the plasma. It is also expected that the phospholipids in the gel state below 35 °C might limit their penetration.[24] Our results showed that the efficacy of loading at 40 and 50 °C was significantly higher than at 30 °C. Encapsulation of orange peel oil in fresh yeast has been reported to be almost independent of the temperatures varying from 6 to 40 °C; however, in line with our findings, the encapsulation rate significantly increased between 40 and 50 °C.[23] Paramera *et al*. (2011) and Ciamponiet *et al*. (2012) also reported that increasing the temperature significantly increases the %EE of active ingredients.[20,24] Nevertheless, different molecules posses different penetration rates according to their temperature dependency models.[20]

In accordance with the findings of previous studies, the interactions between the essential oil, proteins, and polysaccharides, correspond changes in the shape and intensities, as well as the disappearance of some absorbance bands of the EO and yeast in FTIR spectra of the essential oil, yeast cells, and loaded yeast, confirming that the EO was encapsulated into the yeast cells.[21,24]

Stability analysis showed the consistency of EO content in loaded yeast cells during 4 weeks' refrigeration. Berberine loaded yeasts (*S. cerevisiae*) have already been shown to remain stable after one year of production in a previous study.[26] In other words, encapsulation of ZEO in *S. cerevisiae* results in its effective maintenance. However, the stability of the quality of encapsulated ZEO needs to be evaluated in future studies.

Both carvacrol and thymol, as active ingredients in ZEO in our study, showed substantial releases around 60% during the first hour and around 70% during the second hour at two different water temperatures (pH ~7), followed by steady release. Shi *et al*. showed that about 90% of yeast cell encapsulated resveratrol was released within 90 min in simulated gastric fluid (pH: 1.5–3.5).[25] They also showed that more than 95% of chlorogenic acid was released during 2 h in simulated gastric fluid, and the remaining chlorogenic acid was released within the next 5 h.[21] Normand *et al*. showed that release occurs at above the water activity (aw) 0.7, and no flavour release takes place below it.[30] It seems that a burst release happens once the yeasts are suspended in aqueous environment; however, acidity of the fluid will enhance the biodegradation, resulting in increased release. More studies appear to be needed to manipulate and optimize this release profile for various applications.

CONCLUSION

This study has provided information on the encapsulation of Iranian *Zataria multiflora* Boiss essential oil in *S. cerevisiae* yeast cells. Our findings showed that ZEO can be effectively encapsulated in yeast cells, refrigerated without degradation, and released efficiently in aqueous media. Preparation of ZEO encapsulated in yeast cells may be important for the development of new preservatives for the food and drug industry.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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REFERENCES

1 Donsì F, Annunziata M, Sessa M and Ferrari G, Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT - Food Science Technol* **44**:1908–1914 (2011).

2 Sultanbawa Y, Plant antimicrobials in food applications: Minireview, in *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*. Formatex Research Center, Badajoz, Spain (2011) [1084-93].

3 McIntyre L, Hudson J, Billington C and Withers H, Biocontrol of food-borne bacteria: Past, present and future strategies. *Food New Zealand* **7**:25–32 (2007).

4 Iranshahi M, Fata A, Emami B, Mohadesch Jalalzadeh Shahri B and Fazly Bazzaz BS, In vitro antifungal activity of polysulfides-rich essential oil of Ferula latisecta fruits against human pathogenic dermatophytes. *Nat Prod Commun* **3**:1543–1546 (2008).

5 Khatibi SA, Misaghi A, Moosavy M-H, Amoabediny G and Basti AA, Effect of preparation methods on the properties of Zataria multiflora boiss. Essential oil loaded nanoliposomes: Characterization of size, encapsulation efficiency and stability. *Pharm Sci* **20**:141–148 (2015).

6 Shaiq Ali M, Saleem M, Ali Z and Ahmad VU, Chemistry of Zataria multiflora (Lamiaceae). *Phytochemistry* **55**:933–936 (2000).

7 Kfoury M, Landy D, Ruellan S, Auezova L, Greige-Gerges H and Fourmentin S, Determination of formation constants and structural characterization of cyclodextrin inclusion complexes with two phenolic isomers: carvacrol and thymol. *Beilstein J Org Chem* **12**:29–42 (2016).

8 Moosavy M-H, Basti AA, Misaghi A, Salehi TZ, Abbasifar R, Mousavi HAE *et al.*, Effect of Zataria multiflora Boiss. essential oil and nisin on Salmonella typhimurium and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes. *Food Res Int* **41**: 1050–1057 (2008).

9 Jyothi Sri S, Seethadevi A, Suria Prabha K, Muthuprasanna P and Pavitra P, Microencapsulation: A review. *Int J Pharma Bio Sci* **3**: P509–P531 (2012).

10 Paramera EI, Karathanos VT and Konteles SJ, Chapter 23 - Yeast cells and yeast-based materials for microencapsulation, in *Microencapsulation in the Food Industry*. Academic Press, San Diego, pp. 267–281 (2014).

11 Mokhtari S, Khomeiri M, Jafari SM, Maghsoudlou Y and Ghorbani M, Descriptive analysis of bacterial profile, physicochemical and sensory characteristics of grape juice containing *Saccharomyces cerevisiae* cell wall-coated probiotic microcapsules during storage. *Int J Food Sci Technol* **52**:1042–1048 (2017).

12 Sultana A, Miyamoto A, Lan Hy Q, Tanaka Y, Fushimi Y and Yoshii H, Microencapsulation of flavors by spray drying using *Saccharomyces cerevisiae*. *J Food Eng* **199**:36–41 (2017).

13 Sultana A, Tanaka Y, Fushimi Y and Yoshii H, Stability and release behavior of encapsulated flavor from spray-dried Saccharomyces cerevisiae and maltodextrin powder. *Food Res Int* **106**:809–816 (2018).

14 Mokhtari S, Jafari SM, Khomeiri M, Maghsoudlou Y and Ghorbani M, The cell wall compound of *Saccharomyces cerevisiae* as a novel wall material for encapsulation of probiotics. *Food Res Int* **96**:19–26 (2017).

15 da Silva LA and Maciel AP, Mendonça CdJS, Costa Junior LM. Use of encapsulated carvacrol with yeast cell walls to control resistant strains of Rhipicephalus microplus (Acari: Ixodidae). *Ind Crops Prod* **108**:190–194 (2017).

16 Young S, Dea S and Nitin N, Vacuum facilitated infusion of bioactives into yeast microcarriers: Evaluation of a novel encapsulation approach. *Food Res Int* **100**:100–112 (2017).

17 Dadkhodazade E, Mohammadi A, Shojaee-Aliabadi S, Mortazavian AM, Mirmoghtadaie L and Hosseini SM, Yeast cell microcapsules as a novel carrier for Cholecalciferol encapsulation: development characterization and release properties. *Food Biophys* **13**:404–411 (2018).

18 Workman MJ, Gomes B, Weng J-L, Ista LK, Jesus CP, David MR *et al.*, Yeast-encapsulated essential oils: a new perspective as an environmentally friendly larvicide. *Parasit Vectors* **13**:19 (2020).

19 Nakhaee Moghadam M, Movaffagh J, Fazli Bazzaz BS, Azizzadeh M and Jamshidi A, Encapsulation of Zataria multiflora Essential Oil in *Saccharomyces cerevisiae*: Sensory Evaluation and Antibacterial Activity in Commercial Soup. *Iran J Chem Chem Eng* **39**:233–242 (2020).

20 Ciamponi F, Duckham C and Tirelli N, Yeast cells as microcapsules. Analytical tools and process variables in the encapsulation of hydrophobes in *S. cerevisiae*. *Appl Microbiol Biotechnol* **95**:1445–1456 (2012).

21 Shi G, Rao L, Yu H, Xiang H, Pen G, Long S *et al.*, Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *J Food Eng* **80**:1060–1067 (2007).

22 Dini S, Dadkhah A and Fatemi F, Biological Properties of Iranian Zataria Multiflora Essential Oils: A Comparative Approach. *Electron J Biol* **11**: 57–62 (2015).

23 Bishop JR, Nelson G and Lamb J, Microencapsulation in yeast cells. *J Microencapsul* **15**:761–773 (1998).

24 Paramera EI, Konteles SJ and Karathanos VT, Stability and release properties of curcumin encapsulated in *Saccharomyces cerevisiae*, β-cyclodextrin and modified starch. *Food Chem* **125**:913–922 (2011).

25 Shi G, Rao L, Yu H, Xiang H, Yang H and Ji R, Stabilization and encapsulation of photosensitive resveratrol within yeast cell. *Int J Pharm* **349**: 83–93 (2008).

26 Salari R, Rajabi O, Khashyarmanesh Z, Fathi Najafi M and Fazly Bazzaz BS, Characterization of Encapsulated Berberine in Yeast Cells of *Saccharomyces cerevisiae*. *Iran J Pharm Res* **14**:1247–1256 (2015).

27 Xu J, Zhou F, Ji BP, Pei RS and Xu N, The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Lett Appl Microbiol* **47**: 174–179 (2008).

28 Scherrer R, Louden L and Gerhardt P, Porosity of the Yeast Cell Wall and Membrane. *J Bacteriol* **118**:534–540 (1974).

29 Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N and Chalier P, Antimicrobial activity of carvacrol related to its chemical structure. *Lett Appl Microbiol* **43**:149–154 (2006).

30 Normand V, Dardelle G, Bouquerand PE, Nicolas L and Johnston DJ, Flavor encapsulation in yeasts: limonene used as a model system for characterization of the release mechanism. *J Agric Food Chem* **53**: 7532–7543 (2005).

31 Salari R, Bazzaz BS, Rajabi O and Khashyarmanesh Z, New aspects of *Saccharomyces cerevisiae* as a novel carrier for berberine. *Daru Journal Faculty Pharm* **21**:73 (2013).

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