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Characterization of Encapsulated Folic Acid in *Saccharomyces cerevisiae* Cells and Assessment of the Biochemical Stability after Baking and During Storage of two types of Iranian Breads

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Abstract

Background and Objective: Incorporation of folic acid (vitamin B_9) to breads is a solution to avoid folic acid deficiency. However, the vitamin stability is highly concerned, especially at high temperatures. Hence, encapsulation can be a useful protective strategy. In this study, stability of encapsulated folic acid was investigated in *Saccharomyces cerevisiae* after baking and during storage of Iranian breads (lavash and barbari)

.**Material and Methods:** *Saccharomyces cerevisiae* PTCC 5052 was treated via plasmolysis, autolysis and ultrasonication to compare protections of the vitamin. Loading capacity and encapsulation efficiency of the loaded cells were calculated. Thermal behaviors of the vitamin and yeast cells were studied using differential scanning calorimetry. Stability of free and encapsulated folic acid was assessed in the breads after baking and during storage (24 h for barbari and 24, 48 and 72 h for lavash) using high-performance liquid chromatography.

Results and Conclusion: Plasmolyzed, autolyzed and ultrasonicated yeast cells included encapsulation efficiency of 52.50, 56.38 and 47.11% and loading capacity of 18.80, 20.65 and 15.34%, respectively, more than those the intact cells did. Plasmolysis, autolysis and sonication did not lead to significant changes in morphology of the cells. Transmission electron microscopy images verified entrapment of the vitamin within the cells. Differential scanning calorimetry analysis showed endothermic dips at 60–70 °C for the intact and treated cells, which were linked to the protein degradation in the yeast cells. No significant peak/dip was observed in differential scanning calorimetry graphs of the intact and treated yeasts at higher temperatures, showing thermal stability. In the two types of breads, the autolyzed yeast cells showed the best vitamin protection after baking and during storage. Encapsulation of folic acid in *Saccharomyces cerevisiae* is a practical approach in stability of folic acid in foods at high temperatures.

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1. Introduction

During the last two decades, interests in healthy living and eating have increased [1]. Introducing novel food formulations with health benefits can increase health of the consumers [2]. Folic acid or vitamin B_9 is commonly used in fortified foods, especially flours and rice [3]. Production of food supplements and/or fortified foods is a favorable strategy to achieve sufficient folic acid within a day [3,4]. Encapsulated compounds effectively can improve their stability and bioavailability in foods [5]. The wide range of folic acid derivatives, their high sensitivity to light, heat and oxidation make folic acid analysis in foodstuffs quite difficult [6]. Low stability of folic acid is a big challenge for food scientists and nutritionists because inherent degradability of folic acid decreases its bioactivity [2,7–8].

To provide bioactive compounds with a high chemical stability, their encapsulation within protective and stable shells has been interested in recent decades [9]. Yeast cells have successfully been used for encapsulation of bioactive components [10]. These cells can stabilize susceptible bioactive components under harsh conditions. Of yeasts, *Saccharomyces cerevisiae* (baker's and brewer's yeast) has emerged as a promising host for developing novel types of drug delivery systems [11,12,10]. It provides a lipid bilayer with a network of mannoproteins and fibrous β-1,3-glucans [11,12,10]. The β-glucans and mannooligosaccharides are the major structural sugars of the yeast cell wall [11]. This specific structure can protect encapsulated compounds against destructive effects of light, heat, moisture and oxygen [10,11–13]. Due to the hydrophilicity of the yeast membrane, permeation of hydrophilic compounds is mechanistically facilitated [10]. However, encapsulation efficiency of the yeast cells may be affected, especially for fat-soluble compounds [11,12,14].

Modification methods have been developed to increase the yeast encapsulation efficiency and loading capacity [15]. It has been reported that mechanical disruption of cell walls by ultrasonication can lead to increased encapsulation efficiency by discharging the intracellular compounds into the surrounding media [11,13,15]. Use of chemical and biochemical methods is interested for the cell wall treatment. These are included in the utilization of chemicals or enzymes to alter the yeast cell wall. Yeast cell autolysis by endogenous enzymes is a process triggered by activating intracellular enzymes [11,13,15]. Glucanase and proteinase are the major enzymes contributing to disrupt-tion of the cell wall; through which, the cell wall becomes porous and crumpled leading to the release of intracellular compounds into the environment [11,16]. Moreover, plasmolysis is a chemical process, which provides a hyper-tonic environment in the presence of chemicals such as sodium chloride, toluene and ethanol [11,16,17]. It is addressed that pretreat-ment of yeast cells by plasmolysis using NaCl leads to increased encapsulation of cholecalci-ferol (vitamin D_3) [13]. Similar results were observed after plasmolysis of yeast cells for encapsulation of purslane seed oil [15]. In recent years, bread enrichment with folic acid has been carried out as a national strategy in Iran [18]. However, susceptibility of folic acid to high temperatures of bread baking is highly concerned. No study has been carried out on efficiency of treated yeast cells in protection of folic acid under thermal process and during storage of breads. For the first time, this study aimed to treat cells of *S. cerevisiae* using plasmolysis, autolysis and ultrasonica-tion as carrier of folic acid for enrichment of two types of Iranian bread (lavash and barbari). Morphology of the cells, encapsulation efficiency (EE), loading capacity (LC), thermal behaviors and stability of encapsulates during baking and storage of the bread were assessed in the study.

2. Materials and Methods

2.1. Materials

The *S. cerevisiae* cells were provided from Fariman, Mashhad, Iran. All chemicals, including phosphate buffer, were purchased from Sigma Aldrich, USA. Sodium hydroxide and hexane were purchased from Merck, Germany.

2.2. Preparation of non-treated yeast cells

Briefly, 170 g of *S. cerevisiae* cells were washed with phosphate buffer (pH 6.8) and centrifuged at 3984× g for 10 min. Then, cells were rinsed five times with deionized water. Cells were freeze-dried and stored at 4 °C for further experiments [15].

2.3. Yeast cell plasmolysis

Generally, 100 g of the prepared yeast cells were suspended in 1000 ml of NaCl solution (10% w v^{-1}) and incubated at 55 °C for 48 h at 180 rpm. Then, plasmolyzed cells were rinsed with deionized water five times to separate the residual NaCl solution. Washed yeast cells were freeze-dried and stored at 4 °C for further analyses [15].

2.4. Yeast cell autolysis

Yeast cell suspension (15% wv-1) was prepared and pH was adjusted to 5.5 using HCl solution (4 N). To autolyze the yeast cells, 100 ml of the suspension were agitated for 48 h at 55 °C using shaking incubator. Autolyzed cells were centrifuged at 8000× g for 20 min [11].

2.5. Yeast cell ultrasonication

Yeast cell suspension (0.5 g/100 ml) was subjected to an ultrasonic bath (maximum power of 750 W, frequency of 20 kHz) and 20% of power were applied for 60, 180 and 300 s (10 s on-10 s off). Then, cells were accumulated by centrifugation at 4000× g for 5 min at 30 °C. The biomass was collected and assessed for further analyses [11].

2.6. Encapsulation of folic acid within plasmolyzed, autolyzed and ultrasonicated cells

Briefly, 10 g of folic acid were added to 40 ml of deionized water and mixed well using ultra-turrax (Turrax IKA T25-Digital Ultra, Germany) at 11800 g for 5 min. Ice bath was used to control the temperature. Then, intact, plasmolyzed, autolyzed and ultrasonicated yeast cells were separately added to the vitamin solution at 2:1 (w w^{-1}) based on pre-experiences and then incubated at 40 °C for 12 h with a 180-rpm agitation rate. Then, folic acid-loaded yeast cells were centrifuged at 8965× g for 15 min and rinsed with deionized water to eliminate the residual non-loaded vitamin. Folic acid -loaded yeast cells were freeze-dried at -80 °C for 14 h and stored at 4 °C for further uses [19-21].

2.7. Assessment of efficiency and loading capacity

In general, 10 mg of folic acid-loaded yeast cells were suspended in 5 ml of 0.1-N NaOH, stirred for 15 min and then ultrasonicated under output power of 0~400 W and output frequency of 20 kHz with a titanium horn with diameter of 13 mm (Model UHP-400, Lithuania) for 2 min at room temperature (RM) in three cycles of 50% amplitude. Treated suspension was filtered using Whatman filter papers of 11 µm (Whatman, USA). Then, 1 ml of the filtrate was made up to 10 ml with distilled water (DW) and incubated at 37 °C. To assess concentration of encapsulated folic acid, absorbance of the final solution was measured using UV-vis spectrophotometer (WPA S2000 Lightwave, Ireland) at 306 nm. Furthermore, NaOH solution (0.1 N) was used as blank. Concentration of folic acid was assessed using calibration curve. The EE (%) and LC (%) were assessed using Eqs. 1 and 2 as follows:

$$\text{EE}\ (\%) = \frac{\text{Weight of entrapped folic acid (g)}}{\text{Weight of folic acid added to the mixture (g)}} \times 100 \quad \text{Eq. 1}$$

$$\text{LC}\ (\%) = \frac{\text{Weight of entrapped folic acid (g)}}{\text{Weight of folic acid} - \text{loaded yeast cells (g)}} \times 100 \quad \text{Eq. 2}$$

2.8. Microstructure analysis

Microstructure of folic acid-loaded yeast cells was firstly investigated using scanning electron microscopy (SEM) (Oxford Instruments INCA Penta FET × X3, Chapel Hill, USA) [19]. The microencapsulated folic acid powder was adhered to aluminum stand using silver glue. Samples were coated using gold metallizer. Imaging was carried out using electron beam accelerator of 25 kV at ambient temperature. Additional studies on morphological charac-teristics were carried out using transmission electron microscopy (TEM) (Zeiss EM900, Carl Zeiss, Thornwood, USA) [12]. Samples were immerged in glutaraldehyde solution (3% vv^{-1}) overnight. Then, these were washed and centrifuged at 4500× g, followed by suspending in molten agarose (2%). After solidification, agarose gels containing the samples were cut into cubes and transferred into osmium tetroxide (1%) for 60 min, followed by washing with 0.1-M phosphate buffer (pH 7.2). These were dehydrated with ethanol at gradient concentration (25–100% vv^{-1}) and acetone. Then, cubes were embedded in Spurr's resin. Cubes were cut into thin layers with 80-nm thicknesses with a RMC MT-7000 ultramicrotome, stained with uranyl acetate and lead citrate and then studied at 50 kV using TEM [13].

2.9. Thermal analysis

Thermal behaviors of the yeast cells were studied using differential scanning calorimetry (DSC) (Shimadzu DSC-60, Japan) [17]. Samples (6–12 mg) were transferred into aluminum pans, tightly sealed and heated from 25 to 300 °C at a scanning rate of 10 °C min^{-1}. Nitrogen was used as a purge gas at a flow rate of 30 ml min^{-1}.

2.10. Stability of folic acid in breads

Stability of folic acid in breads after baking and during storage (after 24 h for barbari and after 24, 48 and 72 h for lavash) was assessed using high-performance liquid chromatography (HPLC) (Model 7000, Merck-Hitachi, Darmstadt, Germany) equipped with a fluorescence detector (Model 7485, LaChrom, Merck-Hitachi, Darmstadt, Germany). A LiChrosphere100 RP-18 (5 mm) column (Merck, Darmstadt, Germany) was used to separate the compounds. Column was eluted with gradient concentrations of acetonitrile and 30 mmol l^{-1} phosphate buffer at pH 2.2 [potassium phosphate and ortho-phosphoric acid (85%), 10:1] at a flow rate of 0.9 ml min^{-1}. The gradient program was started at 6% acetonitrile. Then, isocratic concentration of 6% was used for 6 min, followed by increasing to 25% acetonitrile until 24 min. At the end of the process, concentration of acetonitrile decreased to 6% after 5 min. Injection volume included 40 ml. The running time was 30 min and the injection interval was 40 min. Fluorescence absorbance at excitation and emission wavelengths of respectively 280 and 350 nm was used to quantify folic acid [16].

2.11. Statistical analysis

Data were analyzed using SPSS software v.22 (IBM, USA). All experiments were repeated three times. Comparison of means was carried out using one-way ANOVA followed by Duncan test to report significant differences at a confidence level of 95%.

3. Results and Discussion

3.1. Effects of plasmolysis, autolysis and ultrasonication on encapsulation efficiency and loading capacity

In general, *S. cerevisiae* has attracted great attentions in microencapsulation [10]. Use of modifications on yeast cells has demonstrated positive effects on the EE [22]. Discharging the contents of yeast cells can increase space needed for entrapping. Plasmolysis, autolysis and ultrasonication can provide necessary space for the entrapment of bioactive ingredients by driving the cell components outside [11,13]. Effects of cell wall treatments on EE and LC are present in Table 1. For folic acid-loaded intact cells, EE and LC were significantly lower than those for treated yeast cells. It was associated with the altering effects of the treatments on the cell wall, leading to leakage of intracellular compounds for providing further spaces for core loading [13]. Chemical treatment of yeast cells leads to the increased permeability [19,23,24]. Due to the intermolecular interactions of mannoproteins, the hydrophobic linkages and disulfide bonds are responsible for the cell wall porosity. Physical and chemical treatments can destroy

these intermolecular network; through which, it promotes permeability of the *S. cerevisiae* cells [23]. In comparison, ultrasonication affects the permeability by rupturing the cell wall through the microstreaming and bubble cavitation, causing shear stress on the cell wall [25]. In a previous study, β-carotene was encapsulated with *Yarrowia lipolytica* cells using improvement of the core entrapment and sonication [25]. Use of ultrasound, as a green physical treatment, significantly increased the β-carotene encapsulation efficiency in yeast [25]. In the current study, autolysis was the most efficient treatment for encapsulation of folic acid, followed by plasmolysis and sonication. Indigenous hydrolyzing enzymes in the yeast cells change the cell wall structure to provide available space for the entrapment of bioactive compounds. As seen in Table 1, EE and LC of autolysis were higher than those of other external chemical (plasmolysis) and physical (ultrasonication) forces.

Table 1. Encapsulation efficiency and loading capacity of folic acid-loaded yeast cells

Sample	EE (%)	LC (%)
Intact cells	43.47 ± 0.69^{d}	13.46 ± 0.30^{d}
Plasmolyzed cells	52.50 ± 1.28^{b}	18.80 ± 0.58^{b}
Autolyzed cells	56.38 ± 0.60^{a}	20.65 ± 0.33^{a}
Ultrasonicated cells	47.11 ± 1.19^{c}	15.34 ± 0.47^{c}

*Different superscript letters indicate significant difference in the columns ($p<0.05$).

3.2. Microstructure and morphology of folic acid-loaded yeast cells

Morphology of folic acid-loaded intact and treated yeast cells was assessed using SEM (Fig. 1). As illustrated in Fig. 1a, intact yeast cells were agglomerated, while plasmolysis, autolysis and ultrasonication increased the cell distances, resulting in separation of the cells (Figs. 1b–d). After plasmolysis, contraction of cell wall resulted in the formation of further spaces between the cells. Furthermore, separation after autolysis was possibly due to the repulsive forces of the cells as a result of accumulation of similar charges on the cell surface in the acidic environment. In addition, the accumulated intact cells were disrupted after ultrasonication through the cavitations process. Nonetheless, discharging cells from internal components possibly led to alteration of the surface and physical hindrance. Relatively, no significant change was observed in integrity of the plasmolyzed yeast cells used for encapsulation of Gallic acid [26]. Similarly, purslane seed oil-loaded non-plasmolyzed, plasmolyzed and plasmolyzed CMC-coated yeast cells included spherical shape and no cracking, deformity or rupture was observed in their SEM images. The authors reported that plasmolysis did not change the integrity of yeast cells [15].

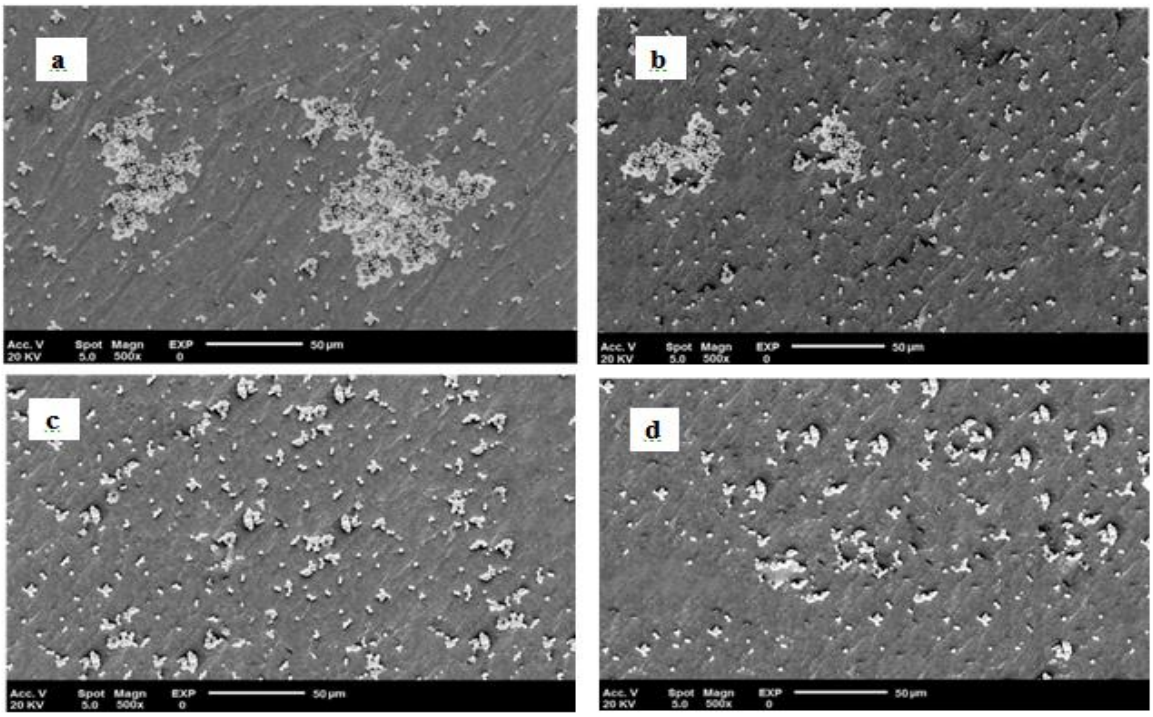


Figure 1. The SEM images of a) intact yeast cells, b) plasmolyzed yeast cells, c) autolyzed yeast cells, and d) ultrasonicated yeast cells

Figure 2 shows TEM images of the non-loaded and loaded intact and treated cells. As seen in the figure, folic acid was successfully entrapped in the yeast cells. The lowest entrapment was observed for the intact cells followed by the ultrasonicated cells (Table 1). Furthermore, treatment of the cells did not change the cell integrity and no inconformity was seen in the treated cells.

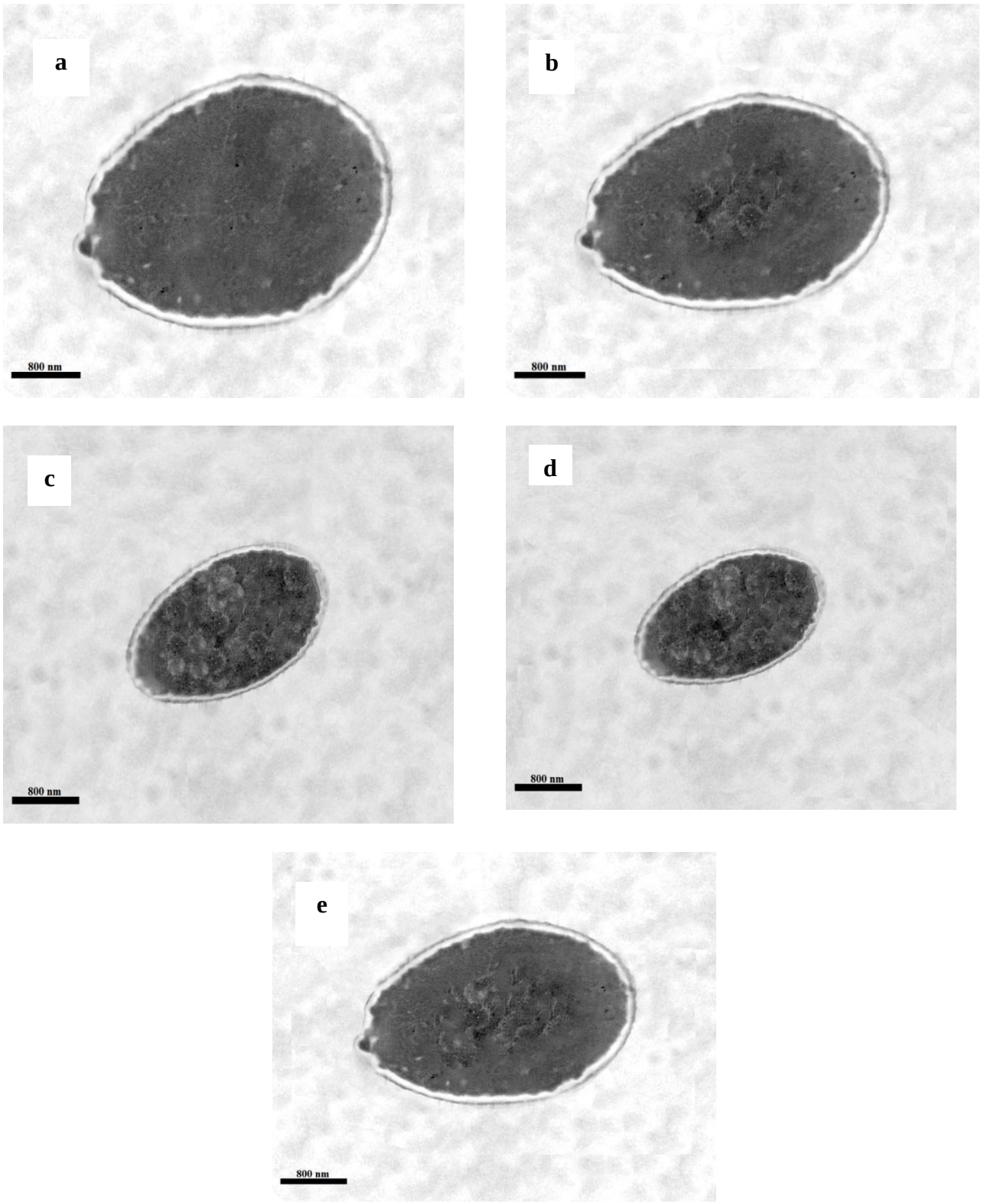


Figure 2. TEM images of a) non-loaded intact yeast cells, b) folic acid-loaded intact yeast cells, c) folic acid-loaded plasmolyzed yeast cells, d) folic acid-loaded autolyzed yeast cells, and e) folic acid-loaded ultrasonicated yeast cells

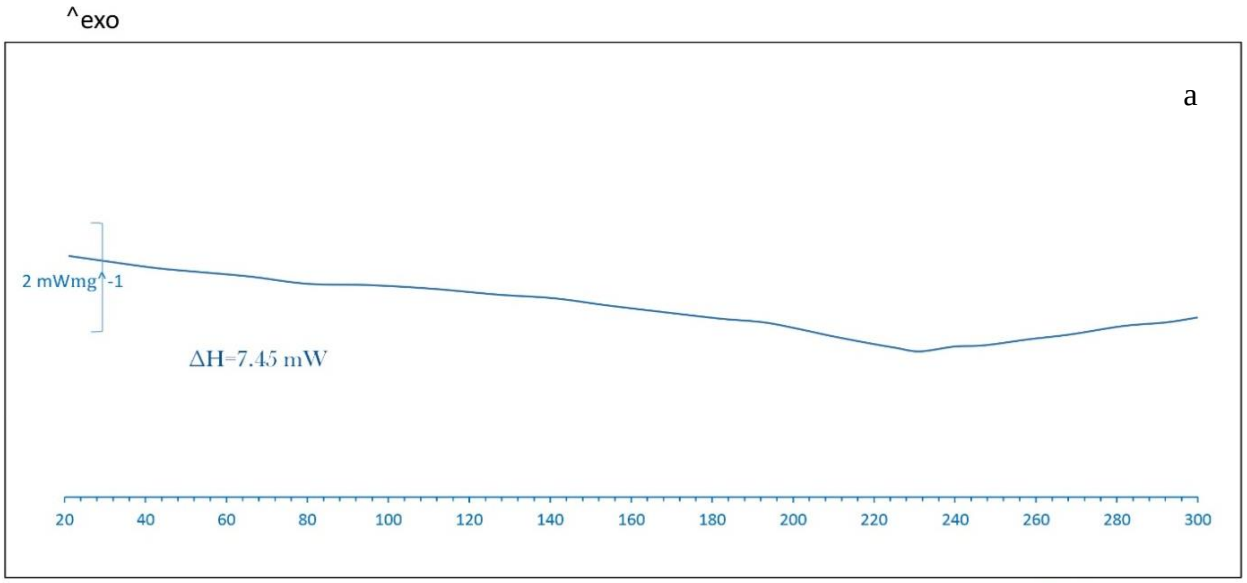
3.3. Differential scanning calorimetry

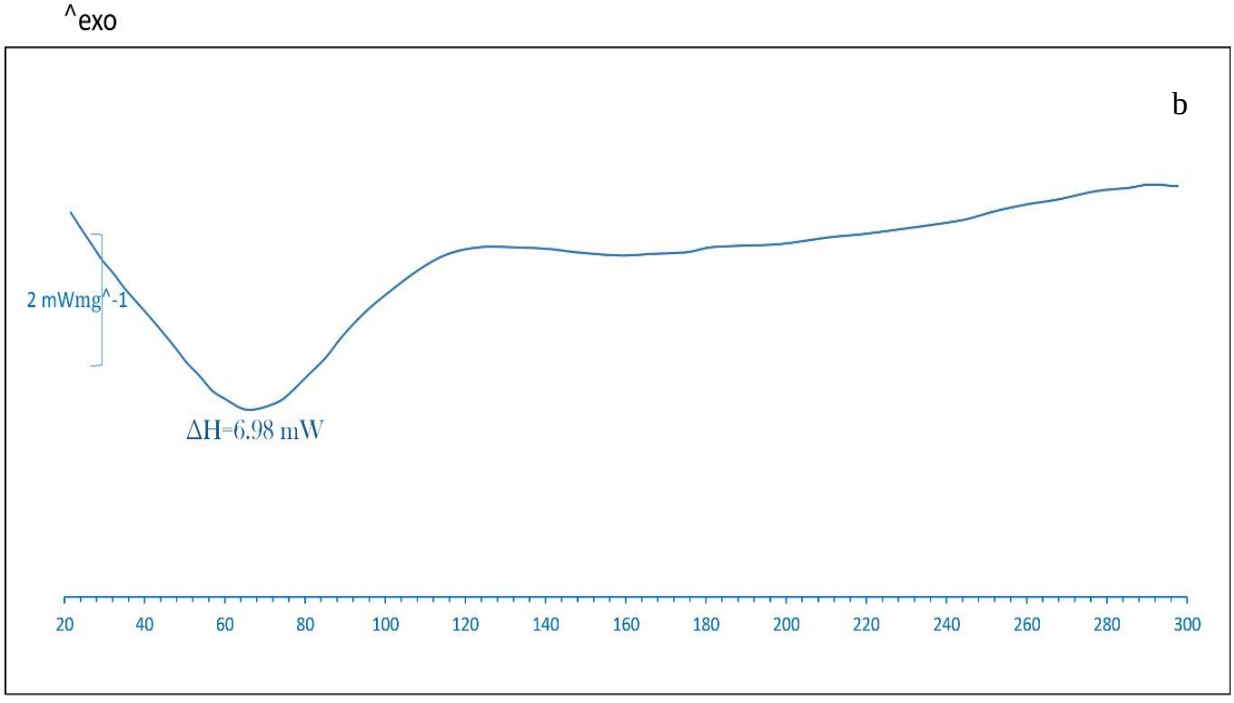
Thermal behaviors of free folic acid and the loaded intact, plasmolyzed, autolyzed and ultrasonicated cells are illustrated in Fig. 3. Folic acid showed a constant thermal behavior until 220 °C and an endothermic dip was detected at 230 °C, which could be linked to the degradation of the vitamin [26]. For the loaded yeast cells, an endothermic dip was detected at 60–70 °C, which was attributed to the protein denaturation. Similarly, an endothermic dip at 67.87 °C was observed by Cruz-Gavia et al. in study of thermal behavior of *S. cerevisiae* cells. The authors believed that protein molecules of the cells denatured in this area [27]. In addition, gelatinization of β-D-glucan might show an endothermic dip at nearly 52 °C [28]. Despite no evidence of degradation at higher temperatures (Figs. 3b–e), degradation of the phospholipid bilayer and yeast cells was usually occurred at higher temperatures (160 and 265 °C) [19]. Interestingly, intact cells showed a sharper endothermic dip in the region (Fig. 3b), compared to the treated cells that might be attributed to its higher protein contents.

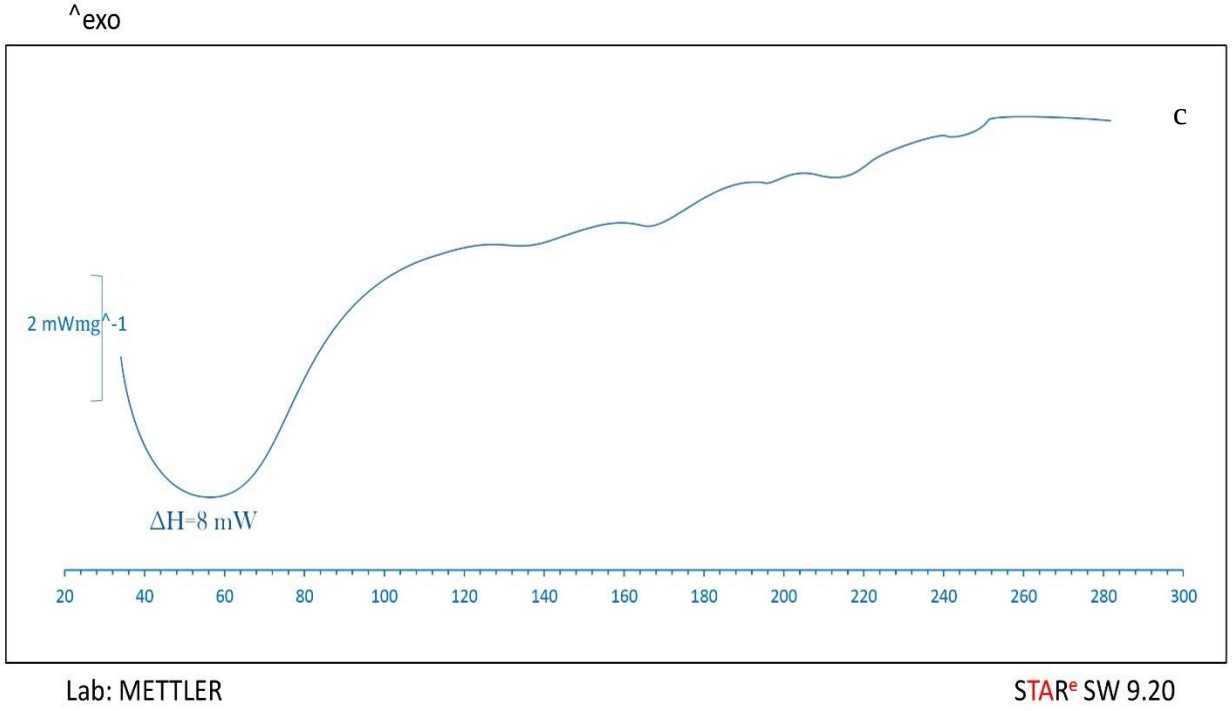
3.4. Vitamin stability

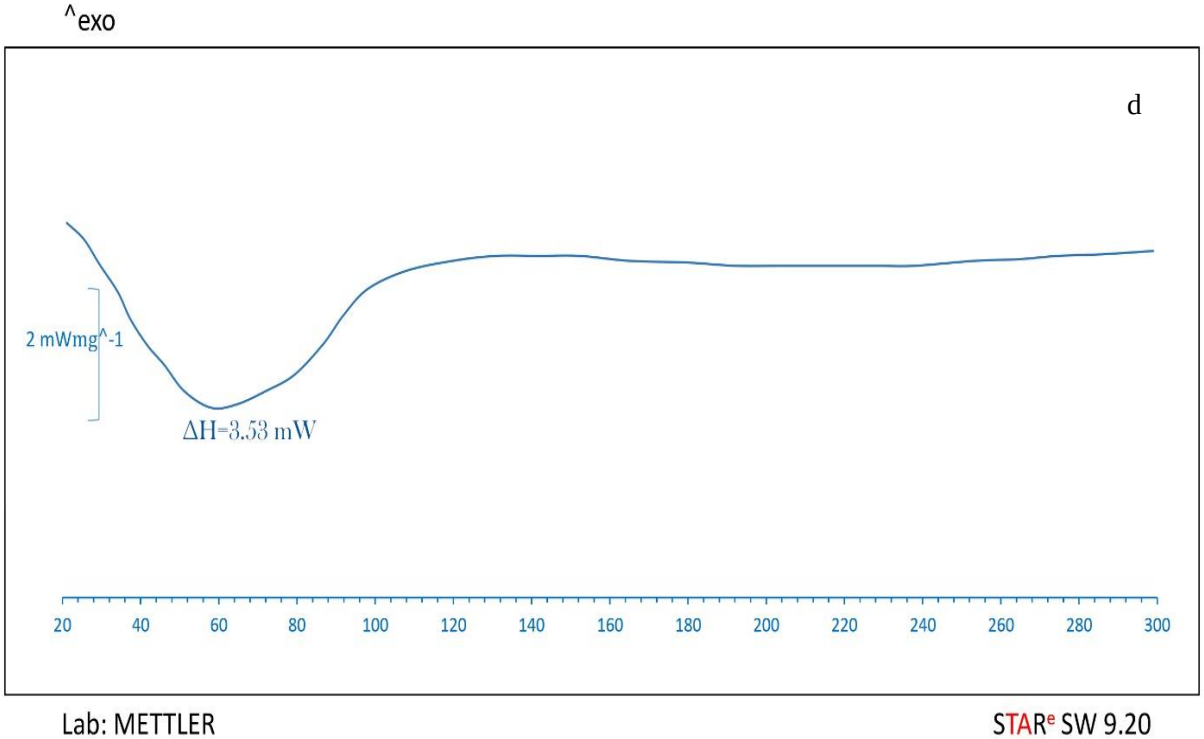
Stability of folic acid within treated and non-treated yeast cells in Iranian breads of lavash and barbari was assessed (Table 2). Breads include various shelf lives in the environment. Barbari is a semi-volume bread and it stales after 24 h at ambient temperature. In comparison, the flat bread of lavash can be stored up to 3 d in the environment. Therefore, the two types of bread were studied under various times. Before baking (dough), the highest quantity of folic acid was preserved in L1, L3 and B1 while the lowest quantity was achieved in L2 and B4. After baking (0 h), the highest folic acid preservation in barbari was achieved in B1 and B2. Similar results were observed after 24 h; thus, breads containing folic acid-loaded autolyzed cells showed the best vitamin preservation. This was similar to the results of lavash breads; in which, folic acid-loaded autolyzed cells included the best vitamin preservation followed by folic acid-loaded plasmolyzed and ultrasonicated cells after baking and until the end of storage. Concentrations of folic acid in dough and breads showed significant differences. This verified the need of protective strategies for better preservations of the vitamin to avoid its significant losses under the processes. Similar results were reported by other studies. Ashkezary et al. enriched Iranian breads with encapsulated riboflavin in plasmolyzed and non-plasmolyzed yeast cells; through which, a better protection of the encapsulated vitamin was seen after baking and during storage of the breads, compared to free riboflavin. In their study, a better protection was detected in the plasmolyzed yeast cells [10]. Microencapsulation of limonene by yeast cells can protect it after drying and heavy washing with hexane [29]. Using the yeast cells for microencapsulation of polyunsaturated fatty acids (PUFA) led to a better protection against high temperatures and oxidation [21]. Similarly, higher preservations of cholecalciferol (vitamin D_3) after exposure to simulated gastrointestinal tract (GIT) was reported through its encapsulation with yeast cells [30]. Neves et al. studied the thermal stability of folic acid in fortified French breads. Based on their results, a less loss of folic acid was seen in breads containing encapsulated vitamin, compared to that observed in breads with free folic acid (degradation started at 100 °C and completed at 155 °C after 30 min or 175 °C after 5 min for free folic acid, while it started at 40 °C and completed at 100 °C after 15 min for encapsulated folic acid). It was suggested that folic acid dispersion on the surface of the microcapsule caused their faster degradation, compared to free vitamins [31].

Villela et al. studied potentials of starch and polyethylene glycol for the encapsulation of folic acid. Accordingly, folic acid was successfully encapsulated within the polymers, but sizes of the particles varied 18–45 µm based on the type of starch (corn, potato and rice). In simulated gastric fluid, a partial degradation of polyethylene glycol (30–37%) was seen, while starch-folic acid core was intact. Moreover, a 50% degradation in polyethylene glycol was observed in simulated intestinal fluid. Degradation of polyethylene glycol provided controlled gradual releases of folic acid in the simulated gastrointestinal environment [32]. Yingleardr-attanakul et al. investigated folic acid fortification of rice vermicelli using various encapsulation techniques of gel particle, complex coacervation and spray drying. In their study, pectin and sodium alginate were used for the entrapment of the vitamin. The authors detected that spray drying was the most appropriate technique for folic acid encapsulation in rice vermicelli because spray dried particles were acid-soluble but water-insoluble; thus, preventing the loss of folic acid during processing [33].









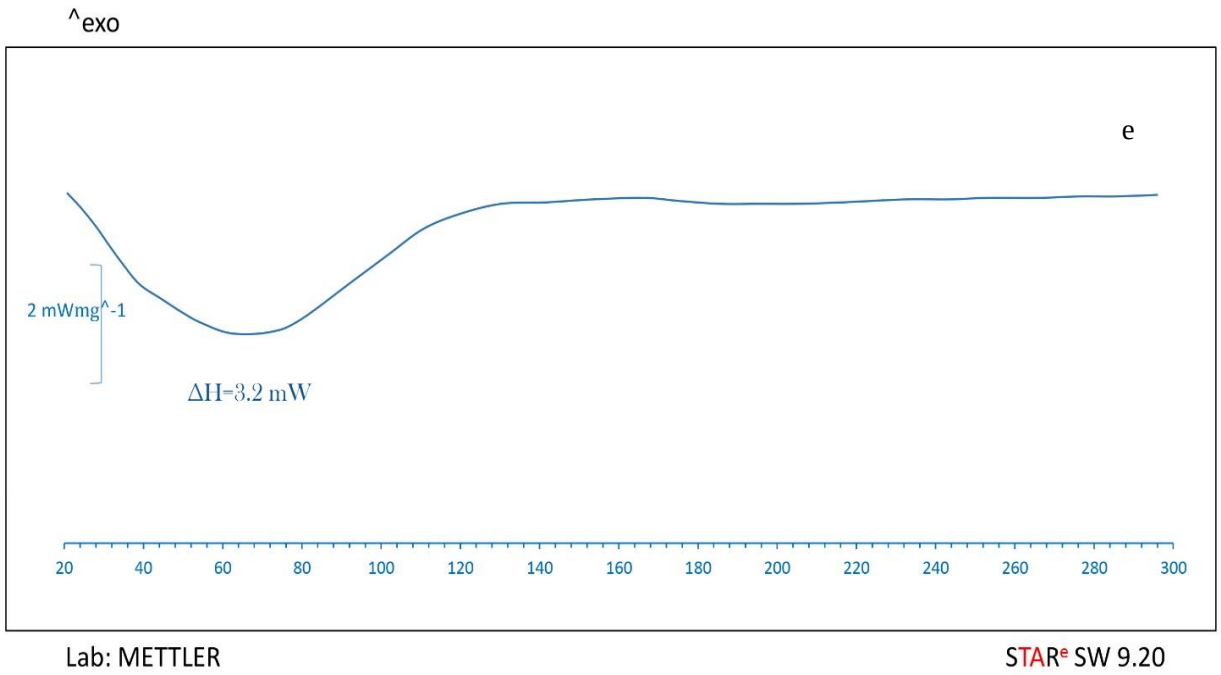


Figure 3. The DSC graphs of a) folic acid, b) loaded intact cells, c) loaded plasmolyzed yeast cells, d) loaded autolyzed yeast cells, and e) loaded ultrasonicated yeast cells

Table 2. Results of folic acid preservation in breads containing free vitamin and folic acid-loaded intact, plasmolyzed, autolyzed and ultrasonicated yeast cells

Sample	Dough	Concentration (mg/kg)			
		Bread			
		0 h	24 h	48 h	72 h
L1	15.90 ± 0.05^{Aa}	3.29 ± 0.07^{Bcd}	3.06 ± 0.10^{Cc}	2.83 ± 0.09^{Db}	2.66 ± 0.07^{Eb}
L2	15.40 ± 0.10^{Acd}	3.48 ± 0.07^{Bbc}	3.26 ± 0.11^{Cb}	3.10 ± 0.05^{Da}	2.98 ± 0.04^{Da}
L3	15.92 ± 0.20^{Aa}	3.06 ± 0.09^{Be}	2.78 ± 0.08^{Cd}	2.55 ± 0.13^{CDc}	2.43 ± 0.11^{Dc}
L4	15.56 ± 0.14^{Abc}	3.01 ± 0.10^{Bd}	2.58 ± 0.09^{Cd}	2.35 ± 0.08^{Dd}	2.15 ± 0.11^{Dd}
LC	15.18 ± 0.25^{Ade}	2.62 ± 0.17^{Bf}	2.12 ± 0.10^{Ce}	1.74 ± 0.05^{De}	1.60 ± 0.08^{De}
B1	15.82 ± 0.17^{Aab}	3.89 ± 0.13^{Ba}	3.69 ± 0.07^{Ba}	-	-
B2	15.37 ± 0.21^{Acd}	4.05 ± 0.10^{Ba}	3.86 ± 0.04^{Ba}	-	-
B3	15.43 ± 0.08^{Acd}	3.63 ± 0.15^{Bb}	3.45 ± 0.18^{Bb}	-	-
B4	15.29 ± 0.20^{Acde}	3.62 ± 0.09^{Bb}	3.37 ± 0.12^{Bb}	-	-
BC	15.02 ± 0.24^{Ae}	3.15 ± 0.02^{Bde}	2.73 ± 0.13^{Cd}	-	-

*L1: Lavash bread containing folic acid-loaded plasmolyzed cell; L2: Lavash bread containing folic acid-loaded autolyzed cell; L3: Lavash bread containing folic acid-loaded ultrasonicated cell; L4: Lavash bread containing folic acid-loaded intact cell; LC: Lavash bread containing free folic acid; B1: Barbari bread containing folic acid-loaded plasmolyzed cell; B2: Barbari bread containing folic acid-loaded autolyzed cell; B3: Barbari bread containing folic acid-loaded ultrasonicated cell; B4: Barbari bread containing folic acid-loaded intact cell; BC: Barbari bread containing free folic acid
**Different small superscript letters indicate significant difference in the columns ($p<0.05$).
***Different large superscript letters indicate significant difference in the rows ($p<0.05$).

4. Conclusion

Microencapsulation of folic acid via plasmolyzed, autolyzed and ultrasonicated yeast cells improved the encapsulation efficiency and loading capacity. Microstructure analysis of the loaded cells showed the dissociation effect of the treatments; therefore, cell dispersity increased in the treated cells, compared to the intact cells. Moreover, TEM images showed that treatment of the cells did not include disrupting effects on the cell structure. Stability experiments showed that encapsulation of folic acid by the treated cells included positive effects on the vitamin preservation after baking and during storage. Autolysis showed the most protection rate, followed by plasmolysis and ultrasonication. It is suggested that further studies use bioactive compounds and encapsulate them inside the yeast cells.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

7. Authors Contributions

AM, Fund acquisition, formal analysis, resource providing, data curation, writing and original drafting; LN, methodology, data curation, validation, investigation, supervision and project administration; MM, conceptualization, data curation, writing, review and editing the final draft and visualization; MH, text editing and manuscript checking for grammar.

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بررسی مشخصات اسید فولیک ریزپوشانی شده در سلول‌های *ساکارومایسس سرویزیه* و بررسی پایداری آن پس از پخت و نگهداری دو نوع نان ایرانی (لواش و بربری)

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چکیده

سابقه و هدف: افزودن اسید فولیک (ویتامین B9) به نان راه حلی برای جلوگیری از کمبود اسید فولیک است. با این حال، پایداری آن به ویژه در دمای بالا نگران کننده است. ریزپوشانی می‌تواند یک استراتژی محافظتی مفید باشد. در این مطالعه، پایداری اسید فولیک ریزپوشانی شده در *ساکارومایسس سرویزیه* پس از پخت و در زمان نگهداری نان ایرانی (لواش و بربری) مورد بررسی قرار گرفت.

مواد و روش‌ها: *ساکارومایسس سرویزیه* PTCC 5052 به روش‌های کافت‌اسمزی[۱](پلاسمولیز)، خودکافت[۲] و فراصوت پیش‌تیمار شد تا میزان محافظت از این ویتامین مقایسه شود. ظرفیت بارگذاری (LC) و راندمان کپسولاسیون سلول های بارگذاری شده محاسبه شد. رفتار حرارتی سلول های ویتامین و مخمر توسط گرماسنجی روبشی تفاضلی (DSC) مورد بررسی قرار گرفت. پایداری اسید فولیک آزاد و ریزپوشانی شده در نان پس از پخت و حین نگهداری (۲۴ ساعت برای بربری و ۲۴، ۴۸، ۷۲ ساعت برای لواش) با HPLC تعیین شد.

یافته‌ها و نتیجه‌گیری: راندمان کپسولاسیون در سلول های مخمر کافت‌اسمزی، خودکافت و فراصوت شده به ترتیب ۵۲/۵۰ ، ۵۶/۳۸ و ۴۷/۱۱ درصد و بارگذاری ۱۸/۸۰، ۲۰/۶۵ و ۱۵/۳۴ درصد بودند که بالاتر از سلول دست نخورده بود. کافت‌اسمزی، خودکافت و فراصوت به تغییر قابل توجهی در ریخت‌شناسی سلول‌ها منجر نشد. تصاویر میکروسکوپ الکترونی عبوری گیرافتادن این ویتامین در سلول‌ها را تایید کرد. تجزیه و تحلیل DSC شیب گرماگیر در ۶۰-۷۰°C را برای سلول‌های دست نخورده و تیمار شده نشان داد که مربوط به تخریب پروتئین در سلولهای مخمر بود. هیچ پیک یا شیب قابل توجهی در نمودار DSC مخمر دست نخورده و تیمار شده در دماهای بالاتر مشاهده نشد که حاکی از پایداری حرارتی آنها بود. در هر دو نوع نان، سلول های مخمر خودکافت شده بهترین محافظت را برای ویتامین پس از پخت و در حین نگهداری نشان دادند. ریزپوشانی اسید فولیک در *ساکارومایسس سرویزیه* یک رویکرد عملی در پایداری اسید فولیک در غذاهای در دمای بالا است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

[۱] Plasmopysis
[۲] Autolysis