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Accumulation of Vitamin C in Yeast under Pulsed Electric Field (PEF) Conditions

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Featured Application: Yeast can be used as microcapsules for sensitive compounds, such as vitamin C, in food technology and biotechnology, and application of a PEF under optimized conditions can increase the efficiency of their accumulation in the cells.

Abstract: Enriching food with vitamin C is a process that challenges food engineers. To prevent the degradation of this vitamin, a microencapsulation can be used in *Saccharomyces cerevisiae* cells. Previous works have shown that applying a pulsed electric field can increase the efficiency of the accumulation of minerals in yeast. The aim of this study was to optimize PEF parameters in order to increase the accumulation of vitamin C in yeast cells, to evaluate the effect of electroporation on biomass and yeast viability, and to assess the effect of storage conditions on the vitamin C content and its antioxidant activity. The most effective accumulation of vitamin C in cells (approx. 1.3 mg/g dry mass) was achieved when a 20-h yeast culture was treated with PEF at a concentration of 5 mg/mL vitamin C in the medium. The optimal PEF parameters were: voltage of 1000 V, pulse width of 10 µs, treatment time of 20 min, and number of pulses, 1200. The process conditions did not affect significantly biomass production nor cell viability. Yeast cells with vitamin C were stored for 7, 14, and 28 days at 20 °C (after prior freeze-drying), 4 °C, and −22 °C. The lowest decrease in vitamin C content was observed for the freeze-dried yeast stored at 20 °C.

Keywords: *Saccharomyces cerevisiae*; pulsed electric field; vitamin C; storage

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1. Introduction

Vitamin C is a water-soluble nutrient which cannot be synthesized in the human body due to the loss of the end enzyme (gulonolactone oxidase) on the biosynthesis pathway. It is an important antioxidant circulating in plasma [1]. Vitamin C acts as an electron donor and has an antioxidant effect, protecting important molecules (proteins, DNA) from oxidation. It also plays a role as a cofactor for enzymes involved in hormone synthesis and metabolic energy production [2]. Vitamin C supports the epithelial barrier function against pathogens. It is necessary for the synthesis of collagen proteins and plays an important role in preventing bleeding and repairing wounds [3]. This vitamin promotes iron absorption and collagen formation. It is often added to food as a nutrient (to supplement technological losses) and antioxidant [4].

A varied and balanced diet should ensure the right amount of all nutrients. A diet poor in nutrients leads to deficiencies. Another reason for deficiencies is food processing that causes a loss of, e.g., vitamin C [5]. Common nutrient deficiencies can be prevented or corrected not only by nutritional advice, but also through the use of food enriched (or fortified) with nutrients or bioactive ingredients [6].

Enriching food with vitamin C is a process that challenges food engineers. This vitamin is easily degraded under the influences of light and temperature, and thus loses its health-

promoting properties [7]. To prevent these unfavorable changes, the microencapsulation process is used [8]. Bioaccumulation of vitamin C in the yeast cells of *Saccharomyces cerevisiae* is one of the encapsulation methods [9], and a pulsed electric field (PEF) can be used to increase the uptake of minerals and vitamins by microorganisms [10,11].

PEF is a method used in food processing to control the microbiological safety and nutritional, sensory, and physicochemical properties of food products that are important from the consumer's viewpoint. It is a simple, non-toxic, and non-thermal method based on the formation of pores under the influence of short pulses of electricity. It is believed that PEF is better than thermal methods of food processing because it does not cause a loss of nutrients; the PEF method maintains the physical and sensory characteristics of food so that the quality of products does not change. PEF causes the formation or growth of membrane pores, i.e., electroporation of the cell membrane, which in turn increases its permeability to small molecules [12]. Electroporation depends on the voltage; the process duration; the number of pulses and their frequency; as well as on the composition of the membrane; the size and shape of the cell; the temperature; and the location of the cell relative to the electric field line [13]. After the action of the electric field, the pores in the membranes are sealed. In this way, the introduced particles or ions are retained in the cell [14].

The innovation of this study is the introduction of vitamin C, which is sensitive to external factors, to cells of yeast *Saccharomyces cerevisiae* with the use of a pulsed electric field. In the scientific literature, only the results of research on the accumulation of vitamins and minerals by yeasts from media enriched with these components are available [15]. There are reports indicating that the PEF method (even a high-intensity mode) has no significant effect on the content and antioxidant properties of vitamin C [16]. Research was also conducted on the ability of PEF treatment to increase the accumulation of metallic elements in yeast and bacteria cells [10–12,17]. The authors obtained the highest accumulation using the following parameters of PEF treatment: electric field strength 3–5 kV/cm, treatment time 5–20 min, pulse width 10–20 us, and pulse frequency 1 Hz, depending on the type of microorganism and the introduced ion. However, there are no reports of the use of a pulsed electric field to increase the bioaccumulation of vitamin C in living yeast cells. So, the aim of this study was to optimize PEF parameters in order to increase the accumulation of vitamin C in yeast cells, to evaluate the effect of electroporation on biomass and yeast viability, and to assess the effect of storage conditions on the vitamin C content and its antioxidant activity. Our research will provide basic data that will help to explain the influence of PEF treatment on the properties of yeast and vitamin C, and it will contribute to the use of yeast as microcapsules for sensitive compounds in food technology and biotechnology.

2. Materials and Methods

2.1. Microorganism and Reagents

The *Saccharomyces cerevisiae* 11 B1 strain (industrial strain) from the yeast factory (Lublin, Poland) was used in the research. The composition of the media used in the study was described by Nowosad et al. [11]. All reagents used in the experiment were of analytical purity.

2.2. Biomass Cultivation

In the first stage of the experiment, yeast was passaged on agar slants and then cultured for 48 h in a thermostat at 30 °C. The agar slants prepared in this way were used to prepare the inoculum by inoculating 150 mL of sterilized medium with one slant. Then, the cultures were transferred to an incubator (NBB 205L, N-BIOTEK Inc., Bucheon-si, Gyeonggi-Do, Korea) and shaken (100 rpm) at 30 °C for 48 h.

After 48 h of culturing, the inoculum was centrifuged and washed three times with sterile water. The pellets from the final centrifugation were collected and suspended in sterile water (final inoculum volume was 300 mL). From the inoculum prepared in this way, an aliquot of 10 mL was taken to inoculate the immersion cultures in 500 mL Erlenmeyer

flasks. Some of the flasks contained 90 mL of medium, and they were control cultures (control culture C1—no vitamin C added); the other flasks contained 80 mL of medium, and 10 mL of vitamin C solution was added to these so that its final concentration in the culture was 5 mg/mL. These flasks were divided into two batches—one batch was not treated with PEF (control culture C2) and the other was treated with PEF. Growth conditions were identical to the inoculum.

2.3. Optimizing the Pulsed Electric Field Process for Vitamin C Accumulation in Yeast

The process parameters were optimized according to the OFAT method (one-factor-at-a-time). The optimized parameters were selected on the basis of the previous studies by Pankiewicz and Jamroz [17]. The following parameters were optimized: voltage (300–2000 V), pulse width (10–100 µs), treatment time (5–20 min), and cultivation time after which PEF was applied (8–24 h). The cultures of *S. cerevisiae* were grown in flasks under continuous agitation and treated with PEF (pulse frequency of 1 Hz) using an ECM 830 unipolar square wave generator (BTX Harvard Apparatus, Holliston, MA, USA). The description of a treatment chamber and operation of the ECM 830 electroporator is presented in Nowosad et al. [11]. Briefly, a 100 mL culture was placed in a PEF treatment chamber consisting of a glass beaker (300 mL) and four parallel stainless-steel electrodes mounted on a removable lid. The electrodes were spaced 5.1 mm apart, and each had an area of 4 cm^2. During the delivery of the pulses, the solution was stirred with a rotating magnet (100 rpm) to prevent cell sedimentation. After covering the beaker with a lid, the electrodes dipped approximately 7.4 mm into the solution.

The initial pulsed electric field parameters were: voltage 1500 V, pulse width 10 µm, treatment time 10 min, and cells were treated after 20 h of cultivation. Then, at each stage, one of the parameters was optimized according to a scheme presented in Figure 1. After each stage of the experiment, the cells were centrifuged, washed several times with deionized water, and then prepared for the determination of vitamin C by HPLC (as described in Section 2.4).

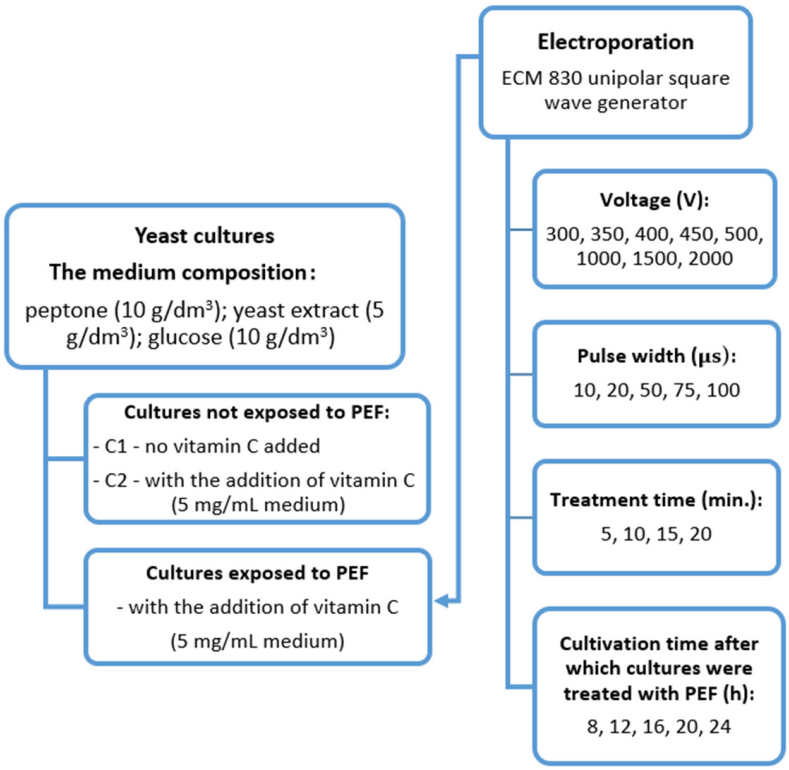


Figure 1. Scheme of optimization of the PEF parameters.

2.4. Determination of Vitamin C

After each parameter optimization step, the yeast samples (1 g) were ground in a mortar with 5 mL of metaphosphoric acid (5%, *w*/*v*) and sand. The samples were centrifuged for 10 min at 3000 rpm and filtered through syringe filters (0.22 µm pore diameter). Vitamin C concentration was determined by a reversed-phase HPLC method (Varian HPLC system, USA) equipped with a diode-array detector (DAD, type 335), an isocratic pump (model 210), an autosampler (model 420), and a column thermostat. Data were controlled and acquired using the Galaxie Chromatography Data System, version 1.9.302. The column used was a Gemini column (150 × 4.6 mm, 3 µm, C18) combined with a Gemini pre-column (C18 4 × 3 mm) from Phonomenex (USA). Technical data of the experiment: injection volume: 20 µL, mobile phase: orthophosphoric acid pH = 2.8, flow rate: 0.6 mL/min, chromatograms were recorded at a light wavelength of 244.0 nm, column temperature: 30 °C. Vitamin C concentration was determined on the basis of the standard curve. Identification of L-ascorbic acid was based on the retention time and UV spectrum of the reference substance [18].

2.5. Determination of Biomass and Viability of the Cells

Measurements of post-cultivation biomass (by determination of optical density at 600 nm) and cell viability (by counting viable cells in a Thoma chamber) were performed at each optimization step of the PEF conditions. The temperature during PEF treatment varied within the range of 24 °C to 26 °C.

2.6. Assessment of the Impact of Yeast Storage on Content and Antioxidant Activity of Vitamin C

In order to evaluate the effect of storage conditions on the vitamin C content in yeast, yeasts from the culture treated with PEF at optimal parameters were stored for 7, 14, and 28 days at 20 °C (after prior freeze-drying in a Labconco freeze dryer, Model 64132, for 3 days), 4 °C, and −22 °C. Vitamin C content was determined in the samples before storage ("fresh yeast") and after each storage period according to the methodology described in Section 2.4. The DPPH assay for yeast extracts was performed according to Brand-Williams et al. [19].

2.7. Data Processing

All measurements were performed in at least three repetitions. Regression analysis and significance tests were performed using the Statistica 13.3. software (StatSoft, Inc., Tulsa, OK, USA). The pos-hoc Tuckey test was employed to determine differences between means. Results of $p < 0.05$ were considered statistically significant.

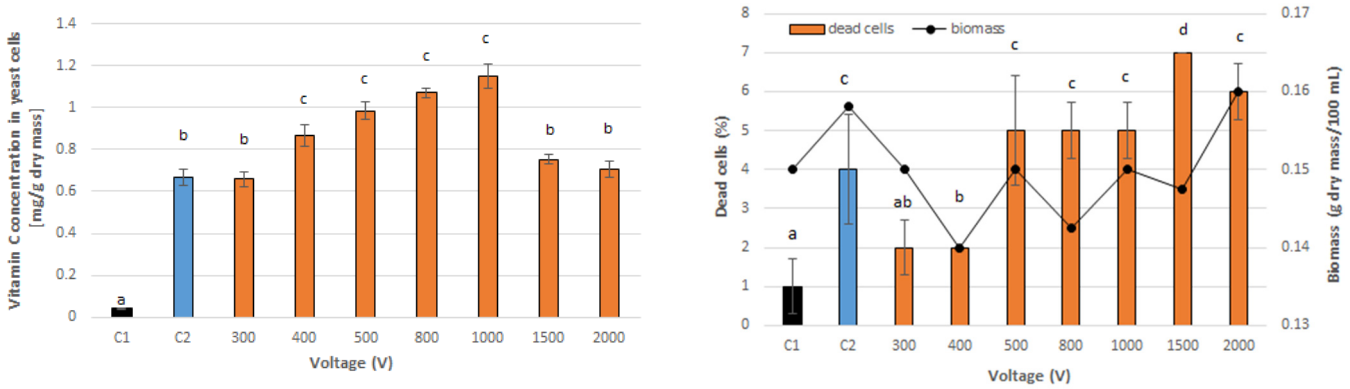
3. Results and Discussion

3.1. Optimal PEF Conditions for Vitamin C Accumulation in Yeast

Yeast is mainly used in the brewing and baking industries. Its cell structure—a thick and mechanically strong wall and a lipid plasma membrane, enables encapsulation of hydrophobic and hydrophilic compounds and ensures high load capacity [20]. Yeast cell wall materials are biodegradable, they protect bioactive compounds against external factors, and the microencapsulation process is relatively simple. PEF treatment can lead to an improvement in encapsulation efficiency [21].

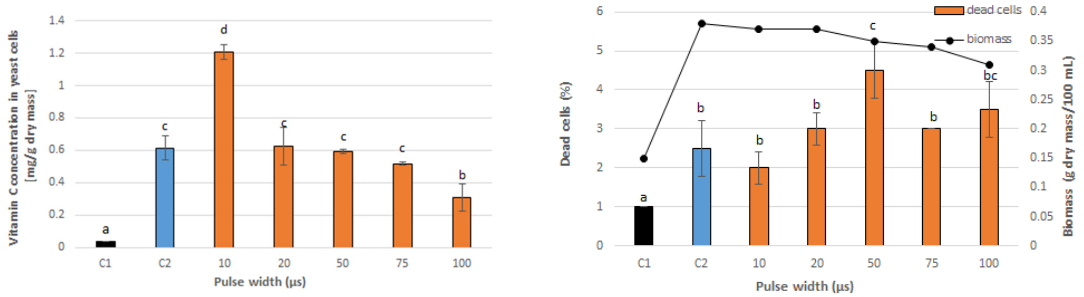
The first stage of the research concerned the optimization of PEF parameters for the effective accumulation of vitamin C in yeast cells and the assessment of their impact on biomass production and cell viability. The results are shown in Figures 2–5. The control sample C1 contained only small amount of vitamin C: 0.04 mg/g dry mass. Yeast cells do not have the ability to produce L-ascorbic acid (L-AA), but contain small amounts of erythroascorbic acid (similar in properties to L-ascorbic acid) [22]. The results of our previous experiments show that PEF treatment significantly increases the accumulation of minerals in yeast cells, e.g., iron increased by 157% compared to the control sample without PEF [11], selenium by 65%, zinc by 100% [23]. So far, the influence of the pulsed

electric field treatment on the accumulation of vitamins in the cells of microorganisms has not been studied due to the high instability of these compounds [24]. The application of a low-voltage electric field (Figure 2a) with a value of 300 V did not cause a significant increase in the amount of vitamin C in yeast cells compared to the control sample C2. The highest accumulation of vitamin C was observed at 1000 V (amounting to 1.15 mg/g of dry mass), and it was 1.72 times higher than in the control sample C2. Higher voltages above 1000 V caused a significant decrease in the content of vitamin C in yeast cells. The optimal value of pulse width was 10 µs (Figure 3a). In this case, the accumulation of vitamin C was two times higher than in the control sample C2. Increasing the pulse width value in the range of 20–100 µs caused a significant and gradual decrease in vitamin C accumulation. In the studies by Pankiewicz and Jamroz [17,25], the highest accumulation of magnesium in *S. cerevisiae* cells was noted at a voltage of 2000 V and a pulse width of 20 µs, while in the case of zinc, it was at 1500 V and 10 µs. The differences in the optimal PEF parameters may result from different sizes of particles introduced into the yeast cells. Figure 4a shows the effect of treatment time on the accumulation of vitamin C in yeast cells. Treatment time studies were conducted over a range of 5–20 min. Vitamin C concentration in *S. cerevisiae* increased with the treatment time, reaching the maximum value after 20 min of PEF action. This value was 1.45 times higher that of the control C2. In our previous study on iron accumulation in *S. cerevisiae*, the optimal treatment time was also 20 min [11], whereas in the studies by Pankiewicz and Jamroz [25], the highest accumulation of zinc in yeast cells was achieved after 10 min of treatment. The final step in optimizing the parameters was determining the optimal cultivation time after which yeast cells would be subjected to PEF (Figure 5a). We took into account the production of biomass and the accumulation of vitamin C in yeast cells. In our study, the optimal cultivation time was 20 h. This is in line with the studies of Pankiewicz et al. who also obtained the optimal cultivation time of 20 h for the highest accumulation of selenium and zinc [23] as well as magnesium and zinc [26] in yeast.



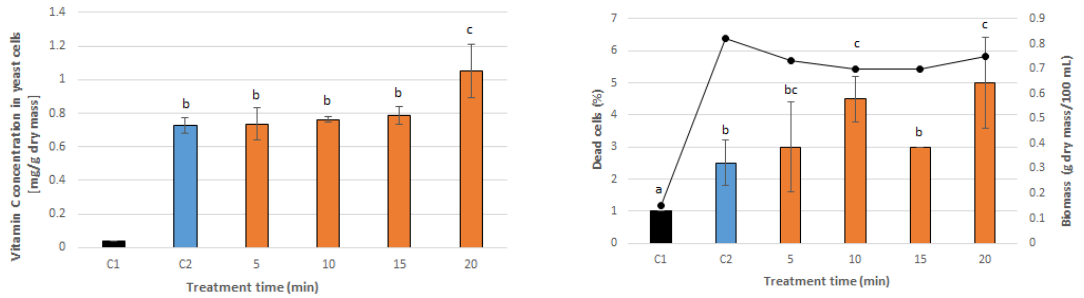
(**a**) (**b**)

Figure 2. Effect of voltage on vitamin C accumulation in yeast cells (**a**). Effect of voltage on the number of dead yeast cells and on biomass (**b**). C1—control culture without vitamin C added to the medium and PEF treatment, C2—control culture with vitamin C added to the medium (5 mg/mL medium) and without PEF treatment, orange bars—cultures treated with PEF (vitamin C, 5 mg/mL medium; pulse width of 10 µs; treatment time, 10 min; number of pulses, 600; after 20 h of cultivation). Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–d) are not significantly different ($p < 0.05$).



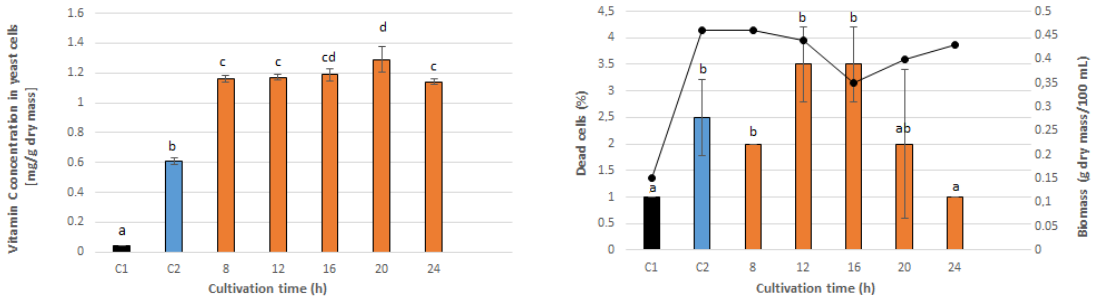
(**a**) (**b**)

Figure 3. Effect of pulse width on vitamin C accumulation in yeast cells (**a**). Effect of pulse width on the number of dead yeast cells and on biomass (**b**). C1—control culture without vitamin C added to the medium and PEF treatment, C2—control culture with vitamin C added to the medium (5 mg/mL medium) and without PEF treatment, orange bars—cultures treated with PEF (vitamin C, 5 mg/mL medium; voltage, 1000 V; treatment time, 10 min; number of pulses, 600; after 20 h of cultivation). Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–d) are not significantly different ($p < 0.05$).



(**a**) (**b**)

Figure 4. Effect of treatment time on vitamin C accumulation in yeast cells (**a**). Effect of treatment time on the number of dead yeast cells and on biomass (**b**). C1—control culture without vitamin C added to the medium and PEF treatment, C2—control culture with vitamin C added to the medium (5 mg/mL medium) and without PEF treatment, orange bars—cultures treated with PEF (vitamin C, 5 mg/mL medium; voltage, 1000 V; pulse width, 10 µs; after 20 h of cultivation). Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–c) are not significantly different ($p < 0.05$).



(**a**) (**b**)

Figure 5. Effect of cultivation time on vitamin C accumulation in yeast cells (**a**). Effect of cultivation time on the number of dead yeast cells and on biomass (**b**). C1—control culture without vitamin C added to the medium and PEF treatment, C2—control culture with vitamin C added to the medium (5 mg/mL medium) and without PEF treatment, orange bars—cultures treated with PEF (vitamin C, 5 mg/mL medium; voltage, 1000 V; pulse width, 10 µs; treatment time, 20 min; number of pulses, 1200). Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–d) are not significantly different ($p < 0.05$).

During the optimization experiment, we also studied the influence of PEF parameters on cell viability and biomass production. Figure 2b shows the effect of voltage on both tested features. The application of 400 V caused a decrease in biomass compared to sample C2, whereas for a voltage in the range 500–1000 V, no significant effect on biomass and cell viability was observed. Martínez et al. [27] reported that the intensity of the pulsed electric field did not significantly affect the viability of *S. cerevisiae* cells. Moreover, other authors confirm that an electric field greater than 10 kV/cm is required to inactivate various types of yeast [28,29]. In some cases, even higher viability of the yeast *S. cerevisiae* was noted with increasing electric field strength and treatment time [30].

In the case of pulse width (Figure 3b), increasing the parameter value did not cause a significant decrease in cell biomass. The highest fraction of dead cells (4.5%) was observed at 50 µs. Simonis et al. [31] showed that yeast cell death is dependent on the parameters of the electric field impulse and increases with increasing electric field intensity and the number of pulses. The duration of the process (Figure 4b) slightly influenced the number of dead cells. We noted the highest share of dead cells when the treatment time was 20 min, but it was only 5%. The biomass production for the same treatment time was only 9% lower than in the control not exposed to PEF. Longer PEF treatment of yeast cultures resulted in a significant reduction in biomass and an increase in the fraction of dead cells. In the case of the cultivation time (Figure 5b) after which yeast cells were subjected to PEF, the greatest decrease in biomass was observed after 16 h and it was lower by 23% compared to the control sample C2. It is assumed that the main mechanism responsible for the inactivation of yeast cells is electroporation, which is a consequence of an increase in transmembrane voltage. The transmembrane voltage threshold value correlates with the cell size. Therefore, the critical electric field required for yeast electroporation is lower than that of bacteria, which are smaller in size, and higher than that required for electroporation of eukaryotic (plant and animal) cells, which are larger in size [32].

Previous studies have shown that PEF treatment can be used to stabilize vitamin C in food products such as juices [33,34] and to increase their antioxidant activity [34]. Application of this technology does not damage vitamin C and even enhances its antioxidant properties [16]. So far, no studies have been published on the use of PEF treatment to increase vitamin C accumulation in yeast cells. This vitamin is a highly unstable compound that poses technological challenges for its incorporation into various food systems. Therefore, microencapsulation is a promising approach to ensure the stability of ascorbic acid and at the same time, improve consumer acceptance of the food carrier [35].

Yeast does not have an endogenous biochemical pathway for vitamin C synthesis. However, appropriately recombinant yeast strains gain the ability to accumulate the vitamin in the culture medium. Budding yeast cells with overexpression of the enzymes d-arabinose dehydrogenase and d-arabinone-1,4-lactone oxidase are able to produce about 100 mg of L-ascorbic acid/L, converting 40% (*w*/*v*) of L-galactose [36].

In a study by Dadkhodazade et al. [37], *S. cerevisiae* cells were used as a new carrier for vitamin D3 encapsulation. The influence of the initial concentration of cholecalciferol (100.000 and 500.000 IU/g yeast) on the properties of the microcapsules was investigated. Vitamin D3 concentration was found to have a significant influence on the encapsulation efficiency. The study showed that yeast cells can be used to extend the stability of compounds such as vitamins.

3.2. The Effect of Storage on the Vitamin C Content in Yeast Cells and Antioxidant Activity of Yeast Extract

The yeast with vitamin C accumulated in the cells was stored for 7, 14, and 28 days under different temperature conditions, and the content of this vitamin was compared with that of fresh yeast (Figure 6). A significant decrease in vitamin C concentration was observed in yeast cells stored at 4 °C; after 28 days its content was 93% lower than that in fresh yeast. The lowest loss of vitamin C was observed in freeze-dried yeast stored at 20 °C. After 28 days of storage, the vitamin C content in cells was lowered by approx. 53%.

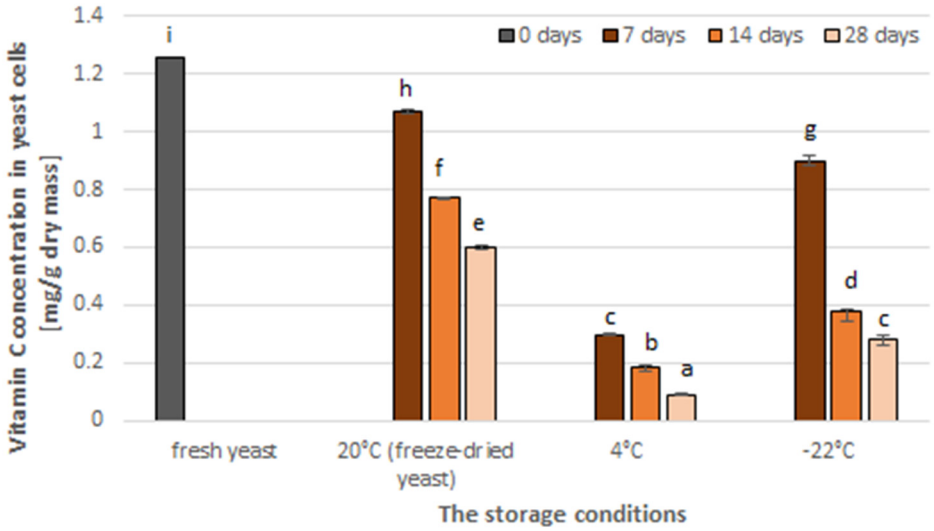


Figure 6. Effect of storage time (0-fresh yeast, 7, 14, 28 days) on vitamin C content in yeast cultures treated with PEF (vitamin C, 5 mg/mL medium; voltage, 1000 V; pulse width, 10 µs; treatment time, 20 min; number of pulses, 1200; after 20 h of cultivation). Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–i) are not significantly different ($p < 0.05$).

Vitamins are reactive chemicals. Their stability during production and storage processes is influenced by many chemical and physical factors, e.g., light, temperature, pH, and oxygen levels. These factors can cause a significant loss of vitamins in food products. Therefore, to prevent loss of vitamin C, microencapsulation is used. Microencapsulation integrates bioactive substances (e.g., vitamins or also enzymes, phenols, or other molecules) in a specific shell to protect these substances against external factors [38,39]. There are no studies in which vitamin C is encapsulated in yeast cells, but studies using a different encapsulating material for vitamin C are available. In the work of Borrmann et al. [40], the content of vitamin C in encapsulated passion fruit juice stored at temperatures 7 and 25 °C was investigated. At 7 °C, the samples retained 77.1% of vitamin C, while at 25 °C it was 71.5%. The storage time was 77 days. Kirby et al. [41] investigated the stabilization of ascorbic acid by microencapsulation in liposomes. Ascorbic acid containing liposomes were prepared by rehydration and the stability of this compound was compared with that of unbound ascorbic acid. The results showed that the free ascorbic acid completely disappeared from the solution after 20 days at 4 °C, and after 6 days at room temperature. In the case of ascorbic acid encapsulated in liposomes at 4 °C, the loss of vitamin C was 40% after 50 days of storage. At room temperature, the loss was greater, but not as fast as for free ascorbic acid. After 50 days, almost 20% of the ascorbic acid was present in microencapsulated form. In the case of the studies by Diachkova et al. [42], long-term storage for 12 and 18 months resulted in reductions in the content of vitamin C (enclosed in a bed of maltodextrins) by less than 2% and by 4%, respectively. Thus, it can be seen that the use of encapsulating materials such as derivatized starch, liposomes, and maltodextrins resulted in better storage of vitamin C, and its content was much higher compared to ascorbic acid encapsulated in yeast. The likely cause was different encapsulating material that more effectively protected vitamin C against degradation.

Figure 7 presents the antioxidant activity of an extract obtained from PEF-treated yeast, fresh and stored for 7, 14, and 28 days. The extract from fresh yeast had the highest antioxidant activity. The storage of yeast with accumulated vitamin C at 20 °C after prior freeze-drying and at −22 °C had only a slight effect on this parameter. A significant reduction was observed in the antioxidant activity of the yeast extract with vitamin C in the samples stored at 4 °C. After 28 days of storage, it was 2.5 times lower than that of the fresh yeast extract.

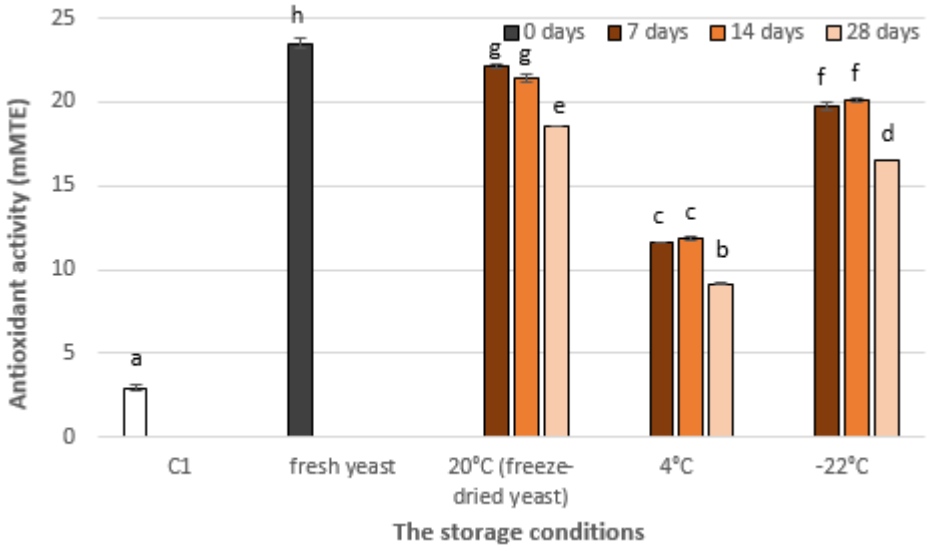


Figure 7. Effect of storage (0-fresh yeast, 7, 14, 28 days) on the antioxidant activity of an extract from yeast treated with PEF (vitamin C, 5 mg/mL medium; voltage, 1000 V; pulse width, 10 µs; treatment time, 20 min; number of pulses, 1200; after 20 h of cultivation) determined by the DPPH radical method. Sample C1 was yeast without vitamin C and PEF treatment. Each value is the mean ± standard deviation (n = 3). Bars with the same letter (a–h) are not significantly different ($p < 0.05$).

Nowak et al. [43] analyzed the antioxidant properties of vitamin C-rich juices. Antioxidant abilities were tested using the DPPH and ABTS radical methods. It was shown that acerola and rosehip juices had the highest antioxidant capacity tested against the DPPH radical (21.4 mM Trolox), followed by Japanese quince juice (17.9 mM Trolox). The lowest antioxidant capacity was found in cranberry (7.9 mM Trolox) and sea buckthorn (9.8 mM Trolox) juices. Our research showed that the antioxidant activity of the extract from yeast subjected to the electroporation process and not stored (23.5 mM Trolox) is comparable to that of juices containing high amounts of vitamin C.

Our research also showed that the yeast extract alone, not enriched with vitamin C (control sample C1), has specific antioxidant activity. The likely reason is that other ingredients present in the yeast exhibit this property. Vieira et al. [44] assessed the nutritional value, antioxidant activity, and phenolic compound profile of the spent brewer's yeast extract. It was shown that the extract from the inner layer of yeast is a rich source of protein, B vitamins, including vitamins B3, B6, and B9, and minerals such as sodium, potassium, calcium, magnesium, and iron. Chromatographic analysis showed that the yeast extract contained phenolic compounds such as gallic acid, protocatechic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid. It can therefore be assumed that thanks to these ingredients the yeast itself has an antioxidant effect and yeast extract may be a potential ingredient for the preparation of functional foods and nutraceuticals.

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