Rapid spectrophotometric method for the determination of iron content in yeast

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Abstract

Introduction. The development of a rapid method for the determination of iron in yeast has become especially important due to the growing demand for functional foods with increased content of this bioavailable microelement.

Materials and Methods. *Saccharomyces cerevisiae* M437 yeast enriched with iron. The determination of total iron content was carried out using a spectrophotometric method based on a colorimetric reaction between ferrous iron Fe (II) and the iron-specific chelator bathophenanthroline disulfonate (BPS). To ensure complete conversion of iron into the Fe (II) form, ferric ions Fe (III) were reduced using sodium ascorbate prior to the reaction.

Results and discussion. The optimal sample preparation conditions for the release of absorbed iron involved treating yeast biomass with 3% HNO_3 at 97 °C. The Fe^{2+}-BPS complex formed stably at pH 5.3–5.4, reaching equilibrium within 3 minutes. A citrate-based buffer system maintained the optimal pH, which is critical for chelate complex stability. The resulting complex solutions remained stable for up to 45 minutes, enabling batch analysis without compromising measurement accuracy.

The method's reproducibility was assessed using three parallel solutions with iron concentrations ranging from 110 to 360 μM; the relative standard deviation (RSD) did not exceed 1.4% across the entire range. Notably, for the 360 μM solution, the RSD was 0.4%, indicating high signal stability at elevated analyte concentrations. Linearity was confirmed over the range of 20–950 μM ($R^2 > 0.99$), allowing for accurate quantification of both low and high iron concentrations.

The iron content in yeast enriched with $FeSO_4$ was 9.7 ± 0.2 mg/g, while enrichment with $FeCl_3$ resulted in 5.2 ± 0.5 mg/g. These values were in good agreement with those obtained using atomic absorption spectrometry (9.3 ± 0.4 mg/g and 5.7 ± 0.6 mg/g, respectively). ANOVA analysis confirmed a statistically significant difference in iron content depending on the type of salt used ($FeSO_4$ vs. $FeCl_3$), whereas the difference between measurement methods (spectrophotometry vs. atomic absorption spectrometry) was not significant ($p = 0.8522$), indicating analytical equivalence between the two methods.

Conclusions. A method for determining iron content in yeast demonstrating high accuracy, reproducibility, and ease of implementation in food quality control and yeast-based biotechnological processes.

Introduction

Iron is one of the key trace elements necessary for the normal functioning of the human body (Piskin et al., 2021). Meanwhile, iron deficiency is the most common cause of anemia worldwide, affecting more than 2 billion people, according to the World Health Organization (World Health Organization, 2008). Therefore, the content of iron in food products is crucial for maintaining public health and forming a balanced diet. There are a number of studies devoted to the issue of functional food fortification with iron to prevent anemia in the population (Hamad and Singh, 2025; Jarzębski et al., 2023; Tsykhanovska et al., 2022, 2023, 2024; Xing et al., 2022; Zielińska-Dawidziak et al., 2023). Yeast enriched with iron is considered a promising source of this trace element, as it is commonly used as a food additive (Jach et al., 2022).

The determination of iron content in biological samples and food products is a multi-stage process. Preliminary sample processing, including deproteinization and the release of iron from protein complexes, is a crucial step that must be optimized depending on the specific characteristics of the sample. Classical approaches involve the use of mixtures of hydrochloric acid and potassium permanganate (Fish, 1988), burning samples to obtain ash with iron (Hailu et al., 2019), but to ensure complete extraction of iron from yeast cells with a strong cell wall, it has been proposed to use more aggressive reagents, such as a 3% solution of nitric acid (Tamarit et al., 2006), a mixture of nitric and perchloric acids (Chen et al., 2024), a 65% solution of nitric and hydrochloric acids (Tafazzoli et al., 2024).

There are several analytical methods for the quantitative determination of iron, each of which is characterized by certain advantages and limitations. Atomic absorption spectrometry (AAS) is one of the most common methods for determining iron content in biological samples, particularly in yeast (Kyyaly et al., 2015; Pirman and Orešnik, 2012). The main advantages of this method are high sensitivity and specificity, as well as the ability to analyze iron concentrations in a wide range. Inductively coupled plasma optical emission spectrometry (ICP-OES) is a highly accurate and sensitive technique that enables the quantitative determination of iron in various biological samples (Mier-Alba et al., 2024; Orlowska et al., 2023). Despite its analytical advantages, including high precision at low analyte concentrations, the practical application of ICP-OES is limited by its technical complexity and the need for specialized laboratory infrastructure. In contrast to the above-mentioned methods, spectrophotometric and colorimetric analyses are more accessible and cost-effective for determining high iron concentrations (over 1 mg/kg), which provide reliability and sufficient sensitivity of the analysis. In colorimetric determinations of iron, methods using colored complexes formed by iron are most often used. Among the most effective chelators used for determining iron in biological objects, ferrozine (Riemer et al., 2004), bathophenanthrolinedisulfonic acid (Gaensly et al., 2014; Tamarit et al., 2006), 1,10-phenanthroline (Herrera et al., 2007), and 2,2'-bipyridyl (Niedzielski et al., 2014) can be highlighted. The application of these reagents provides high sensitivity of the analysis, which makes the spectrophotometric method an optimal choice for routine determination of iron in yeast.

This article presents a rapid and simple spectrophotometric method for determining iron content in yeast, based on the formation of a colored iron complex with the chelator BPS. The main advantages of this method are high sensitivity, ease of implementation, short analysis time and the use of available reagents.

The proposed method for determining iron in yeast can be used for routine control of raw materials and finished products in the food industry, as well as for assessing the effectiveness of iron enrichment of yeast biomass in the process of developing and

implementing biotechnology for obtaining iron-containing biopreparations. This approach is promising for the creation of functional food additives and products with increased content of bioavailable iron, which can potentially contribute to solving the problem of deficiency of this element in the human diet.

Materials and Methods

Equipment

For spectrophotometric analyses, a Specord 210 spectrophotometer (Analytik Jena, Austria) was used, ensuring accurate measurement of the optical density of the iron-chelator complex at a wavelength of 536 nm. To validate the iron content determination, an atomic absorption spectrometer Shimadzu AA-7000 (Shimadzu, Turkey) with flame atomization in an air–acetylene environment was additionally used. The device was equipped with a hollow cathode lamp, and measurements were carried out at a wavelength of 248.3 nm, corresponding to the maximum absorption of iron.

Reagents

The following reagents were used: ferrous sulfate heptahydrate and ferric chloride hexahydrate (Carlo Erba), anhydrous citric acid (Sigma Aldrich), sodium citrate dihydrate (Sigma Aldrich), sodium ascorbate (Sigma Aldrich), 4,7-diphenyl-1,10-phenanthroline disulfonate (bathophenanthroline disulfonate sodium salt, BPS), and Certipur® for MS multi-element standard (Sigma Aldrich) containing iron at a concentration of 1000 ppm, and chromatography-grade water (Millipore).

BPS (bathophenanthroline disulfonic acid) is a water-soluble chelator that specifically binds ferrous ions (Fe^{2+}), forming an intensely colored complex with maximum absorption in the visible spectrum (536 nm). The stoichiometry of BPS binding with iron (Fe^{2+}) is 3:1, meaning that three BPS molecules bind to one Fe^{2+} ion, forming a stable complex $[Fe(BPS)_3]^{6-}$.

Microorganism

The object of the study was *Saccharomyces cerevisiae* M437 yeast, obtained from the Collection of Live Microbial Cultures of the Department of Biotechnology and Microbiology, National University of Food Technologies, and enriched with iron.

Culture preparation

The yeast culture was stored in test tubes on slanted agarized unhopped beer wort (wort agar, WA) at 4 ± 1°C. Before inoculum preparation, the yeast was streaked onto Petri dishes with WA using the depletion method to obtain isolated colonies and cultivated at 30 ± 1°C for 48 hours. Colonies that grew at least 1 cm apart were used for inoculum preparation. These colonies were transferred to tubes with slanted WA and incubated at 30 ± 1°C for 24 hours. All transfers were carried out under aseptic conditions.

Culture medium composition

Saccharomyces cerevisiae M437 was cultivated in a liquid nutrient medium with the following composition (g/L): ammonium sulfate – 1.0, ammonium phosphate – 5.5, potassium dihydrogen phosphate – 5.5, calcium chloride – 0.021, magnesium sulfate – 0.025, glucose – 15.0, yeast extract – 0.5, and casein peptone – 1.5; pH 4.5.

Iron salts (ferric chloride and ferrous sulfate) were added to the medium to reach a final concentration of 100 mM. The control variant consisted of the base medium without the addition of iron compounds.

Cultivation conditions

The inoculum for all variants was prepared by washing a one-day-old culture with sterile saline. Specifically, 5 ml of sterile saline was added to a test tube containing the culture, and a sterile inoculating loop was used to suspend the yeast cells. The resulting inoculum was then transferred with a sterile pipette into a flask containing 50 ml of sterile nutrient medium. The inoculum volume constituted 10% of the medium volume, with an initial cell concentration of 10^4–10^5 CFU/ml. All manipulations were performed under aseptic conditions.

Cultivation was carried out in 250 ml flasks under static microaerophilic conditions at 30°C for 48 hours, until the late exponential growth phase typical of yeast was reached.

Preparation of test solutions

After 48 hours of cultivation, yeast biomass samples were taken from each culture flask. The sample was washed three times with distilled water (centrifuged three times for 10 minutes at 6000 rpm) to remove weakly bound iron fractions from the yeast cell surface. The washed yeast biomass was dried at 80°C for 8 hours and then weighed. To 100.0 mg of biomass, 2.0 mL of 3% (v/v) nitric acid was added, sealed tightly, and heated for 16 hours at 97°C to break down the yeast cell wall and release intracellular iron. After cooling the samples, 8 mL of 3% (v/v) nitric acid was added, and the solution was filtered through a "Red Stripe" filter. 4.0 mL of the filtered solution was diluted to a final volume of 20.0 mL with 3% nitric acid.

Preparation of reference solutions

Iron reference solutions for the calibration curve were prepared by diluting 0.02 M stock solutions of ferrous sulfate (Fe(II)) or ferric chloride (Fe(III)) in 3% nitric acid solution.

Spectrophotometric determination of iron in yeast biomass

0.5 mL of the test solution from the yeast sample or the iron reference solutions was mixed with 4 mL of citrate buffer, followed by the addition of 0.5 mL of 40 mg/mL sodium ascorbate solution and 0.5 mL of 2.0 mg/mL iron chelator (BPS). After 5 minutes, the specific absorbance of the iron-chelator complex was recorded at 536 nm on a spectrophotometer. A compensation solution was prepared similarly using the yeast biomass sample without exogenous iron addition.

Results and discussion

Sample preparation

To break down the yeast cell wall and release iron, solutions of nitric acid with concentrations of 2%, 3%, and 5%, as well as a 2 M hydrochloric acid solution, were tested. Increasing the nitric acid concentration from 2% to 3% resulted in a higher total iron concentration in the samples. However, the use of a 5% nitric acid solution did not lead to further iron release and complicated achieving the optimal pH (5.3-5.4) when adding the buffer solution due to the excessively acidic environment. The high acidity of the 5% solution may reduce the method's sensitivity and also cause corrosion and damage to the equipment. For the same reason, the use of a 2 M hydrochloric acid solution for iron release was also excluded.

Using a 3% nitric acid solution improved the efficiency of iron extraction, allowing more accurate results and enabling simultaneous analysis of the samples using both atomic absorption spectrometry and spectrophotometry.

Choice of iron chelator

BPS (4,7-diphenyl-1,10-phenanthroline-disulfonic acid) was preferred as the iron chelator due to its high solubility in aqueous media, owing to the presence of sulfonate groups, while 1,10-phenanthroline requires organic solvents (e.g., ethanol) for effective dissolution. Moreover, the Fe(II)-BPS complex is highly stable in aqueous solutions, ensuring reproducibility of results. Another important feature of BPS is its lower sensitivity to foreign ions, reducing potential interference when analyzing complex biological samples. The formation of the Fe(II)-BPS complex with 1,10-phenanthroline required heating to 80°C for 10 minutes to achieve sufficient reaction speed. However, BPS also has certain drawbacks. In particular, the formation of the Fe(II)-BPS complex is more sensitive to changes in pH, requiring careful control of this parameter during analysis. However, the chosen buffer solution ensures that the pH remains within the range of 5.3–5.4.

Influence of other metals on iron-BPS complex formation

According to literature (Nilsson et al., 2002; Tamarit et al., 2006), the impact of other metals potentially present in yeast on the formation of the iron-BPS complex was analyzed, specifically manganese, zinc, and copper. The reactivity of BPS with copper was significantly lower than with iron, and no complex formation with zinc or manganese was observed.

Effect of pH on the formation of the iron-BPS complex

The pH of the solution is a key factor affecting the effectiveness of the iron complexation with BPS. The optimal pH range, according to literature, is 5.3–5.4. Experimentally, it was determined that under these conditions, the absorbance of the iron-BPS complex was 0.6010, confirming the stability of the complex and effective chelation. A significant decrease in absorbance to 0.0143 was observed when the pH dropped to 2.3. This can be explained by the oxidation of Fe^{2+} to Fe^{3+} in the strongly acidic environment, which has a lower affinity for BPS. Additionally, protonation of BPS, which reduces its chelation ability, and the competitive binding of hydrogen ions with the chelator may contribute to this decrease.

When the pH was increased to 10.0, the absorbance increased to 0.1052 but remained significantly lower than at the optimal pH. This could be attributed to partial precipitation of iron as $Fe(OH)_2$ or $Fe(OH)_3$, reducing the number of available iron ions for interaction with BPS. In alkaline conditions, the formation of other hydroxy complexes that compete with BPS for iron ions may also reduce the efficiency of the target complex formation.

Thus, the results confirm that the pH range of 5.3–5.4 is the most favorable for stable Fe^{2+}-BPS complex formation, while significant deviations toward acidic or alkaline environments negatively affect the chelation process, as demonstrated by the sharp decrease in absorbance. Citrate buffer solution was added to the aliquot of reference or test solution to achieve a pH of 5.3–5.4, where BPS absorption is maximal.

Method validation

The proposed analytical method for determining iron was validated in terms of specificity, linearity, precision, and stability.

Specificity. The specificity of the method was assessed by recording the spectra of a yeast biomass sample without exogenous iron (control solution) and the test solution (yeast enriched with iron). The spectra are shown in Figure 1. At a wavelength of 536 nm, the formation of the iron-BPS complex was observed, with an optical density value of 0.8418. For the control solution, which did not contain iron, the optical density was 0.0425, indicating no significant background spectral interference. These results confirm the high specificity of the method for determining iron in the analyzed solutions. The control solution was used as a compensation solution in subsequent tests.

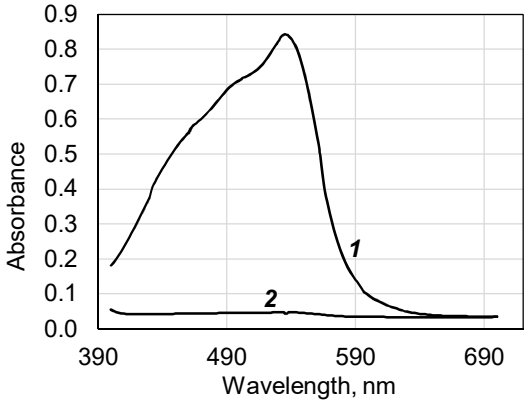

Figure 1. Spectral confirmation of iron-BPS complex formation:
1 – with complex formation (yeast enriched with iron);
2 – without complex formation (control solution).

Linearity. The linearity of the method was assessed based on the calibration curve constructed using a range of analyte concentrations from 20 to 950 μM iron. The corresponding optical density values were recorded in three parallel measurements, confirming the reproducibility of the method. Figure 2 and Figure 3 show the correlation between iron content and light absorbance after complete reaction of iron with BPS. The analysis demonstrated a linear dependence of absorbance on iron content in the range of 20 μM – 950 μM and could accurately determine 20 μM of iron, regardless of whether the iron was in the +2 oxidation state ($FeSO_4$) or +3 oxidation state ($FeCl_3$). This indicates that sodium ascorbate was capable of effectively reducing iron under these conditions.

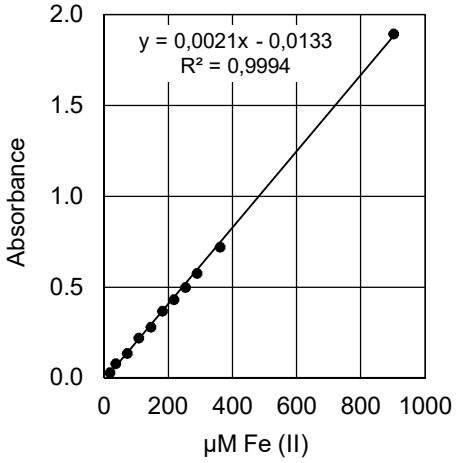

Figure 2. Linear dependence of optical density on iron concentration (from reference solutions of iron (II) sulfate after complex formation with BPS)

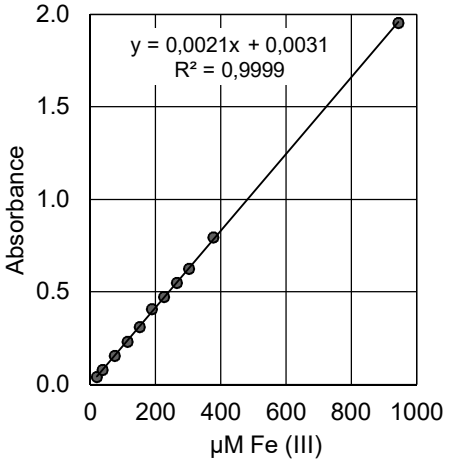

Figure 3. Linear dependence of optical density on iron concentration (from reference solutions of iron (III) chloride after complex formation with BPS)

Stability of the solutions. Previous studies (Nilsson et al., 2002; Tamarit et al., 2006) demonstrated that the formation of the iron complex with 2,2'-bipyridyl-5,5'-sulfonic acid (BPS) occurs within one minute. However, considering that smaller sample volumes were used in those studies, it became necessary to assess the rate of complex formation in a final solution volume of 5.5 mL.

The rate of formation of the iron–BPS complex was evaluated by measuring the optical density of a reference iron solution with a concentration of 300 μM and of the test solution (iron-enriched yeast) over a 5-minute period. The obtained optical density values at different time intervals are presented in Table 1.

Analysis of the data indicates rapid complex formation, as a significant increase in optical density occurs within the first two minutes. From the third minute onwards, the values stabilize, indicating that complexation equilibrium has been reached. Minor fluctuations in optical density after the third minute (0.6239–0.6240) suggest high reproducibility of the process and stability of the formed complex. Thus, the iron–BPS complex forms quickly, and equilibrium is established within 3 minutes, allowing a 5-minute incubation period to be recommended for subsequent measurements in analytical studies.

Table 1

Absorbance values depending on the incubation time of the solution after BPS addition (complex formation study)

Time, min	Absorbance of reference solution	Absorbance of test solution
0	0.5726	0.6765
1	0.6067	0.6877
2	0.6227	0.6963
3	0.6239	0.6983
4	0.6240	0.6984
5	0.6239	0.6984

The stability of the iron-BPS complex solutions was evaluated over time by storing a reference solution containing 360 μM iron and the test solution (iron-enriched yeast) for periods ranging from 5 to 45 minutes. The measurement results are presented in Table 2. Optical density measurements showed only minor changes. Minimal fluctuations in absorbance indicate high stability of the complex within the investigated time range, which allows its use in further analytical studies without significant errors related to the decomposition or transformation of the complex.

Table 2

Absorbance values depending on the incubation time of the solution after BPS addition (stability study)

Time, min	Absorbance of reference solution	Absorbance of test solution
5	0.7276	0.6984
15	0.7245	0.6988
25	0.7275	0.6986
35	0.7280	0.6986
45	0.7286	0.6988

Reproducibility. The reproducibility of the analysis was evaluated by preparing three reference solutions in triplicate on the same day. The measurement results are presented in Table 3.

Table 3

Results of repeated optical density measurements for samples with different iron concentrations

Test number	360 μM Fe	190 μM Fe	110 μM Fe
Measurement 1	0.6010	0.3759	0.2267
Measurement 2	0.5971	0.3710	0.2203
Measurement 3	0.6006	0.3774	0.2231
Average	0.5996	0.3748	0.2234
RSD,%	0.4	0.9	1.4

The obtained values of relative standard deviation (RSD) did not exceed 1.4%, indicating high reproducibility of the method across the entire studied concentration range. The lowest deviation (0.4%) was observed at an iron concentration of 360 μM, which reflects signal stability at higher analyte content. RSD values below 2% generally indicate the reliability and robustness of the method under routine analytical conditions.

To further confirm the accuracy of the spectrophotometric method described in this study, the results were compared with those obtained using atomic absorption spectrometry (AAS). Iron reference solutions for the calibration curve were prepared by diluting a Certipur® multi-element standard for MS. The test samples (solutions obtained from biomass of yeast enriched with iron via the addition of iron (II) sulfate and iron (III) chloride), prepared according to the described procedure, and were analyzed by both methods. The obtained results are presented in Table 4.

Table 4

Comparative table of iron content in yeast determined by spectrophotometry and atomic absorption spectrometry

Sample	Iron content, mg/g DW of yeast, determined by	
	method 1	method 2
Sample (iron enrichment source – $FeSO_4$)	9.7 ± 0.2	9.3 ± 0.4
Sample (iron enrichment source – $FeCl_3$)	5.2 ± 0.5	5.7 ± 0.6

Note:
Method 1 – spectrophotometric method;
Method 2 – atomic absorption spectrometry;
DW, dry weight

The results of the analysis of variance (ANOVA) revealed a significant difference between the two iron sources – iron sulfate ($FeSO_4$) and iron chloride ($FeCl_3$). The F-value for the iron sources was high ($F(1,8) = 243.00$, $p < 0.0001$), indicating a significant difference in iron content depending on the type of compound. This suggests that the form of iron affects its availability or effectiveness under different conditions. Iron in the form of $FeSO_4$ (Fe (II)) is likely better absorbed, as confirmed by the high F-value, compared to $FeCl_3$ (Fe (III)), which requires additional reduction before absorption.

At the same time, no statistically significant difference was found between the measurement methods – spectrophotometry and atomic absorption spectrometry (AAS) ($F(1,8) = 0.037$, $p = 0.8522$). This means that both methods provided similar results, and the choice of measurement method had no substantial effect on determining the iron content in both types of samples. Therefore, under the studied conditions, the choice of method for measuring iron content is not a critical factor.

Conclusions

This study presents a simple and accessible approach for determining iron content in yeast. An effective extraction method was developed that does not require complex or specialized equipment. The proposed method is characterized by ease of execution, reproducibility of results, and suitability for application in a standard laboratory setting. The use of a 3% nitric acid solution was identified as the optimal option for releasing iron from yeast cells, providing high extraction efficiency and compatibility with both spectrophotometric and atomic absorption analyses.

The obtained results highlight the practical value of the proposed method for routine monitoring of iron content in food products, as well as for evaluating the effectiveness of biotechnological processes for yeast iron enrichment and investigating the forms and bioavailability of iron in the development of functional food products.

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