



Research Article

Structural, thermal, morphological, and rheological properties of yeast extracts obtained from *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, and *Kluyveromyces marxianus*

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ARTICLE INFO

Article history

Received: 05 August 2024

Revised: 03 October 2024

Accepted: 07 December 2024

Keywords:

DSC; FTIR; Rheological Properties; SEM; Yeast Extract

ABSTRACT

Yeast extracts, which are mostly used as flavor enhancers in foods, also give to the nutritional value of foods, especially due to their amino acid profiles. In addition to all these properties, yeast extracts have the possibility to supply added technological advantages to the foods they are used in due to their physicochemical properties. However, there are not enough studies investigating the structural, thermal, morphological, and rheological properties of yeast extract powders. To fill this gap and investigate the potential of yeast extracts to supply added benefits to foods, in this study, yeast extract powders obtained from *Saccharomyces cerevisiae* TGM10, *Saccharomyces boulardii* S11, and *Kluyveromyces marxianus* TGM66 structural properties were investigated using Fourier Transform Infrared Spectroscopy, thermal properties using Differential Scanning Calorimetry, morphological properties using Scanning Electron Microscopy and rheological properties using rheometer. The melting points of *Saccharomyces cerevisiae* TGM10, *Saccharomyces boulardii* S11, and *Kluyveromyces marxianus* TGM66 extracts were 129 °C, 128 °C and 132 °C, respectively. It is believed that the Fourier Transform Infrared spectra obtained in the study will contribute to the literature in terms of the production and identification of yeast extracts, and the morphological properties will contribute to the determination of the drying method to be applied to the extracts. It was determined that all three yeast extracts used in the study had the potential to increase the viscosity of the soup, and the yeast extract obtained from *Kluyveromyces marxianus* TGM66 was the most successful sample in this respect. It is thought that this may be related to the fact that this extract has a different carbohydrate and/or amino acid profile than others. Considering the data obtained in the study, it is thought that with a better understanding of the structural, thermal, morphological, and rheological properties of yeast extracts, the possibility of using these additives in foods will expand.

Cite this article as: Demirgöl F, Şimşek Ö, Dertli E, Sağdıç O. Structural, thermal, morphological, and rheological properties of yeast extracts obtained from *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, and *Kluyveromyces marxianus*. Sigma J Eng Nat Sci 2026;44(1):174–183.

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This paper was recommended for publication in revised form by Editor-in-Chief Ahmet Selim Dalkilic



INTRODUCTION

The product obtained by breaking down and removing the yeast cell wall in various ways (such as autolysis, plasmolysis, enzymatic hydrolysis, thermolysis, and mechanical disruption) and consisting of water-soluble constituents of the yeast cell is called yeast extract [1]. The major constituent of yeast extracts is hydrolyzed protein [2], and the protein quantity of yeast extract generally varies between approximately 46% [3] and 74% [4] by dry weight (dw). The fat content of yeast extracts is approximately 1% [5]. Yeast extracts, which are also rich in essential amino acids, are a good source of taking these amino acids into the body [6]. Moreover, yeast extracts contain B group vitamins [4], minerals like magnesium, calcium, iron, and zinc, and phenolic compounds like (\pm)-catechin, gallic acid, protocatechuic acid, ferulic acid, and *p*-coumaric acid [7]. Due to their rich nutritional composition, yeast extracts are used in the nutrition of both humans and various animals. In addition to their nutritional properties, yeast extracts have begun to be utilized in various foods as a natural flavor enhancer another option to monosodium glutamate in recent years, as it has been understood that they cause meat-like flavor and umami taste due to the high amounts of glutamic acid [8], 5'-monophosphate nucleotides [9], and peptide profiles [10] they contain.

In addition to their nutritional and flavor-enhancing properties, yeast extracts may also contribute technologically to the foods they are used in, especially due to the carbohydrates and proteins they contain. As a matter of fact, in a previous study [5] we conducted, we determined that yeast extracts can supply technological benefits to foods due to their water-holding capacity, oil-binding capacity, and emulsion stability. In fact, the yeast cell wall, which is removed in the production of yeast extract, has thickening properties due to its carbohydrate (especially β -glucans) profile [11]. However, there is the potential for slight losses in flavor when yeast cell walls are used in foods [12]. Although there are some studies examining the rheological properties of yeast protein extracts [13, 14] and yeast extract pastes (Marmite™) [15], to our knowledge, there is no study examining the effects of yeast extract powders used as additives in some foods in recent years on the rheological properties of foods such as soup. However, yeast extracts contain approximately 12.9% carbohydrates [7], and due to

their rich protein and carbohydrate content, yeast extracts have the potential to contribute to the rheological properties of foods if used in appropriate foods. In addition, there are not enough studies investigating the structural, thermal, and morphological properties of yeast extracts. However, with a better understanding of their structural and morphological properties, more effective yeast extract powder production can be achieved, and with a better understanding of their thermal properties, the possibilities of using yeast extracts in suitable food systems can be increased. In this context, this study aims to investigate the structural, thermal, morphological, and rheological features of yeast extracts obtained from *S. cerevisiae* TGM10, *S. cerevisiae* var. *boulardii* S11, and *K. marxianus* TGM66 yeasts and define their characteristic properties. Thus, it is targeted to determine whether yeast extracts, which are mostly used in various foods due to their flavor-enhancing and nutritional features, have the potential to provide added benefits to the foods in which they are used.

MATERIALS AND METHODS

Materials

In this study, lyophilized yeast extract powders obtained from *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 strains in our previous study [5] were used. The chemical compositions of the yeast extracts used in the study are summarized in Table 1 [5].

Methods

The experimental framework of the study using three different yeast extracts is summarized in Figure 1.

Structural Properties

Structural properties of yeast extracts were examined using Fourier Transform Infrared (FTIR) spectroscopy (Bruker Tensor 27, Germany). Measurements were done by dissolving yeast extract powders in distilled water and preparing 10% solutions. Before measurement, distilled water was used to ensure that the spectroscopy accepted distilled water as a reference. Measurements of the samples were carried out at room temperature and in the mid-infrared region (in the wavelength range of 500–3500 cm^{-1}).

Table 1. Chemical compositions of yeast extract powders obtained from *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 strains used in the study

Chemical composition (g/100 g dw)	TGM10	S11	TGM66
Moisture (%)	13.93	14.20	15.37
Ash	11.77	12.82	11.15
Protein	69.17	66.16	62.42
Fat	1.36	1.09	1.33
Carbohydrates and other compounds	17.69	19.93	25.10

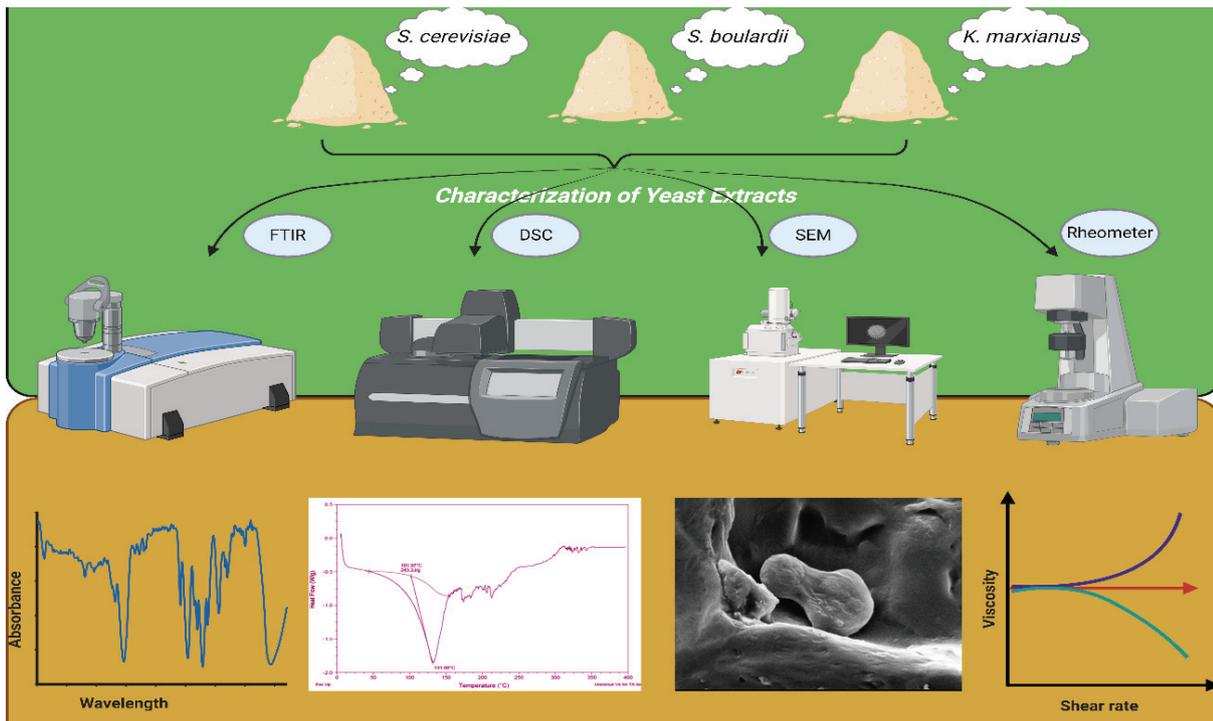


Figure 1. Experimental framework of the study [Figure 1 was figured out by the author (F.D.) using BioRender.com.]

Thermal Properties

Thermal properties of yeast extracts were examined by a Differential Scanning Calorimeter (DSC, Q20, TA Instruments Inc., USA) with some modifications to the method proposed by Kavosi et al. [16]. The samples were weighed as 10-15 mg onto aluminum DSC pans and kept in a desiccator for 20-30 minutes. Then, the hermetically sealed pans were loaded into the DSC calorimeter and heated to 400 °C under high-purity nitrogen gas (20 mL/min) with a heating rate of 10 °C/min. An identical blank pan was used as a control. Melting temperatures of yeast extracts were calculated from the DSC thermograms obtained.

Morphological Properties

Surface morphological properties of yeast extracts were determined by Scanning Electron Microscopy (SEM, Zeiss Evo LS 10, Germany) by slightly modifying the method proposed by İşçi and Hayta [17]. Before analysis, samples were coated with gold-palladium in a gold-plating device (Quorum SC7620, UK) for 120 seconds. Examination of the samples with SEM was carried out at 10 kV.

Rheological Properties

Rheological properties of yeast extracts were examined by making some modifications to the procedure suggested by Zehir-Şentürk et al. [18]. Yeast extracts were dissolved in vegetable soup (Penelope Gurme, Türkiye; consists of semolina, dried broccoli, dried spinach, and dried celery) at two different concentrations (0.5% and 1%) and shear stress (Pa) values against shear rate (s^{-1}) were obtained by measuring 100

points on the rheometer (Anton Paar, MCR-302, Austria) at two different temperatures (25 °C and 50 °C) and the flow behavior of the samples was examined. The obtained data were adapted to the Ostwald de Waele (Power Law) rheological model with the help of the following equation:

$$\tau = K(\dot{\gamma})^n$$

Here, τ represents the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa \cdot s^n$), and n is the flow behavior index.

Statistical Analysis

Statistical analyses were made with the SPSS (PASW 18.0) program, all experiments were made in triplicate. Data were subjected to one-way analysis of variance (ANOVA) and post hoc comparisons were made using the Tukey test. Differences between data were considered statistically significant at the 95% ($p \leq 0.05$) confidence interval. Statistica 14.0 (TIBCO, USA) software was used to evaluate rheological analyses.

RESULTS AND DISCUSSION

Structural Properties

FTIR spectroscopy, a type of vibration spectroscopy, is a technique that can provide easy and fast results that reflect both the molecular structure and the molecular environment, and the fact that each chemical bond has a separate vibration frequency in infrared spectroscopy allows the

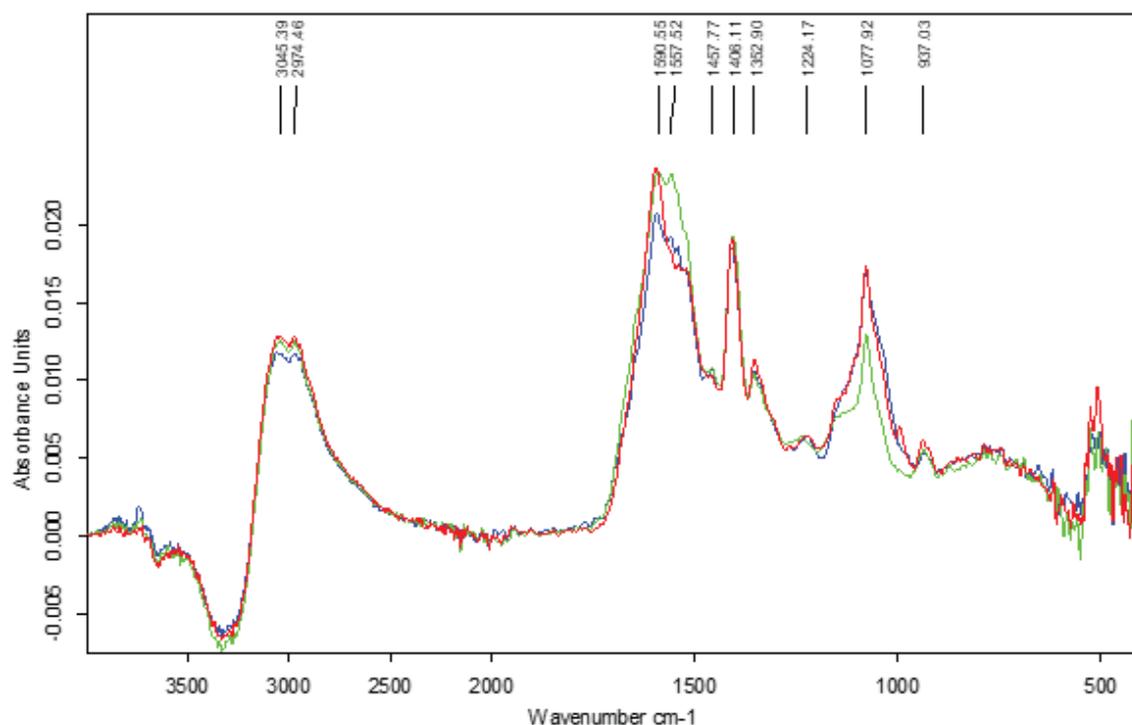


Figure 2. FTIR spectra of three different yeast extracts (the green spectrum belongs to the *S. cerevisiae* TGM10 extract, the red spectrum to the *S. boulardii* S11 extract, and the blue spectrum to the *K. marxianus* TGM66 extract).

identification of chemical groups [19]. For this reason, FTIR spectroscopy was used to perform the structural properties of yeast extracts in this study, and the FTIR spectra of *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts are shown in Figure 2.

Distinct peaks in the FTIR spectra of the yeast extracts were examined, and it was observed that all three yeast extracts gave peaks at similar wavelengths. Yeast extracts are mainly composed of proteins, peptides, amino acids, nucleic acids, carbohydrates, fats, vitamins, minerals and phenolic compounds [8], and the vibrational peaks in the FTIR spectrum of yeast extracts are thought to be associated with these compounds, especially proteins and amino acids. Since there are many overlapping bands in the infrared spectrum, the chemical structure of a protein cannot be inferred from it, but changes in chemical structure can be understood [20].

The bands at approximately 3000 cm^{-1} are generally associated with C-H bonds, and more specifically, the bands to the left of 3000 cm^{-1} (between 3150 cm^{-1} and 3000 cm^{-1}) are attributed to aromatic (between 3010 cm^{-1} and 3050 cm^{-1}) and vinyl CH, while the bands to the right are attributed to aliphatic C-H stretching [21]. Considering this information, the bands at approximately 3039 cm^{-1} and 2975 cm^{-1} ; 3045 cm^{-1} and 2974 cm^{-1} ; 3042 cm^{-1} and 2976 cm^{-1} in *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts, respectively, may be due to the vibration of aromatic and aliphatic C-H bonds. The bands between

1595 cm^{-1} and 1541 cm^{-1} in the spectrum are thought to be related to amino acids and arise from antisymmetric NH_3^+ deformation or antisymmetric COO^- stretching [22]. The bands at approximately 1457 cm^{-1} , 1459 cm^{-1} , and 1457 cm^{-1} in *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts, respectively, may be due to different CH_2/CH_3 bending vibrations in the proteins. The bands at 1406 cm^{-1} (*S. cerevisiae* TGM10), 1407 cm^{-1} (*S. boulardii* S11), and 1408 cm^{-1} (*K. marxianus* TGM66) in the samples are probably related to the $\text{C}(\text{CH}_3)_2$ stretching that generally occurs in proteins. The bands at approximately 1353 cm^{-1} (*S. cerevisiae* TGM10), 1352 cm^{-1} (*S. boulardii* S11), and 1353 cm^{-1} (*K. marxianus* TGM66) may be due to CH_2 deformation in lipids. It is thought that the bands at approximately 1236 cm^{-1} (*S. cerevisiae* TGM10), 1224 cm^{-1} (*S. boulardii* S11), and 1224 cm^{-1} (*K. marxianus* TGM66) may be related to C-O stretching in free nucleotides [23, 24]. The bands at approximately 1076 cm^{-1} (*S. cerevisiae* TGM10), 1077 cm^{-1} (*S. boulardii* S11), and 1077 cm^{-1} (*K. marxianus* TGM66) are probably related to the symmetric stretching of PO_2^- in the RNA [23, 25]. The bands around 900 cm^{-1} and 1000 cm^{-1} are generally associated with carbohydrates, and the bands at approximately 936 cm^{-1} (*S. cerevisiae* TGM10), 994 and 937 cm^{-1} (*S. boulardii* S11), and 933 cm^{-1} (*K. marxianus* TGM66) are thought to be related to vibrations in glycosidic bonds [23, 26].

In recent years, FTIR spectroscopy has become widespread because it is a sensitive, fast, easy, and relatively

inexpensive method for estimating the structural properties of foods. FTIR spectroscopy can be used to estimate the structural properties of yeast extracts, monitor cell growth in yeast biomass production, and monitor the effectiveness of autolysis or other cell lysis methods, which is one of the most important levels of yeast extract production [24].

Thermal Properties

DSC is a useful thermoanalytical approach in which the heat difference required to increase the temperature of a sample, and a reference is measured as a function of temperature, allowing to quickly analyze phase changes caused by some chemical reactions such as water crystallization (melting and freezing), water evaporation, and protein denaturation [27]. In this context, DSC analysis was made to determine the melting temperatures of yeast extracts, and the DSC thermograms of *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts are shown in Figure 3.

When DSC thermograms are examined, endothermic peaks are observed at approximately 129 °C, 128 °C and

132 °C, corresponding to the melting points of *S. cerevisiae* TGM10, *S. boulardii* S11 and *K. marxianus* TGM66 extracts, respectively. It was observed that the thermal stability of *S. cerevisiae* TGM10 and *S. boulardii* S11 extracts were similar to each other, while the thermal stability of *K. marxianus* TGM66 extracts was slightly higher than the other samples.

There are not sufficient studies in literature examining the thermal properties of yeast extracts. However, DSC analysis has a high potential for determining stability parameters in complex systems such as yeast extracts and can be utilized as an easy, rapid, and reliable technique to examine the stability of proteins and fatty acids in particular.

Morphological Properties

With SEM, information can be obtained about the microstructural surface morphologies and chemical compositions of the samples [28]. SEM was used to observe the morphologies of yeast extract powders. Micrographs of *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts examined by SEM are shown in Figure 4.

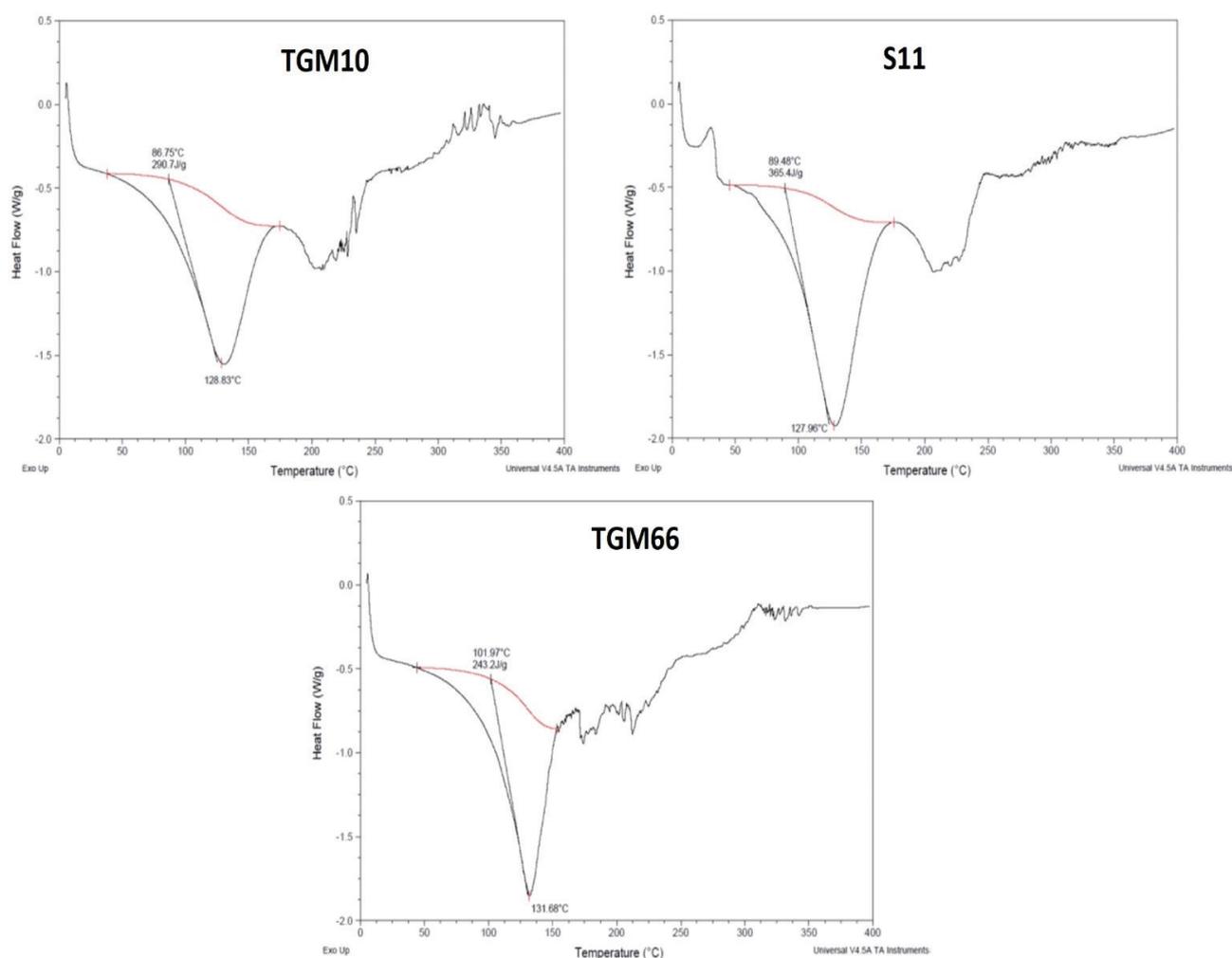


Figure 3. DSC thermograms of *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 extracts, respectively.

The drying process (such as freeze drying and spray drying) applied to liquid samples affects the particle size of the resulting powder particles [29]. In the freeze drying (lyophilization) process, liquid samples are first frozen and then water is removed from the frozen sample by sublimation. Therefore, lyophilized particles break down and become compressed into sheet-like layers with a porous surface [29]. When micrographs of lyophilized yeast extract powders are examined, it is seen that the samples have a porous surface similar to each other and are irregularly distributed. In their study where they examined freeze- and

spray-dried samples of yeast β -glucan, Petravić-Tominac et al. [29] reported that elliptical and oval shapes were observed in spray-dried samples with better preservation of the original structure.

Rheological Properties

The consistency coefficients, flow behavior indices, and R^2 values of yeast extracts dissolved in vegetable soup at two different concentrations (0.5% and 1%) at 25 °C and 50 °C, determined using the Ostwald de Waele model, are shown in Tables 2 and 3, respectively.

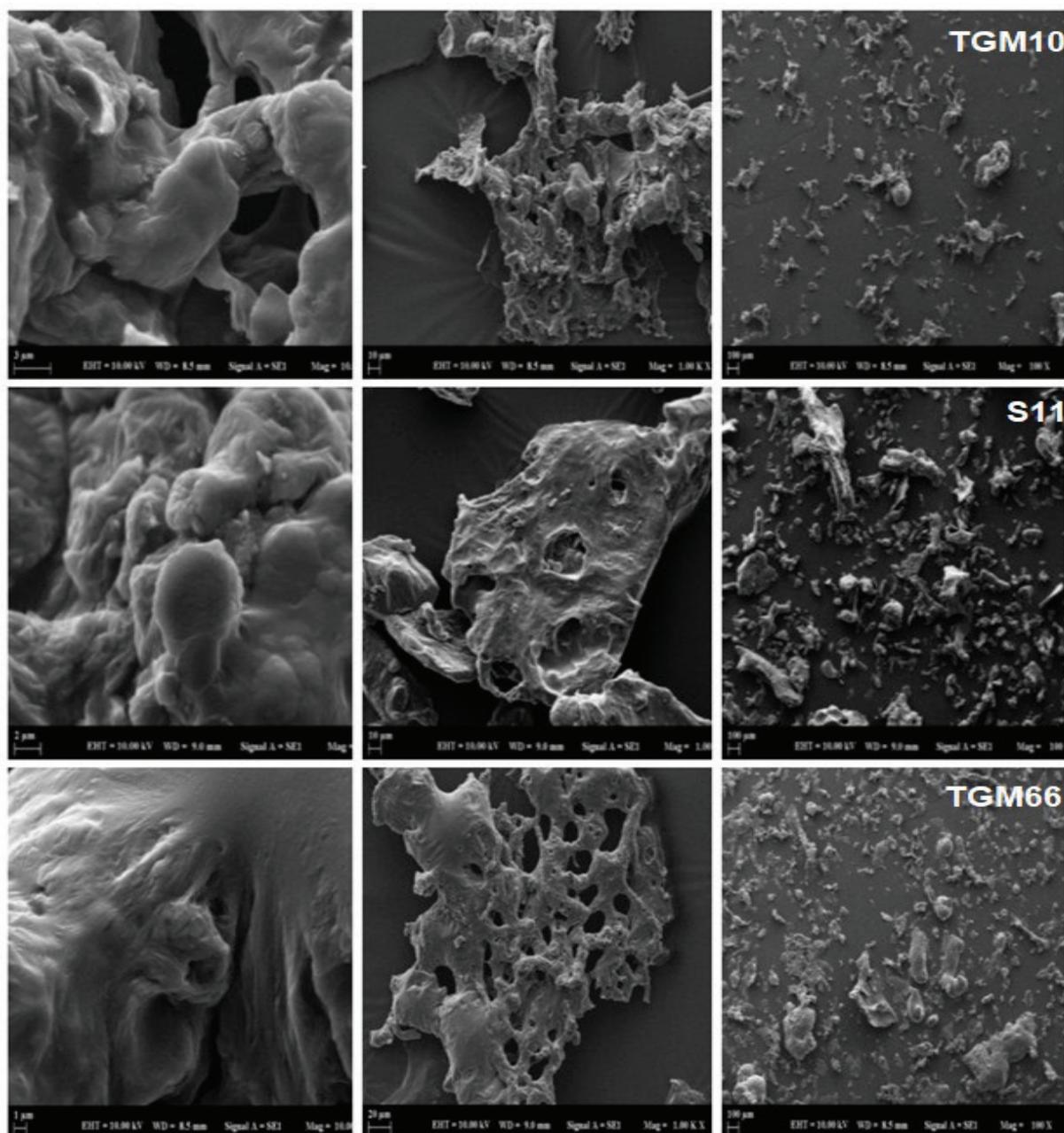


Figure 4. Micrographs of extracts of *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66, respectively.

Table 2. Rheological properties of vegetable soup samples, in which yeast extracts obtained from *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 strains were mixed at two different concentrations (0.5% and 1%), at 25 °C

Samples	Consistency coefficient (Pa.s ⁿ)	Flow behavior index (n)	R ²
TGM10 (0.5%)	2.22 ± 0.14 ^b	0.819 ± 0.056 ^{ab}	0.9923
S11 (0.5%)	2.35 ± 0.46 ^b	0.793 ± 0.023 ^{ab}	0.9893
TGM66 (0.5%)	2.41 ± 0.18 ^b	0.777 ± 0.044 ^{ab}	0.9883
TGM10 (1%)	2.54 ± 0.39 ^{ab}	0.787 ± 0.081 ^{ab}	0.9938
S11 (1%)	3.00 ± 0.51 ^{ab}	0.754 ± 0.034 ^b	0.9884
TGM66 (1%)	3.43 ± 0.44 ^a	0.746 ± 0.035 ^b	0.9899
C-1 (Plain soup)	2.08 ± 0.14 ^b	0.905 ± 0.044 ^a	0.9953

Results are expressed as mean values (n = 3) ± Standard Deviation (SD). Different letters in the same column specify that the difference between the two values is statistically significant (p≤0.05).

In the measurements, the flow behavior indices of all samples were below 1, but close to 1 (between 0.746-0.969) (Table 2 and 3). The flow behavior index being below 1 is classified as pseudoplastic flow behavior [30]. However, since the flow behavior indices are close to 1, it can be said that the flow behavior of all samples shows a weak pseudo-plastic characteristic. In a study by Pacheco and Sgarbieri [13] on brewer's yeast protein concentrates prepared by three different procedures, it was reported that all yeast protein concentrates showed pseudoplastic behavior. In a study [14] investigating the possibility of using yeast protein extracts obtained from *S. cerevisiae* and *Schizosaccharomyces pombe* as an alternative to egg lecithin in mayonnaise sauce production, it was determined that both samples showed similar viscoelastic behavior when compared to mayonnaise containing egg powder. In the study conducted by White et al. [15], according to steady-state shear tests, yeast extract paste (Marmite™) exhibits thixotropic behavior at ambient temperature, while at higher temperatures the extent of thixotropy decreases and approaches Newtonian behavior. The differences between the results are thought to be mainly related to the form, composition, and production

methodology of the products. More research is needed to shed light on the issue.

In addition to the flow behavior index, consistency coefficients are also important for yeast extracts. The consistency coefficient of a fluid gives an idea about the viscosity, and a high consistency coefficient indicates that the viscosity is also high [30]. In measurements made at 25 °C, all samples with added yeast extract increased the consistency coefficient of plain vegetable soup (Table 2), and the most successful sample in this respect was *K. marxianus* TGM66 (1%) (p≤0.05). In addition, all yeast extracts increased the consistency coefficient of vegetable soup by similar (p>0.05) amounts if used at the same concentration. Although the effect of samples other than *K. marxianus* TGM66 (1%) on the consistency coefficient was statistically insignificant (p>0.05), it can be said that yeast extracts will have a positive contribution to the viscosity of foods such as soup at room temperature.

The consistency coefficients of both plain vegetable soup (control) and vegetable soups with yeast extract added at the same concentration decreased significantly (p≤0.05) as the temperature increased from 25 °C to 50 °C. However, the consistency coefficients of all samples with added yeast

Table 3. Rheological properties of vegetable soup samples, in which yeast extracts obtained from *S. cerevisiae* TGM10, *S. boulardii* S11, and *K. marxianus* TGM66 strains were mixed at two different concentrations (0.5% and 1%), at 50 °C

Samples	Consistency coefficient (Pa.s ⁿ)	Flow behavior index (n)	R ²
TGM10 (0.5%)	1.17 ± 0.11 ^{cd}	0.944 ± 0.014 ^a	0.9821
S11 (0.5%)	1.34 ± 0.23 ^{bcd}	0.924 ± 0.029 ^{ab}	0.9795
TGM66 (0.5%)	1.40 ± 0.13 ^{bc}	0.932 ± 0.015 ^a	0.9823
TGM10 (1%)	1.56 ± 0.08 ^{abc}	0.863 ± 0.030 ^{bc}	0.9819
S11 (1%)	1.81 ± 0.21 ^{ab}	0.815 ± 0.011 ^{cd}	0.9761
TGM66 (1%)	1.97 ± 0.14 ^a	0.792 ± 0.032 ^d	0.9797
C-2 (Plain soup)	0.88 ± 0.21 ^d	0.969 ± 0.012 ^a	0.9877

Results are expressed as mean values (n=3) ± SD. Different letters in the same column specify that the difference between the two values is statistically significant (p≤0.05).

extract were higher compared to the control. Similar to measurements performed at 25 °C, all yeast extracts increased the consistency coefficient of vegetable soup by similar ($p>0.05$) amounts when used at the same concentrations. The effect of all samples containing yeast extract at 1% concentration on the consistency coefficient of plain soup was statistically significant ($p\leq 0.05$). However, *K. marxianus* TGM66 sample significantly ($p\leq 0.05$) increased the consistency coefficient of plain soup even when used at 0.5% concentration. In measurements performed at both 25 °C and 50 °C, *K. marxianus* TGM66 extract stands out among all extracts. It is thought that the reason for this situation may be related to the carbohydrate structure and amount and/or amino acid profile of the *K. marxianus* TGM66 extract.

To our knowledge, apart from the present study, there is no study in the literature examining the effects of distinct yeast extract powders on the rheological properties of foods such as soup. Whereas, with a better understanding of the effects of ingredients like yeast extracts and yeast protein extracts on the rheology of different food systems, the possibility of using yeast extract-based products, especially in vegan foods, may increase, as they are generally cheaper than their counterparts and are not animal-based.

CONCLUSION

Although numerous studies have been conducted on the flavor and nutritional properties of yeast extracts, there are not sufficient studies in the literature examining the structural, thermal, morphological, and rheological properties of different yeast extract powders. To fill this gap in the literature, this study investigated whether three different yeast extracts obtained from *Saccharomyces cerevisiae* TGM10, *Saccharomyces boulardii* S11, and *Kluyveromyces marxianus* TGM66 had additional benefits other than their flavor-enhancing and nutritional value-enhancing effects in foods. In this context, structural properties of yeast extracts were examined by Fourier Transform Infrared spectroscopy, and their thermal stability was determined by Differential Scanning Calorimeter. Additionally, surface morphologies of lyophilized yeast extract powders were examined through Scanning Electron Microscopy micrographs. As a result of these investigations, characterizations of three different yeast extracts were provided. It is thought that the Fourier Transform Infrared spectra of yeast extracts obtained from three different yeasts will contribute to the existing literature in terms of the widespread use of Fourier Transform Infrared spectroscopy in the production and identification of yeast extracts, considering that this method is easy, fast, and inexpensive. In addition, it is expected that the determination of the thermal stability of yeast extracts will contribute to the use strategies of yeast extracts in food systems. It is thought that determining the surface morphology of yeast extracts will contribute to the choice of method to be used in drying yeast extracts. In addition, in this study, the effects of extracts obtained from three different yeasts

on the rheological properties of vegetable soup, which was preferred as a model food, were investigated. The findings showed that all yeast extracts slightly increased the viscosity of the soup, but *Kluyveromyces marxianus* TGM66, especially at 50 °C, significantly increased the viscosity of the plain soup at both different concentrations. In light of all these findings, it can be said that the use of yeast extracts in appropriate concentrations, especially in foods that are generally consumed hot, such as soup, and where consistency is an important quality criterion, has the potential to contribute to the sensory properties of foods, such as viscosity. This situation, when considered together with the umami taste and meat-like flavoring properties of yeast extracts, may allow the production of more sensory successful foods by using yeast extracts in appropriate foods and at appropriate concentrations. However, in order to better understand the effects of yeast extracts on viscosity, the chemical profiles of yeast extracts, especially their carbohydrate profiles, need to be elucidated. Further studies to elucidate the effects of yeast extracts on viscosity in different food systems such as sauces as well as different types of soups may pave the way for the increased use of these nutritive additives in foods. In conclusion, with a better understanding of the structural, thermal, morphological, and rheological properties of yeast extracts, the production efficiency of the extracts can be increased and it may be possible to obtain more successful results by using these extracts in food systems for which they are suitable.

AUTHORSHIP CONTRIBUTIONS

F.D.: Conceptualization, Investigation, Methodology, Writing - Original Draft, Writing - Review & Editing. Ö.Ş.: Conceptualization, Investigation, Methodology, Writing - Review & Editing, Supervision. E.D.: Conceptualization, Writing - Review & Editing, Supervision. O.S.: Conceptualization, Writing - Review & Editing, Supervision.

DATA AVAILABILITY STATEMENT

The authors of the manuscript confirm that the data supporting the findings of this study are available in the manuscript. The raw data for the findings obtained in this study can be obtained from the corresponding author upon request.

CONFLICT OF INTEREST

All authors of the manuscript declare that they have no conflicts of interest resulting from the contents of this manuscript.

ETHICS

There is no ethical problem in publishing this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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