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Effects of Ultrasound Application on the Improvement of Probiotic Properties and Antioxidant Activity of *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* var. *boulardii* and *Saccharomyces cerevisiae*

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ABSTRACT

The objective of this study was to ascertain the impact of ultrasound application on the probiotic characteristics and antioxidant activity of yeasts. In this context, the pH changes, probiotic properties and antioxidant activities of *K. marxianus* (Km), *S. boulardii* (Sb) and *S. cerevisiae* (Sc) were determined by ultrasound application at different durations (5, 15, 30 and 60 minutes at 24 kHz). The lowest pH values were determined for cultures of Km (ultrasound non-applied *K. marxianus*), Sb-30 (30 min. ultrasound applied *S. boulardii*) and Sc-5 (5 min. ultrasound applied *S. cerevisiae*) as 4.48, 5.15 and 5.26, respectively. The hydrophobicity values of the yeast strains varied between 6% and 24%, increased with ultrasound application. Although *S. boulardii* had the highest tolerance to low pH and bile salts, the resistance of all yeast to low pH and bile salts decreased with ultrasound application. *K. marxianus* had the least survival under *in vitro* conditions, but ultrasound application increased survival of *K. marxianus* strains and slightly affected the survival rate of *S. boulardii* and *S. cerevisiae*. Increasing of duration time of ultrasound application resulted higher antioxidant activity and so the highest antioxidant activity was determined for Sb-60. Finally, ultrasound application could be used for the development of hydrophobicity and antioxidant properties of yeast cultures.

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Introduction

Live microorganisms that have positive effect on human health are defined as probiotics (Hotel & Cordoba, 2001). Microorganisms should have some major qualifications like aggregation and adhesion abilities, resistance to harsh conditions and non-pathogenic to be used as probiotic (Gut et al., 2018). Although most of the studies on probiotic microorganism are related to *Lactobacillus* and *Bifidobacterium* species (Holzapfel, 2006), some yeast species have also indicated probiotic properties including *Saccharomyces boulardii* (Tomičić et al., 2016), *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Torulaspora delbrueckii*, *Candida krusei*, *Yarrowia lipolytica*, *Pichia fermentase* and *Pichia kudriavzevii* (Kumura et al., 2004; Saber et al., 2017a; Saber et al., 2017b).

Yeasts are eukaryotic microorganisms that have probiotic properties and used as starter or co-cultures in food and biotechnology field and have varied utilization like enzyme industry, pharmaceutical and biotechnology (Sadeghi et al., 2022). Previous studies have demonstrated the probiotic properties of yeasts isolated from a variety of fermented foods, including kefir and cereal-based products (Goktas et al., 2021b; Greppi et al., 2017; Gut et al., 2019). *Saccharomyces cerevisiae* is used as the main fermentation starter culture in the production of alcoholic beverages such as bakery products, distilled spirits, fermented fruit juices and wine. *S. boulardii* is a close relative of *S. cerevisiae* and numerous products are present in the probiotic market containing *S. boulardii* strains as a probiotic agent (Goktas et al., 2021a).

Ultrasound is mostly described as voice of frequency above that to which the human lug be able to answer (Ojha et al., 2017). Ultrasound is applied in the fields of food technology, sonochemistry and medical diagnostic and expressed as green technology (Speranza et al., 2020). Ultrasound technology was firstly applied to investigation of its effect on killing cells in food applications, but in

recent years it has been applied on some biotechnological processes without affecting the cell (Speranza et al., 2020). Many studies have reported that low-intensity ultrasound technology creates repairable harm to cells, alters their viability, and as a result accelerates their proliferation and metabolism (Speranza et al., 2020).

A number of studies have been conducted which examine the effect of ultrasound technology on the properties of yeasts. For example, Lanchun et al., (2003) and Borah et al., (2019) have demonstrated that ultrasound technology has the capacity to enhance the growth characteristics and fermentation kinetics of *S. cerevisiae*. In a separate study, Al Daccache et al., (2020) demonstrated that ultrasound technology reduced the fermentation time of wine and beer and increased ethanol yield. Additionally, Speranza et al., (2020) indicated that the utilisation of ultrasound enhanced the technological attributes of *S. cerevisiae*. Similarly, effect of ultrasound technology on some probiotic properties of lactic acid bacteria was investigated. Ultrasound has promoted the growth of two different strains of *L. plantarum* and improved bioactive properties during fermentation (Hashemi and Gholamhosseinpour, 2020), increased the growth of *L. casei* strains and positively affected the probiotic properties (Yeo & Liong, 2013), and also hydrophobicity and adhesion to Caco-2 cells of *L. reuteri* (Racioppo et al., 2017). The effects of ultrasound application on microorganisms are associated with the formation of pores in the cell wall, an increase in extracellular metabolites and a disruption of protein structures.

The yeast cell wall is a rich source of β-glucan. Furthermore, the yeast cell wall contains a number of antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and catalase. The antioxidant activity of yeast is attributable to these components (Goktas et al., 2021b). Gholamhosseinpour & Hashemi (2019) reported that the application of ultrasound during the process of milk fermentation resulted in an increase in antioxidant activity. In a separate study, Hashemi & Gholamhosseinpour (2020) demonstrated that the application of ultrasound technology enhanced the bioactive properties of goat milk.

Although various studies reported the effect of ultrasound technology on the probiotic properties of lactic acid bacteria, limited number of studies reported for yeast species. So, in this study, the effects of ultrasound treatment on auto-aggregation, hydrophobicity, resistance to low pH, bile salts and *in vitro* conditions, resistance to some fungal antibiotic agents and antioxidant activity of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were investigated. The objective of this study was to investigate the effect of ultrasound treatment on the probiotic properties and antioxidant activity of yeasts.

Material and methods

Yeast Species and Applying Ultrasound Treatment

In this study, *S. boulardii* S10, *K. marxianus* KY-2 and *S. cerevisiae* KY-15 strains, which were isolated in our previous studies and had the best probiotic properties, were selected to determine the effect of ultrasound treatment on their probiotic properties (Goktas et al., 2021a; Goktas et al., 2021b). Yeast extract peptone dextrose (YPD) Broth medium (yeast extract 10 g/L, peptone 20 g/L and dextrose 20 g/L, Difco, USA) was used to propagate yeast cultures. Overnight yeast cultures were inoculated into fresh YPD broth medium at a rate of 1% to apply ultrasound treatment. YPD broth medium inoculated with yeast cultures were placed in an ultrasonic water bath. Ultrasound treatment was performed at 24 kHz and four different times, 5, 15, 30 and 60 minutes. Ultrasound-treated yeast cultures were incubated at 30°C for 24 h and pellets were collected by centrifugation at 7000 rpm for 15 minutes at 4°C. Finally, stock cultures were created by adding 20% glycerol and stored at -80°C until used in probiotic and antioxidant analyses. The following abbreviations are employed in the Results and Discussion sections of the article: Km: Ultrasound non-applied *K. Marxianus*, Km-5: 5 min. Ultrasound applied *K. Marxianus*, Km-15: 15 min. Ultrasound applied *K. Marxianus*, Km-30: 30 min. Ultrasound applied *K. Marxianus*, Km-60: 60 min. Ultrasound applied *K. Marxianus*, Sb: Ultrasound non-applied *S. boulardii*, Sb-5: 5 min. Ultrasound applied *S. boulardii*, Sb-15: 15 min. Ultrasound applied *S. boulardii*, Sb-30: 30 min. Ultrasound applied *S. boulardii*, Sb-60: 60 min. Ultrasound applied *S. boulardii*, Sc: Ultrasound non-applied *S. cerevisiae,* Sc-5: 5 min. Ultrasound applied *S. cerevisiae*, Sc-15: 15 min. Ultrasound applied *S. cerevisiae*, Sc-30: 30 min. Ultrasound applied *S. cerevisiae*, Sc-60: 60 min. Ultrasound applied *S. cerevisiae*.

Determination of the Growth Curves and pH Changes During Incubation Period

The growth curves and pH changes of yeast cultures were determined during the 24 h incubation period at 30ºC. Briefly, the optical density (OD_{600}) values of the twice-activated yeast cultures were set to 1.0 and inoculated at 2% in 100 mL of YPD broth medium. Absorbance values of the cultures were measured (Genesys 10S UV-Vis, Thermo Scientific, USA) at 600 nm by taking 5 mL of the relevant medium at 4-hour intervals. Also, pH values were measured by using a pH meter at 22°C. The growth graphs and pH changes graphs of the cultures were created using the absorbance values and pH values against incubation time, respectively.

Auto-aggregation Profile

Auto-aggregation profile of the yeast cultures was performed on the Unban et al., (2021) methodology, with slightly modifications. Overnight cultures were centrifuged at 4 °C and 7000 rpm for 10 min, washed twice with Phosphate buffered saline (PBS) and 10 mL of the PBS were added to pellets. The mixtures were homogeneously vortexed and 1 mL of the cell mixture was measured by spectrophotometer at 600 nm (A_0). Then, the mixtures were incubated without shaking for 24 h at 37 °C and absorbance values of the mixture's upper phase was measured for 2, 4 and 24 h (A_t). Using following equation auto-aggregation percentage of the yeast cultures was determined:

$$\% = [(A_0-A_t)/A_0]\times 100$$

where A_0 is the absorbance at 0 h, and A_t is the absorbance at 2, 4 and 24 h.

Hydrophobicity Profile

Hydrophobicity profile of the yeast cultures was determined with regard to adhesion to hydrocarbons, with a method presented by Vinderola & Reinheimer (2003). Overnight yeast cultures were centrifuged at the above centrifugal condition, washed twice with 50 mM K_2HPO_4 and suspended with same solution. Absorbance values of the cell suspension were set to 1.0 at 600 nm and then absorbance values of the suspension were recorded at 560 nm (A_1). Three mL of the cell suspension were mixed with 0.6 mL *n*-hexadecane, vortexed for 2 min. and incubated at 37 C for 20 min. After the incubation, upper aqueous phase carefully taken out and absorbance values were recorded at 560 nm (A_2). Hydrophobicity profile was calculated with following mathematical expression:

Hydrophobicity (%) = $[(A_1-A_2)/A_1]\times100$.

Resistance to Low pH and Bile Salts

To determine resistance to low pH previously defined methodology was used with some modifications (Argyri et al., 2013). Briefly, overnight yeast cultures were centrifuged at above centrifugation conditions, washed twice with PBS and resulting pellet resuspended into PBS. The suspension was adjusted to 1.0 at OD_{600} nm. 0.1 mL of the suspension was inoculated into 10 mL of PBS medium adjusted to pH 2 using 1M hydrochloric acid, vortexed and incubated for 3 h at 37 °C. The number of viable cells was counted on YPD agar medium after incubated at 30°C for 48 h and results were expressed as log Colony Forming Unit/mL (CFU/mL). An inoculation was created from OD_{600} adjusted cultures on YPD agar as mentioned above for forming control groups.

Resistance to bile salts of yeast cultures was determined with a method (García-Hernández et al., 2016). 0.1 mL of the OD_{600} adjusted yeast cultures were inoculated to 10 mL of the PBS containing 0.3% bile salts, vortexed and incubated for 3 h at 37 °C. Control groups were created as mentioned above and all cultures were inoculated on YPD agar, incubated at 30°C for 48 h. Finally, the number of viable cells was expressed as log CFU/mL.

Resistance to Fungal Antibiotic Agents

Antibiotic resistance of yeast cultures against some antibiotics were determined with a method specified by Goktas et al., (2021b). OD_{600} values of activated yeast cultures were adjusted to 1.0 at 600 nm and 100 uL of the each culture was spread on the YPD agar. Amphotericin B (AMB, 20 mcg), clotrimazole (CLT, 10 mcg), flucanazole (FLU, 25 mcg), ketoconazole (KTC, 10 mcg), nystatin (NY, 100 U) (Bioanalyse, Türkiye) were placed on the agar medium containing yeast cultures. After the incubation at 37°C for 24 h, formed zone areas measured and results were expressed as in mm.

Resistance to In vitro Conditions

Resistance to *in vitro* conditions of yeast cultures was determined as defined by Minekus et al., (2014). An overnight yeast cultures were centrifuged at above centrifugation conditions, washed twice with PBS and the OD_{600} of the suspension was set to 1.0 with a final volume of 10 mL. 0.1 mL of the this suspension was spread on YPD agar to represent the control group, incubated at 30°C for 48 h and counted. Further steps of resistance to *in vitro* conditions were performed as in the Figure 1. Furthermore, the survival rates of the yeast cultures were calculated in accordance with the specified equations:

$$GS = \frac{(\text{Control log CFU/mL}) - \text{Gastric log CFU/mL})}{\text{Control log CFU/mL}} \times 100$$

$$PS = \frac{(\text{Gastric log CFU/mL}) - \text{Pancreatic log CFU/mL})}{\text{Gastric log CFU/mL}} \times 100$$

$$OS = \frac{(\text{Control log CFU/mL}) - \text{Pancreatic log CFU/mL})}{\text{Control log CFU/mL}} \times 100$$

GS : Gastric Survival (%)
PS : Pancreatic Survival (%)
OS : Overall Survival (%)

Antioxidant Activity

Antioxidant activity of the yeast cell free supernatant was determined according to the method described by Unban et al., (2021) with some modifications. Activated yeast cultures were centrifuged at above centrifugation procedure and supernatants carefully collected with a syringe and filtered through a 0.45 um filters and 0.4 ml of the supernatant was transferred to into new tubes. 0.8 mL of the freshly prepared 0.2 mM of DPPH solution (1,1-diphenyl-2-picrylhydrazyl) in 80% methanol was mixed with filtered supernatant, vortexed for 30 s and incubated at room temperature in darkness for 20 min. Absorbance values of the mixes were recorded at 517 nm (A_2). YPD broth without inoculation of yeast cultures was used as control (A_1). Antioxidant activity of the yeast cultures was determined with following mathematical model:

Antioxidant activity (%) = $[(A_1-A_2)/A_1]\times100$

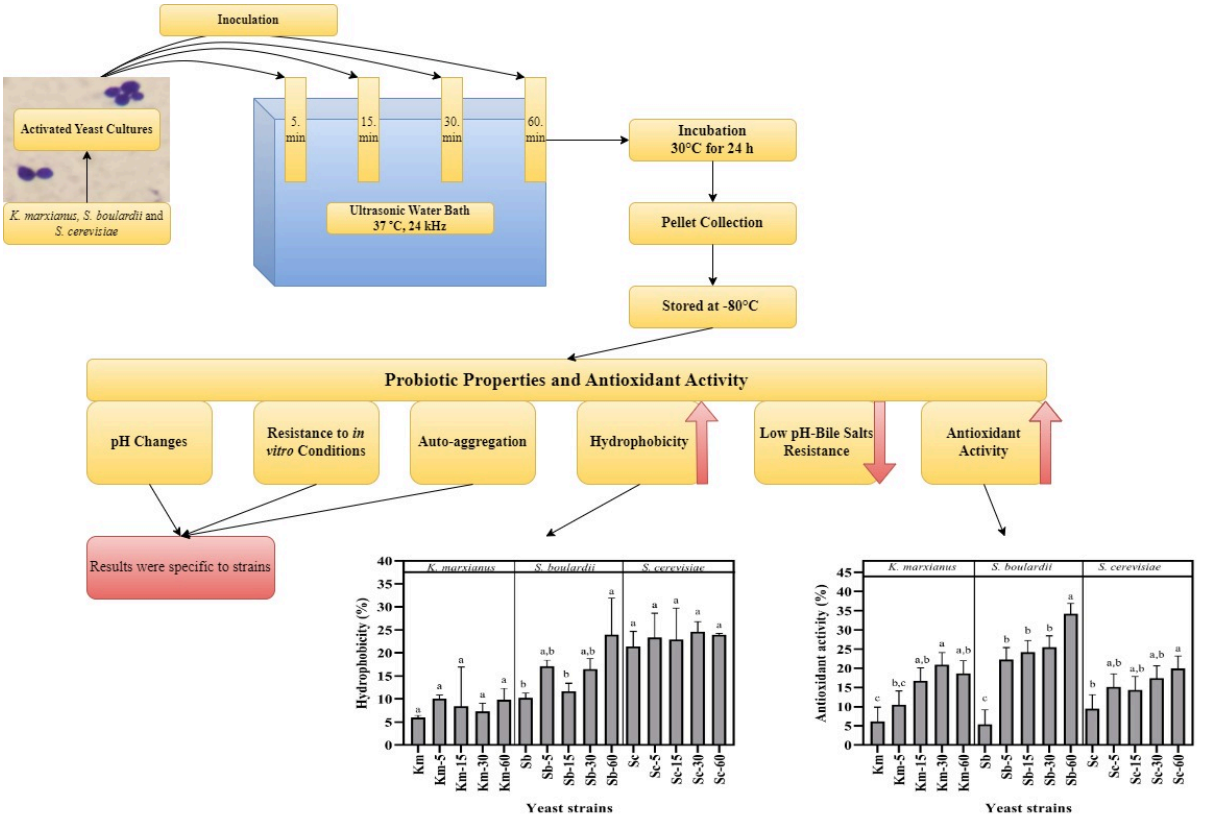
Statistical Analyses

All analyses were carried out duplicate and results were expressed as mean plus standart deviation. Statistical differences were determined with Student's t test at 95% importance level by using statistical programme of JMP software.

Results and Discussion

Growth Curves and pH Changes

Growth curves of ultrasound applied and non-applied strains of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were presented in the Figure 2. Generally, strains showed similar growth curves during incubation period. Km-5 showed slowly growth compared to control *K. marxianus* strain during incubation period and the growth curve of Km-5 remained under control strain. Similarly, Sc-5 and Sc-30 showed slower growth than the control *S. cerevisiae* strain. Also, pH changes of ultrasound applied and non-applied strains of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were determined during incubation period (Table 1). There was no significant change in pH values in the first inoculation of yeast strains into the growth medium. At the end of the 24-hour incubation, the lowest pH values were determined as 4.48, 5.15 and 5.26 for Km, Sb-30 and Sc-5, respectively.



Graphical Abstract

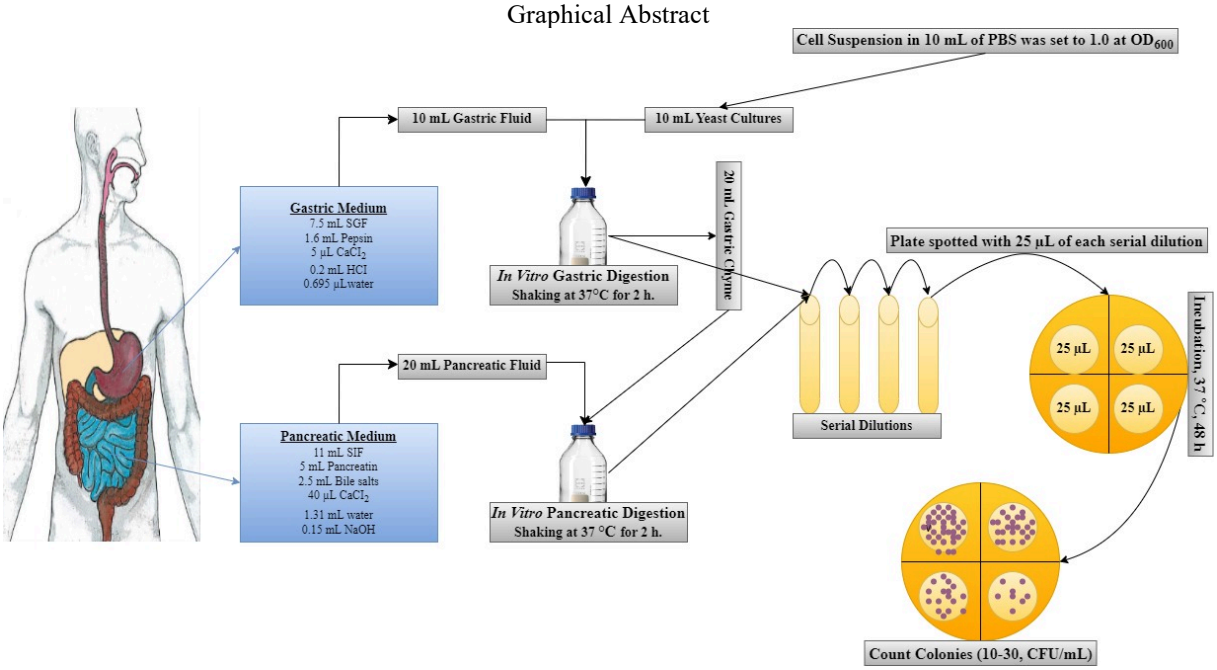


Figure 1. Schematic representation of determination of *in vitro* resistance of yeast strains.

Ultrasound application caused different pH changes for *K. marxianus*, *S. boulardii* and *S. cerevisiae*. At the end of the incubation, the pH values of Km-5, Km-15, Km-30 and Km-60 were found to be higher than Km and the highest pH value was determined for Km-5 due to the showed slower growth as seen in the growth curve. However, ultrasound non-applied strain of *S. boulardii* (Sb) had the higher pH value than ultrasound applied strains of *S. boulardii* (Sb-5, Sb-15, Sb-30 and Sb-60) and ultrasound application resulted lower pH values for *S. boulardii* strains. As stated above, a regular increase or decrease for pH values could not be determined for *S. cerevisiae*. The pH value of Sc-60 was higher than Sc, while the pH value of Sc-5 was found to be lower. This showed that ultrasound application caused different pH changes for these three yeasts at the end of incubation.

Auto-Aggregation

Auto-aggregation is one of the desirable probiotic properties in probiotic selection, as it reflects adhesion to the intestinal tract of the host. Auto-aggregation profile of the ultrasound applied and non-applied strains of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were presented in the Figure 3.

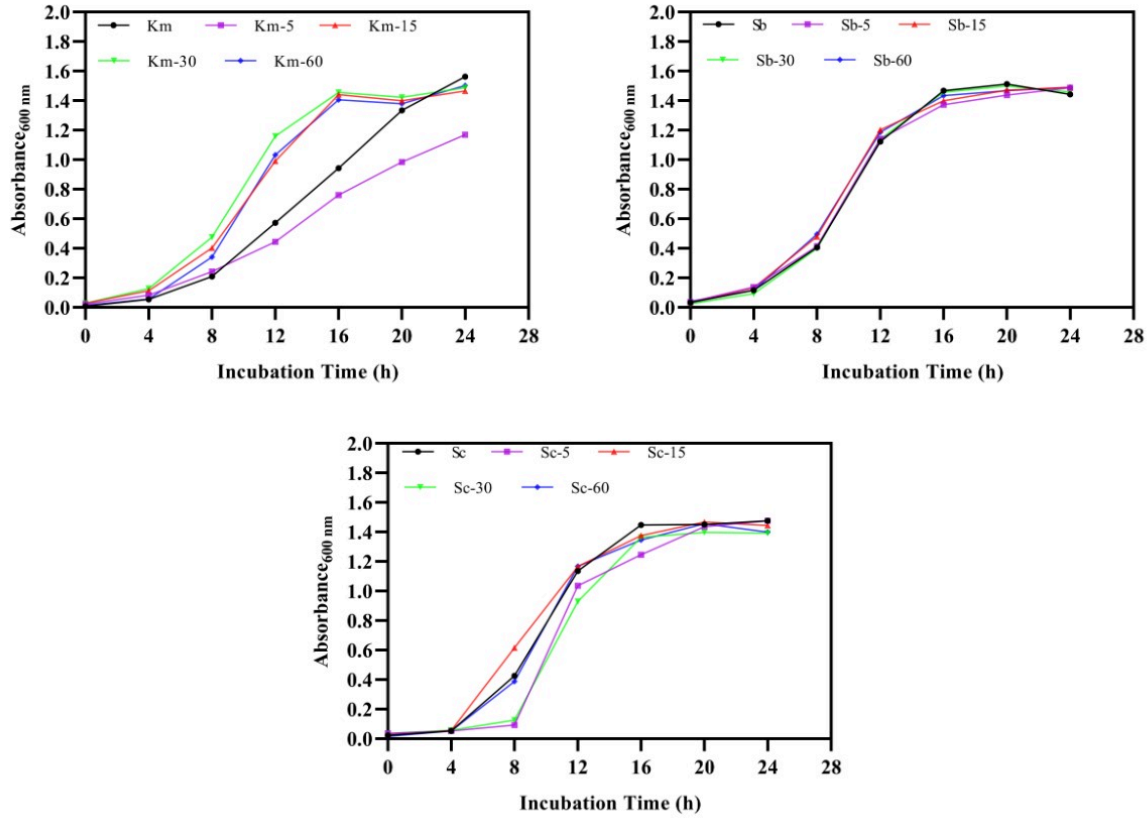


Figure 2. Growth curves of ultrasound applied and non-applied yeast strains

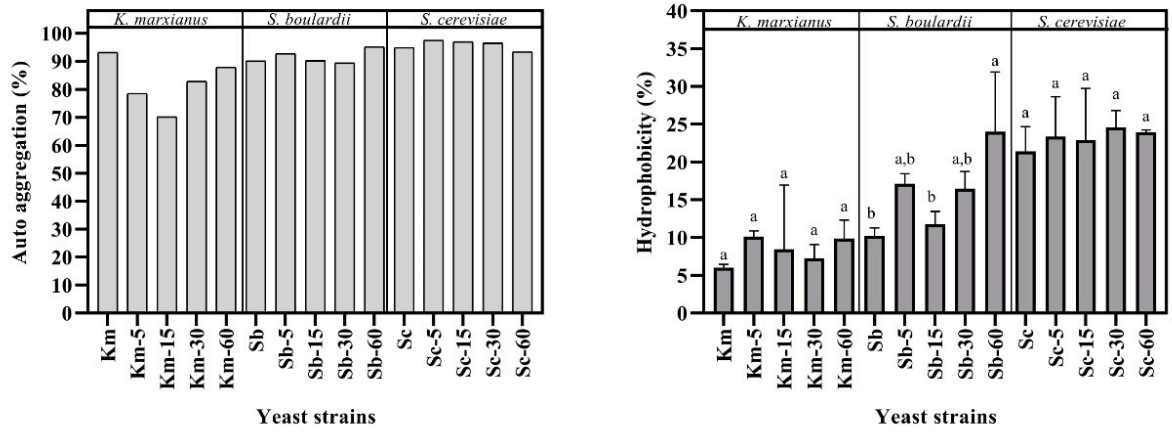


Figure 3. Auto aggregation profile and Hydrophobicity of ultrasound applied and non-applied yeast strains.

All yeast genus of ultrasound non-applied have shown a great auto aggregation profile more than 90% for 2h. However, ultrasound application has revealed different auto-aggregation profile for yeast strains. Auto-aggregation profile of the *K. marxianus* strains decreased with ultrasound application compared to control. The auto-aggregation profile of *S. boulardii* strains increased with 5 and 60 minutes of ultrasound treatment, but decreased with 15 and 30 minutes of ultrasound treatment and was higher than the control. Auto-aggregation profiles of *S. cerevisiae* strains increased with 5, 15 and 30 min ultrasound treatment but slightly decreased with 60 min ultrasound treatment. Ultrasound application created different auto-aggregation profiles for different yeasts. Especially for *S. boulardii* and *S. cerevisiae*, 5 min. ultrasound application increased the auto-aggregation profiles of the strains. 5 min. ultrasound application reduced the auto-aggregation profiles of *K. marxianus* strains. It was reported that ultrasound application affected the auto-aggregation profile of some *S. cerevisiae* strains, while not affected some strains (Speranza et al., 2020). Giordano & Mauriello (2023) stated that 6 and 8 minutes ultrasound application was reduced the auto-aggregation ability of *L. casei* strains by 20 fold. It is possible that ultrasound has affected the cell membrane of yeasts, resulting in alterations to their aggregation properties. These findings revealed that the application of ultrasound may affect the auto-aggregation profiles of different microorganisms at different levels and the applied time should be taken into account, and also ultrasound application may be specific to the microorganism.

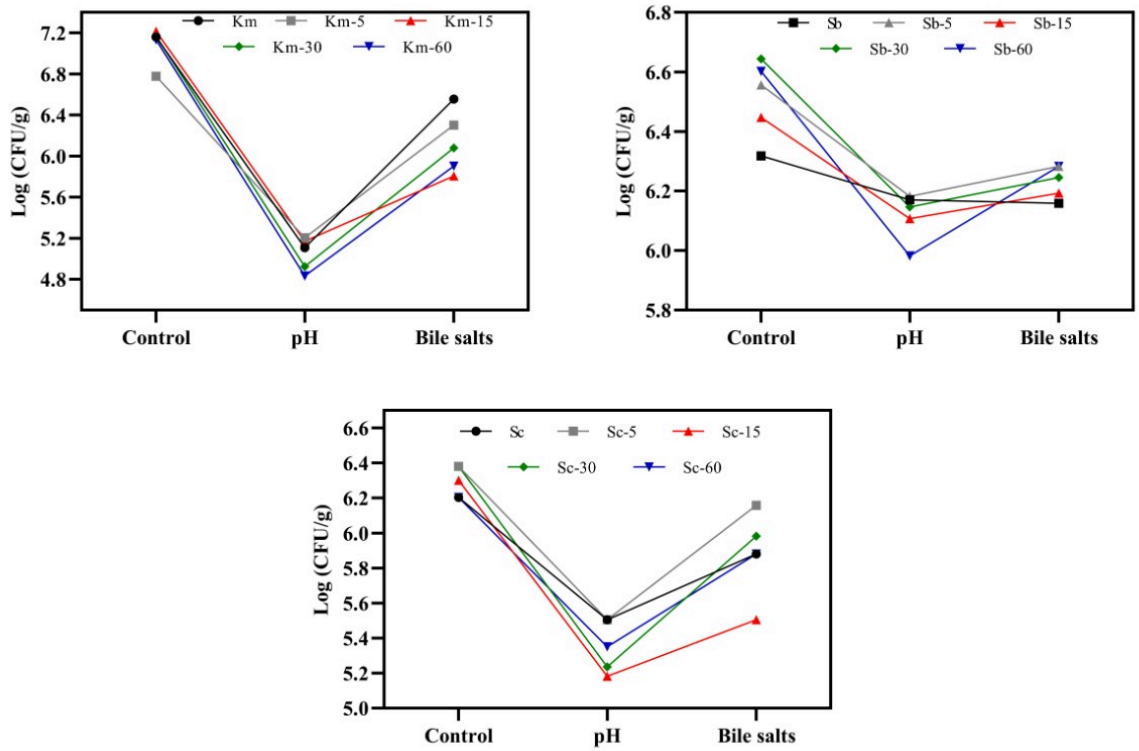


Figure 4. Resistance to low pH and Bile salts conditions of ultrasound applied and non-applied yeast strains.

Hydrophobicity

Hydrophobicity is another *in vitro* probiotic property that reflects the ability of microorganisms to adhere to the intestinal mucosa. As seen in the Figure 3, the hydrophobicity values of the yeast strains varied between 6% and 24%. The lowest hydrophobicity values among all yeast strains were determined for *K. marxianus* and the values were below 10%. However, different hydrophobicity values were recorded for *S. boulardii* strains and the values varied between 10% and 24%. The hydrophobicity values of *S. cerevisiae* strains were found to be over 20% and the highest values were determined for *S. cerevisiae* strains, except *S. boulardii* strain, which was treated ultrasound for 60 min. Ultrasound treatment affected the hydrophobicity values of the yeast strains and the hydrophobicity values for all applied times increased in all three yeast strains compared to the control. 5 and 60 minutes of ultrasound treatment for *K. marxianus* and *S. boulardii* increased the hydrophobicity values of these two yeast strains more than 1.5 fold. Although ultrasound treatment increased the hydrophobicity values of *S. cerevisiae* strains, this increase was not as high as in *K. marxianus* and *S. boulardii*, and was a more limited increase recorded. It has been demonstrated that ultrasound has an impact on the cell membrane of microorganisms (Yeo & Liong, 2013). Consequently, the application of ultrasound caused in a modification of the yeast cell membranes, which in turn led to alterations in the hydrophobic properties. It has been stated in the literature that ultrasound application increased the hydrophobicity values of *L. casei* (Giordano & Mauriello, 2023), *P. freudenreichii* and *A. jensenii* (Bevilacqua et al., 2019) and *L. reuteri* (Racioppo et al., 2017), decreased the hydrophobicity value of *L. plantarum*, but had no effect on *Bifidobacterium* strains (Racioppo et al., 2017). Physical processes or chemical substances can affect the hydrophobicity properties of microorganisms (Racioppo et al., 2017) and ultrasound application affected the hydrophobicity values of microorganisms at different levels, both as in our study and as reported in the literature, and this difference also changed according to the strain.

Resistance to Low pH and Bile Salts

A probiotic candidate must be live at low pH before arriving the gut to offer its healthy effects. During digestion, stomach acidity can decrease to values at pH 1.5, so probiotic viability is important (Yeo & Liong, 2013). At low pH and bile salts conditions, viability of the ultrasound applied and non-applied yeast strains were affected and a decreasing was observed for all strains viability (Figure 4). Increasing of ultrasound application time resulted higher decrease in the viability of all yeast strains at low pH and bile salts conditions compared to control, except Km-5. The least viability was determined for *K. marxianus* strains and increasing of ultrasound application time resulted approximately 2 log units decrease in viability of *K. marxianus* strains, except Km-5. For strains of the Km, Km-5, Km-15, Km-30 and Km-60, a decrease of 2.05, 1.57, 2.04, 2.24 and 2.30 log units occurred, respectively, at pH=2. Increasing ultrasound application time similarly resulted decrease in the viability of *S. cerevisiae* and *S. boulardii* strains and approximately 1 log unit decrease was determined for viability of *S. cerevisiae* strains at pH 2, while less than 1 log unit decrease was determined for viability of *S. boulardii* strains. And so, *S. boulardii* strains were the most resistant to low pH among these three yeasts.

Similarly, a decrease in the viability of ultrasound applied and non-applied yeast strains were detected in the bile salts conditions and increasing duration of ultrasound application resulted decreasing viability of yeast strains.

For viability of *K. marxianus* strains approximately 1 log unit decrease was determined. However, viability of *S. boulardii* and *S. cerevisiae* were better than *K. marxianus* strains in the bile salts conditions and less than 1 log unit decrease was determined for viability of both two yeasts. In summary, the application of ultrasound affected the low pH and bile salt tolerance of the yeasts, and increasing the duration of ultrasound application resulted decreasing in the viability of the yeast strains.

Racioppo et al., (2017) reported that ultrasound application affected the viability of *L. reuteri*, *L. plantarum* and *B. infantis* at different levels: viability of *L. reuteri* was not affected by low pH and bile salts, approximately 4 log units decrease was determined for viability of *L. plantarum* and the viability of *B. infantis* was affected only in the bile salts. In another study, it was reported that *L. casei* strains treated with ultrasound showed better tolerance to acidic conditions and bile salts compared to control (Yeo & Liong, 2013). There are few studies showing the effect of ultrasound application on the viability of microorganisms in acidic conditions and bile salts, but it has been reported that physical processes such as ultrasound application may affect the viability of microorganisms at different levels (Racioppo et al., 2017).

Viability under In vitro Conditions

Enzymes and salts in the gastric and pancreatic environment significantly affect the viability of probiotic microorganisms. Therefore, in order to obtain the desired health benefits from probiotics, their viability in the digestive environment must be at the highest level, and in this regard, viability *in vitro* conditions is one of the most desirable features in the selection of probiotic microorganisms. Viability under *in vitro* conditions of the ultrasound applied and non-applied strains of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were detailed in the Table 1. All strains were showed great viability to gastric conditions and their survivability were more than 92%. Ultrasound application positively affected the viability of the strains to gastric conditions and an increase in the survivability of the strains was observed. Different viability levels were detected for yeast strains to pancreatic conditions. Under pancreatic conditions, the lowest viability rate was observed in the Km strain (71.67%), followed by the Km-5 strain (83.31%). The viability of the other strains under pancreatic conditions was over 94%, and so the application of ultrasound importantly increased the viability of *K. marxinaus* under pancreatic conditions. However, the viability of *S. boulardii* and *S. cerevisiae* in pancreatic conditions was not significantly affected by ultrasound application, and slightly decreases or increases were detected depending on the duration of ultrasound application. The overall viability of yeast strains was similar to pancreatic conditions, and the yeast species most affected by ultrasound treatment was *K. marxianus*.

Antibiotic Susceptibility

Antibiotic administration is important, as maintaining the host's microflora balance may cause intestinal disorders. Therefore, antibiotic resistance of probiotics ensures the preservation of healthy intestinal microbiota (Gotcheva et al., 2002). Yeasts are naturally resistant to bacterial antibiotics and can be applied in the treatment of antibiotic-related diseases. In addition, the resistance of yeasts to fungal antibiotics should be revealed. In this study, resistance to fungal antibiotics was determined for yeast strains that were ultrasound applied and non-applied. All strains were sensitivity against tested antibiotics (Table 1).

The smallest and highest zone diameters against clotrimazole, flucanazole, amphotericin B, ketoconazole and nystatin for ultrasound applied and non-applied strains of *K. marxianus*, *S. boulardii* and *S. cerevisiae* were measured as follows, respectively: for *K. marxianus*: 9.5-12.5, 18.0-20.0, 7.0-7.5, 22.0-24.0 and 17.0. -18.5 mm, for *S. boulardii*, 13.5-16.0, 16.0-20.0, 7.5-7.5, 21.5-22.5 and 16.0-19.0 mm and for *S. cerevisiae*, 17.0-22.0, 21.0-28.0, 7.0-8.0, 22.5-28.0 and 16.5-17.5 mm. Ultrasound application for *K. marxianus* did not have a significant effect on measured zone diameters of antibiotics compared to control ($P>0.05$). However, higher zone diameters were measured for *S. boulardii* strains, which were longer ultrasound applied, against clotrimazole and nystatin, and significant differences were detected compared to control ($P\leq0.05$). As mentioned above, higher zone diameters were measured for *S. cerevisiae* strains, which were longer ultrasound applied, against only ketoconazole, but for Sc-5 smaller zone diameters were measured against clotrimazole and fluconazole. In a study Bevilacqua et al., (2019), it was reported that ultrasound application significantly affected the minimum inhibition concentration of *A. jensenii* against gentamicin and *P. freudenreichii* against gentamicin and erythromycin, and a lower MIC concentration was measured. However, no significant change was reported against other tested antibiotics. Differences in the measured zone diameters may be related to their different behavior under stress conditions because ultrasound stresses the yeasts, or to their growth in agar medium.

Antioxidant Activity

Antioxidant activity of the yeast cultures was presented in the Figure 5. Antioxidant activity of the yeast cultures changed between 5% and 34% and the highest and lowest values were determined for Sb and Sb-60 strains of *S. boulardii*, respectively.

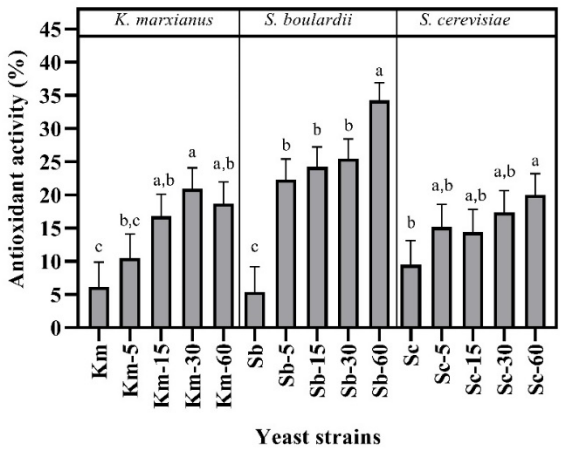


Figure 5. Antioxidant activity profile of ultrasound applied and non-applied yeast strains.

Table 1. pH changes, Survival under *in vitro* conditions and Antibiotic susceptibility of ultrasound applied and non-applied yeast strains.

Yeast Strains	pH Changes During Incubation Period (h)						
	0.	4.	8.	12.	16.	20.	24.
Km	6.22	6.08	6.02	5.92	5.41	4.74	4.48
Km-5	6.20	6.17	6.03	5.94	5.77	5.66	5.56
Km-15	6.22	6.13	5.84	5.55	5.49	5.38	5.33
Km-30	6.27	6.11	5.73	5.48	5.29	5.28	5.25
Km-60	6.31	5.96	5.85	5.54	5.46	5.44	5.30
Sb	6.16	6.04	5.86	5.47	5.42	5.41	5.39
Sb-5	6.24	6.12	5.80	5.44	5.37	5.37	5.35
Sb-15	6.11	6.02	5.76	5.42	5.41	5.33	5.17
Sb-30	6.13	6.02	5.84	5.47	5.44	5.35	5.15
Sb-60	6.14	6.03	5.76	5.45	5.43	5.34	5.21
Sc	6.28	5.93	5.84	5.54	5.50	5.46	5.32
Sc-5	6.28	5.86	5.79	5.61	5.56	5.51	5.26
Sc-15	6.29	5.87	5.80	5.60	5.59	5.41	5.35
Sc-30	6.28	5.91	5.58	5.57	5.56	5.56	5.33
Sc-60	6.02	5.91	5.61	5.59	5.56	5.53	5.47

Yeast Strains	Survival Under *in vitro* Conditions (%)			Antibiotic susceptibility (mm)				
	Gastric Survival	Pancreatic Survival	Overall Survival	CLT	FLU	AMB	KTC	NY
Km	92.53	71.67	66.32	11.0±1.4^{a,b}	18.5±0.7^{a}	7.5±0.7^{a}	24.0±1.4^{a}	18.5±0.7^{a}
Km-5	97.90	82.31	80.58	10.0±1.4^{a,b}	19.0±1.4^{a}	7.0±1.4^{a}	24.0±2.8^{a}	17.5±0.7^{a}
Km-15	99.78	96.15	95.95	9.5±0.7^{b}	18.0±1.4^{a}	7.5±0.7^{a}	22.0±1.4^{a}	17.5±2.1^{a}
Km-30	98.24	95.69	94.01	9.5±0.7^{b}	19.0±1.4^{a}	7.5±0.7^{a}	22.0±0.0^{a}	17.0±0.0^{a}
Km-60	99.79	95.41	95.95	12.5±0.7^{a}	20.0±2.8^{a}	7.5±0.7^{a}	23.0±2.8^{a}	18.0±1.4^{a}
Sb	96.44	99.54	95.99	13.5±0.7^{b}	16.0±0.0^{a}	7.5±0.7^{a}	22.5±0.7^{a}	16.0±0.0^{b}
Sb-5	98.22	96.66	94.95	14.5±2.1^{a,b}	18.5±0.7^{a}	7.5±0.7^{a}	22.0±2.8^{a}	17.0±0.0^{a,b}
Sb-15	97.18	96.73	94.01	16.5±0.7^{a,b}	20.0±2.8^{a}	7.5±0.7^{a}	21.5±2.1^{a}	18.0±1.4^{a,b}
Sb-30	98.93	96.43	95.40	17.5±2.1^{a}	16.0±0.0^{a}	7.5±0.7^{a}	21.5±0.7^{a}	16.5±0.7^{b}
Sb-60	98.22	96.92	95.19	16.0±0.0^{a,b}	16.5±2.1^{a}	7.5±0.7^{a}	21.5±0.7^{a}	19.0±1.4^{a}
Sc	98.87	95.78	94.70	20.0±0.0^{b}	25.5±0.7^{a}	7.5±0.7^{a,b}	22.5±0.7^{b}	16.5±0.7^{a}
Sc-5	99.12	94.49	93.66	17.0±0.0^{c}	21.0±1.4^{b}	8.0±0.0^{a}	26.5±2.1^{a}	17.0±1.4^{a}
Sc-15	96.04	95.51	91.72	22.0±0.0^{a}	25.0±0.0^{a}	8.0±0.0^{a}	26.5±0.7^{a}	17.0±1.4^{a}
Sc-30	99.54	96.12	95.68	20.0±1.4^{b}	25.5±0.7^{a}	7.0±0.0^{b}	25.5±0.7^{a}	17.5±0.7^{a}
Sc-60	98.64	95.27	93.98	20.0±1.4^{b}	28.0±2.8^{a}	8.0±0.0^{a}	28.0±0.0^{a}	17.5±0.7^{a}

Km: Ultrasound non-applied *K. marxianus*, Km-5: 5 min. Ultrasound applied *K. marxianus*, Km-15: 15 min. Ultrasound applied *K. marxianus*, Km-30: 30 min. Ultrasound applied *K. marxianus*, Km-60: 60 min. Ultrasound applied *K. marxianus*, Sb: Ultrasound non-applied *S. boulardii*, Sb-5: 5 min. Ultrasound applied *S. boulardii*, Sb-15: 15 min. Ultrasound applied *S. boulardii*, Sb-30: 30 min. Ultrasound applied *S. boulardii*, Sb-60: 60 min. Ultrasound applied *S. boulardii*, Sc: Ultrasound non-applied *S. cerevisiae,* Sc-5: 5 min. Ultrasound applied *S. cerevisiae*, Sc-15: 15 min. Ultrasound applied *S. cerevisiae*, Sc-30: 30 min. Ultrasound applied *S. cerevisiae*, Sc-60: 60 min. Ultrasound applied *S. cerevisiae*. CLT: Clotrimazole, FLU: Flucanazole, AMB: Amphotericin B, KTC: Ketoconazole, NY: Nystatin. Different letters in the same column show statistical difference (P≤0.05).

The antioxidant activity values of Km, Sb and Sc strains, which were ultrasound non-applied, were determined as 6%, 5% and 9%, respectively, and *K. marxianus* and *S. cerevisiae* showed higher antioxidant activity than *S. boulardii*. However, ultrasound treatment significantly increased the antioxidant activity of yeast strains and thus the highest antioxidant percentage was determined for *S. boulardii* strain. Antioxidant activities of the ultrasound applied strains were more than 10% and ultrasound treatment increased the antioxidant values of the strains perfectly when compared to the control strains. In addition, increasing of the ultrasound application time resulted higher antioxidant activity of the strains and Sb-60 and Sc-60, which applied ultrasound for the longest time, had the highest antioxidant activity, except Km-60. The increase in antioxidant activities of ultrasound applied strains was found to be significant for all ultrasound applied *S. boulardii* strains (P≤0.05). However, this increase was found to be significant for *S. cerevisiae* only in Sb-60, and for *K. marxianus* it was significant for other strains except Km-5 (P≤0.05).

Antioxidant activity of yeasts is associated with the high amount of β-glucan in their cell walls and some enzymes such as superoxide dismutase, which show antioxidant activity (Goktas et al., 2021b). Changes in antioxidant activity of yeast strains may depend on ultrasound processing parameters (power, frequency and process time) or strain-related parameters (differences in cell wall composition, thickness and size). Additionally, ultrasound applied products may release more bioactive compounds (Gholamhosseinpour & Hashemi, 2019).

Conclusion

Ultrasound application has recently gained importance in many other fields, especially in biotechnology applications in the food industry. In this study, the effects of ultrasound application on probiotic and antioxidant

properties of *K. marxianus* and *S. cerevisiae*, which are important yeast in food fermentation, and *S. boulardii*, which is unique probiotic yeast, were investigated. The findings showed that ultrasound application can be used to improve some probiotic and antioxidant properties of yeast strains. The ultrasound treatment resulted in an increase in the hydrophobicity percentage of yeast cultures, enhanced resistance to in vitro conditions, and an increase in antioxidant properties. However, it is important to reveal the effects of ultrasound at different times and powers in future studies. In addition effect of ultrasound application on fermentation conditions should be investigated, and so can be eliminated the deficiencies in the field of food applications.

Declarations

Author Contributions

Hamza Goktas: Project administration. Demet Turali: Methodology. Cansu Agan: Investigation. Osman Sagdic: Supervision.

Conflict of Interest

The writers inform no conflict of interest.

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Availability of Data

The data that assist the results of this work provides from the corresponding writer upon acceptable demand.

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