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Determining some of the quality characteristics of probiotic yogurts manufactured by using microencapsulated *Saccharomyces cerevisiae* var. *boulardii*

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Abstract: Probiotics can be defined as living microorganisms that positively contribute to human health by regulating the intestinal flora of a host and stimulating the immune system. As stated in many studies carried out in recent years, probiotic microorganisms are known to be very useful for human health. *Saccharomyces cerevisiae* var. *boulardii* is the only probiotic yeast that has been patented. In this study, it was aimed to determine the physical, rheological, chemical, and microbiological quality parameters of probiotic yogurts manufactured using this yeast microencapsulated via extrusion method during storage. Black cherry jam was used in order to enrich the sensory properties of products and the products were stored for 21 days. Microbiological characteristics such as total mesophilic aerobic bacteria, total coliform, total lactic acid bacteria, total yeast-mold, and viability of the *S. boulardii* were determined by analyzing the samples. We also investigated sensory properties such as external appearance, consistency (with spoon and by mouth), odor, flavor, and overall acceptability throughout the storage to evaluate the acceptability of the product by consumers.

Key words: Microencapsulation, probiotic, *S. boulardii*, yogurt

1. Introduction

The potential properties of yeasts are not generally taken into consideration, although they play a role as a complement to the microflora in most dairy products (1). The yeasts used for probiotic purposes by humans have been rather limited and have only been used in the past for feeding livestock. *Saccharomyces cerevisiae* var. *boulardii* (or *Saccharomyces boulardii*), having probiotic effects is generally used for feeding cattle, pigs, and poultry in the animal husbandry industry in order to improve yield and product quality (2). *S. boulardii* was first isolated by Boulard from the bark of a tropical fruit growing in the Far East and was identified as a yeast species different from *Saccharomyces cerevisiae* (3–6). Although it is genetically similar to *S. cerevisiae*, *S. boulardii* has significant differences in terms of metabolism and probiotic properties (7). The differences in protein expression levels and the synthesis of stress proteins may contribute to better survival and growth rates of *S. boulardii* at lower pH environments when compared to other species. Moreover, *S. boulardii* is resistant to high temperature levels (about 52 °C) and it grows at 37 °C; however, *S. cerevisiae* grows at 30 °C. Recent studies also showed that *S. boulardii* is more resistant to the digestive system than other species (7,8).

Probiotics are living microorganisms that have beneficial effects on the health and physiology of the human body when taken in sufficient quantities (9). Probiotics consumed together with foods should reach the intestinal system and the foods must contain a minimum of 10^6 log CFU/g living probiotic bacteria or more. It has also been reported that probiotics must be viable during the production and shelf life of the products. The most important factor in the production of probiotic foods is the inability to maintain the stability or the vitality of the microorganisms used. In recent years, it has been reported that microencapsulation technology is one of the new methods that can be used for increasing the technological properties of probiotics (10,11). Microencapsulation can be defined as the preservation of solid, liquid, or gaseous food components, as well as the enzymes, cells, and other substances in protein- or carbohydrate-based miniature capsules (12,13). There are many microencapsulation techniques used now. In the recent studies carried out on microencapsulation, both probiotic microorganisms and prebiotics were used in the same capsule, and in the gastrointestinal tract, both are expected to make a symbiotic effect after emergence (14).

There are very few studies carried out on the use of *S. boulardii* as a probiotic in foods in the literature. *S. boulardii*, the only yeast used as a probiotic, is a microorganism that can also be used in fermented milk and products. The aim of this study is to determine the product quality

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parameters by combining the therapeutic properties of this yeast with a dairy product and obtaining a functional new dairy product alternative. For this purpose, an alternative probiotic dairy product flavored with black cherry jam was manufactured by using microencapsulated *S. boulardii* and its physical, chemical, microbiological, rheological, and sensory properties were determined.

2. Materials and methods

2.1. Materials

Saccharomyces cerevisiae var. *boulardii* strain (Reflor, lyophilized; Sanofi, Beauvais, France) was used as the probiotic culture in the present study. Commercial starter cultures (Yayla yeast, Doğadan Food and Milk Products, Turkey) were used in the production of yogurt samples. Black cherry jam manufactured by TAMEK A.Ş. (Bursa, Turkey) was bought from local markets.

2.2. Microencapsulation of *S. boulardii*

S. boulardii cells were cultured in 250 mL of YPD (yeast extract peptone dextrose; 5 g/L yeast extract, 10 g/L meat peptone, and 20 g/L glucose). *S. boulardii* was microencapsulated using coating materials according to the extrusion technique. The suspension (0.1% peptone and 0.1 mM glycerol) containing 10^{10} CFU/mL probiotic culture was added to the solution containing 2% sterile sodium alginate coating material in a 1/5 ratio to obtain microcapsules at 10^9 CFU/g. The resulting mixture was injected into a 0.05 M sterile $CaCl_2$ solution using a sterile syringe with an 11-mm needle. The resulting capsules were left in the solution for 30 min in order to obtain sufficient hardness and then filtered with Whatman No. 4 filter paper and subsequently kept in 0.1% sterile peptone solution at 4 °C. As a result, the microcapsules to be used in analyses and in the production of probiotic yogurt were directly used without any drying process after filtration with Whatman No. 4 filter paper (15).

2.3. Production of black cherry jam-flavored probiotic yogurt

Raw milk samples used in the study were obtained from districts of Giresun Province. Samples were brought to the laboratory under refrigerated conditions and used in the production. For the production of probiotic yogurt, raw milk, milk powder (3%), and sugar (10%) were heat-treated at 85 °C for 10 min. After the heat treatment, the mixture was cooled to 43 °C and incubated in equal amounts (5%) of starter culture (lyophilized commercial culture; 10^7–10^8 log CFU/mL) and microencapsulated *S. boulardii* (each microcapsule at 10^9 CFU/g) until the pH level of 4.7. At the end of incubation, the product was cooled at 4 °C and matured for 24 h. After maturation, the products (in 150-mL glass bottles) were stored for 21 days at 4 °C with the addition of black cherry jam (15%). As a control group in the study, flavored yogurt samples prepared using only 5% starter culture (lyophilized commercial culture) without microencapsulated *S. boulardii* were used. Yogurt productions were carried out between October and November, and all analysis was carried out in triplicate.

2.4. Analyzing the storage period of probiotic yogurt samples

Titration acidity (in terms of lactic acid, %), SH (Soxhlet–Henkel) acidity values, pH, and serum separation (mL) of the samples were calculated during the storage period (16–18). The L^* (openness), a^* (red-green), and b^* (yellow-blue) color parameters of yogurt samples were measured on a standard white surface using a CR-410 colorimeter (Minolta Chroma, Osaka, Japan) (19).

Microbiological changes that may occur during the storage were investigated. Plate count agar, violet red bile agar, and potato dextrose agar were used for the enumerations of total mesophilic aerobic bacteria (TMAB), total coliform bacteria, and total yeast-mold as described by Harrigan (20). De Man, Rogosa, and Sharpe agar (MRS, Merck, Darmstadt, Germany) was used for the enumeration of lactic acid bacteria (LAB) as described by Vinderola and Reinheimer (21). The agar plates were incubated for 18–24 hours at 37 °C (TMAB), 1 day at 35–37 °C (coliform bacteria), 5–7 days at room temperature (yeast and mold), and 3 days at 35–37 °C in an anaerobic jar (LAB).

2.5. Sensory analyses

The stored yogurt samples were analyzed by five different panelists using a sensory rating scale, evaluating some sensory parameters [external appearance, consistency (with spoon and by mouth), odor, flavor, and overall acceptability)] from 1 (poor) to 5 (excellent) as described by Çakmakçı et al. (22). All yogurt samples were presented to the panelists within the glass jars (150 mL) at 4 °C. The panel of assessors comprised an external group of nonsmokers, who were very familiar with fermented dairy products and who were evaluated for sensory acuity and consistency.

2.6. Statistical analyses

Statistical analyses were performed using SPSS 20.0 for Windows (IBM Corp., Armonk, NY, USA). One-way variance analysis (ANOVA) was used in order to compare the significance of differences between tests, whereas Duncan's multiple comparison test was used in comparing the differences between groups ($P \leq 0.05$) (19).

3. Results

The physical, chemical, rheological, microbiological, and sensory characteristics of black cherry jam-flavored probiotic yogurt samples manufactured using microencapsulated *S. boulardii* (PGS) or only the starter

culture (CGS) were examined on the 1st, 7th, 14th, and 21st days of storage. The physical, chemical, and rheological properties of yogurt samples during the storage period are shown in Table 1. As can be seen in Table 1, there was no significant difference between the yogurt samples in terms of physical, chemical, and rheological properties ($P \leq 0.05$). The microbiological analysis results of PGS and CGS are shown in Table 2. A significant difference was found between the yogurt samples in terms of the number of yeasts and molds. However, no significant difference was found in terms of TMAB, LAB, and number of total coliforms. The number of *S. boulardii* decreased during the storage period; however, it did not fall below 10^6 CFU/g. The color properties of the probiotic and the control group of yogurt samples are shown in Table 3. For each group of samples, color parameters L^* (openness), a^* (red-green), and b^* (yellow-blue) were measured by using a CR-410 colorimeter on a white standard surface. The color properties of yogurt samples were found to be similar for both groups during the storage period and no significant differences were found in terms of color properties ($P \leq 0.05$).

The sensory parameter scores [external appearance, consistency (with spoon and by mouth), odor, flavor, and overall acceptability)] are shown in Figures 1 and 2 for PGS and CGS. Significant differences were observed between yogurt samples in terms of sensory properties ($P \leq 0.05$).

4. Discussion

When the results shown in Table 1 are considered, it can be stated that the pH values of PGS are similar to those of CGS. When the incubation of yogurt samples was completed, there was an increase of lactic acid in the yogurt during storage, as well as a decrease in pH values (23). The lowest pH value in yogurt samples was found on the 21st day in CGS. The pH value decreased during storage but the titration acidity increased (Table 1). The bacterial activity decreased during the storage period, but the enzymatic activity was still in progress. The expected increase in titratable acidity (percent acidity) was observed to be in parallel with the decreasing pH value because the bacterial activity in yogurt samples continued to a certain extent. Studies have shown that yeast is beneficial to fermentation and maturation process when used as the starter culture in the production of dairy products (1). The probiotic and starter culture bacteria interaction had a positive impact on yogurt production in our study. In a study by Ünver (24), similar to our study, the pH values of yogurt samples produced with *S. boulardii* were found to change between 3.85 and 4.18. When the values of our samples were examined in terms of titratable acidity (Table 1), the values observed were similar to those observed in the study of Ünver (24), and the titration acidity values of yogurt samples manufactured using *S. boulardii* ranged between 0.8 and 1.0%. Yogurt samples mostly have SH acidity level of 35–75 (18). The acidity values of SH determined in this study are within these limits. Serum separation, which is an important parameter in determining the textural characteristics of dairy products, determines the stability of the yogurt clot (25). In the present study, the serum separation was observed to increase in PGS. High acidity in yogurts is an important factor increasing the serum separation (26). Low serum separation values obtained in PGS, which has higher pH, confirms this situation (Table 1). In a previous study, Ünver (24) reported that the serum separation levels of yogurt samples manufactured using *S. boulardii* varied between 32.2% and 38%. When the dry matter levels of yogurt samples were examined, it was found that they were lower in PGS. Similarly, Ünver (24) reported that the dry matter levels of yogurt samples manufactured using *S. boulardii* ranged between 9.8% and 12%.

In the present study, it was determined that the microbiological quality properties of yogurt samples were in compliance with the Fermented Dairy Products Communiqué. In our study, the *S. boulardii* level was determined as 6.12–9.16 log CFU/g during the storage period. The level of *S. boulardii* decreased during the storage period but did not fall below 10^6 CFU/g, as desired. Ünver (24), in a similar study, reported that the number of *S. boulardii* in probiotic yogurt samples varied between 5.22 and 6.70 log CFU/g during the storage period. As seen in Table 2, PGS and CGS showed similar LAB numbers. Sömer (27) reported that the number of LAB in yogurt samples varied between <100 and 2.85×10^9 CFU/g. In the same study, the mean number of yeasts and molds in filtered yogurt samples taken from different sites was found to be 6.64 log CFU/g. The development of packed condensed yogurt yeasts can be explained by the high concentration of lactic acid in samples, exposure to unsuitable heat during storage, and the sale of product (28). As shown in Table 3, the difference in color characteristics between the control group and the probiotic yogurt samples is not significant ($P \leq 0.05$). In a similar study by Ünver (24), it was found that L^* values varied between 84.10 and 90.58.

When the mean score of "appearance" was compared between the groups, it was determined that the panelists rated the control group of yogurt samples with higher scores. When the values were examined, PGS had a lower mean score for consistency (mouth) sensory characteristics when compared to CGS (Figures 1 and 2). It was thought to be because *S. boulardii* capsules obtained from the microencapsulation treatment were felt in the mouth. Since panelists were accustomed to CGS sample consumption, they pointed out that the roughened structure resulting from the microencapsulated structure warranted a lower

Table 1. The physical, chemical, and rheological properties of yogurt samples.

Physical and chemical properties	Control group*				Probiotic group**			
	Storage time (days)				Storage time (days)			
	1	7	14	21	1	7	14	21
pH	3.89 ± 0.015^{aA}	3.86 ± 0.015^{bA}	3.84 ± 0.000^{cA}	3.87 ± 0.100^{bA}	3.94 ± 0.011^{aA}	3.93 ± 0.017^{aA}	3.94 ± 0.020^{aA}	3.85 ± 0.005^{bA}
SH	40.46 ± 3.001^{aA}	28.86 ± 2.220^{cA}	31.35 ± 1.056^{bcA}	34.48 ± 2.443^{bA}	30.34 ± 2.563^{abB}	27.08 ± 1.541^{aA}	31.62 ± 1.569^{bA}	44.10 ± 1.786^{cB}
Titration acidity	0.90 ± 0.060^{aA}	0.65 ± 0.050^{cA}	0.70 ± 0.023^{bcA}	0.77 ± 0.050^{bA}	0.68 ± 0.057^{abB}	0.61 ± 0.034^{aA}	0.71 ± 0.036^{bA}	1.00 ± 0.036^{cB}
Dry matter	16.03 ± 0.057^{aA}	17.43 ± 1.497^{aA}	16.97 ± 0.025^{aA}	16.93 ± 0.057^{aA}	15.03 ± 0.115^{aA}	16.94 ± 0.036^{bA}	16.33 ± 0.144^{bA}	16.90 ± 1.044^{bA}
Separation of serum (mL)	6.66 ± 0.152^{aA}	7.16 ± 0.763^{abA}	7.56 ± 0.404^{abA}	7.83 ± 0.288^{bA}	7.93 ± 0.115^{aB}	7.43 ± 0.404^{aA}	8.70 ± 1.178^{aB}	8.08 ± 1.010^{aB}

*Control group: black cherry jam-flavored yogurt samples produced using only the yogurt starter cultures; **Probiotic group: black cherry jam-flavored yogurt samples produced with microencapsulated *S. boulardii* and yogurt starter cultures. Mean and standard deviation: mean values with different lowercase letters indicate significant differences ($P \leq 0.05$) within a column; mean values with different uppercase letters indicate significant differences ($P \leq 0.05$) within a row.

Table 2. Microbiological properties of yogurt samples (log CFU/g).

Microbiological properties	Control group*				Probiotic group**			
	Storage time (days)				Storage time (days)			
	1	7	14	21	1	7	14	21
TMAB	3.72 ± 0.030^{aA}	4.21 ± 0.058^{bA}	5.73 ± 0.478^{cA}	6.07 ± 0.106^{cA}	3.96 ± 0.231^{aA}	6.39 ± 0.350^{bB}	6.59 ± 0.457^{bB}	5.98 ± 0.125^{bA}
Yeast-mold	0.43 ± 0.750^{aA}	1.96 ± 1.738^{abA}	2.82 ± 0.326^{bA}	2.33 ± 0.345^{abA}	3.10 ± 0.065^{aB}	4.25 ± 1.011^{bB}	4.61 ± 0.404^{bB}	3.82 ± 0.305^{abB}
LAB	5.34 ± 0.364^{aA}	6.51 ± 0.164^{bA}	6.79 ± 0.049^{bA}	6.59 ± 0.172^{bA}	6.54 ± 0.211^{aB}	6.49 ± 0.221^{aA}	6.65 ± 0.087^{aA}	6.31 ± 0.457^{aA}
Total coliforms	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	1.65 ± 0.220^{bA}	1.38 ± 0.425^{bA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aA}	0.00 ± 0.000^{aB}	$0.00+0.000^{aB}$
S. boulardii	-	-	-	-	9.16 ± 0.098^{a}	6.92 ± 0.863^{b}	5.59 ± 2.072^{b}	6.12 ± 0.101^{b}

*Control group: black cherry jam-flavored yogurt samples produced using only the yogurt starter cultures; **Probiotic group: black cherry jam-flavored yogurt samples produced with microencapsulated *S. boulardii* and yogurt starter cultures. Mean and standard deviation: mean values with different lowercase letters indicate significant differences ($P \leq 0.05$) within a column; mean values with different uppercase letters indicate significant differences ($P \leq 0.05$) within a row.

Table 3. Color properties of yogurt samples.

Color properties	Control group*			
	Storage time (days)			
	1	7	14	21
*L**	82.96 ± 1.473^{aA}	75.20 ± 2.947^{aA}	81.48 ± 1.770^{aA}	82.21 ± 1.565^{aA}
*a**	7.65 ± 0.184^{aA}	8.31 ± 1.010^{aA}	7.66 ± 0.880^{aA}	7.54 ± 0.514^{aA}
*b**	3.49 ± 0.376^{aA}	3.87 ± 0.235^{aA}	4.37 ± 0.317^{aA}	4.77 ± 0.222^{aA}
Color properties	Probiotic group**			
	Storage time (days)			
	1	7	14	21
L*	80.31 ± 3.614^{aA}	80.40 ± 3.822^{aB}	76.89 ± 5.856^{bB}	80.941 ± 3.853^{bB}
a*	6.93 ± 0.665^{aB}	7.92 ± 0.921^{aB}	7.64 ± 1.653^{aA}	7.40 ± 1.473^{aA}
b*	5.11 ± 0.776^{aB}	4.71 ± 0.926^{abB}	5.87 ± 0.811^{bcB}	6.15 ± 0.509^{cB}

*Control group: black cherry jam-flavored yogurt samples produced using only the yogurt starter cultures; **Probiotic group: black cherry jam-flavored yogurt samples produced with microencapsulated *S. boulardii* and yogurt starter cultures. Mean and standard deviation: mean values with different lowercase letters indicate significant differences ($P \leq 0.05$) within a column; mean values with different uppercase letters indicate significant differences ($P \leq 0.05$) within a row.

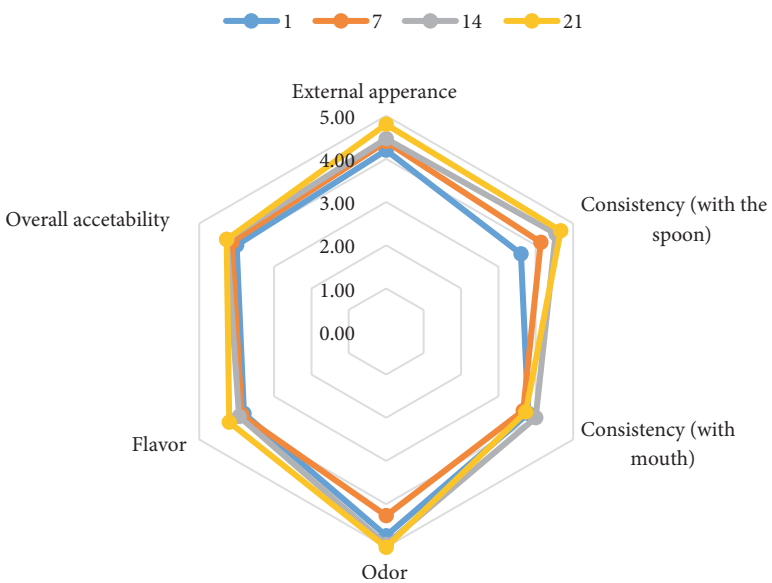

Figure 1. Sensory properties of control group yogurt samples.

score.. At the same time, PGS seems to have a lower mean score for "odor" sensory characteristics in comparison to CGS. For this reason, the probiotic *S. boulardii* capsules are likely to cause a slightly sour odor in the yogurt samples. When the values were analyzed in terms of flavor scores, it was found that PGS had a lower mean score for "taste" sensory properties than CGS. It is thought to be because the probiotic *S. boulardii* capsules cause a slightly sour odor in the yogurt samples and the capsules are felt in the mouth. Finally, the probiotic group samples had a lower mean score for the "overall acceptability" sensory properties than the control group samples. As a result,

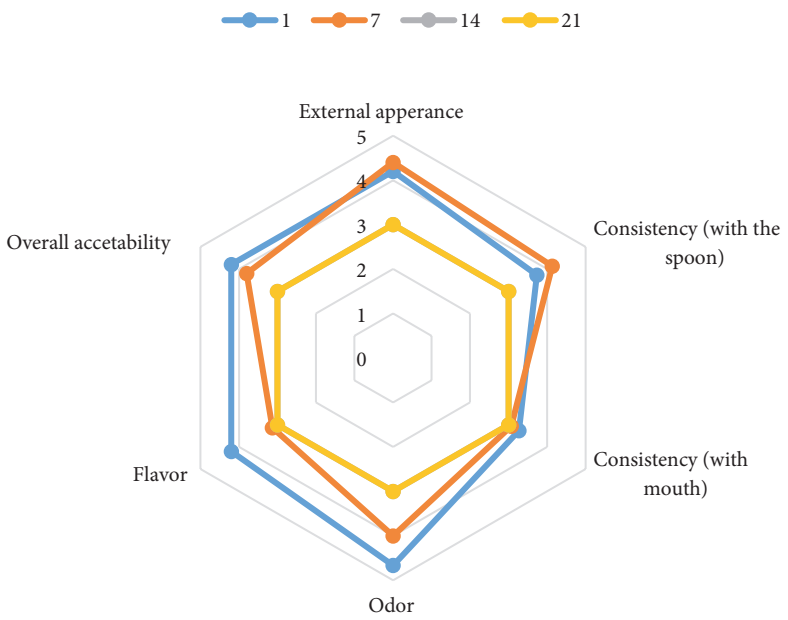

Figure 2. Sensory properties of probiotic yogurt samples.

CGS was more appreciated than PGS, although there were no significant differences between the two groups of yogurt samples in terms of average sensory score points ($P \leq 0.05$). In a similar study carried out by Ünver (24), it was reported that the mean "appearance rating" scores of probiotic yogurt samples ranged between 3.00 and 4.66, mean consistency scores (with spoon) between 3.18 and 4.50, mean consistency scores (by mouth) between 3.12 and 4.58, mean odor scores between 3.16 and 4.50, and mean average acceptability scores between 1.66 and 4.41.

In conclusion, the physical, chemical, rheological, and microbiological properties of probiotic yogurts containing microencapsulated *S. boulardii* aromatized with black cherry jam were within the yogurt standards and the samples were suitable for consumption. In this study, it was aimed to manufacture an alternative functional product in order to increase consumption since the consumption level of probiotic dairy products is significantly low. The product that was developed can be considered as an alternative. As a result of the sensory analyses of the product during 21 days of storage, it was found that the sensory properties of achieved probiotic products were generally appreciated by the consumers in terms of external appearance, consistency (with spoon and by mouth), odor, flavor, and overall acceptability. However, these properties must be further improved for consumer acceptability. Thus, it was determined that the use of *S. boulardii* as a probiotic yeast in milk and milk products yielded positive results in terms of the product quality. The literature on using *S. boulardii* in relation with probiotic fermentation for foodstuffs is limited and further research is needed. This study will contribute to the further studies on this subject.

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