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# Physicochemical and Microbial Characteristics of Yogurt with Added *Saccharomyces Boulardii*

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**Abstract**

The effect of adding *Saccharomyces boulardii* on yogurt quality was studied. Yogurt control was made using whole cow's milk and classic starter culture. Other three treatments of yogurt were made by adding 1%,2% and 3% of *Saccharomyces boulardii* with yogurt starter. pH values and proteolytic activity of all yogurt treatments were determined during fermentation time. Changes in physicochemical and microbial properties of yogurt product were observed during storage time (21 days at 4°C). Yogurt samples with added yeast to starter cultures showed a slight increase in pH values during the 6 hours of fermentation. After fermentation time, pH and proteolytic activity of yogurt with 3% yeast were 4.05 and 250 µg/ml while control sample was 4.22 and 200 µg/ml respectively. pH, TN, WSN, TVFA and WHC values of yogurt with *Saccharomyces boulardii* were slightly increased whereas decreased the STS percentage compare with control yogurt without yeast during storage time. The addition of *Saccharomyces boulardii* improved the survivability of bacterial starter culture. After 21 day, *Saccharomyces boulardii* counts were 5.78, 6.01 and 6.31 Log. CFU/gm for yogurt with 1%,2% and 3% yeast respectively whereas Log. lactic acid bacteria of yogurt with 3% yeast was 7.53 and 7.55 for *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

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**Keywords**

yogurt, *Saccharomyces boulardii,* physicochemical and microbial properties.

## Introduction

*Saccharomyces boulardii* was found by a French microbiologist, Henri Boulard in 1923 when he was in Indochina zone looked for new strains of yeast that could be used in fermenting processes. *Saccharomyces boulardii* isolated from Lychees fruit (*Litchi chinensis*) and Mangosteen fruit (*Garcinia mangostana*) grown in tropics warm as Indochina[1]. It is eukaryotic, single-cell, yeast cell diameter is between 2-3 µm and length is between 2.5-10.5 µm, the yeast cells non-sexual reproduction by budding method or sexual reproduction by cells conjugation, which be Ascus. *S. boulardii* has an optimal high growth temperature at 37°C [2].

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*S. boulardii* yeast is one member of probiotics as a live in human gastrointestinal and used to variety of diarrheal treatment with antibiotic[3]. This yeast secretes many enzymatic proteins, including an enzyme that inhibition for *Clostridium difficile* toxins and phosphatase enzyme (EC 3.1.3.X) that depressed activity endotoxin production from Gram-negative bacteria such as the lipopolysaccharide produced by *E. coli*[4]. The metabolic extract of *S. boulardii* had inhibition activity against 26 species of food related bacteria[5]. Dairy food as the ideal system for delivering probiotics (bacteria and yeasts) to the human digestive system because of an enabling environment that promotes growth and enhances the survivability of these microorganisms. *S. boulardii* used in many food type, produced yeast-acidophilus milk by skim milk and inoculated with 2% probiotic Saccharomyces yeast culture ($10^6$ CFU/g) after fermented at 37 $^0$C for 24 hours[6].

The addition of probiotic yeast in acidophilus milk enhances its demand as probiotic drink because of the supplement of beneficial properties such as quick recovery of diarrhea as well as of tuberculosis[7]. *S. boulardii* and *Lactobacillus acidophilus* used in ice cream, which inoculated at 37 $^0$C for 24 hours. The viability of probiotics was not decreasing after stored at -18 °C to -23 °C for 15 day[7]. The study was conducted to investigate the effect of adding *Saccharomyces boulardii* to yogurt production, and the study of survivability of starter culture bacteria and the probiotic yeast during storage time.

## Materials and Methods

### Microbial Isolates and Cultural Media

The commercial classic yogurt starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1) (Sacco Lyofast companuy, Italy) and *Saccharomyces boulardii* ATCC MYA796TM (Swanson probiotic laboratories, Australia) were used. The starter of yogurt was activated in MRS broth (Hi-media, India) at 42 $^0$C for 16 hour while yeast culture was activated in Potato Dextrose broth (Hi-media, India) at 30 $^0$C for 24 hour.

### Preparation of Yogurt

The yogurt samples were prepared from cow's milk (moisture 87.30%, total protein 3.55%, lactose 4.63%, fat 3.72%, ash 0.77%, total acidity 0.17% and pH 6.68) in Biotechnology Lab. of Department of Food Science, College of Agriculture, University of Basrah. Four yogurt treatments were made using starter culture of yogurt or different percentage of yeast inoculation with starter of yogurt. The first sample of yogurt was manufactured using 5% ($92\times10^8$ CFU/ml) starter culture of yogurt without *Saccharomyces boulardii* yeast as a control whereas other samples were med by starter culture of yogurt with 1%, 2% and 3% of yeast ($43\times10^6$ CFU/ml), incubated at 37°C for completely coagulated, and stored for 21 days at 4 °C. During fermentation and storage time, total acidity of yogurt was measured by titrating 10g of yogurt sample against NaOH (0.5 N) using phenolphthalein indicator to the end point (pink color)[8].

### Determination of Proteolytic Activity

The proteolytic activity of the starter within or without yeast were determined Spectrophotometric method, by additions of 10 ml of Trichloroacetic acid (0.75N) (Sigma company, Germany) and 1mL of distilled water to 5 ml of yogurt sample. The samples were mixed and filtered by Whatman No.1. The filtrate was incubated at 25 $^0$C for 10 min. The ortho-phthalaldehyde (OPA) method described by Church *et al.,*[9] was used to determine free amino group concentration in the samples during fermentation time (6 hour).

### Physicochemical Analyzes of Yogurt

The pH of yogurt was measured by pH-meter (SD300PH, Germany). Total nitrogen of yogurt (TN) was measured using micro kjeldahl method. Total volatile fatty acids (TVFA) and water-soluble nitrogen (WSN) of yogurt were estimated[10]. Water holding capacity (WHC) and susceptibility to syneresis (STS) of yogurt were estimated[11]. The samples of yogurt were analyzed in 1,7,14 and 21 days of storage time.

### Microbiological Tests

The viable cells count of lactic acid bacteria and yeasts were measured during storage time, using MRS agar (Hi-media, India) at pH5.8 and incubated at 48 °C for 48 hours for *Lactobacillus bulgaricus* enumeration while *Streptococcus thermophilus* enumeration was used M17 agar (Hi-media, India) and incubated in aerobic condition at 37 $^0$C for 24-48 hours[12,13]. *Saccharomyces boulardii* enumeration was used Chloramphenicol

glucose yeast extract agar (Hi-media, India) and incubated in aerobic condition at 30 $^0$C for 48-72 hours[5].

**Statistical Analysis**

All data were presented as mean± SD (standard deviation), and were the results of at least three independent experiments with duplicate assays. All statistical analysis were performed using one-way analysis of variance (ANOVA table) followed by least significant difference (LSD) for mean comparison. Statistical significance was established as $p<0.05$.

## Results

**pH Values of Yogurt**

Changes in pH values during the fermentation time of yogurt product are presented in Table 1. The low in pH was slower in yogurt made using starter culture with *Saccharomyces boulardii* compared to the control sample of yogurt. After fermentation time of yogurt product, the pH values were 4.18, 4.12 and 4.05 for added 1%, 2% and 3% of S*accharomyces boulardii* respectively while the pH of yogurt made by yogurt starter without yeast was 4.22%.

**Table 1: pH values of yogurt made with *Saccharomyces boulardii* (Mean ±SD)**

| Treatments | Fermentation time (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | 6.15$^a$±0.05 | 5.68$^a$±0.02 | 5.02$^a$±0.01 | 4.86$^a$±0.03 | 4.45$^a$±0.01 | 4.22$^a$±0.02 |
| 1% yeast | 6.20$^a$±0.03 | 5.60$^a$±0.01 | 4.95$^a$±0.03 | 4.75$^b$±0.01 | 4.35$^a$±0.03 | 4.18$^a$±0.01 |
| 2% yeast | 6.24$^a$±0.02 | 5.66$^a$±0.01 | 4.88$^a$±0.02 | 4.70$^{ab}$±0.07 | 4.28$^b$±0.06 | 4.12$^b$±0.03 |
| 3% yeast | 6.30$^a$±0.03 | 5.69$^a$±0.03 | 4.82$^b$±0.04 | 4.65$^c$±0.09 | 4.22$^c$±0.05 | 4.05$^c$±0.03 |

Figure 1 shows the changes in proteolytic activity of yogurt starter with added 1%, 2% and 3% of *Saccharomyces boulardii* during cow's milk fermentation at 37 $^0$C for 6 hours. The amount of free amino group's released after one hour of milk fermentation were 70, 85 and 95 µg/ml for 1%, 2% and 3% of *S. boulardii* respectively. *S. boulardii* addition to starter cultures was lead to the highest free amino groups released compared with control sample (50 µg/ml). Furthermore, when milk fermentation time increased up to 6 hours, there was a subsequent increase in free amino group's amount to 215, 230 and 250 µg/ml for 1%, 2% and 3% of *S. boulardii* respectively.

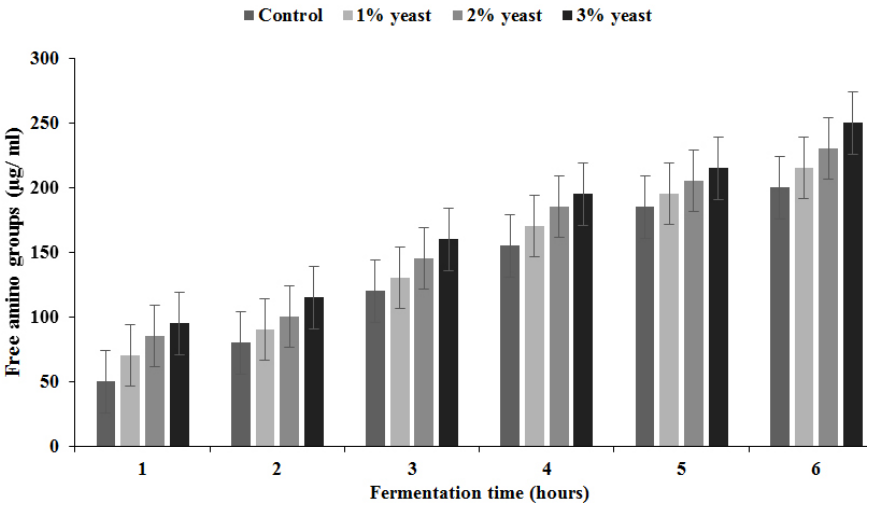



**Fig. 1 : The protelytic activity of yogurt made with *saccharomyces boulardii* during fermentation time**

**Chemical Properties Changes**

As shown in Table 2. addition of *Saccharomyces boulardii* with different percentage decreased pH values of yogurt during storage time. *S. boulardii* can small amounts of lactic acid production. Total nitrogen and water-soluble nitrogen values of yogurt simples during storage time are presented in (Table 2). The percentage of TN in yogurt samples was higher by increasing the concentration of added yeast. This may be due to the yeast cell contain high protein content. TVFA of yogurt produced was increased after *S. boulardii* addition to culture starter compared with yogurt sample without yeast. As storage time (21 days) advanced, WSN and TVFA content of all yogurt samples were increased. This could be attributed to yeast addition to bacteria starter as well as proteolytic and lipolytic of yeast and starter bacteria.

**Table 2: Effect of adding *S. boulardii* with starter culture on pH, TN, WSN and TVFA of yogurt (Mean ±SD)**

| Treatments | Storage time (days) | pH | TN (%) | WSN (%) | TVFA (%) |
|---|---|---|---|---|---|
| Control | 1 | $4.58^{a}$±0.01 | $0.624^{c}$±0.02 | $0.133^{d}$±0.005 | $0.60^{c}$±0.007 |
| | 7 | $4.25^{b}$±0.05 | $0.665^{b}$±0.01 | $0.142^{c}$±0.001 | $0.66^{b}$±0.001 |
| | 14 | $4.11^{c}$±0.01 | $0.682^{a}$±0.01 | $0.145^{b}$±0.005 | $0.71^{a}$±0.001 |
| | 21 | $3.85^{d}$±0.02 | $0.699^{a}$±0.03 | $0.153^{a}$±0.002 | $0.73^{a}$±0.004 |
| 1% yeast | 1 | $4.44^{a}$±0.05 | $0.645^{c}$±0.01 | $0.137^{c}$±0.001$^{e}$ | $0.62^{b}$±0.005 |
| | 7 | $4.12^{b}$±0.04 | $0.678^{b}$±0.01 | 0.144b±0.005$^{d}$ | $0.67^{a}$±0.003 |
| | 14 | $3.91^{b}$±0.09 | $0.703^{a}$±0.03 | $0.153^{a}$±0.001$^{c}$ | $0.73^{a}$±0.003 |
| | 21 | $3.77^{c}$±0.05 | $0.718^{a}$±0.02 | $0.161^{a}$±0.001$^{b}$ | $0.74^{a}$±0.006 |
| 2% yeast | 1 | $4.32^{a}$±0.06 | 0.666c$^{a}$±0.05 | $0.141^{c}$±0.004 | $0.64^{c}$±0.001 |
| | 7 | $4.07^{a}$±0.01 | $0.689^{b}$±0.05 | $0.155^{b}$±0.002 | $0.69^{b}$±0.003 |
| | 14 | $3.81^{b}$±0.04 | $0.712^{a}$±0.04 | $0.162^{a}$±0.001 | $0.74^{a}$±0.002 |
| | 21 | $3.65^{b}$±0.03 | $0.724^{a}$±0.01 | $0.168^{a}$±0.003 | $0.76^{a}$±0.003 |
| 3% yeast | 1 | $4.21^{a}$±0.01 | $0.671^{b}$±0.01 | $0.151^{d}$±0.003 | $0.66^{c}$±0.004 |
| | 7 | $3.91^{b}$±0.01 | $0.701^{a}$±0.02 | $0.165^{c}$±0.001 | $0.73^{b}$±0.005 |
| | 14 | $3.72^{c}$±0.05 | $0.722^{a}$±0.01 | $0.173^{b}$±0.002 | $0.78^{a}$±0.002 |
| | 21 | $3.55^{d}$±0.06 | $0.735^{a}$±0.02 | $0.185^{a}$±0.001 | $0.83^{a}$±0.001 |

**Rheological properties changes**

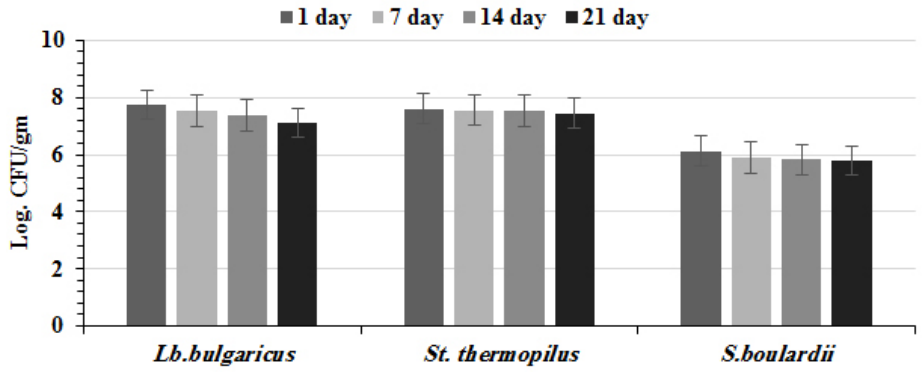
The changes in percentage of WHC and STS during storage time of yogurt samples was stated in Table 3. WHC percentage of all yogurt samples was significantly ($p \leq 0.05$) affected by adding *S. boulardii* with starter culture in yogurt made during 21 days of storage time. Yogurt with yeast had slight increase of WHC moreover, with increased level of *S. boulardii.* After 21 day of storage time, WHC of yogurt with 3% yeast was 50% while yogurt without yeast was 40%. Adding *S. boulardii* with starter culture was displayed low STS percentage compared with yogurt control sample as shown in Table 3. The addition of yeast significantly decreased the rate of STS during storage time (21 days). After 21 day of storage time, The STS percentage of yogurt with yeast was 88, 86 and 85 for 1%, 2% and 3% of yeast respectively. While the control sample was 90%.

**Table 3: The percentage of WHC and STS of yogurt production with *Saccharomyces boulardii* during storage time (Mean ±SD)**

| Treatments | Percentage of WHC Storage time (days) | | | | Percentage of STS Storage time (days) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 1 | 7 | 14 | 21 |
| Control | $50^{c}$±1.0 | $47^{b}$±3.0 | $42^{d}$±1.0 | $40^{b}$±3.0 | $80^{a}$±1.0 | $82^{a}$±3.0 | $85^{a}$±2.0 | $90^{a}$±2.0 |
| 1% yeast | $55^{b}$±2.0 | $50^{b}$±2.0 | $45^{c}$±1.0 | $41^{b}$±4.0 | $80^{a}$±2.0 | $81^{a}$±2.0 | $83^{a}$±3.0 | $88^{a}$±2.0 |
| 2% yeast | $60^{a}$±2.0 | $56^{a}$±2.0 | $50^{b}$±2.0 | $46^{a}$±1.0 | $79^{a}$±2.0 | $80^{a}$±4.0 | $82^{a}$±4.0 | $86^{b}$±1.0 |
| 3% yeast | $63^{a}$±1.0 | $60^{a}$±1.0 | $56^{a}$±2.0 | $50^{a}$±1.0 | $78^{a}$±2.0 | $80^{a}$±1.0 | $81^{b}$±3.0 | $85^{b}$±1.0 |

**Microbiological Tests of Yogurts**

Figure 2. presents the survivability of starter culture bacteria and *Saccharomyces boulardii* of yogurt made during storage time. Increasing percentage of yeast was not effected on viability cells of lactic acid bacteria. Incorporation of yeast showed a synergistic effect by the enhanced growth and viable numbers of bacterial starter in yogurt product. After one day of storage time, Log. numbers of *Lb. bulgaricus*, *St. thermophilus* and *S. boulardii* was 7.74,7.61 and 6.13 CFU/gm respectively in yogurt with 1% of yeast while the viable numbers was 7.12, 7.45 and 5.78 Log. CFU/gm respectively at end of storage time. In yogurt product with 3% of *S. boulardii*, Log. numbers of *Lb. bulgaricus*, *St. thermophilus* and *S. boulardii* was 7.88,7.79 and 6.89 CFU/gm respectively after one day of storage time while the numbers was 7.53,7.55 and 6.31 Log. CFU/gm respectively after 21days of storage time.

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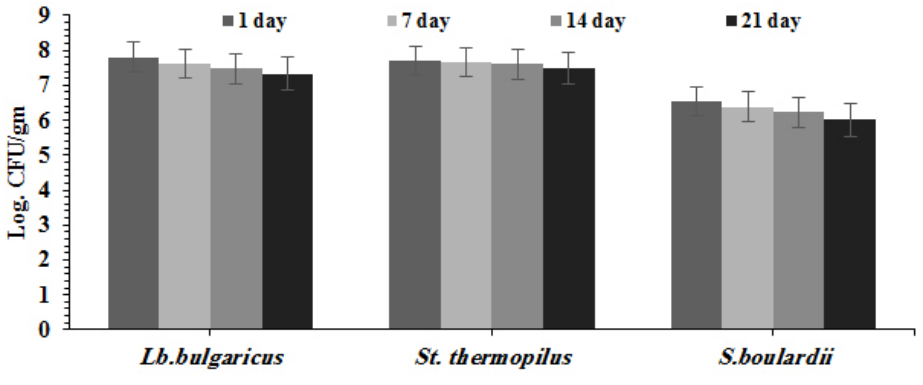


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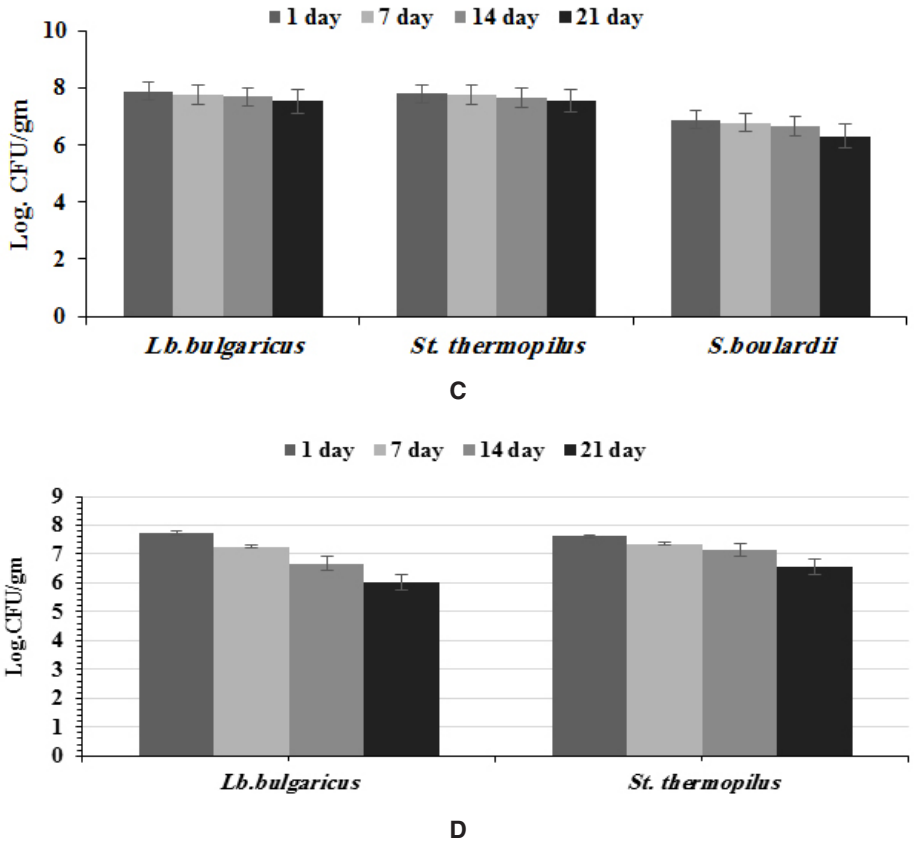


**Fig. 2: The viability of bacterial starter and *S. boulardii* in yogurt product during storage time. A-yogurt with 1% yeast, B-yogurt with 2% yeast, C-yogurt with 3% yeast, D- control yogurt**

## Discussion

*Saccharomyces boulardii* are known to produce several protein enzymes that break down milk protein into peptides that are used as a nitrogen source during starter bacteria growth in milk for yogurt produce[14]. This in turn going to lead to higher starter growth and acidity produced rate in the milk[15]. In addition, the yeast is a main source of B complex vitamins, which need lactic acid bacteria for growth. The yogurt samples with *Saccharomyces boulardii* showed an increase in total acidity percentage during 6 hours of fermentation also, an increase in the *Saccharomyces boulardii* concentration positively affected on the rate of lactic acid production.

In general these results showed that yogurt starter with added *S. boulardii* had higher proteolytic activity compared starter cultures without yeast. *S. boulardii* has proteolytic systems complex, which generate biopeptides. These results agreed with the findings of Buts *et al.*[16] who reported that *S. boulardii* had higher activity of proteolytic enzymes in the pH range 2 to 8. *Saccharomyces cerevisiae* as potential probiotic can produce a variety of enzymes such as protease, cellulose, lipase and amylase[17]. Despite the inability to use lactose, yeast species can use glucose, galactose and organic acids as carbon source which derived from the metabolism of lactic acid bacteria from lactose fermentation are found in dairy products[18].

WSN percentage were higher in yogurt made by starter culture with 2% of *S. boulardii* compared with other treatments because of high proteolytic activity of *S. boulardii*[16]. *Saccharomyces* yeast had phospholipase enzyme which break down for phospholipids and release free fatty acids[19].

Water holding capacity of a protein gel is an important parameter in yogurt production. Reducing

of WHC or separation of whey is due to the irresolute gel network of yogurt production[20,21]. In the present study, *S. boulardii* was added into starter culture during manufacturing. This may also be reason to increasing WHC during 21 of storage time. The addition of yeast lead to increasing total solid of yogurt production could cause increased density of protein gel in yogurt[22]. Very important is the fact that the presence of yeast stimulated the survival of lactic acid bacteria, which in both yogurt and yeast were significantly higher than in control[23].

The survivability of microorganisms in yogurt product has been confirmed in food standards and regulations. These results were confirmed by resulted of Hattingh and Viljoen[18] who pointed that Log. number of *S. boulardii* was 7.6 CFU/ml in yogurt at 5 $^0$C for 29 days of storage time. Incorporation of probiotic yeasts showed a synergistic effect by the enhanced growth and cells viability of lactic acid bacteria in dairy fermentation with increased probiotic effectiveness[24]. The probiotic organisms as *Saccharomyces boulardii* can be inserted into dairy foods to develop therapeutic and functional and foods. Incorporation of *S. boulardii* into yogurts seemed useful in improving the proteolytic activity of yogurts during fermentation. The addition of probiotic yeast to yogurt manufacture has been improved by chemical and rheological properties after 21 days of storage time. Yogurts showed the ability in preserving higher viability of yeast probiotic *S. boulardii* counts in yogurt with 2% and 3% of probiotic yeast were sufficient beneficial organisms yield than threshold of acceptable ($10^6$ CFU/gm).

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