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# EFFECT OF SUPPLEMENTATION WITH OLIVE OIL ON SOME PROPERTIES OF BIO-YOGHURT

**El-Tahra M. A. Ammar[1] --- Ismail, M. M.[2*] --- El-Shazly, A. K.[3] --- Eid, M. Z.[4]**

*[1-3]Dairy Department, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt*

*[2-4]Dairy Technology Department, Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt*

## ABSTRACT

*The effect of fortification with olive oil on yoghurt quality was investigated. Control yoghurt was made using classic yoghurt culture and whole milk. Other four yoghurt treatments were made by ABT-5 culture and whole milk fortified with 0, 1, 2, and 4% virgin olive oil. The sixth treatment was prepared using ABT culture and skim milk with 4% virgin olive oil. Changes in rheological, chemical, microbial and organoleptic properties of yoghurt were monitored during refrigerated storage (4°C) of yoghurt for 15 d. Samples of yoghurt with added olive oil to whole milk showed a slight decrease in titratable acidity during the 180 min of fermentation. Adding of olive oil had no clear effect on coagulation time and curd tension whereas decreased the syneresis of yoghurt. Acidity, TS, fat and TVFA contents of yoghurt supplemented with olive oil were higher than those of control whereas the contents of TN and WSN were similar in both. Yoghurt samples fortified with olive oil had less numbers of TVBC. The addition of olive oil improved the viability of lactic acid bacteria and bifidobacteria. The bifidobacteria counts were sufficient to yield numbers of beneficial organisms that were higher than the accepted threshold ($10^6$ cfu.$g^{-1}$) for a probiotic effect. Also, olive oil adding improved the body, texture and flavor of the yoghurt.*

**Keywords:** Bio-yoghurt, ABT, Viability, Bifidobacteria, Olive oil.

**Abbreviation Key:** LA = *Lactobacillus acidophilus*, WSN = Water soluble nitrogen, TVFA = Total volatile fatty acids, TVBC = Total viable bacterial count, LAB =Lactic acid bacteria, TS = Total solids, TN= Total nitrogen, DM= Dry matter.

## Contribution/ Originality

This study originates new formula for production of bio-yoghurt. Fortification of yoghurt made using ABT culture with olive oil increased the nutritional and health value.



*+ Corresponding author*

## 1. INTRODUCTION

Yoghurt is thought to have originated from the Middle East by an accident when some milk was left to spoil. Instead, it just turned sour and curdled due to some lactic acid bacteria which converted the natural sugar in milk, lactose, to lactic acid and precipitated the proteins [1]. The resultant product, being refreshing in taste and digestible even to lactose intolerant, soon became a traditional food in the Middle East and Balkans. Numerous benefits of yogurt consumption including increased protein digestibility, reduced lactose due to its fermentation, control of intestinal infections, high level of B- group vitamins, anticholesterelemic effect, antitumor activity and high calcium content have been reported by several researchers [2-4] . Today, the growing popularity of yoghurt has encouraged the industry to come up with some of the innovative yogurt based products like yogurts fortified with dried fruits, dietary fiber (e.g. inulin), vegetables and vegetable powders containing natural sources of pectin and vitamin C, carrot pulp, probiotics, calcium, and frozen yogurts [5].

Over the past decade, considerable interest has developed in the use of probiotic organisms [*Lactobacillus acidophilus* (LA) and bifidobacteria] in food, pharmaceutical and feed products. The consumption of probiotic products has increased dramatically in most European, Asia-Pacific and American countries, and >90 products containing *L. acidophilus*, or bifidobacteria or both are available in the market worldwide [6]. For production of probiotic yoghurt, ABT cultures which have LA, bifidobacteria and *Streptococcus thermophilus* as a main fermenting organism are used. Some of the proposed health benefits are thought to be conferred by live bacteria contained in the products. Suggested minimum numbers of probiotic bacteria at consumption are $10^5$-$10^6$ cfu $g^{-1}$.

On the other hand, virgin olive oil is highly appreciated by consumers because of its health benefits and its pleasant flavor. The Mediterranean diet is associated with a lower incidence of atherosclerosis, cardiovascular disease, neurodegenerative diseases and certain types of cancer. The apparent health benefits have been partially ascribed to the dietary consumption of virgin olive oil by Mediterranean populations [7].

To explore the possibility of the potential use of olive oil in probiotic yogurt production and thereby to open a new channel for olive oil utilization, this research project was undertaken. Therefore, the aim of this study was the possibility of increasing the nutritional and health values of bio-yoghurt by adding olive oil and also possibility of using olive oil as a prebiotics for yoghurt cultures.

## 2. MATERIALS AND METHODS

### 2.1. Starter Cultures and Olive Oil

In the present study, a commercial classic yoghurt starter containing *Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbruckii* subsp. *bulgaricus* (1:1) and ABT culture (ABT-5) with mixed strains of *S. thermophilus* (as sole fermenting organism) and LA + *B. bifidum* (as probiotic organisms) (Chr. Hansen's Lab A/S Copenhagen, Denmark) were used. Starter culture

was in freeze-dried direct-to-vat set form. After procurement, the starter cultures were stored at –18°C in the absence of atmospheric air. Extra virgin olive oil was obtained from El-Wadi Company for Food Industries (Wadi Food), Alexandria, Egypt.

### 2.2. Yoghurt Preparation

Yoghurt samples were prepared from fresh buffalo's and cow's milk mixture 1:1 (acidity 0.17%, pH 6.61, fat 5.1, TS 14.56 and total protein 3.87%) in Dairy Laboratory of El-Serw Animal Production Research Station, Animal Production Research Institute, Agriculture Research Center. Six yoghurt treatments were made using classic yoghurt or ABT cultures. The first yoghurt sample was manufactured using yoghurt starter and whole milk as control whereas the treatments from two to five were made by ABT culture and whole milk fortified with 0, 1, 2, and 4% virgin olive oil. The last yogurt sample was prepared by ABT culture and skim milk with 4% olive oil. The whole or skim milk was tempered to 60°C and fortified with 1, 2 and 4% (wt/wt) virgin olive oil. The mix was blended at 2000 rpm for 3 min, reheated to 85°C for 15 min, cooled to 40°C, inoculated with commercial yoghurt culture (0.1 g/L of yoghurt mix), transferred to 100-ml plastic cups, incubated at 40°C for fully coagulation, and stored at 4°C for 15 days. Yoghurt samples were analyzed in fresh and after 7 and 15 days of refrigerated storage. Three replicates of each treatment were conducted.

### 2.3. Chemical Properties

Total solids, fat, TN and ash contents of samples were determined according to AOAC [8]. Titratable acidity in terms of % lactic acid was measured by titrating 10 g of sample mixed with 10 ml of boiling water against 0.1 N NaOH using phenolphthalein indicator to an end point of faint pink color [6]. pH of the sample was measured using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Water soluble nitrogen (WSN) of yoghurt was estimated according to Ling [9]. Total volatile fatty acids (TVFA) were determined according to Kosikowski [10].

### 2.4. Rheological Properties

The curd tension was determined using the method of Chandrasekhara, et al. [11] whereas the curd syneresis was measured as given by Mehanna and Mehanna [12]. For test of coagulation time during yoghurt making, milk was inculcated with starts and incubated at 40°C then coagulation was noticed at 30 min intervals.

### 2.5. Microbiological Analyses

Yoghurt samples were analyzed for total viable bacterial count (TVBC), lactic acid bacteria (LAB), proteolytic, lipolytic, coliform bacteria, moulds and yeast counts according to the methods described by the American Public Health Association [13]. The count of bifidobacteria was

determined according to Dinakar and Mistry [14]. A mixture of antibiotics, including 2 g of neomycin sulfate, 4 g of paromomycin sulfate, 0.3 g of nalidixic acid, and 60 g of lithium chloride (NPNL, Sigma Chemical Co.), was prepared in 1 L of distilled water, filter-sterilized (.22 pm), and stored at 4°C until use. The mixture of antibiotics (5 ml) was added to 100 ml of MRS agar medium. Cysteine-HC1 was added at the rate of 0.05% to decrease the redox potential of the medium. Plates were incubated at 37°C for 48 to 72 h under anaerobic condition.

### 2.6. Organoleptic Analyses

Samples of yoghurt were organoleptically scored by the staff of the El-Serw Animal Production Research Station, Ministry of Agriculture. The score points were 50 for flavour, 35 for body and texture and 15 for colour and appearance, which give a total score of 100 points.

### 2.7. Statistical Analysis

The obtained results were statistically analyzed using a software package [15] based on analysis of variance. When F-test was significant, least significant difference (LSD) was calculated according to Duncan [16] for the comparison between means. The data presented, in the tables, are the mean (± standard deviation) of 3 experiments.

## 3. RESULTS AND DISCUSSION

### 3.1. Changes in Acidity during Fermentation of Yogurt Mix for 180 Min

Changes in acidity during the 180 min fermentation of yoghurt mix are presented in Table 1. The increase in acidity was slower in bio-yoghurt made using ABT culture than that of the control yoghurt (Treatment 1). The ABT cultures are known to produce yoghurt with a fine, mild taste and low post acidification [17]. Highly proteolytic strains of normal starter could produce higher amount of proteinase enzymes which breakdown milk protein into small peptides that are used as a nitrogen source during the growth of the cells in milk [18]. This in turn would lead to higher growth and acidification rate in milk.

Samples of yoghurt with added olive oil to whole milk showed a slight decrease in titratable acidity during the 180 min of fermentation; also, an increase in the concentration of olive oil adversely affected the rate of acid production. After 180 min of incubation time, the acidity percentage was 0.48% for yoghurt made by ABT (treatment 2) and lowered to 0.47 and 0.46% for yoghurt fortified with 2 and 4% olive oil respectively. Conversely, the acidity ratios increased to 0.50% for yogurt prepared from skim milk and supplemented with 4% olive oil (treatment 6). Comparing between results of Tables 1 and 5, it can be observed that however yoghurt made using ABT culture and fortified with olive oil had lower acidity but also contained higher count of lactic acid bacteria than that of control yoghurt. This can be explained by the less proteolytic and acid production of LA and *S. thermophilus*, the main organism responsible for fermentation in ABT

cultures than that of *L. delbrueckii* subsp. *bulgaricus* in normal starter. This refers that the addition of olive oil had no remarkable effect on starter activity.

### 3.2. Changes in Rheological Properties of Yoghurt

The effect of using ABT culture and adding different concentrations of olive oil to buffalo and cow milk mixture on coagulation time, curd tension and curd syneresis was stated in Table 2. Using of ABT starter caused a slight increase in coagulation time. The time taken to reach a complete coagulation was 3 h for the control yoghurt (Treatment 1). It increased to 3.20 h for bio-yoghurt made using ABT culture (Treatment 2). Saccaro, et al. [19] found that growth of probiotic strains, when grown singly or blends with yoghurt cultures affected the fermentation time and the rate of acidification. Curd tension values were similar for both control and ABT yoghurt. Regarding the curd syneresis, it can be observed that there is a little effect of the using of ABT culture in yoghurt production on syneresis values. After 60 min curd syneresis values were 3.97 and 4.14 gm/15 gm of curd for samples 1 and 2 respectively.

**Table-1.** Effect of adding olive oil to buffaloe's and cow's milk mixture on activity of ABT culture (expressed as acidity percentage)

| Treatments | Incubation time [11] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| 1 | 0.15±0.01$^{a}$ | 0.16±0.01$^{a}$ | 0.18±0.01$^{a}$ | 0.23±0.01$^{a}$ | 0.33±0.01$^{a}$ | 0.42±0.01$^{a}$ | 0.52±0.01$^{a}$ |
| 2 | 0.14±0.01$^{a}$ | 0.14±0.01$^{a}$ | 0.16±0.01$^{a}$ | 0.22±0.01$^{a}$ | 0.31±0.01$^{a}$ | 0.39±0.01$^{ab}$ | 0.48±0.01$^{bc}$ |
| 3 | 0.15±0.01$^{a}$ | 0.16±0.01$^{a}$ | 0.18±0.01$^{a}$ | 0.23±0.01$^{a}$ | 0.31±0.01$^{a}$ | 0.38±0.01$^{b}$ | 0.47±0.01$^{bc}$ |
| 4 | 0.15±0.01$^{a}$ | 0.16±0.01$^{a}$ | 0.19±0.01$^{a}$ | 0.22±0.01$^{a}$ | 0.31±0.01$^{a}$ | 0.38±0.01$^{b}$ | 0.47±0.01$^{bc}$ |
| 5 | 0.15±0.01$^{a}$ | 0.15±0.01$^{a}$ | 0.18±0.01$^{a}$ | 0.22±0.01$^{a}$ | 0.30±0.01$^{a}$ | 0.37±0.01$^{b}$ | 0.46±0.01$^{c}$ |
| 6 | 0.16±0.01$^{a}$ | 0.17±0.01$^{a}$ | 0.19±0.01$^{a}$ | 0.23±0.01$^{a}$ | 0.32±0.01$^{a}$ | 0.40±0.01$^{ab}$ | 0.50±0.01$^{ab}$ |

- Yoghurt made using whole milk and commercial starter (*Streptococcus thermophillus* and *Lactobacillus bulgaricus*) (control)
- Yoghurt made using whole milk and ABT (*Lactobacillus acidophilus (A), bifidobacteria (B), and Streptococcus thermophilus (T))*
- Yoghurt made using whole milk and ABT + 1% olive oil
- Yoghurt made using whole milk and ABT + 2% olive oil
- Yoghurt made using whole milk and ABT + 4% olive oil
- Yoghurt made using skim milk and ABT + 4% olive oil

On the other hand, adding different levels of olive oil had no clear effect on coagulation time and curd tension of yoghurt samples. The same trend was observed between yoghurt made from whole or skim milk. Yoghurts supplemented with olive oil had less syneresis values. Further, with increased level of supplementation, the syneresis in yoghurt decreased. This probably, could be attributed to the increasing of yoghurt total solids by adding olive oil. Morris, et al. [20] reported that increasing the level of total solids tends to yield yoghurt with improved syneresis, viscosity, and microstructure.

**Table-2.** Effect of using of ABT culture and adding of olive oil to buffaloe's and cow's milk mixture on yoghurt rheological properties

| Treatments | Coagulation time (hrs) | Curd tension (g) | Curd syneresis (gm/15 gm of curd)* Time [11] | | | |
|---|---|---|---|---|---|---|
| | | | 10 | 30 | 60 | 120 |
| 1 | 3.00±0.1$^{b}$ | 32.55±0.01$^{d}$ | 1.52±0.01$^{b}$ | 2.99±0.01$^{b}$ | 3.97±0.01$^{b}$ | 5.16±0.01$^{b}$ |
| 2 | 3.20±0.01$^{ab}$ | 32.73±0.01$^{c}$ | 1.76±0.01$^{a}$ | 3.11±0.01$^{a}$ | 4.14±0.01$^{a}$ | 5.37±0.01$^{a}$ |
| 3 | 3.10±0.01$^{b}$ | 31.86±0.01$^{e}$ | 1.45±0.01$^{c}$ | 2.85±0.01$^{c}$ | 3.81±0.01$^{c}$ | 4.99±0.01$^{c}$ |
| 4 | 3.15±0.01$^{b}$ | 32.79±0.01$^{b}$ | 1.33±0.01$^{d}$ | 2.69±0.01$^{d}$ | 3.72±0.01$^{d}$ | 4.83±0.01$^{d}$ |
| 5 | 3.30±0.01$^{a}$ | 32.90±0.01$^{a}$ | 1.24±0.01$^{e}$ | 2.55±0.01$^{e}$ | 3.61±0.01$^{e}$ | 4.76±0.01$^{e}$ |
| 6 | 3.20±0.01$^{ab}$ | 31.04±0.01$^{f}$ | 1.11±0.01$^{f}$ | 2.41±0.01$^{f}$ | 3.54±0.01$^{f}$ | 4.66±0.01$^{f}$ |

*Whey excluded (grams) from 15 gm of curd kept at room temperature after 10, 30, 60 and 120min.

### *3.3.* Changes in Chemical Composition of Yoghurt during Refrigerated Storage for 15 Days

The changes in the titratable acidity (% lactic acid), pH, total solids (TS) , fat and ash during the refrigerated storage of yoghurt are presented in Table 3. The values of titratable acidity % and pH values gradually increased and decreased respectively during refrigerated storage of all samples of yoghurt. This may be due to fermentation of lactose, which produces lactic and acetic acid during fermentation and storage period. These findings are in agreement with the findings of Sabbah, et al. [21] and Ozcan and Kurtuldu [22]. At zero time and during storage period, the acidity ratios of control yoghurt (treatment 1) were higher than that of yoghurt made by ABT culture (treatment 2). pH values had the opposite trend. Moreover, the rise in titratable acidity or drop in pH in control yoghurt was more than that observed in the ABT yogurt. On the other side, control yoghurt gave TS, fat and ash results similar to yogurt prepared by ABT starter. These results were confirmed by resulted of Ayad, et al. [23] who stated that TS, SNF, fat, F/DM and protein values in *bifidus* yoghurt-like products were not affected by bifidobacteria incorporation with yoghurt-like products.

The acidity percentages in yoghurt manufactured using ABT and supplemented with olive oil remained slightly higher than that of yoghurt made by just ABT. Yoghurt acidity and pH value were affected ($P<0.001$) by treatments and the interaction of treatment × age. As it is expected, fortification of milk with various concentrations of olive oil increased TS and fat contents of the resulted yoghurt. The fat value of yoghurt raised from 5.8 to 9.1% by adding 4% olive oil to milk. It is not considered a high fat percentage because it consists of animal and vegetarian fats. Tamime and Robinson [5] showed that the fat content of yoghurts manufactured in the different parts of the world can vary from as low as 0.1% to as high as 10%. Also, substitution of milk fat with 4% olive oil (sample 6) kept TS, fat and ash values similar to those in yoghurt made from whole milk (sample 2). The statistical analysis of variance showed that the differences in TS and fat between treatments and the effect of storage time were highly significant ($P<0.001$).

**Table-3.** Effect of using ABT culture and olive oil on the chemical composition of yoghurt

| Treatments | Storage Period (days) | Acidity % | pH Values | TS % | Fat % | Ash % |
|---|---|---|---|---|---|---|
| 1 | 0 | 0.79±0.01$^{a}$ | 4.70±0.01$^{f}$ | 15.47±0.01$^{e}$ | 5.9±0.1$^{d}$ | 0.88±0.01$^{a}$ |
| | 7 | 1.09±0.01$^{a}$ | 4.42±0.01$^{e}$ | 15.56±0.01$^{e}$ | 5.9±0.1$^{d}$ | 0.91±0.01$^{ab}$ |
| | 15 | 1.25±0.01$^{a}$ | 4.21±0.01$^{e}$ | 15.70±0.01$^{d}$ | 6.0±0.1$^{d}$ | 0.95±0.01$^{a}$ |
| 2 | 0 | 0.64±0.01$^{e}$ | 4.98±0.01$^{b}$ | 15.51±0.01$^{d}$ | 5.8±0.1$^{d}$ | 0.86±0.01$^{a}$ |
| | 7 | 0.84±0.01$^{d}$ | 4.76±0.01$^{a}$ | 15.60±0.01$^{d}$ | 5.8±0.1$^{d}$ | 0.89±0.01$^{ab}$ |
| | 15 | 1.01±0.01$^{d}$ | 4.53±0.01$^{a}$ | 15.72±0.01$^{d}$ | 5.9±0.1$^{d}$ | 0.93±0.01$^{ab}$ |
| 3 | 0 | 0.68±0.01$^{d}$ | 4.88±0.01$^{c}$ | 16.21±0.01$^{c}$ | 6.6±0.1$^{c}$ | 0.87±0.01$^{a}$ |
| | 7 | 0.89±0.01$^{c}$ | 4.66±0.01$^{b}$ | 16.34±0.01$^{c}$ | 6.7±0.1$^{c}$ | 0.90±0.01$^{ab}$ |
| | 15 | 1.10±0.01$^{c}$ | 4.49±0.01$^{b}$ | 16.55±0.01$^{c}$ | 6.8±0.1$^{c}$ | 0.94±0.01$^{ab}$ |
| 4 | 0 | 0.71±0.01$^{cd}$ | 4.84±0.01$^{d}$ | 17.04±0.01$^{b}$ | 7.5±0.1$^{b}$ | 0.89±0.01$^{a}$ |
| | 7 | 0.94±0.01$^{b}$ | 4.60±0.01$^{c}$ | 17.14±0.01$^{b}$ | 7.6±0.01$^{b}$ | 0.92±0.01$^{a}$ |
| | 15 | 1.17±0.01$^{b}$ | 4.50±0.01$^{ab}$ | 17.33±0.01$^{b}$ | 7.6±0.1$^{b}$ | 0.94±0.01$^{ab}$ |
| 5 | 0 | 0.73±0.01$^{bc}$ | 4.80±0.01$^{e}$ | 19.05±0.01$^{a}$ | 9.1±0.1$^{a}$ | 0.89±0.01$^{a}$ |
| | 7 | 0.95±0.01$^{b}$ | 4.58±0.01$^{cd}$ | 19.15±0.01$^{a}$ | 9.2±0.1$^{a}$ | 0.91±0.01$^{ab}$ |
| | 15 | 1.20±0.01$^{b}$ | 4.41±0.01$^{c}$ | 19.21±0.01$^{a}$ | 9.3±0.1$^{a}$ | 0.93±0.01$^{ab}$ |
| 6 | 0 | 0.75±0.01$^{b}$ | 7.76±0.01$^{a}$ | 15.37±0.01$^{f}$ | 4.5±0.1$^{e}$ | 0.86±0.01$^{a}$ |
| | 7 | 0.96±0.01$^{b}$ | 4.55±0.01$^{d}$ | 15.51±0.01$^{f}$ | 4.6±0.1$^{e}$ | 0.88±0.01$^{b}$ |
| | 15 | 1.24±0.01$^{a}$ | 4.37±0.01$^{d}$ | 15.60±0.01$^{e}$ | 4.7±0.1$^{e}$ | 0.91±0.01$^{b}$ |

Table 4 shows TN, TN/DM, WSN, WSN/TN and TVFA values of fresh yoghurt and during storage period. TN contents of various yoghurt samples were not significantly ($p>0.05$) affected by either type of starter or addition of olive oil to milk. In contrast, WSN contents were higher in yoghurt made using normal culture (treatment 1) as compared with that made by ABT (treatment 2). This may be due to the high proteolytic activity of *L. delbrueckii* subsp. *bulgaricus*. Similarly, yoghurt made from skim milk supplemented with 4% olive oil (treatment 6) had higher WSN content compared to the yoghurt made from whole milk supplemented with 1, 2 and 4% olive oil (treatments 3, 4 and 5 respectively). Generally, adding of different levels of olive oil to milk had no significant effect on WSN of the produced yoghurt.

A very slight increase in TVFA was observed in yoghurt produced by normal culture compared to yoghurt produced with ABT starter. Significantly higher contents of TVFA ($P<0.05$) in yoghurt supplemented with different concentrations of olive oil were found and the increase was proportional to the percentages added. As storage period advanced, the contents of WSN and TVFA of all yoghurt samples increased. This could be attributed to starter activity especially proteolytic and lipolytic bacteria. These findings are consistent with those of Azzam [24] who found that increasing of TVFA contents of control and yoghurt fortified with iron-whey protein complex during storage period.

### 3.4. Changes in Microbial Counts of Yoghurt during Refrigerated Storage for 15 Days

The total viable bacterial counts (TVBC) and the viable counts of lactic acid bacteria, bifidobacteria, coliform, proteolytic, lipolytic bacteria and moulds & yeasts during storage of

yogurts made using normal and ABT cultures are shown in Table 5. Data revealed that treatments C, D, E and F were free from coliform bacteria at zero time and during storage period whereas they were detected in just fresh samples A and B with counts of 1 x$10^2$ cfu.$g^{-1}$. Moulds and yeasts were not detected at zero time for all samples and found from the second week.

The counts of total viable and lactic acid bacteria were slightly higher in ABT yoghurt than those of control. In contrary, control yoghurt possessed higher proteolytic, lipolytic, moulds and yeasts numbers as compared with that of ABT yoghurt.

**Table-4.** Effect of using ABT culture and olive oil on TN, WSN and TVFA of yoghurt

| Treatments | Storage Period(days) | TN % | TN/ DM % | WSN % | WSN/TN % | TVFA* |
|---|---|---|---|---|---|---|
| 1 | 0 | 0.694±0.001$^{b}$ | 4.48 | 0.1510.001$^{b}$ | 21.75 | 6.0±0.06$^{d}$ |
| | 7 | 0.701±0.001$^{c}$ | 4.50 | 0.1730.001$^{b}$ | 24.67 | 6.7±0.1$^{e}$ |
| | 15 | 0.722±0.001$^{c}$ | 4.59 | 0.185±0.001$^{a}$ | 25.62 | 7.7±0.1$^{e}$ |
| 2 | 0 | 0.697±0.001$^{b}$ | 4.49 | 0.143±0.001$^{c}$ | 20.51 | 5.8±0.1$^{d}$ |
| | 7 | 0.7040.001$^{bc}$ | 4.51 | 0.166±0.001$^{c}$ | 23.57 | 6.4±0.1$^{e}$ |
| | 15 | 0.7240.001$^{bc}$ | 4.60 | 0.1780.001$^{b}$ | 24.58 | 7.3±0.1$^{f}$ |
| 3 | 0 | 0.696±0.001$^{b}$ | 4.29 | 0.142±0.001$^{c}$ | 20.40 | 6.7±0.1$^{c}$ |
| | 7 | 0.705±0.001$^{b}$ | 4.31 | 0.168±0.001$^{c}$ | 22.82 | 7.4±0.1$^{d}$ |
| | 15 | 0.7230.001$^{bc}$ | 4.36 | 0.1800.001$^{b}$ | 24.89 | 8.4±0.1$^{d}$ |
| 4 | 0 | 0.692±0.001$^{c}$ | 4.08 | 0.144±0.001$^{c}$ | 20.80 | 7.0±0.05$^{c}$ |
| | 7 | 0.705±0.001$^{b}$ | 4.13 | 0.167±0.001$^{c}$ | 23.68 | 7.9±0.1$^{c}$ |
| | 15 | 0.726±0.001$^{b}$ | 4.21 | 0.1790.001$^{b}$ | 24.65 | 8.9±0.1$^{c}$ |
| 5 | 0 | 0.6940.001$^{bc}$ | 3.84 | 0.145±0.001$^{c}$ | 20.89 | 7.5±0.1$^{b}$ |
| | 7 | 0.7030.001$^{bc}$ | 3.87 | 0.168±0.001$^{c}$ | 23.89 | 8.6±0.1$^{b}$ |
| | 15 | 0.721±0.001$^{c}$ | 3.95 | 0.1780.001$^{b}$ | 24.68 | 9.4±0.1$^{b}$ |
| 6 | 0 | 0.701±0.001$^{a}$ | 4.56 | 0.156±0.001$^{a}$ | 22.25 | 7.9±0.1$^{a}$ |
| | 7 | 0.713±0.001$^{a}$ | 4.59 | 0.177±0.001$^{a}$ | 24.82 | 9.0±0.05$^{a}$ |
| | 15 | 0.732±0.001$^{a}$ | 4.69 | 0.188±0.001$^{a}$ | 25.68 | 9.9±0.1$^{a}$ |

* expressed as ml 0.1 NaOH 100 $g^{-1}$ cheese

Yoghurt samples supplemented with various concentrations of olive oil had less numbers of TVBC compared to that in control samples. At reverse this trend, the counts of lactic acid bacteria and bifidobacteria were highest in yoghurt supplemented with olive oil (Table 5). As the level of olive oil supplementation increased to 4% level, the viability of these bacteria improved further. This may be indicates that the starter culture is activated by adding olive oil to yoghurt milk. Almost, the numbers of proteolytic, lipolytic and moulds & yeasts were similar in yoghurt samples with and without adding olive oil.

Irrespective of starter culture or ingredient supplementation, there were steady increases in the counts of TVBC, moulds and yeasts during storage in all yoghurt samples and these changes are presented in Table 5. Similar types of results have been reported by Shenana, et al. [25]. Conversely, the numbers of lactic acid bacteria, bifidobacteria, proteolytic and lipolytic bacteria were lowered. This may be attributed to the higher acidity resulted during storage period. The

growth of the bifidobacteria species is retarded below pH 5.0 whereas that of LA is retarded below pH 4.0 [26], Emara, et al. [27] stated that the population of *S. thermophilus*, *L. bulgaricus*, LA and *B. bifidum* declined during 14 days of storage period. The survival of bifidobacteria depends on many factors, such as the strains used, the level of inoculation, the incubation temperature, the availability of nutrients and the dissolved oxygen concentration.

Despite this drop, the recommended level of $10^6$ or $10^7$ cfu.g$^{-1}$ of bifidobacteria as a probiotic was exceeded for all treatments of bio-yoghurt and remained above$10^6$ or $10^7$ cfu g$^{-1}$ until the end of storage period [28]. One of the possibilities of high stability of bifidobacteria at refrigerated storage in these samples could be the absence of *Lb. delbrueckii* ssp. *bulgaricus* which is known to produce post acidification. Post acidification could have further inhibitory effect on the *Streptococcus thermophilus* counts.

**Table-5.** Effect of using ABT culture and adding of olive oil on some microbial groups of yoghurt

| Treatments | Storage Period (days) | TVBC (x $10^5$) | Lactic acid bacteria (x $10^3$) | Bifido-bacteria (x $10^5$) | Coliform bacteria (x $10^2$) | Proteolytic bacteria (x $10^2$) | Lipolytic bacteria (x $10^2$) | Moulds & Yeast (x $10^3$) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 42±1.00$^{bc}$ | 18±1.00$^{d}$ | -±0.00$^{d}$ | 1±1.00$^{a}$ | 6±1.00$^{a}$ | 5±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 128±1.00$^{a}$ | 14±1.00$^{c}$ | -±0.00$^{d}$ | 0±0.00$^{a}$ | 5±1.00$^{a}$ | 3±1.00$^{a}$ | 38±1.00$^{a}$ |
| | 15 | 437±1.00$^{b}$ | 8±1.00$^{b}$ | -±0.00$^{d}$ | 0±0.00$^{a}$ | 3±1.00$^{a}$ | 1±1.00$^{a}$ | 115±1.00$^{a}$ |
| 2 | 0 | 51±1.00$^{a}$ | 21±1.00$^{cd}$ | 28±1.00$^{c}$ | 1±0.00$^{a}$ | 4±1.00$^{a}$ | 4±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 131±1.00$^{a}$ | 18±1.00$^{b}$ | 23±1.00$^{c}$ | 0±0.00$^{a}$ | 3±1.00$^{a}$ | 3±1.00$^{a}$ | 31±1.00$^{bc}$ |
| | 15 | 445±1.00$^{a}$ | 13±1.00$^{a}$ | 17±1.00$^{c}$ | 0±0.00$^{a}$ | 1±1.00$^{a}$ | 1±1.00$^{a}$ | 107±1.00$^{bc}$ |
| 3 | 0 | 45±1.00$^{b}$ | 23±1.00$^{bc}$ | 34±1.00$^{b}$ | 0±0.00$^{a}$ | 4±1.00$^{a}$ | 4±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 124±1.00$^{b}$ | 17±1.00$^{bc}$ | 29±1.00$^{b}$ | 0±0.00$^{a}$ | 2±1.00$^{a}$ | 3±1.00$^{a}$ | 32±1.00$^{b}$ |
| | 15 | 435±1.00$^{b}$ | 13±1.00$^{a}$ | 25±1.00$^{b}$ | 0±0.00$^{a}$ | 1±1.00$^{a}$ | 2±1.00$^{a}$ | 104±1.00$^{cd}$ |
| 4 | 0 | 39±1.00$^{cd}$ | 26±1.00$^{ab}$ | 44±1.00$^{a}$ | 0±0.00$^{a}$ | 3±1.00$^{a}$ | 4±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 118±1.00$^{c}$ | 19±1.00$^{b}$ | 38±1.00$^{a}$ | 0±0.00$^{a}$ | 2±1.00$^{a}$ | 3±1.00$^{a}$ | 28±1.00$^{c}$ |
| | 15 | 426±1.00$^{c}$ | 14±1.00$^{a}$ | 34±1.00$^{a}$ | 0±0.00$^{a}$ | 1±1.00$^{a}$ | 1±1.00$^{a}$ | 110±1.00$^{b}$ |
| 5 | 0 | 35±1.00$^{e}$ | 27±1.00$^{a}$ | 46±1.00$^{a}$ | 0±0.00$^{a}$ | 3±1.00$^{a}$ | 3±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 111±1.00$^{d}$ | 20±1.00$^{ab}$ | 40±1.00$^{a}$ | 0±0.00$^{a}$ | 2±1.00$^{a}$ | 2±1.00$^{a}$ | 30±1.00$^{bc}$ |
| | 15 | 415±1.00$^{d}$ | 14±1.00$^{a}$ | 33±1.00$^{a}$ | 0±0.00$^{a}$ | 0±0.00$^{a}$ | 0±0.00$^{a}$ | 102±1.00$^{d}$ |
| 6 | 0 | 36±1.00$^{de}$ | 29±1.00$^{a}$ | 45±1.00$^{a}$ | 0±0.00$^{a}$ | 4±1.00$^{a}$ | 4±1.00$^{a}$ | 0±0.00$^{d}$ |
| | 7 | 109±1.00$^{d}$ | 23±1.00$^{a}$ | 39±1.00$^{a}$ | 0±0.00$^{a}$ | 3±1.00$^{a}$ | 3±1.00$^{a}$ | 33±1.00$^{b}$ |
| | 15 | 410±1.00$^{e}$ | 16±1.00$^{a}$ | 33±1.00$^{a}$ | 0±0.00$^{a}$ | 2±1.00$^{a}$ | 2±1.00$^{a}$ | 109±1.00$^{b}$ |

### 3.5. Changes in Sensory Evaluation of Yoghurt during Refrigerated Storage for 15 Days

Table 6 presents sensory quality of yoghurt made using ABT culture and fortified with various concentrations of olive oil. Sensory profiles of fresh yoghurts and within storage period suggested that starter type had no significant effect (P>0.05) on the color& appearance, body& texture and flavor scores and thus total scores of all the yoghurts. On the other hand, fortifying of milk with olive oil had no significant effect (P>0.05) on the color and appearance score of fresh bio-yoghurt whereas at the end of storage stage it slightly decreased. The color and appearance ratings were 13, 13 and 13 and reached to 12, 11 and 10 after 15 days of storage for yoghurt samples fortified with 0, 2 and 4% olive oil respectively. The majority of panelists showed that olive oil adding improved the body and texture of the resultant yoghurt. The body and texture of yogurt fortified with 4% olive oil instead of milk fat (treatment 6) were acceptable and had similar

ratings as plain yoghurt (treatment 2). It was noticed from Table 6 that flavor of the product was slightly improved with incorporation of olive oil. At the beginning of storage period, yoghurts made with olive oil supplementation of 1% and 2% resulted into 2% and 4% increase in flavor improvement, respectively. On day 15, a slight drop in the color& appearance, body& texture and flavor scores of all the yoghurt samples was evident. Despite this, olive oil supplemented yoghurts made with ABT starter culture were as good as control yogurts based on total scores. Hanif, et al. [29] found that flavor, body & texture, taste and appearance scores of yogurt decreased after 15 days of storage.

## 4. CONCLUSIONS

Making yoghurt using ABT culture and fortifying with olive oil are of great interest to improve the functionality and create functional foods with health benefits. The addition of olive oil to bio-yoghurt would complement its healthy characteristics. This study has shown that fortifying yoghurt made by ABT starter with 1, 2 and 4% olive oil produced an acceptable product with potential beneficial health effects.

**Table-6.** Effect of using ABT culture and adding of olive oil on organoleptic properties of yoghurt

| Treatments | Storage Period(days) | Color& Appearance (15) | Body& Texture (35) | Flavor (50) | Total (100) |
|---|---|---|---|---|---|
| 1 | 0 | 13±1.00$^{a}$ | 31±1.00$^{a}$ | 45±1.00$^{a}$ | 89 |
| | 7 | 13±1.00$^{a}$ | 31±1.00$^{a}$ | 45±1.00$^{a}$ | 89 |
| | 15 | 12±1.00$^{a}$ | 29±1.00$^{ab}$ | 42±1.00$^{a}$ | 83 |
| 2 | 0 | 13±1.00$^{a}$ | 31±1.00$^{a}$ | 44±1.00$^{a}$ | 88 |
| | 7 | 13±1.00$^{a}$ | 31±1.00$^{a}$ | 44±1.00$^{a}$ | 88 |
| | 15 | 12±1.00$^{a}$ | 28±1.00$^{b}$ | 42±1.00$^{a}$ | 82 |
| 3 | 0 | 13±1.00$^{a}$ | 32±1.00$^{a}$ | 45±1.00$^{a}$ | 90 |
| | 7 | 12±1.00$^{a}$ | 32±1.00$^{a}$ | 45±1.00$^{a}$ | 89 |
| | 15 | 11±1.00$^{a}$ | 30±1.00$^{ab}$ | 42±1.00$^{a}$ | 83 |
| 4 | 0 | 13±1.00$^{a}$ | 32±1.00$^{a}$ | 46±1.00$^{a}$ | 91 |
| | 7 | 12±1.00$^{a}$ | 32±1.00$^{a}$ | 46±1.00$^{a}$ | 90 |
| | 15 | 11±1.00$^{a}$ | 31±1.00$^{ab}$ | 43±1.00$^{a}$ | 85 |
| 5 | 0 | 13±1.00$^{a}$ | 33±1.00$^{a}$ | 45±1.00$^{a}$ | 91 |
| | 7 | 12±1.00$^{a}$ | 33±1.00$^{a}$ | 43±1.00$^{a}$ | 88 |
| | 15 | 10±1.00$^{a}$ | 32±1.00$^{a}$ | 42±1.00$^{a}$ | 84 |
| 6 | 0 | 13±1.00$^{a}$ | 31±1.00$^{a}$ | 46±1.00$^{a}$ | 90 |
| | 7 | 12±1.00$^{a}$ | 31±1.00$^{a}$ | 45±1.00$^{a}$ | 88 |
| | 15 | 10±1.00$^{a}$ | 28±1.00$^{b}$ | 43±1.00$^{a}$ | 81 |

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