

## Impact of using some mutant strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* for production of yoghurt with mild taste

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### Abstract

The mutant strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used to improve of yoghurt quality. The produced yoghurts were kept refrigerated and analyzed when fresh, 7, 14, 21 and 28 days for chemical, microbiological and sensory characteristics. The obtained results revealed that the prolonging of the coagulation time of some treatments to be with a maximum of 7.20 and 6.20 hr for T3 and T6, respectively. No pronounced differences were observed all over the storage period in the chemical composition of yoghurt including T.S, Fat and protein content, T2 and T5 recorded the highest content of acetaldehyde. Moreover, using of the mutant of starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* Subsp. *bulgaricus* improved the organoleptic properties, mild taste and the shelf life of the produced yoghurt compared with the control.

**Key words:** mutant strains, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, yoghurt, mild taste

### Introduction

Yoghurt considers to be the most popular fermented milk, as witnessed by its worldwide distribution. Yoghurt is the fermented milk typically contains a starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* which are normally used. Consumer acceptance of yoghurt depends on physical, chemical attributes, acidity aroma perceptions and textural properties (Yilmaz *et al* 2015). During making of yoghurt the acidity is consequence of lactic acidification obtained at the end of incubation and post acidification during storage. Lactic fermentation is the result of lactose fermentation by the associative growth of two thermophilic, homofermentative lactic acid bacteria, *i.e* *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The fermentation process are influenced by the quality of the milk, the used strain and incubation condition. (Mani-lopez *et al*, 2014).

Introducing of new strains of lactic acid bacteria to yoghurt manufacturer is a good approach in order to produce effective, acceptable and affordable fermented products. However, introducing new strains of lactic acid bacteria to yoghurt starter culture may affects acidity, aroma perceptions and textural properties of the product. Also, the rheological properties affect sensory perceptions and ultimately the acceptance of the product by the consumer. The quality of yoghurt curd, similarly to the other products obtained as the result of the milk fermentation process, depends on the quality and composition of the applied bacterial cultures (Cais-Sokolinska *et al.*, 2004). Appropriate proportion used in the bacterial culture precondition their mutual development and, hence, the proper course of the milk protein coagulation process following the

acidification of the environment resulting in the formation of the casein gel of ordered network structure (Gomes and Malcata, 1999).

Mutation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* expressing a mutant lactose permease, the lactose transport activity of which is modified. These strains, and ferments comprising them, can be used to obtain fermented dairy products having good conservation properties. (van den Bogaard *et al.*, 2000)

Yogurts are conventionally obtained by fermentation of milk with a combination of various lactic acid bacteria, chosen from strains of *Streptococcus thermophilus* and of *Lactobacillus delbrueckii* subsp. *bulgaricus*. During the fermentation these bacteria mainly use lactose as energy substrate, and produce lactic acid which leads to coagulation of the milk. This phenomenon, known as post-acidification, is responsible for degradation of the organoleptic qualities of the product during its storage. Also, the produced lactic acid known as (L) lactic acid and this most useful type for nutrition.

Post-acidification results essentially from the use, by the bacteria, of the lactose remaining in the product at the end of the controlled acidification step. In order to prevent it, it has been proposed to use strains of lactic acid bacteria which do not ferment lactose, or ferment it very little. Gunnewijk and Poolman (2000)

In order to obtain non-post-acidifying strains of lactic acid bacteria, it has therefore been proposed to produce artificial variants, or to select natural mutants, in which the activity of at least one of these enzymes is affected. All of these will give an good characteristics of the final product.

So, the aim of this study was to utilize different mutant lactic acid strains (*i.e* *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp.

*bulgaricus*) in developing a yoghurt of high acceptability with mild taste and their effect on physical, chemical, sensory, microbiology, evaluation properties and shelf life of yoghurt.

## Materials and Methods

### Bacterial strains:

*Streptococcus thermophilus* J 34-6 (St. 6) , *St. thermophilus* J 34-12 (St.12). *Lactobacillus delbrueckii* subsp *bulgaricus* 92063 (Lb.63), *Lb. delbrueckii* subsp *bulgaricus* pH-S-mutant 64 (Lb.64), *Lb. delbrueckii* subsp *bulgaricus* pH-P 11 mutant (Lb.pH 11) and *Streptococcus thermophilus* (ST) & *Lactobacillus delbrueckii* subsp *bulgaricus* (LB) (control) were obtained from the culture collection of the Federal Research Center for Nutrition and Food, Kiel (Germany).

### Manufacture of yoghurt:

Yoghurt was manufactured by using UHT milk according to (Tamime 1978). Milk was pre-warmed to 50°C and divided into seven portions and then inoculated with deferent starter cultures as the following. Original *Streptococcus* and *Lactobacillus*. (Control), St.6 and Lb.63 (T1), St.6 and Lb.64 (T2), St.6 and Lb-pH 11 (T3), St.12 and Lb.63 (T4), St.12 and Lb.64 (T5), St.12 and Lb.pH 11 (T6) at the level of 2% (1% *Streptococcus* + 1% *Lactobacillus*) and incubated at 42°C to complete the coagulation (pH 4.65). Yoghurts were stored at 5°C and analysed when fresh and after 7, 14, 21 and 28 days, respectively.

### Chemical analysis:

Total solids, fat and protein of yoghurt were determined according to the International Dairy Federation (IDF) Standards, 1993, 1991a and b, respectively. PH values of Yoghurts were determined using pH meter (Mettler Delta 320, Germany) according to the method described by BSI (1985). Lactic acid: D-and L-lactic acid and Acetaldehyde

content were determined by using UV-method, Cat. No. 11 112 821 035 and Cat-No 10668613035, respectively of R-BIOPHARM AG, Landwehrstr. 5, D-64293 Darmstadt, Germany.

### Microbiological analysis:

*Streptococcus strains* were counted on M 17 agar medium according to (Terzaghi and Sandine, 1975) and *Lactobacillus strains* counted on MRS agar medium according to (De Man *et al.*, 1960).

### Sensory analysis:

The organoleptic properties were evaluated according to (El-Etriby *et al.*, 1997 and Mehanna *et al.*, 2000) including flavour 60 points; body and texture 30 points and appearance was given score of 10 points. The organoleptic evaluations were done by trained panelists of a sensory group of the Fedral Research Center for Nutrition and Food at Kiel, Germany.

### Statistical analysis:

The statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver. 22). Data were treated as complete randomization design according to **Steel *et al.* (1997)**. Multiple comparisons were carried out applying LSD

## Results and Discussion

### Coagultion time:

Acid development during the coagulation of yoghurt as affected by using different mutant starter culture during incubation at 42°C are presented in Table (1). Starter culture activity for all treatments showed low acid production with different rates than the control. The control yoghurt recorded the lowest coagulation time and recorded 4 hr to reach coagulation followed by T5 and T4 which recorded 4:40 and 5 hr to reach coagulation, respectively.

**Table 1.** Effect of using different starter cultures on the pH of yoghurt during incubation and the time of coagulation.

Incubation time (hr)	Control	T1	T2	T3	T4	T5	T6
0	6.41	6.42	6.42	6.45	6.43	6.43	6.44
1	6.00	6.20	6.27	6.31	6.16	6.18	6.25
2	5.61	6.05	6.12	6.13	5.96	5.90	6.03
3	4.86	5.41	5.46	5.61	5.15	5.05	5.37
4	4.65	4.96	4.98	5.31	4.79	4.70	5.03
5		4.77	4.74	5.09	4.65	4.61	4.88
6		4.65	4.60	4.87			4.70
7				4.74			
Coagulation time (hr)	4:0	6:0	5:30	7:20	5:0	4:40	6:20

However, T3 (Str.6 and Lb.pH 11) recorded the highest coagulation time up to 7:20 hr to reach the pH 4.6. Variability in coagulation time of yoghurt could be attributed to the effect of the mutant strains added to ferment milk and producing acid with different rates. Similar results were reported by Möller *et al.* (2007) who found that yoghurt produced with Lb. pH was characterized by low activity and little post-acidification during storage. Moreover, Lb pH-p11 showed the lowest cell counts, in yoghurt which corresponded with its slower growth during fermentation.

#### **Changes in chemical composition of yoghurt during refrigerated storage:**

##### **Total solids:**

Data of total solids of the produced yoghurts presented in Table (2) showed a slight difference among the control and the other treatments. During storage periods the total solid values of yoghurt from all treatments were slightly increased up to the end of storage (28 days), this may be due to the loss of some moisture during storage. These results are in agreement with El-Nagga and Abd El-Tawab (2012) and Abdou *et al.* (2015).

##### **Fat and protein contents:**

Data of fat and protein contents of the produced yoghurt and other treatments produced by the added mutant starter cultures are presented in Table (2). It's evident that the average content of both fat and protein revealed that there were no differences among the treatments and also among the control when fresh. This indicates that there was no effect on fat, protein and hence T.S. content due to the type of the mutant starter cultures added. Similar results were obtained by Hussein (2010).

At the beginning of storage, a slight increase was observed in all treatments for fat and protein contents. This may be attributed to the limited increase of T.S. due to evaporation of some water during the cold storage. The results are in accordance with Hussein (2010) and Abdou *et al.* (2015).

By the end of the storage period of the produced yoghurt, there was a slight decrease in both fat and protein contents and this could be attributed to the limited lipolytic and proteolytic effect of the added yoghurt cultures. These results are in the same trend with El-Nagga and Abd El-Tawab (2012) and Abdou *et al.* (2015).

##### **Acetaldehyde content:**

Data presented in Table (2) shows the effect of different mutant starter cultures on acetaldehyde

content of the produced yoghurt. These results indicated that the control yoghurt had lower acetaldehyde content than the other treatments followed by T6 and T3, respectively

Moreover, T2 and T5 recorded the highest acetaldehyde content (1.68 and 1.56 mg/100g, respectively). These results are in accordance with El-Nagar *et al.* (2007) and El-Alfy, *et al.* (2011). They observed that the level of acetaldehyde was higher in all bio-yoghurt than the control when it was fresh and all over the storage periods and this may be due to the difference in metabolic activity of the starter cultures. With the progress of storage period, the acetaldehyde content of all treatments was increased. The obtained results are in agreement with Abdou *et al.* (2015). Who found that the acetaldehyde content influenced significantly ( $P > 0.001$ ) by starter culture used and by prolonging storage period. The maximum values of acetaldehyde reached after 7 days of storage then started to decrease up to the end of storage.

##### **pH values:**

The changes of pH values of yoghurt made with different mutant starter cultures during storage presented in Table (2). In fresh yoghurt it could be noticed that the control recorded the lowest pH value (4.21), while T6 recorded the highest pH value (4.97). This may be due to the high acidity of the starter culture in the control. With the progress of storage, the acidity of yoghurt was slightly developed and hence the pH values slightly decreased in all treatments. The pH values of the control were lower than the other treatments. This reflects the effect of using mutant starter cultures on reducing and hence prolonging the shelf life of the yoghurt made with added mutant starter cultures and the produced yoghurt had mild character and acidity.

These results are in agreement with Möller *et al.* (2007) and Gomaa (2015). Who observed that pH values higher than 4.0 after 4 weeks of cold storage apparently corresponded with mild character of the yoghurt produced. Moreover, they showed that yoghurt produced with pH-p11 did not have an intensive yoghurt flavor immediately after manufacture. However, during storage more intensive yoghurt flavors were developed with no defects detected and have little acidity. This yoghurt was comparable to that produced.

**Table 2.** Chemical composition of yoghurt made with the different mutant starter cultures when fresh and during storage at 15° C up to 28 days

Parameter	Treatment	Storage period (days)					Mean	
		0 (fresh)	7	14	21	28		
T.S. (%)	Control	13.32 <sup>aA</sup>	13.36 <sup>cB</sup>	13.37 <sup>abC</sup>	13.42 <sup>abD</sup>	13.45 <sup>abcE</sup>	13.38 <sup>b</sup>	
	T1	13.28 <sup>aA</sup>	13.32 <sup>abB</sup>	13.35 <sup>aC</sup>	13.40 <sup>aD</sup>	13.44 <sup>abE</sup>	13.36 <sup>a</sup>	
	T2	13.30 <sup>abA</sup>	13.34 <sup>abcB</sup>	13.36 <sup>abC</sup>	13.45 <sup>cdD</sup>	13.47 <sup>cdE</sup>	13.38 <sup>b</sup>	
	T3	13.30 <sup>abA</sup>	13.35 <sup>bcB</sup>	13.38 <sup>bC</sup>	13.44 <sup>bdD</sup>	13.46 <sup>abE</sup>	13.39 <sup>b</sup>	
	T4	13.31 <sup>bcA</sup>	13.36 <sup>cB</sup>	13.37 <sup>abB</sup>	13.45 <sup>cC</sup>	13.46 <sup>abcD</sup>	13.39 <sup>b</sup>	
	T5	13.29 <sup>abA</sup>	13.34 <sup>abcB</sup>	13.36 <sup>abC</sup>	13.42 <sup>abD</sup>	13.47 <sup>cdE</sup>	13.38 <sup>b</sup>	
	T6	13.30 <sup>abA</sup>	13.33 <sup>abB</sup>	13.35 <sup>aC</sup>	13.44 <sup>bcD</sup>	13.49 <sup>dE</sup>	13.38 <sup>b</sup>	
	Mean	13.30 <sup>A</sup>	13.34 <sup>B</sup>	13.36 <sup>C</sup>	13.43 <sup>D</sup>	13.46 <sup>E</sup>		
Fat (%)	Control	3.46 <sup>aD</sup>	3.42 <sup>aCD</sup>	3.39 <sup>aC</sup>	3.32 <sup>bcB</sup>	3.27 <sup>aA</sup>	3.37 <sup>a</sup>	
	T1	3.42 <sup>aD</sup>	3.40 <sup>aCD</sup>	3.36 <sup>aC</sup>	3.28 <sup>bB</sup>	3.22 <sup>aA</sup>	3.34 <sup>a</sup>	
	T2	3.45 <sup>aD</sup>	3.44 <sup>aCD</sup>	3.40 <sup>aC</sup>	3.10 <sup>aA</sup>	3.25 <sup>aB</sup>	3.33 <sup>a</sup>	
	T3	3.43 <sup>aB</sup>	3.43 <sup>aB</sup>	3.40 <sup>aB</sup>	3.30 <sup>bA</sup>	3.26 <sup>aA</sup>	3.36 <sup>a</sup>	
	T4	3.44 <sup>aC</sup>	3.42 <sup>abC</sup>	3.39 <sup>aB</sup>	3.29 <sup>bA</sup>	3.26 <sup>aA</sup>	3.36 <sup>a</sup>	
	T5	3.45 <sup>aD</sup>	3.44 <sup>aCD</sup>	3.40 <sup>abC</sup>	3.36 <sup>cB</sup>	3.24 <sup>aA</sup>	3.38 <sup>a</sup>	
	T6	3.43 <sup>aD</sup>	3.41 <sup>aCD</sup>	3.38 <sup>aC</sup>	3.30 <sup>bB</sup>	3.23 <sup>aA</sup>	3.35 <sup>a</sup>	
	Mean	3.44 <sup>C</sup>	3.42 <sup>bC</sup>	3.39 <sup>B</sup>	3.28 <sup>A</sup>	3.25 <sup>A</sup>		
Protein (%)	Control	3.97 <sup>cBC</sup>	3.98 <sup>cBC</sup>	4.00 <sup>cdC</sup>	3.95 <sup>eB</sup>	3.89 <sup>dA</sup>	3.96 <sup>d</sup>	
	T1	3.92 <sup>aC</sup>	3.93 <sup>abCD</sup>	3.96 <sup>abd</sup>	3.91 <sup>dB</sup>	3.82 <sup>cA</sup>	3.91 <sup>ab</sup>	
	T2	3.95 <sup>abcC</sup>	3.96 <sup>bcC</sup>	3.97 <sup>bcC</sup>	3.89 <sup>bcdB</sup>	3.80 <sup>cA</sup>	3.91 <sup>c</sup>	
	T3	3.96 <sup>bcC</sup>	3.95 <sup>abcC</sup>	3.98 <sup>bcdC</sup>	3.90 <sup>cdB</sup>	3.79 <sup>bcA</sup>	3.92 <sup>c</sup>	
	T4	3.97 <sup>cC</sup>	3.98 <sup>cdC</sup>	4.01 <sup>dD</sup>	3.87 <sup>abcB</sup>	3.76 <sup>abA</sup>	3.92 <sup>c</sup>	
	T5	3.93 <sup>abC</sup>	3.94 <sup>abC</sup>	3.96 <sup>abC</sup>	3.85 <sup>aB</sup>	3.75 <sup>aA</sup>	3.89 <sup>ab</sup>	
	T6	3.94 <sup>abcC</sup>	3.92 <sup>aC</sup>	3.93 <sup>aC</sup>	3.86 <sup>abB</sup>	3.75 <sup>aA</sup>	3.88 <sup>a</sup>	
	Mean	3.95 <sup>C</sup>	3.95 <sup>C</sup>	3.97 <sup>C</sup>	3.89 <sup>B</sup>	3.79 <sup>A</sup>		
Acetaldehyde (mg/100 g)	Control	0.41 <sup>aA</sup>	0.48 <sup>aA</sup>	0.49 <sup>aA</sup>	0.48 <sup>aA</sup>	0.41 <sup>aA</sup>	0.45 <sup>a</sup>	
	T1	0.69 <sup>cA</sup>	0.81 <sup>bAB</sup>	0.90 <sup>cB</sup>	0.89 <sup>bB</sup>	0.87 <sup>cB</sup>	0.83 <sup>c</sup>	
	T2	1.68 <sup>dA</sup>	1.82 <sup>dAB</sup>	1.96 <sup>eBC</sup>	2.04 <sup>cCD</sup>	2.19 <sup>eD</sup>	1.94 <sup>e</sup>	
	T3	0.48 <sup>abA</sup>	0.52 <sup>aA</sup>	0.55 <sup>abA</sup>	0.56 <sup>aA</sup>	0.56 <sup>abA</sup>	0.53 <sup>a</sup>	
	T4	0.62 <sup>bcA</sup>	0.67 <sup>abA</sup>	0.69 <sup>abA</sup>	0.64 <sup>aA</sup>	0.64 <sup>ba</sup>	0.65 <sup>b</sup>	
	T5	1.56 <sup>dA</sup>	1.62 <sup>cAB</sup>	1.66 <sup>dAB</sup>	1.71 <sup>bAB</sup>	1.77 <sup>dB</sup>	1.66 <sup>d</sup>	
	T6	0.46 <sup>abA</sup>	0.49 <sup>aA</sup>	0.51 <sup>abA</sup>	0.50 <sup>aA</sup>	0.49 <sup>aA</sup>	0.49 <sup>a</sup>	
	Mean	0.84 <sup>A</sup>	0.92 <sup>A</sup>	0.97 <sup>B</sup>	0.97 <sup>B</sup>	0.99 <sup>cB</sup>		
pH	Control	4.32 <sup>aD</sup>	4.18 <sup>aC</sup>	4.13 <sup>abC</sup>	4.08 <sup>aB</sup>	4.00 <sup>aA</sup>	4.14 <sup>a</sup>	
	T1	4.49 <sup>bC</sup>	4.32 <sup>cB</sup>	4.19 <sup>aA</sup>	4.17 <sup>bcA</sup>	4.15 <sup>bcA</sup>	4.26 <sup>cd</sup>	
	T2	4.36 <sup>aC</sup>	4.22 <sup>abB</sup>	4.15 <sup>aA</sup>	4.13 <sup>abA</sup>	4.11 <sup>ba</sup>	4.19 <sup>ab</sup>	
	T3	4.69 <sup>cD</sup>	4.65 <sup>dD</sup>	4.53 <sup>cC</sup>	4.47 <sup>dB</sup>	4.41 <sup>dA</sup>	4.55 <sup>e</sup>	
	T4	4.52 <sup>bC</sup>	4.38 <sup>cB</sup>	4.25 <sup>ba</sup>	4.23 <sup>cA</sup>	4.20 <sup>cA</sup>	4.32 <sup>d</sup>	
	T5	4.38 <sup>aC</sup>	4.25 <sup>bbB</sup>	4.17 <sup>aA</sup>	4.21 <sup>cAB</sup>	4.18 <sup>cA</sup>	4.24 <sup>bc</sup>	
	T6	4.97 <sup>dD</sup>	4.64 <sup>dC</sup>	4.56 <sup>cB</sup>	4.52 <sup>dAB</sup>	4.50 <sup>eA</sup>	4.64 <sup>f</sup>	
	Mean	4.53 <sup>D</sup>	4.38 <sup>C</sup>	4.28 <sup>B</sup>	4.26 <sup>AB</sup>	4.22 <sup>A</sup>		
Lactic acid (g/100 g)	D	Control	0.270 <sup>dA</sup>	0.304 <sup>ba</sup>	0.424 <sup>dB</sup>	0.442 <sup>cdB</sup>	0.420 <sup>cb</sup>	0.372 <sup>d</sup>
		T1	0.120 <sup>ba</sup>	0.126 <sup>aAB</sup>	0.192 <sup>bc</sup>	0.170 <sup>abABC</sup>	0.174 <sup>abBC</sup>	0.156 <sup>b</sup>
		T2	0.342 <sup>eA</sup>	0.404 <sup>cB</sup>	0.548 <sup>cC</sup>	0.646 <sup>eD</sup>	0.624 <sup>eD</sup>	0.513 <sup>e</sup>
		T3	0.098 <sup>ba</sup>	0.114 <sup>aA</sup>	0.144 <sup>baB</sup>	0.182 <sup>bb</sup>	0.184 <sup>bb</sup>	0.144 <sup>b</sup>
		T4	0.164 <sup>ca</sup>	0.262 <sup>bbB</sup>	0.278 <sup>cB</sup>	0.422 <sup>cC</sup>	0.402 <sup>cC</sup>	0.306 <sup>c</sup>
		T5	0.278 <sup>dA</sup>	0.290 <sup>ba</sup>	0.444 <sup>dB</sup>	0.496 <sup>dBC</sup>	0.500 <sup>dC</sup>	0.402 <sup>d</sup>
	T6	0.022 <sup>aA</sup>	0.074 <sup>ab</sup>	0.078 <sup>ab</sup>	0.118 <sup>ab</sup>	0.114 <sup>ab</sup>	0.081 <sup>a</sup>	
	Mean	0.185 <sup>A</sup>	0.225 <sup>A</sup>	0.305 <sup>B</sup>	0.354 <sup>B</sup>	0.345 <sup>B</sup>		
L	Control	0.760 <sup>fA</sup>	0.980 <sup>fb</sup>	1.180 <sup>fc</sup>	1.260 <sup>gd</sup>	1.424 <sup>ge</sup>	1.121 <sup>d</sup>	
	T1	0.738 <sup>eA</sup>	0.900 <sup>eb</sup>	0.996 <sup>dc</sup>	1.058 <sup>dd</sup>	1.060 <sup>dd</sup>	0.950 <sup>c</sup>	
	T2	0.644 <sup>ca</sup>	0.746 <sup>db</sup>	1.044 <sup>ed</sup>	0.918 <sup>ec</sup>	0.924 <sup>ec</sup>	0.855 <sup>bc</sup>	
	T3	0.642 <sup>ca</sup>	0.642 <sup>aA</sup>	0.740 <sup>ab</sup>	0.842 <sup>ac</sup>	0.846 <sup>ac</sup>	0.742 <sup>a</sup>	
	T4	0.704 <sup>dA</sup>	0.704 <sup>ca</sup>	1.050 <sup>eb</sup>	1.140 <sup>fc</sup>	1.152 <sup>fd</sup>	0.950 <sup>c</sup>	
	T5	0.576 <sup>aA</sup>	0.670 <sup>bb</sup>	0.934 <sup>cC</sup>	1.086 <sup>ed</sup>	1.080 <sup>ed</sup>	0.869 <sup>bc</sup>	
	T6	0.622 <sup>ba</sup>	0.668 <sup>ba</sup>	0.768 <sup>bb</sup>	0.882 <sup>bc</sup>	0.882 <sup>bc</sup>	0.764 <sup>ab</sup>	
	Mean	0.669 <sup>A</sup>	0.759 <sup>B</sup>	0.959 <sup>C</sup>	1.027 <sup>CD</sup>	1.053 <sup>D</sup>		

a, b & c: Superscripts to be compared statistically within the same column. Values with different letter superscripts are significantly different (P<0.05).

A, B & C: Superscripts to be compared statistically within the same row. Values with different letter superscripts are significantly different (P<0.05).

With wild type *Lb. delbrueckii* subsp. *bulgaricus*, however, the later yoghurt gained more acidity during cold storage of such product.

#### Lactic acid:

The presence of lactic acid in dairy products is a reflection of bacterial overgrowth. The D-lactic acid is rare finding in human however, L-lactic acid is a common finding in many foods and dairy products due to the action of fermentation by lactic acid bacteria. The taste and flavour of acid foods and dairy products are due to the presence of L-lactic acid, so, it is important that good starter culture should produce high quantity of L-lactic acid during the fermentation process (Noll, 1988). The average of lactic acid content of fresh yoghurt for all treatments are illustrated in Table (2). The control yoghurt samples recorded the highest acidity than the all other treatments either when fresh or along the storage period. Moreover, the produced L(+) lactic acid isomer is higher than the D (-) isomer either for the control or all the treatments when fresh and during the refrigerated storage period. During storage the L (+) isomer and D (-) isomer of lactic acid were increased up to the end of the storage with a different levels according to the starter cultures used. These results are in agreement with De Noni *et al.* (2004)

#### Microbiological properties of yoghurt:

The Streptococci and Lactobacilli counts in yoghurt made with different mutant starter cultures are presented in Figs. (1 and 2). During refrigerated storage, it was observed that the *Streptococci* and *Lactobacilli* counts were slightly increased then gradually decreased for the control and the other treatments till the end of storage period up to 28 days.

On the other hand, it was reported that the *Lactobacilli* counts of the control and treatments were increased at the commencement of the storage period up to the 7<sup>th</sup> day of storage then the counts were slightly decreased till the end of storage up to 28 days. The gradual decline of both *Streptococci* and *Lactobacilli* counts may be attributed to the effect of refrigerated storage together with development of the acidity. The present results are in agreement with Shalaby *et al.* (2013) and Abdou *et al.* (2015).

#### Organoleptic properties:

Sensory evaluation of yoghurt over the storage period is given in Table (4). In general, the fresh yoghurts either control or treatments' recorded the highest scores including colour, appearance, body and texture and flavor. At the first stage of storage period the different treatments impaired an improvement in the total scores of organoleptic properties. Lee and Lucey (2004) attributed the improved quality of yoghurt during the first stage of cold storage to the flavor compounds and rearrangements of casein particles in the gel network which improve the texture. With storage progress the sensory evaluation degree of the control yoghurt was lowered with a higher rate than the other treatments along the storage period and this could be related to the developed acidity which may impair acid flavour as most of consumer prefers the mild taste of acidity. The taste of yoghurt comes from the acidity, acetaldehyde and flavor compound which affect the sensory properties of the product. The cold storage improves the sensory quality of the produced yoghurt as in table (4). All the produced products were acceptable including the control up to the end of storage period (28 days). The results are in agreement with Basiony *et al.* (2015).

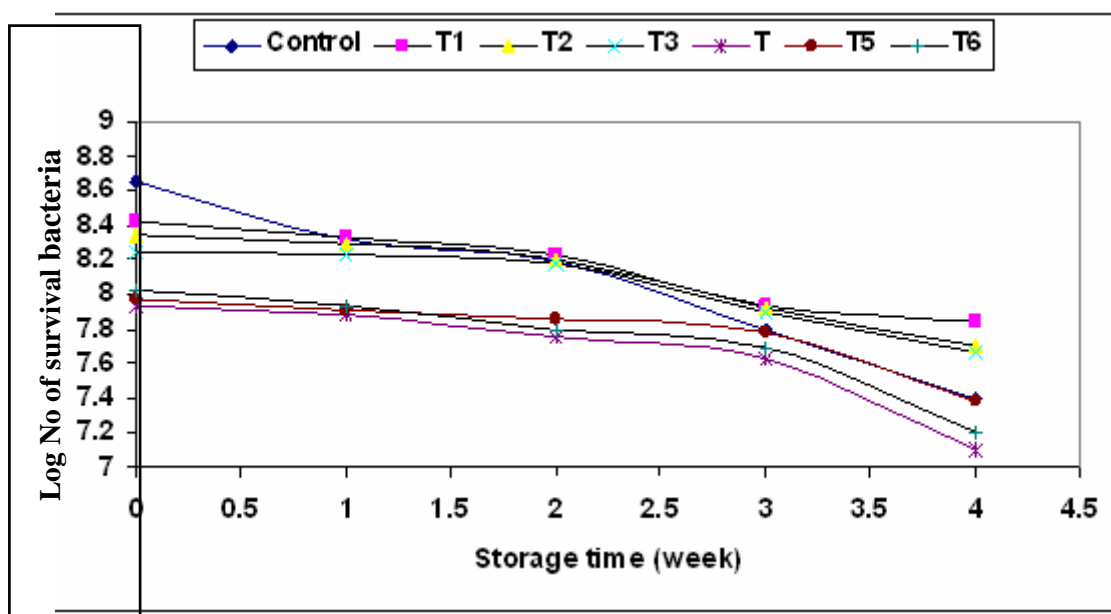


Fig. (1): Viable count of Streptococci in different types of yoghurt made with mutant lactic acid bacteria.

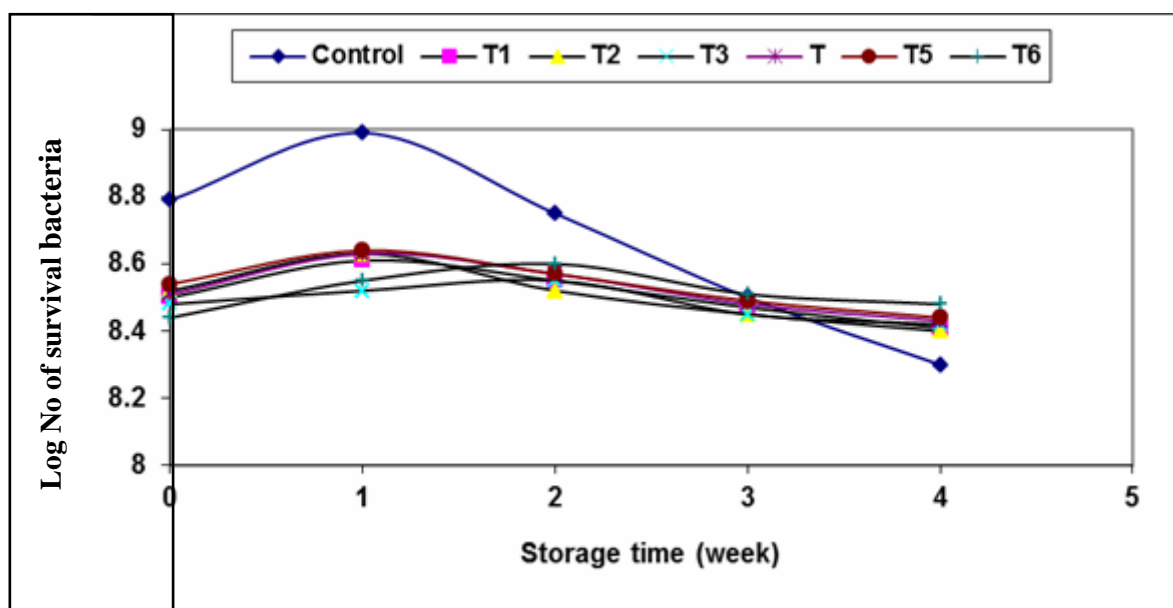


Fig. (2): Viable count of Lactobacilli in different types of yoghurt made with mutant lactic acid bacteria.

Table 4. Organoleptic of fresh yoghurt and during storage at refrigerator.

Properties	Treatments	Storage periods (days)					Mean
		0 (fresh)	7	14	21	28	
Flavour (60)	Control	56 <sup>abcC</sup>	56 <sup>aC</sup>	50 <sup>aB</sup>	50 <sup>aB</sup>	47 <sup>aA</sup>	52.40 <sup>a</sup>
	T1	57 <sup>bcdB</sup>	58 <sup>aB</sup>	57 <sup>bcdB</sup>	57 <sup>bcdB</sup>	54 <sup>bA</sup>	56.80 <sup>b</sup>
	T2	59 <sup>dB</sup>	58 <sup>aB</sup>	57 <sup>bcdB</sup>	57 <sup>bcdB</sup>	54 <sup>bA</sup>	57.20 <sup>b</sup>
	T3	54 <sup>aA</sup>	56 <sup>aAB</sup>	59 <sup>dC</sup>	59 <sup>dC</sup>	57 <sup>cBC</sup>	56.80 <sup>b</sup>
	T4	59 <sup>dB</sup>	58 <sup>aB</sup>	55 <sup>bA</sup>	55 <sup>bA</sup>	54 <sup>bA</sup>	56.60 <sup>b</sup>
	T5	58 <sup>cdB</sup>	58 <sup>aB</sup>	56 <sup>bcAB</sup>	56 <sup>bcAB</sup>	54 <sup>bA</sup>	56.40 <sup>b</sup>
	T6	55 <sup>abA</sup>	56 <sup>aAB</sup>	58 <sup>cdB</sup>	58 <sup>cdB</sup>	58 <sup>cB</sup>	57.00 <sup>b</sup>
	Mean	56.86 <sup>B</sup>	57.14 <sup>B</sup>	56.86 <sup>B</sup>	56.00 <sup>B</sup>	54.00 <sup>A</sup>	
Body and texture (30)	Control	27 <sup>abC</sup>	27 <sup>aC</sup>	26 <sup>aC</sup>	24 <sup>aB</sup>	21 <sup>aA</sup>	25.00 <sup>a</sup>
	T1	28 <sup>abcB</sup>	28 <sup>abB</sup>	28 <sup>bB</sup>	26 <sup>bA</sup>	25 <sup>bA</sup>	27.00 <sup>b</sup>
	T2	29 <sup>cC</sup>	29 <sup>bC</sup>	28 <sup>bBC</sup>	27 <sup>bAB</sup>	26 <sup>bcA</sup>	27.80 <sup>b</sup>
	T3	26 <sup>aA</sup>	27 <sup>aAB</sup>	29 <sup>bC</sup>	28 <sup>cdBC</sup>	27 <sup>cdAB</sup>	27.40 <sup>b</sup>
	T4	29 <sup>cC</sup>	29 <sup>bC</sup>	28 <sup>bBC</sup>	27 <sup>bcB</sup>	25 <sup>bA</sup>	27.60 <sup>b</sup>
	T5	29 <sup>cC</sup>	29 <sup>bC</sup>	28 <sup>bBC</sup>	27 <sup>bcB</sup>	25 <sup>bA</sup>	27.60 <sup>b</sup>
	T6	26 <sup>aA</sup>	27 <sup>aAB</sup>	28 <sup>bBC</sup>	29 <sup>dC</sup>	28 <sup>dBC</sup>	27.60 <sup>b</sup>
	Mean	27.71 <sup>B</sup>	28.00 <sup>B</sup>	27.86 <sup>B</sup>	26.86 <sup>B</sup>	25.29 <sup>A</sup>	
Appearance (10)	Control	10 <sup>aB</sup>	10 <sup>aB</sup>	10 <sup>aB</sup>	9 <sup>aA</sup>	9 <sup>aA</sup>	9.60 <sup>a</sup>
	T1	10 <sup>aB</sup>	10 <sup>aB</sup>	10 <sup>aB</sup>	9 <sup>aA</sup>	9 <sup>aA</sup>	9.60 <sup>a</sup>
	T2	10 <sup>aB</sup>	10 <sup>aB</sup>	10 <sup>aB</sup>	9 <sup>aA</sup>	9 <sup>aA</sup>	9.60 <sup>a</sup>
	T3	10 <sup>aA</sup>	10 <sup>aA</sup>	10 <sup>aA</sup>	10 <sup>bA</sup>	10 <sup>bA</sup>	10.00 <sup>b</sup>
	T4	10 <sup>aB</sup>	10 <sup>aB</sup>	10 <sup>aB</sup>	9 <sup>aA</sup>	9 <sup>aA</sup>	9.60 <sup>a</sup>
	T5	10 <sup>aB</sup>	10 <sup>aB</sup>	10 <sup>aB</sup>	9 <sup>aA</sup>	9 <sup>aA</sup>	9.60 <sup>a</sup>
	T6	10 <sup>aA</sup>	10 <sup>aA</sup>	10 <sup>aA</sup>	10 <sup>bA</sup>	10 <sup>bA</sup>	10.00 <sup>b</sup>
	Mean	10.00 <sup>B</sup>	10.00 <sup>B</sup>	10.00 <sup>B</sup>	9.29 <sup>A</sup>	9.29 <sup>A</sup>	
Overall acceptability (100)	Control	93 <sup>abD</sup>	93 <sup>aD</sup>	89 <sup>aC</sup>	83 <sup>aB</sup>	77 <sup>aA</sup>	87.00 <sup>a</sup>
	T1	95 <sup>bcBC</sup>	96 <sup>aC</sup>	96 <sup>bC</sup>	92 <sup>bB</sup>	88 <sup>bA</sup>	93.40 <sup>b</sup>
	T2	98 <sup>cC</sup>	97 <sup>aC</sup>	96 <sup>bBC</sup>	93 <sup>bcB</sup>	89 <sup>bA</sup>	94.60 <sup>b</sup>
	T3	90 <sup>aA</sup>	93 <sup>aAB</sup>	97 <sup>bC</sup>	97 <sup>cC</sup>	94 <sup>cBC</sup>	94.20 <sup>b</sup>
	T4	98 <sup>cB</sup>	97 <sup>aB</sup>	95 <sup>bB</sup>	91 <sup>bA</sup>	88 <sup>bA</sup>	93.80 <sup>b</sup>
	T5	98 <sup>cD</sup>	97 <sup>cdD</sup>	94 <sup>bBC</sup>	92 <sup>bB</sup>	88 <sup>bA</sup>	93.80 <sup>b</sup>
	T6	91 <sup>abA</sup>	93 <sup>aAB</sup>	96 <sup>bBC</sup>	97 <sup>cC</sup>	96 <sup>cBC</sup>	94.60 <sup>b</sup>
	Mean	94.71 <sup>B</sup>	95.14 <sup>B</sup>	94.71 <sup>B</sup>	92.14 <sup>AB</sup>	88.57 <sup>A</sup>	

a, b & c: Superscripts to be compared statistically within the same column. Values with different letter superscripts are significantly different ( $P < 0.05$ ).

A, B & C: Superscripts to be compared statistically within the same row. Values with different letter superscripts are significantly different ( $P < 0.05$ ).



## Conclusion:

Mutant starter cultures containing St.6+Lb.pH 11 (T3) and St.12+Lb.pH 11 (T6) are interesting strain cultures for commercial production of yoghurt with mild taste. Yoghurt produced with these strains meets international definition of yoghurt. However, the yoghurt produced is characterized by low acidity and low post-acidification during cold storage. The organoleptic properties clear the absence of bitterness and other bad flavours, weak sourness and yoghurt taste typical favourable mild yoghurt.

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### الملخص العربي

تأثير استخدام سلالات بكتريا *Streptococcus thermophilus –Lactobacillus delbreukii subsp bulgaricus* التي بها بعض الطفرات في إنتاج زيادي معتدل الحموضة

الهدف من اجراء هذا البحث هو دراسة تأثير استخدام بعض الطفرات من سلالات بكتيريا *Streptococcus thermophilus* بالاضافة الي المعاملة الكنترول وتم تخزين الزيادي الناتج في الثلاجة لمدة 28 يوم واجري تحليله وهو طازج وبعد 7,14,21,28 يوم كيمابيا وميكروبيولوجيا وحسباً ووضحت النتائج مايلي : التغيرات الكيمابوية للزيادي بسيطة وغير ملحوظة بين المعاملات المختلفة سواء طازج او اثناء التخزين وذلك بالنسبة الدهن والبروتين و الجوامد الكلية اما بالنسبة للحموضة فكانت المعاملة رقم 3 والمعاملة رقم 6 اقل المعاملات في انتاج الحموضة حيث سجلت هذه المعاملات فترة تحضين مقدارها 7-20 ساعة و 6-20 ساعة علي التوالي كما سجلت المعاملة رقم 2 والمعاملة رقم 5 أعلى نسبة في انتاج الاسيتالدهيد. هذا وقد تزايدت أعداد البكتريا بصفة دورية حتي نهاية الاسبوع الاول ثم تقل تدريجياً بطول فترة التخزين حتي 28 يوم ولذلك يمكن استخدام *Str. thermophilus –Lb. delbreukii subsp bulgaricus* التي حدث فيها بعض الطفرات في إنتاج زيادي ذو خواص حسية جيدة و حموضة معتدلة مع اطالة مدة حفظه بالمقارنة بالكنترول