MAKING BIOYOGURT USING NEWLY ISOLATED LACTIC ACID BACTERIA WITH PROBIOTIC FEATURES
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SUMMARY

Bioyogurt was manufactured using the new probiotic LAB strains isolated from infant feces {*Ent. faecium* NM113 (T1), *Ent. faecium* NM213 (T2) and *Lb. casei* NM512 (T3)} in addition to the standard yogurt culture (1:1). The resultant yogurts were stored at ~ 5°C and analyzed when fresh and after 7, 14 and 21 days. The obtained results can be summarized as follows:

Addition of probiotic strains to the yogurt starter cultures increased the coagulation time of bioyogurt with different rates compared to the control yogurt. No significant differences were observed for chemical composition (TS, fat, protein and ash content) between the treatments of yogurt. The acidity percentage of all treatments increased during storage with different rates according to the starter culture used, while the pH decreased. Acetaldehyde content of all treatments significantly increased during the first 7 days of cold storage and then gradually decreased until the end of storage period. Also, there was a significant differences between treatments. The bioyogurts had higher acetaldehyde content than control.

Rheological parameters including firmness and whey synersis of yogurt treatments were affected by the type of starter used and storage period. Viable cell counts of LAB icreased during cold storage through the first 7 days then they decreased thereafter gradually till the end of storage period. Concerning the viable cell counts of the new probiotic isolates, *Lb. casei* NM512 showed the same trend, but the *Enterococci* gradually decreased during storage till the end of the period. The viable cells of the probiotics at the end of storage period remained $>10^7$ cfu g^{-1}. Coliforms and yeasts & moulds of the resultant yogurts were not detected either when fresh or after 21 days of cold storage. All the resultant yogurts were accepted and free from defects and gained high scores when fresh and allover the storage period. The bioyogurt was comparable, but almost higher, in appearance, flavor, texture and overall quality to the standard yogurt. The best treatments were T_2 and T_3.

Keywords: LAB, newly isolates, probiotics, bioyogurt**.**

INTRODUCTION

Lactic acid bacteria are the most important group of microorganisims commercially used for the manufacture of probiotic foods. The health benefits offered by LAB can be nutritional or therapeutic. Although a number of probiotic strains have been isolated and characterized, the search for more effective strains still continue. For example *Ent. faecium* has been considered as essential for the development of organoleptic qualities associated with some varities of cheeses (Bricker *et al*, 2005; Renye *et al*, 2008). Moreover, *Enterococci* are producing powerful bacteriocins which displaying large spectra of inhibition against food-spoiling or pathogenic bacteria (Hogas *et al*, 2003; Leroy *et al*, 2003). These desirable beneficial activities led to the use of *Enterococci* like *Ent. faecium* as a commercial probiotic (Lund & Edlund, 2001).

A number of dairy products are marketed as containing probiotic bacteria. However, the most widely encountered one is yogurt. Bioyogurt is a product that contains live probiotic microorganisms, the present of which may give rise to claimed beneficial health effects. The number of probiotic organisms in a probiotic product should meet suggested minimum of $>10^6$ cfu ml^{-1}, which is the recommended minimum daily intake (Akin *et al.,* 2007). There are many other fermented dairy products in the world which contain probiotic bacteria *e.g.* "Yakult" which is made with selected culture of *Lactobacillus casei.* Yogurt has been used as the most popular vehicle for incorporation of probiotic bacteria. Commercially, it is not feasible to ferment milk using only probiotic organisms owing to the longer time required to reduce the pH of milk and also objectional taste imparted by some of the probiotic bacterial strains (Dave and Shah, 1997; Tamime *et al*, 2005). Most of the probiotic yogurts include live strains of probiotic bacteria in addition to the conventional yogurt organisms, *Str. thermophilus and Lb. delbrueckii* ssp. *bulgaricus* (Tamime *et al*, 2005). So, the objective of this study is to compare the properties of yogurt containing the new isolates (Mansour *et al*, 2014) *Ent. faecium* NM113, *Ent. faecium* NM213 and *Lb. casei* NM512 with the standard yogurt.

MATERIALS AND METHODS

MATERIALS

Fresh milk:

Fresh mixed cow's and buffaloes' milk (1:1) was obtained from the herds of Faculty of Agriculture, Moshtohor, Benha Univ.

Yogurt starter cultures:

Yogurt starter culture contains *Lactobacillus delbreukii* ssp *bulgaricus* and *Streptococcus thermophilus* was obtained from Chr. Hansen, Copenhagen, Denmark, activated and added at a rate of 2g $100g^{-1}$ for the standard yogurt. New probiotic LAB, *Ent. faecium* NM113, *Ent. faecium* NM213 and *Lb. casei* NM512 were isolated from infant feces (Mansour *et al.,* 2014).

Methods:

Manufacture of yogurt:

Yogurt was manufactured according to Tamime (1978). Fresh mixed cow's and buffaloe's milk (1:1) was standardized to~3% fat and heated up to 85°C for 20 min, cooled to 42°C and divided into four portions to make four different treatments with different cultures:
(C): 2% yogurt starter, (*Str. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*).
T1: 1% yogurt starter + 1% *Ent. faecium* NM113
T2: 1% yogurt starter + 1% *Ent. faecium* NM213
T3: 1% yogurt starter + 1% *Lb. casei* NM512

All treatments were filled into plastic cups (120g) and incubated at 42°C until coagulation; then refrigerated at ~ 5°C, as it was analysed when fresh and after 7, 14 and 21 days.

Chemical analysis:

Protein, fat, and total solids of yogurt were determined according to the International Dairy Federation (IDF) Standards, 1993, 1991a and b, respectively. Ash content was derermined according to the method of AOAC (2007). Tritratable acidity was determined according to the methodology mentioned in BSI, (2010). pH value of yogurt samples was determined using a pH meter JENCO Model 1671, USA according to the method described by BSI (1985). Acetaldehyde content was determined according to the method described by Lees and Jago (1969).

Microbiological examination:

Total viable count (TVC), Y&M and coliforms were examined according to the methodology of IDF 1991c, 1990 and A.P.H.A., (1992), respectivily. *Str. thermophilus, Lb. delbreukii* ssp *bulgaricus* , *Lb. casei* and *Ent. faecium* were examined according to the methodology of Ryan *et al*. (1996), Ravula and Shah (1998) and Atlas (1995), respectivily.

Rheological properties:

Whey syneresis of the produced yogurts was determined according to the method of Dannenberg and Kessler (1988) modified by Badawi *et al.* (2004). Firmness of yogurts was measured using the penetrometer Model Koehler Instruments Co., (USA) controller as described by Kammerlehner and Kessler (1980).

Sensory evaluation:

The organoleptic properties included flavour 60 points; body and texture 30 points and appearance was given score of 10 points (El-Etriby *et al.*, 1997 and Mehanna *et al.*, 2000). The organoleptic evaluation was done by 10 experienced Food Scientists Staff at Food Science Department, Moshtohor, Faculty of Agriculture, Benha University and Department of Natural Products Chemistry and Microbiol, National Research Centre, Giza.

Statistical analysis

Statistical analysis for the obtained data was carried out according to the methods described by Clarke and Kempson (1997).

RESULTS AND DISCUSSION

Coagulation time:

Table (1) presents fermentation times to reach coagulation of the prepared yogurts. Data reveal that the control required the shortest coagulation time, followed by T1, T2; while the longest coagulation time was observed in T3. This may be due to the possible inhibition of the starter cultures in the presence of probiotic bacteria. Vinderola *et al* (2002) reported that probiotic bacteria delay the growth of starter cultures. They observed that *Lb. casei* slows the growth of *Str. thermophilus* and *Lb. bulgaricus* in milk. Variability in fermentation time may be due to differences in the ability of lactic acid bacteria to grow and fermenting milk. Similar results were reported by Dave and Shah (1997, 1998). Moreover, (Sodani *et al* 2002) noticed that the addition of *Lb .bulgaricus* in probiotic yogurts reduced about 46% of the fermentation time.

From statistical analysis of coagulation time of the produced bioyogurt data cleared that there is non significant differences between the control and T1. Also, there were non-significant differences between T2 and T3 however; there was significant differences between the control in a side and T2 and T3 on the other side.

Table (1): Coagulation time of the produced bioyogurt

Treatment	Coagulation time/h h:min	Increase %
C	2:10[b]	____
T1	2:12[b]	1.54
T2	2:27[a]	13.80
T3	2:32[a]	16.92

C) : 2% yogurt starter, (*Str. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*).
T1: 1% yogurt starter + 1% *Ent. faecium* NM113, T2: 1% yogurt starter + 1% *Ent. faecium* NM213 and T3. 1% yogurt starter + 1% *Lb. casei* NM512

Chemical composition of the bioyogurt:

Table (2) illustrates the gross chemical compostion of the resultant bioyogurt, for the different treatments.

Data of total solids show that the TS of bioyogurt treatments did not affected by the type of the starter cultures. This agrees with the results of Akalin (1996) and Hussein (2010). However, there was a slight insignificant differences (*P > 0.001)* increase in the total solids of the different treatments with the progress of storage at ~ 5°C. This increase may be due to the evaporation of some water during the cold storage. These results are consent with Farag *et al* (2010) and El-Nagga & Abd-El-Tawab (2012)

The fat content of the produced bioyogurts of the different treatments shown insignificant differences among the yogurt treatments in fat content of the fresh bioyogurt as the fat percentage was standardized before manufacturing to ~3%. However, a slight decrease in all treatments was reported during cold storage which may be due to lipolytic effect of bioyogurt culture (Tamime and Deeth, 1980; El-Nagar and Shenana 1998). Similar results were reported by El-Nagar & Shenana (1998) and El-Nagga & Abd-El-Tawab (2012). However, there were significant differences either between different treatments or between the treatments and the control among the storage up to 21 days (*P > 0.001).*

The average of protein content of fresh yogurt was 4.07 in all treatments (Table 2). As there was no effect on the protein due to the type of starter used insignificant differences (*P > 0.001)* were observed during the storage between treatments. A slight decline of protein content was observed at the end of storage and this may be attributed to the limited proteolytic effect of different bioyogurt cultures (Tamime and Deeth, 1980; El-Nagar & Shenana 1998; Hussein, 2010).

It is evident that the ash content of fresh bioyogurt recorded slight differences (insignificant *P > 0.001)* between treatments. During storage, a slight increase was observed in all treatments. This increase may be due to the limited increase of TS due to the evaporation of some water during storage. However, the type of the starter culture did not significantly affect the ash content. The results are in agreement with Akalin (1996) and Hussein (2010).

From Table (2) it can be seen that the acidity significantly varied (*P > 0.001)* according to the type of starter cultures used. The titratable acidity values of the control and bioyogurt tended to increase during storage (*P > 0.001)*. *Lb. delbreukii* ssp *bulgaricus* and *Str. thermophilus* are responsible for the post acidification of yogurt during cold storage (Donkor *et al*, 2006). The post acidification is due to the slow metabolic activity of the starter cultures. Observed acidity values in the current study are similar to those reported by (Dave and Shah, 1997; Gueimonde *et al*, 2004 and Korbekandi *et al*, 2009) The control was characterized with the highest titratable acidity percentage during the storage period as compared to probiotic yogurts. Moreover, it was found that the majority of *Ent. faecium* strains showed a medium or slow rate of acidification (Ayad *et al*, 2004; Mohamed *et al*, 2009).

Acetaldehyde considers the main component in yogurt flavor. It is realized during the metabolism of microorganism especially lactic acid bacteria.

It is evident that, the acetaldehyde content influenced significantly (*P > 0.001)* by starter cultures used and storage period Table (2). The level of acetaldehyde increased within the first 7 days and then it decreased thereafter gradually in all treatments till the end of storage period. This could be associated with the metabolic activity of the starter cultures, which may be attributed to the demonstrated ability of numerous lactic acid bacteria to reduce acetaldehyde to ethanol (Mehanna and Hefnawy, 1990; Amer *et al.*, 1991; Salama, 1993).

Moreover, the values of acetaldehyde content in this current study are within limits given by Salama and Hassan (1994), EL-Nagar *et al.* (2007) and El-Khatab (2011). It was worthy to observe that the level of acetaldehyde was higher in all bioyogurts than the control when it was fresh and allover the storage period. This may be due to the difference in metabolic activity of the starter cultures.

Table (2): Gross chemical composition(g100g^{-1})., acidity (%), pH and acetaldehyde (mg100g^{-1}) content of the produced bioyogurt during storage periods at ~ 5°C

Treatments	T.S	Fat	Protein	Acidity %	pH	Acet*	Ash
Fresh							
C	13.08^{a}	3.03^{bac}	4.07^{a}	0.88^{feg}	4.53	2.10^{gh}	0.70^{ba}
T1	12.82^{a}	3.08^{a}	4.08^{a}	0.80^{h}	4.49	3.45^{d}	0.69^{b}
T2	12.85^{a}	3.03^{bac}	4.07^{a}	0.82^{h}	4.50	2.80^{e}	0.67^{b}
T3	13.09^{a}	3.07^{a}	4.08^{a}	0.81^{h}	4.53	4.40^{c}	0.68^{b}
7 days							
C	13.18^{a}	2.99^{bdac}	4.13^{a}	0.91^{fe}	4.21	5.00^{b}	0.74^{ba}
T1	12.88^{a}	2.98^{bdec}	4.09^{a}	0.87^{fg}	4.28	5.20^{b}	0.71^{ba}
T2	12.94^{a}	2.99^{bdec}	4.08^{a}	0.88^{fg}	4.29	5.00^{b}	0.70^{ba}
T3	13.15^{a}	2.99^{bdec}	4.11^{a}	0.90^{fe}	4.21	5.80^{a}	0.72^{ba}
14 days							
C	13.22^{a}	2.94^{fdec}	4.01^{a}	0.95^{dc}	3.16	2.00^{gh90}	0.77^{a}
T1	13.05^{a}	2.96^{fdec}	4.05^{a}	0.91^{de}	3.20	3.10^{e}	0.73^{b}
T2	12.98^{a}	2.93^{fde}	4.10^{a}	0.92^{de}	3.20	2.55^{f}	0.73^{b}
T3	13.21^{a}	2.92^{fe}	4.16^{a}	0.96^{c}	3.18	3.50^{d}	0.74^{b}
21 days							
C	13.31^{a}	2.92^{fe}	3.95^{a}	1.24^{a}	3.06	1.95^{h}	0.71^{ba}
T1	13.00^{a}	2.90^{fe}	4.09^{a}	0.95^{dc}	3.09	3.00^{e}	0.74^{ba}
T2	13.10^{a}	2.89^{fe}	4.05^{a}	0.98^{c}	3.11	2.30^{gf}	0.74^{ba}
T3	13.31^{a}	2.89^{fe}	4.00^{a}	1.20^{b}	3.05	3.10^{e}	0.77^{a}

C) : 2% yogurt starter, (*Str. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*). *Acet, acetaldehyde
T1: 1% yogurt starter + 1% *Ent. faecium* NM113, T2: 1% yogurt starter + 1% *Ent. faecium* NM213 and T3: 1% yogurt starter + 1% *Lb. casei* NM512
Values with the same letter in the same column are not significantly different.

Rheological properties:

Bioyogurt firmness:

The firmness of bioyogurt was measured as penetrometer distance in 0.1 mm at 5s. The higher recorded by the penetrometer reading, the less firmness of bioyogurt. The penetrometer reading (Table 3) decreased with different rates during cold storage $P > 0.001$. This means that the firmness of the yogurt increased with cold storage $P > 0.001$. Continuous protein rearrangement, more protein-protein interactions during storage would increase the

viscosity and firmness of yogurt during storage (Isleten & Karagul- Yaccer, 2006). Thus, rheological parameters of yogurt treatments were affected by the type of starter used and storage period. Also, some strains of LAB used in the manufacture of yogurt produce exopolysaccharides (EPS) (Hassan, 2008 and Purohit *et al* 2009). From these strains, *Ent. faecium* was found as EPS producer (Ayad and Shokery, 2011) which used widely in dairy industry as a natural biothickner to enhance the rheological quality of dairy products (Mayra-Makinen and Bigret, 1998).

Whey syneresis:

Syneresis is the separation of the liquid phase from the gel and it is an undesirable feature in yogurt. Whey loss measures the level of collapsed gel and is an indicator for poor quality and stability. Syneresis may be spontaneous or may occur only when the gel is mechanically disrupted by cutting, agitating, or being subjected to a centrifugal force. Furthermore, several adjuvants, solids and stabilizers were added to milk before fermentation took place to reduce syneresis.

Table (3): Curd firmness of the produced bioyogurt during storage periods at ~5°C.*

Storage period (days)	Penetrometer reading (0.1mm/5s)			
	C	**T1**	**T2**	**T3**
0	264^{ba}	263^{ba}	266^{a}	264^{ba}
7	260^{bc}	254^{de}	258^{dc}	258^{dc}
14	254^{de}	250^{f}	253^{ef}	251^{ef}
21	253^{ef}	250^{f}	244^{g}	255^{de}

*See foot note table 2

It is clear that whey separation of all yogurt treatments decreased as storage period progressed. Thus, there was an inverse relationship between the storage period and the susceptibility to syneresis. This is in accordance with Isleten & Karagul- Yaccer (2006). The rate of acid development, rearrangement of casein particles in the gel network, and the rate of solubilization of colloidal calcium particles are the driving force for the syneresis (Lee & Lucey, 2004). However, some strains of LAB used in the manufacture of yogurt produce EPS (Hassan, 2008 and Purohit *et al*, 2009), which affect the syneresis and reduce it.

Microbioliogical properties of Bioyogurt:

Table (5) shows the total bacterial counts of the control and bioyogurt products during cold storage at ~ 5°C for 21 days. It was observed that the total bacterial counts increased up to the 7^{th} day of storage, then decreased till the end of storage period. The decline of bacterial count was probably due to the combined effect of cold storage and development of acidity produced by microbial fermentation. These results are in accordance with Dave and Shah (1997); Kebary *et al* (2009) and Shalaby *et al* (2013).

In making traditional yogurt a specific pure cultures of lactic acid bacteria containing *Lb. delbreukii* ssp *bulgaricus* and *Str. thermophilus* are added to conduct the fermentation process. Moreover, Bioyogurt is yogurt that contains live probiotic microorganisms in addition to the conventional yogurt organisms

Generally, viable cells of *Str. thermophilus* were prevalent in all yogurts made with different starters used, followed by almost *Lb. delbreukii* ssp *bulgaricus*.

The viable counts of *Str. thermophilus* Table (5) was gradually decreased during storage of C and T_1 till the end of the period (21 days), while T_2 slightly increased during the first 7 days then decreased gradually during storage. T_3 decreased after the 7^{th} day till the end of storage. The counts were almost the same at the end of the storage period. The decrease of *Str. thermophilus* during storage in all treatments may be due to its sensitivity to the produced

acidity. Similar trends were obtained by Kebary, *et al* (2010); Hussein (2010); El-Nagga & Abd El-Tawab (2012) and Mani-Lopez *et al* (2014).

Concerning *Lb. delbreukii* ssp *bulgaricus*, Table (5) the viable count was increased during the first 7 days. Thereafter, the viable count decreased gradually till the end of storage period. This may be due to the effect of the developed acidity and the cold storage. The results are in harmony with those obtained by Abd El-salam *et al* (2011) and Servili *et al* (2011). It is common to observe decreasing counts of *Lb. delbreukii* ssp *bulgaricus* in probiotic yogurts with storage. This may be attributed to the secretion of inhibition metabolites (*e.g.* bacteriocins) produced by probiotics (Mani-Lopez *et al*, 2014).

Table (4): Whey syneresis of the produced bioyogurt during storage periods at ~ 5°C ($g100g^{-1}$).

Storage period (days)	Curd syneresis ($g100g^{-1}$)			
	C	**T1**	**T2**	**T3**
15 min				
0	14.75	16.19	15.76	17.35
7	11.49	13.28	12.59	13.00
14	12.05	11.66	11.73	15.60
21	10.52	10.56	11.29	13.70
30min				
0	20.54	22.27	24.07	23.03
7	16.96	18.66	18.69	18.91
14	17.42	16.93	17.12	21.19
21	16.31	15.19	16.73	18.77
45min				
0	24.57	26.16	27.90	26.80
7	20.52	22.13	22.46	22.54
14	21.01	20.41	20.49	24.58
21	20.63	18.46	19.90	21.88
60min				
0	27.69	29.06	30.69	29.61
7	22.97	24.88	25.23	24.94
14	23.71	23.14	23.35	27.22
21	22.86	20.82	22.62	24.42
90min				
0	31.86	33.02	34.59	33.40
7	26.83	28.47	28.95	28.76
14	27.08	26.37	26.99	31.23
21	25.86	24.45	25.99	27.72
120min				
0	34.78	35.52	37.25	35.76
7	29.37	31.01	32.01	31.04
14	30.50	29.85	30.10	33.94
21	27.77	27.48	28.80	29.83

*See foot note Table 2

^

Table (5) Total bacterial count and viable cell counts of *Str .thermophilus* and *Lb. delbruckii* ssp. *bulgaricus* of the produced bioyogurt during storage periods at ~ 5°C (10^7/cfu g^{-1}).

Strains	Storage period (days)	Treatments			
		C	T1	T2	T3
Total Bacterial count	0	0.66	T1	0.97	0.58
	7	1.22	1.19	1.23	0.7
	14	0.8	1.55	0.89	0.66
	21	0.66	1.1	0.5	0.66
Streptococcus thermophilus	0	1.04	0.78	0.7	1.04
	7	0.60	0.79	0.79	1.04
	14	0.23	0.27	0.20	0.25
	21	0.14	0.13	0.14	0.14
Lb. delbrueckii* ssp. *bulgaricus	0	0.65	0.94	0.67	0.62
	7	0.96	1.04	0.81	0.98
	14	0.67	0.88	0.78	0.63
	21	0.52	0.44	0.23	0.44

*See foot note Table 2..

Microbial viability of probiotics during storage:

The survival of probiotic bacteria in fermented dairy bioproducts depends on such varied factors as the strains used, interaction between species present, culture conditions, chemical composition of the fermentation medium (*e.g*. carbohydrate sources), final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, concentration of sugars, dissolved oxygen, level of inoculation, incubation temperature, fermentation time and storage temperature (Young & Nelson, 1978; Hamann & Marth, 1983; Kneifel and Pacher, 1993).

Table (6) clears the changes in probiotic counts during storage of bioyogurts up to 21 days at ~ 5°C. Data reveal that *Ent. faecium* NM113 and *Ent.faecium* NM213 gradually decreased during storage till the end of the period, but remained at the level recommended by FAO/ WHO (2002) $>10^7$ cfu g^{-1} to have beneficial effects of probiotic.

Concerning to *Lb. casei* NM512, it was found that the viable count increased through the first 7 days then the count decreased gradually during cold storage. The decline in the viable count may be attributed to the effect of postacidification (Shah, 2000; Damin *et al*, 2008). Dave and Shah, 1997) addressed an antagonistic effect against peroxide production, which can partially damage the probiotic cells. Gilliland *et al* (2002) reported reduction in *Lb. casei* of 1 log in fermented milks, maintaining populations of 10^5 and 10^6cfu g^{-1}. In general, few studies recorded constant counts of *Lb. casei* in bioyogurts after 21 days to 28 days of storage (Nighswonger *et al*, 1996 and Korbekandi *et al*, 2009).

The results in the current study are in the same line with many previous findings. In general, microbial viability at all three bioyogurt treatments slowly decreased as pH was reduced and acidity was increased, however, they maintained counts of $>10^7$ cfu g^{-1} which is the recommended minimum daily intake (Akin *et al*, 2007) during the 21 days of storage with any of the probiotics (*Ent. faecium* NM113, *Ent. faecium* NM213 or *Lb. casei* NM512). Daily

dietary intake of these probiotics is important because it is a natural commensal bacteria. Bioyogurt that contain *Ent. faecium* NM113, *Ent. faecium* NM213 or *Lb. casei* NM512 should be developed and promoted by the food industry.

Table (6): Probiotic bacterial count of the produced bioyogurt during storage periods at ~ 5°C (10^7/cfu g^{-1}) up to 21 days

Storage periods (days)	Treatments		
	T1 ***Enterococcus faecium*** **NM 113**	**T2** ***Enterococcus faecium*** **NM 213**	**T3** ***Lactobacillus casei*** **NM 512**
0	0.75	0.59	0.60
7	0.58	0.35	0.64
14	0.35	0.27	0.47
21	0.26	0.23	0.31

Coliforms and Yeasts & Moulds:

Coliforms, under the acidity conditions of yogurts should inactivate by the low pH. Furthermore, some species may be susceptible to antibiotics released by the probiotic starter microorganisms. The coliform test of the produced bioyogurts, revealed undetectable organisms either when fresh or during storage periods. Concerning yeast & moulds count, they were not detected also in all yogurt samples allover the experiment.

The above results reflect a good sanitation conditions during making and storing the products, a good quality products and as a warning that the products may constitute a health risks. The results consist with those of Feresu and Nyati (1990), Obi *et al.* (2010), Kebary *et al*. (2010) and El-Nagga & Abd El-Tawab (2012).

Sensory Evaluation:

In recent years, per capita consumption of yogurt has increased dramatically because many consumers associate yogurt with good health. However, scientific approaches to establishing the functional benefits of probiotic foods are still complicated case. Yogurt is characterized as a fermented milk product with a refreshing flavor, a smooth viscous gel, and a slight sour taste (Hekmat and Reid, 2006). These sensory properties offer quality control criteria, and therefore, yogurt should be evaluated for flavor, texture, appearance and overall quality.

The results (Table 7) indicate good acceptability of the different bioyogurt developed. When yogurts were fresh, panelists did not identify flavor or appearance differences among bioyogurts and standard yogurt; while there was slight differences in texture. Similar results were reported by Hekmat and Reid (2006) and Mani-López *et al.* (2014) when they conducted consumer taste panel evaluations to compare sensory properties of probiotic and standard; the appearance, flavor, texture and overall quality of probiotic yogurt were comparable and similar to that of standard yogurt.

Cold storage improves the quality of yogurts through 7^{th} days. This may attributed to the flavor compound (*e.g.* acetaldehyde, some acids) and rearrangements of casein particles in the gel network which improves the texture (Lee & Lucey, 2004). After 14 days of storage, the organoleptic scores of some treatments (C and T_1) revealed some decrease in total scores which may be due to the development of acidity. The highest total scores were gained by T_2 and T_3 (95 and 94).

The results are in accordance with those of Obi *et al*. (2010), Hussein (2010), Abd El-Salam *et al.* (2011) and Mani-López *et al.* (2014).

In conclusion, bioyogurts containing *Ent. faecium* NM113, *Ent. faecium* NM213 and *Lb. casei* NM512 were successfully manufactured and found by sensory evaluation to be comparable, but almost higher, in appearance, texture, flavor and overall quality to the standard yogurt.

Table (7): Organoleptic properties of the produced bioyogurt during storage periods at ~5°C.

Treatments	Flavour (60)	Body & texture (30)	Appearance (10)	Total (100)
Fresh				
C	56	28	9	93
T1	55	27	9	91
T2	56	28	9	93
T3	56	29	9	94
7 days				
C	57	27	9	93
T1	56	27	9	92
T2	57	29	9	95
T3	58	29	9	96
14 days				
C	55	28	9	92
T1	54	27	9	90
T2	56	29	10	95
T3	58	29	10	97
21 days				
C	56	25	8	89
T1	55	24	9	90
T2	56	29	10	95
T3	56	28	10	94

- See foot note Table 2.

CONCLUSIONS

From the foregoing results it revealed that several selected LAB strains could be useful for technological purposes as sources of strains showing probiotic properties. They are currently can be applied to improve some Egyptian dairy products and for new applications/innovation. Thus, a new bioygurt successfully made using the three isolates *Ent. faecium* NM113, *Ent. faecium* NM213 or *Lb. casei* NM512 with a good quality and prolonged shelf life and the final number of viable cells of these strains was within the recommended level 10^6-10^7 cfu g^{-1} for achieving the probiotical count which claimed health benefits.'

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إستخدام عزلات جديدة من بكتريا حامض اللاكتيك والتى لها خواص البروبيوتيك فى صناعة بيوغورت

فى هذا الجزء تم إستخدام عزلات جديدة الداعمة للحيوية *Ent. faecium* NM113, *Ent. faecium* NM213, *Lb.casei* NM512 والمعزولة من براز الأطفال فى صناعة ثلاث معاملات مختلفة من البيويوجورت على أن تكون بنسبة ١:١ مع بادىء الزبادى العادى. وقد أوضحت النتائج إضافة البكتريا الداعمة للحيوية الى بادىء الزبادى العادى أدى إلى إطالة زمن التجبن بنسب مختلفة تبعا لنوع السلالة المضافة.

لم يوجد فروق معنوية بين المعاملات المختلفة بالنسبة للتركيب الكيميائى (جوامد كلية –دهن– بروتين–رماد). وبالنسبة لتأثير فترة التخزين على التركيب الكيميائى فقد وجدت اختلافات بسيطة بتقدم التخزين ماعدا البروتين. تزداد نسبة الحموضة فى جميع المعاملات مع زيادة مدة التخزين بمعدلات متفاوتة تبعا لإختلاف البادىء المستعمل وبالعكس لوحظ إنخفاض ال pH.

لوحظ زيادة نسبة الأسيتالدهيد فى جميع المعاملات خلال السبعة أيام الأولى من التخزين وبعد ذلك أخذ فى الإنخفاض التدريجى حتى نهاية فترة التخزين كما وجدت إختلافات معنوية بين المعاملات المختلفة وكان محتوى البيوغورت من الأسيتالدهيد أعلى من الكنترول. الإختبارات الريولوجية وتشمل صلابة الخثرة ومقدار إنفصال الشرش من الخثرة فقد وجد أنها تتأثر بنوع البادىء المضاف وكذلك بمدة التخزين.

الخواص الميكروبيولوجية

لوحظ زيادة فى أعداد بكتريا حامض اللاكتيك بالتخزين خلال السبعة أيام الأولى ثم حدث إنخفاضا تدريجيا بتقدم التخزين. وبخصوص عدد الخلايا الحية من بكتريا البروبيوتيك المضافة فقد وجد أن *Lb. casei* NM512 تتبع نفس الإتجاه أى الزيادة حتى اليوم السابع ثم الإنخفاض التدريجى أما الـــــ *Enterococci* فقد أخذت فى الإنخفاض التدريجى منذ بداية التخزين ولكن جميع البكتريا الداعمة للحيوية إحتفظت حتى نهاية فترة التخزين بعدد أكثر من ١٠ ^{٧} مستعمرة للجرام من الخلايا الحية وهو العدد المطلوب لكى يظهر تأثيرها المفيد والصحى. لوحظ عدم تواجد مجموعة بكتريا القولون وكذلك الخمائر والفطريات فى جميع المعاملات من اليوغورت المنتج سواء وهو طازج أو خلال فترة التخزين. أوضحت نتائج التحكيم الحسى أن جميع معاملات اليوغورت المصنع كانت مقبولة وخالية من العيوب سواء الناتج بعد التصنيع

مباشرة أو في نهاية فترة التخزين. وقد حصل البيويوغورت على درجات أعلى من الكنترول كما تميز بالمظهر الجيد والنكهة الجيدة طوال فترة التخزين وقد حصلت المعاملات T_2 ، T_3 على أعلى درجات التحكيم.

أوضحت النتائج أنه يمكن إنتخاب وعزل بعض السلالات من بكتريا حامض اللاكتيك النافعة التى يمكن إستخدامها كمصدر للبكتريا الحيوية النافعة (البروبيوتيك). وهذه السلالات يمكن تطبيقها فى تحسين بعض منتجات الألبان المصرية وإنتاج أنواع جديدة من المنتجات الحيوية. ومنهاإستخدام الثلاث سلالات الجديدة المعزولة والتي تنتمي الي بكتريا حامض اللاكتيك وهى *Lb. casei* *Ent.feacium* NM113 NM512, *Ent. feacium* NM213, لإدخالها فى صناعة بيويوغورت وأوضحت النتائج أن البيويوغورت الناتج يتصف بخصائص حسية جيدة بل تفوق على الكنترول وكذلك تميز بطول فترة الحفظ حتي ٢١ يوم. كما وجد أن العدد النهائى للخلايا الحية لهذه السلالات كانت فى حدود الأعداد المطلوبة ($١٠^{٦} - ١٠^{٧}$) مستعمرة متكونة لكل جرام) للحصول على الفائدة والتأثير الصحى المطلوبين.