



## THE PRODUCTION OF NOVEL FUNCTIONAL YOGHURT CONTAINING ANGIOTENSIN I-CONVERTING ENZYME (ACE)-INHIBITORY ACTIVITY

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### ABSTRACT

Novel functional types of yoghurt containing ACE-inhibitory activity were produced by adding *Lactobacillus casei* ATCC 7469, *Lb. paracasei* 6A and kombucha besides the yoghurt starter cultures in order to increase the activity against the ACE. Results showed that the ACE-inhibitory activity increased during the cold storage periods at 4°C for 28 days. Soluble nitrogen was increased during the cold storage periods. The chemical analysis data of the produced novel functional yoghurt were similar to that of control. Same observations were for the microbiological examination. Also, the water holding capacity and firmness of the novel functional yoghurt were good as the control up to the end time of the experimental design.

**Key words:** Novel functional yoghurt, ACE-inhibitory, probiotic, *Lb. casei* ATCC 7469, *Lb. paracasei* 6A and kombucha.

### INTRODUCTION

Yoghurt is one of the most important fermented dairy products. Due to its high nutritional importance, which is well known, also its different types and flavours which gives these products a wide spread between consumers. The beneficial health and functional effects of yoghurt can be increased by different ways based on the peptides that are produced during fermentation and storage of such product. Many peptides with antihypertensive action have been characterized upon fermentation of milk with different microorganisms, or by the action of proteinases on milk proteins. The peptides are not active within the parent protein but can be released and activated with enzymatic hydrolysis (FitzGerald and Meisel, 2003).

Bioactive peptides may act in the body as regulatory components with a hormone-like activity which may modulate specific physiological functions in the human body (Meisel and FitzGerald, 2003). In addition, multi-functional bioactive effects have been identified within

specific casein sequences, for example angiotensin-converting enzymes (ACE)-inhibitory activities (Migliore-Samour and Jolles, 1988). Biologically, active peptides can be produced from precursor milk protein using different ways *i.e.* (a) enzymatic hydrolysis by digestive enzymes (b) fermentation of milk with proteolytic starter cultures and (c) proteolysis by enzymes derived from microorganisms or plants. In many studies a combination of above methods has proven effective in generation of short functional peptides (Korhonen and Pihlanto, 2003).

Many dairy starter cultures are highly proteolytic formation of bioactive peptides can, thus, be expected during the manufacture of fermented dairy products.

Yoghurt bacteria, cheese starter bacteria and commercial probiotic bacteria have been demonstrated to produce different bioactive peptides in milk during fermentation (Gomez-Ruiz *et al.*, 2002; Fuglsang *et al.*, 2003; Gobbetti *et al.*, 2004; Donkor *et al.*, 2005).

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Nowadays, people became more aware about their health *i.e.* (obesity, chronic diseases.. *etc.*). The probiotics and synbiotics have occupied an important sector with the functional dairy foods market. Most of the functional foods are from dairy products (Vasudha and Mishra, 2013). The new trend is to use nondairy probiotics *i.e.*, kombucha (fermented green tea) or in association with dairy products in order to increase the power of probiotics in the products.

The potential health benefits of bioactive milk peptides have been a subject of growing commercial interest in the context of health-promoting functional dairy foods. So far, antihypertensive, mineral-binding and anti carcinogenic peptides have been most studied for their physiological effects (Korhonen, 2009).

Hence, the aim of the present study was to produce novel functional yoghurt, with new properties either during the fermentation or the refrigerated storage and measure the proteolysis and the ACE- inhibitory activity of the novel functional yoghurt.

## MATERIALS AND METHODS

### Materials

#### Raw milk

Cow's raw milk, (4% fat and 13.6% TS) was obtained from a dairy farm (The Ohio State University, Columbus, OH, USA) and used for the novel functional yoghurt making.

#### Starter cultures

Frozen yoghurt starter pellets (F-DVS YoFlex, Mild 2.0) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chr. Hansen) were obtained friendly from Dept. of Food Sci. and Technol., OSU. This starter was added at a rate of 1g/ 1000 ml of milk. *Lactobacillus casei* ATCC 7469 and *Lb. paracasei* 6A were obtained from the culture collection of the Food Microbiology Laboratory of the Ohio State University, Columbus, Ohio and Kombucha was purchased from Kombucha Brooklyn, Brooklyn, NY. *Lb. casei* ATCC 7469, *Lb. paracasei* 6A and kombucha were prepared in skimmed milk (12% TS, heat treated at 121°C for 10 min.) at a rate

of 1% and incubated at 37°C for 3 days for activation the cultures before using in the novel functional yoghurts.

#### Nonfat dry milk (NFDM)

Instant nonfat dry milk was purchased from RYT-way industries LLC. Lakeville, MN, USA.

#### Angiotensin converting enzyme

The Angiotensin converting enzyme was prepared from rabbit lung according to the method described by Cushman and Cheung (1971).

## Methods

### Manufacture of yoghurt

Yoghurt was manufactured according to the method described by Berber (2011) with some modifications as follows: Yoghurt mix was standardized to 4% fat using cream 23% fat and 16% T.S using NFDM, homogenized at 2300 and 500 psi using a Lab 100 M-G homogenizer (LubeckSchlutut, Germany), and then heated at 92°C for 30 sec using an AVP Junior HTST system (Tonawanda, NY). Heated yoghurt mix was cooled to (45 ± 1°C), divided into four portions. The 1<sup>st</sup> portion was served as control, the 2<sup>nd</sup> portion was made with *Lb. casei* ATCC 7469 (T<sub>1</sub>), the 3<sup>rd</sup> portion was made with *Lb. paracasei* 6A (T<sub>2</sub>) and the 4<sup>th</sup> portion was made using kombucha (T<sub>3</sub>). Yoghurt starter was added at a rate of 1g/ 1000 ml of milk in all treatments. The mixtures were filled in 120g plastic cups (sterilized using UV light in biosafety hood for 2 hr.) and incubated at ~ 42 ± 1°C until the pH reached 4.6. All yoghurt treatments were stored at 4°C and analyzed for, chemical, microbiological, rheological and ACE-inhibitory activity properties when fresh and after 7, 14, 21 and 28 days.

### Chemical analyses

Yoghurt treatments were analyzed for fat, total solids (TS) and protein contents according to the International Dairy Federation (IDF) Standards 1991 a, 1991 b and 1993, respectively. pH values of yoghurt samples were measured using a pH meter Mettler Toledo model seven easy, USA according to the method described by BSI (1985).

## Physical Properties

### Penetrometer reading

Penetrometer reading of all the produced novel functional yoghurt was measured according to Dixon and Parekh (1980) using Humboldt MFG testing equipment, laboratory apparatus, USA. Weight of cone is 35 g., cone angle 45° and penetration depth was measured after 3 sec. the recorded penetrometer readings were expressed as 0.1mm/sec.

### Water holding capacity

Water holding capacity of yoghurt treatments was evaluated according to the method described by Sodini *et al.* (2005), as follows, 20g of yoghurt sample (S) were centrifuged for 10 min at 4000 rpm at 4°C. The whey expelled (W) was removed and weighed. The water holding capacity (WHC, g/ kg) was calculated as follows:

$$\text{WHC} = ((S - W) / S) \times 1000.$$

### Evaluation of ACE-inhibitory activity of the novel functional yoghurts crude extract

All the novel functional yoghurt treatments extract were prepared as described by Hernández-Ledesma *et al.* (2004) with some modifications as follows:

20 g of yoghurt were heat treated at 90 °C for 10 min to stop fermentation then cooled at room temperature. The pH was adjusted to 3.8 with 50% lactic acid and centrifuged at 7000 g for 10 min. The pH of the supernatant was adjusted to 8.3 and centrifuged again under the same conditions. The obtained crude extracts were examined for its ability to inhibit the angiotensin converting enzyme using the method of Cushman and Cheung (1971).

### Microbiological examination

Total bacterial counts of all yoghurt samples were determined when fresh and all over the storage periods according to the methods of IDF (1991c).

### Statistical Analysis

Data were analyzed for analysis of variance according to Clarke and Kempson (1997) using Statistical Package for the Social Sciences (SPSS) software (version 19.0).

## RESULTS AND DISCUSSION

### Coagulation Time

Data in Table 1 show the coagulation time of all the manufactured novel functional yoghurt treatments. It can be noticed that the control recorded the shortest time of coagulation followed by T<sub>3</sub>. On the other hand, T<sub>1</sub> and T<sub>2</sub> recorded the longest coagulation time. The (%) increase of coagulation time was 19.92, 19.06 and 11.06 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. Similar results were recorded by Abdou *et al.* (2015), who attributed the increase in coagulation time to the possible inhibition of the starter culture in the presence of probiotic bacteria. Moreover Vinderola *et al.* (2002) observed that probiotic bacteria delay the growth of starter culture.

Statistical analysis of coagulation time showed that, there were non-significant differences between control and T<sub>3</sub>, but there were significant differences between control and (T<sub>1</sub> and T<sub>2</sub>). On the other hand, there were non-significant differences between T<sub>3</sub> and (T<sub>1</sub> and T<sub>2</sub>).

### Total Solids

The results of the total solid contents of all yoghurt treatments (Table 2) of the same storage periods were closed to each other. Also, there was a slightly increase of the total solids for all the treatments along the storage. These results are in agreement with that of El-Nagar and Shenana (1998) and El-Sonbaty *et al.* (2008) who reported that there was a slight increase in the total solids of yoghurt due to loss of some moisture during cold storage.

The main effect of storage periods showed a significant differences for T.S (%) when fresh and after 7 days of storage, but there were non-significant differences at 14, 21 and 28 days of storage. Also, there were non-significant differences between treatments (p=0.741).

### Fat Content

The results of fat content (Table 2) showed that there were non pronounced differences between the control and the other treatments either when fresh or during the different storage periods. Moreover, there was a slight increase in the fat content during the storage periods and

Table 1. Coagulation time of novel functional yoghurts

Treatment	Coagulation time (hrs: min.)	(%) of increase in coagulation time
Control	3:45	----
T <sub>1</sub>	4:41	19.92
T <sub>2</sub>	4:38	19.06
T <sub>3</sub>	4:13	11.06

T<sub>1</sub>= *Lb. casei* ATCC 7469    T<sub>2</sub>= *Lb. paracasei* 6A    T<sub>3</sub>= kombucha

Table 2. Gross chemical composition of the produced novel functional yoghurt samples during storage periods at 4 °C up to 28 days

Storage period (day)	Treatment			
	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
<b>Total solids (%)</b>				
Fresh	16.08 <sup>a</sup>	15.97 <sup>a,b</sup>	16.02 <sup>a</sup>	15.87 <sup>b</sup>
7 days	16.20 <sup>a</sup>	16.14 <sup>a</sup>	16.17 <sup>a</sup>	16.02 <sup>b</sup>
14 days	16.29 <sup>a</sup>	16.26 <sup>a</sup>	16.27 <sup>a</sup>	16.20 <sup>a</sup>
21 days	16.24 <sup>a</sup>	16.25 <sup>a</sup>	16.24 <sup>a</sup>	16.19 <sup>a</sup>
28 days	16.23 <sup>a</sup>	16.22 <sup>a</sup>	16.19 <sup>a</sup>	16.15 <sup>a</sup>
<b>Fat (%)</b>				
Fresh	4.07 <sup>a</sup>	3.83 <sup>b</sup>	4.03 <sup>a</sup>	3.73 <sup>b</sup>
7 days	4.13 <sup>a</sup>	4.03 <sup>a</sup>	4.17 <sup>a</sup>	3.97 <sup>a</sup>
14 days	4.27 <sup>a</sup>	4.20 <sup>a</sup>	4.20 <sup>a</sup>	4.07 <sup>a</sup>
21 days	4.30 <sup>a</sup>	4.23 <sup>b</sup>	4.27 <sup>a</sup>	4.13 <sup>b</sup>
28 days	4.37 <sup>a</sup>	4.30 <sup>a,b</sup>	4.33 <sup>a,b</sup>	4.17 <sup>b</sup>
<b>Protein (%)</b>				
Fresh	4.34 <sup>a</sup>	4.26 <sup>a</sup>	4.29 <sup>a</sup>	4.27 <sup>a</sup>
7 days	4.49 <sup>a</sup>	4.41 <sup>a,b</sup>	4.39 <sup>b</sup>	4.38 <sup>b</sup>
14 days	4.53 <sup>a</sup>	4.54 <sup>a</sup>	4.48 <sup>a</sup>	4.48 <sup>a</sup>
21 days	4.46 <sup>a</sup>	4.44 <sup>a</sup>	4.41 <sup>a</sup>	4.42 <sup>a</sup>
28 days	4.41 <sup>a</sup>	4.37 <sup>a</sup>	4.36 <sup>a</sup>	4.33 <sup>a</sup>
<b>Soluble nitrogen/total nitrogen (%)</b>				
Fresh	1.18 <sup>c</sup>	2.12 <sup>a</sup>	2.27 <sup>a</sup>	1.80 <sup>b</sup>
7 days	1.42 <sup>d</sup>	2.16 <sup>b</sup>	2.67 <sup>a</sup>	1.84 <sup>c</sup>
14 days	1.58 <sup>c</sup>	2.25 <sup>b</sup>	2.81 <sup>a</sup>	2.11 <sup>b</sup>
21 days	1.78 <sup>b</sup>	2.89 <sup>a</sup>	3.10 <sup>a</sup>	2.87 <sup>a</sup>
28 days	1.87 <sup>b</sup>	3.40 <sup>a</sup>	3.52 <sup>a</sup>	3.35 <sup>a</sup>

T<sub>1</sub>= *Lb. Casei*    T<sub>2</sub>= *Lb. paracasei*    T<sub>3</sub> = kombucha

Means at the same row with different superscripts are different (P&lt;0.05)

this could be attributed to the high total solids as a result of some moisture evaporation (Basiony *et al.*, 2015). Parallel trends were showed by Badawi *et al.* (2004), El-Sonbaty *et al.* (2008) and El-Alfy *et al.* (2011).

The statistical analysis showed that there were significant differences between interventions. Moreover, there were significant differences between interventions and storage periods. There were significant differences between all the yoghurt treatments when fresh, 21 and 28 days of storage but there were non-significant differences between interventions at 7 and 14 days of storage.

### Protein Content

Data of protein content (Table 2) of the novel functional yoghurt treatments during the storage periods showed that there was a slight change between control and the other treatments at the same storage period. The protein content of yoghurts was slightly increased after 14 days of storage; and this increase might be due to the increase of the total solids. These results are in agreement with Kebary *et al.* (2004), Mahmoud (2005), Shenana *et al.* (2007) and El-Alfy *et al.* (2011).

By the end of storage period (28 days) a slight decline of protein content was recorded in all the treatments with different rates than the control and this may be due to the proteolytic effect of the added starter culture (Abdou *et al.*, 2015).

Statistically, the storage periods showed significant differences of the protein content between the interval storage periods, but, there were non-significant differences between interventions and storage periods.

### Soluble Nitrogen/Total Nitrogen (SN/TN)

The results (SN/TN) of all the novel functional yoghurts during the storage periods (Table 2 and Fig. 1) revealed that the control had low values in a comparison to other treatments at the same storage periods. This might be due to the ability of the used microorganisms to hydrolyze the protein more than the microorganisms of starter culture resulting higher soluble nitrogen.

The soluble nitrogen content increased during the cold storage periods of all treatments,

but the control recorded the lowest values. These results are in agreement with Papadimitriou *et al.* (2007), Hussien (2010) and Abdou *et al.* (2015).

The storage periods showed significant differences of the interval storage periods, also the different treatments showed that there were significant differences between interventions. Moreover, there were significant differences for the interaction between interventions and storage periods.

### pH Values

The impact of adding other lactic acid bacteria beside the starter culture, *i.e.* *Lb. casei* ATCC 7469, *Lb. paracasei* 6A and kombucha at a rate of 3% on pH values is recorded in Table 3.

The pH values were decreased along the storage periods, which reflect the development of acidity by the activity of the used microorganisms either starter culture or probiotic microorganisms. These results agree with those reported by El-Alfy (1988), El-Sonbaty *et al.* (2008) and El-Alfy *et al.* (2011).

Statistical analysis of the pH data showed the significant differences in the pH levels at different storage periods for all treatments compared with the control, there were non-significant differences between the intervals storage periods of the control and T<sub>3</sub>.

### Water Holding Capacity (WHC)

Water holding capacity (WHC) is a character used as indicator of how much water can be reserved in yoghurt. The most important factors which affect the WHC are the total solids (especially proteins and fat contents), the starter culture (either ropy or non-ropy) and manufacturing conditions of yoghurt (Chandan *et al.*, 2006). From the obtained results it is clear that the WHC was increased with the increase of total solids and decreases with the decrease of total solids. Also the obtained data reflect the structure of the product which depends on the chemical composition (Lucey, 2004).

Statistical analysis for WHC showed that there were significant differences between all the produced yoghurt when fresh, 7 and 14 days of storage, respectively, but there were non-significant differences after 21 and 28 days of storage. Also, storage periods had no significant effect on the WHC for T<sub>1</sub>.

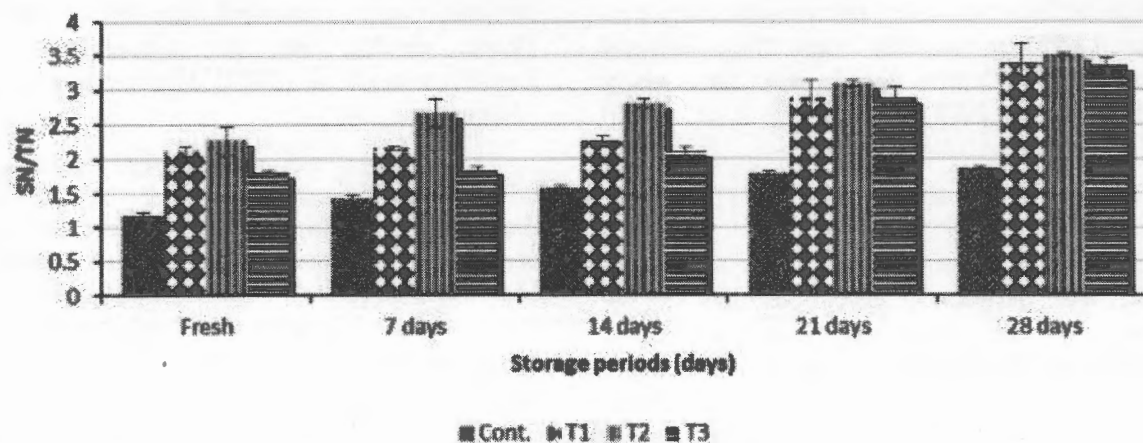


Fig. 1. Change of the soluble nitrogen/total nitrogen (%) of novel functional yoghurts during the storage periods at 4°C up to 28 days

Table 3. Change in the pH values of novel functional yoghurts during storage periods at 4°C up to 28 days

Storage period (day)	Treatment			
	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Fresh	4.62 <sup>a</sup>	4.58 <sup>a</sup>	4.59 <sup>a</sup>	4.60 <sup>a</sup>
7	4.52 <sup>a</sup>	4.47 <sup>a</sup>	4.46 <sup>a</sup>	4.53 <sup>a</sup>
14	4.49 <sup>a</sup>	4.39 <sup>b</sup>	4.38 <sup>b</sup>	4.48 <sup>a</sup>
21	4.46 <sup>a</sup>	4.33 <sup>b</sup>	4.31 <sup>b</sup>	4.41 <sup>a</sup>
28	4.39 <sup>a</sup>	4.28 <sup>b</sup>	4.20 <sup>b</sup>	4.33 <sup>a</sup>

T<sub>1</sub>= *Lb. casei* ATCC 7469 T<sub>2</sub>= *Lb. paracasei* 6A T<sub>3</sub>= kombucha

Means at the same row with different superscripts are different (P<0.05)

Table 4. Change in the WHC of novel functional yoghurts during storage periods at 4°C up to 28 days

Storage period (day)	Treatment			
	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Fresh	790.83 <sup>a</sup>	763.17 <sup>b</sup>	766.67 <sup>b</sup>	795.17 <sup>a</sup>
7	794.00 <sup>a</sup>	765.33 <sup>c</sup>	771.50 <sup>b</sup>	798.50 <sup>a</sup>
14	798.50 <sup>a</sup>	768.67 <sup>b</sup>	773.17 <sup>b</sup>	752.67 <sup>c</sup>
21	791.33 <sup>a</sup>	762.50 <sup>a</sup>	765.83 <sup>a</sup>	767.00 <sup>a</sup>
28	784.67 <sup>a</sup>	770.83 <sup>a</sup>	759.00 <sup>a</sup>	790.17 <sup>a</sup>

T<sub>1</sub>= *Lb. casei* ATCC 7469 T<sub>2</sub>= *Lb. paracasei* 6A T<sub>3</sub>= kombucha

Means at the same row with different superscripts are different (p<0.05)

### Angiotensin Converting Enzyme Inhibition (%) (ACE-inhibition %)

Bioactive peptides could be liberated during the fermentation of milk using different microorganisms of starter cultures which contain several proteolytic enzymes that are responsible for the breakdown of the protein resulting peptides and amino acids depending on the degree of hydrolysis (Meisel *et al.*, 1997).

The hydrolysates of peptides and amino acid sequence (bioactive peptides) have the ability to inhibit the ACE. The maximum ACE inhibition by these bioactive peptides is a result of the hydrolysate (Van der ven *et al.*, 2002). Using of lactic acid bacteria in making some dairy products *i.e* yoghurt one of the famous technique for producing these bioactive peptides.

Table 5 and Fig. 2 show the results of ACE-inhibition (%) of novel functional yoghurts extracts during the storage periods. From the recorded data it can be noticed that there were differences between the control and the other treatments. The control extracts recorded the lowest ACE-inhibition (%), either when fresh or during the interval storage periods in a comparison with the other treatments. Similar results were obtained by Solieri *et al.* (2015). The ACE-inhibition (%) increased during the storage periods. The increase of the ACE-inhibition (%) of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> might be due to the ability of the used microorganisms to liberate bioactive peptides from milk proteins during fermentation process. These results are in agreement with Papadimitriou *et al.* (2007). It is good to mention that the (%) soluble nitrogen was having the trend of increasing during the storage periods, in contrast with storage progress gave a chance to the lactic acid bacteria, the added strains and kombucha to increase protease enzymes which gave more soluble proteins (Table 2) and more active peptides which increase the inhibit of the ACE. The obtained results are in accordance with Ryhänen *et al.* (2001) and Van der ven *et al.* (2002) who found that the ACE-inhibitory activity of liberated bioactive peptides from milk fermented products could be decreased when proteolysis exceeded a certain level. So, the higher bioactive peptides the higher ACE-inhibition (%). From the

forgoing results the produced novel functional yoghurt could be serve as high ACE-inhibitory.

Statistical analysis of (%) ACE-inhibition showed that the (%) ACE-inhibition increased over storage periods, but less in the control. The main effect of storage periods showed significant differences in the (%) ACE-inhibition at different storage periods. Moreover, there were significant differences in the (%) ACE-inhibition between interventions. Also there were statistically significant interaction between interventions and storage periods.

### Firmness

The penetration readings of novel functional yoghurts give indications about the structure, homogeneity, consistency and tension of the produced gel. Results in Fig. 3 show the penetrometer readings (0.1mm/sec.) of yoghurts during the storage periods. From the results it can be observed that the firmness of T<sub>3</sub> was higher than control and other treatments. This result is in agreement with El-Alfy *et al.* (2011).

The penetrometer reading of yoghurts recorded 231, 226, 228 and 216 for control to T<sub>3</sub>, respectively, when fresh. It is going to be decreased among the treatments with different rates along the cold storage up to 14 days of storage according to the changes in the nature and protein content. These results are in accordance with those obtained by Abdou *et al.* (2015) as they attributed the decrease of penetrometer reading and hence the increase of firmness to continuous protein rearrangement and more protein-protein interaction which would increase the viscosity and firmness of yoghurt during the cold storage as affected by the type of starter. Also it can be noticed that there was a little bit increase in the penetrometer reading in 21 and 28 days of cold storage, this may be due to the hydrolysis of casein. These results are in agreement with Shenana *et al.* (2007) and El-Alfy *et al.* (2011).

From statistical analysis of penetrometer readings data, it was found that the main effect of storage periods had statistical significant differences at different storage periods, also the main effect of treatments showed that there were statistically significant differences between

**Table 5. Change of the \*ACE-inhibition (%) of yoghurts extracts during the storage periods at 4°C up to 28 days**

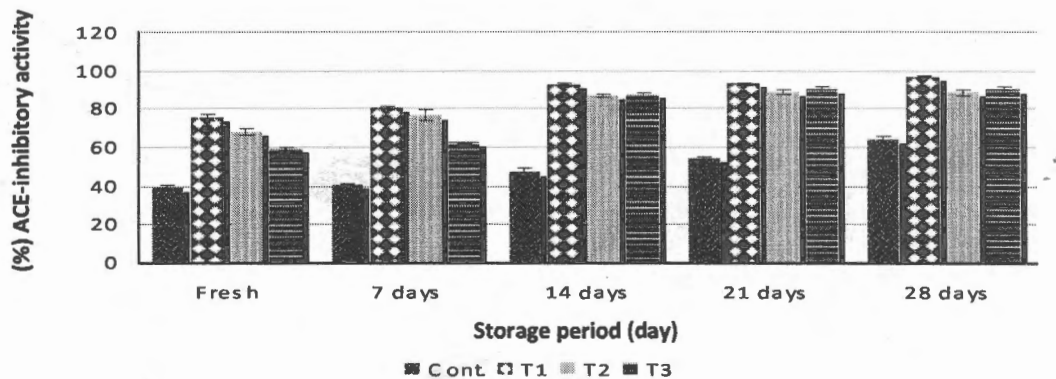
Storage period (day)	Treatment						
	Control	T <sub>1</sub>		T <sub>2</sub>		T <sub>3</sub>	
		(%) Inhibition	** (%) Increase	(%) Inhibition	** (%) Increase	(%) Inhibition	** (%) Increase
Fresh	39.26 <sup>d</sup>	75.76 <sup>a</sup>	48.17	67.98 <sup>b</sup>	42.24	59.20 <sup>c</sup>	33.68
7	40.86 <sup>c</sup>	80.16 <sup>a</sup>	49.02	76.53 <sup>a</sup>	46.60	62.25 <sup>b</sup>	34.36
14	47.14 <sup>c</sup>	92.25 <sup>a</sup>	48.89	86.69 <sup>b</sup>	45.62	87.22 <sup>b</sup>	45.95
21	54.28 <sup>c</sup>	92.97 <sup>a</sup>	41.61	88.55 <sup>b</sup>	38.70	89.92 <sup>b</sup>	39.63
28	63.96 <sup>c</sup>	96.61 <sup>a</sup>	33.79	88.59 <sup>b</sup>	27.80	89.96 <sup>b</sup>	28.90

T<sub>1</sub>= *Lb. casei* ATCC 7469    T<sub>2</sub>= *Lb. paracasei* 6A    T<sub>3</sub>= kombucha

\*ACE= Angiotensin converting enzyme, the activity of the used enzyme is 0.008 unit.

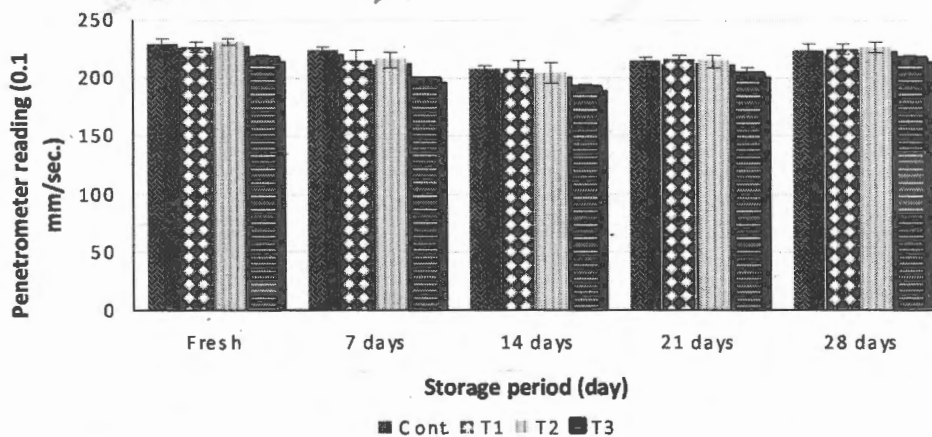
\*\* (%) increase in comparison to the control.

Means at the same row with different superscripts are different (p<0.05)



Cont. = control    T<sub>1</sub>= *Lb. casei* ATCC 7469    T<sub>2</sub>= *Lb. paracasei* 6A    T<sub>3</sub>= kombucha

**Fig. 2. Change of the ACE-inhibition (%) of yoghurts extracts during the storage periods at 4°C up to 28 days**



**Fig. 3. Change in the penetrometer reading (0.1 mm/sec.) of yoghurts during storage periods at 4°C up to 28 days**



interventions, but there were no statistically significant interaction between interventions and storage periods ( $P=0.364$ ). There were no statistical significance differences between interventions at the end of storage periods ( $P=0.068$ ).

#### Total Viable Microorganisms Count (TVC) of the Produced Novel Functional Yoghurt (log cfu/g) During the Storage Periods up to 28 Days

Data in Table 6 and Fig. 4 indicate the microorganisms counts of the produced novel yoghurts during the storage periods. The

recorded counts (log cfu/g) of yoghurts microorganisms increased after 7 days of storage, then decreased up to the end of the storage periods. These results are in agreement with Abd El-Salam *et al.* (1991), Barrantes *et al.* (1994) and El-Alfy *et al.* (2011).

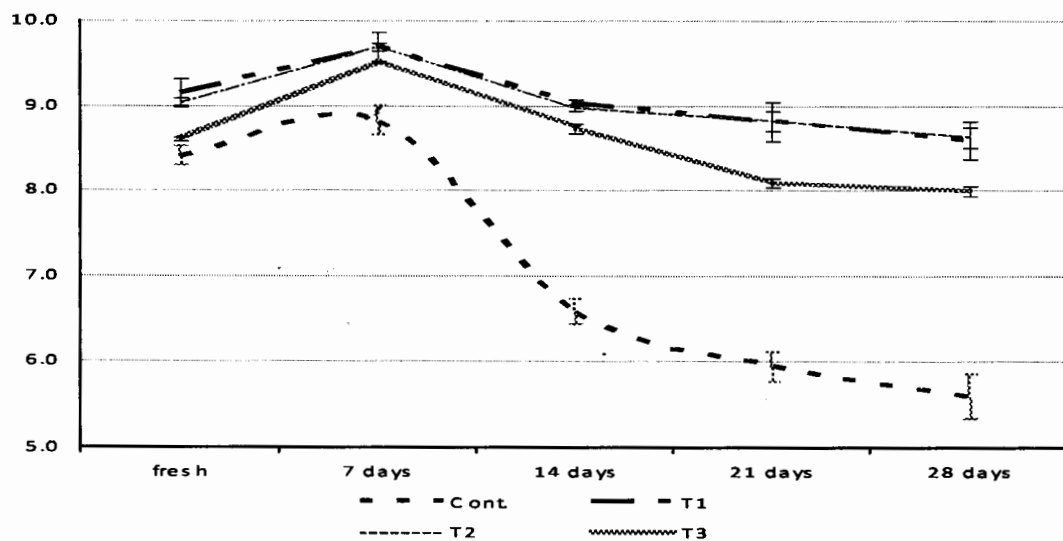
From statistical analysis point of view TVC of the novel functional yoghurts microorganisms showed that the main effect of storage periods had significant differences at different storage periods. Also, there were statistically significant differences between interventions. Moreover there was significant interaction between interventions and the cold storage periods.

**Table 6. Total viable microorganisms count (TVC) of novel functional yoghurts (log cfu/g) during the storage periods at 4 °C up to 28 days**

Storage periods (days)	Treatments			
	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Fresh	8.4 <sup>b</sup>	9.2 <sup>a</sup>	9.0 <sup>a</sup>	8.6 <sup>b</sup>
7	8.8 <sup>b</sup>	9.7 <sup>a</sup>	9.7 <sup>a</sup>	9.5 <sup>a</sup>
14	6.6 <sup>c</sup>	9.0 <sup>a</sup>	9.0 <sup>a</sup>	8.7 <sup>b</sup>
21	5.9 <sup>c</sup>	8.8 <sup>a</sup>	8.8 <sup>a</sup>	8.1 <sup>b</sup>
28	5.6 <sup>c</sup>	8.6 <sup>a</sup>	8.6 <sup>a</sup>	8.0 <sup>b</sup>

T1= *Lb. casei* ATCC 7469      T2= *Lb. paracasei* 6A      T3= kombucha

Means at the same row with different superscripts are different ( $p<0.05$ ).



**Fig. 4. Total viable microorganisms count of novel functional yoghurts (log cfu/g) during the storage periods at 4°C up to 28 days**

## Conclusions

From the forgoing results, we could conclude that the use of *Lb. casei* ATCC 7469, *Lb. paracasei* 6A and kombucha in the manufacture of yoghurt could introduce novel functional types of yoghurt with ACE-inhibitory activity. The chemical, physical and microbiological characteristics of the produced novel functional yoghurts were as good as the control up to the end of storage periods (28 days).

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## إنتاج يوغورت وظيفي يحتوي على مثبط لنشاط الإنزيم المحول للأنجيوتنسين

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تم استخدام *Lactobacillus casei* ATCC 7469، *Lb. paracasei* 6A و kombucha (الكمبوشا) بالإضافة إلى بادئ اليوغورت العادي في إنتاج يوغورت وظيفي له قدرة علي تثبيط انزيم ACE (الإنزيم الذي يحول الأنجيوتنسين I الي انجيوتنسين II) والذي يعتبر المسئول الرئيسي لإرتفاع ضغط الدم. تشير النتائج إلي أن القدرة التثبيطية لليوغورت الوظيفي الجديد إزدادت خلال فترة التخزين المبرد، كما تشير نتائج التحليل الكيميائي إلي أن اليوغورت الوظيفي الجديد له تركيب كيميائي مقارب لعينة المقارنة (الكنترول)، أيضا من نتائج التحليل الميكروبيولوجي لوحظ أن نتائج العدد الميكروبيولوجي بالنسبة للمعاملات المختلفة متقارب مع عينة المقارنة. كما تشير نتائج الاختبارات الريولوجية أن القدرة علي الإحتفاظ بالماء والقوام والتركيب كانت أيضا جيدة في المعاملات المختلفة مثل عينة المقارنة حتي نهاية مدة التخزين (٢٨ يوم).

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