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PROPERTIES OF BIOGHURT DURING STORAGE AS INFLUENCED BY CERTAIN DRIED HERBS AND THEIR OILS

Metry, Wedad A.; Neimat A.H. Elewa; M.O. El-Demerdash and Warda M.A. Ebid
Dairy Department, Faculty of Agriculture, Fayoum University, Egypt.

ABSTRACT

The main target of this study was to know the effect of adding herbs either powder (0.25% of rosemary, chamomile, marjoram or ginger) or their oils (0.025% of rosemary, marjoram or ginger) on some properties of Bioghurt (*Lactobacillus acidophilus* Lab-5 and *Streptococcus thermophilus* were used as starter culture). Microbiological, chemical and organoleptic properties were investigated for all resultant treatments on day one (fresh) and during storage period at 6±1°C for 15 days. The results indicated that, there were significant (P≤0.05) differences for titratable acidity, lactose, flavour compounds and water soluble nitrogen, while non significant differences for total solids, fat and total nitrogen contents were found between treatments and during storage period. The Bioghurt samples containing chamomile, rosemary powder, marjoram or rosemary oils were ranked the highest viability of the probiotic bacteria (*L. acidophilus* Lab-5) during storage at 6±1°C for 15 days and the number of this probiotic bacteria remained above the recommended threshold for a probiotic effect (10^6 cfu/ml) also, these treatments (except chamomile Bioghurt) gained higher sensory evaluation scores than the other treatments and control. Microbiological examination illustrated that, all treatments had a good quality. It could be summarized that the addition of either marjoram oil or rosemary in powder or essential oil to Bioghurt are recommended to improve the viability of *Lb. acidophilus* and organoleptic properties of the resultant Bioghurt.

Keywords: Bioghurt, viability, rosemary, chamomile, marjoram, ginger oils, sensory evaluation and probiotic bacteria.

INTRODUCTION

Lactobacillus acidophilus is a probiotic micro-organism available in milk, yoghurt , toddler formula and dietary supplements which has health-promoting effects, including: alleviation of lactose intolerance, anti-tumor, hypocholesterolemic effects, anti-infection properties and antagonistic effect against food-borne disease agents (Savadogo *et al.*, 2006 and Eied, 2008).Recent scientific investigations have supported the important role of probiotics as a part of a healthy diet for human as well as for animals and may be an avenue to provide a safe, cost effective and 'natural' approach that adds a barrier against microbial infection (Kasimoglu and Akgun, 2004, Parvez *et al.*, 2006 and Millette *et al.*, 2007).

Herbs and spices could be regarded as the first real "functional foods" because they have a long history of medicinal use. Also, spices found to have antimicrobial activities against several species of bacteria, fungi and viruses (Yano *et al.*, 2006 and Busatta *et al.*, 2008), in addition to their flavor and fragrance properties. Most of the food borne bacterial pathogens examined were sensitive to extracts from plants. The antimicrobial compounds in spices and herbs mostly are in the essential oil fraction. The extent of sensitivity varied with the strain and environmental conditions imposed. Certain spices can have a direct effect on the rate of fermentation by stimulating acid production in starter cultures. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in spices (Ceylan and Fung, 2004). Recent studies have demonstrated that the prooxidant activity of essential oils or some of their constituents are very efficient in reducing local tumor volume or tumor cell proliferation by apoptotic and/or necrotic effects, as also that of some polyphenols (Dudai *et al.*, 2005; Tsuneki *et al.*, 2005; Kachadourian and Day, 2006 and Manosroi *et al.*, 2006). In Egypt, many investigators used natural flavoring additives in probiotic dairy products, such as peppermint, anise and caraway herb oil in probiotic liquid fermented products, probiotic labneh and bio-ice milk products (Gooda *et al.*, 2002 and El-Nemr *et al.*, 2004a and b).

Therefore, the objective of this work was to study the effect of adding rosemary, chamomile, marjoram or ginger herbs powder and rosemary, marjoram or ginger herbs oil on some microbiological, chemical and organoleptic properties of Bioghurt stored at 6±1°C.

MATERIALS AND METHODS

1. Milk: Fresh buffaloes' milk was obtained from the herd of animal production farm, Fac. of Agric., Fayoum Univ.

Bacterial strain: *L. acidophilus* - La 5 and *Str. thermophilus* were obtained from Chr. Hansen's laboratories, Copenhagen, Denmark . Herbs: Chamomile (flowers), marjoram (leaves), rosemary (leaves) and ginger (rhizomes) herbs were purchased from the local spices market at Fayoum, while essential oils of the marjoram, rosemary and ginger were obtained from Perfumes and Essences Factories, El-Hawamdia, Giza.

Methods

1. Activation of bacterial strains

Lyophilized culture of *Str. thermophilus* was transferred aseptically into sterilized skim milk and incubated at 37°C until coagulation. Three successive transfers were carried out daily in the same medium for maximum activation. *L. acidophilus* was transferred into sterilized skim milk containing 10 gm dextrose and 1gm yeast extract /L and incubated at 37°C until coagulation. Further activation was achieved by three similar successive transfers in the same medium (Beena and Prasad, 1997).

2. Preparation of herbal Bioghurt.

Preliminary experiments were conducted to select the suitable types and concentrations of herbs and essential oils. The results illustrated that the addition of mint, cinnamon, liquorice and roselle gave a decrease in viability compared with control, furthermore, mint and roselle gave undesirable flavour, also on the addition of chamomile oil led to unacceptable color and therefore the resultant fermented milk was organolyptically refused. On the other hand, the increase in herbs concentration decreased the acceptability of the products of all treatments, the results of sensory evaluation and viability of all herbs revealed that, the most preferable treatments were 0.25%

of chamomile, marjoram, ginger and rosemary powder compared with 0.5 and 1%, but rosemary, ginger and marjoram oil at concentrate 0.025%, gave the preferred score of organoleptic properties compared with 0.05 and 0.075%.

Herbal Bioghurt was made from a standardized (1.5% fat, 0.17% titratable acidity, 5.4% lactose, 4.33 % protein and 12.76% total solids) heat treated buffaloes' milk (90°C/10 min) which divided into two groups as follows: a. The first group was divided into five portions and mixed individually with 0.25% herbs powder(at 90°C) as follows: chamomile Bioghurt ($AT_{ch.p}$), marjoram Bioghurt ($AT_{mar.p}$), ginger Bioghurt ($AT_{gin.p}$) and rosemary Bioghurt ($AT_{ros.p}$), respectively, and filtered after 15 min and AT_c treatment served as control (without herbs). b. In the second group, heat treated buffaloes' milk was divided into three portions mixed with 0.025% rosemary ($AT_{ros.o}$), ginger ($AT_{gin.o}$) or marjoram ($AT_{mar.o}$) oils individually. All treatments were inoculated with 2 % *L. acidophilus* and *Str. thermophilus* (1:1 v/v), distributed, incubated at 40°C until complete fermentation(4-5 h) and stored at 6±1°C up to 15 days. Samples of the resultant herbal powder and oils Bioghurt were subjected to chemical, microbiological and organoleptic properties when fresh (on day one) and during storage period.

3. Method of analysis

Microbiological examinations

L. acidophilus counts of Bioghurt samples were enumerated using MRS agar (Dave and Shah, 1996), total viable counts were enumerated with nutrient agar medium, staphylococci counts of the examined fermented milk samples were p1ated with mannitol salt agar, coliform bacteria group was enumerated on MacConkey's agar medium and Potato dextrose agar medium was used in counting yeasts and moulds as described by Bridson (1990).

N.B.: All counts were expressed as log colony forming unit per milliliter (cfu/ ml).

pH values of fresh milk and resultant Bioghurt samples were measured by using laboratory pH meter with a glass electrode Model pH-(Kent EIL 7020). Titraltab1e acidity expressed as lactic acid (%), fat, moisture, total nitrogen and water soluble nitrogen contents were estimated as described in AOAC (1998). Lactose content was calorimetrically determined as described By Lawrence (1968). Acetaldehyde and diacetyl content were estimated as described by Lees and Jago (1969). Essential oils in herbal Bioghurt samples were analyzed by Hewellet Packard Gas Chromatography (GC) according to Celiktas *et al.* (2007).

Sensory evaluation

The resultant herbal Bioghurt and control samples were organoleptically evaluated according to Bodyfelt *et al.* (1988) by a panel consisting of 18 persons, including 8 staff members and 10 students at the department of dairy science, Faculty of Agriculture, Fayoum University. The panelists judged the samples according the following points: flavour (60 points), body and texture (30 points) and appearance (10 points).

Statistical Analysis

All the experiments were performed in triplicate and the results obtained were analyzed statistically. General linear models (GLM) were performed using SPSS (1999) for windows, version 9 software package. Significant differences among treatments, storage period and the interaction means between them were compared at P≤0.05 level of significance using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Microbiological properties of Bioghurt treatments during storage time at 6 ± 1° C

The viability of *L. acidophilus* in Bioghurt treatments during storage time at 6±1°C illustrated in Fig. (1). These results showed that the addition of herbs powder or oils(chamomile, marjoram, ginger and rosemary) to Bioghurt had stimulatory significant (P≤0.05) effect on the viable counts of *L. acidophilus*. Log counts of *L. acidophilus* were varied from 8.21 to 8.70 cfu /ml in either the fresh powder or oils Bioghurt samples. The highest value was obtained from $AT_{mar.o}$ and $AT_{ros.o}$ treatments while AT_c was the lowest one (8.21 cfu /ml).The statistical analysis of the data obtained clearly illustrated that, each of treatment, storage period and its interaction had significant influences on viable counts of *L. acidophilus* (P≤0.05). However during storage time significant increases were observed in *L. acidophilus* viable counts in all treatments during the first 5-7 days followed by a decrease afterward , except rosemary treatment ($AT_{ros.p}$ and $AT_{ros.o}$), in which had the highest viable count (9.40 log_{10} cfu/ml) at the 9th day whereas of herbal Bioghurt and control. *L.acidophilus* varied from7.11 to 8.45 log cfu/ml at the end of storage period. The partially lowest count was shown in both AT_c and $AT_{gin.p}$ treatments but the highest viable count of *L. acidophilus* was shown for $AT_{ros.p}$ being 8.45 log cfu /ml. Acidic environment is believed to be the most detrimental factor affecting growth and viability of *L. acidophilus* in all treatments particularly after 8 days of storage (Hefny *et al.*, 1995 and Roushdy *et al.*, 1996).

It could be concluded that the additions of herb oils (marjoram or rosemary) and chamomile reflected significantly higher viability than control. On the other hand, all treatments still had the highest *L. acidophilus* viable counts more than 7 log cfu/ml up to 15 days of storage at 6±1°C. The minimum level of probiotic bacteria in fermented milk must be more than 10^6 viable cells per ml products to obtain therapeutic benefits (Kurmann and Rasic, 1991 and Shah and Ravula, 2000).

The viable counts of *S. thermophilus* varied from 7.11 to 7.87 log cfu/ml in all fresh Bioghurt treatments and ranged from 6.13-6.87 log cfu/ml at the end of storage. The use of combined cultures of lactic acid bacteria, viz. *S. thermophilus* and *L. bulgaricus*. *S. thermophilus* alone or mesophilic aromatic cultures, has been advocated as a solution for many such problems, increased growth rates and reduction of fermentation time, absence of certain sensory and texture defects and further improvement of nutritional value of products are advantages brought about by the latter possibility. Adverse effects with respect to viability have, however, been reported for some strains of *L. acidophilus* as reported by Hoier (1992), Gomes *et al.* (1995) , Samona *et al.* (1996) and Dave and Shah (1997).

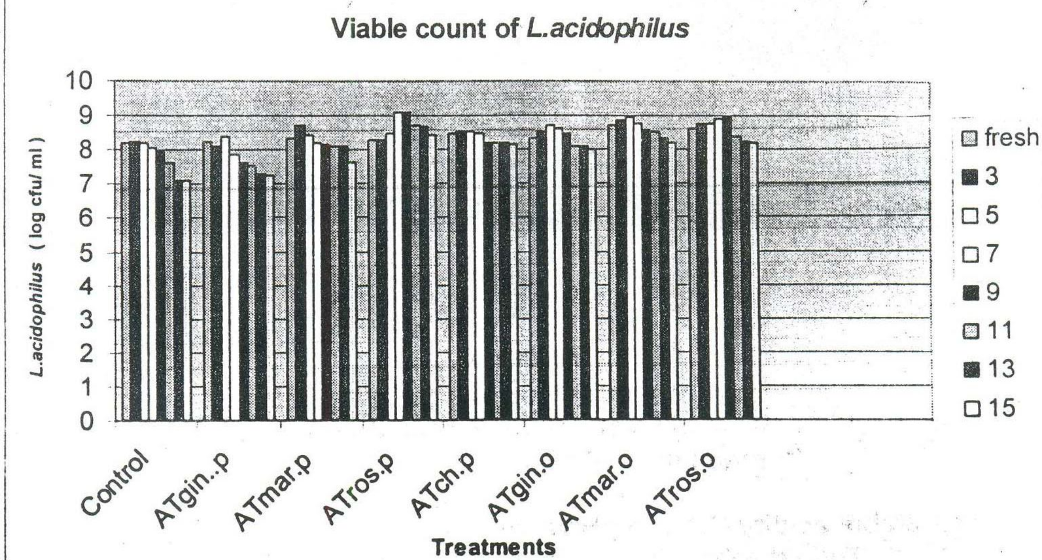

Fig . (1): Viable counts of *L.acidophilus* in herbal Bioghurt and control during storage time at 6 ± 1 °C

The results in Fig. (2) showed the changes occurred in total viable counts (TVC) in control, herbal powder and oil Bioghurt samples stored at 6±1°C. Samples containing herbal powder and the control had significantly ($P \leq 0.05$) higher TVC than those containing herbal oils .The highest count was obtained from both AT_c and $AT_{ch.p}$ treatments., whereas the lowest counts were shown for $AT_{gin.o}$ and $AT_{ros.o}$ treatments. TVC significantly increased during the first 7-9 days, then decreased till the end of storage period. . This may be due to the decrease in pH values during storage (Vinderola *et al.*, 2000).

Staph. aureus, coliform, molds and yeasts were not detected in control and all treatments of herbal powder or oils added to Bioghurt either fresh or stored at 6±1°C. This might be due to the efficient heat treatment of milk which destroy the vegetative cells, also high sanitation and hygenic condition during manufacture and storage . In addition to the effect of acidity of Bioghurt which plays an important role in reducing the rate of growth of *Staph. aureus* and coliform. Also, the antagonistic substances produced by *L. acidophilus* was effective against these microbial groups.These results are in accordance with those reported by El-Nagar and Shenana (1998) and Ammara (2000). Some yeasts and moulds appeared at 15th day of storage in the control sample and after 15 days in other herbal Bioghurt samples .

Chemical composition of fresh and stored Bioghurt at 6 ± 1 °C

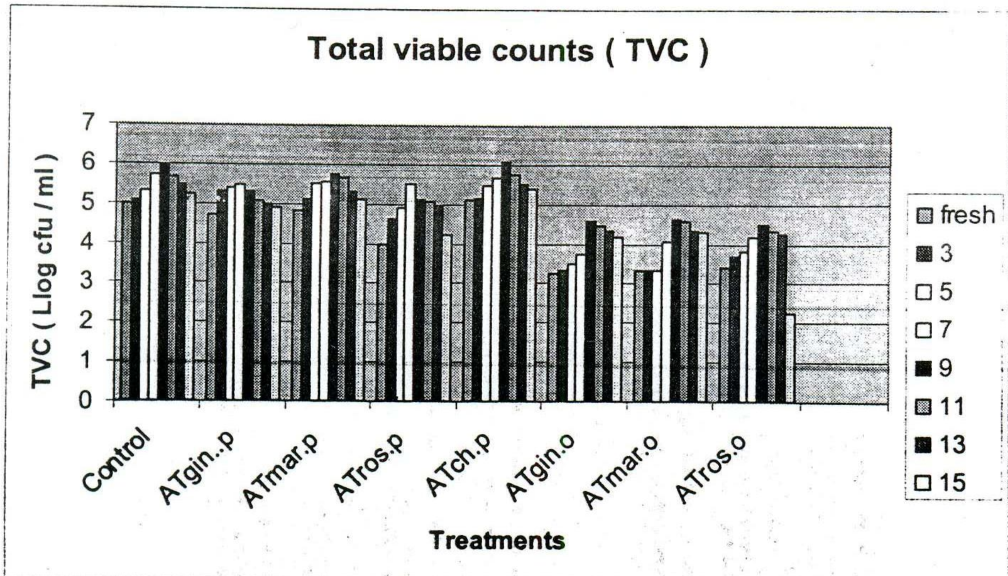

Fig . (2): Total viable counts in herbal Bioghurt and control during storage time at 6 ± 1 ^{0}C

Titratable acidity (%) and pH values

The titratable acidity values were ranged from 0.65 to 0.73 % in the control, herbal powder and oil Bioghurt (Table , 1) . The corresponding pH values varied from 3.95 to 4.23 at the beginning of storage. The highest value of TA and lowest pH value were obtained for the $AT_{ch\cdot p}$ This may be attributed to the strain of starter with chamomile which had the highest activity (Fig., 1).On the other hand, the control samples had lower TA values and higher pH values than other treatments. TA values were gradually increased with simultaneous decrease in the pH values during storage at 6±1°C. At the end of storage , the TA varied from 0.83 to 1.04 % and pH values varied from 3.6 to 3.٩5 , such decrease in pH of Bioghurt may be attributed to lactose degradation and production of lactic acid during storage period.. Statistically, each of treatment, storage period and its interaction had significant influence (P≤0.05) on TA and pH values (Table, 2). During storage time gradual increase in TA with simultaneous decrease in pH values were observed in all treatments. TA varied from 0.83 to 1.04% and pH values varied from 3.60 to 3.75 at the end of storage period. Similar results were reported by Roushdy *et al.*, (1996) .

Table (1): Changes occurred in titratable acidity and PH values of fresh and stored herbal Bioghurt at 6 ± 1 °C

parameters	Storage Period (days)	Treatments								
			Herbal powder				Herbal oils			
		AT_c	$AT_{gin.p}$	$AT_{mar.p}$	$AT_{ros.p}$	$AT_{ch.p}$	$AT_{gin.o}$	$AT_{mar.o}$	$AT_{ros.o}$
Titratable acidity (%)	Fresh	0.62^{B}	0.65^{wxyz}	0.70^{wxyz}	0.68^{zA}	0.72^{tuvwx}	0.69^{zA}	0.71^{yzA}	0.71^{wxyz}
	3	0.69^{zA}	0.71^{wxyz}	0.74^{uvwxy}	0.71^{yzA}	0.75^{tuvwx}	0.74^{uvwxy}	0.73^{wxyz}	0.76^{stuvw}
	5	0.75^{tuvwx}	0.73^{wxyz}	0.77	0.71^{yzA}	0.77^{pqrstuvw}	0.79^{nopqrst}	0.75^{tuvwx}	0.79^{nopqrst}
	7	0.77^{pqrstuvw}	0.76^{stuvw}	0.78^{opqrstuv}	0.73^{wxyz}	0.81^{klmnopq}	0.83^{jklmn}	0.85^{hijkl}	0.84^{ijklm}
	9	0.81^{klmnopq}	0.79^{nopqrst}	0.80^{mnopqrst}	0.74^{uvwxy}	0.82^{klmnop}	0.87^{ghij}	0.88^{fghi}	0.92^{def}
	11	0.82^{klmnop}	0.82^{klmnop}	0.83^{jklmn}	0.78^{opqrstuv}	0.88^{fghi}	0.88^{fghi}	0.90^{efg}	0.96^{bcd}
	13	0.86^{ghij}	0.81^{klmnopq}	0.81^{klmnopq}	0.81^{klmnopq}	0.92^{def}	0.90^{efg}	0.95^{cd}	0.98^{bc}
	15	0.90^{efg}	0.85^{hijkl}	0.83^{fgh}	0.83^{jklmn}	0.92^{def}	0.94^{cde}	1.04^{a}	1.00^{b}
PH values	Fresh	4.23^{bc}	4.05^{efg}	4.01^{efghi}	4.12^{de}	3.95^{ghijklm}	4.25^{b}	4.20^{bc}	4.20^{bc}
	3	4.08^{def}	3.95^{ghijklm}	3.89^{jklmnopq}	3.94^{hijklmn}	3.80^{pqrs}	4.41^{a}	4.15^{bcd}	4.15^{bcd}
	5	4.02^{efghi}	3.85^{mno}	3.85^{mno}	3.90^{jklmnop}	3.80^{pqrs}	4.10^{de}	4.10^{de}	4.05^{efg}
	7	3.98^{fghijk}	3.84^{nopqr}	3.85^{mno}	3.90^{jklmnop}	3.75^{rstu}	3.99^{fghij}	3.95^{ghijklm}	3.99^{fghij}
	9	3.92^{ijklmno}	3.80^{pqrs}	3.83^{opqr}	3.88^{klmnopq}	3.75^{rstu}	3.95^{ghijklm}	3.85^{mno}	3.90^{jklmnop}
	11	3.87^{lmnopq}	3.80^{pqrs}	3.75^{rstu}	3.80^{pqrs}	3.70^{stu}	3.89^{jklmnopq}	3.80^{pqrs}	3.85^{mno}
	13	3.85^{mno}	3.75^{rstu}	3.75^{rstu}	3.79^{qrs}	3.65^{uv}	3.85^{mno}	3.74^{rstu}	3.80^{pqrs}
	15	3.95^{ghijklm}	3.70^{stu}	3.70^{stu}	3.75^{rstu}	3.60^{v}	3.80^{pqrs}	3.65^{tuv}	3.75^{rstu}

AT_c : Control $AT_{gin.p}$: ginger powder Bioghurt , $AT_{mar.p}$: marjoram powder Bioghurt , $AT_{ros.p}$: rosemary powder Bioghurt ,
$AT_{ch.p}$: chamomile powder Bioghurt , $AT_{gin.o}$: ginger oil Bioghurt , $AT_{mar.o}$: marjoram oil Bioghurt
$AT_{ros.o}$: rosemary oil Bioghurt
a, b, ...and I : means within the interaction having different small superscripts are significantly different ($P \leq 0.05$).

Table (2): Means of *L. acidophilus*, Total viable counts (TVC), titratable acidity (TA) and PH of herbal bioghurt as affected by type of addition , treatment and storage period (main effects)

Main effects		*L. acidophilus* (log cfu / ml)	TVC (log cfu / ml)	TA (%)	pH
Type of addition	Oil	8.٤٥^{a}	3.63^{b}	0.79^{b}	3.97^{a}
	Powder	8.٢٠^{b}	5.47^{a}	0.83^{a}	3.86^{b}
Treatment	AT_{c}	8.097^{c}	5.31^{c}	0.77^{c}	3.98^{a}
	ATch	8.54^{a}	7.10^{a}	0.83^{a}	3.75^{d}
	ATgin	8.07^{c}	5.28^{b}	0.80^{b}	3.94^{b}
	ATmar	8.33^{b}	5.50^{b}	0.82^{a}	3.88^{c}
	ATros	8.52^{a}	5.21^{e}	0.81^{b}	3.92^{b}
Storage period (days)	Fresh	8.39^{a}	5.15^{g}	0.70^{h}	4.14^{a}
	3	8.42^{a}	5.23^{f}	0.73^{g}	4.05^{b}
	5	8.39^{a}	5.48^{e}	0.76^{f}	3.97^{c}
	7	8.40^{a}	5.77^{b}	0.80^{e}	3.91^{d}
	9	8.36^{a}	5.89^{a}	0.83^{d}	3.87^{e}
	11	8.10^{b}	5.65^{c}	0.85^{c}	3.82^{f}
	13	8.12^{b}	5.54^{d}	0.88^{b}	3.78^{g}
	15	8.09^{b}	5.46^{e}	0.91^{a}	3.74^{h}

AT_{c} : Control **$AT_{gin.}$: ginger Bioghurt ,**
$AT_{mar.}$: marjoram Bioghurt **AT_{ros}: rosemary Bioghurt ,**
$AT_{ch.}$: chamomile Bioghurt
Means within each effect within the raw having different super scripts are significantly different at ($P \leq 0.05$)

Acetaldehyde, diacetyl and lactose contents

Acetaldehyde and diacetyl are the main volatile compounds responsible for the aroma and plays a considerable role in flavor development in fermented products during storage period. The acetaldehyde and diacetyl content of control and different treatments as affected by adding herbal powder and oils are presented in Figs. (3 and 4). Acetaldehyde content in Bioghurt samples varied from 86.6 to 159.3 ppm and diacetyl content ranged from 81.2 to 98.7 ppm in fresh Bioghurt samples. The $AT_{ch.p}$ treatment had higher values than other treatments; this may be due to the effect of this herb as active factor for *L. acidophilus* growth in fresh, while the lowest one was obtained for $AT_{mar.p}$ treatment. Each of treatment, storage period and its interaction had significant influences ($P \leq 0.05$) on both acetaldehyde and diacetyl contents (Table,3). A significant increase of acetaldehyde and diacetyl was observed in all treatments at the beginning of the storage until 5 days , followed by a gradual decrease till the end of storage period. The decrease in acetaldehyde content during storage is presumably due to the demonstrated ability of *L. acidophilus* to reduce acetaldehyde to ethanol or oxidize to acetic acid and slow reduction of diacetyl to acetone as detected by Driessen and Puhan, (1988), Salama (1993) and Roushdy *et al.*, (1996).

Table (3): Means of chemical analysis and organoleptic properties of herbal bioghurt as affected by type of addition , treatment and storage period (main effects) .

Parameter		Effects										
		Types		Treatments					Storage period (days)			
		oil	powder	AT_c	Ch.	Gin.	Mar.	Ros.	Fresh	5	10	15
Chemical analysis	Acetaldehyde	78.38^b	98.52^a	102.33^b	129.03^a	89.60^c	81.93^e	85.68^d	141.94^a	111.31^b	77.84^c	45.85^d
	Diacetyl	80.81^b	96.05^a	84.10^d	104.89^a	88.44^c	91.32^b	85.45^d	90.12^b	101.46^a	90.66^b	74.89^c
	Lactose	3.47^a	3.45^a	3.52^a	3.37^d	3.49^b	3.41^c	3.44^c	3.98^a	3.68^b	3.35^c	2.81^d
	WSN / TN	0.083^b	0.090^a	0.081^e	0.090^b	0.086^d	0.090^a	0.088^c	0.075^d	0.083^c	0.090^b	0.100^a
Organoleptic properties	Flavour	55.03^a	54.19^b	54.56^c	53.90^d	54.47^c	54.55^b	55.04^a	57.32^a	55.03^b	53.96^c	53.31^d
	Body& texture	27.46^a	26.73^b	26.97^b	26.70^b	26.74^b	27.47^a	27.36^{ab}	27.88^a	27.64^a	26.80^b	26.49^c
	Color & apperanc	8.64^a	7.85^b	8.53^a	7.75^c	8.06^b	7.97^{ab}	8.53^a	9.19^a	8.57^b	8.12^c	7.60^d
	Total score	91.16^a	88.75^b	90.11^a	88.50^c	89.28^b	89.99^b	90.93^a	94.39^a	91.31^b	88.84^c	87.40^d

Means within each effect within the raw having different super scripts are significantly different at ($P \leq 0.05$)
WSN/ TN : Water soluble nitrogen / Total nitrogen
AT_c : Control — $AT_{gin.}$: ginger Bioghurt , — **$AT_{mar.}$: marjoram Bioghurt ,**
AT_{ros}: rosemary Bioghurt , — $AT_{ch.}$: chamomile Bioghurt

Lactose content varied from 4.00 to 4.1% in all fresh samples. Lactose content of Bioghurt samples made with out herbs (control) was higher than other herbal Bioghurt treatments, but the lowest content was obtained by $AT_{mar.o}$ and $AT_{ros.o}$ at the end of storage (Fig. : 5) . This may be due to the higher activity of *L.acidophilus* in the presence of rosemary and marjoram oils than other treatments and control, which was refereed to the lower viable counts of *L. acidophilus* in the former than the other treatments (see Fig., 1). These results confirmed that the herb oil increase the activity of *L. acidophilus* which used more lactose in metabolism. The results indicated that during storage at 6±1°C for up to 15 days, there was significant decrease in all treatments during storage period. Total solids (TS), total nitrogen (TN), fat, water soluble nitrogen (WSN) contents and WSN/TN ratio

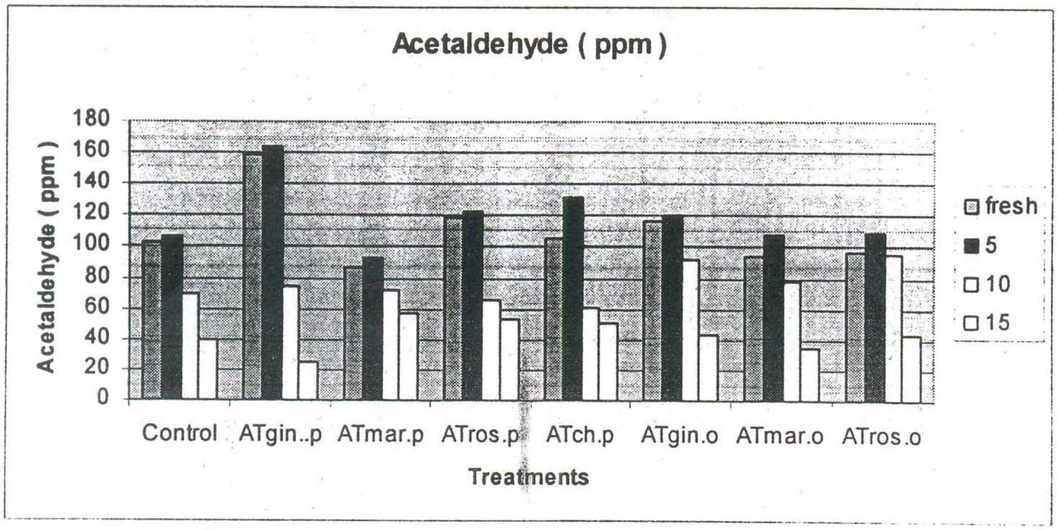

Fig . (3): Changes occurred in acetaldehyde (ppm) of Bioghurt treatments during storage time at 6 ± 1 °C

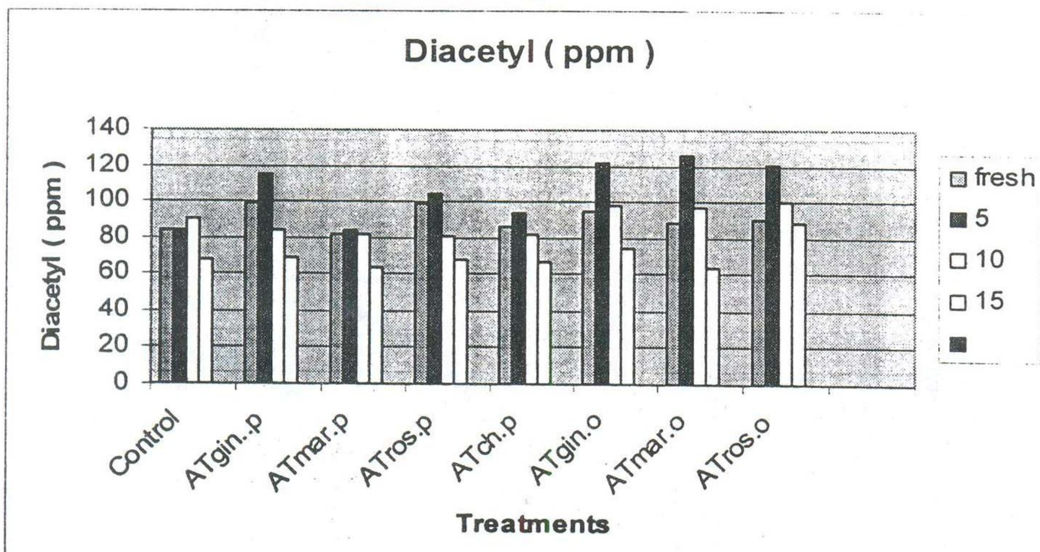

Fig . (4): Changes occurred in diacetyl (ppm) of Bioghurt treatments during storage time at 6 ± 1 °C

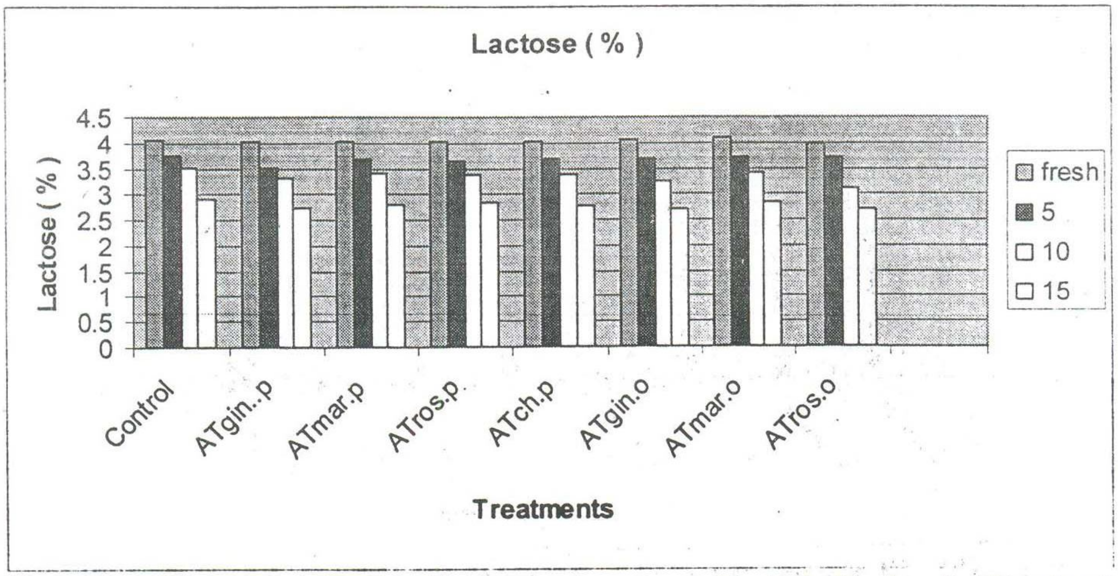

Fig. (5): Changes occurred in lactose content (%) of Bioghurt treatments during storage time at 6 ± 1 ^{0} C

Generally addition of different types of herbal powder and oils to Bioghurt insignificantly affected some chemical properties of the resultant herbal Bioghurt. The results illustrated that no significant changes in each of TS, TN and fat contents of herbal powder and oils Bioghurt either between treatments or during storage time at 6±1°C. TS , TN and fat content ranged from 12.75 to 12.94 , 0.665 to 0.696 and 1.75 to 1.86 , respectively in fresh Bioghurt samples . Whereas they ranged from 12.78 to 13.31 , 0.705 to 0.731 and 1.79 to 1.90 , respectively at the end of the storage period . This may be due to slightly evaporated water during storage.

Also, herbal powder Bioghurt samples had higher WSN/TN content than herbal oils as shown in Fig. (6). The content of WSN/TN in the fresh herbal powder Bioghurt sample ranged from 0.073 to 0.075% and a gradual increase during 15 days of storage were observed in all treatments and varied from 0.089 to 0.121 % at the end of storage. The highest value was obtained for $AT_{mar.p}$ and $AT_{ros.o}$ treatments and the lowest values were obtained for AT_{c1} and $AT_{gin.p}$ treatments. Similar findings were reported by El-Nawawy *et al.* (1998). WSN/TN ratio in herbal oil Bioghurt samples were varied from 0.076 to 0.079 % and from 0.089 to 0.099, respectively in fresh and stored (at 15 days) treatments. The highest value of WSN/TN was recorded by $AT_{ros.o}$ treatment as compared to the control which was the lowest one. However, significant increases (P≤0.05) were observed in WSN and WSN/TN during storage at 6±1°C for 15 days. These results are in agreement with those obtained by Tamime and Robinson (1985) and El-Nawawy *et al.* (1998) .

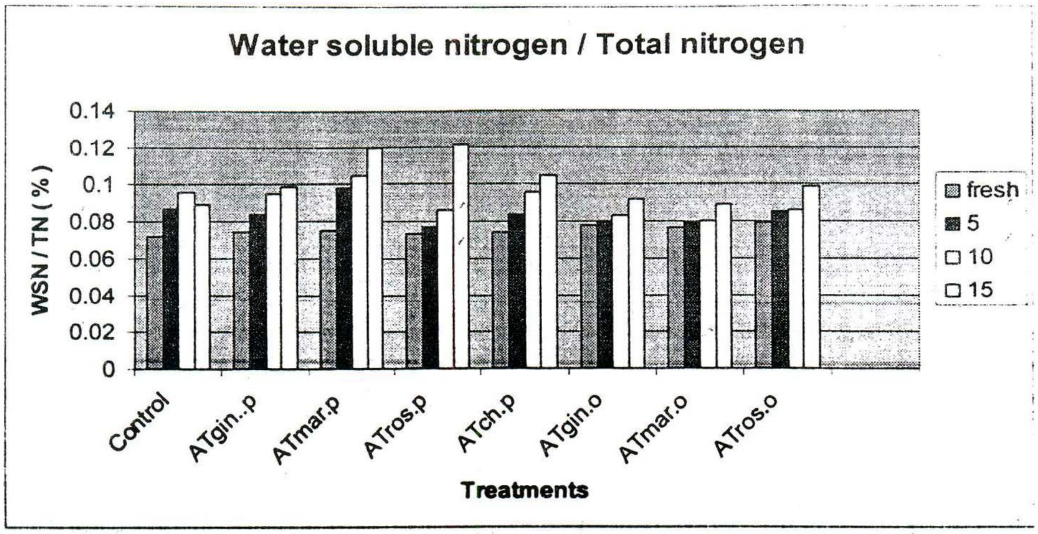

Fig . (6): Changes occurred in water soluble nitrogen / total nitrogen content of Bioghurt treatments during storage time at 6 ± 1 ^{0} C

Essential oils contents

Identification and determination of essential oils by GC in herbal powder and oils Bioghurt samples were investigated. There were differences in the types and ratios of studied herbs essential oils components (Table , 4). In rosemary powder Bioghurt treatment ; the major compounds were alcohols such as camphor (40.002% of all compounds), terpineol (30.614%) and borneol (15.218%) but the minor one was camphene (0.380%). Also , the results clearly illustrated that, the Lemonene, Camphene and β - pinene were the major components in rosemary oil Bioghurt and the minor components were isopropyl acetate and 1,8 Ceneole . This finding agree with that found by Lawrence (1997); Katerinopoulos *et al.* (2005); Wang *et al.* (2008) and Zaouali and Boussaid (2008) . The major components in marjoram powder Bioghurt were alcohols such as, Terpinen-4-ol (60.504%), linalool (26.030%) and phenolic as carvacrol (10.900%), but the components less than 1% were hydrocarbone as α – terpinene, *p*- cymene, alcohol as borneol and engenol. The major components in marjoram oil Bioghurt were carvacrol (17.00%), linalool (9.84), p- cymene (9.46), thymol (7.85) and Genaryl acetate (7.35). Some studies of in vitro antimicrobial activity reported that terpinen-4-ol was the main component of marjoram essential oil (Baratta *et al.*, 1998; Ezzeddine *et al.*, 2001; Vági *et al.*, 2005 and Busatta *et al.*, 2008).

Zingiberol is the main component in ginger powder Bioghurt, it was 27.11%. While curcumene, sesquithujene, geranial and borneol were less than 1%. $AT_{gin.p}$ treatment gave the lowest counts of *L. acidophilus* (see Table 1). These results agree with that reported by Kurata and Koike (1983), who confirmed that the antimicrobial activity of major oil constituents was found to be: alcohols and phenol > ketones > ethers > hydrocarbones. Also the essential oils in ginger oil Bioghurt sample are illustrated in Table (4), the results indicated that, the major components were bisabolene (19.15%) and zingibererol (18.60%) but the lowest component was β- sesquiphellandrol (0.67%) .

Table (4): Identification and determination of essential oils by GC in herbal (powder and oils) Bioghurt

NO.	$AT_{mar.o}$		$AT_{gin.o}$		$AT_{ros.o}$		$AT_{ch.p}$		$AT_{gin.p}$		$AT_{ros.p}$		$AT_{mar.p}$	
	Comp. name	Conc. (%)	Comp name	Conc. (%)	Comp name	Conc. (%)	Comp name	Conc. (%)	Comp name	Conc. (%)	Comp name	Conc. (%)	Comp name	Conc. (%)
1	γ-terpinene	3.91	phellandrene	5.02	α - pinene	3.08	α - pinene	1.24	zingiberene	18.21	α - pinene	1.02	α-terpinene	0.85
2	α-terpinene	0.21	curcumene	8.74	β - pinene	12.60	*p.* cymene	0.23	bisabolene	2.58	β - pinene	1.22	p- cymene	0.63
3	p-cymene	9.46	sesquithujene	3.02	verbenol	4.20	1,8- cineol	0.55	curcumene	0.76	Camphene	0.38	borneol	0.75
4	4-terpinol	2.89	linalool	5.38	terpineol	4.14	Ocimene	0.17	β- sesquiphellan	2.47	Lemonene	1.01	carvacrol	10.90
5	linalool	9.84	camphene	3.35	3-octanone	4.31	Terpinlene	0.42	sesquithujene	0.79	Ceneole	3.57	linalool	26.03
6	thymol	7.85	neral	1.16	Iso propyl acetate	2.90	Caryophyllene	1.09	zingiberol	27.11	Borneol	15.22	Terpinen-4-ol	60.50
7	sabinene	3.81	zingiberene	18.60	Linalool	4.32	Farnesene	46.41	zingiberenol	8.22	Camphor	40.00	engenol	0.39
8	Cis-sabinene	4.53	borenol	1.62	Lemonene	23.37	Cadinene	42.76	sesquisabinene	5.74	Linalool	4.47		
9	Linalyl acetate	3.40	gingerol	1.53	1,8 Ceneole	1.48	Cadinol	0.46	phellandrane	9.59	terpineol	30.61		
10	ocimene	3.65	β-sesquiphellandrene	2.58	Borneol	2.82	Bisabolol oxide β	0.86	geranial	0.18				
11	Genaryl acetate	7.35	zingiberol	14.42	Camphene	18.51	Bisabolon oxide A	0.14	linalool	8.85				
12	borneol	4.81	zingiberenol	2.48	Camphor	2.10	Bisabolol oxide A	4.04	borneol	0.37				
13	cadinene	3.41	Ces-sesquisabinene	1.26			Di cycloether	0.87						
14	carvacrol	17.00	β-sesquiphellandrol	0.67										
15	citral	3.81	bisabolene	19.15										
16	estragole	3.44												
17	eugenol	2.10												

No. : number of compound , comp. : compound , conc. : **concentration of compound (relative percentage)**
$AT_{gin.p}$: ginger powder Bioghurt , $AT_{mar.p}$: marjoram powder **Bioghurt , $AT_{ros.p}$: rosemary powder Bioghurt**
$AT_{ch.p}$: chamomile powder Bioghurt , $AT_{gin.o}$: ginger oil Bioghurt , **$AT_{mar.o}$: marjoram oil Bioghurt** $AT_{ros.o}$: rosemary oil Bioghurt

The major compounds in chamomile Bioghurt were farnesene (46.417%) and cadinene (42.757%) , while the lowest compounds were less than 1% such as p. cymene, 1, 8- cineol, Ocimene, Terpinlene, Cadinol, Bisabolol oxide β, Bisabolon oxide A and Di cycloether. These results are in agreement with those reported by Povh *et al.* (2001) and Szoke *et al.* (2004) who found that the major compounds of the essential oil were γ-cadinene, α-bisabolol oxide B, α-bisabolol, chamazulene, and α and β-farnesene, Organoleptic properties of the herbal Bioghurt during storage period at 6±1°C

To produce healthy products, it must be firstly organoleptically acceptable. Therefore, organoleptic properties of herbal Bioghurt samples whether fresh or during storage were evaluated and scored for flavour, body& texture and appearance. As shown in Fig. (7) all treatments of herbal Bioghurt were acceptable. But, addition of rosemary ($AT_{ros.p}$) , ginger ($AT_{gin.p}$) powder or rosemary and marjoram oils led to significant improve in the judging score for the resultant Bioghurt compared with the other treatments. This may be due to the Egyptian consumer habit to drink ginger and add rosemary in food. Although marjoram and chamomile treatments had partially less score particularly in appearance compared with other treatments or control. Also, the results exhibit that the total scores of sensory evaluation of fresh treatments and control, registered 94.0 , 91.7 , 94.0 , 91.8 , 94.0 , 96.5 , 95.5 and 91.00 for $AT_{gin..p}$ $AT_{mar\cdot p}$,$AT_{ros.p}$,$AT_{ch.p}$,$AT_{gin.o}$ $AT_{mar.o}$, $AT_{ros.o}$, and AT_{c1}, respectively. On the other hand,total score was decreased significantly ($P \leq 0.05$) during storage period at 6±1°C for 15 days. These findings are agreement with results reported by Abo laina (2003).

The total scoring points ranged from 82.8 to 89.2 at the end of storage. This may be due to the acidity development or the production of other microbial exerted metabolites. Also, it should be signed to ginger treatment as the lowest acceptable one, which may be due to the ginger taste and the variation between the panelists acceptability.

Statistical analysis revealed that there were significant differences ($P \leq 0.05$) between treatments , interaction and during storage periods in flavor and total score of herbal Bioghurt (Table 3). It could be concluded that, the preferred treatments were in the following order $AT_{ros.p}$, $AT_{gin.p}$, $AT_{ros.o}$ and $AT_{mar.o}$ according to their higher total score compared to the other treatments. The results revealed that the shelf life of herbal Bioghurt could be extended to 15 days at 6±1°C because there were a drop in organoleptic properties and viability of L. *acidophilus* of all Bioghurt treatments after words.

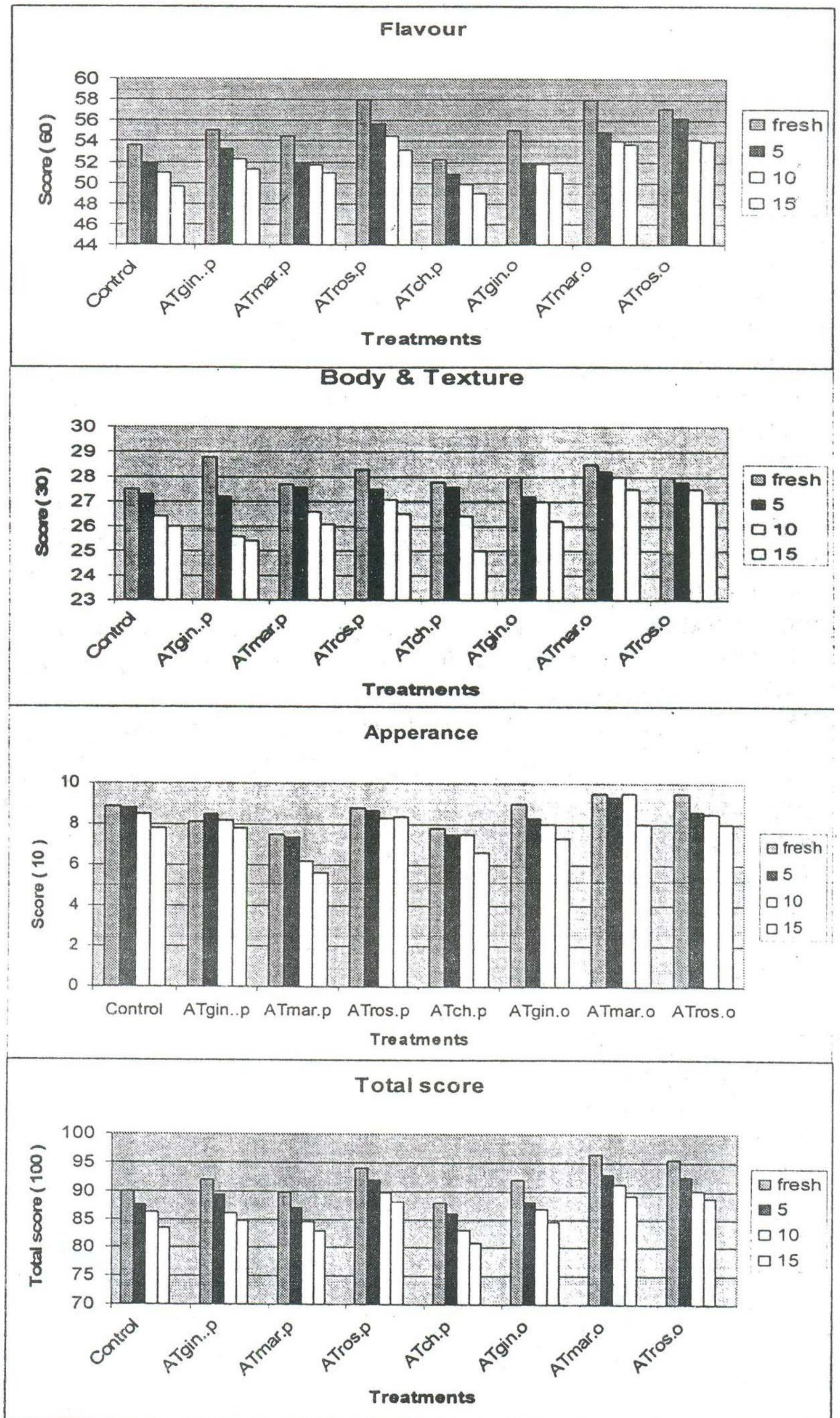

Fig. (7): Organoleptic properties of herbal Bioghurt and control during storage time at 6 ± 1 C

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تأثير بعض الأعشاب المجففة و زيوتها على خواص البيوجورت أثناء التخزين

وداد عـزب متـرى ، نعمـت علـى حـسن ، عمـر الـدمرداش محمـد و وردة مصطفى عبدالتواب عبيد

قسم الألبان – كلية الزراعة – جامعة الفيوم – جمهورية مصر العربية

تهدف هذه الدراسة إلي معرفة تأثير إضافة بعض الأعشاب المجففه أو زيوتها العطرية على خواص البيوجورت أثناء التخزين على درجة حرارة منخفضة (٦±١° م). ولذلك صنعت سبع معاملات بيوجورت بالأعشاب وأستخدم فى التصنيع بادئ يحتوى على بكتريا *Lactobacillus acidophilus* La-5 and *Streptococcus thermophilus* مع إضافة بعض الأعشاب (كلا على حده) إما فى صورة مجففه مثل البردقوش والكاموميل و حصي لبان و الزنجبيل (بنسبة ٠,٢٥ %) أو في صورة زيت مستخلص من البردقوش و الزنجبيل و حصا لبان (بنسبة ٠,٠٢٥ %) وكذلك تم تصنيع البيوجورت بدون إضافات (كنترول للمقارنة). تم دراسة الخصائص الكيميائية والحسيه والميكروبيولوجيه وكذلك حيوية بكتريا البادئ فى اليوم الأول من التصنيع وأيضا أثناء فترة التخزين علي درجة حرارة ٦±١° م . وقد دلت نتائج هذه الدراسة بعد تحليلها إحصائيا على :

أن المعاملات المحتوية علي زيت البردقوش أوحصا لبان سواء في صـوره مجففـه أو زيـت أو الزنجبيـل المجفف قد حصلت على أعلى نتائج التقيـيم الحـسي بينمـا وجـدت زيـادة معنويـة فـى أعـداد بكتريـا *Lactobacillus acidophilus* La-5 بالمعاملات المحتوية علي زيت البردقوش أوحصا لبان سواء فـي صوره مجففه أو زيت أو الكاموميل المجفف أثناء فترة التخزين بالمقارنة بالمعاملات الأخرى. و مـن ناحيـة أخرى وجدت أختلافات معنوية بين المعاملات وكذلك أثناء فترة التخـزين لكـلا مـن الـ % للحموضـة و الأسيتالدهيد و الداى استيل و النيتروجين الذائب و اللاكتوز ، كما وجدت زيادة غير معنوية أثناء التخزين لكـلا من الجوامد الصلبة و الدهن والنيتروجين الكلي فى جميع المعاملات ، كما دلت نتائج التحليل الكرومـاتوجرافى للمنتجات المتحصل عليها أن زيوت هذه الاعشاب تختلف فى محتواها من المركبـات الفينوليـة و الكيتونيـة و الاحماض العضوية و التى لها دور مؤثر فى طعم المنتج و خواصه الميكروبيولوجية .

بناء علي النتائج السابقه وخاصة التحليل الميكروبيولوجي لحيوية البادئ والتقييم الحسي أثناء التخزين علـى درجة حرارة ٦±١° م يوصي بإضافة زيت البردقوش أو حصي لبان مجفـف أو زيـت إلـى البيوجـورت واستهلاك المنتج خلال ١٥ يوم من التصنيع.