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PRODUCTION OF FUNCTIONAL YOGURT: EFFECT OF NATURAL ANTIOXIDANT FROM GUAVA (*PSIDIUM GUAJAVA)* LEAF EXTRACT

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ABSTRACT

The possibility of producing a functional yogurt from a skimmed buffalo's milk using guava (*Psidium guajava)* leaf extract was investigated. Methanol exhibited slightly higher extraction ability for phenolic compounds than ethanol and water. The total phenols were 894, 882 and 877µg/g powder, respectively, when extraction ratio was 1:12. Addition of water extract of guava leaf by different concentrations to a functional yogurt, showed significant changes of pH, titratable acidity during cold storage up to 5 days. The reducing activity of all samples significantly (*P*>0.05) decreased up to the end of storage period , while the inhibition of ascorbate autoxidation significantly increased with increasing of the amount of phenolic compounds till 300 µg phenolic components /100ml yogurt followed by a slight decrease. During storage, the average viable cell counts on MRS increased in yogurt contained guava leaf extract 75 µg phenolic component /100ml from log CFU/ml 9.60 after one day to 10.17 on day 5. Notwithstanding, there was a decline in log CFU/ml on M17 throughout storage. Sensory evaluation data indicated no significant differences (*P*>0.05) between the control and treated samples. Based on the above results, technology can be proposed for productions of a functional yogurt with water extract of guava leaf, as natural antioxidant source.

INTRODUCTION

Intensive oxidative processes occurring in human organism lead to formation of oxygen reactive forms (superoxide anion, hydrogen peroxide and hydroxyl radical), which can damage systemic cells and tissues. It is shown that, body endogenous protective system can be supported in that case by natural antioxidant compounds provided from food. The assessment of food products as the potential sources of antioxidants was performed, taking into consideration the kinds of compounds supplied, and their significance in the diet of different nations (Sikora, *et al.*, 2008).Antioxidants can be defined as compounds (such as ascorbic acid, carotenoids, polyphenols and enzymes) that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction, these dietary phenolic antioxidants have been shown to play important role in delaying the development of chronic diseases such as cardiovascular diseases, cancer, inflammatory bowel syndrome and Alzheimer's diseases (Shetty, 1997 and Akyon, 2002). Phenolic antioxidants are products of secondary metabolism in plant, which could be a good source of natural antioxidants in human diets. Due to the carcinogenic potential of synthetic forms natural phenolic antioxidants are also being targeted as alternative to minimize or retard oxidative deterioration in food and to improve the health-related functional value of the food (Shetty, 1997 and Botsoglou, *et al.*, 2002). Aromatic plants such as herbs and spices are ,especially, rich in their phenolic content, and have been widely used to extend the shelf life of food (Adam, *et al.*,1998 & Botsoglou, *et al.*,2002) and in traditional medicine as treatment for many diseases (Shetty,1997). The extracts from guava leaf were found to possess strong antioxidant activity, the antioxidant mechanisms of guava leaf extracts may be attributed to their free radical- scavenging ability. In addition, phenolic compounds appear to responsible for the antioxidant activity of guava's extract. On these results guava extract from leaf can be used for a variety of beneficial chemo-preventive effects (Chen and Yen 2007 & Tachakittirungrod, *et al.*, 2007).

Fermented milk foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas and textures, which enrich the human diet. Over 3500

traditional, fermented foods exist worldwide (Khurana and Kanawjia, 2007). Fermented foods have been with us since humans arrived on earth and of these fermented milks have long been an important component of nutrition and diet. Originally fermented milks were developed as a means of preserving nutrients (Beena, 2000). The aim of this research was to prepare functional yogurt [plain yogurt with different levels of natural antioxidant from water extract of guava (***Psidium guajava)*** leaf]. In addition, to study the effect of storage period on the changes of some antioxidant activities including inhibition of ascorbate autoxidation and reducing activity in functional yogurt. .

MATERIALS AND METHODS

1. Guava (*Psidium guajava)* leaf extract

Guava leaves were obtained from Kafer El-Dawar district, Behera Governorate, Egypt and were harvested at maturity stage, then dried at 40^{O}C under vacuum. Guava (*Psidium guajava)* leaf powder was obtained by drying and milling of fresh leaves until the whole sample passed through a 0.125 mm sieve. The prepared sample with approximately 1 % moisture content was stored in dark container for further use. The dried guava powder was extracted with various solvents (water, methanol and ethanol) at different ratio (1:6, 1:8, 1:10, 1:12 and 1:14 g/ml) for 48 hours at room temperature, in dark. Among various extracts the highest phenolic containing extracts were chosen for further analysis and antimicrobial activity after filtered through 0.45 μm (Nacalai tesque, Japan) filters, and kept in a freezer at -20^{O}C until use .

2. Starter organisms and functional yogurt manufacture:

Starter yogurt (YO-MIX495 LYO 250 DCU, DANISCO) was used in the manufacture of functional yogurt. A skimmed buffalo's milk (15 g fat L^{-1}) was obtained from Damnhour Agriculture Secondary School, Damnhour, Egypt and mixed skim milk powder (was kindly supplied from Dairy Pilot Plant, Faculty of Agriculture, Alexandria University, Egypt) to obtain 130 g L^{-1} of dry matter. The mixture was subjected to thermal treatment (90^{O}C for 10 min.), then cooled to 45^{O}C in ice bath and poured into 1000 ml flasks. The milk

was fortified with 0, 75,150,225,300 and 375 µg extracted phenolic components /100ml milk. The flasks were inoculated with 2% (v/v) starter yoghurt, followed by dispensing the milk in 100 ml cups. The mixtures were incubated at $42^{O}C$ until coagulation, cooled to ambient temperature, and then stored at $4^{O}C$ in fridge.

3. Microbiological analysis:

For each run, functional yogurt, which enriched with antioxidant, was analyzed after 1, 2 and 5 days of storage at $4^{O}C$. Functional yogurt samples (1ml) were added to 9 ml sterile saline solution (0.85%w/v). Appropriate dilutions were made and subsequently pour-plated in duplicate onto selective media as MRS agar (deMan *et al.* 1960) and M17 agar (Biokar Diagnostics, Beauvais, France), and incubation of the plates were carried out at $45^{O}C$ for 48 h. The selectivity of the growth conditions was confirmed by morphology of cells from single colonies under a microscope.

4. Chemical analysis:

4.1. Percentage of acidity and pH value

About 100 g of prepared yogurt was blended and the pH was measured by a digital pH meter (Model HI 9321, HANNA instrument). Also acidity was determined as lactic acid (%) according to A.O.A.C. (2000).

4.2. Total phenols

The total phenols were estimated according to the Folin-Ciocalteu method (Singleton, *et al.*, 1999). To 50 µl sample, 250 µl of undiluted Folin- Ciocalteu-reagent was added. After 1 min; 750 µl of 20 % (w/v) aqueous Na_2CO_3 was added and the volume was made up to 5.0 ml with distilled H_2O. The controls were run with all the reaction reagents except the extract. After 2 h. of incubation at $25^{O}C$, the absorbance was measured at 760 nm and compared to a tannic acid calibration curve. Total phenols were determined as tannic acid equivalents (µg tannic acid/g extract), and the values are presented as means of triplicate analyses.

4.3. Measurement of inhibition of ascorbate autoxidation :

The method described by Mishra and Kovachich (1984) was used to determine the inhibition of ascorbate autoxidation. A 0.1 ml of sample or distilled water which served as control was mixed with an ascorbate solution (0.1ml, 5.0 mM, Sigma, St. Louis, Missouri,USA) and phosphate buffer (9.8 ml, 0.2 M, pH 7.0). After being placed at $37^{O}C$ for 10 min, the absorbance of this mixture at 265 nm was measured. The ascorbate autoxidation inhibition rate of the sample was then calculated according to the following equation:

Inhibition effect (%) = [$absorbance_{sample}$ / $absorbance_{control}$ - 1] X 100%.

4.4. Measurement of reducing activity:

The reducing activity of sample was determined essentially according to the method of Oyaizu (1986). A sample or distilled water (control) (0.5ml) was mixed with 1.0% potassium ferricyanide (0.5 ml, Sigma, St. Louis, Missouri,USA) and sodium phosphate buffer (0.5ml, 0.02 M, pH 7.0). The mixture was incubated at $50^{O}C$ for 20 min and then 10% trichloroacetic acid (0.5 ml, Ferak, Berlin, Germany) was added. The mixture was centrifuged at 780 xg for 5min.The upper layer (1.5ml) was mixed with 0.1% ferrichloride (0.2ml, Sigma, St. Louis, Missouri,USA) and the absorbance was measured at 700 nm. A higher absorbance of this mixture indicates a higher reducing activity, and the reducing activity of cysteine was used as a standard.

5. Sensory evaluation of functional yogurt:

Fresh prepared yogurt as well as stored samples at different period (1,2 and 5 days) at $4^{O}C$ in a refrigerator were subjected to 20 panelists to evaluate its overall acceptability, color, flavor, and consistency. The panelists were asked to give each item from 1 and 10 points, where as 1 is equal to dislike extremely and 10 is equal to like extremely. Differences in preferences between the conventional functional yogurt using a hedonic scale were recorded. The means of scores obtained by 20 well trained panelists were recorded (Kramer and Twigg, 1962).

6. Statistical analysis method:

Results were analyzed using analysis of variance of the SAS package (SAS, 2000).

RESULTS AND DISCUSSION

1. Total phenolic content:

The total phenolic content of guava leaf extract was determined as a tannic acid equivalent concentration (μg/g) and is shown in Fig., 1. The content of total phenolic compounds in the extract could be arranged in descending order to : methanol extract, ethanol extract then water extract. These results may be due to guava leaf contained a mixture of phenolic compounds at different levels according to the polarity of solvent used in the extraction process (Tachakittirungrod, *et al.*,2007). Chen and Yen (2007) found that the content of total phenolic compounds as (+)-catechin equivalents, were less than those as gallic acid equivalents. This may be affected by the molecular weight of standards.

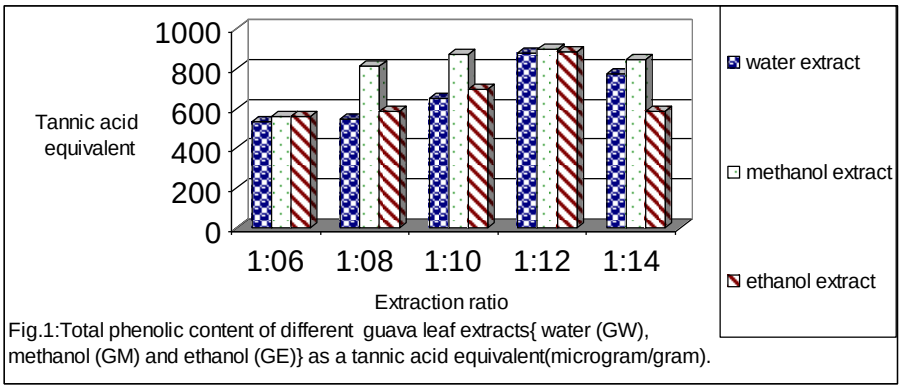

Fig.1:Total phenolic content of different guava leaf extracts{ water (GW), methanol (GM) and ethanol (GE)} as a tannic acid equivalent(microgram/gram).

2. pH and acidity (%) changes during functional yogurt storage:

The results as Fig.,(2) illustrated the changes in pH of the functional yogurt made from buffaloes' milk (control) and/ or that fortified with 75,150,225,300 and 375 μg water extracted phenolic components /100ml functional yogurt. The values of pH were 4.67, 4.60, 4.59, 4.54, 4.49 and 4.54 at zero time (after 1 day), respectively.

It was clear that a gradual decrease in pH value was observed on increasing the level of added phenolic components. Notwithstanding, there was a significant difference ($P< 0.05$) between the pH after 1 day (zero time) and 5 days of storage in fridge across all types of functional yogurts under study (Fig.2). This decline in pH was presumably due to continued fermentation by the starter, especially *Lactobacillus delbreuckii* ssp *bulgaricus*, which usually greatly reduces the decrease in the pH of functional yogurt throughout the shelf life (Kim, *et al*., 1993).

The decrement in the pH of functional yogurt, which reflected the high activity of starter, may be due to the presence of natural antioxidant of guava leaf extracts, which displayed a significant scavenging ability on the peroxyl radicals such as H_2O_2 thus retard the growth (Chen and Yen, 2007).

As shown in Fig.3, in accordance to the pH values, the acidity (%) of functional yogurt samples increased gradually but significant ($P< 0.05$) during storage period up to 5 days and / or within increasing of natural antioxidant of guava leaf extract added. The correlation coefficient between the % acidity and the pH values of yogurt samples understudy was (r= -0.971).However, acidity of all samples lie between that published for ordinary yogurt (plain) (Kailasapathy, *et al.* 2008& Hassanein, *et al.*, 2008).

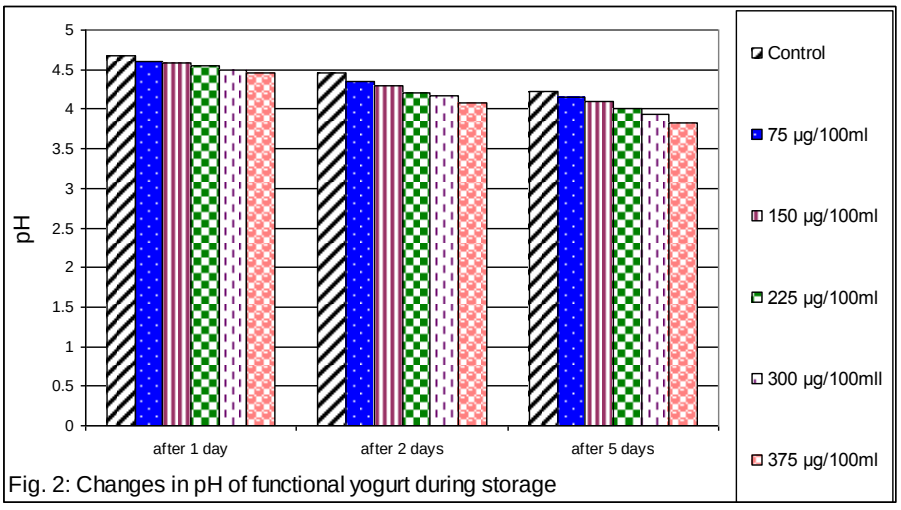

Fig. 2: Changes in pH of functional yogurt during storage

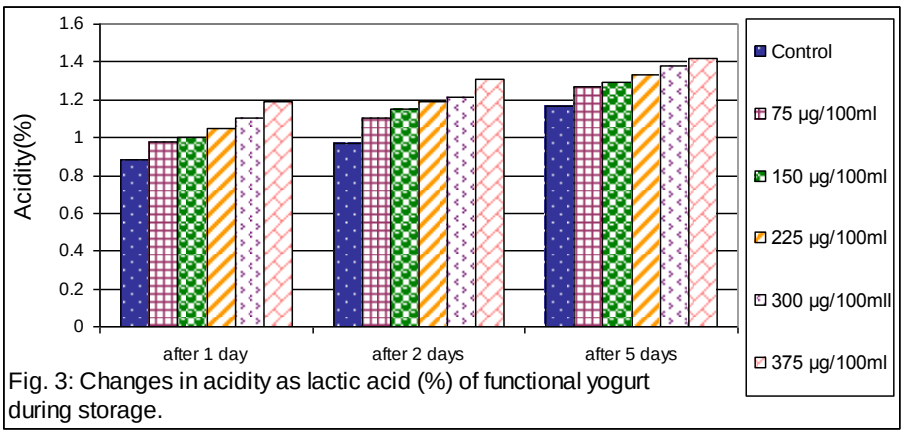

Fig. 3: Changes in acidity as lactic acid (%) of functional yogurt during storage.

3. Reducing activity:

In this study, reducing activity was determined based on the ability of solvent extracts reduced cystiene (µM). As shown in Fig.(4), the reducing activity in control (plain yogurt) was 3.88 µM cystiene after 1 day of storage, which gradually increased as the amount of guava extract increased up to 375 µg phenolic components added, where as, functional yogurt fortified with 75 µg phenolic components /100ml had a reducing activity equal to 169.5 µM cystiene, while the higher reducing activity was 235.5 µM cystiene obtained when 375 µg phenolic components /100ml functional yogurt was added. During 5 days of cold storage the reducing activity of all samples significantly (P>0.05) decreased up to the end of storage period. Lactic acid fermentation was reported to enhance the reducing activity of soymilk (Wang, *et al.* 2006), such an effect was not observed on the lactic acid-fermented vegetable as examined by Sun *et al.*, (2009)

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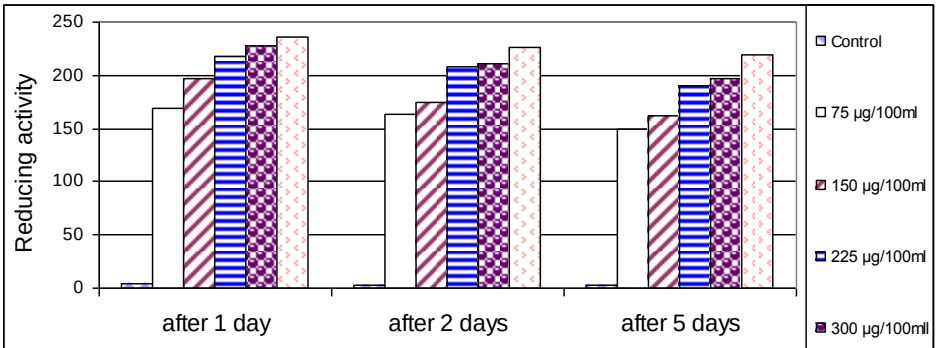

Fig.4: Changes in reducing activity of functional yogurt during cold storage . Activity was determined with 0.1 ml sample and the reducing activity was expressed as equivalent amount of cystiene(micro mole).

4. Inhibition of ascorbate autoxidation:

Fig.5 shows the inhibition of ascorbate autoxidation. Generally, the inhibition of ascorbate autoxidation significantly increased with added of the amount of phenolic compounds up to the level 300 µg phenolic /100ml yogurt followed by a slight decrease. The percentages of inhibition were 22.4, 30.8, 39.0, 48.1 and 46.7% on addition of 75,150,225,300 and 375 µg extracted phenolic components /100ml functional yogurt, respectively. This may be due to the prooxidant effects of guava extracts at high concentrations may be correlated with phenoxy radical formed by the changes of phenolic compounds with phenxy radical react with β-phycoerythrin to participate in radical chain propagation(Bowry, *et al* ., 1992 & Chen and Yen, 2007).

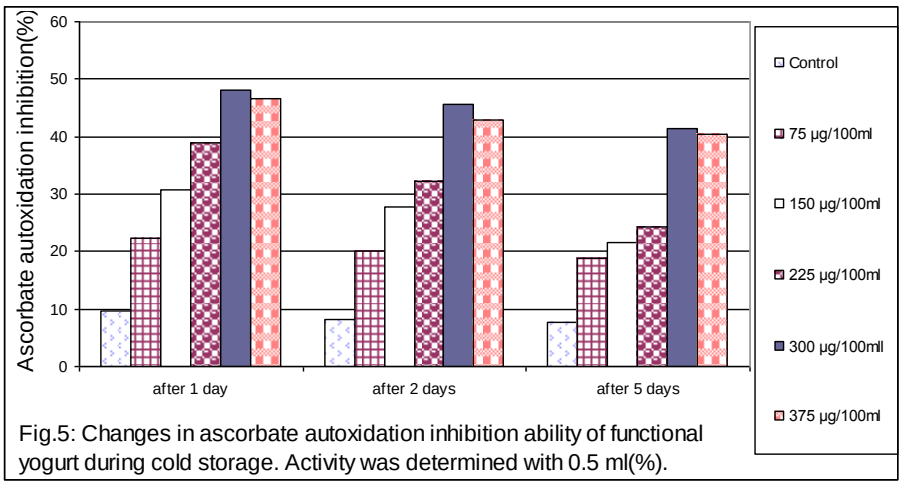

Fig.5: Changes in ascorbate autoxidation inhibition ability of functional yogurt during cold storage. Activity was determined with 0.5 ml(%).

5. Monitoring of lactic acid bacteria on MRS and M17 in functional yogurt:

Lactic acid bacteria were enumerated after 1, 2 and 5 days of cold storage in fridge. Figs.6 and 7 show the changes in counts of lactic acid bacteria for functional yogurt. As shown in Fig.6, the log CFU/ml on MRS in control (plain yogurt) reached to of 9.30 log CFU/ml after 1 day of storage, while in functional yogurt fortified with 75 µg phenolic components /100ml reached to 9.60 log CFU/ml. The higher cell counts were obtained with 375 µg phenolic components /100ml functional yogurt, which was 10.27 log CFU/ml. This result was agree with Rozés and Peres,(1998) who reported that low tannin concentration (0.1 or 0.2 g/l) did not inhibit *L. plantaruml* growth , but high amount of tannin (1 g/l) delayed bacterial growth. During storage the average viable cell counts on MRS increased in yogurt fortified with 75 µg phenolic component /100ml from log CFU/ml 9.60 after one day to 10.17 on day 5. The presence of 9 log CFU/ml of probiotic lactic bacteria is sufficient to ensure the daily intake suggested by Vanderhoof and Young (1998). Notwithstanding, there was a decline in the counts on M17 throughout storage (Fig. 7). This viability loss, which due to pH conditions were not the optimal conditions for the endogenous enzymes (Collins *et al.*, 2003) .

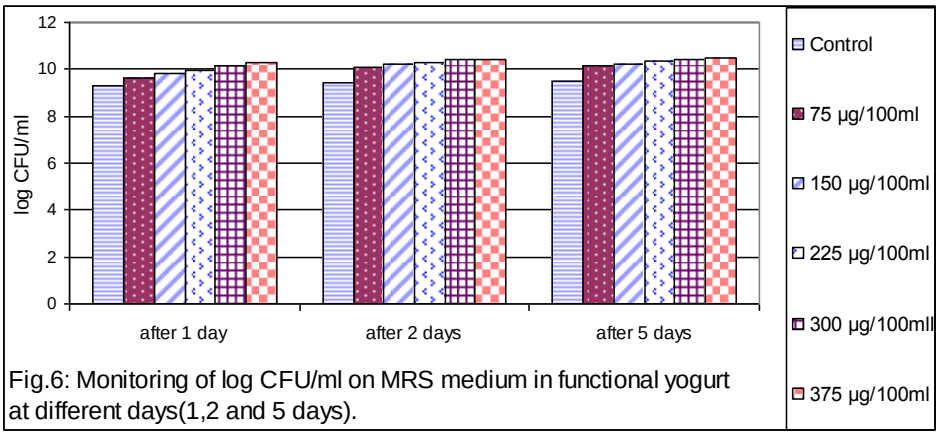

Fig.6: Monitoring of log CFU/ml on MRS medium in functional yogurt at different days(1,2 and 5 days).

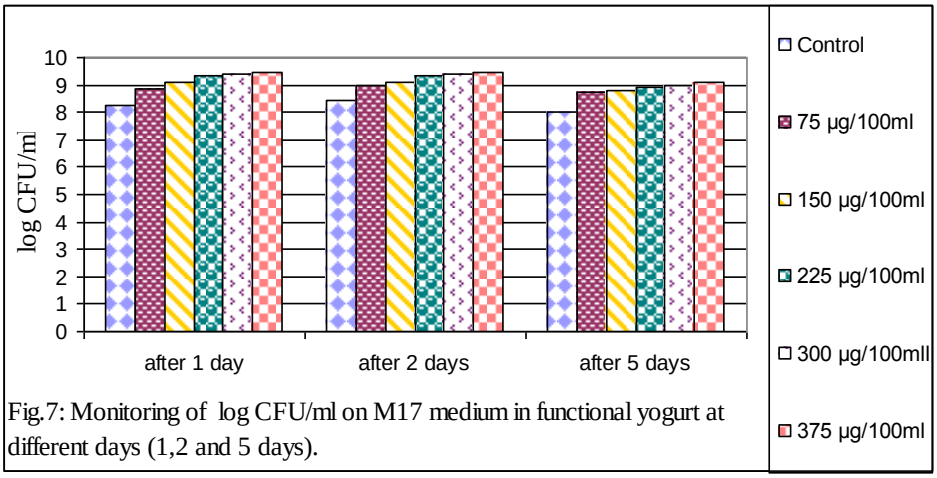

Fig.7: Monitoring of log CFU/ml on M17 medium in functional yogurt at different days (1,2 and 5 days).

6. Sensorial testing:

Data for the organoleptic properties as color, flavor and consistency of functional yogurt fortified with75, 150,225,300 and 375 µg phenolic components /100ml after 1 day, 2 and 5 days are illustrated in Fig.8. There was no significant differences (P>0.05) between the control and samples fortified with different concentrations of phenolic compounds(i.e.75, 150,225,300 and 375 µg phenolic components /100ml) in all sensorial parameters (color, flavor

and consistency) after 1 day, 2 and 5 days of cold storage . Generally, all functional yogurts along with the control are judged as like to like extremely and scored more than 8.4. On other words the scores of color, flavor and consistency ranged between 8.7-9.1; between 8.4-8.9; and between 8.4-8.8, respectively. The point of interest was that addition of guava extract as a source of phenolic compounds and as a natural antioxidant did not influence any deterioration effect in the organoleptic properties, which confirms the ability to introduce such guava leaf extract on dairy products.

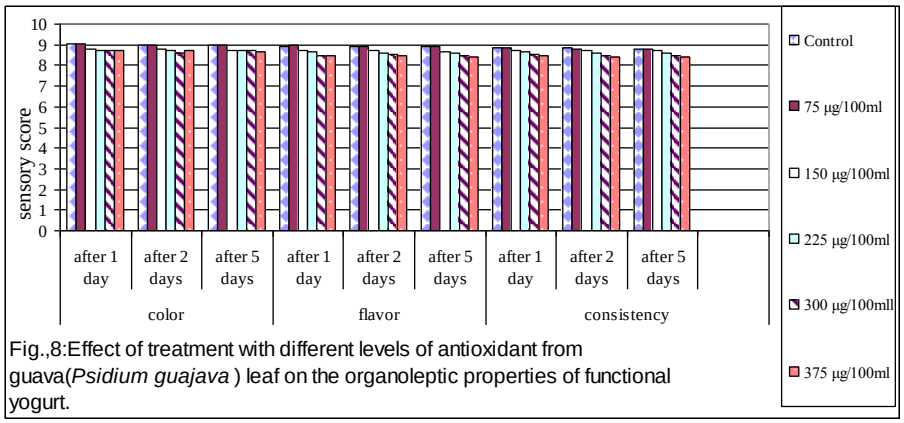

Fig.,8:Effect of treatment with different levels of antioxidant from guava(*Psidium guajava*) leaf on the organoleptic properties of functional yogurt.

CONCLUSION

Since the higher rate consumption of fermented milks is in the form of yogurt so, we can easily used it as a functional food by adding guava leaf extract up to 225 µg phenolic components /100ml yogurt as a natural antioxidant without any deteriorative effect on the sensory properties and the storage ability .

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الملخص العربي

إنتاج زبادى وظيفي : تأثير مضادات الاكسده الطبيعيه من مستخلص أوراق الجوافه

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تم دراسة إمكانية إنتاج زبادى وظيفي من اللبن الجاموسى باستخدام مستخلص ورق الجوافه و قد اتضح ان الميثانول امكنه استخلاص كميه اكبر من المواد الفينوليه مقارنه بالايثانول و الماء حيث كان محتوى الفينولات الكليه 894 و 882 و 877 ميكروجرام /جرام على التوالى و ذلك عند معدل استخلاص 1: 12 (وزن : حجم) . هذا و قد اضيف المستخلص المائى للجوافه الناتج بتركيزات مختلفه اثناء صناعه الزبادى و قد ادى ذلك الى تغيرات معنويه فى كل من ال pH و الحموضه الكليه اثناء التخزين فى الثلاجه لمده 5 ايام و قد اتضح ان النشاط الاختزالى لكل العينات قد انخفض معنويا و بدرجه تدريجيه على النقيض من الارتفاع التدريجى المعنوى لتثبيط الاكسده الذاتيه بحمض الاسكوربيك و ذلك حتى تركيز 300 ميكروجرام مواد فينوليه/ 100 مل زبادى تبع ذلك انخفاض طفيف . هذا و قد ازداد متوسط لوغاريتم عدد الخلايا الحيه / مل على بيئه ال MRS من 9.6 الى 10.17 بعد 5 ايام و ذلك عند استخدام تركيز 75 ميكروجرام مواد فينوليه / 100 مل زبادى و ذلك على النقيض من الانخفاض الحادث فى لوغاريتم عدد الخلايا الحيه/مل على بيئه M17 اثناء التخزين . هذا و قد اتضح من التقيم الحسى عدم وجود اختلافات معنويه بين جميع المعاملات و الكنترول مما يعكس امكانيه انتاج زبادى وظيفى محتوى على مستخلص اوراق الجوافه كمصدر لمضادات الاكسده الطبيعيه.