

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF POMEGRANATE (*Punica granatum L.*) FRUIT PEELS EXTRACT ON SOME CHEMICAL, MICROBIOLOGICAL AND ORGANOLEPTICAL PROPERTIES OF YOGHURT DURING STORAGE.

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ABSTRACT

Pomegranate (*Punica granatum*) fruit peels was investigated for its phenolic compounds, antioxidant and antioxidative activity on L.A.B in yoghurt during cold storage . Results of chemical composition being 7.15 %, 1.22 % 0.65 % and 85.04 % for moisture, protein, crude fat and carbohydrates respectively. Total phenolic compounds were 42.62 mg gallic acid / g. Data of HPLC revealed that ten compounds were identified namely catechin , gallic and -chlorogenic acid being 138.71, 111.68 and 92.39% , Moreover catechol and caffeine were 84.36 and 44.47 % and other trace compounds namely coumarin, cinnamic , chrysin, caffeic and ferulic . Radical scavenging activity DPPH for the aqueous extract was 90.97 %, compared with TBHQ 94.1% as synthetic one. Pomegranate peel extract explored considerable inhibitory effect of each of lactic acid bacteria, coliform group and *Staph. Sp.*

The increase aqueous extract of pomegranate peel from 1 to 5 % lead to an inhibitory effect on LAB and other tested pathogenic bacteria. 1 % of extract was appropriate for the viability of Lactic acid bacteria which was above 30×10^6 c.f.u / ml. In addition, using of 1 %, 0.75, 0.5 and 0.25% on yoghurt processing was acceptable for the chemical, microbiological and organoleptical properties of the resultant yoghurt. Moreover, the ratio of 0.75 % was more acceptable, comparing with other added doses.

Keywords: Yoghurt, fermented milk, *Punica granatum*, and Pomegranate peel.

INTRODUCTION

Plants are always rich source of compounds that do not appear essential for primary metabolism, including thousands of secondary metabolites and several macromolecules, such as peptides, proteins, enzymes, lignin and cellulose. Phytochemicals are often referred to non-nutritive compounds thought to be produced by plants as means of protection against such dangers as harmful ultraviolet radiation, pathogens and herbivorous predators.

Yogurt is one of the oldest fermented milk products known. Fermentation of milk involves the action of microorganisms, principally the lactic acid bacteria. Yogurt gels are built of clusters of aggregated casein particles formed as a result of gradual fermentation of lactose by lactic acid

bacteria (Horne, 1999, 2003). The Food and Drug Administration (FDA, 2008) standard of identity for yogurt drinks specifies >8.25% milk solids-not-fat and fat levels to satisfy nonfat yogurt (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients (Chandan et al., 2006). Yogurt is among the most common dairy products consumed around the world (Saint-Eve et al., 2006). As the popularity of yogurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer et al., 2010).

Punica granatum (Punicaceae), commonly called pomegranate, recently described as nature's power fruit, is a plant used in folkloric medicine for the treatment of various diseases (Abdel Moneim et al., 2011; Ajaikumar et al., 2005). It is widely cultivated in the Mediterranean region. Pomegranate has strong antioxidant and anti-inflammatory properties, recent studies have demonstrated its anti-cancer activity in several humancancers (Adhami and Mukhtar, 2007; Longtin, 2003). In addition, pomegranate peel extract with an abundance of flavonoids and tannins has been shown to have a highantioxidant activity (Abdel Moneim et al., 2011).

Natural antioxidants may be useful in retarding oxidative deterioration of food materials especially those with high lipid content Hence there is a tendency towards the use of natural antioxidants of herbs and to replace these synthetic ones. The application of natural antioxidants to prevent edible oil rancidity had been studied (Xiong, *et al.* 2001).

This research was carried out to study the effect of using aqueous extract pomegranate peel as food by product to extended the shelf life of yoghurt as functional dairy products

MATERIALS AND METHODS

Pomegranate fruits were collected at November from the local market, EL Mansoura city, Egypt.

Row cow's milk was obtained from dairy station, faculty of agriculture, mansoura University, El mansoura, Egypt.

Freez dried yoghurt starter was obtained from Danisco Com., el Denmark. *Staph. aureus* , coliform groupe and Lactic acid bacteria, were obtained from Dairy Department, Faculty Agriculture, Mansoura, Univeristy, Egypt. .

All pure chemicals and media were purchased from El-Gomhorea Company, Egypt.

Pomegranate peel fruits were washed in sterilized water and drained. They were manually cut up, and the outer leathery skin were removed , then the peels were carefully manually removed, cleaned by sterilized water, dried at 50° for 8 hours with air circulation dryer model (Officine specializzate, GARBUIO, Essiccatoi, TREVISO, ITALY) .The dried materials were ground in a domestic mill (Braun ,German). Then sieved and packaged in plastic air tight polyethylene .The samples were stored in domestic refrigerator at 3 ±2°C until the extractions were performed and powdered.

Yoghurt was made from row cow's milk by the method described by Tamine and robinson (1989) and FAO (1990).

Agar wells-diffusion method was used and the antimicrobial test was performed according to the method of Wan *et al.* (1998) with some modification. Briefly, 0.5ml of fresh overnight cultures of the tested bacteria (containing 10^6 – 10^7 cfu/ml) was spread on nutrient agar in sterile Petri dishes (9cm). Wells were created using a 8mm cork borer. Each well was filled with 30µl of pomegranate fruit peels (*Punica granatum*) hot water extract, (0, 0.25, 0.50, 0.75 and 1.00%w/v) samples. The dishes were lifted for 1h at 4 ± 1 °C to allow better diffusion of the extracts into the media prior to incubation at 37 °C for 24h. The inhibition zones were observed as no growth of bacteria and the inhibitory activity was recorded in millimeter (mm). All experiments were conducted in duplicate and the results are expressed as average values of inhibition.

Pomegranate aqueous extraction was analysed for Moisture, protein, ash, fat and total volatile basis TVB-N according to A.O.A.C. (2000).

Organoleptic, chemical and microbiological analysis were carried at zero time and after, 7 and 14 days, respectively. Ten trained panelists from the staff members of the Dairy Department of Faculty of Agriculture, Mansoura University evaluated of each yoghurt sample and used a quality rating score card for evaluation of flavor (50 points) and body and texture (40 points) and appearance (10 points) as described by farahat *et al.* ,(1974).

Curd tension was determined by using the method of Chandrasekhare *et al.* (1957).

Lactic acid bacteria were determined using L.A.B medium as described in the Standard Methods for Examination of Dairy products (SMEDP, 1985). The plates were incubated at 32 ± 1 °C for 2 days.

Staphylococcus sp. was counted by using staphylococcus medium 110. The plates were incubated at 37°C for 24-36 hrs and examined for orange colonies. Coliform bacteria were counted by using MacKonky agar, which was prepared by using 48 g / liter. The plates were incubated at 37°C for 24 hours.

Moulds and yeasts were counted by using potato dextrose agar medium acidified to pH 3.5 with sterile lactic acid (37%). The plates were incubated at 22°C for 72 hours.

Yoghurt samples were analyzed for Titratable acidity as percent lactic acid (AOAC, 1990; Connor, 1995), total solids, total protein, soluble protein, non-protein nitrogen, total fats (Werner-Schmid method) as described by Egan *et al.* (1981) and ash (AOAC, 1990). The pH of the yoghurt samples were measured using () pH-meter. The total microbial loads of the yoghurt samples were enumerated in freshly prepared 0, 7, 14 and 21 days old yoghurt as described by Matalon and Sandine (1986) and Singleton (1999). Inoculated plates were incubated for 48 h at 38°C.

Extractions of phenolic compounds of peels were carried out according to the method described by Wojdylo *et al.*, (2007) and were determined according to the method described by Waskmundzka *et al.*,(2007), Which calculated as mg Gallic acid /100g of dry weight material. Phenolic compounds of peels were identified using high performance

liquid chromatography (HPLC) , "HP1050" . Food Technology Research Institute , Giza, Egypt .

Radical scavenging assay DPPH radical was determined according to Mau *et al.*, (2004) with minor modifications . The extracts 100,200, 250,500, 1000 µg) in methanol (1 mL) was mixed with 4 mL of 0.004% methanolic solution of DPPH . The mixture was shaken vigorously and left to stand for 30 min in dark at 30°C , and the absorbance was then measured at 517 nm. using Spekol 11, Carl Zeiss Jena, German.The percent of DPPH discoloration of the samples was calculated according to the equation:-

Antiradical Activity%: Absorbance of control – absorbance of sample / absorbance of control Turbidity test was carried out by using spectrophotometer at wave length 650 nm according the described method by Ezzat and El shafei 1988.

Regarding the Statistical analysis values represented are the means and standard error, significance was used at $p \leq 0.01$,(ANOVA) was done using (SPSS 2007) program for windows.

RESULTS AND DISCUSSION

Data in Table (1) showed the chemical composition of dried pomegranate peels , Moisture content was 7.15 ± 0.05 , protein content was 1.22 ± 0.01 , Crude fat and ash were 0.65 and 3.11, respectively ,and carbohydrates was 85.04 ± 0.01 . These results are in accordance with those given by Yasoubi *et al.*, (2007).

Table (1) Gross chemical composition of pomegranate peels.

Chemical constitutes	Pomegranate peels				
	Moisture	Ash	Protein	Carbohydrates	Crude FAT
	7.15 ± 0.05	3.11 ± 0.02	1.22 ± 0.01	85.04 ± 0.01	0.65 ± 0.02

Each value is the mean of three replicates \pm SD

Results in Table (2) showed the total phenolic compounds. The concentration of total phenolic in the aqueous extracts, expressed as gallic acid was dependent on the polarity of solvent and method used in the extraction as shown in table(2) . The amount of phenolic compounds in the aqueous extract being 42.62, which were in accordance with those given by Yasoubi *et al.*(2007), who stated that extraction methods were the highest (aqueous 40.0 and acetonic 35.0 % at $p < 0.05$)respectively , followed by methanol (34.5 ethanol (25.3 and ethyl acetate extracts 0.2 %).

Data in Table (3) presented the phenolic acids in pomegranate peel showed were gallic, chlorogenic , catechic as the value being 111.68, 92.39 and 138.71 , respectively while coumaric, cinnamic chrysin were nearly the same .

The results were in parallel with the fact of the pomegranate is a good source of anthocyanins (delphinidin, cyanidin, and pelargonidin) and other phenolic compounds (including hydrolyzable tannins such as punicalin, pedunculagin, punicalagin, gallagic,and ellagic acid), organic acids, and antioxidant activity (Noda *et al.* , 2002 and Li *et al.*, 2006).

Table (2): Total Phenolic Content (TPC) in pomegranate peel extract as mg/g Gallic acid.

Phenolic Extract	mg/g Gallic acid
Pomegranate Extract (PE)	42.62

Table (3) phenolic compounds of pomegranate peel extract (mg/100g)

Phenolic Compounds	Pomegranate Extract (PE)
1-Catechin	138.71
2- Gallic	111.68
3- Chlorogenic acid	92.39
4- Catechol	84.36
5- Caffein	44.47
6- Ferulic	3.76
7- coumarin	0.41
8-cinnamic	0.51
9-chrysin	0.08
10-Caffeic	6.34

Table (4): Free radical of scavenging activity of pomegranate peels aqueous extract.

Antioxidants	Concentration				
	1000 µg/ml	500 µg/ml	250 µg/ml	200 µg/ml	100 µg/ml
TBHQ	94.1±2.08	89±7.36	74±2.92	70±2.56	68.3±3.19
Pomegranate aqueous r extract	90.97±2.01	77.47± 2.03	67.81±1.03	59.87±2.03	45.33± 1.05

All values are means of three replicates ± SD

** Means with a column are significantly different with control $p \leq 0.05$

DPPH is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples (Amarowicz et al., 2004). The free radical scavenging activity determined by DPPH was expressed as mg/ ml (the effective concentration of extract required to inhibit 50% of the initial DPPH free radical). Results are shown in Table 4. Results showed that aqueous extract displayed good antioxidant activities for all parts of the plants. Being 90.97, 77.47, 67.81; 59.87 and 45.33 comparing with synthetic antioxidants TBHQ which could be due to different essential components that could have an antioxidant effect .

The diameter of inhibition zone depends on the ability of the test substance to uniformly diffuse through an agar medium (Friedman et al., 2002). The inhibitory effect of pomegranate fruit peels extract used at different concentrations against various microorganisms using well diffusion methods are presented in Table (5) and Fig.(1).The data showed that the pomegranate peels extract inhibitory activity against on all bacteria. the inhibitory effects were

augmented with increase in extract concentrations. which reason of might be due to the high content of phytochemical and phenolic compounds in pomegranate peels hot water extract . These results are in agreement with (*Glazer et al., 2012*), who reported that The antimicrobial potency of pomegranate peels could be attributed to a high content of hydrolysable polyphenols, mainly consisting of gallotannins and ellagitannins, such as punicalagins, punicalins and ellagic acid . Furthermore , the present results showed that *St. aureus* was the most sensitive bacteria followed by *lactic acid bacteria* and *E .coli* . *Melendez and Capriles (2006)* tested the antimicrobial properties of a number of tropical plants from Puerto Rico using the disc diffusion method against *E. coli* and *St.aureus*. They demonstrated that pomegranate extract produced inhibition zone sizes of 11 and 20 mm, for *E.coli* and *St. aureus* respectively. Thus, their results contrast to the present study in that a smaller zone of inhibition for *Staph. aureus* was observed along with antimicrobial activity against *E.coli* .

Table(5): Diameters of inhibition zones (mm) around wells filled with pomegranate fruit peels hot water extracts at different concentrations

Microorganisms	Diameter of inhibition zone (mm)				
	0%	0.25%	0.50%	0.75%	1%
Lactic acid bacteria	8	11	13	15	17
<i>Sapht. aureus</i>	8	10	11	13	14
Coliform grope	8	12	13	16	19

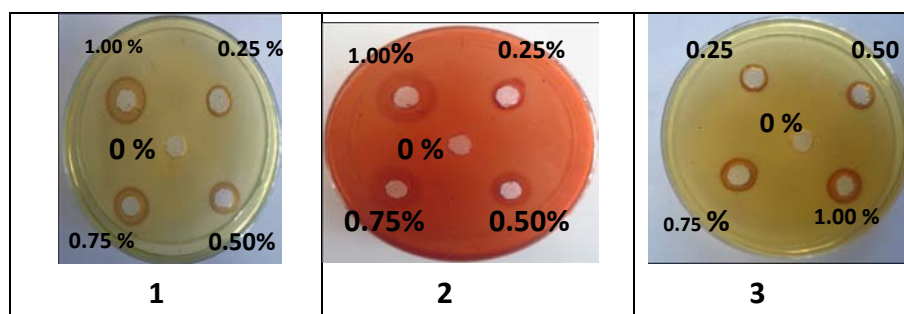


Fig. (1): The antimicrobial activity of pomegranate fruit peels hot water extracts on some bacteria. (1) Lactic acid bacteria, (2) Coliform group and (3) *Staph. aureus*.

Table (6): Count of yogurt strains in skim milk powder reconstituted and reinforcement pomegranate peels aqueous extraction at different ratios.

Pomegranate extraction ratio	Turbidity nm/ hours			Count of L.A.B in M.R.S media × 10 ⁶ c.f.u/ ml at high turbidity
	2 hours	4 hours	6 hours	
0.0 %				86
1%	0.781	0.873	1.234	30
2%	0.420	0.833	1.022	23
3%	0.411	0.802	0.945	20
4%	0.403	0.800	0.893	17
5%	0.391	0.671	0.871	10

Data in Table (6) indicate that the control M.R.S medium inoculated with pure strains of *streptococcus thermophilus* and *lactobacillus bulgaricus* by 1:1 ratio either in the beginning or the end of incubation periods. These data came in computable with those stated by (Glazer *et al.*, 2012) they reported that there were an inhibitory compounds in pomegranate peel aqueous extraction which had an inhibitory effect on all microorganisms growth viability.

Table (7): Chemical composition of yoghurt during storage in refrigerator

Test	Treatment	Storage periods		
		Zero time	7 days	14 days
pH	Control	4.85	4.78	4.74
	T1 (0.25%)	4.73	4.69	4.63
	T2 (0.5%)	4.76	4.71	4.68
	T3 (0.75)	4.79	4.74	4.70
	T4(1.0%)	4.86	4.82	4.78
Acidity	Control	0.71	1.30	1.53
	T1	0.95	1.18	1.28
	T2	0.88	1.03	1.10
	T3	0.81	0.92	0.97
	T4	0.75	0.84	0.87
T.S	Control	16.88	17.48	19.55
	T1	16.92	17.52	19.62
	T2	16.97	17.65	19.79
	T3	17.10	17.75	19.88
	T4	17.15	17.81	19.92
Fat %	Control	6.7	6.9	7.1
	T1	6.7	6.8	7.0
	T2	6.7	6.8	7.1
	T3	6.7	6.8	7.1
	T4	6.7	6.8	7.1
S. N %	Control	0.175	0.210	0.245
	T1 (0.25%)	0.140	0.175	0.210
	T2 (0.5%)	0.140	0.175	0.210
	T3 (0.75)	0.105	0.140	0.175
	T4 (1.0%)	0.105	0.140	0.175
T. P%	Control	0.60	0.64	0.72
	T1	0.60	0.64	0.68
	T2	0.61	0.63	0.69
	T3	0.61	0.63	0.73
	T4	0.61	0.63	0.74
N. P. N%	Control	0.028	0.035	0.042
	T1	0.021	0.028	0.035
	T2	0.029	0.021	0.028
	T3	0.019	0.021	0.028
	T4	0.019	0.021	0.028
Curd tension	Control	19.13	22.41	25.53
	T1	19.00	20.30	21.43
	T2	18.88	20.21	21.31
	T3	18.70	20.11	21.27
	T4	18.70	19.98	21.25

Data in Table (7) indicates the differences in the pH values of control and treated yoghurt. These data indicates that treatment (T4) was of the highest pH values (4.86) among other treatments and control yoghurt ,at the same time treatment (T1) have the lowest pH values (4.73),compared with

other treatments or control yoghurt. These data indicates that the low concentrations of pomegranate aqueous extraction have a stimulatory effect on the growth of starter bacteria, On the other hand the gradual increase of the pomegranate extraction have an inhibitory effect on the growth and vitality of starter bacteria, but all treatments within 1% gained pH value lower than those in control yoghurt and give good characteristics on the final product. These data came in computable with those reported by *Bansode (2012)*. Data in the same Table indicated and reinforce the previous data in pH values, where, the acid values were in adverse trend to the pH values and increased with the low concentrates of pomegranate extraction solution but it returned to increasing again with the high concentrations and this might be due to the inhibition effect the phenolic compound in the extraction of pomegranate aqueous solutions. In addition, all treatments gained an increasing in the acid content during storage periods but this increase was differed from one treatment to another according to the concentration of pomegranate extraction, where, the treatments with high concentration had lower acidity among other treatments or control yoghurt either in fresh or stored product.

Data illustrated in table (7) shows the changes on the total solids content of control and treated yoghurt. Slight differences were observed among all treatments and control yoghurt at the beginning of storage until the end of storage. At the same time, these data indicates that there are gradual increase in the total solids content in all treatments and control yoghurt and this might be due to the loss of some moisture content during cold storage. These data are in agreement with *Muhammad et al., (2009)*.

The curd tension of all treatments and control yoghurt have small differences and gained gradual increase through storage periods, where, this gradual increase might be as a result to the increase in the total solids content, which delay the get out of the knives.

Data in table (7) indicates that there isn't any difference among control or treated yoghurt in fresh or stored product. Also, these data shows that there were gradual increasing in fat content during storage period in all treatments and control yoghurt but there isn't great differences among treatments or control. This gradual increase in fat content during storage might be due to the gradual increase in total solids content which came from the loss of some free water during the refrigerated storage.

As in total solids and fat content there very small differences among all treatments and control yoghurt in the T.N content. In addition, the T.N content has gradual increase with the progress in storage period which might be resulted from the gradual increase in total solids content.

The soluble nitrogen content (S.N) and non-protein nitrogen (N.P.N) take the same trend which was an increasing in treatments which have low concentration of pomegranate aqueous extraction and this increase was more than control yoghurt. This increase of both (S.N) and (N.P.N) in T1 and T2 might be due to the more activity of starter bacteria which was enhanced by the addition of low concentration of pomegranate aqueous extraction. On the other hand, treatments with high concentration of pomegranate gained low content of both (S.N) and (N.P.N) and this also might be resulted from the inhibitory effect of high concentration of pomegranate aqueous extraction.

Moreover, the same status was found among all treatments and control yoghurt either in fresh or during all storage periods. Previous data came in agreement with El Owni and Mahgoub (2012).

Table (8): Growth and activity of bacteria and mould and yeast in yoghurt during storage in refrigerator.

Test	TREATMENT	STORAGE PERIODS		
		Zero time	7 days	14 days
Count of L.A.B in M.R.S media × 10 ⁶ c.f.u/ml	Control	73	98	114
	T1	65	80	95
	T2	56	61	72
	T3	45	59	66
	T4	38	42	47
Moulds& yeasts × 10 ³ c.f.u/ml	Control	ND	ND	ND
	T1	ND	ND	ND
	T2	ND	ND	ND
	T3	ND	ND	ND
	T4	2	ND	ND
Coliform &staph. Sp. × 10 ³ c.f.u/ml	Control	ND	ND	ND
	T1	ND	ND	ND
	T2	ND	ND	ND
	T3	ND	ND	ND
	T4	ND	ND	ND

Tata presented in table (8) indicates the effect of gradual increase of the pomegranate aqueous extraction on the growth of lactic acid bacteria in traditional yoghurt starter which expressed by C.F.U/ml multiple in 10⁶. These data indicates that the low concentration of pomegranate have an enhancing effect on the viability of lactic acid bacteria but this enhancing effect was disappeared with the increasing of pomegranate (0.75 and 1.00%). Moreover, the total bacterial counts in T1 and T2 were more than those in control yoghurt. The total bacterial counts was increased until the 7 days of storage period followed by a decrease through remain period of storage. In addition, all treatments and control yoghurt were free from Coliform group, *staphylococcus aureus*, mould and yeast either in fresh or stored product instead of T4 which showed presence of moulds and yeasts at the beginning of storage period but these shoots were disappeared as soon through the storage periods.

Table (9): Organoleptic properties of yogurt fortified with different levels pomegranate peels .

Item	Treatment	Storage Periods		
		Zero time	7 days	14 days
Appearance (10)	Control	7	8	7
	T1 (0.25%)	6	7	8
	T2 (0.5%)	5	6	7
	T3 (0.75)	4.5	6	8
	T4 (1.0%)	4	5	6
Flavor (50)	Control	47	47	30
	T1	46	46	32
	T2	45	45	30
	T3	46	46	38
	T4	47	47	35
Body & texture (40)	Control	36	36	30
	T1	36.5	34	32
	T2	36	34	32
	T3	34	35	32
	T4	35	34	38
Total score (100)	Control	90	91	67
	T1	88.5	87	72
	T2	86	85	69
	T3	86.5	87	81
	T4	86	86	69

Data in table (9) indicates that all treatments and control yoghurt gained high scores in flavor, where, these scores ranged between 45-57 from total scores 50. On the other hand all scores decreased through the progress in storage periods. In addition, the treatments which have high concentrations of pomegranate aqueous extraction gained the high scores among other treatments and control yoghurt, which, might be due to the effect of the phenolic compound in high concentration of pomegranate. In general, there aren't obvious differences among all treatments during the first and second stage of storage but was obviously in the end of storage period. In addition, all treatments and control yoghurt had a lot of 60 % (30/50) from the total scores of flavor at the end of storage period and were still acceptable for the judgments.

Treatments with high concentration of pomegranate extraction (0.75 and 1.00%) had lower scores of appearance among other treatments and control yoghurt in the beginning of storage, but these scores were enhanced through the progress of storage. this might be resulted from the high percent of anthocyanin pigments in the high percent of the extraction. On the other hand, all treatments had more than 60% (6/10) of total scores for the appearance at the end of storage and were accepted.

In addition, the scores for body and texture not affected by the increasing of the pomegranate extraction on the beginning of storage, where, all treatments and control yoghurt had a lot of 75 % (30/40) of total score through storage period. Moreover, the treatments with high concentration of the pomegranate extractions gained the high scores among other treatments

and control. These results might be due to the inhibition effect of the phenolic compounds on the metabolic activity of starter bacteria, which, decrease the analysis of milk compounds and preserves the body and texture of the treated yoghurt.

In general, there were slight differences among all treatments and control yoghurt at the total scores of organoleptic evaluation and these scores were decreased through the progress of storage period. In addition, the control yoghurt gained the highest total scores at the beginning of storage. On the other hand, the treatment which have (0.75%) of pomegranate extraction gained the highest total scores on the end of storage periods and this might be its high scores in the body and texture and flavor when compared with other treatments or control yoghurt in the end of storage periods.

All treatments and control yoghurt were acceptable for all judgment members either it was fresh or stored product. This means that the pomegranate extraction can be use in the making of yoghurt to enhance its functional and healthy properties.

CONCLUSION

The results of the present investigation are of practical value. The use of Pomegranate peels with yoghurt was advantageous due to inhibitory effect on *staphylococcus sp.*, Coliform, moulds and yeasts. In addition a Pomegranate peel is safe for public health. The results highlighted the possibility of the treatment (0.75%).The developed treatment was evaluated and proved to be of good quality, long shelf life and could be kept at °C for 14 days without significant microbial growth or loss of the product color & texture during manufacture and storage.

REFERENCES

- A.O.A.C. (2000). Official Methods of Analysis, 17th Ed. Association of Official Analytical Chemists, Washington, D.C., USA
- Allgeyer L, Miller M, Lee S. 2010. Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. J. Dairy Sci. 93: 4471-4479.
- AOAC, 1990. Official Methods of analysis.15th Edn.,AOAC, Washington,DC., USA., pp: 200-210.
- Bansode V.V. (2012). Studies on Effect of Initial pH, Storage Temperature and Preservatives on Shelf Life of Yoghurt. Indian Journal of Dairy Science, vol.(65), No. 6.
- Chandrasekhare, M.R.; M. S. Bhagawan and Subrahman (1957) The use of mammalian milk and processed milk foods in the feeding of infants. Indian J. Child. Health, D70L.
- Chandan RC , White CH, Kilara A and Hui YH. 2006. Manufacturing Yogurt and Fermented Milks. Blackwell Publishing, Ames, IA.

- Egan, H., R.S. Kirk and R. Sawyer, 1981. Pearson's Chemical Analysis of Foods. 8th edn., Churchill Livingstone, Edinburgh, pp: 511- 536.
- El owni, O. A. O. and Mahgoub, M. S. A. (2012). The effect of storage on chemical, microbial and sensory characteristics of goat's milk yoghurt. J Nov . Appl Sci., 1 (2): 63-67.
- Ezzat, N.; El-Soda, M. and El-Shafei, H. (1988). The cell-bound proteinase system of *Lactobacillus casei*-purification and characterization. Inter J. Food Microbiol., 6: 327.
- FAO,1990. The technology of traditional milk products in developing countries. FAO Animals Production and Health paper No. 85, FAO of the United Nation, Rome, Italy, pp:1-318.
- Farahat, S. M. ;W. M. , Abou-EL-Ella; G. A. Mabbran and A. A.,Hofi.(1974). The use of soy milk in production of fermented milk (zabadi) .Zagazig J. Agric. Res. (1):187-194.
- FDA. 2008. Food Labeling Guide: Appendix B: Additional Requirements for Nutrient Content Claims. <http://www.cfsan.fda.gov/~dms/2lg-xb.html> Accessed Mar., 2011.
- Friedman, M. ; Henika, P.R. and Mandrell, R.E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.*, 65: 1545–1560
- Glazer, I., Masaphy, S., Marciano, P., Bar-Ilan, I., Holland, D., Kerem, Z., Amir, R.J.,(2012). Partial identification of antifungal compounds from *Punica granatum* peel extracts. *J. Agric. Food Chem.* 60, 4841–4848
- Horne DS. 1999. Formation and structure of acidified milk gels. *Int. Dairy J.* 9:261–268.
- Horne DS. 2003. Casein micelles as hard spheres: Limitations of the model in acidified gel formation. *Colloids Surf. A Physicochem. Eng. Asp.* 213:255–263.
- Li, Y.; Guo, C.; Yang, J.; Wei, J.; Xu, J.and Cheng, S.(2006):Evaluation of antioxidant properties of pomegranate peel extract in comparison with the pomegranate pulp extract. *Food Chemistry* 96, 254–260.
- Matalon, M. E. and W. C. Sandine, 1986. Improved media for differentiation of rods and cocci in yoghurt. *J. Dairy Sci.*, 69:2569-2576.
- Mau ,L.J. , Chang, C.N., S.J. and Hung, C.C., (2004). Antioxidant properties of methanolic extracts from *Gifola frondosa*, *Marchella esculenta* amd *Terminotomyces albuminous mycella*. *Food Chemistry*, 94:415-419
- Melendez , P.A. and Capriles, V.A. (2006). Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine*, 13: 272- 276.
- Muhammad B.F; abubakar M.M. and Adegbola T.A.,(2009). Effect of period and condition of storage on properties of yoghurt produced from cow milk and soymilk materials. *Research journal of dairy science*,3(2), pp:18-24.
- Noda, Y.; Kaneyuki, T.; Mori, A .and Packer, L. (2002): Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin, pelargonidin. *Journal of Agricultural and FoodChemistry*, 50, 166–171

- O'Connor, C.B., 1995. Rural Dairy Technology. International Livestock Research Institute, Ethiopia, ISBN: 92-9146-000-1, pp:153.
- Saint-Eve A, Levy C, Martin N, Souchon I. 2006. Influence of proteins on the perception of flavored stirred yogurts. J. Dairy Sci. 89:922–933.
- Standard methods for the examination of dairy products(S.M.E.D.P) (1985):Published by the American public health association APHA.IN.C.15Th E d. NEW York
- Singleton, P., 1999. Bacteria in Biology, Biotechnology and Medicine. 5th Edn., John Wiley and Sons Ltd., West Sussex, ISBN: 0471988774, pp: 334-454.
- SPSS, (2007). Statistical Package for Social Science program version 17 for Windows, SPSS Inc, Chicago , IL , USA
- Tamine, A. Y. and R. K. Robinson, 1989. Yoghurt Science and technology. 1st Edn., pergamon Press, New York,pp:413.
- The shelf life of chicken meat under refrigerated storage (4 °C). Meat SCIENCE (88) , 409-414 .
- Vaithyanathan . S; Naveen B.M; Muthukumar M; Girish P.S., Kondaiiah N.(2011) . Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on
- Wan, J. ; Wilcock, A..and Coventry,M.J. (1998).The effect of essential oils of Basil on the growth of *Aeromonas hydrophila* and *pseudomonas Juorescens*. J. Appl. Microbiol.84, 152 .
- Waskmundzka, M. ; Wianowska, D. ; Szewczyk, K. and Oniszczuk, A. (2007). Effect of sample-preparation methods on the HPLC quantitation of some phenolic acids in plant materials. Acta Chromatographica (19)- 227-237.
- Williams, A.A., 1982. Scoring methods used in the scoring analysis of foods and beverages at Long Ashton Research Station. Food Technol., 17: 163-175.
- Wojdylo A. ; Oszmianski J. and Czemerys R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs . Food Chemistry 105, 490-494.
- Yasoubi , P. ; . Barzega, M Sahari, M.A , Aziz ,M.H. (2007). Total Phenolic Contents and Antioxidant Activity of Pomegranate (*Punica granatum* L.) Peel Extracts. J. Agric. Sei. Technol. VoL 9: 35-42
- Yehia ,H. ; Elkhadragey ,F. and Abdel Moneim , E. (, 2011).Antimicrobial activity of pomegranate rind peel extracts . African Journal of Microbiology Research Vol. 4(22), pp. 3664-3668
- Zeyada, N., Zeitoun M. and Barbary O. (2007). Extraction, Identification and evaluation of antioxidant activity in some herbs and spices .Alex.J.Fd.Sci, and Technology

النشاط المضاد للاكسدة و الميكروبي لمستخلص قشور الرمان على بعض الخواص الكيميائية و البكتريولوجيه و الحسيه بالزبادى اثناء التخزين .
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اجريت الدراسة علي المستخلص المائي لقشور الرمان بغرض التعرف علي المركبات الفينولية بجانب كلا من النشاط المضاد للاكسدة والنشاط الميكروبي . اوضحت نتائج التركيب الكيماوي احتواء مستخلص قشور الرمان علي 7.15 و 1.22 و 0.65 و 85.04 % من الرطوبة والبروتين والدهون و الكربوهيدرات . كما اظهرت نتائج الفينولات الكلية تواجدها بنسبة مجم حامض جاليك /جم . كما اظهرت نتائج الفصل بواسطة جهاز الفصل الكروماتوجرافي عالي الكفاءة HPLC وجود 10 مركبات فينولية اهمها Catechin و Gallic و Chlorogenic بنسب 138.71 – 111.68 – 92.39% علي التوالي علاوه علي Catechol و Caffein بنسب 84.36 و 44.47 % بلاضافة لبعض المركبات الاخرى ك coumarin و cinnamic و chrysin و Caffeic و Ferulic . واتضح من النتائج ان لمستخلص الرمان نشاط عالي كمضاد اكسدة حيث اعطي 90.97 % مقارنة بالـ TBHQ 97.1 % كمضاد اكسدة صناعي . واعطت ايضا مستخلص قشور الرمان نشاط ميكروبي ضد السلالات الميكروبية التالية lactic acid bacteria و Coliform grope و Staph . aureus .
وعند اضافه المستخلص المائي لقشور الرمان الي الالبان المتخميره (الزبادى) المصنع باضافه بكتريا حامض لاكتيك معلومه التكوين بنسب من 1: 5 % وجد انها تؤثر على معدل النشاط كلما زاد تركيز المستخلص بالاضافه الي تاثيرها المثبط لبكتريا القولون و الاستاف و ولما كان التركيز 1% اقل تاثيرا على معدل نشاط بكتريا حامض الاكتيك مع احتفاظه بقدرته على تثبيط بكتريا القولون و الاستاف تم عمل عدده تخفيفات منه (25 و 50 و 75 و 1%) للوصول الي النسبه الاكثر قبولا لدى المستهلك و التي تحافظ على الخواص الطبيعيه و الكيميائيه و البكتريولوجيه للزبادى فكانت النسبه 75 و 0 % مقارنا بالنسب الاخرى .

قام بتحكيم البحث

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