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Preparation of Functional Probiotic Dairy Beverages Fortified with Pomegranate Juice Concentrate

Mohamed Nour- Eldin Farid Hamad[1], Hayam Mohamed Abbas Ramadan[2*], Samaa M. El-Sayed[3]

[1]Dairy Department, Faculty of Agriculture, Damietta University, Damietta, Egypt.
[2]Dairy Department, National Research Centre, Dokki, Giza. Egypt.
[3]Food Science &Technology Department, Faculty of Home Economic, Al-Azhar University, Tanta , Egypt.

THE main target of the present study is to prepare probiotic drinkable yogurt fortified with Pomegranate Juice concentrate (PJC). Three ratios of PJC were used (0.8, 1.0, and 1.2%; (w/w) vs. control using cow milk. *Lactobacillus acidophilus* LA-5 (A), *Bifidobacterium bifidium*, and *Streptococcus thermophiles* were used. All resultants samples were stored at 5-7 °C for 14 days and were analyzed during 14 days of storage period. The results indicated that the pH values of the beverages fortified with PJC were decreased during 14 days of storage at 5-7 °C. The levels of total solids , water soluble nitrogen, and ash contents were increased as the level of fortification increased. Fat contents showed no clear differences between control and treatments. The values of total nitrogen were increased as the level of PJC increased, while their values were decreased through the storage period. The total volatile fatty acids levels were increased as the fortification ratio and storage period increased. Microbiological examination revealed that *St. thermophiles, L. acidophilus,* and *Bifidbacterium bifidium* counts in fortified samples were decreased as PJC ratio increased and storage period progressed rather than control. The organoleptic properties of fortified samples showed that the color and appearance degrees were low in treated samples rather than control. However, T1 and T2 possessed the favorite color. The scores for body and texture were clear varied either in treated samples or stored samples. Flavor scores indicated that sample T2 had the favorite flavor. The total acceptability indicated that control samples had the highest degrees followed by T2.

Keywords: Probiotic dairy beverages, Pomegranate, *Bifidobacterium*, *L. acidofilus.*

Introduction

The dairy beverages marketplace is a competitive and growing category in the dairy industry. They are defined as beverages that are drinkable in a liquid form and may or may not include fruit or fruit flavoring. Over the last two decades, consumers have become more aware of the relationship between food intake and good health, especially from natural foods such as fruits and vegetables Dairy beverages are delicious products which consumed by all ages, they have high nutrition value as they mainly supplemented with fruits and benefit microorganisms. (Heng et al., 2018 and Hamad et al., 2019 and 2020). Probiotic dairy beverages are functional products appeared in world markets in recent time because they possessed high health value (Champagne, et al., 2018; Ertem & Cakmak , 2018 and Fazilah, 2018). In addition ; food manufacturers are attracted to the usage of probiotics due to high margins and growing consumer interest in functional foods (Bimbo et al., 2017; Hamad et al., 2019; Turkmen et al., 2019; El-Sayed & Ramadan, 2020 and Garcia et al., 2020). So, preparation of probiotic dairy products fortified with fruits is a recent and argent need. In other view, Pomegranate (*Punica granatum* L.) is one of the edible fruits widely grown in many tropical and subtropical countries. It is described as nature's power fruit

*Corresponding author: E-mail: prof.hayamabbas@yahoo.com, Tel. +2 01220634193
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which used in folkloric medicine for treatment of various diseases. Pomegranate fruit which is renowned for its health benefits has become very popular worldwide over the last few years (Eccles, 2009; Arjemand et al., 2012; Abd El-Aziz et al., 2013; Hassanein et al., 2014 and Ali, 2016). On another side, yogurt is one of the oldest fermented milk products known as the popularity of yogurt continues to grow; the researchers and manufacturers are continuously investigating value added ingredients to entice health conscious consumers (Allgeyer et al., 2010; Ali, 2016 and Hamad et al., 2019 & 2020). So, this paper dealt with preparing functional dairy beverages inoculated with probiotic microbial strains and fortified with pomegranate juice concentrate at three ratios. Their chemical, microbiological and sensory properties were estimated through two weeks of storages at refrigerator (5-7 °C).

Materials and Methods

Milk

Fresh raw cow milk was collected in the winter season from the local market in Damiatta Governorate.

Pomegranate Juice concentrate (PJC) was obtained from Alnada factory in Damietta El-Jadida city, Damietta Governorate, Egypt. Its analyses are shown as follows in Table 1:

TABLE 1. The physicochemical properties of raw pomogranete juice concentrate(PJC).

Item	Results
Appearance	Turbid dark-red color
Physical state	Viscous liquid
Organoleptic properties	Conform to standard
pH	2.83 (more acidic)
Total dissolved solids (brix0)	69.00
Total acidity %	3.16 (as citric)

Sugar was purchased from the local market in Damietta was used. Starter: "ABT-5 culture" probiotic yogurt culture which consists of *Lactobacillus acidophilus* LA-5 (A), *Bifidobacterium bifidium* and *Streptococcus thermophilus* CHCC 742/2130 (T) (Chr. Hansen's Lab A/S Copenhagen, Denmark) were used, cultures were in freeze-dried direct-to-vat set form and stored at –18 °C until used. MRS agar medium (Tharmaraj and Shah, 2003) was composed of Dextrose 20.0g, Yeast extract 4.0 g, Bacteriological peptone 10.0 g, Ammonium citrate 2 g, Beef extract 8 g, Magnesium sulphate 0.29 g, Sodium citrate 5 g, Manganese sulphate 0.05 g, Agar 15 g, Di potassium phosphate 2 g, Tween 1 ml and distillation water 1000 ml (pH 6±0.2 at 25 °C). Sterilized in the autoclave at 121 °C for 15 minutes. The medium was used for counting *Lactobacillus acidophilus* counts. M17 agar medium (Tharmaraj & Shah, 2003): The counting of *Streptococcus thermophillus* was determined using M17-lactose agar medium which has the following composition: Tryptone 5 g, Soya peptone 5 g, Meat digest 5 g, Magnesium sulphate 0.25 g, Di-sodium-glycerophosphate 19 g, Agar 15 g and Distillation water 1000 mL (pH 6.9±0.2 at 25 °C). Bifidobacterim medium (Dinakar & Mistry, 1994): This media was composed of: Neomycin sulfate 2 g, Nalidixic acid 0.3 g, Paromomycin sulfate 4g and Lithium chloride (NPNL, Sigma Chemical Co.) 60 g. It was prepared in 1 Liter of distilled water, sterilized in the autoclave at 121 °C for 15 min and stored at 4 °C until use.

Preparation of dairy beverage samples

Four treatments of dairy beverages or drinkable yogurt were made from cow milk and Pomegranate Juice Concentrate (PJC) as follows: C: cow milk (control), T1: control + 0.8% PJC, T2: control + 1.0% PJC and T3: control + 1.2% PJC.

After heating milk samples to 85 °C for 15 min, milk of various treatments were immediately cooled to 45 °C. Control milk (C) was sweated with 5% sugar, inoculated with 0.1 g/L of mix cultures (ABT-5), incubated at 45 °C for fully coagulation and stored at 5-7 °C overnight. In other treatments, 5% sugar was mixed with cow's milk then the 0.8, 1.0, and 1.2% (w/w) of PJC were added to serve three treatments (T1, T2, and T3) and individually blended at 2000 rpm for 3–4 min. Samples were inoculated with "cultures ABT-5" (0.1 g/L of milk mix cultures), incubated at 45 °C for fully coagulation and kept at 5-7 °C overnight. All samples were preserved at 5-7 °C for two weeks. Dairy beverage samples were analyzed when fresh and after 7 & 14 days of refrigerated storage.

Chemical analysis of beverages

Total solids (TS), fat, ash, total nitrogen (TN) and water-soluble nitrogen (WSN) contents

as well as titratable acidity (TA) of beverages were determined according to AOAC (2012). The pH values of samples were measured using a laboratory digital pH–meter equipped with a glass electrode (model H 18418; Hanna Instruments, Padova, Italy). (Corning pH/ ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Total Volatile Fatty Acids (TVFA) were determined according to Kosikowski (1978).

Microbial examination

Cultivation methods

Dairy beverage samples were examined for *Streptococcus thermophiles* and *Lactobacillus acidophilus* counts according to the methods described by Tharmaraj & Shah (2004). The counting of *Streptococcus thermophiles* was determined using M17-lactose agar medium. Enumeration of *Lactobacillus acidophilus* was done by using MRS-sorbitol agar medium. The counting of *bifidobacterium bifidium* was determined according to Dinakar & Mistry (1994).

Sensory Evaluation: Samples of dairy beverages were organoleptically scored by 25 members of the staff of the Dairy Department, Faculty of Agriculture; Damietta University. The score points were 50 for flavor, 35 for body and fluidity, and 15 points for color and appearance, which give a total score of 100 points.

Results and Discussion

Acidity percent and pH value

Figures. 1 & 2 reflected the pH values and acidity percent of the beverages fortified with pomegranate juice concentrate (PJC). It could be observed that pH value of control was 4.42 in the fresh sample decreased to 4.21 and 4.17 and 4.09 when the samples fortified with 0.8, 1.0, and 1.2% PJC, respectively. During 14 days of storage; the values were normally decreased as a result of fermentation. At the first 7 days, their values become 4.21, 4.14, 4.06, and 3.86 for control, T1, T2, and T3, respectively. While after 14 days their values reached 4.16, 3.97, 3.89, and 3.71 in the same order. For acidity data; it was clear that their percent took an opposite trend of pH. The acidity was increased in two directions. The first as a result of adding PJC because it is an acidic ingredient; and the other as the effect of storage as a result of starter action. Acidity values for the control sample were 0.843, 0.978, and 1.117 % at fresh and after 7 & 14 days, respectively. The corresponding values for T1 were 1.014; 1.053 and 1.101 against 0.918; 1.065 and 1.143% for T2 and 1.053; 1.074 and 1.173% for T3. These results were in confirming with that obtained by Hassanein et al. (2014) who reported that the addition of concentrated pomegranate caused a significant decrease in pH, while titratable acidity was increased. Titratable acidity percent were also increased during the storage of the yogurt. They added that the pH values of fresh yogurt samples fortified with 1.0, 1.25, and 1.50 % were 4.46, 4.43, and 4.20, while their corresponding acidity were1.02, 1.03, and 1.05%. These data also agreed with those of Bansode (2012); Abd El-Aziz et al. (2013) and Ali (2016).

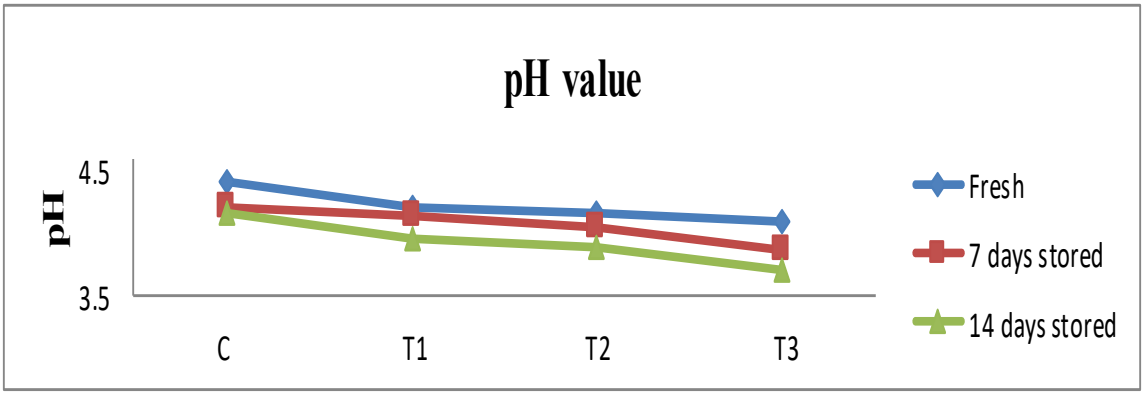

Fig. 1. The average pH value of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

C: Control; T1: contained 0.8% PJC; T2: contained 1.0% PJC; T3: contained 1.2% PJC

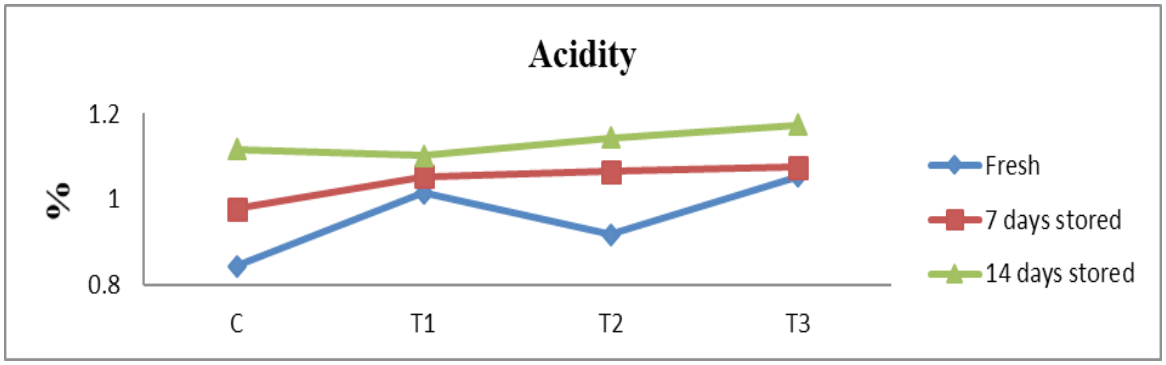

Fig. 2. The average acidity as lactic acid % of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatments : See footnote of Table (1)

Total solids content (TS)

The contents of TS of probiotic dairy beverages samples fortified with different ratios of pomegranate juice concentrate (PJC) were increased as the proportion of fortification increased as shown in Fig. 3. The increases were parallel to the level of pomegranate juice concentrate added. However, the contents of TS were increased during storage as a result of moisture loss and whey-off. Control sample gained 16.02; 16.51 and 17.29% at fresh and after 7&14 days of storage, respectively. The corresponding values for T1 were 16.77; 17.27 and 18.06% vs. 17.18; 17.46 and 18.25 % for T2 and 20.64; 17.67 and 18.46% for T3, respectively. The obtained data agreed with Hassanein et al. (2014); Abd El-Aziz et al. (2013); Ali (2016) and Hamad et al. (2019 & 2020). They showed that slight differences were observed among all treatments and control yogurt at the beginning of storage until the end of storage. At the same time, they indicated that there was a gradual increase in the total solids content in all treatments and control yogurt and this might be due to the loss of moisture content during cold storage.

Fat content

The values of fat content of probiotic dairy beverages samples fortified with different ratios of pomegranate juice concentrate were also presented in Fig. 4. It could be observed that their values were 3.5, 3.4 and 3.3% for the control sample at fresh and after 7 & 14 days of storage. No clear differences were noticed between control and T1 where they contained the same ratio. T2 and T3 possessed 3.6, 3.5 and 3.4%. It could be noticed that pomegranate is a fruit poor in fat content, so, there were no clear differences in fat content among treatments. Abd El Aziz et al. (2013) and Hassanein et al. (2014) confirmed these data where they mentioned that the addition of pomegranate resulted in no noticeable differences between control and fortified-yogurt-samples in their fat contents but a slight increase in fat contents during storage period in all treatments were observed as a result of the loss of water. Obtained data were consistent with that obtained by Hamad et al. (2019 & 2020).

Ash content

Figure 5 reflected the ash content of various probiotic dairy beverages samples fortified with different ratios of pomegranate juice concentrate. It could be noticed that ash percent increased as the level of fruit increased in fresh samples. Their values were 1.18, 1.20, 1.22, and 1.24% for control, T1, T2, and T3, respectively.

This result is logical for adding fruit which considered a good source of minerals. The values were generally increased during storage as a result of increasing the TS. The present data were in harmony with that obtained by Hassanein et al. (2014) and Ali (2016) .

Total Nitrogen content (TN)

Figure 6 showed the TN content values of dairy beverages fortified with pomegranate juice concentrate. No clear differences in the TN contents were observed as a result of the fortification of the pomegranate juice concentrate. Control sample gained 0.466% at fresh while T1 possessed 0.490 and T2 gained 0.492; however T3 contained 0.513%. These observations as results of the low protein content of pomegranate itself. The values of TN were normally decreased through storage. These findings agreed with Abd El-Aziz et al. (2013); Hassanein et al. (2014) and Ali (2016) who reported that it is obvious that the addition of concentrated pomegranate had a negligible effect on protein percentage of the entire resultant yogurt.

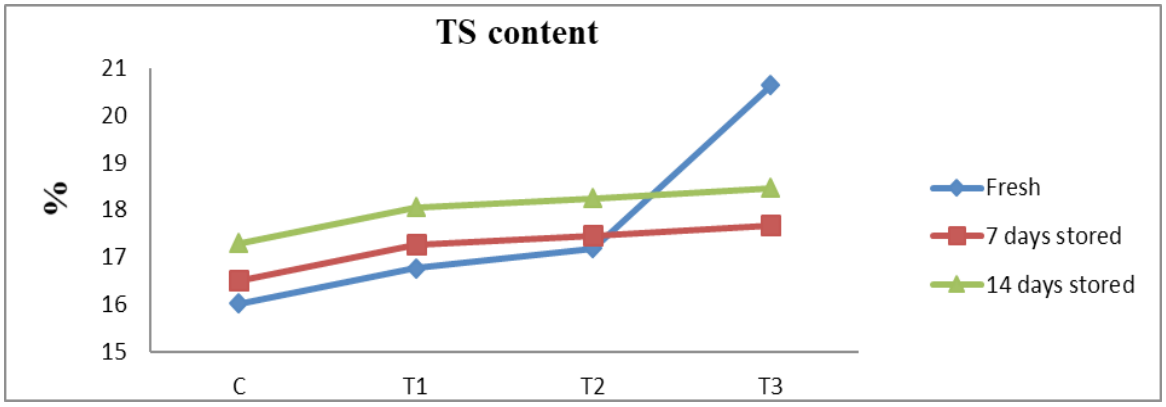

Fig. 3. The average (TS) content of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatments: See footnote of Table (1)

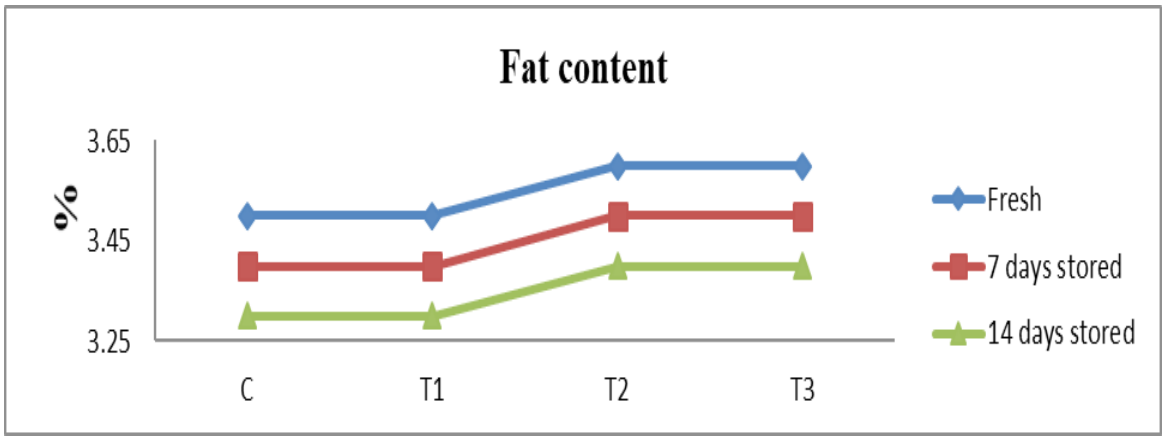

Fig. 4. The average fat content of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatments : See footnote of Table (1)

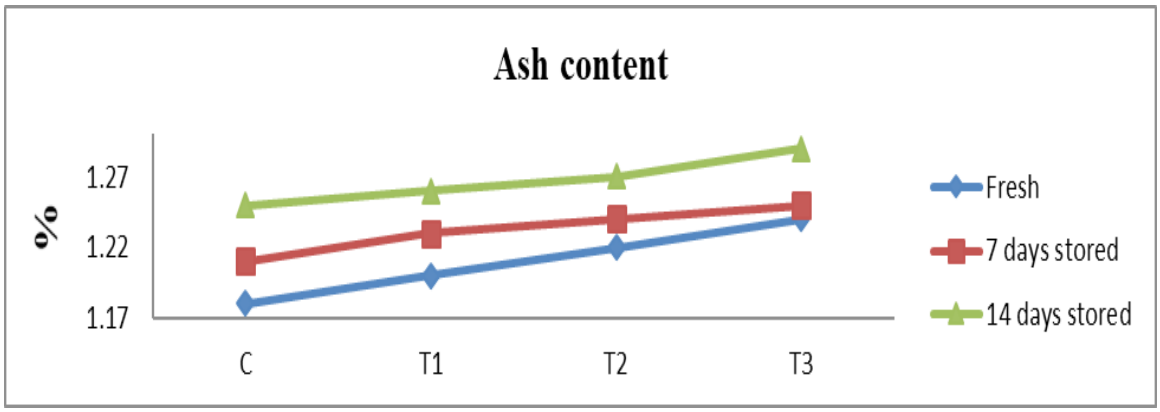

Fig. 5. The average ash content of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatment : See footnote of Table (1)

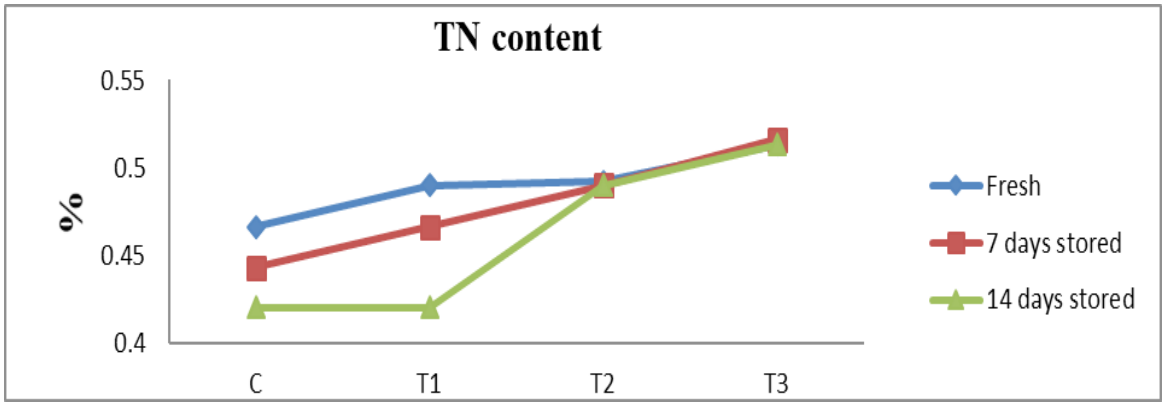

Fig. 6.The average TN content of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.
Treatments : See footnote of Table (1)

Water soluble nitrogen content (WSN)

As shown in Fig. 7; the WSN was noticeably increased as the level of pomegranate juice concentrate ratio increased. Their values were 0.0163; 0.0186; 0.0210 and 0.0233% for Control; T1; T2 and T3 respectively in fresh. During storage, all values were considerably increased as a result of proteolysis as a starter action. The pomegranate juice concentrate contains nutrients which enhance the action of starter and probiotic bacteria. These results agreed with Arjmand et al. (2012). Abd El-Aziz et al. (2013) used pomegranate aqueous extraction and they indicated that the soluble nitrogen content (SN) and non-protein nitrogen (NPN) increased in fortified samples. This increase of both SN and NPN 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 a high concentration of pomegranate gained low content of both SN and NPN and this also might be resulted from the inhibitory effect of high concentration of pomegranate aqueous extraction.

Total volatile fatty acids content (TVFA)

Data listed in Fig. 8 showed the values of the total volatile fatty acids produced in probiotic dairy beverages samples fortified with pomegranate juice concentrate. It clear that their values were considerably increased as juice ratio increased as well as the storage period also increased. Control samples had 8.50; 9.80 and 10.60 (mL 0.I Na OH/10 g sample). These increased as a result of lipolysis during storage. The addition of pomegranate juice concentrate increased the rate of lipolysis and this increase was parallel to the added- ratio. Fresh T1-sample had 9.10 reached to 10.80 and 11.60 after 7 & 14 days of storage. For T2 sample; the values were 9.30, 11.10, and 11.80 at fresh and after 7 days & after 14 days. The corresponding values for T3 were 9.60, 11.30, and 12.00 in the same order.

Microbiological examination

Counts of St. thermophiles

Figure 9 reflected the growth of *St. thermophiles* in probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period. It was clear that the counts were decreased as pomegranate juice concentrate ratio increased and also as the storage period progressed. The control sample had 33, 23, and 19 cfu×10^6. The corresponding counts for T1 were 28, 17, and 8 cfu×10^6. While their counts for T2 were 17, 8, and 4 cfu×10^6, respectively. Samples labeled T3 contained 9, 5, and 2 cfu×x10^6. Abd El-Aziz et al. (2013) reported that the low concentration of pomegranate aqueous extract had a stimulatory effect on the growth of starter bacteria, while the increases of pomegranate concentration had an inhibitory effect on the growth and vitality of starter. It could be explained this phenomenon as mentioned by Ali (2016). He reported that control-yogurt significantly presented the high value of total plate counts; while fortified-samples had the lowest counts. He explained the reduction of bacterial growth to the antibacterial effect of pomegranate as a result of the presence of a high concentration of phytochemicals. These data came incomputable with those of Bansod (2002) and Opera et al. (2009) .

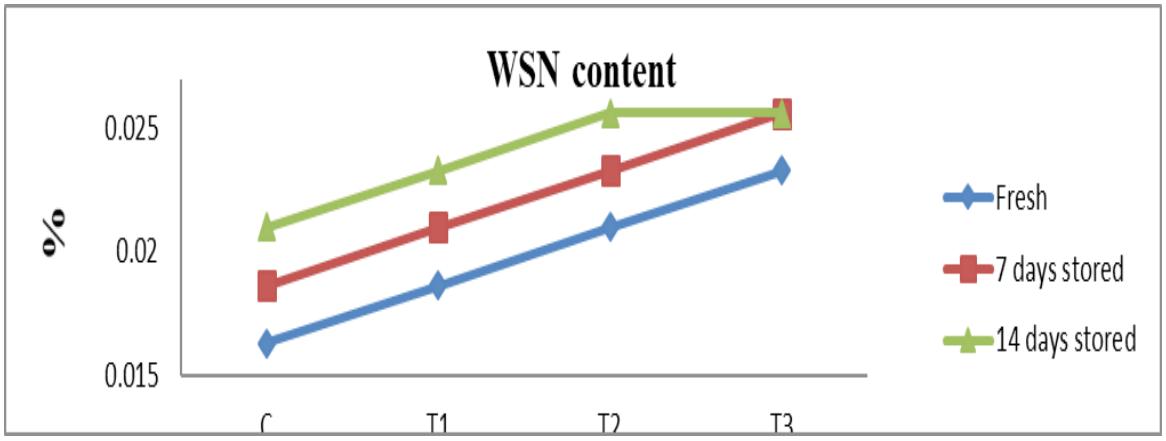

Fig. 7. The average WSN content of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.
Treatments: See footnote of Table (1)

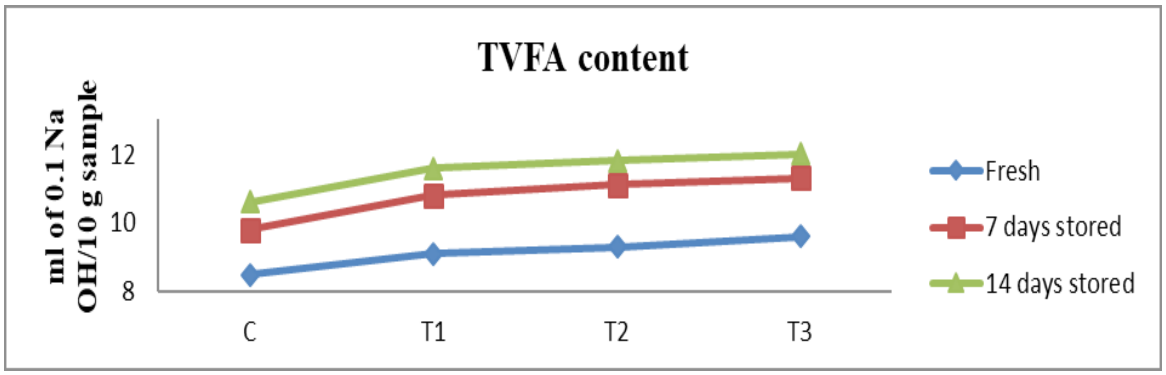

Fig. 8. The average TVFA content* of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.
Treatment: See footnote of Table (1)

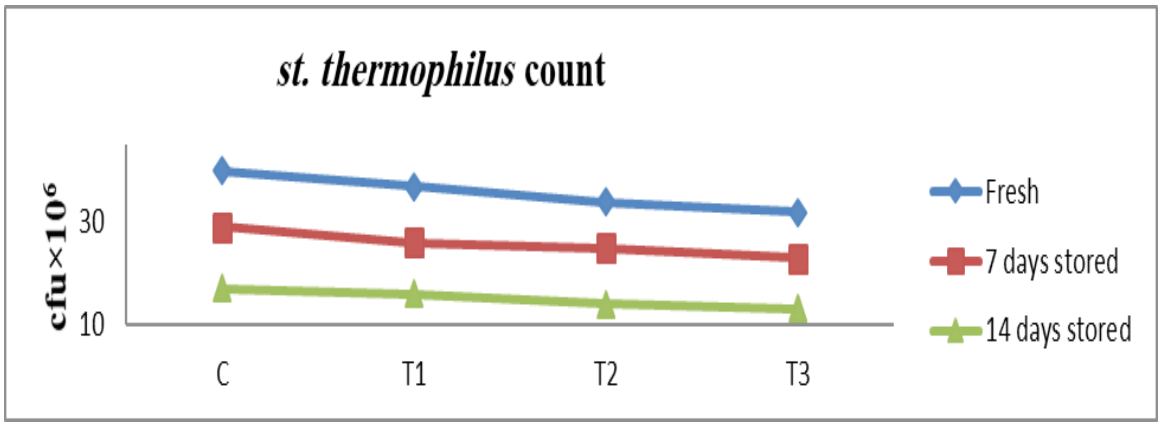

Fig. 9. The counts of *St. thermophilus* of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.
Treatment: See footnote of Table (1)

Counts of L. acidophilus

Figure 10 reflected the growth of *L. acidophilus* in probiotic dairy beverages samples fortified with different ratios (w/w) of pomegranate juice concentrate during the storage period. It was clear that the counts were decreased as pomegranate juice concentrate ratio increased and also as the storage period progressed. The control sample had 39, 30, and 23 cfu×10^5. The corresponding counts for T1 were 35, 27, and 18 cfu×10^5. While their counts for T2 were 31, 24, and 21 cfu×10^5, respectively. Samples labeled T3 contained 30, 22, and 13 cfu×10^5. It could be explained this phenomenon as mentioned by Amadou et al. (2016) who reported that control yogurt significantly presented the high value of total plate counts; while treated fruits-supplemented-samples had the lowest counts. Abd El-Aziz et al. (2013) cleared that pomegranate peel extract explored the considerable inhibitory effect of each of lactic acid bacteria, *coliform* group, and *Staph. Spp.* 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 the extract was appropriate for the viability of Lactic acid bacteria which was above 30×10^6 cfu/ml. Moreover, the ratio of 0.75% was more acceptable, comparing with other added ratios.

Counts of Bifidbacterium bifidium

Figure 11 reflected the counts of *Bifidbacterium bifidium* in probiotic beverages fortified with pomegranate juice concentrate. It could be observed that the counts were decreased in fortified samples rather than control. The counts of fresh samples were 31, 24, 23, and 20 **(**cfu×10^5**)** in C, T1, T2, and T3, respectively. The counts decreased during storage to reach 26 and 23 **(**cfu×10^5**)** for control and 22 and 19 **(**cfu×10^5**)** for T1, while it became 20 and 18 (cfu×10^5**)** for T2. The third sample (T3) gained 20, 17 and 15 (cfu×10^5), when fresh and after 7 and 14 days.

Sensory evaluation

Figure 12 showed the organoleptic properties of the probiotic beverages fortified with pomegranate juice consecrate. The panel test showed that the color and appearance degrees were low in treated samples rather than control. Fresh control had 15 degree for appearance while T1, T2, and T3 had 13, 14, and 10 degree. So, T2 possessed the favorite color within treatments. T3 had an odd color. The scores for body and texture were clear varied either in treated samples or stored samples. Adding of pomegranate lead to produce none homogenized body and little coagulated particles as the effect of acidic action of pomegranate juice consentrate. So this fortification led to an unacceptable body. Flavor scores indicated that the favorite sample was T2, where it possessed 49, 48, and 46 points when fresh and after 7 and 14 days, respectively. However, the total acceptability indicated that control samples had the highest degrees. Abd El-Aziz et al. (2013) mentioned that there were slight differences among all treatments and control at the total scores of organoleptic evaluation. They added that control gained the highest total scores at the beginning of storage. However, Hassanein et al. (2014);reported that the highest scores were obtained in control followed by treatment containing 0.25% concentrated pomegranate (the lowest ratio). Increasing the levels of concentrated pomegranate rather 1%; negatively affected the flavor scores. Similarly, the addition of concentrated pomegranate influenced the body and texture of the yogurt samples. No significant differences were found in the appearance score of samples.

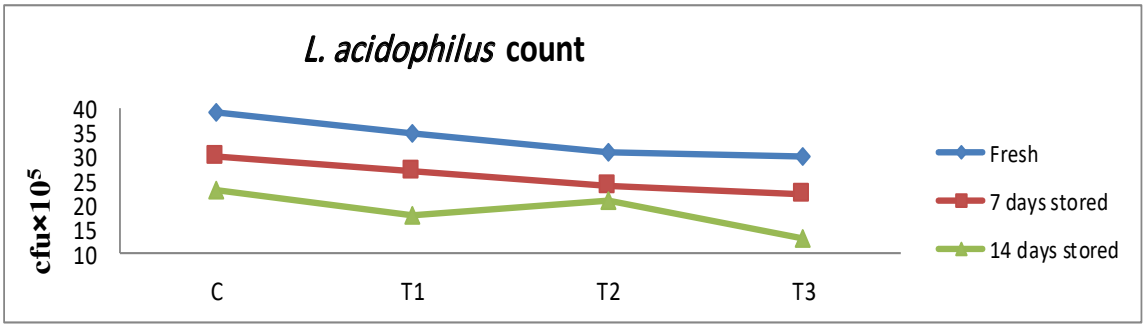

Fig.10. The counts of *L. acidophilus* of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during storage period.

Treatment : See footnote of Table (1).

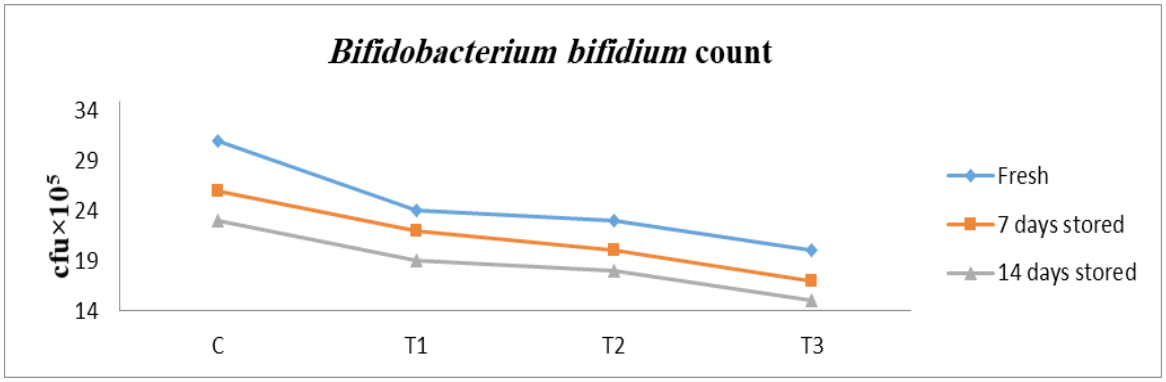

Fig. 11. The counts of Bifidbacterium bifidium of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatments: See footnote of Table (1)

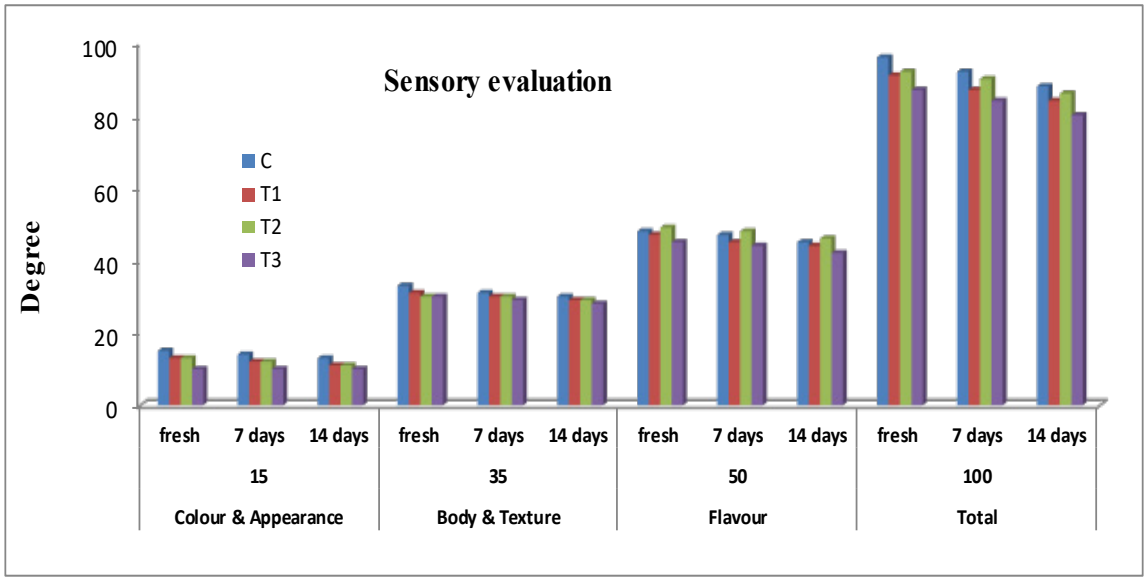

Fig. 12. Sensory evaluation (degree) of probiotic dairy beverages samples fortified with different ratios (w/w) of PJC during the storage period.

Treatment: See footnote of Table (1)

Conclusion

The evaluation of organoleptic properties of the probiotic dairy beverages fortified with pomegranate juice concentrate illustrated that T2 (1.0%) was the favorite treatment. The scores for body & texture were clear varied either in treated samples or stored samples. Flavor scores indicated that the favorite sample was T2. The total acceptability indicated that control samples had the highest degrees followed by T2. It could be concluded that fortification of pomegranate juice resulted in producing undesirable color or body.

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