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## Physicochemical, Microbiological Evaluation of Probiotic Milk Beverages Fortified with Concentrated Apple Juice

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THE preparation of dairy beverages rich in probiotic microorganism and fortified with concentrated apple juice (CAJ) is the goal of this study. Culture (ABT-5) c*ontained Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacterium bifidium* were used to prepare the dairy beverages. Sweetened cow milk which inoculated with cultures (0.1g/L of milk) was used as a control beverage sample (C). Samples were fortified with 8, 10 and 12% (w/w) CAJ to create treatments T1, T2, and T3, respectively. The chemical, microbial and sensory evaluation of samples were acheived fresh and during storage at 52±ºC. The results revealed that the pH values decreased, but the total solids, ash, water-soluble nitrogen (WSN), and total volatile fatty acid (TVFA) contents increased as the level of CAJ increased. During the storage period ;the pH value also decreased, while WSN and TVFA in all dairy beverages increased. Fat contents showed slight increases between control and fortified samples, however, slight decrease was noticed in their values during storage period. The counts of *St.thermophilus and Bifidbacterium bifidium* in for tified samples were decreased as CAJ ratio increased and as storage period progressed, while the counts of L.acidophilus were increased as theratio of CA Jincreased; else, they decreased as storage period increased. Organoleptic properties of samples fortified with CAJ during 14 days of storage recorded obvious variations in all evaluated properties. The total acceptability indicated that T2 samples (10% CAJ) had the highest degrees and possessed favorite properties with probiotic and healthy behavior.

**Keywords:** Milk beverages, Applejuice, Physiochemical properties, Organoleptic properties

## Introduction

Beverage sector plays an important role in consumers' daily diet. Recent declines in fruit beverage and soft drink consumption may reflect changing consumers' lifestyles and perceptions (Heng Yan et al., 2018).Probiotics are defined as viable microorganisms which can be consumed separately or with foods, which assist dietary and microbial balance by regulating the mucosal & systemically immunity which has beneficially affect the consumer's health. A great variety of food-products contain probiotic bacteria are produced by different means and various procedures (Kanmani et al., 2013; Lollo et al., 2013; Champagne, 2018; Ertem & Cakmak, 2018 and Fazilah, 2018). Food manufacturers are attracted to the usage of probiotics due to the projected market growth, high margins and growing consumer interest in functional foods. Probiotic foods enhance health after consumption and contain micro-organisms which are viable, specific and effective on main systems of nutritional physiology (Fernandes et al., 2013; Bimbo et al., 2017 and Nazli et al., 2019). In Japan, probiotics are consumed as frozen culture tablets; whilst in Europe, probiotics are only consumed through the inclusion of probiotics

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to foods (Vaughan & Mollet, 1999; Shah, 2001; Meyer et al., 2007 and Soccoll et al., 2010). Probiotic bacteria consist of many species, such as *Lactobacillus acidophilus,* Bifidobacterium ssp and *Streptococcus thermophiles* and fermented milks have long been used as the main vehicles for probiotic strains (Ertem & Cakmak, 2018; Champagne et al., 2018 and Fasilah et al., 2018). Traditional fermented foods and beverages have been made for centuries in many regions of Europe, Asia, America and Africa. They are obtained through spontaneous fermentation or by starter or probiotic addition. Addition of active bacterial cultures can be considered by the consumer as an added value and a marker of high quality. Historically, the use of probiotics in dairy beverages has been widely investigated, whereas it was less documented in other matrices (Garcia et al., 2020). On the other side, dairy beverages are delicious products consumed by all ages; they have high nutrition value as they mainly supplemented with healthy food additive such as fruits. Beverages based on fruits and milk products are currently receiving considerable attention as their market potential is growing.(Castro et al., 2013; Hamad et al., 2019; Rayan et al., 2020 and El-Sayed & Ramadan, 2020). Acidophilus yoghurt further acts as prebiotic, thus, the yogurt containing both fiber (prebiotic) as well as *Lactobacillus acidophilus* and Bifidobacterium longum (probiotic) are considered as symbiotic to impart benefits of prebiotic as well as probiotic (Guarner et al., 2005). Dietary guidelines around the world recommend increasing the intake of fruit and vegetables for the prevention of chronic diseases. Fruit and vegetables are naturally rich in carbohydrates, dietary fibers, vitamins minerals, polyphenols and phytochemicals (Garcia et al., 2020). Apple is a good source of fiber with well-balanced proportion between soluble and insoluble fractions (Gorinstein et al., 2001) based on whether they form a solution when mixed with water soluble, or not insoluble (Ajila and PrasadaRao, 2013). They may be particularly useful in places where there is inadequate nutrition, which could lead to nutritional deficiency diseases (Shukla et al., 2003). Fernandes et al. (2013) mentioned that Cashew apple juice is considered a good source of organic acids, polyphenols, reducing sugars (fructose and glucose), minerals, and some amino acids and vitamins. Cashew apple juice has about six times more vitamin C than orange juice and the cashew agroindustry is socioeconomically important in the Brazilian Northeast due to the high production of cashew apples and its use in the food industry. Moazzem et al. (2019) reported also that wood apple is one of the most useful medicinal fruits of the Indian subcontinent. It is considered to be a natural source of antioxidants due to its potential radical scavenging activity of various phytochemicals and it also has hypoglycemic, antitumor, larvicidal, antimicrobial, and hepato-protective activity. In addition, it has anti-diabetic and antioxidant potential in terms of reducing levels of blood glucose and malondialdehyde. Therefore, this study aims to produce a functional dairy beverage fortified with probiotic bacteria and concentrated apple juice and to evaluate its chemical, mechanical, and sensory properties during storage at 52±ºC for 14 days.

## Materials and Methods

*Materials*

Fresh cow milk was obtained from the local market in Damiatta Governorate, Egypt. Concentrated applejuice (CAJ) was obtained from the Alnada factory in Damietta El-Jadida city, Damietta Governorate, Egypt.The total soluble solids, acidity content and pH value of CAJ were 70.40 Brix, 1.62% (as a citric acid) and 3.59, respectively.Probiotic bacteria, *Lactobacillus acidophilus* LA-5 (A), and *Streptococcus thermophilus* CHCC 742/2130(T) as well as *Bifidobacterium bifidium,* were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. Sugarproduced by El-Fayrouz Food Packaging & Distribution Company; Damietta was used. All chemical reagents used in the present study were analytical fine grade and were obtained from El-Jomhoria Chemical Company, Mansoura, Egypt.

Preparation of dairy beverage samples:Fresh cow milk was heated to 85°C for 15 min, cooling to 45°C and then sweetened with 5% sugar. Sweetened cow milk was inoculated with 0.1% probiotic starter cultures (1:1:1, *L. acidophilus: St. thermophilus: B. bifidium),* and divided to 4 equal portions. The CAJ was added at the rate of 8, 10, and 12% to create three treatments; T1, T2, and T3, respectively. The fourth portion had no CAJ and served as a control (C). All treatments were incubated at 45°C until a uniform coagulation was formed and stored at (5±2°C) overnight. All samples were stored at 5±2°C for 14 days. Milk beverage samples were analyzed when fresh, after 7 and 14 days of refrigerated storage.

*Chemical analysis*

Calibrated pH-meter (HANNA Instruments, pH 211, Italy), with a glass electrode, was used for directly measuring the pH value of the milk beverages. Acidity content, expressed as percentage lactic acid, was determined by using 0.10 N NaOH to the phenolphthalein end poin. Total solids, fat, total nitrogen (TN) and ash contentswere determined as mentioned by AOAC (2012). The protein content was obtained by multiplying the percentage of TN by 6.38. Water Soluble Nitrogen (WSN) content of dairy beverageswas also determined by the macro-kjeldahl method according to AOAC (2012). Total Volatile Fatty Acids (TVFA) was determined according to the method described by Kosikowski (1978).

*Microbial analysis*

The viable cell count *of Lactobacillus acidophilus* and *Streptococcus thermophillus* in milk beverage samples were determined on MRS agar and M17 agar as described by Tharmaraj & Shah (2003), respectively. *The Bifidobacterium bifidium* was counted in milk beverage samples according to the method described by Dinakar and Mistry (1994).

*Sensory evaluation*

Probiotic milk beverages fortified with CAJ were organoleptic 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 & fluidity and 15 points for color & appearance, which give a total score of 100 points.

## Results and Discussion

*Acidity content and pH value*

As shown in Fig. 1, the pH values of the probiotic milk beverages samples fortified with concentrated apple juice (CAJ) were decreased as level of fortification and storage period increased. The pH values control samplewas 4.49, 4.25 and 4.20 at fresh,7 and 14 days of storage, respectively. Also, T1 had 4.22, 4.13 and 3.82 at the same orders. The corresponding values for T2 were 3.84, 3.71 and 3.49; while the values for T3 were 3.69, 3.42 and 3.13. The acidity content of all milk beverage samples had an opposite trend of pH values. for the acidity content of control sample was0.792, 0.969 and 1.113% against 0.873, 1.017 and 1.149 % for T1; vs. 0.906, 10.53 and 1.182% for T2 at fresh,7 and 14 days, respectively. Sample labeled T3 had 0.963, 1.128 and 1.197%. These results were in agreement with those of Mahmood et al. (2008) who recorded that there were significant changes in pH and acidity of yogurt samples when they fortified with apple pulp at 8 and 16 percent levels. They recorded that the increase in acidity of fruit-stirred-yogurt might be due to the acidity of apple fruit. Pratap et al. (2015) mentioned that apple was incorporated in the frozen yogurt in the quantity of 5%, 10%, 15% and 20% in the form of pulp. The addition of fruit increased the acidity of fruit flavored frozen yogurt and acidity was increased with the increase in the amount of fruit pulp added. The lowest acidity for the sample was 0.46%. Moeiny et al. (2017) and El-Sayed & Ramadan (2020) confirming also these data.

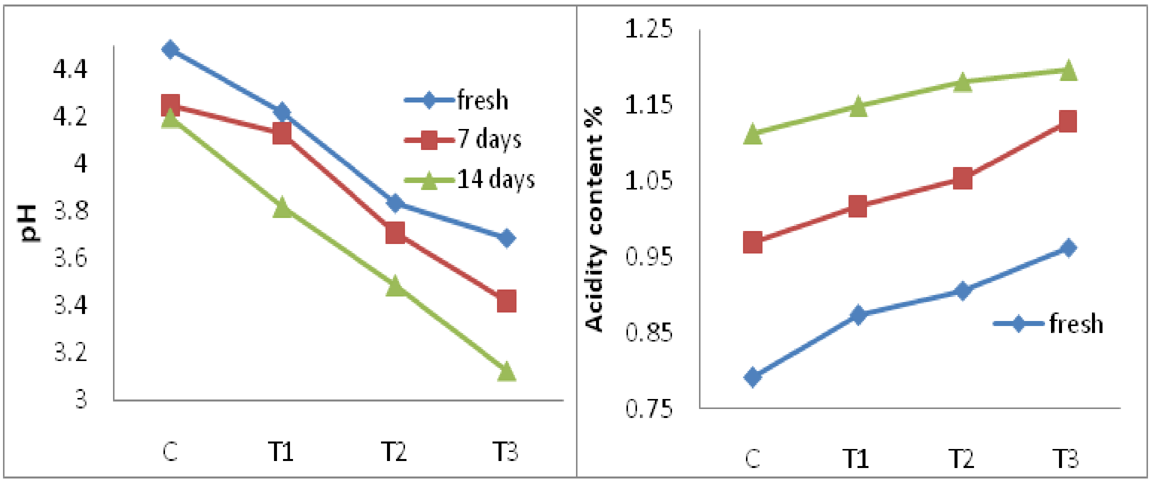


**Fig. 1. The pH values and the acidity content of probiotic dairy beverages samples fortified with different levels (w/w) of concentrated apple juice (CAJ) during storage at 5±2°C for 14 days.**

C, Control;T1, 8% CAJ; T2, 10% CAJ; T3, 12% CAJ.

*Total solids content (TS)*

Figure 2 reflected the total solid contents of probiotic milk beverages samples fortified with CAJ. It was clear that adding the CAJ increased the TS% as the level of addition increased. Control sample possessed 15.69, 16.41 and 17.22%, while T1 had 20.68, 21.29 and 22.05% at fresh,7 and 14 days, respectively. However, T2 gained 21.96, 22.68 and 23.38, and T3 had 22.98, 24.36 and 24.89%. The increases in TS as a result of TS of CAJ added. The TS% increases also during storage as result of evaporation and wheying off. Pratap et al. (2015) indicated that apple pulp was incorporated in the frozen yogurt in the level of 5%, 10%, 15% and 20%. They observed that the addition of apple pulp decreased the TS of frozen yogurt and therefore increased the moisture content. However, Gangwar et al. (2016) mentioned that total solids content was increased in all treatments of yogurt fortified apple. Mahmood et al. (2008); Fernandes et al. (2013) and Moeiny et al. (2017) data were in harmony with the present results.

*Fat content*

Data represented in Fig. 3 reflected the fat content of probiotic dairy beverages samples fortified with CAJ. It was obvious that the differences in fat content were not pronounced. The control had 3.6, 3.5 and 3.4 at fresh, 7 days and14 days of storage. While T1 had 3.7, 3.6 and 3.5 at the same period. Samples of T2 and T3 gained the same contents (3.8, 3.7 and 3.6%). The decreases during storage as result of evaporation. The little increase in fat content in all treatment was due to little content of apple fruit itself. These data were consistent with Mahmood et al. (2008) and Pratap et al. (2015).

*Ash content*

The values of ash content of probiotic dairy beverages samples fortified with CAJ were presented in Fig. 4. It could be observed that their values were 1.15; 1.20 and 1.23 % for control sample at fresh, 7,and 14 days of storage. T1 contained 1.34, 1.39 and 1.40% after the same periods. T2 and T3 possessed 1.47, 1.42 & 1.45% and 1.51, 1.49 and 1.50%, respectively. It could be noticed that apple as all fruits is rich in minerals which led to increase the ash content. The rate of increase was parallel with the ratio of CAJ added. These results were in harmony with that of Mahmood et al. (2008) and Pratap et al. (2015). However, Gangwar et al. (2016) added that ash content was decreased at all concentrations of apple-juice yogurts than plain yogurt.

*Total nitrogen content (TN)*

Figure 5 reflected the data of TN contents of the resultant samples. Clear differences in the TN content values were observed as a result of fortification of CAJ. Control sample had 0.491% TN at fresh, while T1 possessed 0.536 and T2 had 0.568% TN; however T3 contained 0.583% TN. During storage, the contents became 0.490, 0.533, 0.583 and 0.606% after 7 days. After 14 days, the values became 0.466, 0.513, 0.568 and 0.630 % for control, T1, T2 and T3, respectively. Mahmood et al. (2008) recorded that there were significant changes in protein contents. The addition of fruit caused an increase in protein content of yogurt as the amount of fruit increased. Pratap et al. (2015);Gangwar et al. (2017) and Moeiny et al. (2017) confirming these data.

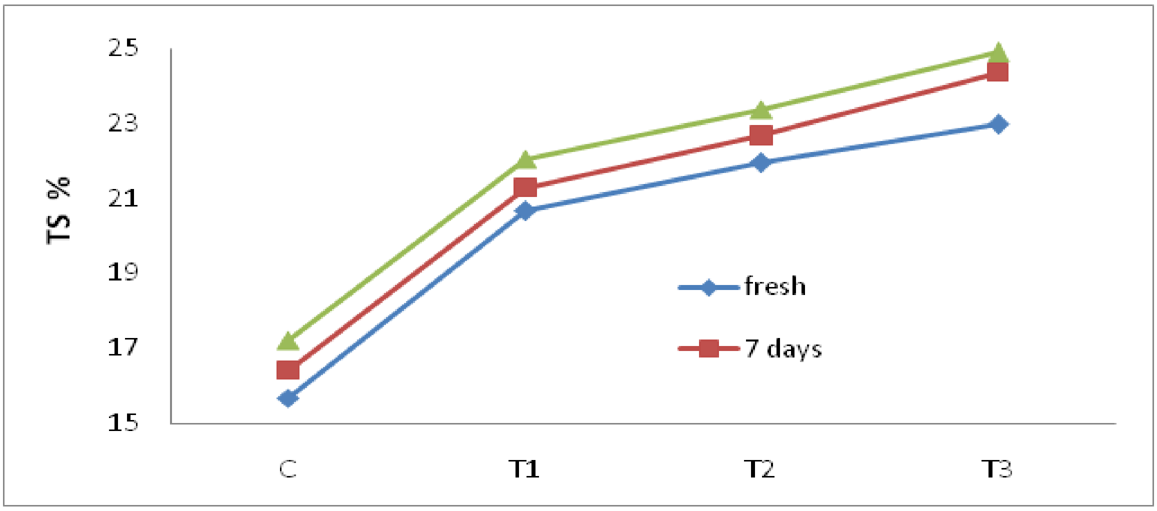


**Fig. 2. TS content of probiotic dairybeverages samples fortified with different levels (w/w) of CAJduring storage at 5±2°C for 14 day.**

See foot note of Fig (1).

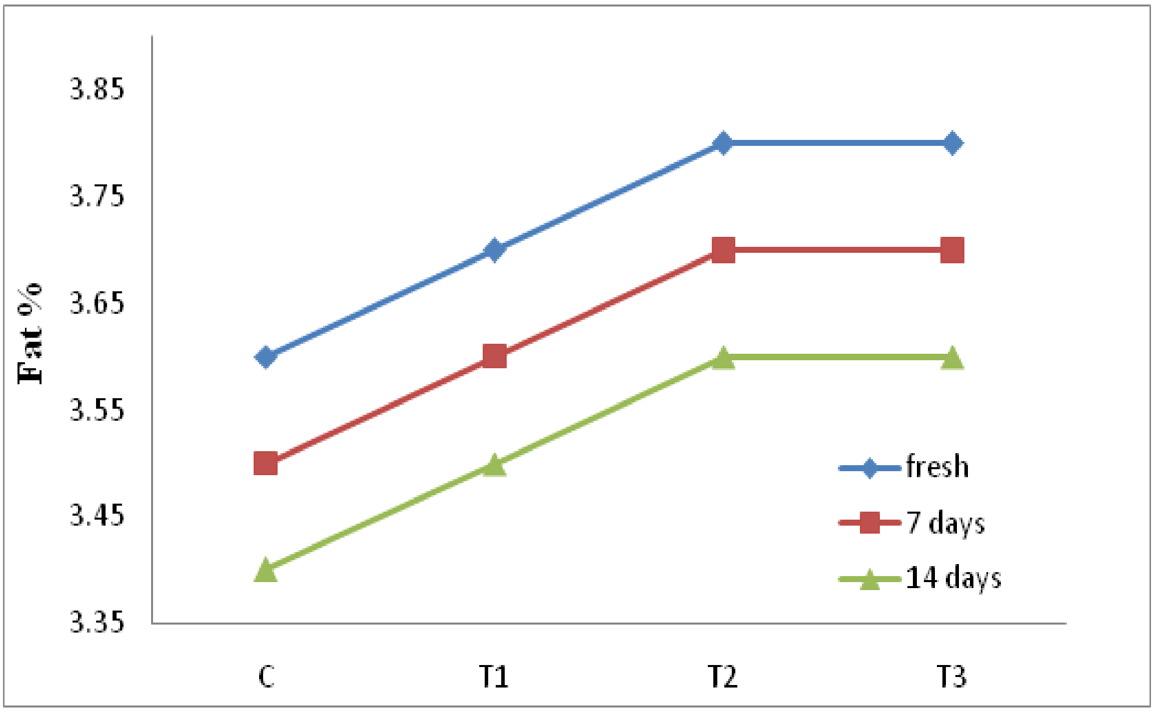


**Fig. 3. Fat content percent of probiotic dairy beverages samples fortified with different lecels (w/w) of CAJ during storage at 5±2°C for 14 day.**

*See foot note of Fig. (1).

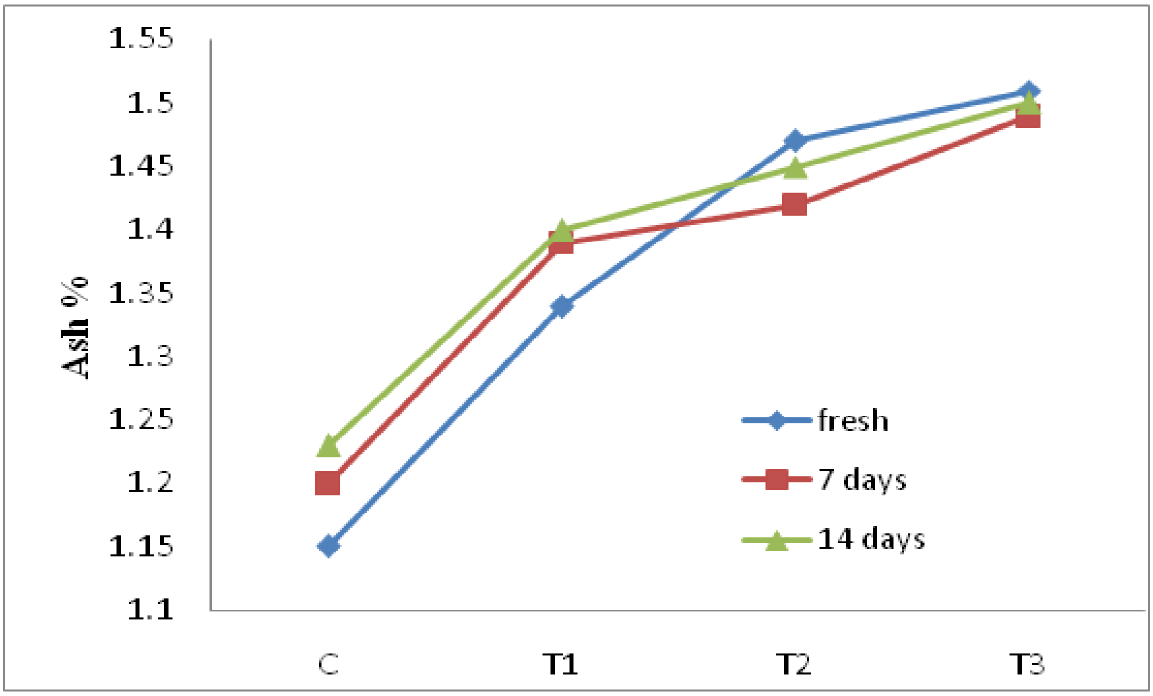


**Fig. 4. The ash content percent of probiotic dairy beverages samples fortified with different levels (w/w) of CAJ during at 5±2°C for 14 day.**

See foot note of Fig. (1).

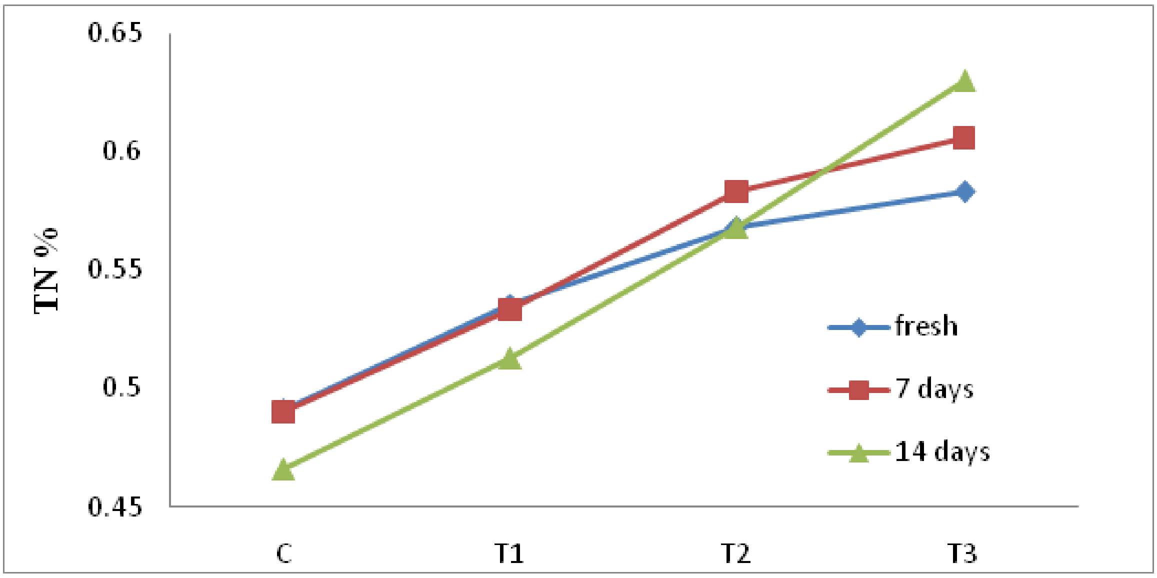


**Fig. 5. The total nitrogen (TN) percent of probiotic dairy beverages samples fortified with different levels (w/w) of CAJ during storage at 5±2°C for 14 day.**

See foot note of Fig (1).

Water soluble nitrogen content (WSN):Data presented in Fig. 6 represented the WSN content of probiotic dairy beveragesfortified with CAJ. The values of WSN were increased as the level of CAJ and storage periods increased. Control samples contained 0.0210, 0.0218 and 0.0220% when fresh and after 7 & 14 days of storage. T1 had 0.0261, 0.0285 and 0.0297%. The corresponding values for T2 were 0.0280, 0.0297 and 0.0318% However, T3 contained 0.0303, 0.0335 and 0.0356 % in the same order. It could be concluded that WSN contents were noticeably increased as storage period increased; this is due to the progress of proteolysis.

*Total volatile fatty acids content (TVFA)*

As shown in Fig. 7, the values of TVFA were pronouncedly increased as level of CAJ added and as storage days increased. Fresh control sample had 7.40mL 0.I NaOH/10g sample; increased to 10.30 and 12.70 after 7 and 14 days. The corresponding values for T1 were 9.20, 12.80 and 15.40mL0.I Na OH/10 g. The values for T2 were 12.30, 16.00 and 18.40 mL. However T3 possessed 15.40, 18.40mL 0.I Na OH/10g and 22.66 mL. 0.I NaOH/10 g sample. The progress in TVFA during storage is a result of lipolysis.

*Counts of St. thermophiles*

Figure 8 reflected the growth of St. thermophiles in probiotic dairy beverages samples fortified with different ratios (w/w) of CAJ during storage period. It was clear that the counts of bacteria were decreased as fortification ratio increased and also as storage period progressed. Control sample had 39, 28 and 19 cfu×$10^6$. The corresponding counts for T1 were 36, 25 and 17cfu×$10^6$. While their counts for T2 were 28, 23 and 15 cfu×$10^6$, respectively. Samples labeled No. 3, contained 24, 20 and 14 cfu×$10^6$. It could be observes that AJC had an inhibitory effect on LAB. Mohamedet al. (2015) recorded that during storage the number of viable count decreased in apple-yogurt samples. Lactobacillus was identified in all yogurt samples. These data came in computable with those of Thumrongchote, 2014 and Fazillah et al., 2018.

*Counts of L. acidophilus*

Figure 9 reflected the growth of L. acidophilus in probiotic dairy beverages samples fortified with different ratios (w/w) of CAJ during storage period. It was clear that the count values were increased as CAJ ratio increased and the count values were decreased as storage period increased. Control sample had 36, 27 and 20 cfu×$10^5$ in fresh, after 7 days and after 14 days of storage. The corresponding counts for T1 were 40, 32 and 23 cfu×$10^5$.While their counts for T2 were 45, 34 and 28 cfu×$10^5$ in order. Samples labeled T3 contained 49, 37 and 26 cfu×$10^5$. Thumrongchote (2014), reported that the decline of probiotic bacteria cells was differed according the type of fruit were in pineapple fruit yogurt and mango fruit yogurt were significantly greater than papaya fruit yogurt. Present data were confirmed by Ertem and Cakmakc (2018).

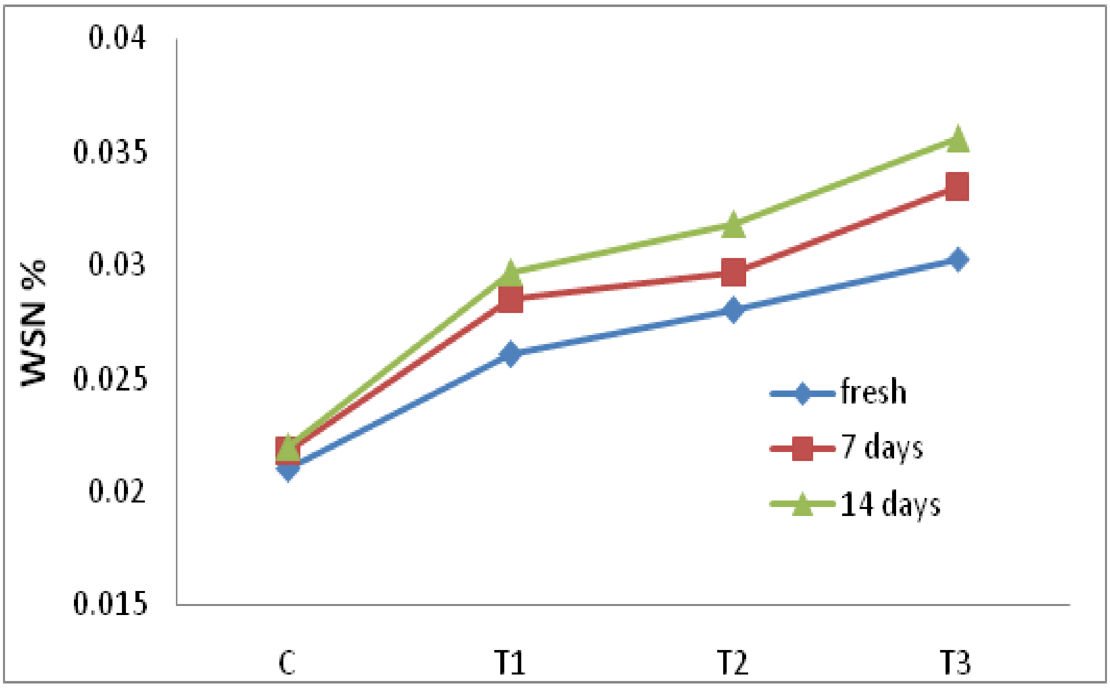


**Fig. 6. The water soluble nitrogen (WSN) percent of probiotic dairy beverages samples fortified with different levels (w/w) of CAJduring storage at 5±2°C for 14 day.**

See foot note of Fig. (1).

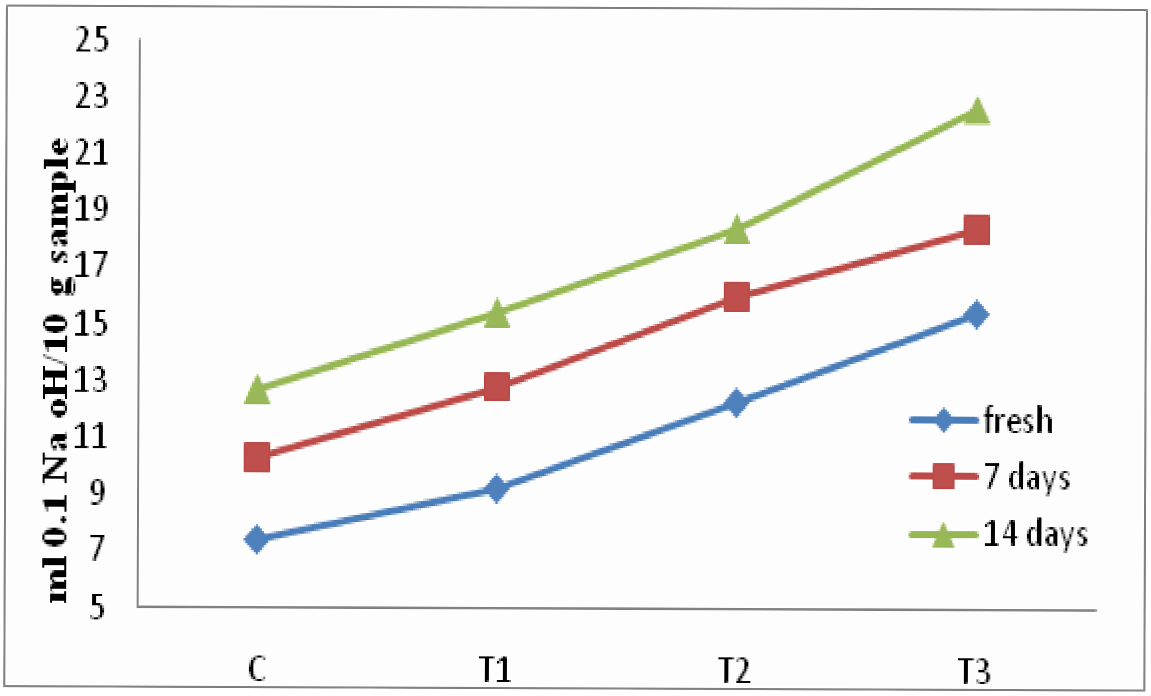


**Fig. 7. The total volatile fatty acids (TVFA)* content of probiotic milk beverages samples fortified with different levels (w/w) of CAJduring storage at 5±2°C for 14 day.**

See foot note of Fig. (1).

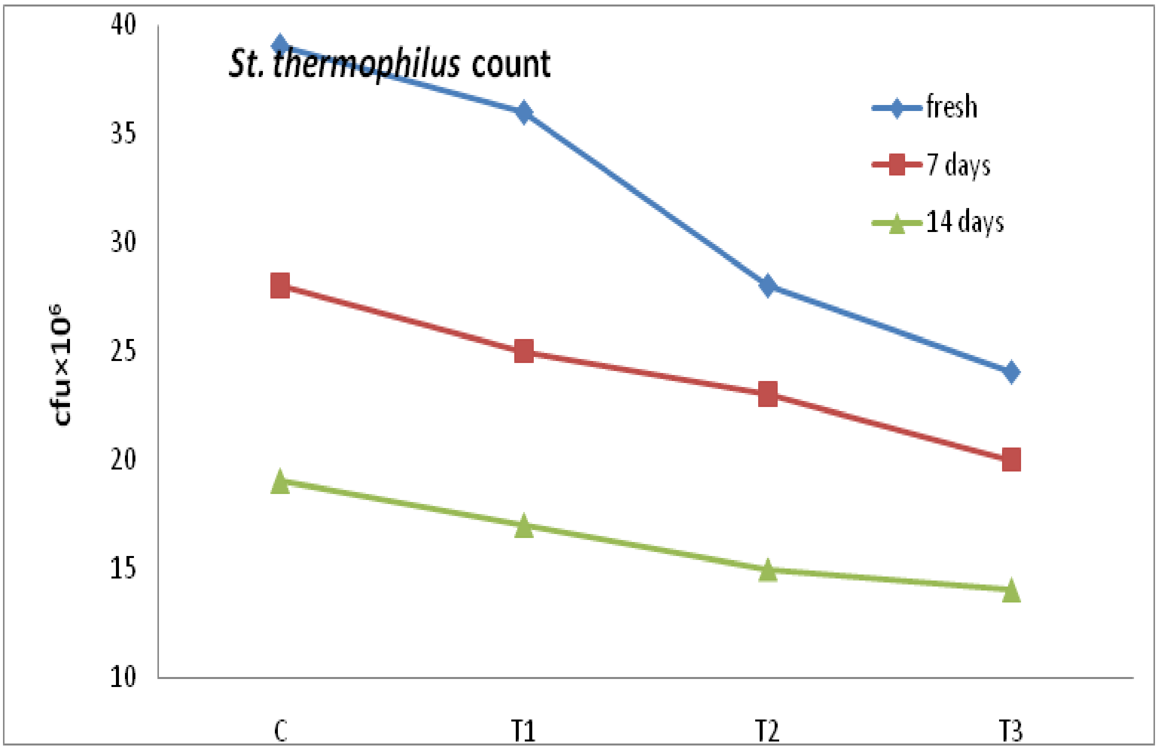


**Fig. 8. The count (cfu×10⁶) of *St. thermophiles* of probiotic milk beverages samples fortified with different levels (w/w) of CAJ during storage at 5±2°C for 14 day.**

* See foot note of Fig. (1).

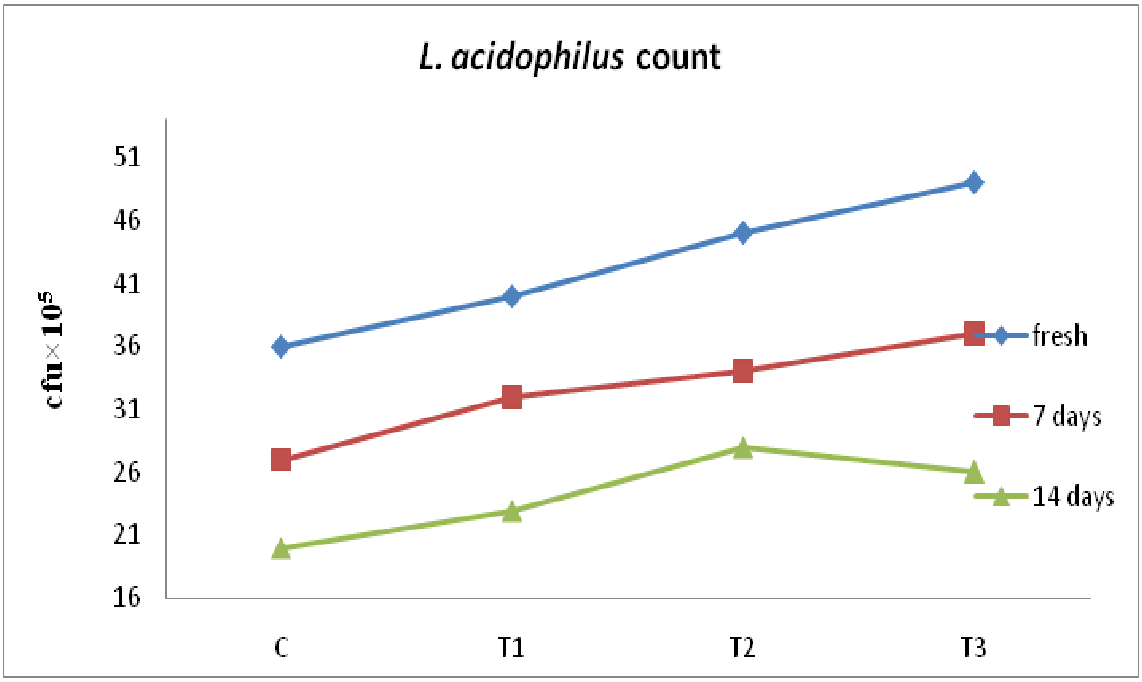


**Fig. 9. The count (cfu×10⁵) of *L. acidophilus* of probiotic milk beverages samples fortified with different levels (w/w) of CAJ during storage at 5±2°C for 14 day.**

* See foot note of Fig. (1).

*Counts of Bifidbacterium bifidium*

Figure 10 reflected the counts of Bifidbacteriumbifidium in probiotic milk beverages samples fortified with different ratios (w/w) of CAJ. It could be noticed an increase in their count as level of CAJ increased, while the counts decreases as storage periods increased. Control sample contained 34, 26 and 19 cfu×$10^5$ when fresh and after 7& 14 days. The corresponding counts for T1 were 39, 28 and 21. However their counts were 46, 37 and 27 for T2 and 48, 38 and 29 for T3. It could be concluded that the apple juice concentrate had a stimulatory effect on the growth of Bifidibacterium bifidium bacteria. Thumrongchote (2014) studied the effect of Thai fruit on probiotic bacteria viability (Lactobacillusacidophilus and Bifidobacteriumaninals) in yogurt samples after storage at a temperature of 52±°C for a period of three weeks. The average viability of probiotic bacteria decreased from 3.37×$10^9$ cfu/g at fresh to 8.17×$10^7$ cfu/g on day 21, and all stored yogurt contained a higher amount of probiotic bacteria than the recommended level ($10^6$-$10^7$ cfu/g). The viability of probiotic bacteria in papaya yogurt was 1.47×$10^8$ cfu/g on day 21, which was the highest count. The decline in bacterial counts may be due to the decline in the pH value of yogurt.

*Sensory evaluation*

Figure 11 recorded the organoleptic properties of probiotic dairy beverages samples fortified with different ratios (w/w) of CAJ during 14 days of storage. It noticed obvious variations in all evaluated properties. For color & appearance; T2 gained the highest scores either fresh or during storage (14, 12 & 11, respectively), while T3 gained the lowest scores where its color was dark. Body & texture degrees indicated that control had the highest scores followed by T1, while T3 possessed the lowest scores in body & texture after 14 days. Flavor scores reveled that T2 had the favorite score either fresh or after 14 days of storage. It could be concluded that T2 gained the all over acceptability.Shukla et al. (2003) prepared beverages by blending apple juice at four different concentrations (100, 200, 300 and 400 g/L) with separated and reconstituted skim milk. They concluded that organoleptic evaluation of the beverages showed that apple juice could be blended at up to 300 and 100 g/L in milk products with acceptable properties. Mohamed et al. (2015) mentioned that the apple pulp was added at 8 and 16 percent levels. Yogurt with 8% apple pulp was liked in all sensory attributes; it can be concluded that addition of apple enhanced the quality and favor of yogurt. The overall sensory quality of these yogurts was rated as good to very good. Gangwar et al.(2016), indicated that yogurts which contained 10% apple had better quality and yogurts fortified with 5 and 10% apple juice were good in smell and taste; 10% apple juice yogurt being the best among all fruit yogurts.

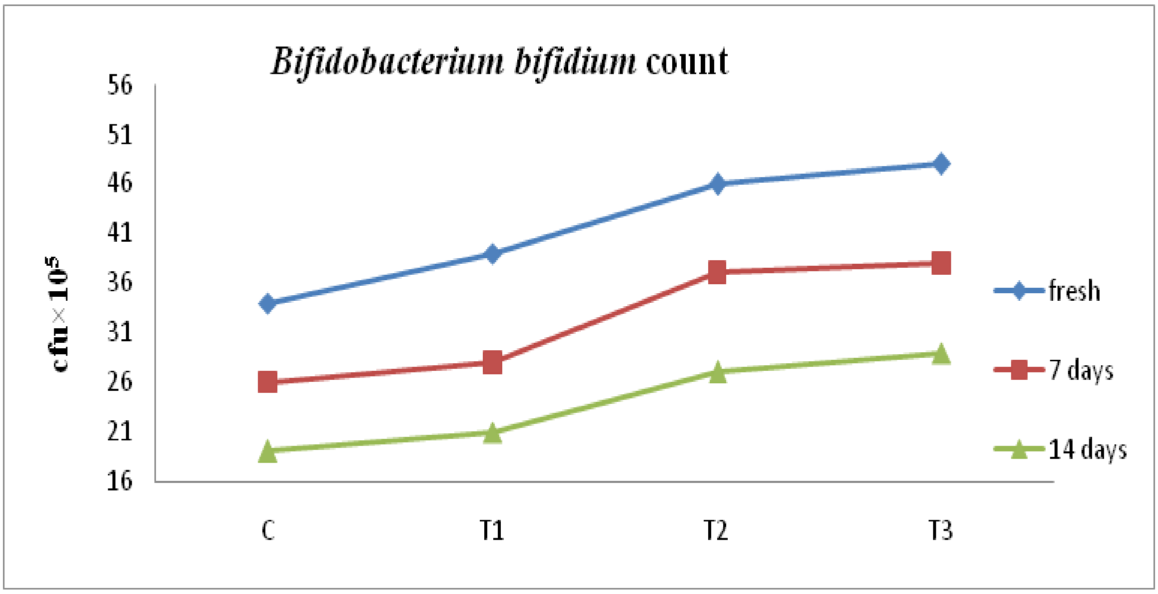


**Fig. 10. The count (cfu×$10^5$) of Bifidbacterium bifidium of probiotic milk beverages samples fortified with different levels (w/w) of CAJ during storage at 5±2°C for 14 day.**

*See foot note of Fig (1).

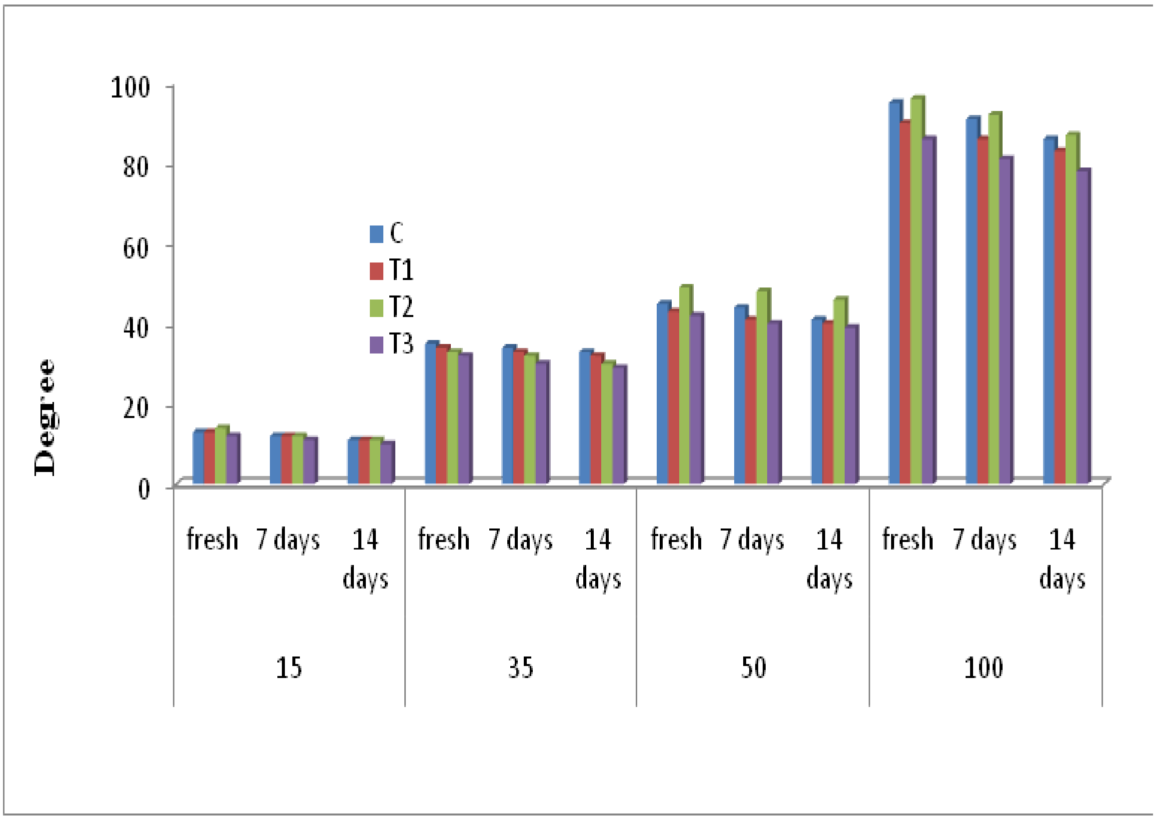


**Fig. 11. Sensory evaluation (degree) of probiotic milk beverages samples fortified with different ratios (w/w) of CAJ during storage at 5±2°C for 14 day.**

See foot note of Fig. (1).

## Conclusion

The obtained results concluded that it could be prepared dairy beverages fortified with probiotic bacteria and concentrated apple juice with acceptable properties to raise their healthy benefit. Theorganoleptic properties of apple-juice-dairy beverages samples during 14 days of storage recorded obvious variations in all evaluated properties. Total acceptability of T2 which contained 10% (w/w) concentrated apple juice gained the highest scores either fresh or during storage.

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