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IMPACT OF COLD STORAGE ON ANTIOXIDANTS ACTIVITY AND VITAMIN C IN MILK FLAVORED BY SUBTROPICAL FRUITS

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ABSTRACT

However, Milk is considered the superior food. The addition of fruits into milk is to improve its functionality. In this study four subtropical fruits; Annona (Annona squamosal), Avocado (Persea Americana), Kiwi (Actinidia deliciosa), and Pomegranate (Punica granatum) were mixed with milk in concentrations of 20, 30, and 50%. The concentration of total antioxidants (TAC) and vitamin C of samples were measured colorimetrically across 7 days of cold storage. The highest values of TAC were for annona and pomegranate flavored milk samples at day 0 (126.12 and 125.62 mM/L) respectively, while by 7th day all types of flavored milk samples losses its contents of antioxidants dramatically except the avocado milk sample which kept the highest contents (118.76 mM/L). The amount of vit. C is dropped across cold storage in an analogous manner of TAC. Sensory properties and over all acceptability of samples were at the side of annona and avocado flavored milk. Subtropical fruits must be added to milk under scientific control to avoid interaction with casein. The fruit flavored dairy should be consumed fresh to avoid destructive effect of cold storage on antioxidants.

Key words: flavored milk, antioxidants, vit C, ascorbic acid.

INTRODUCTION

Milk is considered the superior food for children as it contains all the nutrients necessary for human growth and health in balanced ratios. In addition to proteins, minerals and vitamins in milk, antioxidants play a vital role in protecting the body from free radicals and maintaining cell vitality. Milk is rich in both fat soluble antioxidants (conjugated linoleic acid, α-tocopherol, β-carotene, vitamins A and D3, phospholipids) and water soluble antioxidants (proteins, peptides, minerals and trace elements.

Antioxidants are antitoxic protective substances dynamically overcome the destructive effects of the free radicals on human health as well as elongating shelf-life of foods by delaying its spoilage through inhibition of microbial and enzymatic activities which accelerate rancidity. The efficiency of antioxidants in human body cells is due to their capability to bind free radicals so preventing their destructive oxidant activity (Blasa *et al.*, 2010).

There are various natural and synthetic antioxidants, with the natural ones being safer and demanded by consumers. Natural antioxidants (tocopherols, flavonoids & phenolic acids) could be found besides milk in fruits, vegetables, grains and herbs.

Great antioxidant power was possessed by vitamin C in which it is easily converted from its reduced form (ascorbic acid) to its oxidized form (dehydroascorbic acid). Ascorbic acid or Vit C is rare in milk of all species except camel milk; (El-Hatmi *et al.,* 2006) but fruits and vegetables supplied about 90% of a person's dietary requirement of vitamin C. (Salunkhe *et al.,* 1991).

Vit. C attracts the attention of the research community and consumers as a nutrient with a broad biological activity and importance for human health. It supports the absorption of iron and the formation of collagen. Vit C is added to foods not only as a nutrient but also as an antioxidant. (Kirby *et al.,* 1991).

Ascorbic acid intercepting in eliminating free radicals so, it minimizes damage to lipids, protein and nucleic acids (Zanini *et al.,* 2018). Important functions in the body are performed by ascorbic acid like building and maintaining strong tissues especially, connective tissues (bones, cartilage, dentin, collagen, etc.), forming strong capillary walls for blood vessel, tissue building, resistance to infection, helps in the absorption of calcium, so it ensures the health of bones, haemoglobin synthesis

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by increase the bioavailability of iron, wound healing infections and fever recovery. It is important for tissue synthesis and naturally it is needed in growth stages of life. It helps in tying up free radicals so it is considered as strong reducing agents so it protects the body from their deleterious effects (Sumati *et al.,* 2003). Although it has a great importance for metabolism due to its oxidation Vitamin C is a member of water soluble vitamins with molecular formula is C6H8O6 and its molar mass is 176.13 g/mol and found in nature as isomer L (Blasa *et al.,* 2010).

The core purpose for the consideration of fruits (organic natural additives) in milk is to increase its antioxidant activity and functionality. Taking in consideration the mood swings of children, their tastes may differ from milk. So many trails to improve and change milk taste are developed to suit all tastes and ensure the interest of children and adults to eat it. One of the best ways to improve the taste of milk is milk based beverages by inclusion of different fruits with different taste to provide a wide base of different tastes. The fortification of milk with fruiters is not only to improve the taste, but also improving its functionality, in that they provide all essential and non-essential amino acids but, are less acidic than plain fruit juices (Dalim *et al.,* 2012). Also, added fruits found to have a significant effect on the milk content of antioxidants especially, fruits rich in antioxidants bioactive phytochemicals such as tropical annona, avocado, pomegranate, and kiwi. Therefore new ingredients in milk beverage industry will refine their nutritional quality.

This experiment was designed to detect the fluctuations in antioxidants and ascorbic acid levels and manner in milk as a result of mixing four sub-tropical fruits Annona (*Annona squamosal*), Avocado (*Persea Americana*), Kiwi (*Actinidia deliciosa*) Pomegranate (*Punica granatum*) as well as evaluation of the developed product sensory characters and consumer acceptability.

MATERIALS AND METHODS

1. Raw materials

1.1. Milk

UHT whole cow milk was used in this experiment. The production date was in the first week of the beginning of the self-life to minimize the probable damage in antioxidants activity.

1.2. Sub-Tropical Fruits

Four types of sub-tropical fruits (Annona, Avocado, Kiwi, and Pomegranate) were purchased from Aswan markets in late summer season in September 2018. The samples were fresh, healthy and intact.

1.3. Fruit extract

The extract was obtained by crushing 500 g of each type of fruits individually by a sterile mixer in a clean 1000 ml capacity stainless container. Then filtrated twice through a sterilized muslin cloth to minimizing the amount of impurities and then collected in glass beakers.

1.4. Flavored milk processing

Different amounts of fruits extracts were inoculated into milk and mixed well to obtain concentrations of 20, 30, and 50%. Neither preservatives nor sugars were used. Next Table pronounces the different formulae and abbreviations.

Code	Formulae
Control	Plain UHT milk sample
ANN20	Annona milk juice 20%
ANN30	Annona milk juice 30%
ANN50	Annona milk juice 50%
AVD20	Avocado milk juice 20%
AVD30	Avocado milk juice 30%
AVD50	Avocado milk juice 50%
KW20	Kiwi milk juice 20%
KW30	Kiwi milk juice 30%
KW50	Kiwi milk juice 50%
PMG20	Pomegranate milk juice 20%
PMG30	Pomegranate milk juice 30%
PMG50	Pomegranate milk juice 50%

2. Experiment design

All formulae as well as control samples of plain whole UHT milk were kept in refrigerator (4 ºC) and examined in days 0, 5, and 7 for antioxidants and Vit C contents.

3. Antioxidant measurement

Antioxidant activity was detected colorimetrical by Total Antioxidant Capacity kits (Biodiagnostic Lab) according to Koracevic and Koracevic (2001) using 3 reagents R1 Substrate, R2 Chromogen, R3

Enzyme - Buffer. The blank as well as the sample were read against distilled water at 505 nm.

4. Ascorbic acid measurement

Ascorbic acid level was detected colorimetrically by Ascorbic acid kits according to Harris, and Ray (1935) in which 0.1 ml of sample was mixed with 0.5 ml buffer and 1.0 ml 2, 6-dichlorophenol indophenol (DCPIP) and measured against distilled water at wave length 520 nm.

5. Sensory evaluation

5.1. Physical properties

The pH value of each formula was measured by pH meter (HANNA-pH 210, Germany).

5.2. Rheological properties (Meilgaard *et al.*, 2007)

The Taste, flavor, texture and over all acceptability of formulae were tested by 11 non experienced panelists (6 men and 5 women) aged between (20: 40 years). The panelists were the doctors and employee in Aswan University. They were asked to score the quality of coded dairy beverages samples on a 5 grades score card in which grade 1 means very bad, 3 = fair and 5 = very good.

RESULTS

Table 1: Total anti-oxidant capacity (mM/L) in flavored milk samples across 7 days of cold storage.

	Day 0 ± SE	Day 5 ± SE	Day 7 ± SE
Control	109.56 ± 0.617	114.22± 0.779	101.27± 0.721***
ANN20	113.23± 0.34	102± 0.09	91.46± 1.33***
ANN30	123.94± 0.837	116.82± 0.671	90.35± 2.165***
ANN50	126.12± 0.646	121.31± 0.326	113.4± 0.653
AVD20	109.56± 0.9238	103.34± 0.2935	104.47± 0.665***
AVD30	121.95± 1.818	122.87± 0.876	118.76± 1.587
AVD50	124.74± 0.5484	121.55± 2.93	114± 0.88
KW20	110.52± 1.167	101.32± 0.587	97.33± 1.097***
KW30	116.08± 0.635	109.62± 0.626	103.55± 1.1835
KW50	120.39± 1.2438	109± 1.1547	100± 1.1547***
PMG20	120.45± 1.39	86.21± 1.1837	66.84± 0.733***
PMG30	121.74± 1.01036	93.31± 0.7505	72.74± 1.010***
PMG50	125.62± 0.682	101.11± 0.875	87.75± 1.181***

***$p < 0.001$ indicate statistically significant differences compared to the control at day 0.

Table 2: pH values of flavored milk samples across 7 days of cold storage.

	Day 0 ± SE	Day 5 ± SE	Day 7 ± SE
Control	6.58 ± 0.06	6.47 ± 0.01	6.52 ±0.01
ANN20	6.2 ± 0.05	6.0 ±0.02	5.5 ±0.06
ANN30	6.3 ± 0.05	6.2 ±0.05	6.0 ±0.03
ANN50	6.3 ± 0.11	6.3 ± 0.02	6.3 ± 0.01
AVD20	6.2 ± 0.01	6.0 ± 0.01	6.0 ± 0.01
AVD30	6.2 ± 0.02	5.8 ± 0.01	5.9 ± 0.12
AVD50	6.27 ± 0.03	6.34 ±0.01	6.27 ±0.01
KW20	3.22 ± 0.06	3.00 ±0.03	3.03 ±0.06
KW30	3.4 ± 0.057	3.4 ±0.04	2.9 ±0.06
KW50	3.59 ± 0.057	3.2 ±0.057	3.0 ±0.057
PMG20	3.13 ± 0.057	3.00 ±0.01	2.9 ±0.057
PMG30	2.93 ± 0.01	2.5 ±0.057	2.5 ±0.057
PMG50	3.20 ± 0.06	2.4 ±0.057	2.3 ±0.057

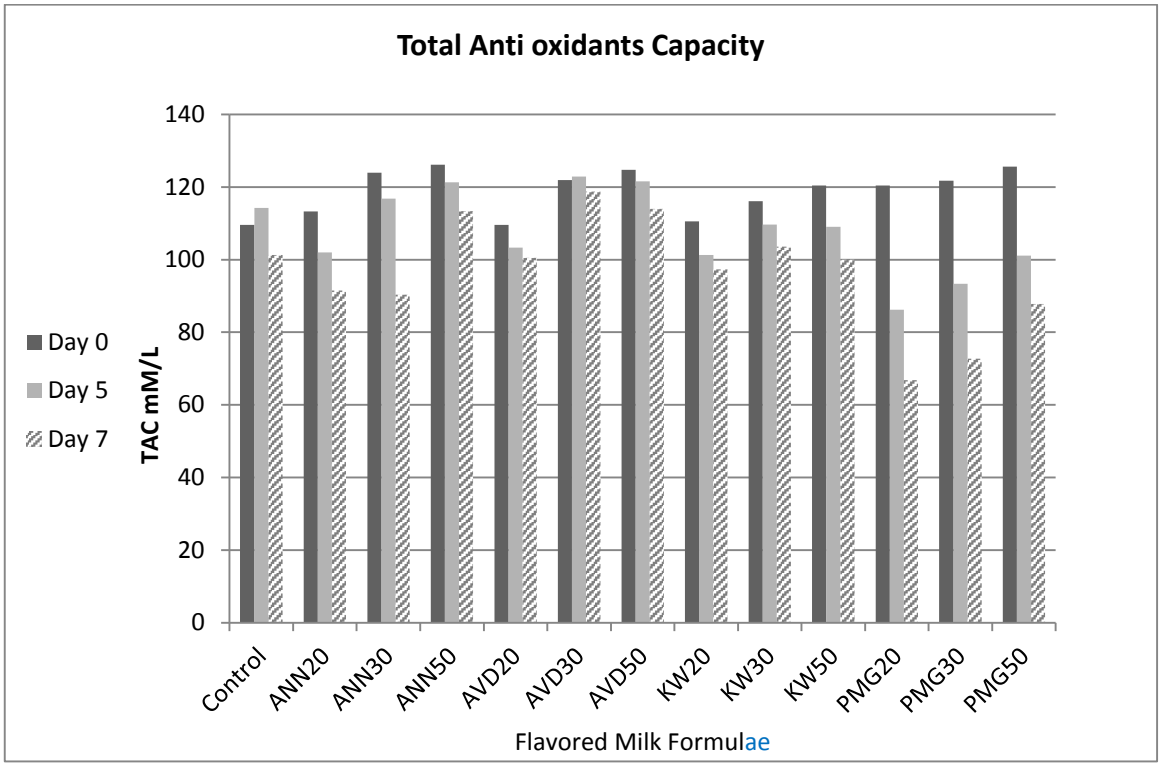

Fig. 1: Total Anti-oxidants capacity of flavored milk samples across 7 days of cold storage

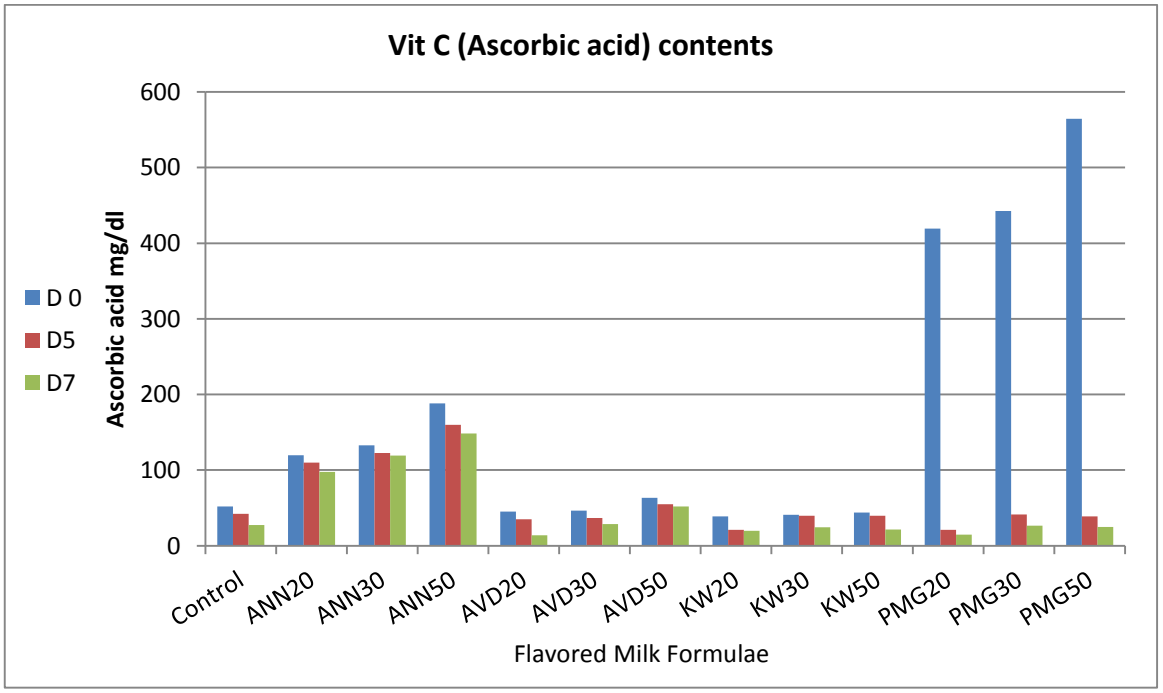

Fig. 2: Vitamin C (Ascorbic acid) contents in flavored milk samples across 7 days of cold storage

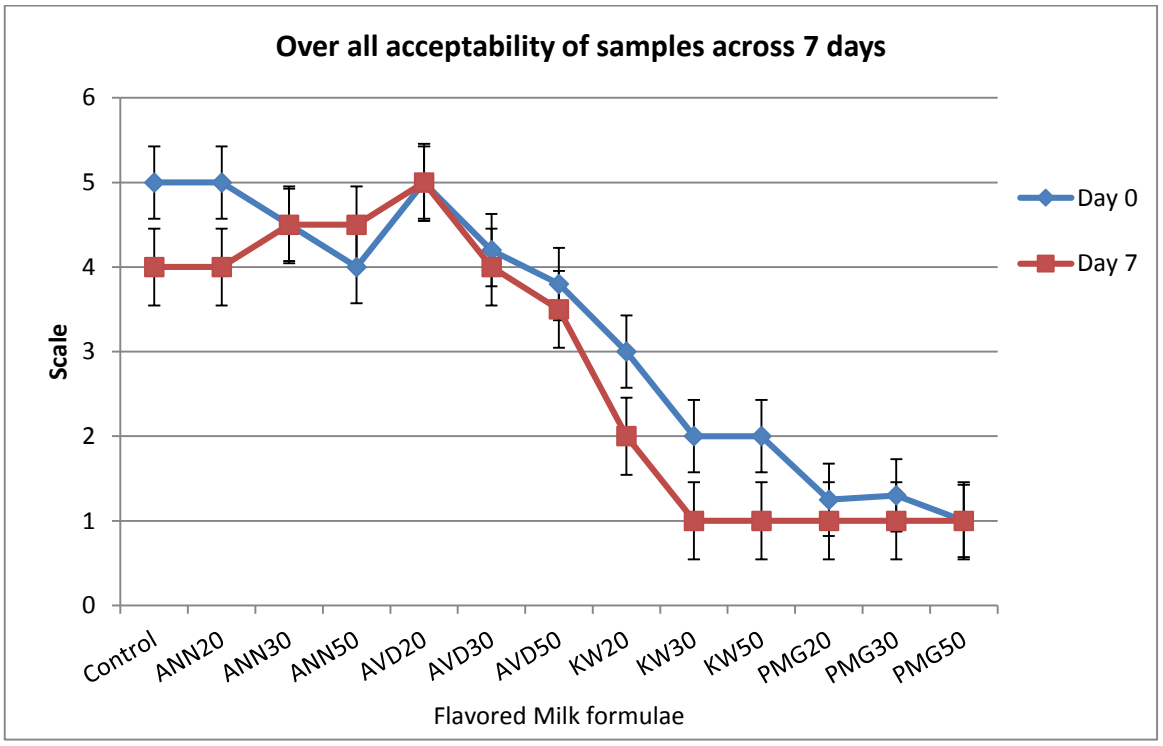

Fig. 3: Over all acceptability of flavored milk samples across 7 days of cold storage

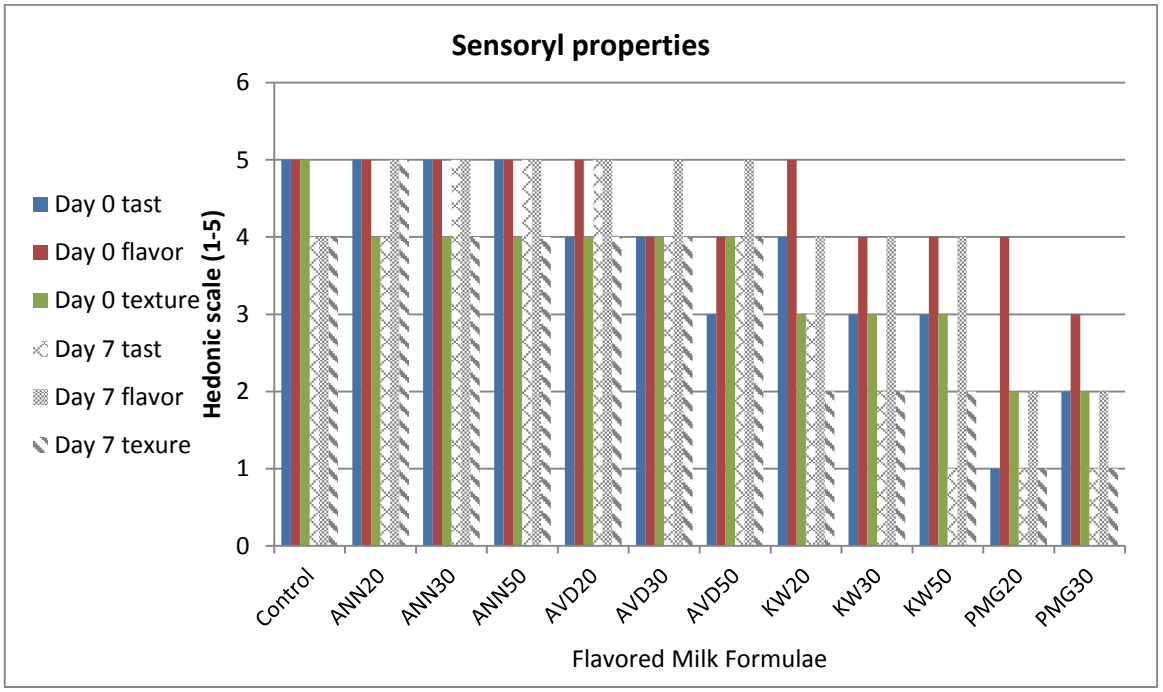

Fig. 4: Sensory properties of flavored milk samples across 7 days of cold storage

DISCUSSION

The purpose of milk inoculation with fruits is to make a superior functional food rich in natural antioxidants and fibers. Also milk is very rich in both lipophilic and hydrophilic antioxidants, but addition of fruits will multiply this capacity and improving taste and dietary fibers content.

1. Antioxidant levels in milk beverages

The significance of antioxidant activity in the dairy beverages is conveying both the shelf life and human body cell protection against oxidative damage.

Table (1) and fig. 1 showed that the Total antioxidant capacity (TAC) of the control sample

(UHT skim cow milk) in day zero of the experiment was 109.56 mM/L. Higher values (456) to (356) µmol/L were measured by oxygen radical absorbance capacity (ORAC) assay in (UHT) cow milk, by Chen *et al.* (2003) and Zulueta *et al.* (2009a). This could be attributed to many variables, such as methodology of extraction, reduced fat contents which carry a significant part of lipophilic antioxidants and variances in methodology. The type of plant also may be responsible for the concentrations of bioactive compounds beside other factors as genetic factors, maturity stage, environmental. (Ddriozola-Serrano *et al.,* 2008; Albuquerque *et al.,* 2016).

Total antioxidant capacity in all milk samples fortified with the fruits was higher than the plain milk in day 0. Specially the concentration of 50% annona (ANN50) and pomegranate (PMG50) in which the antioxidant capacity of pomegranate juice was shown to be higher due to the free radicals scavenging activity, and iron reducing capacity of the juice (Gil *et al.*, 2000).

By the 5th day all samples showed decline in its content of TAC except avocado beverages which kept TAC balanced between day 0 and 7 (121.95 and118.76) for beverage of 30% concentration and 124.74: 114 for 50% concentration, respectively. This is may be due to that avocado is rich in lipophilic antioxidants components such as carotenoids, unsaturated fatty acids including oleic, linoleic, palmitoleic acid which with long storage are subjected easily to oxidative stress. (Ding *et al.*, 2007).

Moreover, Gómez *et al.* (2014) recorded high TAC of avocado parts in a dose-dependent manner. High redox power and oxygen chelation of edible plant extracts was due to its high contents of phenols and antioxidants Adedapo *et al.* (2008). The kiwifruit had significantly higher radical scavenging activities at the start of fruit development, but it decreased as the fruit matured (Ramesh *et al.,* 2015). This explains the drop of TAC in Kiwi flavored milk samples in this study. Also, a desirable change that occur during ripening of pomegranate in which the fruit losses its acidity due to reduce the levels of phenolic compounds (Fawole and Opara, 2013) may be the cause of losing large value of TAC in pomegranate milk samples by cold storage.

In this study it was obvious that for all flavored milk types the total antioxidant capacity increased by the increasing of fruits concentration up to 30% and then no significant effect were observed. El-Said *et al.* (2014) recorded similar observation in stirred yogurt fortified with pomegranate peel extract.

The recorded decline in TAC in most examined dairy beverages may be due to interactions between phenolic compounds of fruits and lipids, carbohydrates and proteins of milk. Numerous previous studies found that milk diminished the antioxidants capacity of beverages by about 30%. This impact is thought to happen in light of the fact that the milk casein has affinity power for antioxidants, lessening their capacity to battle unsafe free radicals. (Bourassa *et al.,* 2013, Serafini *et al.*, 2009. Jakobek 2015).

2. Ascorbic acid contents

Ascorbic acid (Vit C) is the major hydrophilic antioxidant. It is raising the human body immunity, vessels elasticity, reducing circulation disturbances and bleeding due to capillary damage and therefore in food ascorbic acid protects from or at least delay spoilage and rancidity.

It is well known that bovine milk is not a rich source of Ascorbic acid; on the other hand fresh fruits are recommended as vit C suppliers.

Fig. 2 declared that Ascorbic acid (AA) in control sample dropped significantly between day 0 and day 7 of cold storage from 51.9 to 27.3 mg/dl, respectively. This is may be due to that Vit C is hydrophilic in nature and is very sensitive to dryness resulted from long storage. Similar consequences were recorded by Silva *et al.* (2018) in infants and children foods.

In all samples of fruits beverages the vit C degradation after 5 days of cold storage at 5 C° was noticeable. In Samples of pomegranate beverages PMG 20 was sharply dropped from 442.8 to 26.7 mg/ dl across 7 days of storage. Quirós *et al.* (2009) found similar pattern of vit C degradation in cold stored foods. Galani *et al.* (2017) recorded significant decrease in vit C contents in fruit foods by cold storage. While, milk samples with avocado and Annona Vit C contents degraded gradually. The wide changes in the degradation levels in different beverages formulae could be recognized to many causes, such as fruit nature, milk components interaction with fruit, AA contents and nature in each fruit, and concentration of fruits in beverage. Also vitamin binding proteins in milk could react with Vit C and developing of complexes (Claeys *et al.,* 2013). Moreover, other vitamins (B1, B2 and B12) and metal (Fe, Cu and Zn) contained in milk are able to interact with vitamin C, thus increasing its degradation rate.

3. Determination of pH

The normal cow milk pH is slightly acidic ranged between 4.4 and 4.8. Table (3) showed the plain UHT milk sample has 4.58 pH value and remain constant across 5 and 7 days of cold storage with non-significant drop (4.44 and 4.52), respectively. Although kiwi and pomegranate have more acidic reactions. By the day 5 the pH of formulae PMG2,

PMG30 and PMG50 were sharply dropped to reach 3.00, 2.5, 2.4, respectively and showing the lowest pH (2.3) among all formulae at day 7. Zulueta *et al.* b (2013) recorded significant decrease in pH of acidic fruit milk beverages by cold storage at day 7. This fall in pH value of milk is due to acidity of pomegranate peel. So, pomegranate is not proper to inoculated into milk as a beverage of storage period due to sever drop of acidity which leads to watery appearance of milk. But pomegranate may add to milk for immediate consumption only.

On the other hand, annona and avocado fruit beverages showed a less acidic pH. Annona beverages have a pH ranged between 6.0 and 6.3 and the drop of value was very narrow from day 0 to day 7. Hassan *et al.* (2015) recorded parallel results in pH of fruit dairy beverages during refrigerated storage.

Concerning, avocado milk formulae, the pH values ranged from 5.8 to 6.58. The highest concentration formulae AVD50 the pH was 6.85 and kept balanced in cold storage with minor decreases.

4. Sensory properties of dairy beverages

The sensory evaluation was presented in Fig. (3, 4). In respect of beverage flavor, panelists evaluated all samples as good to very good. But according to texture, pomegranate samples (PMG10, PMG20, PMG30) were classified as very bad due to weakness of texture and separation of liquid, while, annona beverage (ANN20, ANN30, ANN50) and avocado (AVD20, ADV30) were classified as very good for texture due to good viscosity, while AVD50 was classified as bad to texture this may be due to AVD50 contain large amount of avocado pulp 50% which is responsible for thick creamy texture. (Concha-Meyer *et al.,* 2016).

In respect to overall acceptability (OAA) in Fig. (3) Samples of ANN20, ANN30, ANN50, AVD20 were scoring between good and very good. Kiwi samples coded KW20, KW30, KW50 were moderately acceptable (3, 2, 2 respectively) while, PMG samples were recorded as very bad and recommended to used freshly to avoid beverage separation.

According apparent descriptions the samples of ANN have white color like creamy milk and sweetly taste, AVD samples were greenish in color and slightly bitter in taste. KW samples were yellowish white and have banana like taste with kiwi seeds. PMG were pale brown in color and pomegranate taste.

CONCLUSIONS

Mixing milk it with sub-tropical fruits must be under scientific control not industrial design to avoid interaction between casein and plant components, and to achieve complete benefits. Also the long storage of dairy beverages over 5 days is not recommended to avoid degradation of antioxidants and Vit C content.

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تأثير التبريد على مضادات الأكسدة وفيتامين سي في اللبن المطعم بالفواكه المدارية

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يعتبر اللبن أكمل الأغذية قيمة وأكثرها شعبية، ويتم اضافة الفاكهة له لزيادة محتواه من الالياف ومضادات الاكسدة. وقد تم في هذه الدراسة اضافة أربعة أنواع مختلفة من الفواكهة المدارية التي تباع في مدينة اسوان وهي القشطة والافوكادو والكيوي والرمان بتركيزات مختلفة ٢٠ و٣٠ و٥٠% ، وتم قياس نسب مضادة الاكسدة وفيتامين سي في كل نوع وكل تركيز على مدار سبعة أيام في درجة حرارة الثلاجة. وفي اليوم الأول كانت نسب مضادات الاكسدة أعلى ما يمكن في عينات القشطة والرمان حيث بلغت (١٢٦,١٢ و ١٢٥,٦٢) مل /مول على التوالي ومع الحفظ في الثلاجة تناقصت النسب بشكل ملحوظ في كل أنواع العينات عدا الافوكادو. ولم يكن معدل فيتامين سي بأحسن حالا من مضادات الاكسدة إذ تعرض للانخفاض الشديد بنفس النهج تقريبا. وباختبار الخواص الحسية للمنتجات وجد أن الحليب بنكهة الافوكادو والقشطة هما الأفضل بينما كان الحليب بالرمان مرفوضا تماما خاصة بعد الحفظ بالثلاجة. وعليه فإن اضافة الفواكه المدارية أو غيرها للحليب يجب أن يتم باشراف علمي حتى لا يتفاعل الكازين في اللبن مع مكونات الفاكهة ويؤدي لخسارة مضادات الاكسدة، كما يفضل استهلاك الألبان الممزوجة بالفاكهة في الحالة الطازجة حفاظا على مضادات الاكسدة من تأثير البرودة المدمر.