

Journal of Food and Dairy Sciences

Journal homepage: www.jfds.mans.edu.eg
Available online at: www.jfds.journals.ekb.eg

Production of Nutraceutical Probiotic Whey-Based Beverage Fortified with *Annona squamosa* L. (Custard apple) Fruit

Safaa A. M. El-Aidie; H. H.Omar*; Amal M. Ewis and Amal M. M. El-Nimer



Dairy Technology Research Department, Animal Production Research Institute, Agricultural Research Center, Egypt.

ABSTRACT

Probiotic whey-based custard apple beverages (P.C.A.B.) were prepared with different combinations of whey and custard apple pulp juice, PJ (75:25, 50:50 and 25:75, weight :weight) and inoculated with 1% probiotic bacteria *Lactobacillus casei*. Beverages were evaluated for physico-chemical, microbiological and sensory properties, during cold storage. Total solids and the apparent viscosity were increased in (P.C.A.B.) treatments as the level of P.J. increased, whereas values of titratable acidity (T.A) were decreased, compared to control treatment. The pH values of all treatments behaved reverse trend to T.A, through the storage period. Total antioxidant compounds, vitamins A & C and minerals were found higher in fresh beverage contained 25% P.J. than control one. The rate of increase (%) in the antioxidants, vitamins A & C were 9.09, 135.30, and 173.30, respectively. Minerals were increased also in (P.C.A.B.) but in a variable ratios. Counts of probiotic lactobacilli were gradually decreased as the storage period increased and as the level of P.J. increased. The probiotic strain was still viable, in all treatments, with counts more than 10^6 cfu/ml, while coliform group & spore-forming bacteria were absent in all treatments, during storage. Control treatment was less acceptable, at the 21 days of storage as a result of growing yeast & molds with counts reached to 30×10^2 cfu/ml. The beverage prepared from 75% whey : 25% P.J. was found superior organoleptically than the other ones, along the storage period. It has clean acid flavor and long keeping quality up to 40 days of storage.

Keywords: Beverage, Custard apple, Probiotic bacteria, *Lactobacillus casei*.



INTRODUCTION

Whey is the main by-product produced in a large quantities from the dairy and cheese processing industries. Wrong disposal of whey causes major environmental problems due to its increased production and high content of organic materials (Lehn *et al.*, 2018). Several efforts have been done during the recent years to produce value-added products from whey to utilize advantage of their nutrients and decrease the environmental pollution (Magalhães *et al.*, 2011). Whey proteins are distinguished by their high biological importance compared to other proteins such as egg, soy and milk casein (Pescuma *et al.*, 2010). It contains around 20% of the total protein of milk. Whey contained, also precious nutrients including whey proteins, lactose, milk salts and most of water-soluble vitamins (Sakhale *et al.*, 2012), as well as, α -lactoalbumin, β -lactoglobulin, bovin serum albumin, caseinomacropetides, immunoglobulins, lactoferrin, lysozyme, which are associated with health benefits, e.g. enhance immunity, anticancer, antiviral, antimicrobial and antihypertensive properties. It is, moreover, used to treat a wide variety of conditions such as arthritis, anemia and hepatic complaints (Chavan *et al.*, 2015).

Due to the former enormous health benefits, whey-based beverages can be used as a functional food or nutraceutical food and can compensate the body with organic components such as vitamins, minerals, lactates and amino acids which lost from body fluids as a result of effort, temperature or age (Sakhale *et al.*, 2012). Because

of the low overall solids content of liquid whey (about 6% by weight), the mouth feeling of whey-based beverages appear to be weak and watery, compared to whey-based fermented milk beverages, and this case required either the use of exopolysaccharide-producing starters or addition of hydrocolloids or fruit juice, (Legarová and Kouřimská, 2010). Adding fruit juice can increase the total solids content, nutrients as well as dissimulation the fresh whey flavor (Nursiwi *et al.*, 2017).

Annona squamosa L. (Custard apple, CA) is a tropical fruit belonging to the Annonaceae family and had a unique aroma and flavor (Olagunju and Sandewa, 2018). *Annona Squamosa* L. is cultivated around the tropical world, named in India, as sitaphal seureuba, sugar apple, gishta, sweet apple, zimtapfel, sharifa, sitaphal, matomoko, aati, atis and anoda, and considered as one of the delicious fruits because of its good taste, mild fragrance, smooth, sugary, juicy and granular pulp (Bala *et al.*, 2018). CA is a nutritional healthy food, made up nutrients; protein, carbohydrates, sugars, minerals, vitamins, very little fat content and energy. Consumption of fresh fruit extracts or beverages is growing worldwide, due to the belief that they contribute to a balanced diet and are a good source of antioxidants, vitamins (C, A, B2, and B6) and minerals (potassium, calcium, phosphorus, magnesium, and iron). It also has anti-cancer, anti-inflammatory and anti-tumor capabilities and the potential to minimize the risk of many diseases due to its biological active compounds such as polyphenols and acetogenins (Virgen-Ceceña *et al.*, 2019).

* Corresponding author.

E-mail address: Hatemhelmy1412@gmail.com

DOI: 10.21608/jfds.2020.106390

The general definition, describes beverages as a suitable beverage for human consumption, providing nutrition compounds, refreshment, and has appealing taste and flavour. Functional beverages offer also other health benefits (Nursiwi *et al.*, 2017). The functionality of these beverages lies in the presence or strengthening of certain bioactive or functional components. The beverages can be classified into a few main groups: unfermented and fermented beverages, probiotic drinks, healthy soft drinks, alcoholic drinks, nutritional drinks, high-protein sports drinks, direct-consumption drinks and powdered drinks (Chavan *et al.*, 2015). Fermented whey beverages that contain probiotics have received much attention in recent times due to the many health benefits that have been linked to their consumption (El-Aidie *et al.*, 2017).

Prebiotic is defined as an indigestible nutrient that positively affects the host by stimulating the growth and activity of one or a limited number of beneficial bacteria in the colon, thus improving the host's health (Vasiljevic and Shah, 2008). Probiotics act as an inhibition of intestinal carcinogens due to their ability to balance the host intestinal microflora (Senadeera *et al.*, 2018). Fermented beverages containing probiotics are not just a source of nutrients but also a source of live microorganisms which enhancing the properties of the beneficial indigenous microbiota (Mudgil and Barak, 2019). *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus reuteri*, and *Bifidobacterium sp.* are among the most popular probiotic bacteria used in the production of fermented whey beverages (Reid, 2008). Probiotics have been shown to strengthen the immune system, decrease blood pressure and blood cholesterol and exhibit antitumor activity. They prevent gastrointestinal inflammation, control infection occurs during pregnancy, the obstacle of urinary tract infection, allergic diseases and other medical complications (Iqbal *et al.* 2017).

Take into consideration from the above, fruits are an excellent source of antioxidants, prebiotic fibers and nutrients. Consuming fruits, probiotic bacteria and whey together as a beverage can provide additional nutritional-physiological value, as well as a synergistic effect on human health. Since the literatures lack data on probiotic fermented whey beverage enrichment with *Annona Squamosa L.*, the current study was conducted to produce a nutraceutical probiotic whey beverage enrichment with custard apple pulp juice of (*Annona squamosa L.*), and analyzing the resultant product chemically, microbiologically and organoleptically, during cold storage.

MATERIALS AND METHODS

Materials

Mozzarella cheese whey was acquired from the processing dairy technology unit, Faculty of Agriculture, Cairo University and composed of total solid (6.3%), protein (0.43%), lactose (5.12%), fat (0.1%) and ash (0.5%). Mature Custard apple (*Annona squamosa L.*) fruit, sugar and Carrageenan (stabilizer) were purchased from the local market in Egypt. Commercial probiotic culture *Lactobacillus casei* was obtained from Chr.Hansen laboratories, Hoersholm, Denmark.

Custard apple pulp contained high amounts of nutrients (moisture, sugar, total carbohydrates, crude fiber, crude protein, ash, and acidity) and minimum amounts of fat content as shown in Table (1) . These important constituents of the custard apple pulp may be used to enhance the processing and the nutritional aspects. The fruit showed presence of important minerals like calcium, potassium, sodium, magnesium in a moderate quantity which holds the potential to meet the daily requirement from such type of processed beverages. The other minerals like iron, copper, manganese were present in low quantity but these quantities may be nutritionally superior than wheat flour, rice flour, bean flour and green banana flour. High amount of sugar, vitamin C, calcium and potassium in custard apple based products could be considered a functional source of nutraceutical and flavouring agents, Bala *et al.*, (2018).

Table 1. Nutritional parameters of custard apple pulp of *Annona squamosa L.*

Parameters	Parameters			
	Chemical composition (%)		Minerals	mg/kg mg/100g
Moisture	74.6	Calcium	642.5	64.25
Crude protein	2.8	Potassium	4280	428
Fat	0.39	Sodium	627.5	62.75
Total soluble solids	26.0	Magnesium	545	54.5
Crude fiber	3.30	Manganese	17.5	1.75
Acidity	0.29	Iron	28	2.8
Total sugar	20.90	Copper	27.5	2.75
Ash	1.05	Arsenic	-15.5	-1.55
Fiber	3.1			
Vitamin C (mg/100g)	32.5			

C.F. : Bala *et al.* (2018)

Preparing of custard apple pulp juice (P.J.) .

The fresh fully ripened fruits were soaked in warm water for 15 minutes, cutting manually, scooped with stainless steel spoon for separation of seeds, and blending the pulp in electric blender. To improve the blending process, sterile distilled water was added in a ratio of 1:2 (w/v, pulp/water).

Preparing of probiotic whey-based custard apple beverage (P.C.A.B.).

The experimental plan or formula used for preparing 1Liter of whey- based custard apple beverages from whey and custard apple pulp juice is shown in Table (2). Three formulations were prepared, using the following proportions (whey weight/ pulp juice weight): 75/25, 50/50 and 25/75 , namely B1, B2 and B3 in order . By heating to 60°C, the sugar 10% and 0.1% (w/v) carrageenan powder were dissolved in whey and then filtered through the muslin cloth.

Table 2. Formula applied for preparing P.C.A.B.

Ingredients	Control	B1	B2	B3
		25 %	50%	75%
		P.J.	P.J.	P.J.
Custard apple pulp juice , P.J. (g)	-	250	500	750
Sweet whey (g)	899	649	399	149
Sugar (sucrose) (g)	100	100	100	100
Stabilizer (carragenan) (g)	1	1	1	1
<i>Lactobacillus casei</i> (ml)	1%	1%	1%	1%

The formulations were blending well to ensure perfect homogeneity and analysed through 21 days for physico-chemical , and microbiological properties and within 40 days for sensory evaluation . The flow diagram for the production of P.C.A.B is shown in Fig. (1)

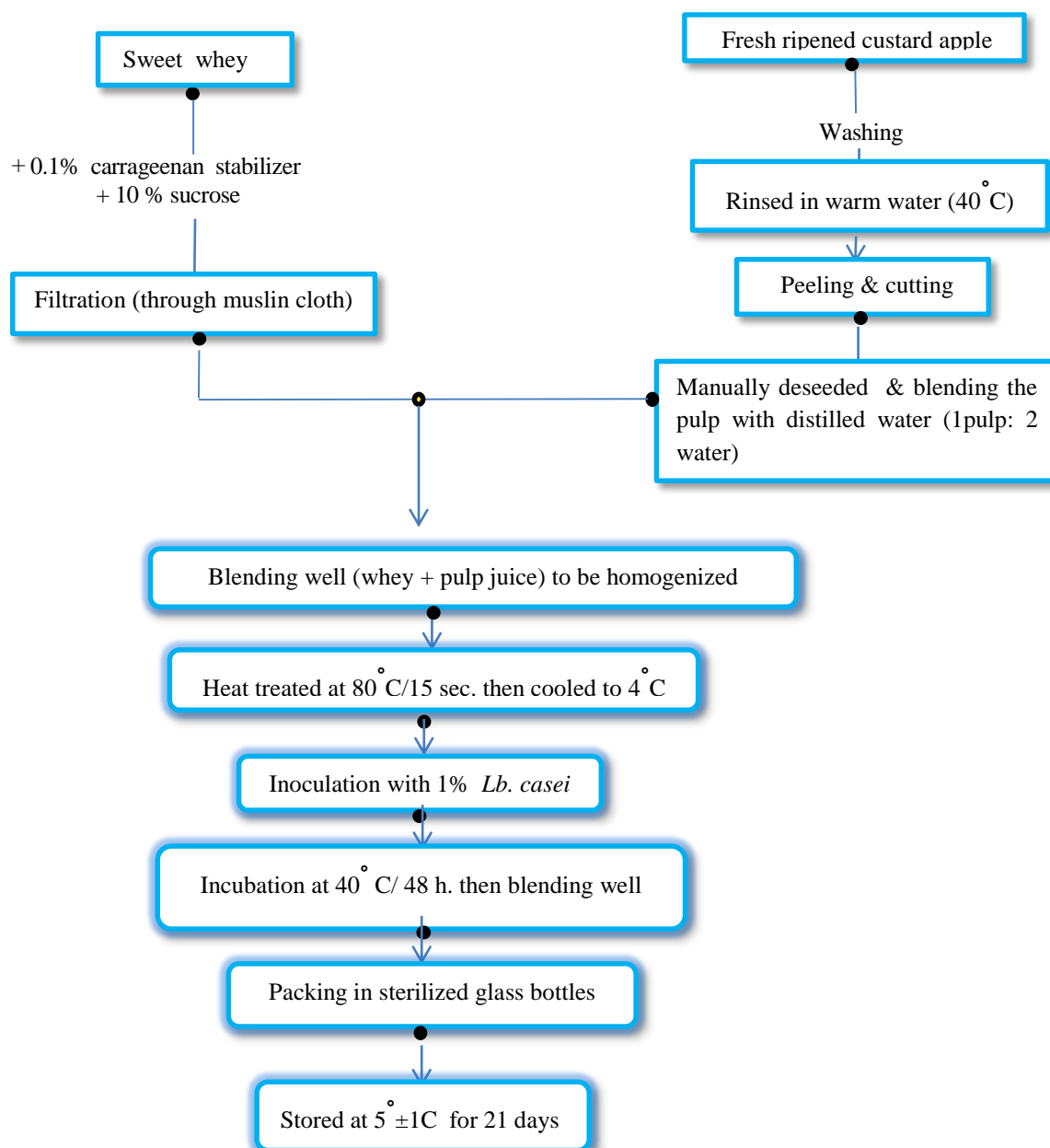


Fig. 1. Flow diagram of preparing probiotic whey-based custard apple beverage.

The method of Panghal *et al.*, (2007) was used for preparing the probiotic whey-based custard apple beverage.

Methods

Physicochemical analysis

Titrate acidity (T.A.), pH value (measured in triplicates using an electric pH meter with combined glass electrode, jesenway 3305, England), total fibers and total solids were determined according to the AOAC official methods (AOAC, 2012). Viscosity was determined using Brookfield viscometer, Brookfield Engineering Labs., USA.

Minerals, vitamins and antioxidant

Minerals of (P.C.A.B.) were determined using inductively coupled plasma atomic absorption spectrometry (ICP-AAS). The content of Ca, K, Mg, Fe and Zn in sample was determined from calibration curve by plotting absorbance peak area against concentrations. The calibration curve is prepared using concentrations of

standard solutions within working range (as described by Amarteifio and Mosase, 2006). vitamin A was determined according to the method of NÖll *et al.*, (1996), and vitamin C was determined by spectrophotometric method (Raghmrammluet *et al.*,1983). Total antioxidant compounds was determined using the phosphomolybdenum method (Aguilar Urbano *et al.*, 2013).

Microbiological Analysis

Counts of *Lactobacillus casei* were determined using MRS agar medium (incubated at 37°C for 48 h), Coliform bacteria (violet red bile glucose agar medium, incubated at 37°C for 24 h), Spore-forming bacteria (plate count agar medium, incubated at 32°C for 48 h) and Mold and Yeasts counts (potato dextrose agar medium, incubated at 25°C for 72 h) as recommended by the American Public Health Association (APHA, 2004).

Sensory Evaluation

Freshly manufactured beverages and stored ones were evaluated , through 40 days of cold storage , for its

sensory attributes by 10 staff members of Dairy Technology Department, Animal Production Research Institutes. The attributes evaluated were appearance, flavour, mouthfeel and aftertaste. Panelists scored their liking for each sample using the nine-point hedonic scale (1- extremely dislike, 2 - dislike very much, 3 - moderately dislike, 4- slightly dislike, 5-dislike, 6-likes lightly, 7-like moderately, 8- like very much and 9- like extremely) according to (Bala et al., 2018) method.

RESULTS AND DISCUSSION

Chemical composition and viscosity :

Results in Table (3) indicated that the total solids (T.S.) and the apparent viscosity were increased in(P.C.A.B.) treatments (B1, B2, and B3) as the level of P.J. increased, whereas the values of titratable acidity (T.A) were decreased, compared to control treatment. Sigdel et al. (2018) confirmed that and attributed the differences in the T.S noticed among the soursop yoghurt-based beverages to the differences in the T.S content of the CAP used in preparing the formulations plan of the experiment. Nazli Turkmen et al., (2019), furthermore attributed the decrease in the apparent viscosity to the variable levels of sweet whey used. This means that as the level of whey increased, the viscosity decreased. The highest values of T.S (24.64%) and viscosity (3420 C.P.) , observed in Table (3) , were found in treatment B3 (25:75 % whey to P.J.) compared to control treatment which recorded 17.1% T.S and 33 C.P viscosity, in order. The high level of T.S present in C.A.P (approximately 25.4%, Bala et al., 2018) and the low T.S. content of sweet whey (6.5%, De Wit, 2001) may be responsible for that.

T.A and pH value are commonly used as quality indicators in dairy products (Virgen-Ceceña et al. 2019). Results, also showed that T.A of P.C.A.B treatments were decreased as the rate of P.J. increased, and this may be due to: 1- The direct relationship between the amount of phytochemicals present in CAP and the levels of P.J. used. 2- The decrease in the lactose contents among the P.C.A.B treatments as a result of using different amounts of sweet whey in the preparation of the formulations plan. pH values of all treatments behaved reverse trend to T.A., through the storage period. Variation in pH values may be due to metabolic activity in the fermentation medium which may lead to breaking down of the fruit sugar to produce organic acid (Stephani et al., 2017). Data in Table (3) indicated, also that treatment B1 recorded the best values of T.S, viscosity, T.A and sensory properties than the other treatments, along the storage period. The percent of P.J. used may be considered the superior or favorable level for the activation and growth of probiotic bacteria. The use above this percent may affect on the former parameters.

As the storage period advanced, the values of T.S and viscosity were decreased, while values of T.A were increased. The main reason for decreasing the viscosity was the decrease of T.S and the increase of T.A, during storage. Senadeera et al., (2018) attributed the increase of T.A in CAB treatments than control treatment to the higher availability of carbohydrate sources from fruit juice to the metabolic activity of the starter, resulting in higher levels of organic acids.

Table 3. Proximate composition and viscosity of P.C.A.B. during 21days of cold storage.

Parameters	Control	B ₁ (25%P.J.)	B ₂ (50%P.J.)	B ₃ (75%P.J.)
			Fresh	
Total solids %.....	17.10	19.21	21.40	24.64
Acidity %	0.42	0.62	0.55	0.50
pH value	4.72	4.50	4.61	4.68
Viscosity (cp)	33	113	373	3420
RPM 50 & Spindle 5			7 days	
Total solids %.....	16.70	18.75	20.80	24.10
Acidity %	0.56	0.82	0.73	0.66
pH value	4.57	4.33	4.50	4.55
Viscosity (cp)	28	72	325	3115
RPM 50 & Spindle 5			14 days	
Total solids %.....	16.20	18.15	20.30	23.46
Acidity %	0.76	1.0	0.89	0.84
pH value	4.46	4.21	4.27	4.33
Viscosity (cp)	24	46	275	2825
RPM			21 days	
50 & Spindle 5	15.88	17.64	19.50	22.91
Total solids %	0.85	1.04	0.98	0.91
Acidity %	4.31	4.16	4.20	4.25
pH value				
Viscosity (cp)	20	40	200	2560
RPM 50 & Spindle 5				

Total antioxidants, vitamins, minerals and fibers in fresh beverages.

In recent years, consumers have searched for healthier and functional foods with natural antioxidants, dietary fiber, minerals, vitamins and metabolites with healthy biological activity; and therefore, the development of new dairy product formulations with the addition of ingredients with functional or nutraceutical properties are being explored. *Annona squamosal L.* is considered a healthy fruit because of its bioactive compounds such as

polyphenols and acetogenins that offer attractive health benefits such as anti-inflammatory, anti-diabetic, and anti-tumoral activities (Coria-Te llez et al., 2018).

It has been demonstrated that antioxidants may act via multiple mechanisms of radical- scavenging activity such as metal scavengers, electron transfer or by donating ion hydrogen (Suntornsuk et al., 2002). Results in Table (4) revealed that fresh beverage of treatment B1(25% P.J.) had higher percent of total antioxidant compounds (190.82 mg/100g, ascorbic acid equivalent) than the control

treatment (174.92 mg/100g). The rate of increase was 9.09 %. The high level of phytochemicals in CAP may be the responsible factor for that. These findings are in agreement with Virgen-Cesena *et al.*, (2019) who mentioned that the addition of custard apple pulp to whey-based beverages is an effective way to improve their functional characteristics, in particular by the presence of phytochemicals such as polyphenols & flavonoids which provide excellent antioxidant activity properties.

Vitamins and minerals playing important or vital role in the physiological processes taking place in the human body. It participate in the synthesis of numerous compounds e.g. hormones, enzymes, hemoglobin, proteins, fats, immune substances etc. and treated much of diseases. Data in Table (4) showed that vitamins A & C were found higher in (P.C.A.B.) treatment (B1) than that in control one. The rate of increase in vitamins A & C were 135.30 and 173.30 %, respectively. The high percent of Vitamin C in C.A.P of *Annona squamosa L* (32.50 % mg/100g, Bala *et al.*, 2018) and the low content of vitamin C in sweet whey (1.50 mg /100g, De Wit, 2001) were the responsible reasons for that.

Data concerning the macro-elements (calcium, Ca, potassium, K and magnesium, Mg) and the micro-elements (iron & zinc) present in Table (4) showed higher levels of these minerals in fresh PC.A.B (treatment B1) than in control one, because C.A.P contained appreciable amounts of these elements than sweet whey. The rate of increase in Ca, K and Mg, iron and zinc were 0.52, 9.31, 29.27, 10.38 and 26.67%, respectively. It was noticed that the percent of these elements were increased below 11%, with the exception of Mg & zinc which increased above that level. Ca content was found approximately equal in treatment B1& control. Jonas *et al.*, (2019) found same results of these elements in soursop whey-based beverage. The values of iron in our study were less than the level of 4.81mg /100g of custard apple juice reported by Bhardwaj *et al.*, (2014), and the contribution of iron by this fruits in the diet is poor. However, this fruit and its products contain less iron but it could be superior than other foods such as milk (0.6 mg/100g), wheat flour (0.8 mg /100g) oat meal (2.09 mg/100g) and corn flour (1.1 mg/100g) (Pedron *et al.*, 2016).

Treatment B1 had 0.82 % dietary fiber while control one was free from it. The rate of increase was found 100%. Erkaya *et al.*, (2012) stated that the presence of dietary fiber in the prepared dairy foods enhanced their nutritional value.

Table 4. Total antioxidants, vitamins, minerals, and fibers of fresh beverages.

Parameters	Control	B1 (25% P.J.)	% of increase
Antioxidants (mg/100g)	174.92	190.82	9.09
Vitamins			
A (mg/100g)	0.85 ± 0.03	2.0±0.09	135.29
C (mg/100g)	1.50	4.10	173.30
Minerals (mg/100g)			
Calcium	51.47	51.74	0.52
Potassium	145	158.5	9.31
Magnesium	9.67	12.50	29.27
Iron	0.71	0.82	10.38
Zinc	1.05	1.33	26.67
Fiber (%)	-	0.82	100

Microbiological evaluation :

Table (5) shows the microbiological evaluation of all beverages, during 21days of storage. Concerning the counts of lactobacilli group, it was found that these counts were gradually decreased as the storage period increased and decreased, also as the level of P.J. added increased. The main reasons for that were the continuous increase of acidity as well as the highest total solids in P.C.A.B treatments. This trend of continuous decrease in the lactobacilli counts during storage was noticed previously by Senadeera *et al.*, (2018) in probiotic yoghurt incorporated with pulps of various *Annona* species. Results in Table (5), also showed that counts of lactobacilli in fresh beverage of B1 treatment (contained 25% P.J.) was found the highest one being 131.5×10^6 cfu /1ml compared with control and the other treatments B2 (50% P.J.) and B3 (75% P.J.), along the storage period. These count of B1 treatment was decreased to 50.50×10^6 cfu/ml after 21 days of storage. The corresponding counts of control treatment were 25.5 and 8.40×10^6 cfu/ml, in order. Lactobacilli counts in B3 treatment was found lower than that of the control treatment, during storage period. Results show, furthermore that the probiotic strain of *Lb. casei* was still viable in all samples during 3 weeks of storage. The viability ($\times 10^6$ cfu/ml) ranged from 131.5 - 50.50 for B1, 51.5 - 18.0 for B2 and 5.5 - 1.10 for B3, respectively. The viable cell counts of *Lb. casei* in the P.C.A.B treatments were higher than 10^6 cfu/ml after 3 weeks of cold storage and this fact was confirmed by (Akpeji *et al.*, 2017). Shah (2001) added that it is important to have significant number of viable lactic acid bacteria (L.A.B) in the product for maximum health benefits. He added, also that the viability of probiotic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature. Zoeliner *et al.*, (2009), moreover, found that whey and fruit beverages can be used as a carrier for probiotic bacteria as well as increasing the viability of probiotic bacteria .

Coliform group and spore-forming bacteria were absent in all treatments during storage. Control treatment was slightly altered , at the day 21 of storage as a result of growing the yeast & molds , which recorded counts reached to 30×10^2 cfu/ ml.

Table 5. Changes in the viable counts of beverages, during 21 days of cold storage.

Microorganism Groups	Storage period (Days)	Control	B1	B2	B3
	Fresh	25.5	131.5	51.5	5.5
Lactobacilli ($\times 10^6$ cfu/ml)	7	17.25	100.0	25.0	3.20
	14	12.10	68.20	22.5	2.50
	21	8.40	50.50	18.0	1.40
Yeast & Mold ($\times 10^2$ cfu/ml)	21	30	ND	ND	ND
Coliform	ND	ND	ND	ND	ND
Spore-forming bacteria	ND	ND	ND	ND	ND

ND: not detect

Organoleptic properties

As a general, the use of beverages with fruits can improve the sensory properties and gave more refreshing taste to the products. Scoring process, in our study, revealed that P.C.A.B (25% P.J.) or B1 treatment was found the best one compared to the control and the other

treatments B2 & B3, along the storage period. Treatment B3 (75% P.J.) was less acceptable by the judging panel owing to its viscous or gel-like body & the high intensity of flavor. B2 treatment (50% P.J.) was superior than control and B3 treatment. It was noticed that as the level of (P.J.) increased, the intensity of the sensory properties was increased, but to a limited extent. Scoring evaluation, moreover, was conducted to the day 40 and treatments of B1 & B2 still, as a general, kept its overall-acceptability and showed slightly less sensory differences along the storage period (40 days), while control treatment was less acceptable at the day 21 of storage, owing to the growth of yeast & molds. No sedimentation was found in the bottled of the P.C.A.B treatments, during the storage period. Caplice and Fitzgerald (1999), said that the preservatives action of L.A.B, antimicrobial substances and metabolites produced during fermentation process playing important role in the quality of the resultant product and acquired it hygienic safety, storage stability and attractive sensory properties.

CONCLUSION

Results indicated that the use of custard apple fruit increased both the antioxidant compounds and the nutritional value of the probiotic whey-based custard apple beverage due to the inclusion of phytochemicals such as polyphenols & flavonoids, as well as numerous nutrients such as crude proteins, total soluble solids, vitamins, minerals and fibers. In addition, the use of *Annona squamosa* L (custard apple or sugar apple) resulted in increasing the counts of the viable probiotic bacteria (*Lactobacillus casei*) in the product. This fruit may be considered as a prebiotic ingredient and it has the potential to stimulate the former probiotic bacteria. Moreover, the addition of *Lactobacillus casei* as a probiotic bacteria inhibited the growth of spoilage microorganisms and extending the beverage shelf life. The probiotic strain viable counts of all treatments were found adequate during the storage period and exhibit the beverage clean acid flavor, good sensory properties and overall acceptability by consumers. Finally, it could be considered that beverage as a functional source of a probiotic, phytochemicals, nutrients and flavouring agents.

REFERENCES

Aguilar Urbano, M., Pineda Priego, M. and Prieto, P. (2013). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry* 269 (2): 337–41.

Akpeji, S.C., Adebayo-Tayo, B.C., Sanusi, J.F. and Alao, S.O. (2017). Production and properties of probiotic soursop juice using *Pediococcus pentosaceus* Lbf2 as starter. *International Journal of Biochemistry Research & Review*, 17(2), pp.1-10.

Amarteifio, J.O. and Mosase, M.O. (2006). The chemical composition of selected indigenous fruits of Botswana. *Journal of Applied Sciences and Environmental Management*, 10 (2).

American Public Health Association, APHA. (2004). *Standard Methods for the Examination of Dairy Products*. 16th ed. Washington, DC: APHA Inc. USA

Association of official Analytical Chemists, AOAC (2012). *Official Methods of Analysis*, Association of Official Analytical Chemists, 19th ed.; AOAC: Washington, DC, USA.

Babu, C., Devi, P.R. and Kombiah, P. (2014). Screening of biological actives extracts from *Annona squamosa*. *Int J Biol Res*, 2(1), pp.18-20.

Bala, S., Nigam, V.K., Singh, S.S., Kumar, A. and Kumar, S. (2018). Evaluation of Nutraceutical Applications of *Annona squamosa* L. based Food Products. *J Pharmacogn Phytochem*, 7, pp.827-831.

Bhardwaj, A., Satpathy, G., Gupta RK. (2014). Preliminary screening of nutraceutical potential of *Annona squamosa*, an underutilized exotic fruit of India and its use as a valuable source in functional foods. *Journal of Pharmacognosy and Phytochemistry* ,3 (2):172-180.

Caplice, E. and Fitzgerald, GF., (1999). Food fermentation: Role of food microorganisms in food production and preservation. *Int. J. Food Microbiol.*, 50:113-149.

Chavan, R.S., Shradha, R.C., Kumar, A. and Nalawade, T., (2015). Whey- Based beverage: its functionality, formulations, health benefits and applications. *Journal of Food Processing & Technology* , 6 (10) , p.1.

Coria-Teñe AV, Montalvo-González E, Yahia EM, Obledo-Vázquez EN. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arab. J. Chem.* 11: 662–691 (2018) Dias DDRCC, Barros ZMP, Carvalho CBO, De Honorato FA, Guerra .

De Wit J.N. (2001). *Lecture's Handbook on Whey and Whey Products*. 1st ed. Published by European Whey Products Association, 14, Rue Montoyer 1000 Brussels, Belgium.

El-Aidie, S.A.A., El-Dieb, S.M., El-Nawawy, M., Emara, E. and Sobhy, H., (2017). Nutraceutical Food Based on Cereal and Probiotic Fermented Milk. *International Journal of Dairy Science*, 12, pp.377-384.

Erkaya, T., Dağdemir, E. and Şengül, M. (2012). Influence of Cape gooseberry (*Physalis peruviana* L.) addition on the chemical and sensory characteristics and mineral concentrations of ice cream. *Food Research International*, 45(1), pp.331-335.

Guimarães, J.T., Silva, E.K., Ranadheera, C.S., Moraes, J., Raices, R.S., Silva, M.C., Ferreira, M.S., Freitas, M.Q., Meireles, M.A.A. and Cruz, A.G. (2019). Effect of high-intensity ultrasound on the nutritional profile and volatile compounds of a prebiotic soursop whey beverage. *Ultrasonics sonochemistry* , 55, pp.157-164.

Harrigan, W.F.; MacCance, M.E. (1976). *Laboratory Methods in Food and Dairy Microbiology*, 1st ed.; Academic Press: London, UK.; pp. 25–29.

Iqbal, M.W., Mu, W., Khan, I.M. and Mohsin, A. (2017). Development of Probiotic Soft Cheese with *Lactobacillus casei* as Adjunct Culture. *Journal of Academia and Industrial Research (JAIR)*, 6(1), p.1.

Legarová, V. and Kouřimská, L. (2010). Sensory quality evaluation of whey-based beverages. *Mljekarstvo* , 60 (4), p.280.

Lehn, D.N., Esquerdo, V.M., Júnior, M.A.D., Dall'Agnol, W., dos Santos, A.C.F., de Souza, C.F.V. and de Almeida Pinto, L.A. (2018). Microencapsulation of different oils rich in unsaturated fatty acids using dairy industry waste. *Journal of Cleaner Production*, 196, pp.665-673.

- Magalhães, K.T., Dragone, G., de Melo Pereira, G.V., Oliveira, J.M., Domingues, L., Teixeira, J.A., e Silva, J.B.A. and Schwan, R.F., (2011). Comparative study of the biochemical changes and volatile compound formations during the production of novel whey-based kefir beverages and traditional milk kefir. *Food Chemistry*, 126(1), pp.249-253.
- Mudgil, D. and Barak, S. (2019). Dairy-Based Functional Beverages. In *Milk-Based Beverages* (pp. 67-93). Woodhead Publishing.
- Nazli Turkmen Nazli Turkmen, Ceren Akal, Barbaros Özer (2019). Probiotic dairy-based beverages: A review. *Journal of Functional Foods*, 53 : 62-75 .
- NÖll, G. N. (1996). High performance liquid chromatographic analysis of retinal and retinoid isomers. *Journal of Chromatography.A*, 247-259.
- Nursiwi, A., Nurhartadi, E., Utami, R., Sari, A.M., Laksono, P.W. and Aprilia, E.N. (2017). Characteristic of Fermented Whey Beverage with Addition of Tomato Juice (*Lycopersicon esculentum*). In *IOP Conference Series: Materials Science and Engineering* (Vol. 193, p. 012009).
- Olagunju, A.I. and Sandewa, O.E. (2018). Comparative physicochemical properties and antioxidant activity of dietary soursop milkshake. *Beverages*, 4(2), p.38.
- Panghal, A., Sharma, K., Setia, S. and Ray, A. (2007). Studies on Utilization of whey for preparation of ready to serve (RTS) Beverages. *Beverage Food World*, 34(8), pp.77-79
- Pedron, T., Segura, F.R., da Silva, F.F., de Souza, A.L., Maltez, H.F. and Batista, B.L. (2016). Essential and non-essential elements in Brazilian infant food and other rice-based products frequently consumed by children and celiac population. *Journal of Food Composition and Analysis*, 49, pp.78-86.
- Pescuma, M., Hébert, E.M., Mozzi, F. and De Valdez, G.F. (2010). Functional fermented whey-based beverage using lactic acid bacteria. *International Journal of Food Microbiology*, 141(1-2), pp.73-81.
- Raghuramulu, N., K, Madhavan Nair and Kalyanasundaran. (1983). A manual of laboratory techniques. National Institute of Nutrition, ICMR, Jamai-Osmania, Hyderabad, pp. 124.
- Reid, G. (2008). Probiotics and prebiotics—progress and challenges. *International Dairy Journal*, 18(10-11), pp.969-975.
- Sakhale, B.K., Pawar, V.N. and Ranveer, R.C. (2012). Studies on the development and storage of whey based RTS beverage from mango cv. Kesar. *Journal of Food Processing and Technology*, 3(3), pp.1-4.
- Senadeera, S.S., Prasanna, P.H.P., Jayawardana, N.W.I.A., Gunasekara, D.C.S., Senadeera, P. and Chandrasekara, A., (2018). Antioxidant, physicochemical, microbiological, and sensory properties of probiotic yoghurt incorporated with various *Annona* species pulp. *Heliyon*, 4(11), p.e00955.
- Shah, N.P. (2001). Functional foods from probiotics and prebiotics: Functional Foods from Probiotics and Prebiotics. *Food Technology* (Chicago), 55(11), pp.46-53.
- Sigdel, A., Ojha, P. and Karki, T.B. (2018). Phytochemicals and syneresis of osmo-dried mulberry incorporated yoghurt. *Food Science & Nutrition*, 6(4), pp.1045-1052.
- Srivastava, P., David, J., Rajput, H., Laishram, S. and Chandra, R. (2017). Nutritional Information of Custard Apple and Strawberry Fruit Pulp. *Chem. Sci Rev Lett*, 6(24), pp.2337-2341.
- Suntornasuk, L., Gritsanapun, W., Nilkamhank, S. and Paochom, A. (2002). Quantitation of vitamin C content in herbal juice using direct titration. *Journal of Pharmaceutical and Biomedical Analysis*, 28(5), pp.849-855.
- Turkmen, N., Akal, C. and Özer, B. (2019). Probiotic dairy-based beverages: A review. *Journal of Functional Foods*, 53, pp.62-75.
- Vasiljevic, T. and Shah, N.P. (2008). Probiotics—from Metchnikoff to bioactives. *International Dairy Journal*, 18(7), pp.714-728.
- Virgen-Ceceña, L.J., Anaya-Esparza, L.M., Coria-Téllez, A.V., de Lourdes García-Magaña, M., García-Galindo, H.S., Yahia, E. and Montalvo-González, E., (2019). Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen dessert. *Food Science and Biotechnology*, 28(5), pp.1337-1347.
- Zoellner, S.S., Cruz, A.G., Faria, J.A.F., Bolini, H.M.A., Moura, M.R.L., Carvalho, L.M.J. and Sant'ana, A.S. (2009). Whey beverage with acai pulp as a food carrier of probiotic bacteria. *Australian Journal of Dairy Technology*, 64(2), p.177.

إنتاج مشروب شرش حيوي مغذي مدعم بفاكهة (ثمرة القشدة) *Annona squamosal L*

صفاء عبد العليم محمد العايدى ، حاتم حلمي عمر عرفات ، أمال محمد عويس و أمل مجاهد محمد النمر

مركز البحوث الزراعية - معهد بحوث الانتاج الحيواني -- قسم تكنولوجيا الالبان

تم تحضير مشروب من الشرش الحلو المدعم بعصير ثمار فاكهة *Annona squamosal L* وذلك بأخذ لب الثمرة وتخفيفه بالماء المقطر المعقم بنسبة 2 ماء : 1 فاكهة وضربه في الخلاط جيدا وإضافة إلى الشرش بالنسب التالية (عصير : شرش) 75:25 & 50:50 & 25:75 (وزن:وزن). تم إضافة السكر بنسبة 10% ومثبت القوام Carrageenan بنسبة 0.1% ومعاملة المشروبات الناتجة حراريا على 80 م° / 15 ث ثم التبريد إلى 40 م° وإضافة البادئ الحيوي *Lb. casei* بنسبة 1 % والتخصين لمدة 48 ساعة لتنشيط وزيادة أعداد البادئ. تم حفظ المشروبات الناتجة في التلاجة (5 ± 1 م°) وتقييمها حسيا خلال 40 يوم بالإضافة إلى تحليلها فيزيوكيميائيا ؛ ميكروبيولوجيا؛ وهي طازجة وخلال 21 يوم من التخزين وكانت أهم النتائج ملى : * إزدادت الجوامد الصلبة الكلية واللزوجة في المعاملات المحتوية على عصير الفاكهة بينما إنخفضت الحموضة بزيادة نسبة العصير وذلك طوال مدة التخزين مقارنة بعينة المقارنة. * إتخذت قيم الـ pH إتجاها معاكسا للحموضة في جميع المعاملات. * إزدادت نسبة المواد المضادة للأكسدة & فيتامين A و فيتامين C والمعادن الكبرى (الكالسيوم؛ البوتاسيوم؛ المغنسيوم) والمعادن الصغرى (الحديد؛ الزنك) في المشروب الطازج المدعم بـ 25 % من عصير الفاكهة مقارنة بمشروب المقارنة وبلغت النسبة المنوية للزيادة (%) 9.09 ؛ 135.30 ؛ 173.30 في المواد المضادة للأكسدة؛ فيتامين A و فيتامين C على التوالي. * إنخفضت أعداد البكتريا الحيوية بتقدم مدة التخزين و بزيادة نسبة العصير المضافة وقد بلغت أعدادها في نهاية مدة التخزين أكثر من 10⁶ / مل . * لم تظهر بكتريا القولون أو البكتريا المكونة للجراثيم في جميع المعاملات. * كان المشروب المحتوى على 25% عصير الفاكهة الأفضل حسيا وتميز بنكهة وطعم حمضى نظيف ومدة حفظ طويلة (أكثر من 30 يوم) في حين كان مشروب عينة المقارنة أقل قبولا عند اليوم الـ 21 من التخزين بسبب نمو الخمائر.