RESEARCH ARTICLE

Physico-chemical and microbiological quality changes in cocoa and whey protein enriched functional dairy drink during storage

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Abstract: Cocoa and whey protein enriched functional dairy drink was subjected to storage studies at refrigeration temperature 4±1° C in the present study. The samples were studied for microbial and physicochemical tests during storage. The results in this study suggest that the product can be stored upto 18 days at 4±1° C. A decrease in pH from 7.01 to 6.49 for whey protein enriched product and from 6.94 to 6.41 in case of cocoa & whey protein enriched product was observed at the end of shelf life. The FFA increased from 0.72 to 1.46 meq./ml at the end of shelf life of 18 days in case of whey protein enriched product and from 1.16 to 2.02 meq./ml in case of cocoa & whey protein enriched product. An increase in the HMF content of the functional drink was recorded from 59.84 to 134.56 µ mol/l at the end of 18 days of storage in case of whey protein enriched product and from 1.16 to 2.02 µ mol/l in case of cocoa & whey protein enriched product. The total bacterial counts and yeast and mould counts were increased from 3.14 to 6.48 log cfu/ml and 1.14 to 2.10 log cfu/ml respectively in whey protein enriched functional drink. The coliform count was observed to be nil in both the variants of the functional dairy drink of throughout the storage period.

Keywords: Functional dairy drink, physico-chemical and microbiological characteristics, Cocoa and WPC

Introduction

Milk is an important component of diet which provides various vital nutrients to the consumers. Milk is a rich source of fat, protein, lactose and essential minerals specially the calcium. It has also got micronutrients and functional compounds e.g. vitamins, amino acids, phospholipids, etc. (Bhat and Bhat, 2011; Pouliot and Gauthier, 2006).

A functional beverage is defined as product that is non-alcoholic, ready to drink and includes in its formulation non-traditional ingredients such as herbs, vitamins, minerals, amino acids or additional raw fruit or vegetable ingredients, so as to provide specific health benefits that go beyond general nutrition. Functional beverage is the fastest growing sector. Development of a ready to drink milk beverage with whey protein concentrate (WPC), cocoa powder and other ingredients in order to produce a stable product of high protein and antioxidant potential drink with appealing, refreshing taste could be the ideal mode to introduce functional drink beverage to suit Indian palate (Jayaprakasha and Brueckener, 1999; Korhonen and Pihlanto, 2006; Oliveira et al., 2016).

Cocoa solids in chocolate relish our taste buds. Incorporation of these solids to the beverage improves the acceptability of the product. In addition, cocoa solids also enhance functionality of the beverage with its inherent properties of flavonoids and polyphenolics, which exhibits antioxidant and anti-inflammatory activities in the human biological system. Combining the whey protein and cocoa solids and stabilizing the product will be the greater challenge to the food industry (Ludvigsen, 2010; Oliveira et al., 2015).

Realizing the need for healthy nutrition for Indian population, a WPC [illegible] was developed. The current [illegible] changes occurring in physicochemical and microbiological quality during storage of the functional dairy drink.

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Materials and Methods

Raw materials

Buffalo milk was procured from Experimental Dairy of N.D.R.I., Karnal for the preparation of functional dairy beverage. The milk was adjusted to 1.5, 3.0 and 4.5% fat level using skim milk.

Standardised milk samples were then pasteurized and kept under refrigerated conditions. WPC 70 was procured from Modern Dairies Limited, Karnal and was used for optimizing functional dairy drink. Carrageenan was sourced from Sigma Aldrich Co., and was predominately containing k-Carrageenan.

Preparation of functional dairy drink:

The dairy drink was prepared as per the method described by Gupta et al. (2017). The drink was prepared using varying levels of WPC (0.5-5.0%), Sugar (6-8%), Carrageenan (0.2-1.0%) and Milk fat (1.5-4.5%). The experiment comprised of 30 trials. Regression analysis and analysis of variance (ANOVA) was conducted for fitting the models and to examine the statistical significance of the model. The adequacy of the models were determined using model analysis, lack-of fit test and R^2 (coefficient of determination) analysis as outlined by Lee *et al* (2003).

Determination of pH

The pH of milk was determined electrometrically with the pH meter (Phan Labindia Labtek Engg. Pvt. Ltd., India) by method described in IS: SP (Part XI, 1981). About 50 ml of sample at 20°C was taken for pH determination. The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and 7.0 at 20±0.1°C.

FFA and HMF

The FFA content was estimated using the method suggested by Deeth, *et al,* (1975) while the total HMF content was determined following the method prescribed by Keeney *et al,* (1959).

Microbiological analysis

The functional drink was analysed for standard plate counts (SPC), coliform counts, and yeast and mould counts using various agar medium (Hi-media Laboratories Pvt. Ltd. Mumbai).

Standard plate count

The total numbers of viable bacteria in the protein enriched milks were enumerated by the method described by Houghtby *et al.* (1992) using Plate Count Agar (pH-7.0±0.1) as nutrient medium. The prepared plates were incubated at 37ÚC for 48 hours. After the period of incubation the plates were observed for appearance of colonies and were counted. The results were expressed as log cfu per g of sample as follows:

log cfu per g = log (Average no. of colonies x dilution factor)

Total coliform counts

Total coliform counts in protein enriched functional dairy drink were determined as per standard procedure described in IS: 5401

Table 1: Changes in physico-chemical and microbiological attributes of whey protein and cocoa & whey protein enriched functional dairy drink packed in LDPE pouch and stored at 4±1

Physical Attributes	pH		FFA (meq./ml)		HMF (µ mol/ lt)		SPC (log cfu/ml)		Y & M (log cfu/ml)	
StorageDays	WPC	Cocoa	WPC	Cocoa	WPC	Cocoa	WPC	Cocoa	WPC	Cocoa
0	7.01 ± 0.41	6.94 ± 0.58	0.72 ± 0.25	1.16 ± 0.12	59.84 ± 0.22	62.79 ± 0.41	3.14± 0.52	3.82± 0.45	1.14± 0.02	1.16 ± 0.07
3	6.95 ± 0.38	6.89 ± 0.45	0.74 ± 0.12	1.20 ± 0.18	70.02 ± 0.32	70.38 ± 0.35	3.90± 0.35	4.12± 0.21	1.20± 0.10	1.21± 0.12
6	6.88 ± 0.29	6.82 ± 0.56	0.80 ± 0.15	1.30 ± 0.14	76.95 ± 0.41	78.20 ± 0.52	4.92± 0.17	5.23± 0.24	1.39± 0.17	1.39± 0.24
9	6.80 ± 0.32	6.77 ± 0.25	0.82 ± 0.08	1.36 ± 0.15	83.95 ± 0.36	85.79 ± 0.61	5.26± 0.21	5.61± 0.39	1.56± 0.14	1.49 ± 0.15
12	6.70 ± 0.41	6.67 ± 0.54	1.06 ± 0.07	1.54 ± 0.09	90.47 ± 0.32	93.98 ± 0.45	5.75± 0.23	6.10± 0.08	1.20± 0.30	1.98± 0.25
15	6.59 ± 0.36	6.58 ± 0.45	1.22 ± 0.08	1.78 ± 0.11	108.53 ± 0.51	113.03 ± 0.43	6.12± 0.20	6.30± 0.32	1.80± 0.36	1.92± 0.32
18	6.49 ± 0.25	6.41 ± 0.36	1.46 ± 0.11	2.02 ± 0.14	134.45 ± 0.52	138.68 ± 0.68	6.48± 0.24	6.72± 0.08	2.10± 0.24	2.25± 0.12

(1969) using violet red bile agar (Sisco Research Laboratories Pvt. Limited, Mumbai). The plates were incubated at 37± 0.5 or at 30±0.5ÚC for 18 to 24 hours followed by enumeration.

Yeast and Mould counts

Yeast and mould counts in whey protein enriched functional drink were determined as per standard procedure described in IS: 5401 (1969) using potato dextrose agar (Sisco Research Laboratories Pvt. Limited, Mumbai). The plates were incubated at 37± 0.5 or at 30±0.5ÚC for 18 to 24 hours followed by enumeration.

Results and Discussion

Changes in physico-chemical attributes of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1ºC

pH

Both treatment and storage period were found to affect significantly the pH of the product during storage. Average pH value was found higher and statistically different in whey protein enriched functional dairy drink (6.77) than cocoa & whey protein enriched functional dairy drink (6.72). It was observed that a highly significant ($p<0.01$) decrease (Table 1) in pH value was noticed during 18 days storage period from 6.97 to 6.45. The pH of functional dairy drink as affected by storage period has been presented in Fig. 1 A. The pH decreased from 7.00 to 6.49 at the end of 18 days of storage in case of WPC enriched product and from 6.93 to 6.41 in case of cocoa & whey protein enriched product. However the interaction between treatment and storage period was found to be non- significant (Table 2). Edmund *et al*, (1999) reported similar results of decreased pH values during storage of UHT treated milk. The decrease in pH was attributed to proteolytic activity of enzymes and microbial activity. A definite effect of storage time on pH has been reported in the past for the similar products. Richards, Buys, and De Kock (2016) suggested that the pH decrease can be linked to age gelation of the milk because of destabilization of the casein micelles in milk below pH 6.7.

Free Fatty Acids (FFA)

Both treatment and storage period were found to affect significantly the FFA content of the product. Average FFA value was found higher and statistically different in cocoa & whey protein enriched functional dairy drink (1.48) than whey protein enriched functional dairy drink (0.97). It is observed that FFA content of functional dairy drink was significantly ($p<0.01$) affected by the storage period from 0.94 (at 0 day) to 1.74 (at 18 day) and is depicted in Fig. 1 B and Table 1. However, the interaction between treatment and storage period was found to be non-significant but FFA increased from 0.72 to 1.46 meq./ml at the end of storage period of 18 days in case of WPC enriched product and from 1.16 to 2.02 meq./ml in case of cocoa & whey protein enriched product (Table 3). The results are in agreement with the research finding of Pradyuman *et al*. (2004). Increased FFA content is recorded during storage of mango soy fortified yoghurt powder which was attributed to hydrolytic reactions occurring during storage that can cause fat cleavage resulting in liberation of free fatty acids.

Hydroxy Methyl Furfural (HMF)

HMF is considered as an index of browning in milk and milk products. It was observed that the HMF content of the product increased significantly ($p<0.01$) during storage (Table 4) from 61.31 µ mol/ l. to 136.57 µ mol/ l. (Fig. 1 C.) However, HMF contents was found significantly higher in cocoa & whey protein enriched functional drink (91.83 µ mol/ l.) than WPC enriched functional drink (89.17 µ mol/ l.). The interaction between treatment and days was found to be non –significant in case of HMF. However, HMF content of the product increased from 59.84 to 134.46 µ mol/ l. at the end of storage period of 18 days in case of WPC enriched product and from 62.79 to 138.68 µ mol/ l. in case of cocoa & whey protein enriched product (Table 4). Alok *et al,* (2014) also reported increase in HMF content during storage of *lal peda*. Increase in HMF content can be linked with browning reaction occurring continuously even during storage. In the presence of oxygen, during storage -SH groups are converted into S-S group leading to increase in HMF value. Similar results were reported by Richards, Buys, and De Kock (2016), wherein increase of both total and free HMF content was reported during storage of UHT milk. It was concluded that a drop in the pH is concomitant to increase in browning reactions that take place during storage of the milk.

Effect of storage on microbiological attributes of whey protein and cocoa & whey protein enriched functional dairy drink

Standard Plate Counts (SPC)

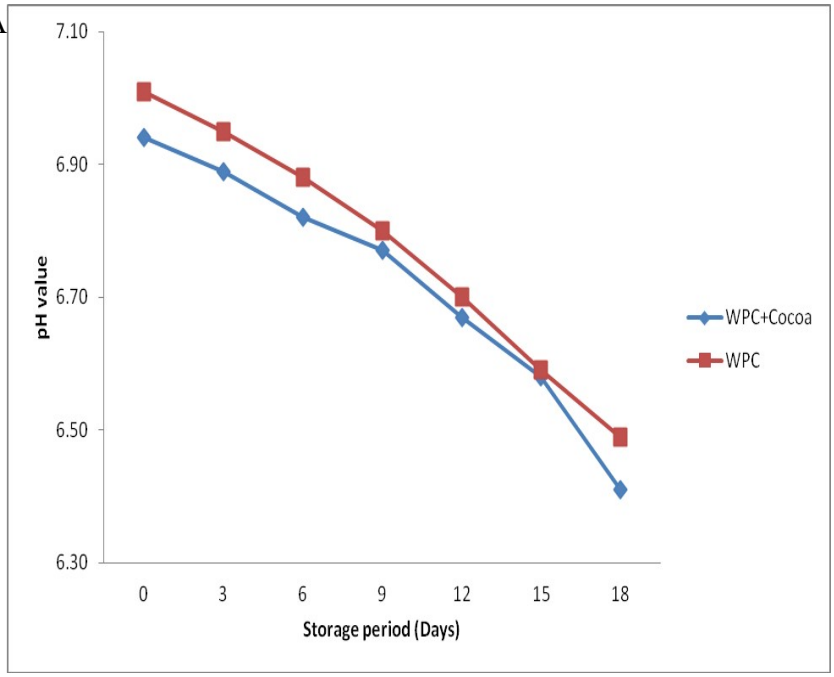
It was observed that a significant ($p<0.01$) increase (Table 5) in the total bacterial counts of functional dairy drink of both the variants during the storage. There was a sharp increase in total bacterial count from an initial count of 3.14 to 6.48 log cfu/ml at the end of shelf life of 18 days in case of WPC enriched functional drink and in case of cocoa enriched product the counts were increased from 3.82 to 6.72 log cfu/ml (Fig. 2 A). These results are in agreement with Nagarajappa and Battula (2017). They also reported in increase in microbial load of fortified milk during the storage.

Yeast and Mold Counts

It was observed that an increase in the yeast and mold counts of functional dairy drink of both the variants during the storage (Table 5). There was a sharp increase in yeast and Mold count

Fig. 1: Changes in physico-chemical attributes of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1°C. (A, B, & C)

A



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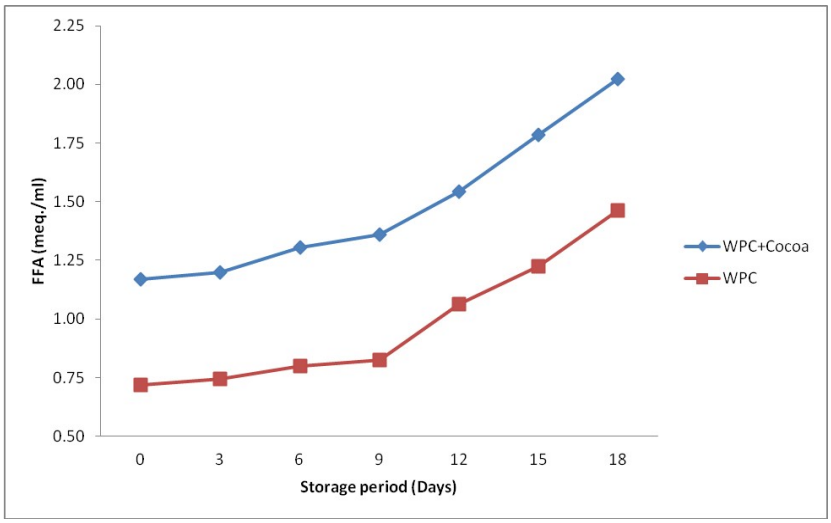

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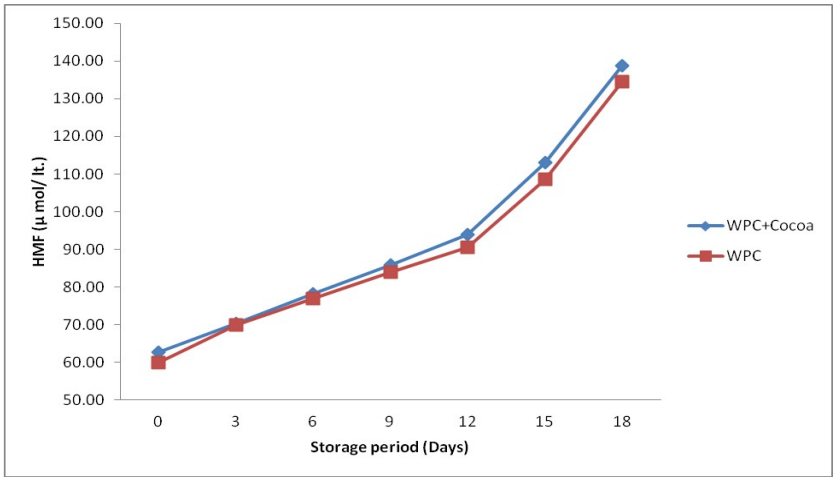

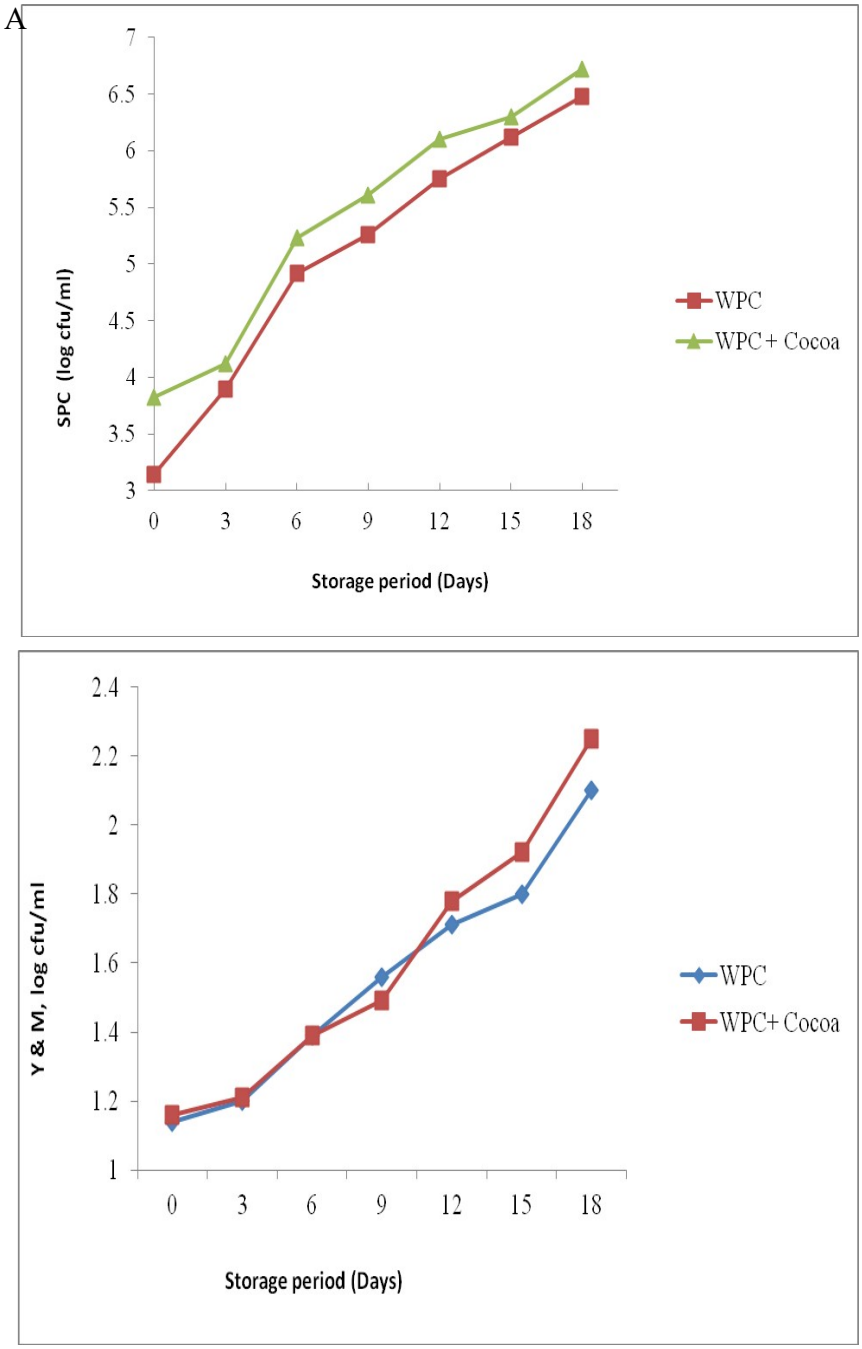

Fig. 2: Changes in microbiological quality of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1°C. (A & B)

Table 2: Average pH values of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1ºC

Treatment	Days							Overall Mean
	0	3	6	9	12	15	18	
WPC	7.0067	6.9533	6.8767	6.8000	6.7000	6.5933	6.4900	6.7743
Cocoa+WPC	6.9367	6.8900	6.8200	6.7667	6.6733	6.5833	6.4133	6.7262
Overall Mean	6.9717	6.9217	6.8483	6.7833	6.6867	6.5883	6.4517	

C.D. (Treatment) = 0.0179
C.D. (Days) = 0.0336
C.D. (Treatment x days) = NS

Table 3: Average Free Fatty Acids (FFA) values of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1ºC

Treatment	Days							Overall Mean
	0	3	6	9	12	15	18	
WPC	0.7200	0.7467	0.8000	0.8267	1.0667	1.2267	1.4667	0.9790
Cocoa+ WPC	1.1733	1.2000	1.3067	1.3867	1.5467	1.7867	2.0267	1.4895
Overall Mean	0.9467	0.9733	1.0533	1.1067	1.3067	1.5067		1.7467

C.D. (Treatment)=0.0893

C.D. (Days) =0.1671

C.D. (Treatment x days)=NS

Table 4: Average Hydroxy Methyl Furfural (HMF) values of whey protein and cocoa & whey protein enriched functional dairy drink during storage at 4±1ºC

Treatment	Days							Overall Mean
	0	3	6	9	12	15	18	
WPC	59.8453	70.0240	76.9587	83.9510	90.4697	108.5357	134.4583	89.1775
Cocoa+ WPC	62.7913	70.3807	78.2083	85.7977	93.9820	113.0297	138.6843	91.8391
Overall Mean	61.3183	70.2023	77.5835	84.8743	92.2258	110.7827		136.5713

C.D. (Treatment) =0.7828

C.D. (Days)=1.4646

C.D. (Treatment x days)=NS

Table 5: ANOVA of changes in physico-chemical and microbiological attributes of whey protein and cocoa enriched functional dairy drinks packed in LDPE pouch and stored at 4±1°C

Source	Dependent Variable	Type III sum of squares	df	Mean Square	F-value
	pH	0.02	1	0.02	30.09**
	FFA	2.74	1	2.74	137.07**
Treatment	HMF	74.38	1	74.38	48.48**
	SPC	1.320	1	1.320	128.07*
	Y & M	0.48	1	0.48	2.58
	pH	1.25	6	0.21	258.36**
	FFA	3.25	6	0.54	27.14**
Days	HMF	23993.89	6	3998.98	2606.49**
	SPC	9.245	6	1.540	58.05*
	Y & M	2.65	6	0.44	1.065
Treatmentx Days	pH	0.01	6	0.006	1.16
	FFA	0.02	6	0.006	0.19
	HMF	21.87	6	3.65	2.38
	SPC	0.512	6	0.085	0.32*
	Y & M	0.244	6	0.040	0.12

* Significant at p<0.0.5 ** Significant at p<0.0.1

from an initial count of 1.14 to 2.10 log cfu/ml at the end of shelf life of 18 days in case of WPC enriched functional drink and in case of cocoa enriched product the counts were increased from 1.16 to 2.25 log cfu/ml (Fig. 2 B). The results are in agreement with the finding of Evangelia *et al* (2002).

Coliform counts

The coliform count was nil in functional dairy drink of both the variants throughout the storage period. Absence of coliform in all the milk samples indicates hygienic practices followed during processing and storage. Nagarajappa and Battula (2017). also reported similar results during storage of fortified milks.

Conclusions

WPC and WPC-Cocoa enriched functional dairy drink were found to be suitable upto 18 days of storage at at 4±1°C. Both FFA and HMF content of the both variants of functional drink increased during storage, indicating deteriorative changes in physic-chemical characteristics of the product. A continuous increase in total bacterial count and yeast and mould counts from 3.14 to 6.48 log cfu/ml and 1.14 to 2.10 log cfu/ml respectively in whey protein enriched functional drink was observed. The same trend was also noticed in the cocoa & whey protein enriched functional drink. The coliform count was found to be nil in both the variants of the functional dairy drink of throughout the storage period.

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