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Microbial quality evaluation of *Aloe vera* and coconut water based whey beverages prepared from camel and goat milk

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Abstract
The milk products and byproducts may be contaminated intentionally/unintentionally at different level at the time of production, processing and storage. Therefore, the present study was conducted with an objective to assess Microbial Quality Evaluation of *Aloe vera* and Coconut Water based Whey Beverages prepared from Camel and Goat Milk. The samples were collected hygienically and subjected to microbiological analysis. The control and treatment whey beverages were examined for the standard plate count (SPC), coliform count, yeast and mold count during storage for 12 days at refrigeration temperature for interval of 0, 3, 6, 9 and 12 days. The statistical analysis of data related to SPC count revealed a highly significant ($P<0.01$) increase of the all three samples (T_0, T_1 and T_2) under study on the 3rd, 6th, 9th and 12th day of refrigerated storage. The coliform count was not observed at day 0 to 6 day of storage whereas yeast and mould count was not observed from day 0 to day 9 of storage study, it was only observed on day 12 of storage. Therefore it is concluded that the Whey Beverages based on *Aloe vera* and Coconut Water prepared from Camel and Goat Milk were highly safe to consume from fresh condition till six days of storage period. The microbiological quality of these beverages was showing a declining pattern after six days of storage period. After nine days of storage, it should not be recommended for consumption as it v is almost deteriorated by yeast and mold.

Keywords: *Aloe vera*, coconut water, camel, goat milk

Introduction
Whey is a rich source of nutritious components and has proven its effects in the treatment of various chronic diseases such as cancer, cardiovascular disease, HIV, etc. It can also be used in baby, geriatric and athletic foods because it is too nutritionally dense. Whey protein has the potential to contribute to body weight regulation as a functional food component by providing satiety signals that influence the regulation of short-term and long-term food intake (Khamrui and Rajorhia, 1998) [7].

camel milk is unique in terms of antioxidative factors, antibacterial, antiviral, antifungal, anti-hepatitis, Paratuberculosis care, hypoglycaemic function, anticancer, aging prevention and autoimmune disease remedy (Sharma *et al*., 2014) [10]. In the management of type 1 diabetes, camel milk proved successful supplementation as there was a substantial decrease in insulin doses along with increase in BMI, quality of life for diabetes, but no shift in lipid profile and insulin levels (Agarwal *et al*., 2003) [1].

Goat milk proteins have been recognized as special lipids and proteins with unique health benefits. For adults suffering from gastrointestinal disorders and ulcers, the soft curd of goat milk can be useful. Goat milk is vital for the prevention and immune stimulation of cardiovascular diseases, cancers, allergies and microorganisms. For children, elderly and convalescent people, goat milk is recommended (Zenebe *et al*., 2014) [17].

Aloe vera (*Aloe barbadensis*) has antioxidant, anti-inflammatory, antidiabetic, sunburn relief, immune boost, anti-aging and anti-cancer properties (Langmead *et al*., 2004). *Aloe vera* has the ability to prevent and treat gastric ulcers, also has anti-inflammatory properties, healing effects, mucus relaxation and gastric secretion control (Suvitayavat *et al*., 2004**)** [14].

A variety of nutritional and therapeutic properties are present in coconut water. It can help for regulating the level of blood sugar and prevent diabetes. Coconut water can improve cardiovascular health and lower the risk of serious cardiovascular disease by lowering the cholesterol/triglyceride levels.

Coconut water is rich in calcium, which helps to reduce oxidative stress and improve antioxidant status and bone density (Bhagya *et al*., 2010) [2].

Looking at the health benefits of *Aloe vera* juice, coconut water and whey, the aim of study was to produce whey beverages that are nutritious as well as palatable with different proportions of aloe vera juice and coconut were developed with microbiologically safe manner.

Material and Methods

Whey obtained during process of paneer preparation by using 70% of camel milk and 30% of goat milk was used to prepare control whey beverage (T_0) and treatment whey beverages. The following treatment combinations were formulated:

T_0 (Control) Whey beverage contains 94% plain whey,5% honey and 1% black salt.

T_1A_1- *Aloe vera* based whey beverage contains 89% whey, 5% *Aloe vera* juice,5% honey and 1% black salt.

T_1A_2- *Aloe vera* based whey beverage contains 84% whey, 10% *Aloe vera* juice, 5% honey and 1% black salt.

T_1A_3 - *Aloe vera* based whey beverage contains 79% whey, 15% *Aloe vera* juice, 5% honey and 1% black salt.

T_2C_1- Coconut water based whey beverage contains 89% whey, 5% coconut water, 5% honey and 1% black salt.

T_2C_2- Coconut water based whey beverage contains 84% whey, 10% coconut water, 5% honey and 1% black salt.

T_2C_3- Coconut water based whey beverage contains 79% whey, 15% coconut water, 5% honey and 1% black salt.

Microbial study of prepared whey beverages

The control and treatment whey beverages were examined for the SPC count, coliform count, yeast and mold count during storage for 12 days at refrigeration temperature for interval of 0, 3, 6, 9 and 12 days. Microbial counts were determined by using pour plate method. Standard plate count (SPC) was determined with plate count agar medium, and the plates of different dilutions were incubated at 37 °C for 24 hours. The total number of colonies per log (cfu/ml) was determined. Violet Red Bile (VRB) Agar medium was used for determination of coliform bacteria and the plates of different dilutions were incubated at 37 °C for 24 hours by pour plate method, and the number of dark red colonies was calculated. Yeast molds (Y-M) count take place by Potato dextrose agar (PDA) medium by pour plate method and the plates of different dilutions were incubated at 37 °C for 24 hours, for yeast and mold count. The microbial count of each sample was measured at day 0, 3, 6, 9 and 12 from preparation.

Standard plate count of prepared whey beverages

The standard plate counts of whey beverage samples was evaluated by using method described in IS: 5402 (1969) [4]. The 11 g of whey beverage sample aseptically weighed and transferred into a sterile 99 ml dilution blank and mixed well. The samples were properly diluted by serial dilution by using 9 ml phosphate buffer. Then from 2nd, 3rd and 4th dilution of whey beverage samples were used for plating. One ml diluent from each sample was taken in duplicate into the sterile petriplates with the help of sterile pipettes. Then the standard plate count agar media was added to these Petriplates and properly rotated so as to mix the content well. The plates were allowed to solidity. All the solidified plates were incubated at 37 °C for 48 hrs in an incubator in an inverted position and the number of colonies developed was recorded as cfu/g.

Coliform counts of prepared whey beverages

The serial dilutions prepared for standard plate count were used for coliform count. The diluents from 1st and 2ndilutions of whey beverage samples were used for plating. The 1 ml diluents from each was taken in duplicate in petriplates and then 10-15 ml violet red bile agar media was added and mixed well. The plates were allowed to solidify. The plates were again overlaid with the same violet red bile agar media and allowed to solidify. Then the plates were incubated at 37oC for 24 hrs in an incubator. The number of coliform colonies was recorded as cfu/g. The coliform colonies were with dark red cantered and pinkish periphery.

Yeast and mould count of prepared whey beverages

The yeast and mould count of whey beverage samples were determined by using method described in IS: 5403 (1969) [5]. The serial dilutions prepared for standard plate count were used for enumeration of yeast and mould count. The diluents from 1st and 2nd dilutions of whey beverage samples were used for plating. One ml each was taken in duplicate in petriplates and the Potato Dextrose Agar (PDA) was used by adjusting pH 3.5 by using 10% sterilized tartaric acid solution. After solidification, the agar plates were incubated at 25ºC for 5 days. At the end of incubation period count of the colonies of yeast and mould were recorded as cfu/g.

Result and Discussion

Microbial analysis of prepared whey beverages

All the microbial counts done in the present study (standard plate count, coliform count, yeast and mould count) has been shown in table 1 under refrigerated storage condition (0, 3, 6, 9 and 12 days).

A. Standard plate count (SPC) of prepared whey beverages

The data related to standard plate count (SPC) of whey based beverage has been shown in Table 1 and depicted in figure 1. The minimum SPC was observed at day 0 for sample T_0, T_1 and T_2 i.e. 5.06 ± 0.014 log (cfu/ml), 5.24 ± 0.017 log (cfu/ml) and 5.33 ± 0.020 log (cfu/ml) respectively but it was found maximum at day 12 i.e. 7.15 ± 0.023 log (cfu/ml), 7.35 ± 0.014 log (cfu/ml) and 7.44 ± 0.012 log (cfu/ml) respectively for sample T_0, T_1 and T_2. The SPC of sample T_2 was significantly higher than sample T_0 and T_1.

The statistical analysis of data shown in Table revealed that a highly significant (P<0.01) increase was observed Between period, between treatment and Interaction between treatment and period in the standard plate count of whey based beverages of the all three sample T_0, T_1 and T_2 under study on the 3rd, 6th, 9th and 12th day of refrigerated storage. The SPC of prepared whey beverages in the present investigation was variable with the findings of Ismail *et al*. (2011) [6], Yonis *et al*. (2014) [16], Mohamed *et al.* (2014) [8], Sharma *et al.* (2020) [11]. Tak (2017) [15], Singh (2017) [13], Gorachiya (2018), Prajapat (2019) [9], Singh (2020) [12], reported the similar trend of significant increase (P<0.01) in the standard plate count with increase of storage period.

B. Coli form count of prepared whey beverages

The data related to coliform count of whey based beverage has been shown in Table 1 and depicted in figure 2 The coliform count was not observed at day 0 to 6 day of storage in all the samples i.e. T_0, T_1 and T_2, respectively. The mean coliform count at day 9 of sample T_0, T_1 and T_2 were observed

to be 2.34 ± 0.024 log (cfu/ml), 2.68 ± 0.048 log (cfu/ml), and 2.56 ± 0.015log (cfu/ml), respectively whereas at day 12 of sample T_0, T_1 and T_2 were observed 2.54 ± 0.018log (cfu/ml), 2.85 ± 0.035 log (cfu/ml), and 2.74 ± 0.030log (cfu/ml), respectively **(**Gorachiya, 2018) observed significant increase in the coliform count with increase of storage of whey beverages. Whereas Singh (2020) [12] observed no coliform count during 15-day storage study of khoa burfi.

C. Yeast and mould count of prepared whey beverages

Yeast and mould count of all treatment beverages has been shown in Table 1 and depicted in figure 3. The yeast and mould count was not observed from day 0 to day 9 of storage study in all the three samples. Yeast and mould count was first observed on day 12 of storage. The mean yeast and mould count at day 12 of T_0 was 3.74 ± 0.021 log (cfu/ml) whereas in sample T_1 it was 3.55 ± 0.018 log (cfu/ml) and in sample T_2 it was 3.45 ± 0.023 log (cfu/ml). The present findings of Yeast and mould count are in accordance with the findings of Gorachiya (2018) who observed yeast and mold count only on 12th day of storage of whey beverages.

Table 1: Standard plate count log (cfu/g) (mean ± SE) of prepared whey beverages

Parameter	Storage period (days)	Samples		
		T0	T1	T2
**Standard plate count log (cfu/ml)	Day 0	5.06^{aA} ± 0.014	5.24^{aB} ± 0.017	5.33^{aC} ± 0.020
	Day 3	6.15^{bA} ± 0.017	6.26^{bB} ± 0.020	6.23^{bB} ± 0.026
	Day 6	6.33^{cA} ± 0.014	6.45^{cB} ± 0.020	6.55^{cC} ± 0.011
	Day 9	6.84^{dA} ± 0.024	6.90^{dB} ± 0.017	6.95^{dB} ± 0.015
	Day 12	7.15^{eA} ± 0.023	7.35^{eB} ± 0.014	7.44^{eC} ± 0.012
Coli form count** (CFU/ml)	Day 0 to 6	nil	nil	nil
	Day 9	2.34 ± 0.024	2.68 ± 0.048	2.56 ± 0.015
	Day 12	2.54 ± 0.018	2.85 ± 0.035	2.74 ± 0.030
Yeast and mould count (CFU/ml)	Day 0 to 9	nil	nil	nil
	12	3.74 ± 0.021	3.55 ± 0.018	3.45 ± 0.023

** = Significant at 1% (P<0.01). Means bearing different superscript in a column (capital letter) and in a row (small letter) differ significantly.
T_0- Control whey beverage, T_1- *Aloe vera* based whey beverage (ABWB) and T_2-Coconut water based whey beverage (CBWB)

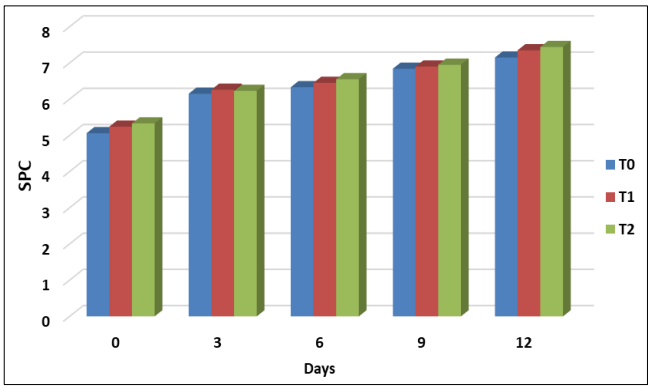

Fig 1: Standard plate count of prepared whey beverages

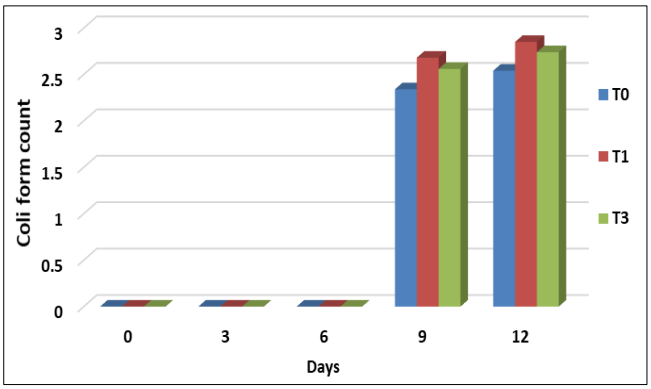

Fig 2: Coliform count of prepared whey beverages

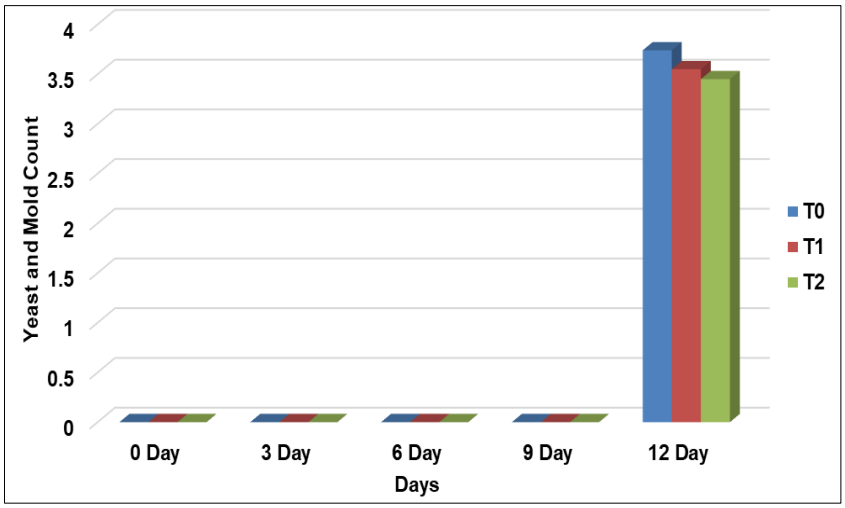

Fig 3: Yeast and mould count of prepared whey beverages

Conclusion

From present study, It is concluded that the *Aloe vera* and Coconut Water based Whey Beverages prepared from Camel and Goat Milk were hygienic and microbiologically safe to consume in fresh condition till six days of storage period under refrigeration conditions. The technologies generated

may be explored for value addition to paneer for preparing a safe ready to serve (RTS) product from the byproduct whey. The gradual declining pattern of microbiological quality of these whey beverages shows increased utilization and cost benefits from the byproduct sector of dairy industry particularly from paneer sector.

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