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Proximate and Microbial Analysis of Developed Hydrolyzed Carbonated Whey Beverage with Pomegranate Peel Extract and Guava Leaves Extract

Fathimath Naurin K.A.[1*], Marx Nirmal R.[2], Ayyavoo Preamnath Manoharan[2] and Karthikeyan N.[2]

[1]College of Food and Dairy Technology,
Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai (Tamil Nadu), India.
[2]Department of Food Process Engineering, College of Food and Dairy Technology,
Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai (Tamil Nadu), India.
[3]Department of Dairy Microbiology, College of Food and Dairy Technology,
Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai (Tamil Nadu), India.

(Corresponding author: Fathimath Naurin K.A.)*
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ABSTRACT: The present study aim on the proximate and microbial analysis of developed carbonated beverage using lactose hydrolyzed whey by lactase enzyme at pH 6.6. Proper utilization of whey can be considered as a big challenge in dairy industry. Whey beverages were prepared by blending sugar (10%), pomegranate peel extract (3%) and guava leaves extract (3%) to the hydrolyzed whey. Carbonation was done by the injection of CO_2. The proximate analysis such as moisture, protein, fat, total solids, lactose and ash (%) content in the developed whey beverage was analyzed and the values were 89.94, 0.98, 0.064, 11.12, 0.79 and 0.53 respectively. Proximate analysis of product shows that the developed beverage is a protein rich, low fat and low lactose. Microbial analysis was carried out for carbonated whey beverage and results showed that there was no growth of aerobic bacteria up to 28th day of storage, no growth of yeast and mold up to 14th day of storage and no growth of coliform throughout the storage period at 4°. The low microbial growth in the product may be attributed by the effect of carbonation and antimicrobial properties of pomegranate peel and guava leaves extract. The developed beverage is protein rich with low lactose and low fat and the antibacterial properties of pomegranate and guava leaves helped in extending the shelf life. This study will be helpful in proper utilization of whey in dairy industry.

Keywords: Pomegranate peel extract, Guava leaves extract, Proximate analysis, Protein, Microbial analysis, Coliforms, Whey beverage.

INTRODUCTION

Beverages fulfill the human needs and their role is vast in thirst quenching ability. In consumption of Beverages, other than its thirst-quenching ability it is important in its "food value" also.

The manufacture of different value-added dairy products creates large quantities of whey with potential source of nutrients which is not being fully utilized in dairy industry (Shiby *et al*., 2013). Whey is a rich source of high-quality proteins, minerals, vitamins and lactose that can be utilized and transformed in to numerous valuable products (lactose, protein concentrates, lactalbumin, lactoglobulin, galactose, glucose, etc.) through various processes such as concentration or fractionation, drying, fermentation or hydrolysis (Caric and Milanovic 1995).

By improving the energizing and sparkling nature of beverages by carbonation can make it more acceptable to everyone and its development is increasing globally as well, largely because of the thirstiness satisfying and energizing properties of these products and since carbonation is usually a cheap and harmless procedure. Carbonation can stop the development of microbes in the beverage and can improve the shelf life (Silva *et al.*, 2018).

Pomegranate (*Punica granatum* L) fruit is considered as "food medicine" because of the presence of several bioactive compounds in it. Each and every part of pomegranate fruit is important in the nutrition point of view and other than its fruit portion, seed and peel are also used as a source of bioactive compounds in several food products. Pomegranate peel attracts attention due to its apparent wound healing properties (Chidambara *et al*., 2004), immune modulatory activity and antibacterial activity (Navarro *et al*., 1996).

The cheaply available guava leaves are considered as a folk medicine because of its antibacterial, antihyperglycemic, antitumour and anti-cancerous effects (Tran *et al*., 2020). The flavonoids present in guava leaf extract chiefly determine their antibacterial activity, while quercetin, which is the most principal flavonoid of guava leaves, exhibits strong antidiarrheal activities (Kumar *et al*., 2021).

This study was anticipated to develop a protein rich carbonated hydrolysed whey beverage and analyze the proximate and microbial qualities.

MATERIAL AND METHODS

Materials. Whey (paneer whey) was obtained from Incubation centre of College of Food and Dairy Technology, Chennai, Tamil Nadu. The β-galactosidase (Maxilact® L 5000 Lactase) enzyme was purchased from DSM Food specialties, Denmark. Good grade sugar was purchased from local market-Redhills, Chennai. Pomegranate peel was obtained from local market-Redhills Chennai. Fresh Guava leaves were collected from guava trees of CFDT college compound, Alamathi. Standard plate count agar, Red Bile Agar and Potato dextrose agar was purchased from M/s. Himedia Labs Ltd Mumbai.

Methods. Fresh whey obtained from channa or paneer making was filtered with muslin cloth and cream separated using cream separator. Then it was pasteurized at 80º C for 10 minutes and cooled to 37ºC. The pH of whey was adjusted to 6.6 using 1N KOH for the lactose hydrolysis. Lactase enzyme (Maxilact® L 5000 Lactase enzyme) was added at 0.3ml/litre. Then the whey was incubated at 40º C for 3 hours in the incubator. After incubation the enzyme was inactivated by heating at 80º C for 5 minutes. The hydrolyzed whey was cooled to 37ºC and added the other ingredients like Sugar, 3% aqueous extracts of Pomegranate peel and Guava leaves. Then again, the whey was pasteurized at 80º C for 10 minutes and immediately cooled to 5ºC. The resulting beverage was carbonated at 15 psi pressure, filled in glass bottle, crown corking and kept for storage at refrigerated temperature (4°).

Proximate Analysis. Moisture and ash were estimated by the procedure given in AOAC (1995). Fat was analyzed by Gerber method as per IS: SP 18 (Part XI, 1981) and Protein by Kjeldhal method as per AOAC (1990). Lactose was analyzed as per Musallam *et al*. (2017) and total solids estimated with the method described by Ranganna (2005).

Microbiological analysis. Microbiological analysis *viz*, standard plate count, coliform count and yeast and mould counts was carried out as per the standard technique described in IS: 1981, SP: 18 (Part XI).

Statistical analysis. Statistical analysis was performed using one way analysis of variance (ANOVA). Statistical analysis was conducted with IBM SPSS® 20.0 for Windows® software program.

RESULTS AND DISCUSSION

Proximate analysis of control and carbonated whey beverage. Proximate analysis of control and carbonated whey beverage and their Analysis of variants are presented in Table 1, Fig. 1 and 2.

The moisture content of control and carbonated whey beverage was 94.21 and 89.94 respectively. A high significant difference was found in the moisture content of control and carbonated whey beverage. The moisture content in the product is reduced by added extracts in the product as compared to the control. A similar result was obtained in whey mango-based beverage developed by Ahmad *et al*. (2018) and they reported a moisture content of plain whey is 94.2% and whey mango beverage is 82.6 percent.

The mean values of protein content of control and carbonated whey beverage was 0.82 and 0.98 respectively. There is a high significant (P≤0.01) difference in protein content between control and the carbonated whey beverage. Addition of plant extract increased the protein content in the carbonated whey beverage, and this is comparable with the results of protein (0.8per cent) in the whey beverage added with mint leaves extract developed by Rizk (2016). The mean values of fat content of control and product was 0.065 and 0.064 respectively. The fat content is almost same in both control and whey beverage because the addition of both extracts will not increase the fat content. A minimum fat percentage (0.05) percent was obtained in the lactose hydrolyzed carbonated beverage using fat separated whey reported by Rajendrabhai and Prajapati (2017).

The mean of total solids content of control and product was 5.91 and 11.12 respectively which indicate that TS content is more in product when compared to the control due to addition of extracts. The results are comparable with the results of Singh *et al*. (2014). The mean of lactose content of control and product was 0.8 and 0.79 respectively. Rajendrabhai and Prajapati (2017) showed the same value of lactose (0.8per cent) in hydrolyzed whey. Hydrolysis is reducing the lactose content in the whey.

The mean of ash content of control and product was 0.49 and 0.53 respectively. Highly significant difference (P≤0.01) in the ash content of control and product were observed. The results are similar with the findings of Rizk (2016).

Table 1: Proximate analysis (Mean±SE) of control and Carbonated whey beverage.

Parameter (per cent)	Sample		t-value
	Control	Carbonated whey beverage	
Moisture	94.21±0.017[b]	89.94±0.022[a]	**152.597****
Protein	0.82±0.005[b]	0.98±0.006[a]	**18.459****
Fat	0.065±0.006[a]	0.064±0.007[a]	**0.102** [NS]
Total solids	5.91±0.005[b]	11.12±0.013[a]	**355.540****
Lactose	0.800±0.005[a]	0.79±0.013[a]	**0.682**[NS]
Ash	0.49±0.006[b]	0.53±0.007[a]	**4.219****

@ Average 6 trials, ** highly significant (P≤0.01); * significant (P ≤0.05); NS – No significant difference (P>0.05)

Table 2: Microbiological analysis (Mean±SE) [@] of carbonated whey beverage during storage at 4°C.

Parameters	Storage days	Sample		t value
		Control	Carbonated whey beverage	
Standard plate count (log_{10}cfu/mL)	**0th**	ND	ND	-
	14th	ND	ND	-
	28th	0.18±0.01	ND	-
	42nd	0.79±0.02	0.75±0.02	**1.509NS**
	56th	1.21±0.09	1.11±0.06	**0.871NS**
	F value	**82.923****	**98.787****	-
Coliform count(log_{10}cfu/mL)	**0th**	ND	ND	-
	14th	ND	ND	-
	28th	ND	ND	-
	42nd	ND	ND	-
	56th	ND	ND	-
	F value	-	-	-
Yeast and mold (log_{10}cfu/mL)	**0th**	ND	ND	-
	14th	ND	ND	-
	28th	0.66±0.04	0.59±0.04	**1.099NS**
	42nd	1.31±0.07	1.18±0.05	**1.378NS**
	56th	2.67[d]±0.14	2.00[d]±0.08	**4.057***
	F value	**80.988****	**89.033****	-

@ Average 6 trials, ** Highly significant (P≤0.01); * Significant (P ≤0.05); NS – No significant difference (P>0.05). ND-Not Detected, Means bearing various superscripts in the same column differs highly significantly.

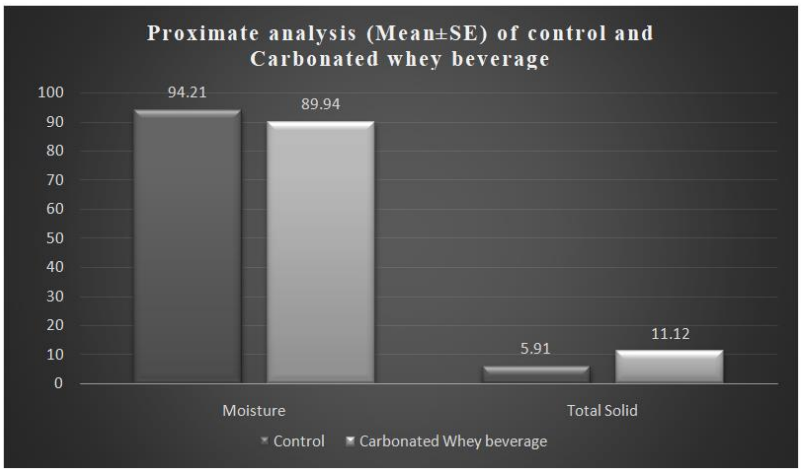

Fig. 1. Proximate analysis of control and carbonated whey beverage.

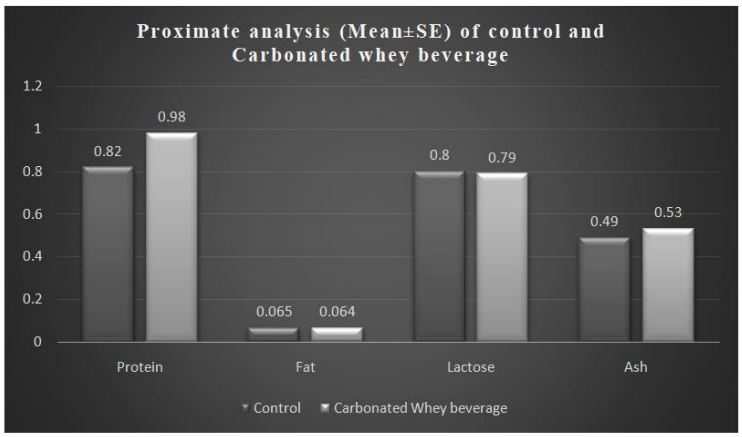

Fig. 2. Proximate analysis of control and carbonated whey beverage.

Microbiological analysis of control and carbonated whey beverage. The mean of SPC in log cfu/mLof control on the 0th, 14th, 28th, 42ndand 56th day of storage at 4° was ND, ND, 0.18, 0.79 and 1.21 respectively. The mean of SPC in log cfu/mL of carbonated whey beverage on the 0th, 14th, 28th, 42ndand 56th day of storage at 4° was ND, ND, ND, 0.75 and 1.11 respectively. The absence of initial growth is due to the antimicrobial properties of pomegranate peel and guava leaves extract. The results are correlated with results of Rajendrabhai and Prajapati (2017) in carbonated lemon whey beverage, who observed that there was no growth of SPC up to 21 days of storage of beverage at 4° and then it increased to1.21 log cfu/mL.

No coliform count was detected in control and carbonated whey beverage throughout the storage period. The results are correlated with results of Rajendrabhai and Prajapati (2017) in carbonated lemon whey beverage, who observed that no coliform growth was observed in beverage throughout storage at 4°. Absence of coliforms and absence of initial growth of SPC in the carbonated whey beverage is due to the presence of antibacterial activity of pomegranate peel extract and guava leaves extract (Sandhya *et al.* 2018).

The mean of yeast and mold counton log cfu/mL of control in the 0th, 14th, 28th, 42ndand 56th day of storage at 4° was ND, ND, 0.66, 1.31 and 2.67 respectively. The mean of yeast and mold in log cfu/mLof carbonated whey beverage in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° was ND, ND, 0.59, 1.18 and 2.00 respectively. The absence of initial growth is due to the antimicrobial properties of pomegranate peel and guava leaves extract. Sandhya *et al.* (2018) found that curd samples added with Pomegranate peel extracts shows resistance against microorganisms due to antimicrobial action of pomegranate peel extract.

CONCLUSIONS

The proximate and microbial analysis showed that the developed beverage is a protein rich and nutritious and acceptable by lactose intolerant people and it as shelf stable upto 56 days of storage at 4°. The antibacterial property of the pomegranate peel and guava leaves enhanced the microbial quality of the product. The developed product is healthy protein rich. Low microbial growth in the product may be attributed by the effect of carbonation and antimicrobial properties of pomegranate peel and guava leaves extract. Since carbonated beverages are new trend in beverage industry, the project will have vast scope.

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Conflict of Interest. None.

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