

Evaluation of Cheddar Cheese Whey and Paneer Whey as a Growth Medium for Lactic Acid Bacteria

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(Received: 20 February 2016; accepted: 04 April 2016)

Whey is the major by-product obtained during the manufacture of cheese, casein, paneer and chhana. The total solids, fat, total protein, lactose and ash content were 6.77, 0.72, 1.11, 4.88 and 0.43% in Cheddar cheese whey and paneer whey 6.37, 0.59, 0.51, 4.57 and 0.46 %, respectively. The average values of specific gravity, viscosity, surface tension, refractive index and electrical conductivity of Cheddar cheese whey were 1.026 at 20°C, 1.28 cp, 49.75 dynes/cm, 1.3435 and 4.24 ms respectively, while, that for paneer whey were 1.021 at 20°C, 1.16 cp, 50.14 dynes/cm, 1.3431 and 4.38 ms respectively. The mean values of pH, titratable acidity and acid degree value of Cheddar cheese whey were 6.71, 0.12 % LA and 6.59 milliequivalents of alkali per 100 g of fat, whereas, that of paneer whey were 5.81, 0.14 % LA and 5.76 respectively. After 48 h of incubation drop in pH by *L. helveticus* MTCC 5463, *S. thermophilus* MTCC 5461, *L. mesenteroides* and *L. lactis* were 3.56, 3.68, 4.05 and 3.61 in cheddar cheese whey whereas in paneer whey 3.54, 3.68, 3.89 and 3.64 respectively. Highest mean viable counts were obtained after 12 h of incubation. Counts (log cfu/ml) of all four lactic acid bacteria were 8.25, 9.19, 8.66 and 9.80 in paneer whey and 8.32, 9.16, 8.72 and 9.82 in cheddar cheese whey respectively.

Keywords: Cheddar cheese whey, Paneer whey, Lactic acid bacteria.

Whey is transparent watery liquid which remains after removal of fat and casein from milk. Technically whey is termed as milk serum, however, practically it is greenish-yellow residual fluid obtained on coagulation of milk. In dairy industry whey is obtained as by-product during the manufacture of coagulated milk products such as paneer, chhana, cheese, casein, etc. There are two types of whey; acid whey (pH 4.4 to 4.6) and sweet whey (pH 5.9 to 6.3) (Agustriyanto and Fatmawati, 2009).

Whey is produced in very large amount and its utilization has been a continuing challenge for dairy industry. BOD and COD values of whey are very high, e.g. acid whey has BOD value of 35000 to 45000 ppm and COD values of ranging

from 55000 to 70000 ppm (Mawson, 1994). Because of this high BOD value and the insufficient amount of protein, whey disrupts the biological operation of sewage disposal plants (Cavit Atkin *et al.*, 1967). In many countries including India, most of the whey is discarded as waste creating severe environmental pollution problems due to its high BOD and COD (Vishwakarma, 2010). The safe disposal of whey results in increased operating costs of the effluent treatment plants due to high consumption of electrical energy. The estimated expenditure for treating 1 liter of whey to the required discharge standards is about 35 paise, which is considered expensive (Mallik and Kulkarni, 2009). The way of whey disposal as a waste will lead to the loss of valuable milk nutrients. This in turn affects the profitability of dairy plant. Hence, utilization of this valuable by-product leads to the financial advantage in dairying, as well as, it reduces the organic load and treatment costs on the effluent

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treatment plant by reducing the consumption of electrical energy (Mallik and Kulkarni, 2010).

The high nutritional value of whey makes it an interesting substrate for the development of fermented foods (Pescuma *et al.*, 2012). This by-product is a rich substrate that has been suggested for many applications including solid enrichment in cheese manufacture, bacterial or yeast growth medium to produce biomass, animal nutrients supplement, source of added value proteins (Aguirre-Ezkauriatza *et al.*, 2010).

Most lactic acid bacteria (LAB) are facultatively anaerobic, catalase-negative, non-motile and non-spore forming. Expensive culture media, which contain natural complex organic nitrogen sources such as yeast extract, malt extract and/or polypeptone, are necessary for the cultivation of lactic bacteria because nutritional requirement of lactic bacteria is very complicated (Tanaka *et al.*, 1995).

Whey has mainly been used to grow LAB for purposes of lactic acid production, its potential as a growth medium for biomass production has not been explored. Therefore, looking into the nutritional value of whey, it can be a good ready to serve nutrient to for the growth of beneficial LAB.

MATERIALS AND METHODS

Paneer whey and unsalted cheddar cheese whey were collected from Amul Dairy, Anand.

Gross Composition

Fat, total solids, protein, lactose and ash of Cheddar cheese whey and paneer whey samples were determined as per procedures given in (IS: SP 18: part XI, 1981). The lactose content of the whey sample was determined using Lane Eynon Method. Total solids content of the whey samples was determined gravimetrically. The fat content of the whey samples was estimated by Gerber method using skim milk butyrometer. Kjeldahl digestion unit and Kelplus distillation unit of Pelican Instruments, Chennai, were used to estimate nitrogen content of whey samples for determination of protein. The procedure outlined in BIS Handbook (IS: SP 18: part XI, 1981) was followed for determination of ash content in the whey samples.

Physical Properties

Specific gravity, Viscosity and Surface tension of whey samples were determined by

procedures as prescribed in BIS Handbook (IS: SP 18: Part XI, 1981).

Refractive index

Refractive index was determined using Abbe Refractometer (Atago, Japan) at 20°C.

Electrical conductivity

Electrical conductivity was analyzed by Electrical conductivity meter (Systronics Ltd. Ahmedabad) at 20°C.

Chemical Characteristics

pH

pH was determined using pH meter (Oaklon pH 700)

Titrateable acidity

The titrateable acidity of whey was determined by the method prescribed in BIS Handbook (IS: SP 18: part XI, 1981).

Free fatty acids (acid degree value)

Free fatty acid was measured by the extraction - titration procedure (Frankel and Tarassuk, 1955).

Growth of Lactic Acid Bacteria in Whey

The pure strains of *Lactobacillus helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Lactobacillus bulgaricus* were acquired from the culture collection of Dairy Microbiology Department, Anand Agricultural University, Anand, Gujarat. The strains were activated from its frozen form (stored in 10% glycerol at -80 °C) by giving one transfer in respective broths. This was followed by 2 successive transfers into sterile respective broths under incubation conditions of 37°C for 12 h.

Two types of whey were used, i.e., Paneer whey and Cheddar Cheese whey. Both whey were heated at 95°C for 1 min. After cooling down to around 37°C, pH of paneer whey was adjusted to 6.5 by addition of 0.1 N NaOH whereas adjustment was done by 0.1 N HCL in case of cheddar cheese whey. Subsequently lactic cultures i.e., *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis* were inoculated at the rate of 2%. All flasks were incubated at 37°C. 10 ml of samples were withdrawn after 0, 4, 8, 12, 24, 32 and 48 h of incubation. Samples were analyzed for change in pH and total viable count.

RESULTS AND DISCUSSION**Gross composition**

The mean values of gross composition of cheddar cheese whey and paneer whey samples were analysed and the results are presented in Table 1.

The mean value of total solids of cheddar cheese whey and paneer whey were found to be 6.77 % and 6.37% respectively. The mean values of other constituents like fat, protein and ash for cheddar cheese whey were 0.72, 1.11 and 0.43%, whereas, for paneer whey were 0.59, 0.51 and 0.46%.

The higher value of protein in cheddar cheese whey may be attributed to release of whey proteins and GMP (glycomacropeptide) in whey. Mineral content of paneer whey is higher than that of cheddar cheese whey as colloidal salts are released into whey during coagulation by acid.

The mean lactose content in cheddar cheese whey and paneer whey were 4.88% and 4.57% respectively. Divya (2007) in her study, analyzed the composition of cheddar cheese whey and paneer whey. She reported that differences exist between paneer and cheese whey with regard to protein and ash contents. The protein and ash

Table 1. Gross composition

Whey	TS (%)	Protein (%)	Lactose (%)	Fat (%)	Ash (%)
Cheddar cheese whey	6.77	1.11	4.88	0.72	0.43
Paneer whey	6.37	0.51	4.57	0.59	0.46
Source of Variation					
SEm	0.06	0.01	0.01	0.02	0.01
Test (P < 0.05)	*	*	*	*	NS
CD	0.19	0.04	0.03	0.06	0.04
CV%	3.78	6.28	0.77	12.32	11.06

Table 2. Physical properties

Whey	Viscosity (cp)	Specific Gravity	Refractive Index	Electrical conductivity (ms)	Surface Tension (dynes/cm)
Cheddar cheese whey	1.28	1.026	1.3435	4.24	49.75
Paneer whey	1.16	1.021	1.3431	4.38	50.14
Source of Variation					
SEm	0.01	0.00	0.00	0.07	0.21
Test (P < 0.05)	*	*	NS	NS	NS
CD	0.02	0.00	0.00	0.21	0.60
CV%	2.23	0.11	0.16	6.53	1.61

Table 3. Chemical properties

Whey	Acidity (%LA)	pH	Acid Degree Value(milliequivalents of alkali per 100 g of fat)
Cheddar cheese whey	0.12	6.71	6.59
Paneer whey	0.14	5.81	5.76
Source of Variation			
SEm	0.00	0.03	0.21
Test (P < 0.05)	*	*	*
CD	0.00	0.08	0.62
CV%	4.42	1.79	13.46

contents of paneer whey were 0.38 and 0.60%, whereas those in cheese whey were 0.81 and 0.39% respectively. The mean value of fat, lactose and total solids of paneer whey 0.52%, 5.12% and 6.39% whereas that of cheese whey were 0.28%, 4.83% and 6.66%. Goyal and Gandhi (2009) also studied chemical composition of paneer whey and cheese whey. In their study, they reported mean values of total solids, fat, total protein and lactose of paneer whey as 5.8, 0.01, 0.41 and 4.5% respectively whereas of cheese whey these values were 6.3, 0.2, 0.53 and 5.0% respectively. Rupnar, *et al.* (2009) analyzed composition of paneer whey and reported the values of total solids, total protein and reducing sugar as 6.25, 0.09 and 4.52% respectively. Baljeet, *et al.* (2013) reported the mean values of total soluble solids, fat and proteins of whey as 5.63 °Brix, 0.01% and 0.18% respectively. Our results for gross composition of paneer whey and cheddar cheese whey are in similar lines.

In another study, normal acid and sweet whey samples were collected from six national dairy plants located in Amman city, Jordan. The whey samples analyzed after concentration using vacuum scraper concentrator. The obtained results revealed that the concentrated acid whey (CAW) had more dry matter (68.98%), salt (14.56%) as compared with the concentrated sweet whey (CSW). On the other side, CSW had more lactose (36.40%) and protein (8.25%) compared to CAW (Ali K. Alsaed, *et al.*, 2013).

Physical Characteristics

Cheddar cheese whey and paneer whey were compared with respect to their physical properties, i.e. specific gravity, viscosity, surface tension, refractive index, electrical conductivity and redox potential. The mean values of these properties are shown in the Table-2.

The mean values of specific gravity, viscosity (cp), surface tension (dynes/cm), refractive index and electrical conductivity (ms) of cheddar cheese whey were 1.026, 1.28 cp, 49.75 dynes/cm, 1.3435 and 4.24 ms respectively, while, that for paneer whey were 1.021, 1.16 cp, 50.14 dynes/cm, 1.3431 and 4.38 ms respectively. The higher values of specific gravity and viscosity of cheddar cheese whey is attributed to higher total solids in the cheddar cheese whey. However, higher refractive index and electrical conductivity values of paneer whey is probably due to more

salt content of paneer whey. The surface tension value of paneer whey is higher than that of cheddar cheese whey. The lower value of redox potential of cheddar cheese whey might be due to the addition of starter culture during manufacture of cheddar cheese (Kavimandan and Sharma, 2015).

The surface tension of various whole whey, solutions of component whey proteins, UF fractions, and the effect of heating on the surface tensions of these solutions were determined using the Wilhemy plate method. The mean surface tension of three commercial cottage cheese whey, a commercial cheddar cheese whey, and a laboratory rennet whey was found to be 41.7 ± 1.2 dyne/cm (25°C) and did not vary significantly with the type of whey despite differences in both pH and protein content. The surface tensions of aqueous solutions of individual pure protein fractions of whey (serum albumin, β -lactoglobulin, α -lactalbumin, and gamma globulins), in concentrations approximating normal whey contents, were significantly different and greater than for the whole whey (Roehl and Jelen, 1988).

Chemical Characteristics

The chemical properties of cheddar cheese and paneer whey are given in Table 3.

The mean value of pH and titratable acidity of cheddar cheese whey were found to be 6.71 and 0.12% lactic acid, whereas, that of paneer whey is found to be 5.81 and 0.14% lactic acid respectively. Divya (2007) reported mean pH values of cheese and paneer whey to be 6.66 and 5.6 respectively. Rupnar, *et al.* (2009) also reported acidity and pH values of paneer whey as 0.21% Lactic acid and 4.96 respectively. Jayalakshmi (2011) reported the pH values of fresh paneer whey to be 4.9. Sagar (2011) in his study determined pH and acidity value of paneer whey as 4.9 and 1.29% lactic acid whereas for hydrolysed paneer whey 5.8 and 1.16% lactic acid respectively. Baljeet, *et al.* (2013) also reported acidity and pH values of whey as 0.39% Lactic acid and 5.0 respectively. The mean value of acid degree value of cheddar cheese and paneer whey were found to be 6.59 and 5.76% ADV respectively.

Growth characteristics of Lactic acid bacteria in whey

To evaluate suitability of paneer and cheddar cheese whey for growth of four lactic cultures (*Lb. helveticus* MTCC 5463,

Table 4. Changes in pH during growth of lactic cultures

Incubation period (h)	<i>Lactobacillus helveticus</i> MTCC 5463		<i>Streptococcus thermophilus</i> MTCC 5461		<i>Leuconostoc mesenteroides</i>		<i>Lactococcus lactis</i>	
	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey
0	6.25	6.28	6.43	6.47	6.36	6.42	6.34	6.37
4	5.31	5.35	5.31	5.31	6.11	6.17	5.78	5.83
8	4.57	4.60	4.69	4.63	5.76	5.43	5.19	5.17
12	4.31	4.31	4.42	4.40	4.74	4.56	4.67	4.79
24	3.90	3.84	4.05	4.03	4.19	4.28	3.98	4.04
32	3.69	3.66	3.86	3.84	4.04	4.18	3.85	3.85
48	3.54	3.56	3.68	3.68	3.89	4.05	3.64	3.61

Source of Variation	<i>Lactobacillus helveticus</i> MTCC 5463		<i>Streptococcus thermophilus</i> MTCC 5461		<i>Leuconostoc mesenteroides</i>		<i>Lactococcus lactis</i>	
	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)
SEm	0.09	0.17	0.06	0.12	0.04	0.07	0.04	0.07
Test (P < 0.05)	NS	*	NS	*	NS	*	NS	*
CD	NS	0.49	NS	0.35	NS	0.20	NS	0.22
CV%	NS	9.19	NS	6.39	NS	3.33	NS	3.82
Interaction (T X P)	0.24	0.17	0.17	0.10	0.10	0.10	0.04	0.11

Table 5. Changes in total viable count during growth of lactic cultures

Incubation period (h)	<i>Lactobacillus helveticus</i> MTCC 5463		<i>Streptococcus thermophilus</i> MTCC 5461		<i>Leuconostoc mesenteroides</i>		<i>Lactococcus lactis</i>	
	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey	Paneer whey	Cheddar cheese whey
0	6.88	7.06	6.59	6.48	6.34	6.27	7.33	7.43
4	7.85	7.78	8.43	8.27	7.56	7.38	8.02	7.94
8	8.15	8.00	9.07	8.62	8.18	8.07	9.43	9.33
12	8.25	8.32	9.19	9.16	8.66	8.72	9.80	9.82
24	7.76	7.69	8.99	9.09	8.29	8.25	8.91	8.68
32	7.68	7.65	6.61	6.92	7.46	7.49	8.80	8.39
48	6.27	6.38	6.41	6.23	7.03	7.20	7.69	7.17

Source of Variation	<i>Lactobacillus helveticus</i> (T X P)		<i>Streptococcus thermophilus</i> (T X P)		<i>Leuconostoc mesenteroides</i> (T X P)		<i>Lactococcus lactis</i> (T X P)	
	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)	Treatment (T)	Incubation period(P)
SEm	0.23	0.43	0.27	0.50	0.18	0.34	0.12	0.22
Test (P < 0.05)	NS	*	NS	*	NS	*	NS	*
CD	NS	1.24	NS	1.44	NS	0.97	NS	0.64
CV%	NS	13.85	NS	15.50	NS	10.76	NS	6.42

Streptococcus thermophilus MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis*) were used.

The data revealed (Table-4) that treatment has no significant effect on drop in pH of paneer whey and cheese whey during growth of *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis* up to 48 h. After 48 h of incubation growth of *Lb. helveticus* MTCC 5463 resulted in pH of 3.54 and 3.56 in paneer whey and cheese whey respectively. Same way after 48 h of incubation growth of *Streptococcus thermophilus* MTCC 5461 resulted in pH of 3.68 for both paneer whey and cheese whey. In case of *Leuconostoc mesenteroides* 48 h of incubation growth resulted in pH of 3.89 and 4.05 in paneer whey and cheese whey respectively. After 48 h of incubation growth of *Lactococcus lactis* resulted in pH of 3.64 and 3.61 in paneer whey and cheese whey respectively. For all four cultures both the treatments were statistically non-significant. As incubation period increased pH decreased significantly. However, interaction between treatment and period was again non-significant.

Data shown in Table 5 indicates that treatment has no significant effect on total viable count. Growth of all four lactic cultures followed almost similar pattern in both cheese as well as paneer whey. It can be seen that log cfu/ml reached maximum at 12 hours of incubation period and the mean viable count for *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis* were 8.25 log cfu/ml, 9.19 log cfu/ml, 8.66 log cfu/ml and 9.80 log cfu/ml in paneer whey and 8.32 log cfu/ml, 9.16 log cfu/ml, 8.72 log cfu/ml and 9.82 log cfu/ml in cheddar cheese whey respectively. Then after it started decline. The cessation of growth might be owing to the depletion of some essential nutrients in the medium, the accumulation of some toxic products of the organism in the medium, or a combination of both. Throughout the incubation period change in count was statistically significant. However, interaction between treatment and period was again non-significant.

Fermentation of whey by Lactic Acid Bacteria (LAB) usually focuses on the production of lactic acid. Alternatively, whey or whey permeate has the potential as a culture medium for the

propagation of dairy cultures. Whey or UF whey permeate are cheap and readily available sources for use as fermentation media. (Parente and Zottola, 1991)

Bhuvaneshwari and Sivasubramanian (2011) investigated the treatment of the organic wastes using microbiological process for effective usage of waste and to develop value added products from it. The organic wastes used in this processes were domestic wastes, vegetable wastes, fruit wastes, bakery wastes and whey. They used *Lactococcus lactis subsp lactis* for synthesis of lactic acid. The optimal production of lactic acid and bacterial growth were 35.45 g/l and 1.34 g/l respectively from whey by *Lactobacillus rhamnosus*.

Lavari et al. (2014) explored double use of cheese whey (culture medium and thermoprotectant for spray drying of lactobacilli) for their capacity to produce biomass of *Lb. paracasei* JP1, *Lb. rhamnosus* 64 and *Lb. gasseri* 37. All the cultures were found to ferment the media and at highest biomass production, the viability of the cultures ranged between 8-9 log cfu/g of biomass.

Above referred studies are in corroboration with the results obtained in this study. Both cheddar cheese whey and paneer whey can be used as a growth medium for lactic acid bacteria. Amongst these two whey used, cheddar cheese whey contains significantly higher amount of protein. Therefore, further enhancement in growth of lactic cultures can be done by hydrolysing these whey proteins as a nitrogen source.

CONCLUSION

Cheddar cheese whey and paneer whey were differing in their composition, physical properties and chemical characteristics. Growth parameters of lactic acid bacteria in Cheddar cheese whey and paneer whey were almost at par. Highest drop in pH was observed after 48 h of incubation for all four lactic cultures i.e., *L. helveticus* MTCC 5463, *S. thermophilus* MTCC 5461, *L. mesenteroides* and *L. lactis* same way the mean bacterial counts were highest after 12 h of incubation. Therefore, whey is a cheap and readily available source for use as fermentation media.

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