Chemical and microbial quality of whey from cow milk using calf rennet, microbial rennet, lactic acid and papaya latex

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Abstract

The purpose of the study was to determine the quality of whey obtained from cow milk using different types of coagulants (microbial rennet, calf rennet, lactic acid and papaya latex). The quality of whey was evaluated with the help of chemical and microbial tests. Results revealed that maximum whey yield (765 mL/L milk) was obtained from lactic acid coagulation which was 17 mL higher than that of the microbial rennet coagulation (p=0.000). The pH was found significantly (p<0.05) higher when the microbial rennet was used as coagulants. Whey obtained by the lactic acid coagulation was superior in terms of chemical quality compared to the whey obtained by coagulating action of other coagulating agents (p=0.000-0.001). Total solids and lactose content was found highest (7.2% and 4.8%, respectively) in lactic acid whey which was significantly higher (±1%) than that of the other types of whey (p=0.000). In addition, protein content was also found higher (p<0.05) in whey obtained by lactic coagulation. On the other hand, efficiency of fat and ash recovery in curd was found lowest in calf rennet (p=0.000) among the studied coagulants. However, phosphorus content was highest (p=0.005) in calf rennet whey and the whey obtained by microbial rennet coagulation had the lowest (p=0.000) calcium concentration. Microbial load was found maximum (117 cfu × 10^3/mL) in whey drained out in microbial rennet coagulation, whereas, papaya latex whey had the lowest count (78 cfu × 10^2/mL), and the microbial count of other two whey was intermediate of them (p=0.000-0.001). In general, considering all the quality attributes, lactic acid whey was found better followed by calf rennet and papaya latex. It indicates more nutrient recovery in the curd produced by the microbial rennet.

Keywords: Whey, coagulants, microbial rennet, calf rennet, lactic acid, papaya latex

INTRODUCTION

Whey is the watery part of the milk obtained during cheese or curd making which is rich in lactose, minerals, water soluble vitamins and contains lactalbumin and traces of fat (Bylund, 2003). To the western world, it originates from cheese, casein and yoghurt industry whereas, in Bangladesh sweetmeat industry is the main producers. Whey is one of the most promising nutritional by-products of the dairy sector, particularly in the cheese, chhana, sweetmeats, and paneer industries (Islam et al., 2021). The whey presents greenish yellow color and according to Kumar et al. (2016) the pH of the whey can vary from 4.0 to 6.6 depending on the coagulation types of the milk. From 10 kg of milk used for the cheese production, almost 9 kg of whey- the main by product is obtained (Prazeres et al., 2012). Approximately 180 to 190 tons of whey per year was produced worldwide (Mollea et al., 2013), but, unfortunately only about 50% of this valuable product is utilized into various food and feed products. Rest of the unutilized whey is usually disposed in sewerage, rivers and other water bodies that causes foremost polluting effects on environment (Smithers, 2015). Wastage of this whey causes huge nutritional and economic loses as well. However, environment control regulations are more aware of its cheap disposal directly to the environment owe to both of its high biological and chemical oxygen demand.

Whey proteins are recommended due to their benefits in boosting immunity and lowering the risk of heart disease and cancer (Li et al., 2005).
Whey has been used to make whey protein concentrate (WPC), whey powder, lactose, lactic acid, whey pastes, and other products all over the world. Opportunities for wide-range applications of whey and whey constituents in the food and animal feed industry has been enhanced due to the presence of several nutritionally important constituents having excellent functional characteristics that improve the nutritional status and immunity of the body. Hence, the development of technology to utilize whey for the manufacture of a variety of new food products as well as for the replacement of comparatively expensive food ingredients is regarded as an important research and development activity of food industry (Mathur and Shahani, 1979). Aslam (2017) prepared a probiotic beverage using whey and orange juice. Islam et al. (2021) also prepared a whey pineapple juice blended probiotic beverage.

Milk processing techniques are the primary determinants of the type and composition of whey. The most common type of whey originates from the cheese manufacturing industry based on coagulating the casein by rennet or by other coagulating enzymes. The use of coagulants other than the calf rennet is attributed to the high demand (compare to the availability) and price of the calf rennet, religious point of view (Islam and Judaism), food habits (e.g. vegetarian) and consumer preference (e.g. organic acids for acid curd and sweetmeats in Indian sub-continent). The major coagulants types are aspartate, serine and cysteine proteases that are different in their proteolytic actions. Finally, leads to the variation in the milk gel formation and thus the drainage to the whey. Cheese composition, nitrogen fractions, degradation pattern of αs1-casein, β-casein and textural properties during ripening are directly associated with the coagulants used (Sengul, 2014). According to Mamo and Balasubramanian (2008), lipolytic enzymes contained in the rennet extracted from a crude abomasum attributed to characteristic flavor of whey and cheese. Among the different coagulants used, the domain of papaya latex is poor compared to the others and the crude calf rennet has been used since time immemorial for the production of artisanal cheese in different parts of the Bangladesh, especially, in the Ostogram and Kulicharch of Kishoreganj district. The use of coagulants are found different around the globe depending on the market demand of the types of manufactured dairy product and hence, necessitates the reporting of whey quality in response of the use of different milk coagulant.

Therefore, in this work we attempt to report comparatively the chemical and microbial quality of the whey produced by coagulating the cow milk using calf rennet, microbial rennet, lactic acid and papaya (Carica papaya) latex.

**MATERIALS AND METHODS**

**Site of the study**

The study was conducted at the Dairy Chemistry and Technology Laboratory, Department of Dairy Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. In addition, protein test was carried out in the Animal Nutrition Laboratory, BAU and minerals were analyzed in the Agricultural Chemistry Laboratory, BAU.

**Collection of fresh milk and coagulants**

Milk (Table 1) was collected from Bangladesh Agricultural University Dairy Farm. Six litters of fresh whole milk were collected for each trial. Preparation of different coagulants are presented in Table 2. An abomasum of a calf of 11 months old was collected from Ostogram, Kishorganj district. Papaya was collected from on campus plantation of BAU. Commercial preparation of lactic acid (Merck, D6100 Darmastadt, F. R. Germany, concentration 90%) and commercial rennet (Chr. Hansen A/s, Denmark) was also used as coagulant.

**Preparation of whey**

The freshly collected milk was heated up to 90 °C, followed by cooling to 40 °C. After that, 2% undefined composite lactic starter culture was added to the measured amount of milk and kept at 40 °C for 1 hr. After incubation, milk was divided into 4 equal parts (= 1.2 L in each part) in different beakers. Calcium chloride solution was added to each beaker @ 0.02%. Followed by mixing and adding calf rennet 100 mL, microbial rennet 10 mL, lactic acid solution 120 mL and papaya latex 120 mL to 4 different beakers separately and then waited up to coagulation (Checked by spatula cutting of the curd). After complete coagulation, the curd was cut and whey was drained out by muslin cloth. Then it was kept in refrigerator until further analyses.
Quality of whey using different coagulants

Table 1: pH, acidity (%), compositional (%) and microbial features (log cfu/mL) of the raw milk.

| Measurements       | Quantity |
|--------------------|----------|
| pH                 | 6.25     |
| Acidity            | 0.16     |
| Total solids       | 12.7     |
| Protein            | 3.34     |
| Fat                | 3.56     |
| Calcium            | 0.10     |
| Phosphorus         | 0.06     |
| Magnesium          | 0.04     |
| SPC                | 7.00     |
| Coliform           | 3.00     |

*SPC, Standard Plate Count; cfu, Colony forming Unit.

Table 2: Preparation of different types of coagulant.

| Types of coagulants | Preparation of coagulants |
|---------------------|---------------------------|
| Calf rennet         | 750 ml water + 250 ml milk + 100 g abomasum (kept overnight) |
| Microbial rennet    | 1000 ml water + 7 g microbial rennet |
| Lactic acid         | 120 ml water + 1.5 ml lactic acid |
| Papaya latex        | 120 ml water + 6 drops papaya latex |

Chemical tests

The milk and whey pH was measured with the help of pH meter-215 (ciba coming diagnostic limited). Acidity was determined by titrating with N/10 sodium hydroxide. Total solids was determined by oven drying and ash of the samples was determined by the ignition of the oven dried sample in muffle furnace according to AOAC (2004). Fat test was done by Babcock method. Protein was estimated by Kjeldhal method and 6.38 was used to convert the N into crude protein. Calcium content was assayed by EDTA titrimetric method in the presence of calcon indicator. Phosphorus content was determined by spectrophotometric (TG-80U, Germany) method using SnCl₂ as reducing agent and sulphomolybdic acid. The color intensity was measured at 660 nm. EDTA titrimetric method was used for the determination of Mg concentration in the presence of eriochrome black T (EBT) indicator.

Microbiological test

Standard plate count method was used to determine the total bacterial count. The SPC agar (HiMedia, India) was used and the incubation temperature was 34 °C for 48 hrs in an oven (J. P. SELECTA, Barcelona, Spain). The coliform count was done in a VRB agar (HiMedia, India) plate. An incubation temperature of 32 °C was used for 24 hrs in the oven (J. P. SELECTA, Barcelona, Spain).

Statistical analysis

The grouping of the recorded variables was done based on coagulants used. All the analyses were done in triplicate. One way ANOVA was obtained from the Minitab 16 where Tukey’s test was done for mean separation.

RESULTS AND DISCUSSION

Yield of whey

Maximum whey yields (763-765 mL/L) were obtained from the calf rennet, lactic acid and papaya latex coagulation of the milk (p>0.05). The yield of whey from the microbial rennet of the milk was significantly (p=0.000) lower (17 mL/L) than that of those three coagulants. Jayaprakasha (1992) reported 80 to 90% whey yield during the cheese or casein preparation on the basis of original milk used. In the present study, we found 74 - 76% which is not too far from the report. According to Jelen (1979) 1 kg cheese production yields 8.7 - 9 kg of whey production. Hill and Ferrer (2021) and Bylund (2003) also mentioned the similar pattern of whey yield during the cheese production.

pH and acidity

The average pH was found 5.48-6.18 in whey using different coagulants. Bund and Pandit (2005) found that pH content of sweet whey is 5.8-6.6 which is 4.0-5.0 in acid whey. Yadav et al. (2015) reported a different pH range of 3.57 - 4.34 and 6.02-6.58 for acid and sweet whey, respectively. The most commonly encountered type of whey drained out from manufacture of cheese and/or certain casein products, where the mechanism of processing is based on the casein
coagulation by the action of rennet. An industrial casein-clotting preparation generally comprising of chymosin or other casein-coagulating enzymes. Walstra et al. (2006) mentioned that the optimum pH for mozzarella and cottage cheese are 5.2 - 5.3 and 4.6 to 4.8, respectively. So, the requirement of the main product is the pre-determined factor to set the pH of the whey.

The highest (0.12%) acidity content was found when microbial rennet used as coagulant and the lowest (0.07%) value was found in calf rennet whey. These are not consistent with the results on pH. Bund and Pandit (2005) reported that acidity content of sweet whey is 0.10-0.20% and the acid whey was 0.20-0.40%. These results are also different from the present study.

**Total solids**

The highest total solids content was found in the lactic acid coagulated whey (7%) followed by the

| Variables                  | Microbial rennet | Calf rennet | Lactic acid | Papaya latex | p-value |
|----------------------------|------------------|-------------|-------------|--------------|---------|
| Yield of whey (mL/L)       | 747.67±2.52      | 762.67±2.52 | 764.67±2.52 | 763.00±2.00  | 0.000   |
| pH                        | 6.18±0.020       | 5.48±0.08   | 5.62±0.06   | 6.06±0.05    | 0.000   |
| Acidity (%)               | 0.12±0.005       | 0.07±0.005  | 0.08±0.005  | 0.11±0.01    | 0.000   |
| Total solids (%)          | 6.22±0.030       | 6.25±0.02   | 7.16±0.03   | 6.13±0.02    | 0.000   |
| Protein (%)               | 0.93±0.02        | 0.91±0.02   | 0.99±0.02   | 0.92±0.01    | 0.001   |
| Fat (%)                   | 0.50±0.10        | 0.77±0.03   | 0.63±0.01   | 0.52±0.03    | 0.001   |
| CHO (%)                   | 4.26±0.01        | 3.96±0.04   | 4.83±0.03   | 4.13±0.02    | 0.000   |
| Ash (%)                   | 0.53±0.02        | 0.74±0.02   | 0.64±0.02   | 0.54±0.02    | 0.000   |
| Calcium (%)               | 0.06±0.002       | 0.09±0.005  | 0.09±0.009  | 0.08±0.005   | 0.001   |
| Phosphorus (%)            | 0.03±0.005       | 0.06±0.00   | 0.04±0.01   | 0.04±0.005   | 0.005   |
| Magnesium (%)             | 0.01±0.003       | 0.02±0.003  | 0.02±0.008  | 0.02±0.002   | 0.173   |
| SPC (cfu × 10⁴/ml)        | 117.33±6.43      | 91.00±3.61  | 44.33±4.04  | 77.67±2.52   | 0.000   |
| Coliform (cfu × 10⁴/ml)   | nil              | nil         | Nil         | nil          | -       |

Means with the different superscripts in a row differed significantly; SPC, Standard plate count; Cf, colony forming unit.

**Protein content**

The whey obtained from lactic acid coagulation showed significantly (p<0.001) higher crude protein content (0.99%) which was 0.06 - 0.08% higher than the other three types of whey who among them were found statistically similar (p>0.05). According to Ganju and Gogate (2017) the protein content of whey may range from 0.80 to 1.0%. Khamrui and Rajorhia (1998) found highest protein in the buffalo milk cheddar cheese whey (0.98%) and that of buffalo milk chhana whey was 0.89%. They also observed 0.30 and 0.38% protein in paneer whey and shrikhand whey, respectively. All others were ranged
between 0.7 - 0.9%. All these reported data support the present results and indicates the significance of types of milk used and milk processing from where the whey is obtained.

**Fat content**

Calf rennet has the lowest efficiency in the recovery of fat content in cheese as a result highest percent of fat was found 0.77% in the calf rennet whey whereas, the lowest (0.50%) fat percent was found in microbial rennet whey. Khamrui and Rajorhia (1998) found that fat content of sweet whey of cow milk was 0.06% and buffalo milk was 0.34%. And the acid whey of cow milk was 0.10% and buffalo milk was 0.10%. Kosikowski (1999) also reported that fat content of sweet whey is 0.5% and acid whey is 0.04%. All these studies support the result of present study. Bylund (2003) reported 0.05% fat both in cheese and acid whey, however, Hill and Ferrer (2021) mentioned that 0.04% - 0.06% fat in whey after cream separation. These variations are related with several different factors like technologies applied (Aldalur et al. 2019), Milk coagulation properties (Vacca, et al. 2020) and types of cheese (Margolies et al. 2017) or product.

**Ash content**

The ash content of different whey differed significantly (p=0.000). Whey from calf rennet coagulation had the maximum (0.74%) ash content which is 0.20% higher than the whey obtained after microbial rennet and papaya latex coagulation and 0.10% higher than the lactic acid type. Gupta (2000) found that the ash content of sweet whey is 0.60% and that of the acid whey is 0.70%. On the other hand, Yadav et al., (2015) found 0.61% ash in the whey. Khamrui and Rajorhia (1998) demonstrated the differences between the milk types and whey types. According to them, the sweet whey obtained from cow and buffalo milk contains 0.59% and 0.54% ash, respectively. Which is 0.57% and 0.44%, respectively for the acid whey. However, all these are in a reasonable range.

**Mineral content**

The average calcium content was found 0.06-0.09% and phosphorus content was found 0.03-0.06% in experimental types of whey. Khamrui and Rajorhia (1998) found that the calcium content of sweet whey of cow milk is 0.04% and buffalo milk is 0.05%. In case of acid whey these are 0.05% and 0.04%, respectively. They also reported that the phosphorus content of sweet whey of cow and buffalo milk are same (0.04%) and buffalo milk acid whey has 0.01% more phosphorus. All these are in line with the results of the present study. The magnesium content was ranged between 0.01 to 0.02% which differs non-significantly among the whey types. Morr (1984) reported that magnesium content of whey is 0.013%. Bund and Pandit (2005) found the magnesium content of whey as 0.023%. Which also support the result of present study.

**Microbiological count**

The maximum standard plate count was observed in microbial rennet whey which was 117.33 × 10^4/ mL. This was followed by calf rennet whey which is 26 × 10^4/ mL less than the microbial rennet whey. Lactic acid whey has a count half of the calf rennet while the papaya latex whey is intermediate of calf rennet and lactic acid whey with 40 × 10^4/mL less value than the microbial rennet whey. Coliform count was found nil in all types of the whey. Deosarkar (2004) reported that Escherichia coli should not be present in a good and well-prepared cheese as high acidity of the fermented product should restrict their survival. Slow acidity development can allow sufficient buildup of E. coli to give a “taint” or “off flavour” to this retail product which is a clear indication of gross contamination.

**CONCLUSIONS**

This study was conducted to know the chemical composition and microbial quality of whey from fresh cow milk using microbial rennet, calf rennet, lactic acid and papaya latex. Variations were found in the yield of whey and concentration of different milk constituents in the whey including calcium and phosphorus. This indicates the importance of nutrient recovery from the whey and effects of different coagulant in the preparation of milk curd. However, the milk nutrient loss in whey or curd yield and recovery of milk constituents in curd is a multifactor variables and need to consider all to make a conclusive statement.

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