Formulation of hypotonic electrolyte re-hydration whey drinks from paneer and cheese whey

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ABSTRACT

In the present study, formulation of electrolyte drink from paneer and cheese whey was done by enzymatic hydrolysis of lactose to simple sugars and adjusting the salt levels to meet the World Health Organization requirements (245 mOsm/L) for Oral Rehydration Salt. Complete hydrolysis of lactose was achieved in whey using Lactozyme enzyme (3000L) used at level of 2 ml/L and incubated at 40°C for 2 h at neutral pH. Lactose hydrolyzed whey was diluted (1:2.3, v/v) using potable quality demineralised water (Total dissolved solids: 60 ppm) to achieve reduction in osmolarity of 7 5 mosm/l. The salt concentration was adjusted by adding 3.25 g sodium chloride and 0.8 g potassium chloride for paneer whey (per L) and 2.5 g sodium chloride, 0.9 g of potassium chloride and 1.5 g of trisodium citrate for cheese whey (per L) to meet sodium, potassium and citrate levels in the resultant whey drink.

Key words: Cheese whey, Electrolyte, Lactose hydrolysis, Osmolarity, Paneer whey, Rehydration drink.

INTRODUCTION

Rehydration or electrolyte drink contains water, electrolytes and carbohydrates in ideal concentrations to maintain the osmolarity of the body fluid. This is a special type of drink required or recommended by medical practitioners for those persons who are dehydrated. Dehydration is a condition of the body wherein much loss of water and depletion of electrolytes takes place causing the personnel to be fatigued, prone to muscle cramps and poor performance (Wagner, 2013). People suffering from many illnesses like vomiting, diarrhea, food poisoning, severe burns needs to be rehydrated under medical supervision. Rehydration is also necessary for persons involved in strenuous occupation like sports, body building, competitive athletics, persons working in outdoors at higher tempera tures or heat. Replenishing of water with electrolytes and sugar through suitable drinks is very much essential to keep the body hydrated, restore electrolyte balance, prevent muscle cramps and to boost up the endurance of the performers (von Duvillard et al., 2008; Rodriguez et al., 2009 ; Maughan, 2015).

Electrolyte solutions contain salts of sodium, potassium, citrates and simple sugars. Electrolytes allow the body to stay hydrated, help the brain and nervous system to trans mit and receive important signals, help the body’s cells generate energy and allow the muscles to contract and relax. Sodium level in body is critical for maintaining fluid balance, nerve function, muscle contractions and acid-base balance. The sodium level in blood of minimum 45 mEq/L is critical in maintaining fluid balance during dehydrated conditions. Excessive losses of sodium (via sweat) can lead to muscle cramps and in some cases hyponatremia (low blood sodium) both can lead to a decrease in performance and can cause major health complications (Ryan, 2012). Potassium in conjunction with sodium helps to alleviate and prevent muscle cramps. High potassium levels in the drink (> 30 mEq/L) might be harmful to individuals suffering from kidney or cardiac disfunction and may be undesirable even for normal individuals after extreme exercise (Wagner, 2013; Bruce et al., 2016).

During the past 20 years, a numerous studies have been undertaken to develop an ‘improved’ Oral Rehydration Salts (ORS) to avoid possible adverse effects of hypertonicity on net fluid absorption. This was done by reducing the solution’s glucose and salt (NaCl) concentrations in the old ORS formulation. As per the technical recommendations made by several studies, WHO and UNICEF launched a new ORS solution with reduced osmolarity which is safe and effective against rehydration (WHO and UNICEF, 2006). The new formulation for reduced ORS includes sodium (75 mmol/L), chloride (65 mmol/L), glucose, anhydrous (75 mmol/L), potassium (20 mmol/L) and citrate (10 mmol/L) with a total osmolarity of 245 mOsm/L.

Sodium bicarbonate or sodium citrate are alkalinizing agents added to electrolyte drink decrease incidence of acidosis, it also provide some energy and buffers lactic acid in the blood. Few researches have shown that they can help in delaying the onset of fatigue and enhance endurance capacity (Ryan, 2012). Simple sugars in the drink
facilitate water absorption from intestine and help to prevent fatigue by giving immediate energy to the body cells. Calcium and magnesium is critical for healthy muscle function and can be included in next generation sports drinks. Calcium (240 mg/L) in rehydration drink is essential for muscle contraction, while magnesium (500 mg/L) aids in muscle relaxation (Clark, 2008; Perderson et al., 2016).

Several studies concluded that rehydration drinks having lower osmolarity provide faster hydration with lower stomach discomfort. A person ingesting concentrated fruit juice possessing high osmolarity, the components of fruit juice does not get absorbed in human body quickly, hence it is required to be diluted to a lower osmolarity. So these hyper osmolar drinks can lead to nausea, stomach pain and gastrointestinal distress and is not recommended for immediate recovery of the dehydrated persons (James et al., 2012). So in the production of rehydration drinks, osmolarity is one such factor to be considered during the formulations. As per WHO recommendations, Oral Rehydration Solution (ORS) electrolyte drinks, of hypo osmolar type, should possess osmolarity of 245 mOsm/L which is lesser than the blood osmolarity (~295 mOsm/L) (Shirreffs et al., 2004).

Whey is a by-product obtained during the production of cheese, chhana and paneer. Whey constitutes about 45 - 50 % of total milk solids, 70 % of the milk sugar (lactose), 20 % of the milk proteins and 70 – 90 % of the milk minerals and almost all the water soluble vitamins originally present in milk (Horton, 1995). The paneer and cheese whey differ mainly in mineral composition due to the differences in conditions of process preparation hence, formulation was carried out to adjust mineral content, accordingly in both the products. The present study was undertaken to formulate hypo osmolar electrolyte whey drink by hydrolyzing lactose, formulating the salt and sugar concentrations in paneer and cheese whey.

MATERIALS AND METHODS

Fresh whey was obtained from Student’s Experimental Dairy Plant, Dairy Science College, KVAFSU, Hebbal, Bengaluru. Paneer and cheese whey were subjected to centrifugation (Froilabo, SW9, France) at 3000 g/ 10 min to remove residual fat and fine particles of colloidal caseinate. Commercially available Lactozyme (3000L, HP-G) manufactured by NOVO NORDISK, Denmark was used to hydrolyze lactose present in whey. Sodium chloride, sodium citrate and citric acid were of food grade (99 % purity) and purchased from standard companies of India like Ranbaxy and S.D. Fine chemicals Ltd. India. Demineralized water was obtained by filtering water (Total dissolved solids: 250 ppm) by RO purification system (ZERO B Technology Ltd., Mumbai, India) to obtain water with lower TDS (60 ppm).

The processing parameters viz, enzyme levels, temperature and pH were optimized to obtain the maximum hydrolysis of lactose in whey by Lactozyme. The effect of enzyme level on lactose hydrolysis was tested by incubating whey samples at 40°C and pH 7.0 with the addition of enzyme at the rate of 1 ml/L (3U/ml of enzyme), 2 ml/L (6 U/ml of enzyme) and 3 ml/L (9U/ml of enzyme) and incubated for 3 hours (Martinez-Villaluzenza et al., 2008). To know the effect of temperature on hydrolysis, the pH of whey was adjusted to 7.0 using 1 N NaOH and the enzyme was added at the rate of 2 ml/L and samples were incubated at 30°C, 40°C and 50°C for a period of 1 h, 2 h and 3 h. The effect of pH on lactose hydrolysis was tested by incubating the whey sample at 40°C, by adding enzyme at the rate of 2 ml/L and the pH was adjusted to 6.5, 7.0 and 7.5. The samples were drawn at regular intervals of one hour and analyzed for glucose content (Roy and Sen, 1991) to determine the degree of hydrolysis (%).

Lactose content in whey was estimated by Lane Eynon method as described by Ranganna (1986). Lactose hydrolysis yielded two monomers viz., glucose and galactose units by the action of lactozyme enzyme. So the presence of glucose in the sample is an indication of breakdown of lactose into equimolar concentrations of glucose and galactose. Glucose content in whey samples was determined by using modified Barfoed’s reagent and phosphomolybic acid, using standard glucose curve (Roy and Sen, 1991). Degree of hydrolysis (DH) of lactose, expressed in per cent was calculated by comparing the colour intensities spectrophotometrically (optical density at 620 nm) of lactose hydrolysed samples with unhydrolysed sample (control), taking 5 % standard glucose solution as maximum hydrolysis (100 %).

The sugar and salt concentrations were adjusted in whey to meet the requirements of WHO recommendation as in ORS rehydration drink formula which contain Dextrose anhydrous (13.5 g/L), sodium chloride (2.6 g/L), potassium chloride (1.5 g/L) and sodium citrate (2.9 g/L) with an osmolarity of 245 mOsm/L.

The contribution of sugars (glucose and galactose) towards osmolarity was high (250 mOsm/L) in lactose hydrolyzed whey when compared to sodium salts. So the sugar content was reduced from 45 g/L to 13.6 g/L (paneer whey and from 48 g/L to 14.5 g/L (cheese whey) by diluting them with demineralised potable water (1: 2.3 v/v; whey:water) to obtain osmolarity of 75 mOsm/L. Dilution of whey brought down the concentrations of salt elements (Na, K and citrate). So, calculated amount of Na, K and citrate salts were added to adjust their levels in whey.

The Na and K content were adjusted to requisite level (Na: 75 mOsm/L ; K: 20 mOsm/L) by adding 3.25 g of NaCl and 0.8 g of KCl per L of diluted paneer whey and 2.5 g of NaCl and 0.9 g of KCl per L of diluted cheese whey. The citrate content in paneer whey was reduced to 2.3 g/L upon dilution and so 1.9 g trisodium citrate per L was added to cheese whey to obtain desired citrate content (10 mOsm/L).
Calculation of osmolarity of whey: Osmolarity was calculated by substituting the concentration of each electrolyte (sugar and salts), their molecular weight and the number of species they dissociate in solution form.

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\text{mOsm/L} = \frac{\text{Weight of substance (g/L) x Number of dissociated particles x 1000 Molecular Weight (g)}}{1000}
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The whey (paneer and cheese whey) samples were analyzed for their various constituents like total solids, lactose, fat, protein, ash and major mineral composition. Total solids, ash and acidity were determined by standard method (BIS, 1981). Fat content was determined by Rose – Gottlieb method (AOAC, 2000). Protein content was determined by the micro Kjeldhal method (AOAC, 1995). Lactose content was estimated by Lane – Eynon method (Ranganna, 1986). Sodium and potassium in whey sample were estimated by flame photometer-128 (SYSTRONICS India Ltd., Ahmedabad, India). Calcium and magnesium in whey/formulated whey drink was analyzed by Atomic Absorption Spectrometer (PERKIN ELMER AAS 200, Waltham, USA). Chloride content of whey was estimated by Mohr’s method (Mahindru, 1987). Citrate content of whey was estimated by spectrophotometrically (Indyk and Kurmann, 1987). pH of samples were measured by digital pH meter (ELICO Pvt. Ltd., Hyderabad, India).

Statistical analysis: Each treatment was replicated thrice and recorded data were statistically analyzed at 5 % level of significance. The analysis was performed using “R” statistical software (R-Version 3.1.3 R Foundation for Statistical Computing). The data are presented as mean ± SE.

RESULTS AND DISCUSSION

Chemical composition of paneer and cheese whey: Paneer and cheese whey were analyzed for their chemical composition and mineral content and the data are presented in Table 1. The results showed that the total solids, protein and lactose content of cheese whey were slightly greater than paneer whey but ash content of cheese whey was lower. Higher ash content in paneer whey might be due to solubilization of calcium and other salts at low pH (5.3) of coagulation. The major minerals present in paneer whey viz. Na, K, Ca, Mg and chloride concentrations were higher when compared to those in cheese whey. Citrate content in paneer whey was high due to addition of citric acid as coagulant during the preparation of paneer. The results are in agreement with the observations reported by Goyal and Gandhi (2009) with regard to the composition of paneer and cheese whey composition.

Lactose hydrolysis in whey: Lactose hydrolysis in whey was carried out at different pH, temperature and enzyme concentrations, expressed as percent degree of hydrolysis (% DH) (Table 2). It was observed that % DH was maximum (100 %) when whey was incubated at 40°C, neutral pH for 2 h added with 2 ml of lactoymzme enzyme per L. Even though maximum hydrolysis could be achieved at different enzyme levels and incubation period (1 ml -3 h, 2 ml -2 h and 3 ml -2 h), 2 ml of lactozyme and 2 h of incubation period was selected considering least enzyme level and shortest incubation period (i.e. 2 h). Singh and Singh (2012) observed 80 % DH of lactose in whey (prepared from skimmed milk in laboratory) using 2 ml/L of immobilized β - galactosidase enzyme (isolated from yeast culture Kluyveromyces marxianus in laboratory incubated for one h.

Osmolarity of electrolyte whey drink: The osmolarity contributed by sugars and salts in fresh whey, hydrolyzed whey and formulated whey drink are given in the Table 3. The osmolarity values of hydrolyzed paneer whey (250 mOsm/L) and cheese whey (266 mOsm/L) due to sugars were nearly double than that of unhydrolyzed paneer whey (125 mOsm/L) and cheese whey (133 mOsm/L); each dissociated molecule (i.e. glucose and galactose) contributes...
to osmolarity of the solution. Dilution of lactose hydrolyzed whey (1:2.3, whey: deionized water, v/v) during formulation brought down the osmolarity (mOsm/L) of solutions from 250 to 75 in and 266 to 79 in case of paneer whey and cheese whey, respectively. Slightly higher values of osmolarity in whey was 14.08, 30.94, 38.92 and 9.37, respectively. Osmolarity (mOsm/L) contributed by Na, K, Cl and citrate content were found to be higher for paneer whey than for cheese whey. Therefore, in our study, enhanced osmolarity which were lower than that of blood (295 mOsm/L). Hypotonic osmolar drinks containing electrolytes and carbohydrates facilitate faster water absorption and prevent fatigue as compared to isonic or hypertonic drinks. James et al. (2012) concluded that hypertonic drinks cannot be absorbed quickly, until it is diluted to a lower concentration and the fluid retention was greater in the body after ingestion of a solution comprising a blend of carbohydrate and milk protein than ingesting a solution of solely carbohydrate.

The osmolarity (mOsm/L) contributed by Na, K, Cl and citrates in paneer whey was 14.08, 30.94, 38.92 and 9.37 whereas for cheese whey the corresponding values were 14.35, 28.34, 37.88 and 9.37, respectively. Osmolarity (mOsm/L) due to Na, K, Cl and citrates in the reformulated product (i.e. lactose hydrolyzed whey formulated by dilution with water and incorporation of necessary salts) were 14.35, 28.34, 37.88 and 9.37 for paneer whey and 75, 20.08, 10.64 and 2.57 for cheese whey, respectively. Such values of osmolarity nearly met with the requirements of WHO formula (75, 20, 65 and 10 mOsM/L, respectively for Na, K, Ca, Mg, Cl and citrate).

The total osmolarity (mOsM/L) of fresh paneer whey and cheese whey was 261 and 245 respectively; mainly determined by lactose content. The total osmolarity of hydrolyzed whey was more than that of unhydrolyzed fresh whey due to breakdown of lactose to simple sugars (paneeर whey and cheese whey had values of 370 and 356 mOsM/L, respectively). The total osmolarity (mOsM/L) of electrolyte paneer whey drink and cheese whey drink was 249 and 245 respectively; such values could be considered hypotonic osmolarity which were lower than that of blood (295 mOsM/L). Hypotonic osmolar drinks containing electrolytes and carbohydrates facilitate faster water absorption and prevent fatigue as compared to isonic or hypertonic drinks. James et al. (2012) concluded that hypertonic drinks cannot be absorbed quickly, until it is diluted to a lower concentration and the fluid retention was greater in the body after ingestion of a solution comprising a blend of carbohydrate and milk protein rather than ingesting a solution of solely carbohydrate.

### Table 3: Osmolarity of untreated whey, lactose hydrolyzed whey and electrolyte whey drink.

| Constituents (Minerals and sugars) | Untreated whey | Lactose hydrolyzed whey | Electrolyte whey drink | WHO formula for ORS drinks |
|-----------------------------------|---------------|-------------------------|------------------------|---------------------------|
| Sodium                           | 14.08         | 14.08                   | 14.35                  | 75.00                     |
| Potassium                        | 30.94         | 30.94                   | 28.34                  | 20.08                     |
| Chlorides                        | 38.92         | 38.92                   | 37.88                  | 59.23                     |
| Citrates                         | 35.93         | 35.93                   | 9.37                   | 10.64                     |
| Total Osmolarity                 | 261.00        | 232.00                  | 356.00                 | 245.00                    |

The chemical composition including mineral content of paneer and cheese whey drinks are presented in Table 4. TS content of cheese whey (2.71 %) was slightly higher than paneer whey (2.57 %) and the minerals in terms of Na, K, Ca, Mg, Cl and citrate content were found to be higher for paneer whey drink as compared to cheese whey drink. The chemical composition of whey depends on the initial mineral content of whey and due to the difference in the preparation method for paneer (acidification) and cheese (enzymatic coagulation using rennet) making; heat treatment of milk in both cases are also quite different. Goyal and Gandhi (2009) reported that the chemical composition of paneer whey and cheese whey depends on the composition of milk used for preparation. While Jindal et al. (1993) and Baba et al. (2016) attributed the variation in composition of whey to the processing conditions meted out to milk in such product (paneer or cheese) making. James et al. (2014) and Hobson and James (2015) reported that incorporation of whey protein isolate to a carbohydrate based electrolyte (60:20) neither enhanced nor inhibited rehydration property in human being. Therefore, in our study, paneer and cheese whey drinks containing 0.17 g and 0.18 g of whey proteins, respectively might benefit in recovery of protein after exercise, without possibly affecting rehydration property.

### Table 4: Chemical composition and mineral content of whey based electrolyte drink.

| Constituents/parameters | Paneer whey (Mean±SE) | Cheese whey (Mean±SE) |
|-------------------------|------------------------|-----------------------|
| Total Solids (%)        | 2.57±0.16              | 2.71±0.11             |
| Fat (%)                 | 0                      | 0                     |
| Protein (%)             | 0.17±0.012             | 0.18±0.015            |
| Glucose (%)             | 1.36±0.015             | 1.45±0.014            |
| Ash (%)                 | 0.45±0.01              | 0.43±0.009            |
| Sodium(mg/L)            | 1200±1.52              | 1170±1.82             |
| Potassium(mg/L)         | 412±1.02               | 394±0.90              |
| Calcium(mg/L)           | 172±0.78               | 102±0.91              |
| Magnesium(mg/L)         | 29±0.08                | 25±0.085              |
| Chloride(mg/L)          | 20±0.18                | 1930±1.56             |
| Citrates(mg/L)          | 2300±1.89              | 2325±1.67             |
CONCLUSION
An attempt was made to formulate rehydration electrolyte drink utilizing byproduct whey in order to salvage the nutritious whey solids and to prepare value added whey drink products. Lactose hydrolysis in preparation of whey drinks can help lactose intolerant persons and increase the sweetness of drink. Formulation of paneer and cheese whey by adjusting sugar to salt ratio can be useful for persons who need to be hydrated with drinks having hypooosmolality.

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