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Formulation of Milk Permeate for Utilization as Electrolyte Beverages

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Abstract: This study was conducted to design electrolyte beverages utilizing milk permeate. The permeate was pretreated by going through several processes, which included; heat treatment, fermentation, and clarification to produce a low lactose and electrolyte rich base for electrolyte beverages. Two types of electrolyte beverages namely; permeate thirst quencher and permeate endurance performance were appropriately formulated using this base. The electrolyte composition of each permeate based-beverage was compared to the number of dissolved electrolytes per liter that labeled in the commercial sport drinks. Sensory properties of the permeate based-beverages was also evaluated. The result revealed that, milk permeate based-beverages had electrolyte compositions superior to those of their commercial counterparts. Sensory evaluation indicated that the two permeate based-beverages had good acceptability which is maintained under refrigeration for three weeks.

Key words: Milk permeate, Sports drinks, Electrolyte beverages.

INTRODUCTION

Milk permeate, which penetrates the membrane during UF process of milk has been regarded as waste product, although it contains high level of lactose, soluble proteins, vitamins and minerals. However, because of its high nutrient content and its disposal can pose environmental problems, there is interest in finding a value-added utilization for it.

The electrolyte beverage industry is a growing market sector, especially for sports drinks, which fall into the functional beverage category (Williams, 2001). Several attempts have been developed numerous of electrolyte beverages from milk permeate (Geilman *et al.*, 1990; Geilman *et al.*, 1992) and from whey permeate (Girsh, 1999; Djuric *et al.*, 2004; Beucler *et al.*, 2005). However, these beverages have not found their way to the beverage market, possibly because of their improper formulation.

Properly formulated sports drinks encourage voluntary fluid consumption, stimulate rapid fluid absorption, improve performance, augment physiological response, and deliver rehydration (Murray and Stofan, 2001). Most of sports drinks are formulated to fit these functions. Sport drinks are one of the responsive researched food products. A slight change in product formulation can alter the physiological and performance responses during exercise (Maughan and Murray, 2001).

Gatorade is the most researched sports beverage in the world. Sports drinks, such as Gatorade are formulated to supply appropriate amounts of carbohydrates and electrolytes needed to maintain the optimal performance (Williams, 2001).

Therefore, the present study was conducted to utilize milk permeate in formulating two versions of sports beverages comparable to their commercial counterparts of Gatorade.

MATERIALS AND METHODS

Milk Permeate:

UF milk permeate was obtained from Snow White Co., El-Oubor City, Industrial region, Egypt. The permeate was a by-product from the ultrafiltration of cow's skim milk at 50 oC using spiral-wound module membrane supplied by APV Pasilac, Denmark. The obtained permeate contained 6.1% total solids (about 5.3% lactose, 0.26% protein and 0.54% ash) and had a pH of 6.12. The permeate was immediately heated in a water bath at 80°C for 5 min and cooled to 4 oC, and kept frozen at -20 oC until use.

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Milk Permeate Pretreatment:

The frozen permeate was thawed at 4°C before use and cultured with (0.5 g per 100 mL) of thermophilic lactic acid culture in the form of freez-dried for direct vat set (DVS) containing *Lactobacillus helveticus* (LH 100) supplied by Chr. Hansen (Chr. Hansen's Lab, Copenhagen, Denmark). This process was aimed to reduction of lactose content in permeate. The inoculated permeate was next placed in a water bath at 42°C with constant stirring at 60 rpm/min. The pH was checked every 30 min for the first four hours and every hour thereafter. Food grade ammonium hydroxide was added to permeate (0.5 ml/650 ml) whenever the pH dropped below 5.0 until the pH reached approximately 6.0. After 24 hours, the fermentation process was terminated by heating the permeate at 80°C for 5 min. Activated charcoal powder (El-Nasr pharmaceutical chemicals Co., Egypt) was added to the fermented permeate at the rate of 0.01 g/L for clarification followed by centrifugation for 30 min at 3000 rpm. The mix was then filtered until a completely clear liquid was obtained. The clarified permeate was again heated at 80°C for 5 min.

Formulation of Permeate Based- Beverages:

To estimate the upper limits for replacement of electrolytes with permeate based beverages, the mineral composition of permeate was first compared with sweat electrolyte losses. Then two versions of permeate based-beverages were formulated. One version was designed as a thirst quenching formula and the other was for endurance use. The formulation process as later described in details comprising dilution of permeate with appropriate amount of double deionized water followed by mineral re-adjustment to obtain permeate based-products which meet the label data of their corresponding counterparts of Gatorade. A combination of sugars was further added to achieve a carbohydrate level typical as isotonic fluid. Orange flavor and color were later added to the product. The flavored beverages were then heated at 85°C for 5 min, filled while hot into 250-ml sterile glass bottles which crown corked and cooled to 5°C prior to refrigeration.

Chemical Analysis:

pH of the permeate was measured using a pH-meter (Orion model 410A, Boston, MA, USA). Total solids were determined by the drying oven. The protein content was determined by the semi-micro Kjeldahl method. Ash content was determined by incineration at 600°C in a muffle furnace. Lactose was calculated by difference. Minerals (Na, K, Ca, Mg and Zn) were determined using the atomic absorption spectrophotometer (Instrumentation Laboratory Spectrophotometer, USA). Phosphorus was determined using colorimetric method. Chloride was titrated according to the method of IDF, (1988). All chemical analyses were performed in triplicate.

Sensory Evaluation:

The two permeate based-beverages were sensory evaluated when fresh and during storage period for 3 weeks by a panel taste of five judges.

RESULTS AND DISCUSSION

Electrolytes:

The average electrolyte composition of milk permeate, expressed as mg per liter was as follows: sodium, 450; potassium, 1450; calcium, 357; magnesium, 78; phosphorus, 346; chloride, 1020; and zinc, 0.175. This was compared with electrolyte composition of human sweat quoted from Konopka, (2001). The comparison can be discerned simply side-by-side in Fig. (1). Surprisingly, the milk permeate resembles human sweat in terms of electrolyte composition. The electrolyte composition of both may be relatively different but comprises of almost the same elements. The main difference between them is primarily due to the differences in the individual electrolyte levels. Sodium level of sweat is greatly higher than permeate. Unlike sodium, the levels of most other electrolytes in milk permeate are generally higher than those in sweat. Potassium, in particular, is dramatically higher. The higher electrolyte levels of milk permeate than those of human sweat offer an advantage, but the high potassium level might be harmful to individuals suffering from kidney or cardiac disfunction, and may be undesirable even for normal individuals after extreme exercise. Thus, it was advisable, to download the potassium level of permeate by dilution with appropriate amount of deionized water. Hirokawa *et al.* (2007) showed that sweat composition varies greatly within and between the different individuals depending on the quantity secreted, fitness levels and the state of heat acclimatisation.

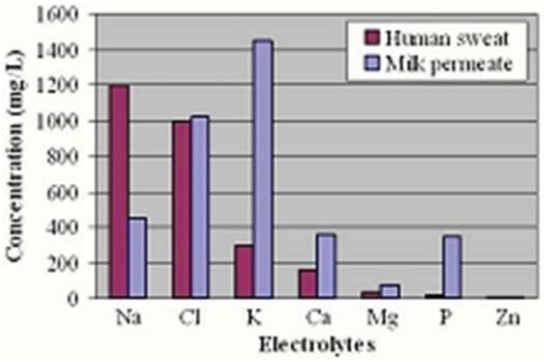

Fig. 1: A Comparison of the electrolyte composition of milk permeate versus that of human sweat under normal circumstances.

Since, the primary purpose of electrolyte beverages ingesting is to replace electrolytes lost through sweat, it was appropriate to compare the electrolyte composition of milk permeate to the electrolyte loss in sweat. The two fluids have never been compared in a comparative study. This electrolyte comparison chart is constructed to characterize the differences in the type and amount of electrolytes of milk permeate compared to those of sweat secreted under normal circumstances. Both the type and amount of electrolytes are key factors in a properly formulated electrolyte beverage. These differences are important to determine the upper limit for replacement of an electrolyte with permeate-based drinks. In a systematic investigation of the relationship between sweat sodium losses and the effectiveness of beverages with different sodium levels in restoring fluid balance, Shirreffs and Maughan (1998) showed that rehydration is achieved when the sodium intake is greater than the sweat sodium loss, although not all studies have reported similar findings (Mitchell *et al.*, 2000). It is suggested that the electrolyte levels in sports drinks should not exceed the upper limit. Adding excessive levels of electrolytes to products could be degenerative. Therefore, the formula must replenishing the loss of electrolytes through sweat in a proper way.

Milk Permeate-based Formulation:

Research in the field of sport's nutrition points out to the need to create products that are based on proper physiological responses. It also depends on whether the product is intended for practice or competition, and if it is for an endurance athlete in a sporting event or resistance training session. In view of that, Gatorade has developed two types of sports drinks, each of which contains various levels of electrolytes. Gatorade thirst quencher is a conventional sports drink formula contains sodium, chloride and potassium; the key electrolytes lost through sweat, and it is designed for most athletes during a typical workout. Gatorade endurance formula is another one with a blend of five electrolytes, contains the same chloride, nearly twice the sodium, and three times the potassium of Gatorade thirst quencher, along with calcium and magnesium, to more replaces what athletes lose during longer and more intense workouts. Accordingly, this study was conducted to create a formula that impact short term performance and another for long term performance as follows:

Permeate Thirst Quencher:

To be taken following normal exercise as a thirst quencher formula, the permeate was diluted with double deionized water about 1:10, to provide a potassium level approximated that of Gatorade thirst quencher (125 mg/L). This led to a decrease in potassium from the initial level of 1450 before dilution to 131 mg/L after dilution. Permeate after such extreme dilution, became too much low in sodium. To get enough sodium in the permeate (458 mg/L as Gatorade thirst quencher), sodium chloride (0.5g/L) and sodium citrate (1g/L) were added to the diluted permeate. This increased the sodium level of the permeate to 473 mg/L, from the estimated level of 41 mg/L after dilution. Addition of sodium chloride also increased the chloride level from 92 to 395 mg/L (nearly that of Gatorade thirst quencher; 375 mg/L), as it is inherently linked to sodium. The use of sodium citrate with sodium chloride instead of sodium chloride alone was advantagous as it increased the sodium level without excessive increasing the level of chloride. Oopvik, *et al.* (2003) also showed that use of sodium citrate (approximately 37 grams) improved running performance over 5 kilo meters by 30 seconds.

A comparison of electrolyte composition of permeate thirst quencher versus label data of Gatorade thirst quencher is shown in Fig. (2). Gatorade thirst quencher contains only sodium (458 mg/L), potassium (125 mg/L) and chloride (375 mg/L). Permeate thirst quencher had these three electrolytes in levels of 473, 131 and 395 mg/L, respectively, along with other readily occurring electrolytes, essentially calcium (32.4 mg/L), magnesium (7.1 mg/L) and phosphorus (31.5 mg/L). Because zinc was found only in a minimal amount in milk permeate before dilution, the zinc was diminished to undetectable levels after dilution.

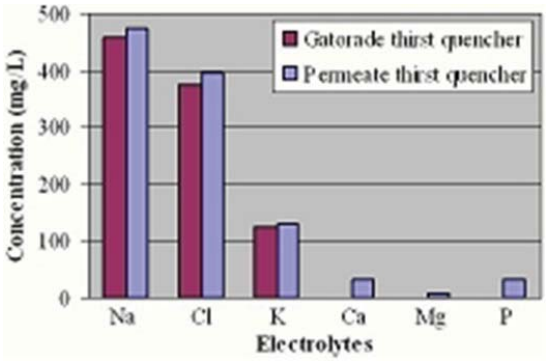

Fig. 2: A comparison of the electrolyte composition in permeate thirst quencher versus label data of Gatorade thirst quencher.

Sodium is the most important electrolyte in sports drinks because it is the most one lost in sweat and has the most powerful effect on stimulating rehydration. It also assists in muscle contraction and nerve impulse transmissions. Chloride is another electrolyte works synergistically with sodium and potassium. It controls electrolyte and fluid balance. Intake adequate amounts of sodium chloride, is crucial to maintaining the volume and balance of fluids outside the body's cells, including in the blood. In addition to its beneficial effect on enhancing water uptake from the intestine, the salt in sports drinks works in the brain to stimulate thirst and encourage drinking and minimize urine formation, thus improving the body's ability to hold water (Passe *et al.,* 2000).

During exercise, the body loses fluids and sodium through sweat. This causes a decrease in the blood volume, thereby increasing sodium and chloride levels in the blood. This what triggers the thirst mechanism. So restoration of blood volume is critical for the prevention of dehydration. Water consumption is effective in increasing the blood volume, however there is a consequential dilution of sodium in the blood due to the increased blood volume, so electrolyte replenishment is the key. Studies have found that the fluid from an 8 ounce serving of a sports drink with about 110 mg of sodium absorbs into the body faster than plain water. Unfortunately, most sports drinks are comprised mainly of sodium and potassium and lack the other valuable minerals, which the body uses for a variety of physiological functions (Maughan and Murray, 2001). However, calcium, for example, plays a central role in both the synthesis and breakdown of muscle and liver glycogen in addition to its role in bone formation and maintenance. Low serum blood levels of calcium can cause a muscular cramping (Rosenbloom, 2000). In addition to calcium intake, athletes should be aware that weight- bearing exercise is beneficial the maintenance of a healthy skeleton. Non-weight bearing sports like bicycling and swimming have been associated with bone mass similar to or below that of normal sedentary people (Duncan *et al.*, 2002; Warner *et al.*, 2002). Magnesium, too, is an important element when it comes to exercise. It is responsible for many metabolic processes required for exercise. Individuals who have low magnesium levels could suffer from muscle spasms during exercise (Rosenbloom, 2000). Zinc also is associated with optimal energy metabolism, endurance and tissue regeneration. Moderate levels of magnesium and zinc were shown to improve strength and muscle metabolism (Lukaski, 2000). Phosphorus also is an important component of the high-energy compounds essential for muscle function, as well as compounds that participate in oxygen delivery to muscle. Recent studies have shown that phosphate loading improves endurance performance (Folland *et al.*, 2008).

Permeate Endurance Formula:

For use as an endurance formula, to replace electrolytes lost during extreme exercise particularly in hot and humid climates, the permeate was diluted with double deionized water (1:3), to achieve a potassium level of 375 mg/L, as that of Gatorade endurance formula. This decreased the potassium level of permeate from the orignal level of 1450 before dilution to 363 mg/L after dilution. The dilution also decreased the sodium level to 112 mg/L. Thus, it was appropriate to raise the sodium level of the permeate by adding 0.2 gram sodium chloride and 2.75 gram sodium citrate per liter. This increased the sodium level from the basic level of 112 mg/L after dilution to 836 mg/L (comparable to that of Gatorade endurance formula; 833 mg/L). The addition of sodium chloride also increased the chloride from 255 to 376 mg/L (375 mg/L in Gatorade endurance formula).

A comparison of electrolyte composition in permeate endurance formula versus label data of Gatorade endurance formula is shown in Fig. (3). Beside sodium, potassium and chloride, Gatorade endurance formula contains also calcium and magnesium, but in levels far less than those in permeate endurance formula. Moreover, phosphorus is readily found in permeate endurance formula, Gatorade endurance formula don't include this nutritionally valuable mineral.

In extreme exercising conditions the consumption of a complex sports drink with electrolytes is recommended. Athletes who do not consume electrolytes under these conditions risk of overhydration (or hyponatremia) (Sawka and Montain, 2000 and Burke, 2001).

Carbohydrates:

As a result of fermentation process, the lactose content was reduced by approximately 50% in the fermented permeate. A further reduction of lactose was achieved as a result of subsequent dilution of fermented permeate when down the potassium load to the desired level. The lactose content was reduced to less than 1% in permeate endurance formula which has been diluted with water e.g 1:3, while almost complete lactose disappearance was found in permeate thirst quencher because of excessive dilution (1:10), leaving it virtually lactose-free.

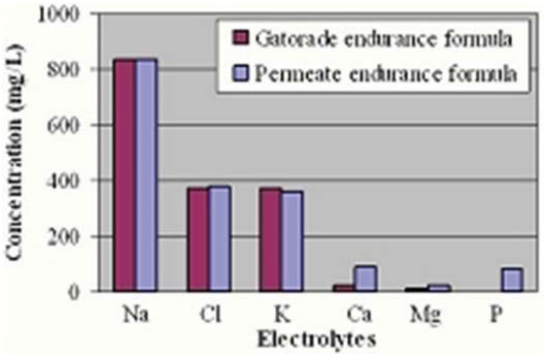

Fig. 3: A comparison of electrolyte composition in permeate endurance formula versus label data of Gatorade endurance formula.

Lactose in each milk permeate-based formula was replaced by adding 6% (typically as isotonic fluid) carbohydrates blend of selected sugars; sucrose, glucose and fructose 2:1:1, respectively. These sugars are appropriate types of carbohydrates for inclusion in sports drinks. Carbohydrates in sports drinks provide the most benefit during and after exercise because they help maintain blood glucose and muscle glycogen levels, which enhances performance (McConell *et al.,* 2000). Glucose is the body's preferred source of energy that is readily absorbed and therefore gives rise to reduced fatigue and faster recovery following exercise. Fructose takes longer time to convert into blood glucose and may be more beneficial in post-exercise drinks rather than during exercise (Anderson *et al,* 2002). Each type of sugar enhances fluid absorption from the intestine by a different manner, and therefore different carbohydrates create different blood sugar response, which is largely responsible for total energy output and intensity. Ultimately, this will allow longer exercise duration and the ability to increase the intensity at the end of the exercise bout.

The amount of carbohydrate in a beverage affects taste, fluid absorption and performance. Research has shown that 6% carbohydrate solution strikes the optimal balance in taste, rapid fluid absorption, and delivery of carbohydrate energy to fuel working muscles (Passe *et al*., 2000). Research indicates that sports drinks should be isotonic or hypotonic to ensure rapid gastric emptying. Isotonic drinks with a carbohydrate level of 6-8% are emptied from the stomach at a rate similar to water. Levels beyond this have been shown to decrease the rate of fluid absorption by decreasing gastric emptying and increasing the risks of gastrointestinal discomfort at no benefit to performance (Maughan and Murray, 2001). Rogers *et al*., (2005) indicated that lowering the carbohydrate level of a sport drink from 6% does not enhance gastric emptying, intestinal water absorption, or time trial performance, but reduces carbohydrate and solute absorption. Pollard *et al.* (2006) reported that a properly formulated sports drink should contain 4-8 percent carbohydrates, electrolytes (sodium and potassium), and water. Most of sports drinks are formulated to meet these criteria.

Sensory Evaluation:

A taste panel found the taste of permeate thirst quencher was bland and milder orange-tasting but pleasant. Permeate endurance formula was a slightly tart orange-tasting but very palatable, and not salty. Sodium citrate possesses a saline, mildly tart flavor, which does not have the same salty taste as sodium chloride. The use of citrate along with choloride rather than chloride alone elevated the sodium level of the permeate without sacrificing desired taste. The two permeate based-beverages maintained under refrigeration for three weeks without changes of the sensory properties during storage period. Additional research, using physically active individuals, is needed to assess the impact of the experimental beverages on short- and long-term performance.

Conclusion:

Milk permeate is a rich source of essential electrolytes, particularly those lost in sweat. It has the potential to appropriately formulate into electrolyte beverages. The two electrolyte beverages made from milk permeate were as good as or better than Gatorade because they provide a number of other readily occurring electrolytes such as calcium, potassium, magnesium, and phosphorus, which may be benefit for an athlete's health. The resulting electrolyte beverages may be taken as sports drinks after normal or vigorous exercise, to replace sweat lost. The proper formulation of permeate-based electrolyte beverages may open a new avenue to the beverage market.

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