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Studies on standardization of whey based isotonic sports beverages

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Abstract
The objective of the study was framed to develop whey based isotonic fruit drinks to provide maximum effectiveness to the sports persons by preventing the dehydration during performance. The paneer whey was used for the formulation of drinks. The preliminary study was conducted to select the best sample from 18 formulated samples. Their osmolality ranged between 202.02–278.8mOsm/kg H_2O. The selected sample has 226.83mOsm/kg of osmolality. From the selected sample, three variations such as T1 (pineapple + whey), T2 (peach + whey) and T3 (pomegranate + whey) were further formulated. The TSS and osmolality of isotonic samples (T1, T2 and T3) were 6.8±0.12^{0}B, 7.2±0.06^{0}B, 6.4±0.11^{0}B and 231mOsm/kg, 239 mOsm/kg, 232mOsm/kg respectively. The energy values (kcal/100g) of formulated drinks are T1 (36.98±0.52), T2 (29.01±0.29), T3 (26.66±0.67). The physiochemical parameters such as pH, TSS, acidity, Osmolality, moisture, protein, lactose, fat, ash and total carbohydrates of accepted sample T1 are 4.41±0.06, 6.8±0.12^{0}Brix, 0.25±0.00%, 231 mOsm/kg, 92.2±0.75%, 0.12±0.00%, 1.07±0.03%, 1.26±0.0%, 0.13±0.00% and 6.29±0.13% respectively.

Keywords: fluids, isotonic drink, osmolality, sports nutrition, whey

1. Introduction
The main objective behind the formulation of sports drink is to provide readily available form of nutrients mainly fluids, carbohydrates and electrolytes. It was consumed by athletes and other physical workers to prevent them from dehydration and heat exhaustion. (Rauj *et al*., 2017) [1]. The intake of protein that was widely accepted for endurance and power athletes has exceeded recommended daily allowance. Proteins that are of inferior content and digestibility are important to recognized and restricted or limited in the diet. The major sources of protein are animal protein and vegetable protein. (Las Vegas, 2005) [7]. Sports drinks supplements are becoming integral parts of professional and amateur exercise enthusiast and athletes. Carbohydrate-Electrolyte (CE) drink was gaining focus on improving performance lasting one hour or above. (Francesca Burton, 2011) [4].There is difference in nutrient content of paneer whey and cheese whey is mainly due to manufacturing process. The result indicated significantly higher concentration of sodium, potassium, calcium and chloride in paneer whey than cheese whey. So, paneer whey is best to utilized as electrolytic drink. (Nupur *et al*., 2009) [2]. The composition of liquid and dried acid and sweet whey and their concentrates differed in nutritionally important minerals. Calcium was three times greater and zinc was twenty times greater in acid whey as compared to sweet whey (Wong *et al*., 1978) [5]. Instant energy Ready To Serve (RTS) whey beverage was prepared by hydrolyzing lactose with immobilized β-galactosidase enzyme, isolated from yeast culture *Kluyveromyces marxianus*, for hard working group of people. The energy value of prepared beverage was 322±3.08 KJ per 100 mL of product. (Alak *et al*., 2012) [3]. The new isotonic drink was designed with lemon juice and anthocyanins rich-berries gained attractive color and consumer acceptance. The result showed significantly higher anti-oxidant content, in comparison with commercial isotonic beverage. (Amadeo *et al*., 2012) [6]. The study was investigated the effectiveness of isotonic drink and water consumption of the elite wrestlers during the exercise. The result concluded the post exercise sodium level was lower than pre-exercise and there was no significant difference between sodium values of the isotonic and water consumption group. And the study suggested that water should be replaced for sports drink during shorter activities. (Bilal *et al*., 2015)[8].The sports and energy (S/E) were used by high-school athletes of United States. The pilot study revealed that, 59.5% of athletes were at least occasional users of sports drinks, and more than 37.3% were at least occasional users of energy drinks. The most common motivation for consumption of sports drinks was to rehydrate (84.1%) and of energy drinks was to gain energy (61.8%). The 49.5% drank their first sport drink at ≤ 8 years and 41.3% consumed their first energy drink ≤ 11–12 years of age. (Sarah *et al*., 2015)[9].The isotonic drinks are more and more popular among the teenagers, sportsmen, elderly and fitness-oriented people. The proper osmolality of

isotonic drinks should be of 300 mOsm kg^{-1} of water±10%. The study result revealed that the osmolality of the majority of the drinks available in Tricity market (the area of three cities: Gdańsk, Sopot, Gdynia) was within the recommendations of European Union – from 270 to 330 mOsm kg^{-1} of water. Hypotonic drinks contain lower concentration of solute per unit volume than blood, as in the case of water which is recommended for use before exercise. It contains 4-6% sugar (Maughan *et al*., 2010) [11] Isotonic drinks contains simple sugars and electrolytes (sodium), giving the same osmotic pressure as blood. Generally, it was used during exercise. The characteristic of isotonic drinks are sugar content (6% to 9%) and osmolality (200-330mOsm/l). (Jeukendrup *et al*., 2011) [12] Hypertonic drinks contain higher concentration of solutes, simple sugars, and/or sodium per unit volume than blood, usually recommended after exercise. It contains 9-10% sugar. (Evans *et al*., 2009) [13] This study is therefore mainly focused on development of isotonic beverage using paneer whey, which will be a value addition to the byproduct which is usually discarded in the manufacturing process of paneer and also deliver a nutrition rich ready to drink beverage.

2. Materials and Methods

This research was carried out in Cavinkare Research Centre, Chennai-Tamil Nadu. The commercially available standardized milk was purchased from the local market near to the Research Centre. The analytical food grade acetic acid, glucose, sucrose, sodium chloride, sodium benzoate, sodium citrate, citric acid, pectin, pineapple juice concentrate, peach juice concentrate and pomegranate juice concentrate were sourced from several food ingredient suppliers. The paneer whey was prepared by using acetic acid as a coagulant. Then, it was pasteurized (85±2^{0}C) and stored at refrigerated temperature (4 ±1^{0}C) until further experimentation (less than 12 hours).

2.1. Experimental design – preliminary test

In this study was 18 different combinations of whey based carbohydrate-Electrolyte (C/E) using 3 variables such as amounts of carbohydrates (2%, 3%, 4%); concentration of sodium (18.66, 41.51 mmol/L) and percentage of whey (75%, 50%, 25%) was developed for sensory analysis. (Lorna *et al*., US Patent) Table 1.shown the variables used for preparation of isotonic drinks for sensory. The paneer whey was prepared and measured into pre-sterile stainless steel vessel, required amounts of ingredients were added and the total volume was made up to 100ml with addition of RO water. The solutions were pasteurized at 85^{0}C for 5 minutes and hot filled (75±2^{0}C) in PET bottles. The samples were kept at refrigerated temperature (4±1^{0}C) until evaluation was carried out. The sensory evaluation was conducted for the 18 different combinations of carbohydrate-electrolyte drinks. The Sensory evaluation was conducted for 18 different combinations of carbohydrate-electrolyte drinks using 9 point hedonic scale by the untrained panelists. Sweetness, tartness, saltiness, mouthfeel and overall acceptability were the parameters that were considered for sensory evaluation. The best sample was selected out of 18 samples at the end of the preliminary study.

Table 1: Variables used to develop the whey isotonic drink samples for the sensory analysis

Amount of Carbohydrates %	Concentration of sodium (mmol/L)	Percentage of whey (%)
2	18.66 41.51	75
3		50
4		25

2.2. Product development process

The composition of new isotonic beverage contained 25% of whey, 3g of carbohydrate (1g glucose; 2g sucrose), NaCl, sodium benzoate as permitted class II preservative (Spanish Law, Royal Decree 142/2002), sodium citrate, citric acid, pectin, pineapple juice concentrate (Equivalent to 10% fruit juice content) and RO water. In addition to this, two more variations of drinks such as peach and pomegranate based whey isotonic drink were prepared using the same propositions shown in Table. 2. One isotonic drink was assayed without fruit juice concentrate as control. The samples were pasteurized and filled in PET bottles.

The protocol of product development is showed in figure.1. The samples were labeled as follows: T0-control (without fruit), T1- whey + pineapple juice concentrate, T2 - whey + peach juice concentrate, T3 - whey + pomegranate juice concentrate.

Table 2: Formulation of different isotonic drink from the selected sample

Product descriptions				
Treatments	**Percentage (%) of CHO used**	**Concentration of sodium (mmol/L)**	**Percentage of Whey (%)**	**10 percentage (%) Fruit juice concentrate used**
T0	3	41.51	25	-
T1				Pineapple
T2				Peach
T3				Pomegranate

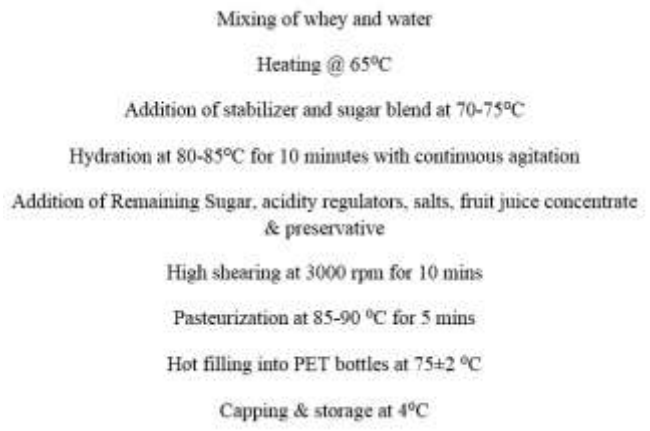

Fig 1: Steps involved in whey based fruit isotonic drink development

2.3. Proximate analysis
The pH, Titrable Acidity and total soluble solids (TSS) were analysed as quality indexes, following the method of Mena *et al*. (2011) [14].The Titrable acidity was expressed as gram of citric acid in sample and the TSS in ^{0} Brix. The fat content was analysed by Gerber method. (IS 1479 - Part I/1961) [15].The lactose (milk sugar) content in milk products was measured by Fehling's test. The Carbohydrate content of the samples was determined according to the following formula [16]. The % Available carbohydrate = 100 – (Protein% + Fat % + Fiber% + Ash% + Moisture %). Total energy of the samples was determined by the Atwater formula where carbohydrate, protein and fat supplied were 4, 4 and 9 kcal/100g respectively [16]. Total Energy (kcal/100g) = Carbohydrate% x 4+ Protein% x 4 + Fat% x 9. The protein content, total ash content, moisture content were determined according to AOAC method [17]

2.4. Sensory evaluation
A panel of 30 untrained judges was involved for the Sensory evaluation of the prepared samples by using nine-point hedonic scale, the sensory evaluation was conducted based on the sensory attributes such as color, taste, odor, mouthfeel, overall acceptability and the best sample was selected as the final product.

2.5. Storage stability study
The physical and microbiological parameters were checked once a week. The pH, Acidity (%), TSS and turbidity of the drink was checked once a week under ambient temperature and refrigerated condition (4±1^{0}C). These studies were conducted weekly to check if there are any significant influences to sensory and visual parameters of the drinks.

2.6. Microbiological analysis
The total plate count (TPC), Yeast & mold count (YMC), Coliform count and Flat sour count (FSC) were analysed on 7th, 14th and 21st day of storage period.

2.7. Statistical analysis
The collected data was analyzed by using AGRES Software statistical tool. The a, b, c and d was denoted as follows, ^{a} - 1st best treatment, ^{b} - 2nd best treatment, ^{c} - 3rd best treatment, ^{d} - 4th best treatment.

3. Result and discussion
3.1. Osmolality and tonicity of the carbohydrate-Electrolyte (C/E) drinks-preliminary test
Drinks with an osmolality similar to that of blood are called isotonic solution [12]. The Table 3.showed the osmolality and tonicity of the 18 Carbohydrate-Electrolyte drinks. Tonicity was graded as >330mOsm/kg H_2O (hypertonic); 200-330 mOsm/kg H_2O (isotonic) and <200 mOsm/kg H_2O (hypotonic) [18]. The casein incorporated CHO isotonic beverage (7% CHO+2%Pc) and whey protein incorporated CHO isotonic beverages (7% CHO+2%Pw) showed different physiological effects than the only CHO drink (9% CHO). The muscle glycogen re-synthesis is enhanced when protein are added to the CHO isotonic beverages [19].

Table 3: Osmolality and Tonicity of the samples

Amount of CHO %	Concentration of sodium (mmol/L)	Percentage of whey (%)	Osmolality (mOsm/kg)	Tonicity
2	18.66	75	202.02	Isotonic
		50	179.23	Hypotonic
		25	156.45	Hypotonic
	41.51	75	243.18	Isotonic
		50	220.40	Isotonic
		25	197.61	Hypotonic
3	18.66	75	231.23	Isotonic
		50	208.45	Isotonic
		25	185.76	Hypotonic
	41.51	75	272.40	Isotonic
		50	249.61	Isotonic
		25	226.83	Isotonic
4	18.66	75	260.45	Isotonic
		50	237.66	Isotonic
		25	214.98	Isotonic
	41.51	75	301.61	Isotonic
		50	278.83	Isotonic
		25	256.04	Isotonic

(Jeukendrup *et al*., 2011)

3.2. Osmolality and tonicity of further developed products
The Table 4.showed the osmolality and tonicity of sample, after adding the fruit juice concentrate and additives into the selected sample. The fruit juice concentrate contributed to the total soluble solid content which may exceed the carbohydrate content of isotonic drink (>9%). And also, lactose in whey leads to increase the total soluble solid content. To avoiding this condition, among the 18 variations, 3% CHO+41.51 mmol/litre of sodium+25% of whey sample was selected as a standard for further development of fruit incorporated whey isotonic drinks. The result showed that, osmolality and CHO content of developed samples, T1 (231mOsm/kg, 6.8%), T2 (239.4mOsm/kg, 7.2%), T3 (232.14mOsm/kg, 6.8%) respectively. These samples fall under the isotonic drink category. [12] The addition of fruit juice concentrates significantly altered the final pH, % acidity and TSS (^{0}B). Table 4.2 shows the TSS (^{0}B) of drinks T0 (5.0), T1 (6.8), T2 (7.2), T3 (6.4), pH of drinks T0 (5.1), T1 (4.41), T2 (4.24), T3 (4.4) and acidity (%) of drinks T0 (0.2), T1 (0.22), T2 (0.25), T3 (0.24) respectively.

Table 4: Osmolality and tonicity of fruit incorporated whey isotonic drink

Treatments	CHO (%)	Sodium (mmol/L)	Whey (%)	TSS (^{0}B)	Osmolality (mOsm/kg)	pH	Citric acid (%)	Tonicity
T0	3	41.51	25	5.0±0.07	226.83	5.1±0.05	0.20±0.00	Isotonic
T1				6.8±0.12	231.00	4.41±0.06	0.22±0.00	Isotonic
T2				7.2±0.06	239.40	4.24±0.13	0.25±0.00	Isotonic
T3				6.4±0.11	232.14	4.4±0.09	0.24±0.00	Isotonic

Value represents mean of four replication readings.

3.3. Nutrient composition of developed whey based fruit isotonic drinks

The nutritional composition of formulated drinks showed Table 5. The percentage protein content was higher in the samples T1, T2 and T3 compared to T0. This was due to the contribution of protein content of different fruits. After addition of fruits, the protein was significantly increased. T3 has highest protein content (0.23±0.00%). The moisture and lactose were not significantly differing as they were not altered by the incorporation of fruit in the whey isotonic drink. T1 had the highest fat content (1.26±0.01%), T2 had highest ash content (0.15±0.00) and T2 had the highest total carbohydrate content (7.01±0.03%). The energy value (kcal/100g) of drinks T0, T1, T2 and T3 were 22.95±0.56, 36.98±0.52, 29.01±0.29 and 26.66±0.67 respectively. Pineapple incorporated whey isotonic drink (T1) has the highest energy value (36.98±0.52). The results showed that, inclusion of fruits significantly affected the total carbohydrates, fat, protein and ash and energy values of samples T1, T2 and T3.

Table 5: Nutritional composition of formulated isotonic drinks

T	Moisture %	Protein %	Lactose %	Fat %	Ash %	Total CHO %	Energy (kcal/100g)
T0	94.2±0.64	0.07±0.00^d	1.07±0.01	0.03±0.00^a	0.1±0.00^d	5.60±0.11^c	22.95±0.56^d
T1	92.2±0.75	0.12±0.00^c	1.07±0.03	1.26±0.01^d	0.13±0.00^c	6.29±0.13^b	36.98±0.52^a
T2	93.0±2.40	0.13±0.00^b	1.07±0.03	0.05±0.00^b	0.15±0.00^a	7.01±0.03^a	29.01±0.29^b
T3	93.4±1.58	0.23±0.00^a	1.07±0.00	0.14±0.00^c	0.14±0.00^b	6.12±0.15^b	26.66±0.67^c

Value represents mean of four replication readings. Value with the same superscript letter(s) within the same column is not significantly different (p>0.05).

3.4. Storage stability of formulated beverages

Acidity and TSS

The formulated beverages were kept at both room temperature (30±2^{0}C) and refrigerated temperature (4±1^{0}C) for testing the stability of samples upto 30 days of storage periods. The incorporation of fruit juice concentrates (pineapple, peach, and pomegranate) was altered the pH, acidity and total soluble solids content of the samples (T1, T2, T3) from the control (T0). The Table 6 showed the acidity and TSS content of samples under room temperature (30±2^{0}C). The acidity in terms of percentage of citric acid of sample was increased T0 (0.20-0.24%), T1 (0.22-0.25%), T2 (0.25-0.28%) and T3 (0.24-0.27%) upon storage. The total soluble solids (^{0}Brix) of samples were slightly increased T0 (5-5.6), T1 (6.8-7.4), T2 (7.2-7.6) and T3 (6.4-6.8) during period of storage (21 days). [17] Mina *et al*., (2016) reported that during storage, TSS values significantly fluctuated. Mean TSS values of drinks significantly decreased on the 1st, 3rd, and 7th day, but on the 14th day, the TSS of the drinks significantly increased.

The Table 7 showed the acidity and total soluble content of formulated drinks under refrigerated condition. The acidity was increased of the samples T0 (0.2-0.22%), T1 (0.22-0.24%), T2 (0.25-0.26%), T3 (0.24-0.25%) from an initial period to till the end of storage period. TSS content also was fluctuated T0 (5-5.5), T1 (6.8-7.3), T2 (7.2-7.5), T3 (6.4-6.7) during the storage. The result of % of acidity was increased during storage under room temperature compared to refrigerated condition [20]. Chatterjee *et al*., (2015) reported that titrable acidity was increased under room temperature from 0.11-0.14% was comparatively highest than non-refrigerated condition from an initial value of 0.107% to 0.128%. [21] Sikder *et al*., (2001) reported that production of organic acids and amino acids lead to an increase in acidity thereby a decrease in pH, as also reported for mango based beverages and the TSS was fluctuated and increased [22]. Barwal *et al*., (2005) reported that the possibility for the increment in TSS content could be due to hydrolysis of sucrose to invert sugars. The results showed that when the samples were stored at room temperature, it was accepted upto 10 days and refrigerated, it was acceptable upto 21 days.

Table 6: Acidity and TSS content of formulated fruit incorporated whey isotonic drink under room temperature (30±2^{0}C)

Days of storage	Acidity (%)				TSS (^{0}B)			
	T0	T1	T2	T3	T0	T1	T2	T3
1	0.20^a	0.22^a	0.25^a	0.24^a	5.0^a	6.8^a	7.2^a	6.4^a
7	0.22^b	0.24^b	0.25^a	0.24^a	5.1^b	6.8^a	7.3^b	6.5^b
14	0.23^c	0.25^c	0.27^b	0.25^b	5.1^b	7.1^b	7.3^b	6.6^c
21	0.24^d	0.25^c	0.28^c	0.27^c	5.5^c	7.3^c	7.5^c	6.7^d

Value represents mean of four replication readings. Value with the same superscript letter(s) within the same column is not significantly different (p>0.05).

Table 7: Acidity and TSS content of formulated fruit incorporated whey isotonic drink under refrigerated condition (4±1^{0}C)

Days of storage	Acidity (%)				TSS (^{0}B)			
	T0	T1	T2	T3	T0	T1	T2	T3
1	0.20^a	0.22^a	0.25^a	0.24^a	5.0^a	6.8^a	7.2^a	6.4^a
7	0.21^b	0.22^a	0.25^a	0.24^a	5^a	6.8^a	7.2^a	6.4^a
14	0.21^b	0.23^b	0.25^a	0.24^a	5.1^b	7^b	7.2^b	6.5^b
21	0.22^c	0.24^c	0.26^b	0.25^b	5.3^c	7.1^c	7.4^c	6.7^c

Value represents mean of four replication readings. Value with the same superscript letter(s) within the same column is not significantly different (p>0.05).

3.5. Turbidity

Table 8 showed the turbidity changes during storage period which was only visually observed. The results of turbidity showed that, there was no turbidity up to the end of the 21st

day under refrigerated temperature. But, there was slight cloudiness was occurred up to the end of the 21st day under room temperature. Rauj *et al*., (2017) reported that all ingredients and desirable sensory attributes have to be preserved in sports drink during its shelf life. If only one ingredient or sensory attribute do not fulfill desired requirements, in spite of well microbiological findings, shelf-life should be shortened.

Table 8: Turbidity changes during room temperature and refrigerated temperature

Condition	End of 1st week	End of 2nd week	End of 3rd Week
Under room temperature	No turbidity	No turbidity	No turbidity
Under refrigerated temperature	No turbidity	No turbidity	No turbidity

3.6. Microbial results

The four treatments (T0, T1, T2 and T3) were subjected to microbial analysis such as total plate count (TPC), Yeast and mold count (YMC), Coliform count and flat sour count (FSC) at refrigerated storage condition (4±1^{0}C). Fungi such as yeast and mold, which are very resistant to low pH, can grow in formulated sports drinks. (Mina *et al*., 2016)[17]. The Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods (Food and Drugs Administration-Philippines, 2013) pointed out that samples with TPC that exceeds 10^{2}cfu/mL should be rejected as this indicates a potential health hazard or imminent spoilage. Table 8 showed that all samples with TPC except T0 were less than 10^{2}cfu/ml upto 21th day of storage. Foods with low pH values (4.5 and below) usually are not readily spoiled by bacteria but are more susceptible to spoilage by yeast and molds. For product stability and safety, Food and Drugs Administration-Philippines (2013) reported that YMC of sports drinks should not exceed 1 cfu/ml. Table 4.7 showed that YMC was <1 cfu/ml. And coliform count was <1 cfu/mL for all samples. The results showed that the flat source count was <1 cfu/ml for all the samples, it was fulfilled the specification of FSSAI standard for beverages. The microbial study was revealed that the developed samples are safe and acceptable upto 21 days at refrigerated condition.

Table 9: Microbial analysis of developed samples at refrigerated condition (4±1^{0}C)

T	TPC (cfu/ml)			YMC (cfu/ml)			CC (cfu/ml)			FSC (cfu/ml)		
	7th	14th	21st	7th	14th	21st	7th	14th	21st	7th	14th	21st
T0	<1	<1	3	<1	<1	2	<1	<1	<1	<1	<1	<1
T1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
T2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
T3	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

3.7. Sensory evaluation

Fig.2.shows the sensory evaluation of formulated drinks T0, T1, T2 and T3. T1 scored highest color acceptability, T1 and T2 scored highest taste acceptability, T1 scored highest odor acceptability and T0, T1, T3 scored highest in mouth feel. T1 was scored the highest overall acceptability among all other formulated drinks. This is due to pineapple juice concentrate chemical parameters.

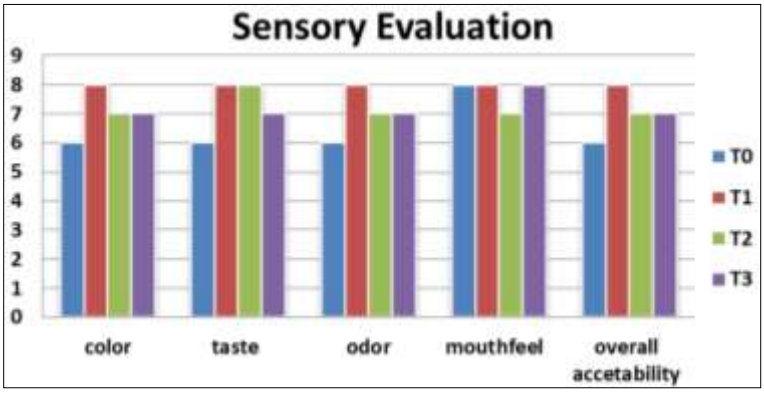

Fig 2: Sensory evaluation of formulated drinks

4. Conclusion

In spite of nutritive value, the whey is not properly utilized for the consumption and it is not familiar among peoples. Hence, the whey based isotonic drinks could possibly increase the consumption especially among physically active peoples. The formulated drinks had a similar appearance to available the market product. It also helps in reducing the organic pollution due to decomposition of whey nutrients into the soil. And also, it helps enhancing the by-product utilization in dairy industries. These isotonic drinks are low cost nutritious products especially sports persons compared to commercially available isotonic drinks. Therefore, from this study it was concluded that this drink can meet the requirement similar to any commercial available isotonic drink with enhanced nutritional content. The product can further be iterated with the addition other fruit and vegetable juices, herbal extract and suitable flavor and color based on consumer likeability.

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6. Authorship

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Conflict of interest

The authors declare no conflict of interest.

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