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Effect of menthol *(Mentha arvensis)* and beet root extract on physico-chemical properties of *paneer* whey based beverage

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Abstract
This study was carried with incorporation of *Mentha arvensis* extract (2-6%) to prepare herbal whey beverage. These experiment have four different treatment combinations viz., T-1 (80% whey with 20% beetroot extract), T-2 (80% whey with 20% beetroot extract and 2% *M*entha extract), T-3 (80% whey with 20% beetroot extract and 4% *M*entha extract) T-4 (80% whey with 20% beetroot extract and 6% *M*entha extract). The amount of sugar was fixed at 7 percent in all in 100 ml of beverage in all treatments. All the samples were chemically analyzed using standard procedures. It was also observed that as the *Mentha* extract increased, there was decrease in acidity, total solids and fat content of herbal whey beverage and increased in pH, protein, ash, total sugar and moisture content of herbal whey beverage. Development of such herbal whey beverages based upon whey, beetroot and menthol to exhaust nutritional, therapeutic as well as medicinal properties of beetroot and menthol.

Keywords: *Paneer* whey, *Mentha* extract, beetroot extract, beverage and physico-chemical parameters

Introduction
The development of health promoting food is one of the set targets in food process engineering. Research during the last two decades has shown that the combination of routine food with medicinal herbs having any special health beneficial effect can be an excellent source for development of functional food. The conversion of whey into beverage by adding different food ingredient is one of the most attractive avenues for the utilization of whey for human consumption. By realizing the functional properties of whey, many industries targets upon utilizing whey as the functional food ingredient. Kamte (2015) [19] developed whey drink by using beet root extract. But it was deficient in thrust quenching and chilling effect which is expected from any drink. This deficiency can be overcome by adding menthol extract to whey beverage.

Menthol (*Mentha arvensis*) which belongs to the family Libeaceae is a common edible and aromatic perennial herb which is cultivated throughout the India. Its common name is *pudina*. The physico-chemical properties of menthol are melting point 43^0C (106-109^0F), freezing point is 27-28^0C, boiling point is 212^0C (414^0F). Molecular formula $C_{10}H_{20}O$ and molecular weight is 156.27 g/mol. It has an antioxidant, antimicrobial, cytotoxic and analgesic activities of *Mentha arvensis* extract (Nripendra *et. al*., 2014) [22]. Herbal extract of *Mentha arvensis* has preventive and curative value. It is used to treat sour throat, gastric problems and other problems related to gastrointestinal tract (Campbell *et al*., 1973; Jamal *et al*., 2006) [19, 18]. The aromatic leaves widely used for flavouring foods and beverages. Whey based mango herbal beverage prepared with 2% *M*entha extract has been found to be highest overall acceptability on the day of preparation as well as after 30 days of storage (Sirohi *et al.* 2005). In beverages menthol is used for the cooling effect and flavouring, (Yadav *et al*, 2010) [28].

Beetroot (*Beta vulgaris*) is botanically classified as an herbaceous biennial from Chenopodiaceous family and has several varieties with bulb colors ranging from yellow to red. Deep red-coloured beetroots are the most popular for human consumption, both cooked and raw as salad or juice. The roots and leaves of the beet have been used in folk medicine to treat a wide variety of ailments (Grubben and Denton, 2004) [13]. Beetroot juice lower the blood pressure (BP) in men when consumed as a part of normal diet in free-living healthy adults. There is growing interest in the use of natural food colours, because synthetic dyes are becoming more and more hazardous (Manoharan *et al*., 2012) [21]. But in food processing, as compared with anthocyanin and carotenoids, betalains are less commonly used, although these water-soluble pigments, they are stable between pH 3 and 7. To improve the red colour of tomato pastes, sauces, soups, desserts, jams, jellies, ice creams, sweets and breakfast cereals, fresh beet/beet powder or extracted pigments are used (Roy *et al*. 2004) [26]. It also contributes to consumer's health and wellbeing because it is known to have antioxidants because of the presence of nitrogen pigments called betalains, mainly comprise of red–violet-coloured betacyanins (betanin, isobetanin, probetanin and neobetanin) and yellow–orange-coloured betaxanthyns (Singh and Hathan 2014) [27].

Whey is a by-product of the manufacture of cheese, *paneer* and casein and has several commercial uses. It contains 45-50 per cent of total milk solids, 70 per cent of milk sugars (Lactose), and 20 per cent of milk proteins, 70-90 per cent of milk minerals and almost all water soluble vitamins present in milk (Horten, 1995) [14]. Considerable work has been done throughout the world to utilize whey for production of whey

protein concentrates (WPC), whey protein isolates and whey powder, Lactose, Lactic acid, whey paste etc. (Panesar *et al*., 2009) [23]. Whey and its biological components have proven its effects in treatments of cervical chronic diseases like cancer, cardiovascular, HIV etc. As it is nutritionally too rich it can also be used in beverages of infants; gastric and athletic foods (Devera, 2005) [7]. Hence, the conversion of whey into beverage is one of the most attractive avenues for utilizing whey for human consumption (Goyal and Gandhi, 2009) [12].

Material and Methods

The study was carried out in the Department of Animal Husbandry and Dairy science, College of Agriculture, Latur (M.S.) in the year 2016. All the raw materials sugar, beetroot. Milk, *Mentha arvensis* etc were collected from the local market of Latur. The damaged and off type fruits and *Mentha arvensis* leaves were discarded.

Preparation of beet root extract

For the preparation of beetroot extract, fresh and healthy beetroot was used. Beetroot were sorted without injury and washed thoroughly under the tap water, cleaned and dried using cloth. After cleaning beetroot were peeled manually and cut into small pieces. Further, pieces were ground in mixer grinder with *paneer* whey (1:1 proportion) and extract were collected in 250 ml beaker.

Preparation of *Paneer* whey and mentha extract

The milk was heated in a stainless steel vessel to 86°c and cooled to 76°c at room temperature. The hot milk was acidified by addition of citric acid 0.5 per cent with continuous stirring, resulted in complete coagulation of milk protein (casein). The liquid (*paneer* whey) was filtered through muslin cloth. Mentha extract was prepared from fresh leaves. The leaves were washed, ground in a mixer grinder with whey at proportion (1: 0.5) and filtered using muslin cloth.

Preparation of herbal whey beverage

For preparation of herbal whey beverage as per the treatment combination. The *paneer* whey was heated at 45^0C temperature. Then added the cane sugar @ 7 per cent was maintained in all treatments. After that the beetroot and *Mentha arvensis* extract was added as per the treatment combinations. Simmering was done at 70^0C for 2-3 min and filtered through (whatman No.1) filter paper and filled into pasteurized glass bottles (100 ml) and sealed. Pasteurization of filled bottles was done in boiling water for 30 min (Lal *et al*., 1998) [10] and cooled to room temperature and stored at refrigerated condition (5^0C).

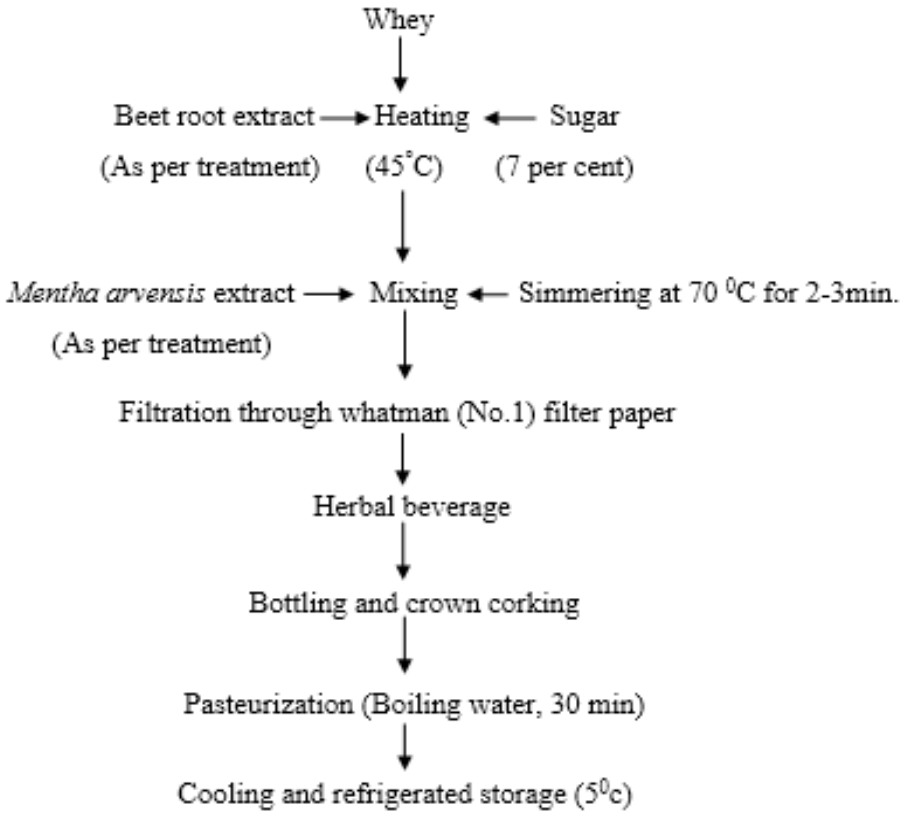

Fig 1: Preparation of herbal whey beverage.

Result and Discussion

Physico chemical analysis of finished product

The finished product was subjected for the proximate analysis *viz.* fat, protein, total sugar, ash, moisture, and total sugar. The results obtained on account of this parameter are presented in forthcoming table.

Table 1: Physicochemical properties of beverage

Treatment	Acidity	pH	Fat	Protein	Total Sugar	Moisture	Total Solids	Ash
T_1	0.37^{a}	4.34^{a}	0.45^{a}	0.57^{a}	12.00^{a}	87.00^{a}	13.00^{a}	0.46^{a}
T_2	0.36^{ab}	4.35^{ab}	0.45^{a}	0.59^{b}	12.06^{ab}	87.08^{b}	12.92^{b}	0.47^{a}
T_3	0.35^{ab}	4.36^{ab}	0.44^{ab}	0.60^{b}	12.08^{b}	87.12^{b}	12.88^{b}	0.49^{b}
T_4	0.35^{b}	4.36^{b}	0.43^{b}	0.62^{d}	12.09^{b}	87.14^{b}	12.86^{b}	0.50^{b}
S.E. ±	0.0066	0.0053	0.0029	0.0057	0.0213	0.0221	0.0207	0.0055
C.D.at 5 %	0.0206	0.0164	0.0091	0.0176	0.0657	0.0681	0.0638	0.0170

a. Acidity

The table indicates that in acidity each successive treatment non significant differences were observed between treatments and significant differences was observed between T_1 and T_2. It indicates that up to four part of menthol extract not affected on acidity. The average acidity was 0.37, 0.36, 0.35 and 0.34 per cent for treatment T_1, T_2, T_3 and T_4, respectively. The overall effect of menthol extract on acidity of beverage was decreasing as the menthol increased in formulation of beverage. This might be due to the low per cent of acidity in the menthol i.e 0.33 per cent. The acidity recorded in the present investigation was agree with the results observed by, Yadav *et.al* (2010) [28] studied development and storage studies on whey based banana herbal (*Mentha arvensis*) beverage with incorporation of *Mentha arvensis* extract 0-4%. The acidity P_4 was 0.50, 0.38, 0.32, 0.35 and 0.36, of fresh beverage P_0 (control), P_1, P_2, P_3 and respectively. Further, Babar *et al*. (2008) [3] also observed the higher acidity but same reducing trend in their study in utilization of pomegranate juice for the preparation of *chakka* whey beverage and recorded the acidity for treatments 0 (T_1), 10 (T_2), 15 (T_3) and 20 (T_4) per cent with of pomegranate juice with 10 per cent sugar was 0.77, 0.74, 0.71, 0.67 per cent. This might be the different fruits, proportion and acidity of that fruit, they used to prepared beverage.

b. PH

The above table indicates that the average pH of herbal whey beverage samples were 4.34, 4.35, 4.36 and 4.36 for treatment T_1, T_2, T_3 and T_4, respectively. Treatment, T_4 was higher pH from other treatments. It could be revealed from the content of table that there is not wide variation in pH due to the addition of two parts of *Mentha arvensis* extract, varies only more than four part observed in T_1 and T_2. It was also observed that as the quantity of *Mentha arvensis* extract in the beverage increased, pH of the herbal beverage was increased. Higher pH values than this results were observed by Baljeet *et al.* (2013) [5] for freshly prepared WPBH (whey-based pineapple (*Ananas comosus*) and bottle gourd (*Lagenaria siceraria*)) beverage samples were 4.99, 4.96, 5.01, 5.02 and 5.01, respectively in which used *Mentha arvensis* extract (0 to 4 per cent). Chaudasama and John (2014) [10] also studied in preparation of whey based mango mint beverage (WBMMB) with the incorporation of *Mentha arvensis* extract (0% to 3%) and measured pH as 5.58, 5.8, 6.02 and 6.14 respectively. The slight variation between above mentioned research workers with our findings may be due to different ingredient used by them but generally observed acceptable in all types of drinks. As was seen from the contents of table and discussed above, the acidity and pH is affected same way, indicate that menthol's effect in all supported by Dubey *et al*. (2007) [8].

c. FAT

The average fat content in herbal whey beverage was found to be 0.45, 0.45, 0.44 and 0.43 per cent for treatment T_1, T_2, T_3 and T_4, respectively. The highest and same fat content was record for treatment T_1 and T_2 i.e. 0.45 per cent. The lowest fat content was recorded for treatment T_4 i.e. 0.43 per cent. The fat content in all treatments were not differ significantly except T_1 and T_4. Above observations clearly indicates that, as the incorporation of *Mentha* extract to whey beverage was increased, the fat content in the finished product was decreased. The fat content in the finished product was decreased due to the less amount of fat in *Mentha* extract. i.e. 0.60 per cent. Bhavsagar *et al*. (2010) [4] studied the manufacture of pineapple flavored beverage from *chhana* whey and recorded the same decreasing trend for fat content. This beverage was prepared with the addition of 5, 10 and 15 per cent of pineapple pulp in *chhana* whey. The values recorded for fat in the whey beverages prepared by Bhavsagar *et.al* (2010) [4], Perasiriyan *et al*. (2013) [24] and Kamte (2015) [19] were comparable with the values observed present research work.

d. Protein

The average protein content of the finished product was found to be 0.57, 0.59, 0.60 and 0.62 per cent for treatment T_1, T_2, T_3 and T_4, respectively. The T_4 treatment was significantly higher content of protein i.e. 0.62 per cent than other, whereas lowest protein content was recorded for treatment T_1 i.e. 0.57 per cent. This might be due to the incorporation of *Mentha arvensis* extract content high protein (4.8 per cent) than whey, increased the protein content in finished product increased significantly at 5 per cent level of significant. The values and remark recorded for protein in the present research work were agreed with the pattern observed by following workers. Babar *et al*. (2008) [3] studied the utilization of pomegranate juice for the preparation of *chakka* whey beverage observed the average protein content were as 0.394, 0.468, 0.504 and 0.544 per cent in treatment T_1, T_2, T_3 and T_4, respectively. Gaikwad *et al.* (2010) [11] studied on preparation of *chhana* whey beverages using sapota pulp. The beverage was prepared by using 0, 5, 10 and 15 per cent of sapota pulp. They observed an average protein 0.38, 0.40, 0.41 and 0.42 per cent for treatment T_0, T_1, T_2 and T_3, respectively. Chaudasama and John (2014) [10] studied the development and evaluation of whey based mango mint beverage. Per cent protein of prepared WBMM beverage samples measured as 0.672, 0.706, 0.764 and 0.796 respectively.

The above mentioned worker observed different protein

content in whey beverages, but noted same increasing trend of protein content in their studies, may be the used of different fruit juice used i. e. pomegranate juice by Babar *et al*. (2008) [3], sapota pulp by Gaikwad *et al.* (2010) [11] and mango by Chaudasama and John (2014) [10] have variation in protein content.

e. Total Sugar

From the content of table it could be seen that the average total sugar content ranged in between 12.00 to 12.09 per cent. Next, it is noticed from table that the developed treatments showed higher content of sugar attributed by menthol only. Total sugar content of 12.00 (T_1) in the control sample increased to 12.09 (T_4), when *Mentha* extract concentration was increased to 6%. The least value of total sugar content was recorded for T_1 treatment, 12.00 per cent. The values of total sugar content in menthol used treatments were non significantly differ with in each other and differ with control except T_2.

Baljeet *et al*. (2013) [5] studied on development and storage of whey based pineapple (*Ananas comosus*) and bottle gourd (*Lagenaria siceraria*) mixed herbal beverage and observed the total sugar content of freshly prepared WPBH beverage samples of H_0, H_1, H_2, H_3 and H_4 were 13.12, 13.44, 13.35, 13.48 and 13.15, respectively, which was more than this findings might be due to the high sugar of pineapple supported by Bhavsagar *et al.* (2010) [4] and Revathi and Vinita (2012).

f. Moisture

It is easily observed from the contents of table the addition of menthol significantly contributed to the moisture content in whey beverages in treatments T_2, T_3, and T_4 over the control beverage. The average moisture content of the product was found to be 87.00, 87.08, 87.12 and 87.14 per cent for the treatments T_1, T_2, T_3 and T_4, respectively. The increasing trend for moisture was observed in successive treatment. The treatment T_4 had significantly higher moisture content (87.14 per cent) as compared to the rest of the treatments. The *Mentha arvensis* extract (84.90 per cent) are strongly affected on the moisture content of developed product confirmed by the findings of Damunupola *et al.* (2014) [6] in goat milk yogurt incorporated with beetroot juice. They observed the moisture content of beetroot yogurt was 79.08 ± 0.60 per cent. This was increased due to addition of beetroot juice in yogurt which content more moisture than normal yogurt (75.04 ± 0.43 per cent). But the controversial results was coated by Kamte, 2014 in her study in which she developed whey beet root beverage and observed reducing in moisture content in her study, might be due to the quality of beet root, water used to extraction of juice from beet (in this study the whey was used to extract the beet root juice).

g. Total Solids

It clearly indicated from above table, that the average total solids content of the control and developed beverage samples were found to be 13.00, 12.92, 12.88 and 12.86 for treatment T_1, T_2, T_3 and T_4, respectively. The highest total solids content was recorded for control treatment T_1 i.e. 13.00 and lowest for treatment T_4 i.e. 12.86. All the developed beverage samples were non significantly different between each other at five per cent level of significant but significantly differ with control. Baljeet *et al*. (2013) [5] in their study observed comparable TSS content of freshly prepared WPBH beverage samples of H_0 (control), H_1, H_2, H_3 and H_4 were as 13.93, 14.03, 13.97, 13.77 and 13.73, respectively, in the preparation of beverages the volume of pineapple and bottle gourd juice 10 (T_0), 10 (T_1), 10 (T_2), 10 (T_3) and 10 (T_4) per cent and 0, 1, 2, 3 and 4 per cent *Mentha arvensis* extract respectively with 8 per cent sugar was mixed in *chakka* whey; Kamte (2015) [19] recorded the total solid content in her studied on development of *paneer* whey as a nutritional beverage by using beetroot extract was 13.70 per cent. And lower total solid were recorded by Goyal and Gandhi (2009) [12] in electrolyte whey drink from Indian *Paneer* and cheese whey i.e. 5.8±0.13; Perasiriyan *et al*. (2013) [24] i.e. 6.5 per cent; might be due to the absence of fruits or different fruits in preparation.

h. ASH

Table indicates that the mean ash content in the finished products was found to be 0.46, 0.47, 0.49, and 0.50 per cent for treatment T_1, T_2, T_3 and T_4, respectively. The treatments T_1 and T_2 were found to be significantly different from T_3 and T_4 and at par between themselves. The values recorded were found to be in increasing order from 0.46 to 0.51 for treatment T_1 to T_4. This may be due to the incorporation of *Mentha arvensis* extract in increasing level content more mineral matter. The use of fruits and vegetables for the development of whey as beverages studied by many workers i. e. Baber *et al.* (2008) [3], Bhavsagar *et.al* (2010) [4], Chaudasama and John (2014) [10] and Kamte (2015) [19] were conclude unanimously that addition of fruit and vegetables for preparation of whey beverages increased the ash content than control. Bhavsagar *et al*. (2010) [4] studied the manufacture of pineapple flavoured beverage from *chhana* whey. This beverage was prepared with the addition of 5, 10 and 15 per cent of pineapple pulp in *chhana* whey and observed ash as 0.44, 0.60, 0.76 and 0.78 per cent, respectively. Whereas, Chaudasama and John (2014) [10], observed in whey based mango mint beverage as 0.466, 0.502, 0.582 and 0.632 respectively.

As seen from the discussion and data presented in table 1 in respect to physico-chemical parameters, it would like to conclude that the physical and compositional properties of any developed or formulated food strongly depends on the quality of raw material used to prepared and method followed for its preparation.

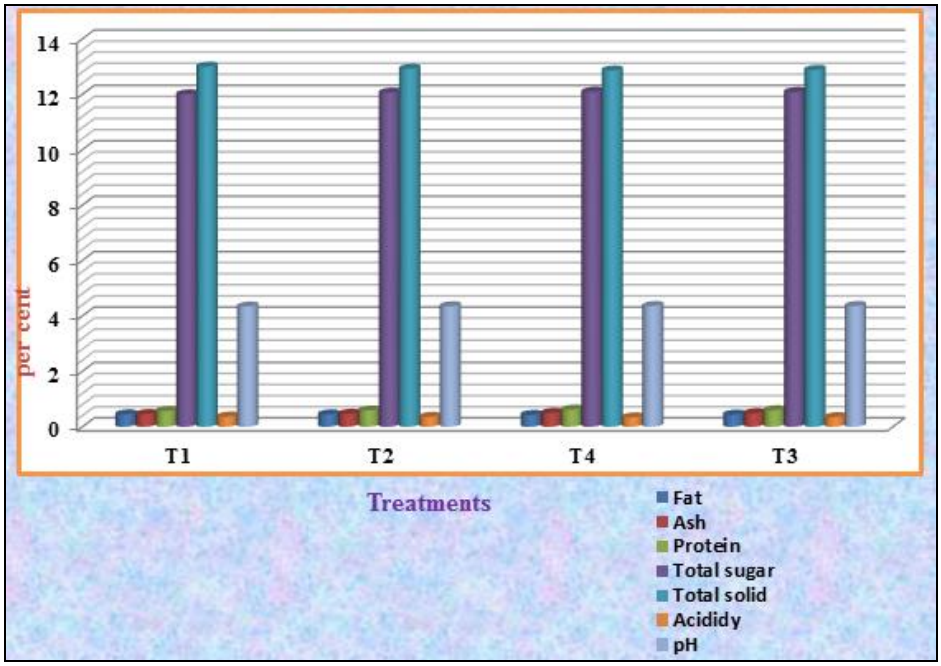

Fig 2: Physico chemical properties of herbal whey beverage

Cost structure of herbal whey beverage

Cost of herbal whey beverage prepared from buffalo milk *paneer* whey in combination with different proportions of *Mentha arvensis* extract was worked out as per the prevailing market prices of ingredient for the year 2015-16 and presented in further table.

Table 2: Cost structure of herbal whey beverage

Sr. No.	Particulars	Cost (Rs./kg)	T_1		T_2		T_3		T_4	
			Qty. (per lit)	Amt. (Rs)	Qty. (per lit)	Amt. (Rs)	Qty. (per lit)	Amt. (Rs.)	Qty. (per lit)	Amt. (Rs.)
1.	Whey (ml)	10	800	8.00	800	8.00	800	8.00	800	8.00
2.	Beetroot extract (ml)	60	200	12.00	200	12.00	200	12.00	200	12.00
3.	Mentha extract (ml)	160	-	-	20	3.2	40	6.4	60	9.6
4.	Sugar (gm)	34	70	2.38	70	2.38	70	2.38	70	2.38
5.	Labour and fuel charges	-	-	08.00	-	8.00	-	8.00	-	8.00
6.	Product obtained (lit)	-	1	-	1.20	-	1.40	-	1.60	-
7.	Total cost for obtained product	-	-	30.38	-	33.58	-	36.78	-	39.98
8.	Total cost per lit	-	-	30.38	-	32.92	-	35.36	-	37.71

While calculating the cost of finished product, the cost of ingredients used in the preparation of whey beverage i.e. cost of whey, beetroot, menthol and sugar used for whey beverage were accounted. In addition to these, the approximate cost of fuel, labour charges and paper cup on account of processing the product were also taken into consideration.

It was seen from table that the cost of herbal whey beverage ranged between Rs. 30.38 to 37.71 per 1000 ml for treatment T_1 to T_4 respectively. The cost of treatment T_1, T_2, T_3 and T_4 was 30.38, 32.92, 35.36 and 37.7 1per 1000 ml, respectively. The highest cost was recorded for treatment T_4 i.e. Rs. 37.71 where in 20 per cent beetroot extracts was used and 6 per cent *Mentha arvensis* was incorporated in it. The lowest cost was recorded for treatment T_1 i.e. Rs. 30.38 where in the *Mentha* extracts was not fortified. The per cent cost required to developed herbal beverage by using menthol was 3.03, 3.29, 3.53 and 3.77 per cent, respectively for the treatments T_1, T_2, T_3 and T_4.

The overall cost recorded for different blends was comparatively lower than market prices of different beverages. Further, it has better scope in future and may capture better popularity due to its and being a dairy based drink. The *paneer* whey can convert in to energetic and nutritional beverage by using beetroot and menthol.

Babar *et al*. (2008) [3] studied the utilization of pomegranate juice for the preparation of *chakka* whey beverage. In the preparation of beverages the volume of pomegranate juice 0 (T_1), 10 (T_2), 15 (T_3) and 20 (T_4) per cent with 10 per cent sugar was mixed in *chakka* whey. They reported the cost of whey beverage per litre as Rs. 4.44, 12.39, 16.36 and 20.34 for the treatment T_1, T_2, T_3 and T_4, respectively.

Chaudasama and John (2014) [10] studied the development and evaluation of whey based mango mint beverage. For preparation whey based mango mint beverage (WBMMB) with the incorporation of *Mentha arvensis* extract (0% to 3%). They reported Cost (Rs.) of WBMM beverage samples of different treatments and control it was noted that the highest mean cost was of the whey based mango mint beverage

sample of T3 (14.62) followed by T2 (12.64) and T1 (10.66) and then T0 (8.68).

Fig 3: Cost structure of herbal whey beverage

Conclusion

From present investigation it can be concluded that the *Mentha* extract can be very well utilized for preparation of palatable, energetic, nutritional herbal whey beverage. By using 6 per cent *Mentha* extract in 80 per cent *paneer* whey and 20 per cent beetroot extract on weight basis the development of health promoting food is possible, which might be countable for prevailing cold drinks present in market. It was observed that as the adding of *Mentha* extract increased in pH, protein, ash, moisture and total sugar whereas decreased in acidity, fat and total solid content of herbal whey beverage. Product developers seeking out functional and nutritional attributes of whey to tap the tremendous growth opportunities in the beverage industry can move forward for the development of such herbal whey beverages based upon whey, beetroot and menthol to exhaust nutritional, therapeutic as well as medicinal properties of beetroot and menthol.

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