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Study of Formulation, Sensory Evaluation, Antioxidant Potential and Storage Study of Watermelon Based Whey Beverage from Camel and Buffalo Milk

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ABSTRACT

Present study was based on whey, obtained by acid coagulation of camel and buffalo milk, mixed in a ratio of 70:30. Further this whey was used to prepare watermelon based; ready-to- serve (RTS) whey beverage using different levels of watermelon juice in which sugar (4.5%) and black salt (1.0%) was added replacing whey and on the basis of whey, 4 different treatments viz. T_1, T_2, T_3 and T_4 respectively were prepared. Control treatment (T_0) was the whey. Sensory evaluation was carried out by a group of panellists on the basis of 8 point hedonic scale. The proportion which has 74.5% whey and 20% watermelon juice (T_4) had highest overall acceptability. On the basis of sensory evaluation, further storage study was carried out for the treatment T_4 with different parameters like pH, TA (Titratable acidity), DPPH (2, 2′-diphenyl-1-picrylhydrazyl), ABTS(2, 2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) and TBA (2-Thio Barbituric Acid) over a period of 12 days. Among storage period increase in TA and TBA whereas decrease in pH, DPPH and ABTS value were noted. Results of storage revealed that whey beverage have its highest functional properties and accessibility when consume in fresh state.

Keywords: Camel milk, Watermelon, Whey

Whey is a valuable by-product obtained during coagulation of milk by using acid and/or rennet during physico-chemical process for the preparation of cheese, paneer, chhana, chakka and casein. The main biological activities of whey proteins are suggested to include cancer prevention, increase of glutathione levels, antimicrobial function and increase of satiety response (Madureira *et al.,* 2007). However, in spite of being a nutritive product, whey is still little used in human diet (Drgalic *et al.,* 2005). Previously, whey was used mainly in animal feed or surplus. With advances in technology and recent discoveries of functional and bioactive roles of whey proteins, whey and whey components are now viewed as precious ingredients. The recognition of whey as a source of unique physiological and functional attributes provides opportunity for the food industry to incorporate whey and whey components into a variety of foods. Whey beverages have been recognized as a genuine thirst quencher, light, refreshing, healthful and nutritious (Prendergast, 1985).

Considerable work has been done throughout the world to utilize whey for production of whey protein concentrate (WPC), whey powder, lactose, lactic acid, whey paste, etc. The conversion of whey into beverages through fermentation or without fermentation is one of the most attractive avenues for the utilization of whey for human consumption. Beverages based on fruit and milk products are currently receiving considerable attention as their market potential is growing. Besides being delicious, these beverages are highly nutritious. In terms of functionality, whey protein enhances protein content of beverage while improving its quality.

By adding some simple ingredients in the whey, like sugar, colour and flavour to it, results in improvement of

its nutritive value, taste and acceptability. So utilization of such whey for the conversion into best beverage would be one of the important ways to utilize it. There is a lot of scope to explore the possibility of its utilization in beverage industries (Sakhale *et al*., 2012).

Consumers are usually looking for ways to improve their health whether it's changing their diet, lifestyle. Whey is enriched by biologically active ingredients or valuable organic complements gained from nature's resources e.g., nutritious protein source which is a high-quality that provides all of the essential amino acids necessary for good health (Kimball and Jefferson, 2001).

Watermelon (*Citrullus lanatus*) is one of the most abundant and cheap fruits that is available in India. It is available throughout the year, but production is highest in the summer. Watermelon production contributes to 6–7 % of overall fruit production in the world (Reddy *et al.,* 2008). This fruit is a rich natural source of lycopene, a compound responsible for its red colour (Perkins-Veazie *et al*., 2001). Intake of lycopene containing-products has been associated with a reduced incidence of coronary heart disease and some types of cancer (Giovannucci, 2002).

Looking towards all of the health benefits of watermelon juice an attempt was made in the present study to utilize whey in combination with different proportions of watermelon juice to develop naturally flavoured whey beverages which are nutritious as well as palatable.

MATERIALS AND METHODS

In the present study 70% of camel milk and 30% of buffalo milk was used for the preparation of good quality whey. Fresh buffalo milk was obtained from buffaloes maintained under the project "Establishment of live demonstration models of diversified livestock production systems for motivating adaption to enhancing agricultural income (RKVY-15)" C.V.A.S., RAJUVAS, Bikaner and fresh camel milk was collected from camel dairy maintained at ICAR-NRC on Camel, Bikaner. Watermelon, sugar and black salt obtained from the local market. Both camel and buffalo milk was mixed well and heated at 80°C and milk was coagulated using 2% citric acid solution followed by continuous stirring resulted in complete coagulation of milk protein (casein). The liquid (whey) was filtered using muslin cloth and stored for further use. For the extraction of juice watermelon was peeled and cut into small pieces. After separating the seeds the fruit pieces were grinded in a mixture and the pulp was then filtered through a double layered muslin cloth and a clear watermelon juice and stored.

Preparation of formulated beverage

The blended beverage was prepared using different ratios of concentrated whey and watermelon juice with constant level of 4.5% sugar and 1% black salt (Table 1). All the ingredients are mixed with a shaker, filtered (Muslin cloth), bottled and finally corked. Before storing the beverages, bottles are pasteurized at a temperature of 65-70°C for 15 minutes and then cooled to room temperature. The storage stability of the optimized beverage sample is determined at room refrigeration temperature (5 ± 1ºC) without addition of preservative. Formulated beverages of various combinations are chilled before evaluation. The samples were subjected to sensory evaluation on 8 point hedonic scale by a panel of eight semi-trained members from academic staff and students of the department for various sensory attributes viz., appearance, flavour, taste and overall acceptability using 8 point descriptive scale, where '8' denotes 'Excellent' and '1'denotes 'extremely poor'.

Analytical tests

The determination of pH is carried out using a digital pH meter (HANNA instrument USA). Titratable acidity expressed as percentage of lactic acid, was determined by using 10 ml of sample titrating with 0.1 N NaOH solution using phenolphthalein as an indicator to an end-point of faint pink color. The ability to scavenge 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical by added antioxidants in samples was estimated following the method of Brand-Williams *et al.* (1995). The spectrophotometric analysis of ABTS radical-scavenging activity was determined according to method described by Salami *et al.* (2009). The TBA values were determined according to the extraction method described by Witte *et al.* (1970).

Statistical analysis

All the experiments of study were repeated three times and samples were drawn in duplicate. Data collected during

the present investigation were subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Chochran (1994).

RESULTS AND DISCUSSION

Formulation of beverage

The different formulations as indicated in Table 1 are analyzed for sensory properties by a group of panel. All formulas are prepared in triplicate and these triplicate scores are averaged for each panellist.

Table 1: Formulation for Preparation of 100ml of Watermelon Based Whey Beverage (WBWB)

Treatment	Whey (%)	Watermelon juice (%)	Sugar (%)	Black salt (%)
T_0 (Control)	94.5	00.0	4.5	1.0
T_1	89.5	05.0	4.5	1.0
T_2	84.5	10.0	4.5	1.0
T_3	79.5	15.0	4.5	1.0
T_4	74.5	20.0	4.5	1.0

The treatments (beverages) in Table 2 indicate that the panelists, on average, prefer the treatment T_4 with 74.5% whey, 20% watermelon juice, 4.5% sugar and 1% black salt from these, T_4 is preferred on average for, appearance/ colour, flavour, taste and overall acceptability. To summarize, it can be concluded that T_4 made with 74.5% whey, 20% watermelon juice, 4.5% sugar and 1% black salt is the most appreciated. Hence this formula is used to evaluate its nutritional profile and storage stability of the beverage at refrigeration temperature.

Table 2: Sensory evaluation of naturally flavoured whey beverages (Mean+SE?)

Treatment	N	Appearance/ Colour	Flavour	Taste	Overall acceptability
T_0	6	6.11 ± 0.01	6.11 ± 0.02	6.01 ± 0.01	6.07 ± 0.03
T_1	6	6.65 ± 0.03	6.50 ± 0.04	6.80 ± 0.04	6.65 ± 0.08
T_2	6	6.70 ± 0.05	6.75 ± 0.06	6.70 ± 0.07	6.88 ± 0.15
T_3	6	7.13 ± 0.02	7.21 ± 0.03	7.33± 0.02	7.22 ± 0.05
T_4	6	7.53 ± 0.02	7.32 ± 0.02	7.49 ± 0.03	7.45 ± 0.06

Table legends, n=6, meaning for T0, T1 etc.

T_0 (Control) - Whey beverage contains 94.5% plain whey, 4.5% sugar and 1% black salt; T_1- Watermelon based whey beverage contains 89.5% whey, 5% watermelon juice, 4.5% sugar and 1% black salt; T_2- Watermelon based whey beverage contains 84.5% whey, 10% watermelon juice, 4.5% sugar and 1% black salt; T_3- Watermelon based whey beverage contains 79.5% whey, 15% watermelon juice, 4.5% sugar and 1% black salt; T_4- Watermelon based whey beverage contains 74.5% whey, 20% watermelon juice, 4.5% sugar and 1% black salt.

Storage stability of formulated beverage

On the basis of sensory evaluation beverage/ treatment T_4 is stored at refrigeration temperature (5 ± 1ºC) to determine its storage stability without addition of preservative. During storage the changes in pH, titratable acidity, DPPH activity, ABTS activity and TBA were measured and analyzed at 0, 3, 6, 9 and 12 days of storage at refrigeration temperature. The data of pH has been shown in Table 3. The initial pH of 4.77 ± 0.009 decreases to 4.48 ± 0.008 after 12 days of storage. Decline in pH during storage is observed which may be due to the action of citric and ascorbic acid on the sugar and protein component of the product. 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 (Kalra *et al.,* 1991; Sikder *et al.,* 2001). The whey based watermelon beverage has an initial titratable acidity of 1.73 ± 0.008% in terms of citric acid and increases to 2.85 ± 0.008% citric acid after 12 day of storage. The data of titratable acidity has been shown in Table 3 and has been presented in Fig 1. Similar results of significantly increase in titratable acidity were also observed in the study conducted for utilization of pomegranate juice for the preparation of chakka whey beverage by Babar *et al*. (2008). Naik *et al.* (2009) also reported a significant increase in titratable acidity in whey based watermelon beverage.

The changes in DPPH (2, 2′-diphenyl-1-picrylhydrazyl) radical-scavenging antioxidant activity, ABTS Activity (% inhibition) (2, 2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging antioxidant activity

of beverage during storage at refrigeration temperature has been shown in Table 3. The initial DPPH antioxidant activity significantly decreases from 12.08 ± 0.337 to 4.19 ± 0.217 after 12 days of storage. The similar results were obtained for DPPH activity by Ujjala, (2012) who had also reported that antioxidant properties of polyphenols (fruit) extract fortified whey beverages with storage significantly decreased. Tak (2017) and Singh (2017) also reported that the DPPH activity of fermented camel and buffalo milk yoghurt was significantly decreased with respect to increase in the storage period. For ABTS activity similar results of significant decrease of the antioxidant activity with respect to the storage period were reported by Tak (2017) and Singh (2017).

TBA (2-Thio Barbituric Acid) value of the beverage during storage at refrigeration temperature is indicated in Table 3. The initial TBA (2-Thio Barbituric Acid) value of watermelon based whey beverage 0.06 ± 0.0028 increases to 0.28 ± 0.0057 after 12 days of storage. However elevation of TBA value during storage was due to change in its physicochemical as well as microbial properties of whey beverages. Tak (2017) and Singh (2017) also observed similar results of highly significant increased TBA value with increase of storage period of fermented camel and buffalo milk yoghurt.

Table 3: Storage study (Mean ± SE) of formulated beverage

Parameter	Day 0	Day 3	Day 6	Day 9	Day 12
pH**	4.77^{e} ± 0.009	4.72^{d} ± 0.008	4.69^{c} ± 0.004	4.61^{b} ± 0.009	4.48^{a} ± 0.008
TA**	1.73^{a} ± 0.008	2.02^{b} ± 0.011	2.23^{c} ± 0.006	2.63^{d} ± 0.016	2.85^{e} ± 0.008
DPPH**	12.08^{e} ± 0.337	10.27^{d} ± 0.224	8.15^{c} ± 0.283	6.04^{b} ± 0.339	4.19^{a} ± 0.217
ABTS**	21.96^{e} ± 0.293	19.21^{d} ± 0.349	16.02^{c} ± 0.239	13.15^{b} ± 0.299	9.95^{a} ± 0.278
TBA**	0.06^{a} ± 0.0028	0.11^{b} ± 0.0007	0.14^{c} ± 0.0031	0.20^{d} ± 0.0039	0.28^{e} ± 0.0057

** = Significant at 1% (P<0.01).

CONCLUSION

In this study, whey is successfully utilized to develop a watermelon-based fruit beverage with optimum sensory and nutritional properties as well as good storage stability. The beverage possesses high colour, flavour and stability properties. A nutritious beverage with better storage life is developed with the addition of whey, watermelon juice, sugar and black salt in appropriate proportion. In view of the functional properties arising from bioactive constituents present in fruit and whey, it is proposed that watermelon based whey beverages with excellent nutritional, sensory and storage properties could be an interesting product in the constantly growing market for functional foods. Since the beverage is prepared from discarded whey water, it is contributing to lower the cost and thus proved to be economically viable.

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