www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(1): 254-259

www.thepharmajournal.com
Received: 28-11-2022
Accepted: 31-12-2022

Navdeep JP
G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar Campus, Sardarkrushinagar, Gujarat, India

Ramesh V
G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar Campus, Sardarkrushinagar, Gujarat, India

Amit Kumar
G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar Campus, Sardarkrushinagar, Gujarat, India

Smita Singh
College of Basic Science and Humanities, S.D. Agricultural University, Sardarkrushinagar, Gujarat, India

Manoj Kumar Jaipal
College of Food Technology, S.D. Agricultural University, Sardarkrushinagar, Gujarat, India

Ashish Dixit
College of Food Technology, S.D. Agricultural University, Sardarkrushinagar, Gujarat, India

Corresponding Author:
Navdeep JP
G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar Campus, Sardarkrushinagar, Gujarat, India

Development of protein enriched whey-bael beverage and its evaluation for antioxidant potential

Navdeep JP, Ramesh V, Amit Kumar, Smita Singh, Manoj Kumar Jaipal and Ashish Dixit

DOI: https://doi.org/10.22271/tpi.2023.v12.i1c.19197

Abstract
The study was carried out to develop and to evaluate the antioxidant potential of whey-based bael beverage fortified with whey protein. Bael fruit pulp was analysed for physico-chemical characteristics and antioxidant activity. The TS, TSS, pH, titratable acidity and vitamin C content of pulp was 40.26%, 38.4°Brix, 4.82, 0.32% as citric acid and 65.32 mg/100 g, respectively. The ABTS, DPPH and TPC content of bael fruit pulp was 11.63 µmol TE/g, 7.28 µmol TE/g and 14.16 µmol GAE/g, respectively. The antioxidant activity of hydrophilic extract measured by ABTS and DPPH method was 8.65, 5.32 µmol TE/g, respectively, which was significantly (p<0.05) higher than lipophilic extract (2.98, 1.96 µmol TE/g). For optimization of the level of bael fruit pulp, whey-bael beverages were prepared by incorporation of bael pulp at 6.0, 8.0, 10.0, 12.0, 14.0%, keeping sugar constant at 10.0%. The protein content of the whey was adjusted to 1.0% using WPC 80. The sensory analysis revealed that the beverage prepared with 14.0% bael pulp and 10.0% sugar secured highest scores for sensorial attributes. The TS, protein, fat, ash, carbohydrate, TSS, pH and titratable acidity of the developed beverage was 21.29, 1.23, 0.54, 0.87, 18.65%, 19.8^{0}brix, 4.0 and 0.35% as citric acid, respectively. Thus, it may be concluded that bael fruit has potential to be incorporated in protein enriched healthy whey-based beverage.

Keywords: Whey, underutilized fruit, hydrophilic, lipophilic, antioxidant

Introduction
Whey is obtained during the manufacture of paneer, cheese, casein, chhana and shrikhand. In India, the major source of whey is from production of chhana and paneer (Macwan *et al.*, 2016) [11]. Whey is also a most potent pollutant among all dairy wastes, as it possesses high organic content contributing to high biological oxygen demand (BOD) and chemical oxygen demand (COD). Further, the treatment of whey before disposal is very costly. To overcome problem, various technological methods had been employed to convert whey into value added products (Krolczyk *et al.*, 2016) [9].

Fruits and vegetables contain various bioactive compounds and a great number of these naturally occurring substances have been recognized to have antioxidant potential. Flavonoids and phenolic compounds are among the major contributors to antioxidant activity. These compounds may work independently or synergistically (Kang *et al.*, 2003) [7]. The underutilized fruits viz., bael, karonda, ber and wood apple are cheap but highly nutritious. Exploitation of underutilized fruits in the development of value added food products has been gaining considerable interest because of high nutritional and health benefits of these fruits (Singh and Nath *et al.*, 2004; Vino, 2016; Jadhao *et al.*, 2018) [16, 19, 5].

Currently, the potential health benefits of the phytochemicals and their ability to be incorporated into dairy foods as nutraceuticals has received considerable interest in dairy and food industry. Recent studies have shown these compounds to be good contributors to the total antioxidant capacity of the foods that contain them. Milk and milk products including whey combined with fruit preparation will be a good source of antioxidants provided the antioxidant capacity of these preparations are not depleted through oxidation-reduction reactions upon mixing, processing and storage of the products.

Bael (*Aegle marmelos* L.) also known as stone apple is one of the most widely used medicinal fruit. It contains vitamins like Vitamin A, Vitamin C, thiamine, riboflavin, niacin, and minerals like calcium and phosphorous (Bag *et al.*, 2011) [1]. Wijewardena *et al.* (2015) [20] also studied the antioxidant activity of dried bael fruit powder based on the ability of fruit extract to scavenge DPPH radical scavenging activity.

Bael is such a fruit which may preferred by the consumer in the form of value added products than consumed directly because of bitterness of its seeds present in the pulp.

Development of a ready to drink whey-fruit beverage from whey, whey protein concentrate, fruit concentrate and other ingredients is one of the ways for utilization of whey and whey proteins in the development of protein enriched beverages with high antioxidant potential.

In-spite of high nutritional and medicinal value, bael fruit is not utilised much in the development of value-added dairy products. The development of protein enriched whey drinks admixed with bael fruit rich in vitamins, minerals and antioxidant components is expected to provide a healthy nutritive drink and may also enhance the antioxidant status of the body when consumed, thus preventing the life style related diseases due to oxidative stress. In this research, a healthy and nutritious whey-based beverage was prepared by the incorporation of bael fruit pulp and whey protein Concentrate (WPC) and evaluated for physico-chemical and antioxidant activity.

Materials and Methods

Raw materials and Chemicals

Pasteurized paneer whey was obtained from Banas dairy, Palanpur, Gujarat. Fresh and ripened bael fruits were procured from Horticulture Farm, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Whey protein concentrates supplied by Charotar Casein Company Pvt. Ltd., Nadiyad, Gujarat. Food grade citric acid was supplied by Jay chemicals, Palanpur. Fine crystalline sugar was obtained from the local market. All the chemicals used in the research work were of analytical grade (AR) and HPLC grade.

Preparation of bael fruit pulp

The fresh ripened bael fruits were thoroughly cleaned with potable water to remove adhering dirt. The shell was broken, the pulp with seeds and fibers were scooped. The pulp was obtained by sieving to remove seeds. The pulp was packed in 150 ml sterile plastic container and stored at -20 °C till further use.

Hydrophilic and lipophilic extracts of bael fruit pulp for analysis of antioxidant activity and TPC content was prepared according to the method described by Nilsson *et al*. (2005) [13].

Physico-chemical analysis of whey and bael Pulp

Physico-chemical analysis of paneer whey was carried out as per the method of BIS (1981) [3]. Bael pulp was analysed for total solids (TS), total soluble solids (TSS), pH, titratable acidity (TA) and ascorbic acid by the method of Ranganna (2012). Total phenolic content (TPC) of bael pulp and its hydrophilic and lipophilic extracts was determined by the method of Kahkonen *et al.* (1999) [6].

Antioxidant activity of bael pulp

Hydrophilic and lipophilic extracts of bael pulp were analysed for antioxidant activity by ABTS radical scavenging assay as described by Re *et al*. (1999) [15] and DPPH radical scavenging assay as described by Brand Williams *et al.* (1995) [2] with slight modification.

Optimization of level of bael pulp for development of protein enriched whey-bael beverage

The whey-bael beverage was prepared by incorporating 6, 8, 10, 12, 14% bael pulp keeping sugar concentration constant at 10%. The protocol followed for the preparation of whey-bael beverage are shown in the fig-1.

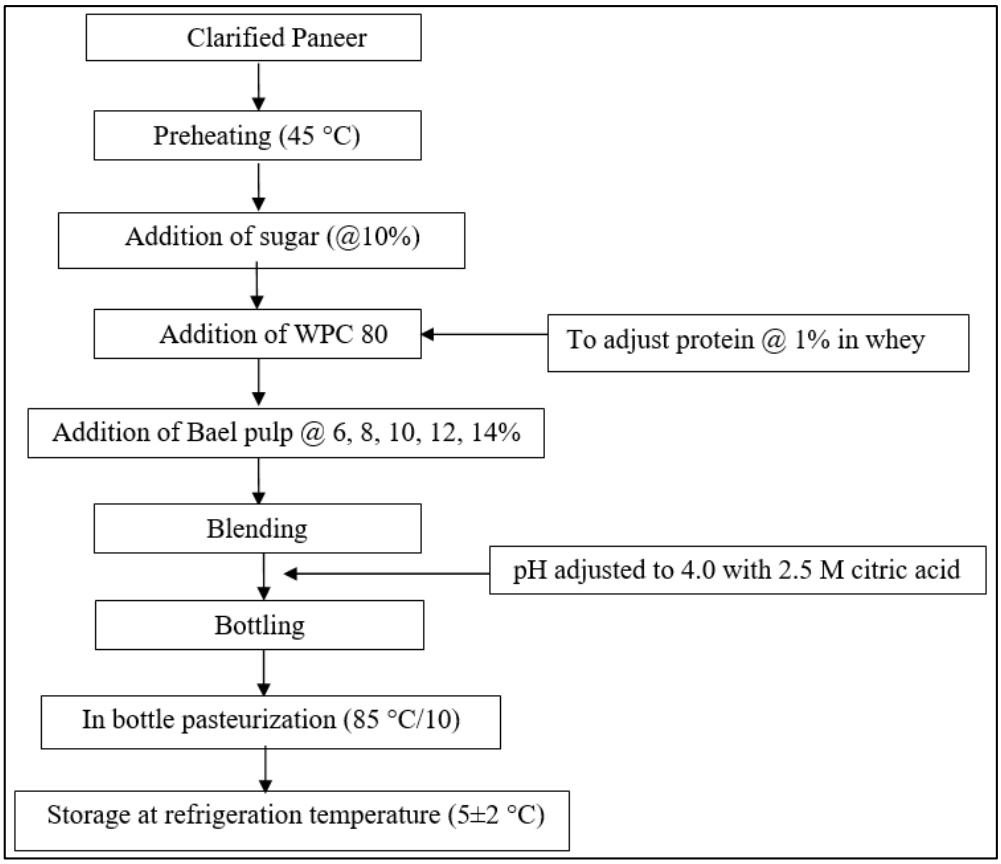

Fig 1: The protocol followed for the preparation of whey-bael beverage

Sensory evaluation of the beverages

The sensory evaluation of beverage samples with different levels of fruit (bael) pulp were done by a panel of 7 judges using 9-point hedonic Scale. During each replication, samples were judged for various sensory attributes like taste, aroma, mouthfeel, colour and appearance and overall acceptability.

Analysis of protein enriched whey-bael beverages

The beverage samples were analysed for TS, TSS, pH, titrable acidity, Ascorbic acid content and TPC. The protein and fat contents were determined using semi-micro Kjeldahl and gravimetric method, respectively as per FSSAI (2016) [4] manual. Antioxidant activity was analysed by ABTS and DPPH radical scavenging assay.

Statistical analysis

The data related to physico-chemical and sensory characteristics of beverage samples were analysed using statistical design CRD with equal number of observations as per the method of Snedecor and Cochran (1994) [17].

Results and Discussion

Physico-chemical characteristics of paneer whey

The gross composition and physico-chemical characteristics of paneer whey were determined and the data is presented in table-1. The total solids (TS), fat, protein, lactose and ash content of paneer whey were 6.27, 0.35, 0.56, 4.74 and 0.62%, respectively. The titratable acidity (TA) and the pH of the whey were 0.21% lactic acid (LA) and 5.60, respectively.

Table 1: Proximate composition and chemical characteristics of paneer whey

Proximate composition (%)	
Constituents	**Values**
Total solids	6.27±0.03
Fat	0.35±0.02
Protein	0.56±0.03
Lactose	4.74±0.05
Ash	0.62±0.02
Chemical characteristics	
Characteristics	**Value**
Acidity (% Lactic acid)	0.21±0.03
pH	5.60±0.08
Data are presented as means±SD (n=3)	

The chemical composition, pH and acidity of paneer whey were in close proximity with the values reported by Jadhao *et al.* (2018) [5] and they found that moisture, TS, fat, protein, ash and lactose as 93.50, 6.50, 0.50, 0.41, 0.60 and 5.0%, respectively. Manasiya (2016) [12] reported pH and acidity values as 5.6 and 0.19% lactic acid which was close to the values obtained in this study.

Physico-chemical characteristics of bael fruit pulp

The bael fruit pulp was analysed for its chemical characteristics *viz.*, TS, total soluble solids (TSS), titratable acidity (TA), pH, ascorbic acid, the results are presented in table-2.

Table 2: Physico-chemical characteristics of bael fruit pulp

Bael fruit pulp	
Physico-Chemical characteristic	**Values**
Total Solids (%)	40.26±0.58
Total Soluble Solids (^{0}Brix)	38.4±0.30
pH	4.82±0.03
Titratable Acidity (% Citric acid)	0.32±0.04
Ascorbic Acid (mg/100 g of pulp)	65.32±0.72
Data are presented as means±SD (n=3)	

The TS, TSS, pH, TA and Ascorbic acid contents of bael fruit pulp were 40.26%, 38.4^{0}Brix, 4.82, 0.32% as citric acid and 65.32mg/100 g of pulp, respectively. The physico-chemical characteristics are comparable with the studies carried out by Kaur and Kalia (2017) [8] wherein, they observed the TA (%) and pH as 0.30 and 4.95. Tarsem and Gehlot (2006) also reported TSS (°Brix), TA (%) and pH of 36.3, 0.46 and 4.5, respectively.

Antioxidant potential of bael fruit pulp

To assess the contribution of water soluble and lipid soluble components present in the bael fruit pulp to antioxidant activity, the hydrophilic and lipophilic extracts were prepared from bael fruit pulp and analysed for antioxidant activity and total phenolic content and depicted in table-3. The total antioxidant capacity of pulp was determined by the sum of hydrophilic and lipophilic antioxidant values.

Table 3: Antioxidant activity of bael fruit pulp

Bael fruit pulp	**Antioxidant capacity (µmol *TE/g)**		**Total phenolic content (µmol GAE**/g)**
	ABTS	**DPPH**	
Hydrophilic Extract	8.65±0.03^b	5.32±0.02^b	10.32±0.15^b
Lipophilic Extract	2.98±0.01^c	1.96±0.04^c	3.84±0.15^c
Total antioxidant activity	11.63±0.02^a	7.28±0.03^a	14.16±0.14^a
Means with different superscripts in each column (a, b, c) were significantly different from each other. Data are presented as means±SD (n=3) *TE- trolox equivalent **GAE- gallic acid equivalent			

As shown in Table 3, the antioxidant activity of hydrophilic extract measured by ABTS and DPPH method was significantly (p<0.05) higher as compared to lipophilic extract. The total antioxidant activity obtained by adding the antioxidant activity of both extract was 11.63 mmol and 7.28 Trolox equivalent/g of the sample measured using ABTS and DPPH, respectively. Similar trend was also observed with phenolic content of both extract. The high antioxidant activity of hydrophilic extract could be attributed to the presence of high concentration of polyphenolic compounds and other water-soluble components in the hydrophilic extract.

Optimization of levels of fruit pulp for preparation of protein enriched whey-bael beverage

The beverage was prepared using different levels of bael fruit pulp, 10% sugar and WPC for adjustment of protein in the final product at the level of 1.0%. All the beverages were subjected to sensory analysis on 9-point hedonic scale. The sensory scores obtained from panel of sensory analysts are presented in table-4.

Table 4: Sensory scores for protein enriched whey-bael beverages prepared using different levels of bael pulp

Bael pulp (%)	Scores on 9 point hedonic scale				
	Taste	Aroma	Mouthfeel	Colour & appearance	Overall acceptability
6	7.5±0.26^{a}	7.2±0.22^{a}	7.1±0.21^{a}	7.5±0.26^{a}	7.6±0.21^{a}
8	7.8±0.21^{b}	7.5±0.14^{b}	7.9±0.23^{b}	8.0±0.27^{b}	7.8±0.28^{b}
10	8.0±0.29^{b}	7.8±0.15^{c}	8.2±0.05^{c}	8.5±0.19^{c}	8.1±0.16^{c}
12	8.4±0.17^{c}	8.3±0.16^{d}	8.5±0.17^{d}	8.5±0.18^{c}	8.5±0.18^{d}
14	8.6±0.18^{c}	8.4±0.15^{d}	8.6±0.16^{d}	8.7±0.05^{c}	8.6±0.14^{d}

Means with different superscripts in each column (a, b, c, d) were significantly different from each other. Data are presented as means±SD (n=3)

It is evident from the results that whey-bael beverage prepared with the increase in the bael pulp concentration from 6.0 to 14.0%, results in significant (p<0.05) increase in the taste, aroma, mouthfeel and overall acceptability scores up to 12.0% of addition of bael pulp. Above 12.0%, no significant difference in these sensorial scores was observed. However, the colour and appearance score were increased significantly (p<0.05) only from 6.0 to 10.0% addition of bael pulp, thereafter no significant difference was observed upto 14.0% addition of bael pulp. On the contrary, Landge *et al.* (2020) [10] observed decrease in the sensory scores with increase in the supplementation of bael fruit pulp in the preparation of ready to serve whey beverage. Further, to select the optimized level of addition of bael pulp in the beverages, the antioxidant activity of all the beverages were also determined by ABTS and DPPH assay.

Antioxidant activity of protein enriched whey-bael beverage

The antioxidant capacity of beverages prepared with different levels (6.0, 8.0, 10.0, 12.0 and14.0%) of bael-pulp are presented in table-5. The ABTS radical scavenging activity of beverage prepared at different levels of bael pulp were 5.93, 7.41, 8.95, 10.26 and 11.86 whereas, DPPH radical scavenging activity values were 2.69, 3.43, 4.26, 4.77 and 5.80, respectively. It is evident from the Table 5 that as the level of bael pulp increased from 6.0 to 14.0%, there was a significant (p<0.05) increase in the ABTS and DPPH radical scavenging activity. Based on both sensorial scores and antioxidant activity, beverage with 14% level of addition of bael pulp and 10% addition of sugar, having 1.0% protein was found optimal. Singh and Nath (2004) [16] also prepared bael whey beverage that also used acidic polysaccharides (carboxy methyl cellulose and pectin), they found that superior quality of beverage was obtained using 25% bael pulp, 16°Brix, pH 3.9 and 1.75% whey protein level of CMC-WPC complex.

The best product obtained in the study on the basis of sensorial scores and antioxidant activity was further evaluated for its gross composition, physico-chemical properties and antioxidant activity.

Table 5: Antioxidant Activity of protein enriched whey-bael beverages.

Bael pulp (%)	Antioxidant activity (mmol TE/ml)	
	ABTS	DPPH
6	5.93±0.02^{a}	2.69±0.03^{a}
8	7.41±0.01^{b}	3.43±0.02^{b}
10	8.95±0.04^{c}	4.26±0.02^{c}
12	10.26±0.05^{d}	4.77±0.03^{d}
14	11.86±0.02^{e}	5.80±0.01^{e}

Means with different superscripts in each column (a, b, c, d, e) were significantly different (LSD test, p<0.05) from each other. Data are presented as means±SD (n=3)

Gross composition and physico-chemical properties of optimised protein enriched whey-bael beverage

The gross composition and physico-chemical properties of optimized beverage are presented in table-6. The values of TS, protein, fat, ash and carbohydrate of the optimized protein enriched whey-bael beverage were 21.29, 1.23, 0.54, 0.87 and 18.65%, respectively. The TSS content, pH and titratable acidity of the product were found as 19.8°Brix, 4.0 and 0.35% as Citric acid, respectively.

Table 6: Gross composition and physico-chemical properties of optimized protein enriched whey-bael beverage

Gross Composition	
Total Solids (%)	21.29±0.02
Protein (%)	1.23±0.02
Fat (%)	0.54±0.01
Ash (%)	0.87±0.03
Carbohydrate* (%)	18.65±0.4
Physico-Chemical Characteristics	
Total soluble solids(°Brix)	19.8±0.02
pH	4.0±0.01
Titratable acidity (% Citric acid)	0.35
Data are presented as means±SD (n=3) *By difference	

Antioxidant activity, TPC and Vitamin C content of optimized protein enriched whey-bael beverage

The antioxidant activities of whey-bael beverage were determined using ABTS and DPPH assay and compared with that of whey. The results are presented in table-7. The total antioxidant capacity of optimized whey-bael beverage were found as 11.86 and 5.80 µmol TE/ml based on ABTS and DPPH methods of assay, respectively, while it was 0.34 and 0.12 µmol TE/ml only for whey. The results showed that the ABTS value of whey-bael beverage was 34.88 fold higher and DPPH values were found to be 48.33 fold higher, respectively than paneer whey.

Table 7: Antioxidant capacity, total phenolic content and Vitamin C of protein enriched whey-bael beverages

Product	**Antioxidant capacity (µmol *TE /ml)**		**Total phenolic content (mg GAE**/100ml)**	**Vitamin C (mg/ml)**
	ABTS	**DPPH**	**TPC**	
Whey-Bael beverage	11.86±0.02	5.8±0.02	38.54±0.02	1.43±0.03
Whey	0.34±0.02	0.12±0.01	12.6±0.03	NA
Data are presented as means±SD (n=4), NA = Not analyzed *TE- Trolox Equivalent **GAE- Gallic Acid Equivalent				

The beverage was also analyzed for total phenolic content and Vitamin C content. The TPC content of the beverage was 38.54 mg GAE/100ml which was about 3 times higher than that of whey. The vitamin C content was found in the optimised beverage at the level of 1.43 mg/ ml. All these parameters together make the product antioxidant rich and health promoting.

Conclusion

Protein enriched and antioxidant rich whey-bael beverage can be prepared using different concentration of bael pulp. As the level of bael pulp increased from 6% to 12%, the sensorial scores significantly increased, however, addition of 14% pulp did not affect the sensorial scores significantly. The antioxidant activity of beverages significantly increases with increase in the addition of the bael pulp. Based on the sensorial attributes and the antioxidant activity, beverage prepared with 14% bael pulp, 10% sugar and 1% protein in the whey was optimal. The nutrient content of the beverage also increased with the addition of bael pulp and whey protein. Thus, protein enriched whey beverage with enhanced antioxidant activity and high acceptable sensory attributes could be developed by incorporation bael pulp in the whey. Further, antioxidant activity through *in-vivo* studies needs to be carried out for validation of the health benefits of developed whey-bael beverage.

References

1. Bag SK, Srivastav PP, Mishra HN. Optimization of process parameters for foaming of Bael (*Aegle marmelos* L.) fruit pulp. Food Bioprocess Technology. 2011;4:1450-1458.
2. Brand Williams W, Cuvelier M, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology. 1995;28(1):25-30.
3. Bureau of Indian Standard, Handbook (BIS: Pa-XI. Handbook of food analysis, Dairy products. Indian standard institution, manak bhavan, New Delhi; c1981.
4. FSSAI, Manual of method s of analysis of foods, microbiological testing. Food safety and standards authority of India ministry of health and family welfare government of India, New Delhi; c2016.
5. Jadhao MU, Shegokar SR, Wankhede PV, Kashid PS. Studies on Sensory Evaluation of jamun juice based paneer whey beverage. Bulletin of Environment, Pharmacology and Life Sciences. 2018;8(2):70-74.
6. Kahkonen MP, Hopia AI, Vuorela HJ. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry. 1999;47(10):3954-3962.
7. Kang DG, Yun C, Lee H. Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea. Journal of Ethnopharmacology. 2003;87(2-3):231-236.
8. Kaur A, Kalia M. Physico chemical analysis of bael (*Aegle marmelos* L.) fruit pulp, seed and pericarp. Chemical Science Review and Letters. 2017;6(22):1213-1218.
9. Krolczyk JB, Dawidziuk T, Janiszewska-Turak E, Sołowiej B. Use of whey and whey preparations in the food industry: A review. Polish journal of food and nutrition sciences. 2016;66(3):157-165.
10. Landge BD, Landge SN, Gaikwad SM, Nira VV. Studies on preparation of Ready to Serve Whey Beverage using whey and bael fruit (*Aegle marmelos* L.). Asian Journal of Applied Science. 2020;13:28-31.

11. Macwan SR, Dabhi BK, Parmar SC, Aparnathi KD. Whey and its utilization. International Journal of Current Microbiology and Applied Sciences. 2016;5(8):134-155.
12. Manasiya RA. Process standardization for utilization of paneer whey in jelly like confection. M.Tech. (Dairy chemistry) Thesis, Anand agricultural university, anand, 2016.
13. Nilsson J, Pillai D, Onning G, Persson C, Nilsson A, Akesson B. Comparison of the 2,2'-azinobis-3-ethylbenzotiazoline- 6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) methods to assess the total antioxidant capacity in extracts of fruit and vegetables. Molecular Nutrition and Food Research. 2005;49(2):239-246.
14. Ranganna S. In: Handbook of analysis and quality control for fruit and vegetable products, 2nd ed. Tata Mcgraw-hill publishing company limited, New Delhi, India, 2012.
15. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical biology and medicine. 1999;26(9-10):1231-1237.
16. Singh AK, Nath N. Development and evaluation of whey protein enriched bael fruit (*Aegle marmelos*) beverage. Journal of Food Science and Technology-Mysore. 2004;41(4):432-436.
17. Snedecor CW, Cochran WG. Statistical Methods. 6th Edn., The Iowa State University Press, Ames, Iowa, USA. 1994.
18. Tarsem C, Gehlot R. Utilization of bael (*Aegle marmelos Correa*.) for preparation and preservation of pulp, Research on Crops. 2006;3:887-890.
19. Vino SA. Underutilized fruits in India. Indian Food Industry. 2016;35(2):45-46
20. Wijewardana RMNA, Nawarathne SB, Wickramasinghe I, Gunawardane CR, Wasala WMCB, Thilakarathne BMKS. Retention of physicochemical and antioxidant properties of dehydrated bael (*Aegle marmelos*) and palmyra (*Borassus flabellifer*) fruit powders. Procedia food science. 2016;6:170-175. https://doi.org/10.1016/j.profoo.2016.02.041