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Development of Whey Drink Enriched with Vitamin C & Antioxidants

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Abstract: ***The present investigation was aimed to develop whey drink with incorporation of amla juice, honey and basil juice, was assessed on the sensory quality and physio-chemical parameters of drink. The whey-amla drink was prepared by using four different levels of amla juice, honey and basil leaves juice. Formulated samples were analysed by sensory evaluation and the beverage sample with 70ml whey, 20ml amla juice, 7ghoney and 3ml basil leaves juice were found to be superior. However, sensory parameters viz. colour, flavour, smell, mouth feel and overall acceptability of different formulations were significantly affected by variation of whey and amla juice. In bottle pasteurization was done at 85°C for 15min and stored in refrigeration (4°C). Antioxidant activity of the amla juice, basil leaves juice and whey-amla drink was assayed by DPPH method.***

Keywords: whey, amla juice, vitamin C, antioxidants, sensory attributes, beverage

1. Introduction

In order to create various food products with additional value, the food industry is currently searching for ingredients that could offer strong functional and nutritional features. The food business is being forced to look for these components by customers' growing awareness of nutrition, health, and food quality as well as by the fierce competition in the market. A number of unique functional food items have been produced in recent years in this regard. Dairy products make up more than 40% of these functional meals, while fermented drinks made with milk whey are the main functional beverages (Turkmen, N. *et al.*, 2019).

The milk serum, or yellowish liquid fraction of the milk that remains after curd or other coagulated products have been formed as a result of acid or a proteolytic enzyme. It is the principal by-product that is produced after the production of cheese, casein, paneer, channa, and other products. Just 10-20% of the milk used to make these goods is recovered as an end product, leaving around 80-90% whey behind. Whey contains between 45 and 50 percent of all milk solids, 70 percent of milk sugar (lactose), 20 percent of milk protein, 70 to 90 percent of milk minerals, and nearly all of the water-soluble vitamins found in milk (Wit, 1995).

The food processing business has seen a propensity to replace components in recipes for numerous goods throughout the years. It relates to low-fat and low-sugar foods, as well as vegetarian and lactose-intolerant food products (Bolumar*et al.*, 2015; Serna *et al.*, 2014). In addition to improving consumer health, whey and its preparations may help many businesses financially by cutting manufacturing costs by lowering the cost of raw materials (Bozanic*et al.*, 2014).

There is still significant opportunity to explore the potential of whey usage in the beverage sector, despite the numerous attempts to incorporate it into the composition of various dairy products. The manufacture of whey drinks with the inclusion of various components has been the subject of multiple patents recently. For instance, Whey-based drinks have already been created using a range of citrus fruits, tropical fruits, and other fruits like berries, apples, cherries, pear, apricot, or melon (Djuri´c, M. *et al.*, 2004).

Amla is known for its distinct flavour and aroma, and is a good source of vitamin C (478.56 mg/100 mL). When the fruit's juice was removed, the vitamin content of amla increased even more, containing calcium, phosphorus, iron, carotene, thiamine, riboflavin, and niacin. It also includes a lot of chemical components, giving it a very high antioxidant value.

Honey is a natural substance secreted by *Apis mellifera* honeybees and is used for food and medicine since ancient times. It is currently produced by less than 1% of the world's total sugar production and is the only commonly available sweetener and a significant source of carbohydrates. Honey contains a variety of small components, many of which are recognised for their antioxidant qualities, including enzymes, amino acids, and phenolic acids and flavonoids. Amino acids, trace amounts of vitamin B, vitamin B6, vitamin C, niacin, folic acid, minerals, iron, zinc, and antioxidants are all present in honey. Honey is often utilised as a bacterial, anti-inflammatory, and antioxidant agent.

Tulsi, commonly known as "The Extraordinary One, " "Mother Medicine of Nature, " and "The Queen of Herbs, " is an annual herb with a pungent flavour native to India. It has high levels of minerals and vitamin A, but low amounts of vitamin C and other vitamins, protein, fibre, and minerals. Basil has substantial health advantages due to its strong antioxidant and mineral content, with 8% of the plant containing eugenol, β element, caryophyllene, and germacrene.

2. Material and Methods

Source of raw material and sample preparation:
The various raw materials for the preparation of Whey-Amla drink such as milk, amla, honeywas procured from the local market of Mehsana. Basil leaves were collected form the campus garden of Ganpat University, Mehsana.

Preparation of Whey:
Milk was used for the preparation of good quality whey. Milk was heated to 85°C and when temperature decreased to 80°C, citric acid solution (40-50 ml per liter of milk) gradually added followed by continuous stirring which resulted in complete coagulation of milk protein (casein). The liquid (whey) was filtered using muslin cloth and stored for further use.

Preparation of Amla juice:
Whole amla fruit were used for the preparation on amla juice. Properly wash the amla and peel off black marks. Cut the amla in small pieces and discard the seeds. Make a blend paste of amla by adding small amount of water in mixer grinder. Strain out the juice from paste using muslin cloth. Pour the juice in clean, sterilized preheated bottles and seal the bottle accurately. Then in bottle sterilization was done at 85-90°C for 15 min and immediately cooled and keep the bottle in refrigerated temperature for further use.

Preparation of Basil leaves juice
Freshly harvested basil leaves were used for the preparation of basil leaves juice. Basil leaves were washed properly and stems from the leaves were removed. Make a paste of leaves by adding few drops of water in mortar-pastel. Filter the juice by using strainer from the paste for further use.

Method of Preparation:
Different formulation of whey beverage was prepared by adding of honey, amla juice and basil leaves juice as shown in flow chart. After proper mixing, it was filtered through clean muslin cloth to obtain whey-amla drink and filled into bottle. Then in bottle sterilization was done at 85-90°C for 15 min and immediately cooled and subjected to sensory evaluation to find out best formulation which was stored in refrigeration and ambient temperature for further study. The complete process flowchart for the preparation of beverage is presented in **Fig 1**.

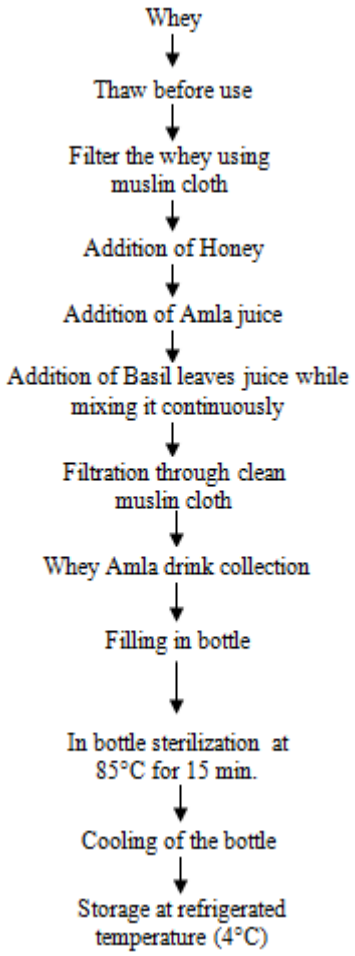

Figure 1: Flowchart for preparation of Whey Amla drink

Formulation:
For the preparation of 100 ml Whey-Amla drink, following four different formulations were used.

Table 1: Whey amla drink formulation

Ingredients	A (Control)	B	C	D	E
Liquid whey (ml)	100	60	65	70	75
Amla juice (ml)	0	30	25	20	18
Honey (g)	0	03	06	07	05
Basil leaves juice (ml)	0	07	04	03	03

Physio-chemical analysis:
The liquid whey, amla juice and whey-amla drink were analyzed for different physicochemical characteristics by applying standard methods. Total soluble solids were determined with Hand Refractometer (0-32) and the values were expressed as °Brix. Titratable acidity was calculated in terms of lactic acid for whey and citric acid for amla juice and whey-amla drink by titrating against 0.1N NaOH according to AOAC method (AOAC, 2019). Protein content of whey sample was determined by Kjeldahl method (KjelTRON Apparatus; Tulin) for nitrogen estimation, using factor of 6.38 for conversion of nitrogen into protein (Mariotti et al., 2008).

Fat content of liquid whey and whey-amla drink were determined by Gerber centrifuge method (AOAC, 2019). Digital pH meter (SYSTRONICS Digital pH Meter 335) was used for pH measurements. The ascorbic acid content of amla juice and whey-amla drink were determined according

to a redox iodometric titration using starch as an indicator (University of Canterbury College of Science, 2017).

DPPH Antioxidant assay:
1 ml sample of amla juice, basil juice and whey-amla drink were taken in a beaker to that 100 ml of 80% methanol was added. After that the content was transferred to a centrifuge tube and it was kept in a centrifuge tube and it was kept in centrifuge for 20 minutes at 3000 rpm. The extracted supernatant was collected in a beaker.

The DPPH (1, 1-Diphenyl2-picryl hydrazyl) Radical Scavenging Assay was performed according to Chang *et al.* (2001). The antioxidant activity of the extracts was compared with the natural antioxidant, ascorbic acid. Stock solution of DPPH assay was prepared by dissolving 4 mg DPPH with 100 ml methanol and then stored at 4°C for further use. Five different concentration of extract solution were prepared in 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml. A blank solution was prepared only with 3 ml of 80% methanol.

All the test tube containing different concentrations of standard ascorbic acid, extract, control as well as blank were allowed for stand for 30 minutes in dark cupboard. Next, all the test tube containing different concentrations of standard ascorbic acid, extract, control and blank were subjected to UV visible analysis in a dark condition. Approximately 2 ml content of each test tube was transferred into the quartz cuvette and measured for the absorbance at 517 nm in UV-Visible spectrometer. The absorbance values of each different concentrations of standard ascorbic acid, extract, control and blank were recorded. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\boldsymbol{Inhibition}\,(\%) = \frac{(\boldsymbol{Absorbance_{Control}} - \boldsymbol{Absorbance_{Sample}})}{\boldsymbol{Absorbance_{Control}}} \times \mathbf{100}$$

Sensory analysis:
Five different formulations of beverage samples were exposed for sensory analysis using 10 semi-trained penalists. The beverage samples were evaluated for appearance, taste, smell, mouthfeel and overall acceptability. The panelists were asked to record their observations on the sensory score card based on 9-point Hedonic rating scale ranging from 9: 'Like extremely' to 1: 'Dislike extremely'.

Statistical analysis:
The data was carried out using M. S. Excel (computer software), statistical tests such as Mean and standard deviation.

3. Results and Discussions

Physicochemical analysis of Raw Materials (Whey Liquid and Amla Juice):
The liquid *paneer* whey and amla juice were assessed for total solids, protein, moisture, fat, titratable acidity, pH, Total Soluble Solids (TSS), and ascorbic acid content as shown in **Table 2**. According to the results, whey showed 7.57% of total solids, 5.77 pH, 0.423% acidity (measured as percent lactic acid), 0.753% protein and 0.26% of fat. The composition of paneer whey presented and discussed above justifies with the study made by Lievore P. *et al.*, 2013.

Table 2: Chemical composition of liquid whey and amla juice

Sr. No.	Parameter	Whey	Amla juice
1.	Moisture (%)	92.43 ± 0.02	89 ± 0.015
2.	TSS (°Brix)	11.2 ± 0.10	15.8 ± 0.17
3.	pH	5.77 ± 0.01	4.35 ± 0.23
4.	Titratable Acidity (%)	0.423 ± 0.18	2.56 ± 0.31
5.	Protein (%)	0.753 ± 0.06	0.236 ± 0.05
6.	Ash (%)	0.51 ± 0.09	0.43 ± 0.027
7.	Fat (%)	0.26 ± 0.005	-
8.	Ascorbic acid (mg/100 ml)	-	597 ± 0.42
9.	Total Solids (%)	7.57 ± 0.02	11 ± 0.019

The amla juice showed 15.8 °Brix Total Soluble Solids (TSS), 2.56% acidity (measured as percent citric acid) and 4.35 pH which was lesser than pH of whey. The ascorbic acid content (597 mg/100 ml of sample) which was justify the range of the previously reported range of 387.53 to 729 mg/100 ml (Fenn, B. N., 2010; Kothari, C. and Bhatnagar, V., 2010).

Sensory analysis of different formulations of Whey-amla Drink:
Five different formulations of beverage samples were evaluated for sensory attributes namely appearance, taste, smell, mouthfeel and overall acceptability. It was observed from Table 3 that whey drink with 20 ml amla juice (formulation D) has scored the highest mean scores for all the sensory attributes such as appearance (7.81), taste (7.36), smell (7.27), mouthfeel (7.81) and overall acceptability (7.54).

The lowest mean scores for almost all sensory attributes were obtained by control sample indicating that control has the lowest consumer acceptance. Also, according to the statistical analysis effect of five treatment levels on all sensory characteristics were different.

Finally, the present study sensory evaluation data indicates that the good colour, peculiar flavour, good smell and pleasant mouthfeel were observed in formulation D also appreciated by the panel of judges.

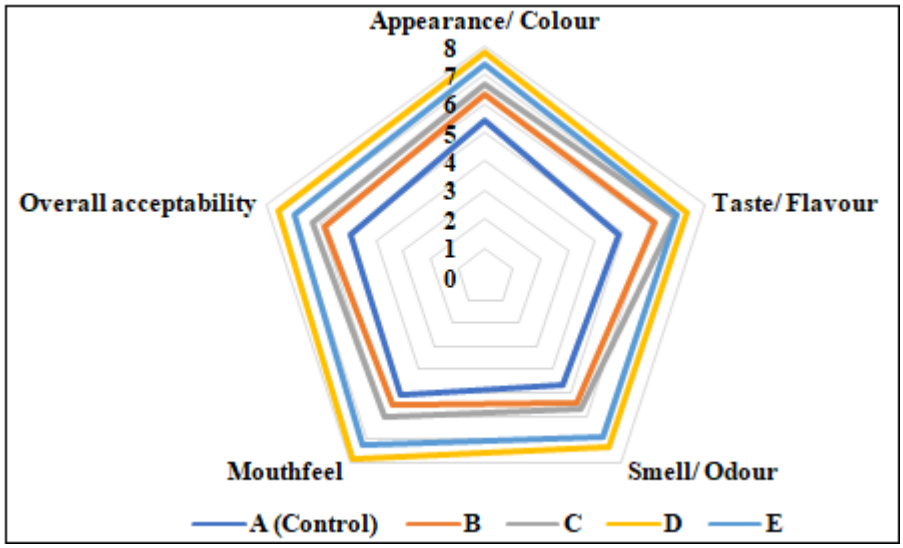

Figure 2: Changes in mean scores of sensory attributes among different formulations

Table 3: Sensory scores of different formulations

Formulation	Appearance/ Colour	Taste/ Flavour	Smell/ Odour	Mouthfeel	Overall acceptability
A (Control)	5.49 ± 0.892	4.89 ± 0.253	4.63 ± 0.452	5.03 ± 1.752	4.92 ± 0.591
B	6.38 ± 1.692	6.21 ± 0.785	5.37 ± 0.955	5.45 ± 1.894	5.87 ± 1.436
C	6.71 ± 1.236	6.91 ± 1.678	5.61 ± 0.237	5.95 ± 1.069	6.32 ± 0.612
D	7.81 ± 0.715	7.36 ± 0.979	7.27 ± 1.354	7.81 ± 0.935	7.54 ± 0.782
E	7.39 ± 2.541	6.98 ± 2.01	6.89 ± 1.689	7.22 ± 1.578	6.97 ± 2.598

Physicochemical analysis of final product (formulation-D):

The sample D with whey 70% (V/V) and amla juice 20% (V/V) is rated superior in the sensory evaluation. The chemical composition of the final product is presented in **Table 4.** According to the results, Whey-amla drink had a lower acidity 0.64 and pH 4.30. The protein content of the drink was 1.05% that is including whey protein and amla juice protein. Total soluble solids (TSS) of the drink was obtained 16 °Brix. The drink contains higher amount of ascorbic acid i. e.160 mg/ 100 ml. Vitamin C is an antioxidant which has led to its endorsement by some researchers as a complementary therapy for improving quality of life (Yeom*et al.*, 2007).

Table 4: Chemical composition of final product

Sr No.	Parameter	Whey-Amla drink
1.	Moisture (%)	72.83 ± 0.014
2.	TSS (°Brix)	16 ± 0.19
3.	pH	4.30 ± 0.26
4.	Titratable Acidity (%)	0.64 ± 0.31
5.	Protein (%)	1.05 ± 0.10
6.	Ash (%)	0.73 ± 0.06
7.	Fat (%)	0.45 ± 0.08
8.	Ascorbic acid (mg/100 ml)	160 ± 0.25
9.	Total Solids (%)	27.17 ± 0.09

Antioxidant activity:

The antioxidant activity of amla *(Emblica officinalis)* juice, basil leaves (*Ocimumbasilicum)* juice and whey-amla drink extract were assessed by DPPH scavenging method. The DPPH antioxidant assay was conducted according to the procedure described. Ascorbic acid was served as a reference standard for this experiment. The results obtained are given in the **Table 5.** The different calibration curves for DPPH assay from different solvents (Ascorbic acid, amla *(Emblica officinalis)* juice, basil leaves (*Ocimumbasilicum)* juice and whey-amla drink extract) are shown in (**Fig: 3-6**), respectively. **Fig: 7** represents the per cent inhibition of DPPH radical of different extracts of the amla *(Emblica officinalis)* juice, basil leaves (*Ocimumbasilicum)* juice and whey-amla drink and standard at different concentrations.

Table 5: % DPPH free radical scavenging activity of extracts

Concentration (mg/ml)	% Inhibition			
	Ascorbic acid (Standard)	Amla juice	Basil leaves juice	Whey-amla drink
0.5	97.44 ± 0.051	86.99 ± 0.072	75.87 ± 0.044	87.23 ± 0.069
1	97.95 ± 0.083	87.49 ± 0.028	79.62 ± 0.025	88.96 ± 0.017
1.5	98.12 ± 0.039	89.53 ± 0.057	83.79 ± 0.061	91.05 ± 0.024
2	98.63 ± 0.092	91.78 ± 0.015	86.96 ± 0.087	92.63 ± 0.054
2.5	99.14 ± 0.067	93.05 ± 0.049	88.12 ± 0.018	93.98 ± 0.076

In this study, % inhibition of standard ascorbic acid recorded between **97.44 ± 0.051** to **99.14 ± 0.067**. Whereas, % inhibition of amla *(Emblica officinalis)* juice methanolic extract resulted between **86.99 ± 0.072** to **93.05 ± 0.049**. % Inhibition of basil leaves (*Ocimumbasilicum)* juice methanolic extract obtained between **75.87 ± 0.044** to **88.12 ± 0.018**. % Inhibition of whey-amla drink (final product) extract ranges from **87.23 ± 0.069** to **93.98 ± 0.076**.

Antioxidant properties by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of standard ascorbic acid were ranges between **81.23 ± 0.009** to **96.84 ± 0.009** and % Inhibition of methanolic extract of amla *(Emblica officinalis)* recorded from **50.51 ± 0.028** to **96.49 ± 0.003** (Joey Teh Rou Xin *et al.*, 2022). DPPH values of basil leaves (*Ocimumbasilicum)* of different samples varied from **89.22 ± 2.5 %** to **69.33 ± 3.4%** (Aburigal, Y. A. A. *et al.*, 2017).

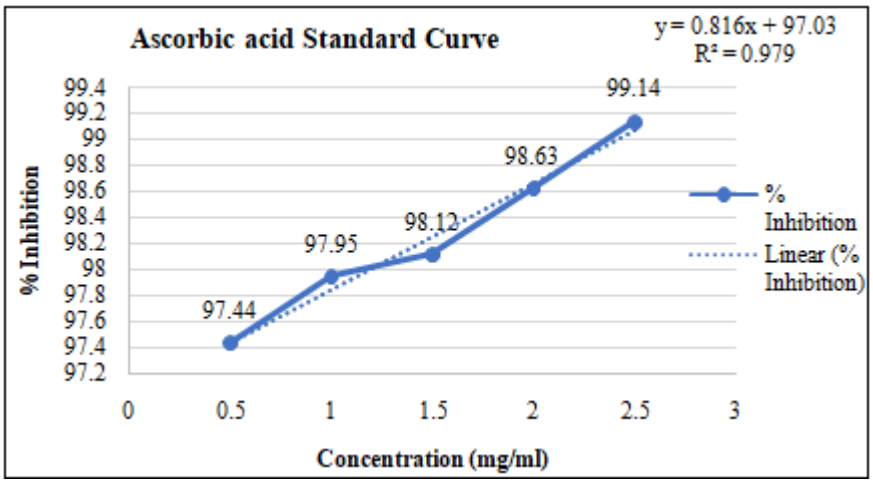

Figure 3: Calibration curve of ascorbic acid (standard)

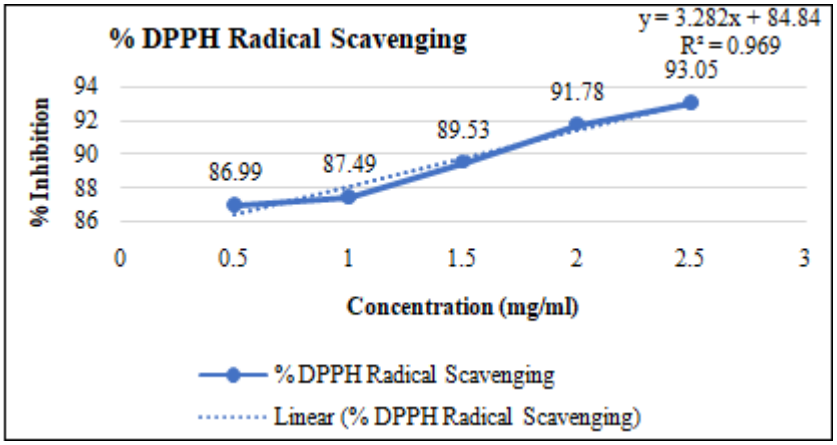

Figure 4: Calibration curve of amla *(Emblica officinalis)* juice methanolic extract

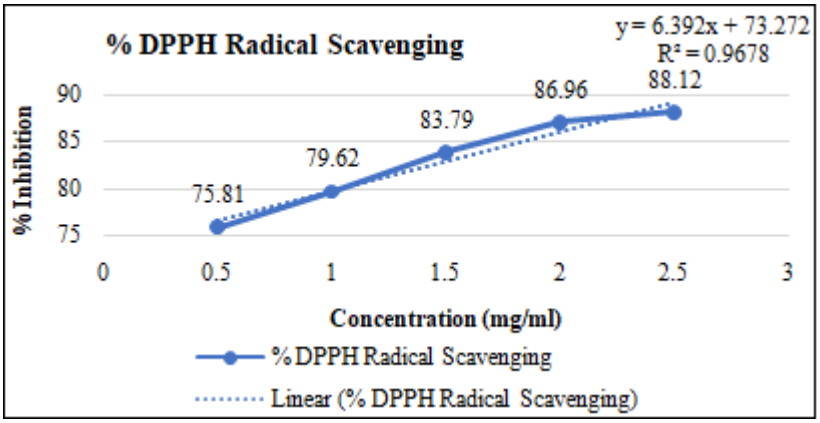

Figure 5: Calibration curve of basil leaves (*Ocimumbasilicum)* juice methanolic extract

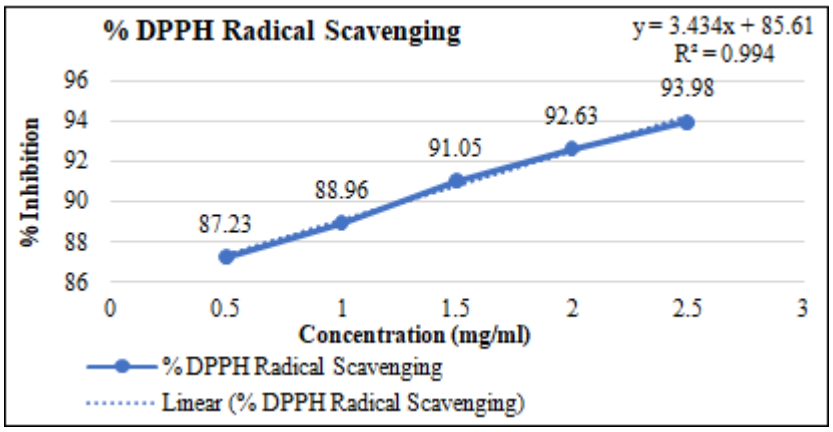

Figure 6: Calibration curve of whey-amla drink (final product) methanolic extract

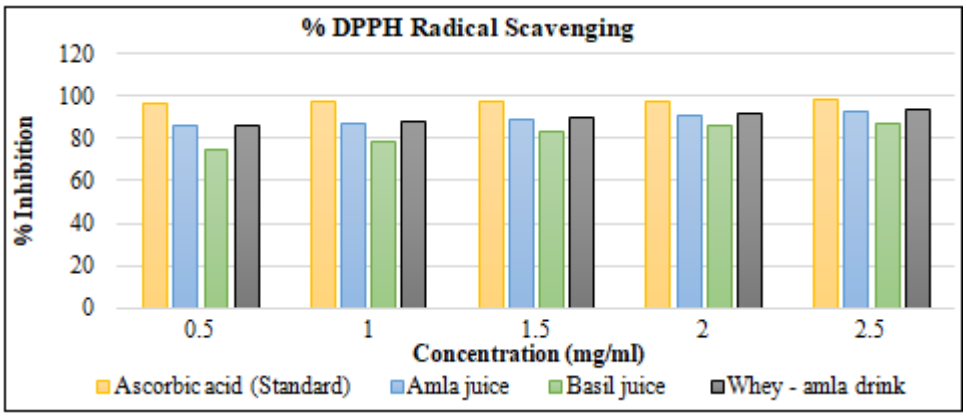

Figure 7: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition. Free radical scavenging activity of amla *(Emblica officinalis)* juice, basil leaves (*Ocimumbasilicum)* juice and whey-amla drink (final product) at different concentration level

4. Conclusions

From the results obtained based on the sensory analysis, it can be concluded that the incorporation of amla juice in blends of whey up to 20 ml proportion was found acceptable and more suitable without affecting the sensory characteristics significantly. The blending of amla juice enhanced the level of protein, total solids, ascorbic acid content and decreased the level of pH. The variation in proximate compositional constitute was proportional and significant. Fresh amla juice utilization up to 20 ml in whey produce good quality value added beverage. This will protect the interest of dairy industries with best utilization of whey.

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