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Optimization of Nutritious Mulberry Blended Herb Juices using Response Surface Methodology

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Abstract—*Blended juice from Mulberry, Ginger and herbs like Basil and mint leaves were formulated to enhance the nutritional and functional properties of blended juice using Response Surface Methodology (RSM). In this study the level of juice (3-9ml ginger juice, 4-12ml basil juice and mint juice) and degree of sweetness were optimized using RSM. The product was optimized on the basis of physico-chemical, therapeutic components and organoleptic attributes. The addition of basil, mint and ginger juice increases the therapeutic nutrient value of the juice blend as well as provides health benefit to the consumer. Therefore, ingredients composition of the juice blend was optimized by a statistical design tool in which a composite rotatable design (CCRD) has been used. Out of 30 suggested formulations, it could be concluded that a formulation having ranges of sweetness and major ingredients used were 16.6%, 3%, 11.97% and 12% in brix, Ginger, Basil and Mint respectively was the best among all combinations. The maxima obtained in the plot was considered as optimum formulation with expected outcome to be 8.21, 1096.34 mg of AA/100gm, 631.43 mg GAE/100gm and 87.65 mg/100gm in overall acceptability, Antioxidant activity by FRAP, TPC and Vitamin C respectively. Therefore, the ingredient composition of ginger %: basil %: mint % = 3%:11.97%:12% with 20% mulberry pulp maintain brix as 16.6 was selected as optimum.*

Keywords*: RSM, CCRD, organoleptic attributes etc.*

1. INTRODUCTION

The shelf life of fruit is very short at room temperature. In view of its limited shelf-life, it must be processed to assure availability of its produce and also minimize the glut in the market in its peak season of production. People consume beverages for their nutritional value, thirst-quenching properties, stimulating effect and/ or their medicinal values. Fruit beverages are well relished by all age groups of the society. Fruit beverage is produced and consumed all over the world and over a very long life span thus prevents nutrition related diseases. It can help to reduce high cholesterol levels in our blood. Functional beverages are drinks that have enhanced with added ingredients to provide specific health benefits and diseases preventing property beyond general nutrition. The total production of fruit juices in the year 2010 was 230 million litres and the production of packed fruit juices was 3.4 million litres (Bharadwaj, 2011). The common nutritious juicy fruits are high acidity, astringency and such other factors in some of the fruits, the utilization of these fruits for preparation of various products become limited, despite having their high nutritional quantities. Therefore, blending of fruit juices is practiced to overcome the high cost of some exotic fruit juices, scarcity or seasonal availability, balancing of strong flavors, improving and stabilizing color (FAO, 2001). Nutritional or phytochemical properties can be improved by blending which offers to adjust sugar/acid ratios and compensate undesirable juice consistency alternative for its utilization in order to have some value added fruit drinks, which are of high quality in respect of both sensory and nutritional aspect.

Mulberry *(Morusindica)* is a perennial and woody plant, belongs to the family Moraceaeand genus *Morus*, native of China. Mulberry plants showing fast growth and short proliferation period, a deciduous plant grows under various environmental conditions i.e., tropical, subtropical and temperate. The mulberry fruits are often harvested by spreading a sheet on the ground and shaking the branches. The deep colored mulberry fruits are rich in phenolic compounds, including flavonoids, anthocyanins, and carotenoids (Sofia*et al*., 2014). Mulberry fruit has pigment named anthocyanins. Anthocyanins are pigments which hold potential use as dietary modulators of mechanisms for various diseases and as natural food colorants. Anthocyanins are responsible for the attractive colors of fresh plant foods, producing colors such as red, purple, black, and blue.Ginger, a herb known as *Zingiberofficinale*belongs to the family Zingiberaceae.The rhizome (underground stem) is used as a spice and also as a medicine. Ginger is commonly used to treat various types of stomach problems, including motion sickness, upset stomach, gas, diarrhea, nauseacaused by cancer treatment, nausea and vomiting after surgery, as well as loss of appetite.Other uses include pain relief from arthritis or muscle soreness, menstrual pain, upper respiratory tract infections, cough, and bronchitis. In foods and beverages, ginger is used as a flavoring agent.

Basil (*Ocimumbasilicum*), the holy herb belongs to the family of *Lamiacea*. It has shown reduction in the cholesterol levels and blood glucose levels, prevents radiation poisoning and cataracts due to its high antioxidant content. It is also very useful for respiratory disorders (Khogare and Lokhande, 2011). Peppermint (*Mentha* × *piperita*) is a hybrid mint, a cross between water mint and spearmint. Peppermint oil also contains small amounts of many additional compounds including limonene, pulegone, caryophyllene and pinene. RSM is a method to optimize the process parameters. It reduces the number of trials needed to evaluate multiple parameters and their interactions. Central composite randomized design was used as a tool to design an optimized formulation for enhancing the nutritional and functional properties of blended juice.

2. MATERIALS AND METHODS:

2.1 Procurement of raw material:

Fully matured ripened, fresh fruits of mulberry, well developed ginger rhizome and laves of basil and mint were procured from local area of Burla, Sambalpur, Odisha. The study was carried out in the year 2017-18 at Sambalpur University, JyotiVihar, Burla, Sambalpur.

2.2 Juice extraction and Preparation of RTS beverages:

Mulberry, ginger and leaves were washed with clean running water to remove dust and to reduce the microbial load. The juice from mulberry fruits was extracted mechanically by crushing and pressing. The juices were kept for 30min at 4±2°C for sedimentation. Then the clear juice was siphoned off. The juice was filtered through muslin cloth, after that clear juices were blendedto standardize the proportion.

2.3 Experimental Design

Response surface methodology (RSM) is an effective statistical technique of process optimization. Central Composite rotatable design (CCRD) with quadratic model is employed to study the combined effect of four independent variables which influence on physiochemical and sensory characteristics of low calorie therapeutic beverages of fruits beverage. The central composite rotatable composite a box behnkendesign (CCRD), (Lee,2006) was adopted to predict responses based on few sets of experimental data in which all factors were varied within a chosen range. Numerical optimization technique of the Design–Expert software (8.0.3) was used for simultaneous optimization of the multiple responses. The design of experiments is mathematical and statistical techniques for designing experiments and evaluating the effects of factors. The experiments were performed and responses were fitted in the design. After each individual experiment, responses were analysed to assess the effect of independent variables on them. The first order or second order polynomial equation examines the statistical significance of the model and the following form was fitted to the responses:

$$Y = \beta_o + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_i X_i^2 + \sum_{i=1}^{3} \sum_{j=i}^{4} \beta_j X_i X_j$$

Where,

Y = response variable

βo, βi ,βi&βij = regression coefficient

Xi, Xj&Xij = coded independent variables

Response surface methodology was applied to the experimental data using a commercial statistical package for the generation of response surface plot and optimization of process variables. The experiments were conducted according to Central Composite Rotatable Design (CCRD). Quadratic polynomial regression model was used for fitting modal for the standard score and predicting individual Y with responses by employing at least square technique. RSM involves design of experiments, selection of levels of variables in experimental runs, use of fitting mathematical models and finally selection of variables levels by optimizing the response was employed in the study. Thirty sets of experiments were performed taking into account 4 factors viz., levels of TSS, ginger, basil and mint juice. The variables taken for present research work were TSS of blended juice, concentration of ginger, basil and orange juices, in the range of 12-18%, 3-9 ml, 4-12 ml and 4-12 ml, respectively. There were 6 experiments at centre point to calculate the repeatability of the method. Responses obtained after each trials were analyzed to visualize the interactive effect of various parameters on physico-chemical, sensory attributes and textural properties of blended juice. It studies the interaction between all factors and quickly arrives at the optimum conditions of factors for desirable responses.

2.4 Sensory evaluation

A sensory score card suggested by with little modification was adapted to analyze the sensory characteristics of the developed blended juice were done at 27°C.Sensory were evaluated by a panel of 20semi-trained members from Department of Food Science Technology and Nutrition, Sambalpur University, for colour, flavour and over all acceptability of the blended therapeutic beverages. The tests were performed using9-point hedonic scale, where 9 shows 'like extremely' and 1 was 'dislike extremely (Ranganna,2010).

2.5 Physico-chemical analysis:

The determination of total phenol content, vitamin C and anti-oxidant activity of blended juice were done. The phenol content was determined by folin-ciocalteau method and antioxidant activity by frap method. The vitamin C was determined by visual titration method with 2, 6-dichloro phenol indophenols dye solution by the standard procedure given in AOAC and expressed as mg/100gm.

2.5.1 Determination of Total Phenolic Content

The total phenol content of samples were obtained by using ciocalteu reagent.0.1ml of diluted extact was taken and mixed

with0.5ml of folin-ciocalteu reagent. Then the mixure was allowed to stand at room temperature for 5min.Then 2ml of prepared 20%Na_2CO_3(w/v)solution was added to the above mixture. The reaction mixture was then incubated at boiling water bath exactly 1minute, and then cooled at room temperature. The absorbance was measured at 765nm by taking distilled water as blank with the help of a spectrophotometer. Different concentration (10, 20, 30, 40, 50) µ/mg of Gallic acid solution was taken to make standard calibration curve. The curve was used to determine the total phenolic content of the sample and the results were expressed as mg of Gallic acid equivalent/g.

Calculation:

mggallic acid eq/100 g= C x V

M

Where,

C= Conentration determined from standard curve

V= Volume used during the assay(ml)

M= Mass of the extract used during the assay(gm)

2.5.2 Determination of Antioxidant Activity(FRAP method):

Antioxidant assay was determined by ferric reducing antioxidant power method. To the 2.5ml of extract, 1ml of 0.2M phosphate buffer pH 6.6 and 1ml of 1% potassium ferricyanide was added. The reaction mixture was incubated in water bath at 50°C for 20 minutes. Afterward reaction mixture was rapidly cooled and 2.5 ml of 10%trichloroacetic acid was added to stop the reaction and was centrifuged for 10 minutes. 2.5ml of distilled water and .5ml of 0.1% ferric chloride solution was added. The colour changes to green. The mixture was allowed to stand for 10 minutes and absorbance was measured at 593nm using UV- visible spectrophotometer. The blank was performed using reagent blank and solvent. Ascorbic acid was used as standard. The extracts were performed in triplicate.

3. RESULT AND DISCUSSION:

3.1 Proximate Nutritional composition of juice:

The nutritional qualities of juices were improved with the blending of ginger, basil and mint juice. The average contents of TSS, pH, acidity, Reducing Sugar, Vitamin C, TPC, TFC, Antioxidant activity and Anthocyanin contents of the blended juice were 16.6%, 3.56, 0.256%, 3.48%, 90mg/100g, 716..22mg GAE/100g, 164.12mg QE/100g, 1195.62mg AA/ 100g, 821.39 mg/L of cyanidine-3-glucoside.

Table 1: Nutritional content of raw ingredients:

Sr. No.	Constituents	Mulberry juice	Ginger Juice	Basil juice	Mint juice
1	Moisture	87.4%	81.9%	96%	95%
2	TSS	12 ° Brix	2.4 ° Brix	1.6	2.2
3	pH	3.37	5.9	7	7.5
4	Acidity	1.24%	0.6%	0.075	0.088
5	Carbohydrate	9.88gm	12.77gm	1gm	1.2gm
6	Protein	1.24gm	1.82gm	0.8gm	1gm
7	Vitamin C	32.4mg	5mg	16.6mg	28.8mg

3.2 Sensory Analysis

Color is an important factor influencing consumer acceptability of a product. It can indicate high-quality products such as the magenta or purple. Color also influences flavor recognition. Color increases the attractiveness. Aroma/flavour of a food can be recognized as a tantalizing and detectable to eating by nose and palate, but not always as an invaluable part of flavor. It is also defined as fragrance. The appearance of juice represents the clarity or turbidity nature of juice. The turbidity is only due to the suspended particles that makes haze formation. Taste is a sense that detected through the solution of soluble compounds in the saliva or in the food juices and the contact of those dissolved compounds with the taste buds. Mouth feel is a sensory perception, which means that only the human can perceive, describe and quantify it. It is a characteristic involved with touch sensation as well as after taste effect. It is generally a multi parameter attribute, usually associated with mechanical, geometrical and acoustic parameters (Szczesniak, 1998). The overall acceptability in respect to color, taste, flavor and texture were taken into consideration.

Theunliked blend was with low ginger that include Mulberry, water, sugar and mint leaves. It may be due to the strong smell and taste of mint leaves. The strong taste of sample was because of mint leaves contain menthol up to 50% which gave strong taste to drink. It was because of menthol that mint was used as mouth freshner and for soothing effects. (Ellen*et al*, 2011). The results showed that strong effect of mint leaves was reduced by the smell of ginger, mainly the terpenoids. The pungent taste of ginger is because of gingerols and shogaols which made the blend slightly pungent. The overall acceptability at the top as it contained complete blend of Mulberry, mint leaves, fresh basil juice and ginger. In this blend the strong taste of mint leaves was overcome by the ginger juice and ginger was only used to give little taste so this was most liked by the panel.

3.3 Optimization of juice combination;

The study was carried out for the optimization and the effect of these juices on nutritional properties of blended juice. The factors chosen for experimental design with different independent variables were include brix of juice, amount of

ginger, basil and mint juices, final responses also decided This was based on the physico-chemical (TPC, Vitamin C, Antioxidant activity) and sensory (OAA) characteristics of the blended juice. The design-expert software had represented 30 different sets of compositions as presented in Table 1. The results for central composite rotatable designs were used to fit second order polynomial equation. The response surface plots of these models have been plotted as a function of four variables where the effect of all the variations in levels of independent factors in designs on different responses can be visualized for each response.

The maxima obtained in the plot was considered as optimum formulation (brix: ginger %: basil %: mint % =16.6:3%:11.97%:12% expressed as %v/v).The expected outcome were found to be OAA=8.21, Antioxidant activity=1096.34 mg of AA/100gm, TPC= 631.43 mg GAE/100gm and Vitamin C= 87.65 mg/100gm. Therefore, the ingredient composition of ginger %: basil %: mint % = 3%:11.97%:12% with 20% mulberry pulp maintain brix as 16.6 was selected as optimum. The constraints for optimization and validation of the optimized formulation for mixed fruit beverage had given in Table 2.

Table 2: Experimental runs and actual values of factors used in central composite rotatable design (CCRD)

	Factors				Responses			
Run	**A:Brix Degree**	**B:Ginger Extract %**	**C:Basil Extract%**	**D:Mint%**	**OAA (9)**	**Antioxidant Activity**	**TPC(mg GAE/100g)**	**Vit. C (mg/100g)**
1	15.00	6.00	13.66	8.00	7.00	1401.459	551.25	80
2	15.00	6.00	8.00	8.00	8.055	982.481	472.54	77.5
3	15.00	6.00	2.34	8.00	6.333	1160.583	560.14	72.5
4	15.00	10.24	8.00	8.00	6.555	1119.708	511.50	78.5
5	18.00	9.00	12.00	12.00	7.722	1163.832	476.46	90.00
6	15.00	6.00	8.00	13.66	6.722	1207.298	519.61	92.5
7	15.00	1.76	8.00	8.00	6.388	1211.678	571.65	70.5
8	18.00	3.00	4.00	4.00	6.944	1213.138	588.91	60.0
9	15.00	6.00	8.00	8.00	7.833	998.540	470.71	77.5
10	15.00	6.00	8.00	8.00	8.088	989.146	474.37	75.0
11	18.00	9.00	4.00	12.00	7.50	1051.094	295.50	85.0
12	12.00	3.00	4.00	4.00	6.166	1258.394	587.34	60.0
13	15.00	6.00	8.00	8.00	8.222	1105.109	756.27	67.5
14	18.00	9.00	12.00	4.00	8.111	1205.839	602.51	62.5
15	15.00	6.00	8.00	2.34	7.333	1268.613	721.75	60.0
16	15.00	6.00	8.00	8.00	6.888	944.525	628.12	77.5
17	12.00	3.00	12.00	4.00	6.277	807.299	542.88	60.5
18	12.00	3.00	12.00	12.00	7.777	1345.985	675.20	87.5
19	18.00	3.00	12.00	4.00	7.111	817.518	545.50	62.5
20	19.24	6.00	8.00	8.00	5.777	966.423	480.18	77.5
21	15.00	6.00	8.00	8.00	8.333	978.342	478.24	77.5
22	12.00	9.00	12.00	4.00	6.777	1229.197	623.43	65.0
23	12.00	9.00	4.00	4.00	5.50	1192.700	607.21	62.5
24	10.76	6.00	8.00	8.00	5.222	975.684	478.13	77.5
25	18.00	3.00	12.00	12.00	6.444	1456.935	672.59	87.5
26	12.00	9.00	4.00	12.00	6.055	1001.459	296.81	85
27	18.00	9.00	4.00	4.00	7.166	1153.285	614.53	62.5
28	18.00	3.00	4.00	12.00	8.5	1129.927	717.05	85.0
29	12.00	9.00	12.00	12.00	7.166	1147.445	481.17	90.0
30	12.00	3.00	4.00	12.00	7.722	1110.948	698.74	85.0

Effect of ingredient variables on nutritional properties of blended juice:

Overall acceptability: The quadratic equation obtained by the RSA of the data showing the effect of brix, Ginger, Basil and Mint extracts on overall acceptability (OA) score is shown below:

Overall Acceptability = -18.63326 +3.06396* Brix +0.072694* Basil Extract +0.48296 * Mint -0.025833* Brix * Mint -0.092667 * Brix^2

The response surface plot presented as Figure 1 shows the effect of brix and mint on OA score of blended juice. Increasing the levels of brix produces a significant effect on OA score as well as higher the amounts of mint juice had the positive effect on OA score. The overall acceptability of the blended juices was found to be superior as compared to juices prepared from individual and combination of two. The quadratic model for antioxidant activity was found to be significant (p<0.05). The OAA was mainly affected by the level of mint juice and sweetness.

Antioxidant Activity: The quadratic equation obtained by the RSA of the data showing the effect of brix, Ginger, Basil and Mint extracts on antioxidant activity (AA) score is shown below:

Antioxidant Activity = +443.14114 +127.41031 * Ginger Extract -6.81350 * Basil Extract -4.91417 Ginger Extract * Basil Extract -4.39845 * Ginger Extract^2 +3.97055* Basil Extract^2

The response surface plot presented as Figure 2 shows the effect of basil extract and ginger extract on Antioxidant Activity score of blended juice. Increasing the levels of both extracts produces a significant effect on Antioxidant activity score. Mint juice also enhances the antioxidant activity. Thus the extracts addition was found to have a synergistic effect in antioxidant activity.

Total Phenol Content: The quadratic equation obtained by the RSA of the data showing the effect of brix, Ginger, Basil and Mint extracts on TPC score is shown below:

TPC = +487.50831 +17.64325 * Ginger Extract -13.97997 * Basil Extract +35.16332 * Mint +2.75724 * Ginger Extract * Basil Extract -7.29526 * Ginger Extract * Mint

The response surface plot presented as Figure 3 shows the effect of mint extract and ginger extract on TPC score of blended juice. Increasing the levels of both extracts produces a significant effect on score.

Vitamin C Content: The quadratic equation obtained by the RSA of the data showing the effect of brix, Ginger, Basil and Mint extracts on Vit. C score is shown below:

Vitamin C= +49.86148 +0.091958 * Basil Extract +2.77140 * Mint +0.037109 * Basil Extract * Mint

The figure shows that vitamin C increases as the level of mint juice increases in the blended juice. The vitamin content of the blended juice was found to be 60-90mg/100ml (Table 3).

Table 3: Levels of responses fixed for optimization of blended juices

Constraints	Goal	Lower Limit	Upper Limit	Importance
Brix	Is in range	12	18	3
Ginger Extract	Is in range	3	9	3
Basil Extract	Is in range	4	12	3
Mint	Is in range	4	12	3
Overall Acceptability	Maximize	5.22	8.21	5
Antioxidant	Maximize	771	1401.46	4
TPC	Maximize	292.81	721.75	3
Vit C	Maximize	60	92.5	5

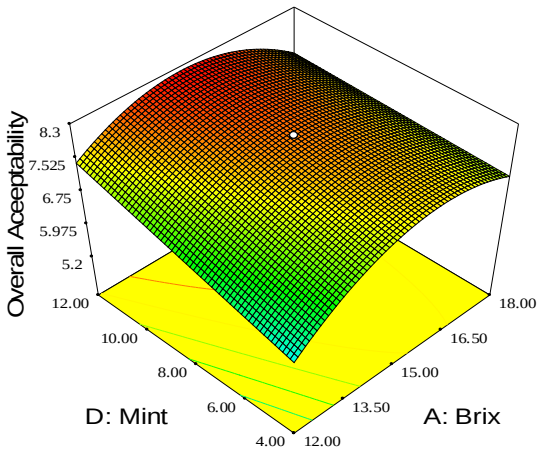

Fig. 1 Effect of ingredients variables on OAA ofblendedjuice

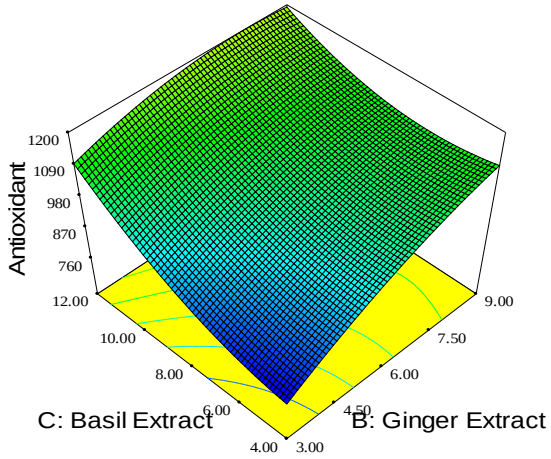

Fig. 2 Effect of ingredients variables on Antioxidant Activity of blended juice

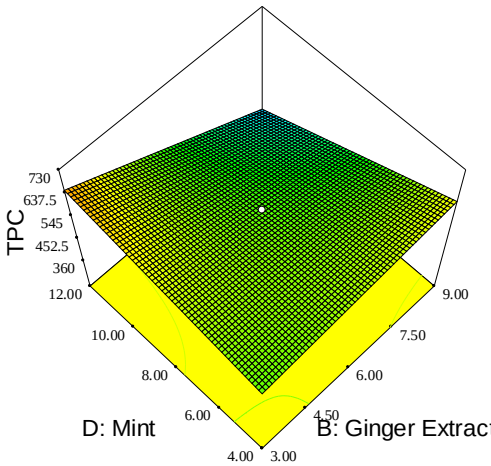

Fig. 3 Effect of ingredients variables on TPC of juice

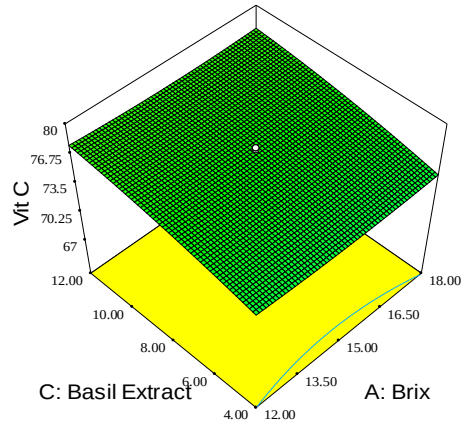

Fig. 4 (a) Effect of Basil extract on Vit. C

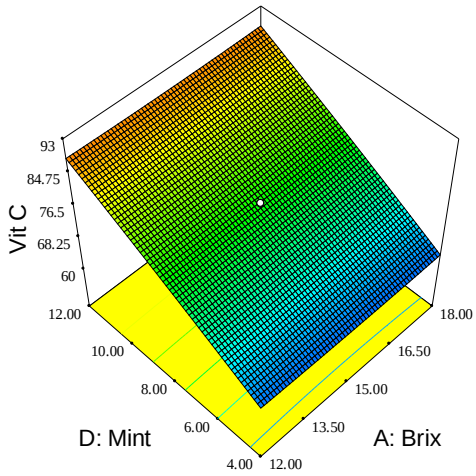

Fig. 4 (b) Effect of Mint Extract on Vit.C

4. CONCLUSION

The optimized level of sweetness, ginger, basil and mint juice for the manufacture of the nutritional and functional was predicted based on score of over al acceptability, antioxidant activity, vitamin C and total phenol content using RSM. Out of 30 suggested formulations, the optimum formulation having brix: ginger %: basil %: mint % =16.6:3%:11.97%:12% expressed as %v/v. The maxima obtained in the plot was considered as optimum formulation with expected outcome to be 8.21, 1096.34 mg of AA/100gm, 631.43 mg GAE/100gm and 87.65 mg/100gm in overall acceptability, Antioxidant activity by FRAP, TPC and Vitamin C respectively. Therefore, the ingredient composition of ginger %: basil %: mint % = 3%:11.97%:12% with 20% mulberry pulp maintain brix as 16.6 was selected as optimum.

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