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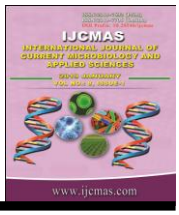

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Process Optimization for Flavoured Milk Added with *Piper betel* leaves

Vaibhav S. Kamble, Dnyaneshwar D. Patange*, Dinakar K. Kamble, Karishma S. Kamble and Sharad J. Patil

Faculty member of Division of Animal Husbandry and Dairy Science, College of Agriculture, Kolhapur-416004, India

**Corresponding author*

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A B S T R A C T

Considering the increasing demand to flavored milk and nutritional, therapeutic and post meal mouth freshener property of *Piper betel* leaves the present study was undertaken with object to optimize the levels of *Piper betel* leaves for developing flavoured milk and to study the sensory qualities as well as physico-chemical properties of *Piper betel* flavoured milk. Initially fresh cow milk was standardized to 2% fat and aqueous extract of piper betel leaves (PBLE) of different verities were prepared. The level of PBLE and sugar were optimized using CCRD of RSM. It was found that the flavoured milk prepared by the addition of the PBLE of Calcutta variety had obtained maximum scored for all the sensory qualities among other two varieties under study. In RSM trial the results showed that the colour and appearance, sweetness and overall acceptability score of milk was recorded maximum for formulation, having 5 per cent PBLE and 10 per cent sugar. The best solution exerted through the software, contained PBLE and sugar at 5.15 and 10.30 per cent, respectively. The validation of the prediction was done by actual observations recorded for sensory score. The optimized formulation had 8.49, 8.50, 7.40, 8.30 and 8.15 score for colour and appearance, flavour, consistency, sweetness and overall acceptability, respectively. Consumers as a whole liked the product 'moderately' to 'very much' with an average score of 7.70.

Introduction

It is estimated that about 46 per cent of milk is used as market milk while 54 per cent of total milk is converted into various milk products. Market milk means that milk, which is sold into the market and directly consumed by the individual (David, 2006). Market milk includes cow milk, buffalo milk, standardized milk, toned milk, double toned milk, skim milk, sterilized milk, full cream milk and flavored milk of which flavoured milk caters to the desire of the consumers for variety and a different experience in flavour. Some consumers do not like the flavour of natural milk but appreciate the nutritional value of milk in the form of flavour milk (Bisig, 2011). As per FSSR, (2011) flavoured milk, by whatever name called, may contain nuts (whole, fragmented or ground) chocolate, coffee or any other edible flavour, food colours and cane sugar. Flavoured milk shall

be pasteurized, sterilized or boiled. The type of milk shall be mentioned on the label. Bisig (2011) defined flavoured milk as are ready to drink products made from unfermented milk of different fat contents mixed with ingredients like sugar or other sweetener, cocoa powder, fruit juice, coffee, aroma agents, and /or other ingredients and additives. Flavoured milk can also encourage children to consume more milk, and some milk is used in school milk programmes.

Flavoured milks provide another option for meeting the recommended intakes of dairy products. The most popular flavour used in the flavoured milk are chocolate, coffee, vanilla, strawberry, rose, pineapple, mango, almond, banana etc., these flavoring materials are mostly synthetic or animal origin. Addition of synthetic flavour and color in food products may results in hyperactivity and behavioral problems in children and due to their toxic nature, manufactures are driving towards the natural color.

More and more people are interesting in herbal or natural way of life and for that they have used natural flavoring and colour material in preparation of foods (Palthur *et al*., 2014).Here is the official FDA definition of natural flavoring: "Natural flavor is the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, Several natural flavors and colors are available in the market such as caramel, carotenoids, carmine and Anthocyanin.

The deep green heart shaped *Piper betel* leaves are popularly known as Paan in India. It is also known as Nagaballi, Nagurvel, Saptaseera, Sompatra, Tamalapaku, Tambul, Tambuli, Vakshapatra, Vettilai, Voojangalata etc. in different parts of India. *Piper betel* leaves is an aromatic leaves belonging to Piperaceae family commonly used as masticatory as it is rich nutritionally and is known medicinally as a stimulant and carminative, an antiseptic and an expectorant.

It is composed of essential oils (0.7-2.6 per cent), and other constituents viz., carbohydrate (0.5-6.1 per cent), fat (0.4 -1.0 per cent) protein (3.0 -3.5 per cent), fiber (2.3 per cent), minerals (2.3-3.3 per cent), water (80-90 per cent) with good source of water and oil soluble vitamins (Guha, 2006 and Ramamurthi and Usha, 2012). The leaves also contain the enzyme like diastase and catalase besides a significant amount of all the essential amino acids except lysine, hystidine and arginine.

The leaves contain aromatic volatile oil composed by a phenol called chavicol which has powerful antiseptic properties. The alkaloid arakene is stimulant of muscular and mental efficiency. It is also used as mouth freshener after meal (Kumar, 1999).

Considering the increasing demand to flavored milk and flavoring, nutritional, therapeutic and post meal mouth freshener property of *Piper betel* leaves, the present investigation was carried out.

Materials and Methods

Materials

Fresh milk of crossbreed cow was procured from Dairy Farm, College of Agriculture, Kolhapur. Whereas, *Piper Betel* leave of Calcutta, Banaras and Maghai variety and crystalline cane sugar were procured from local market of Kolhapur city. Carboxy-methyl cellulose (E466) of Bijur Scooper Foods Pvt. Ltd. make was used as stabilizing agent.

Methodology

Preparation of *Piper betel* leaves extract

For the preparation of extract, fresh piper betel leaves were washed under running tap water, of which 10 g leaves were crushed with 100ml distilled water in a grinder for 2-3 min. The leaves juice was filtered through two folded muslin cloth and were used in the preparation of flavoured milk.

Preparation of flavoured milk

Flavoured milk was prepared as per the method suggested by Palthur *et al.,* (2014) for the preparation of ginger flavoured milk with slight modification (Fig. 1). Milk was pre-heated to 40ºC and filtered through a muslin cloth followed by standardiation to 2 %fat level, furthur it wasalllowed to heat at 65°C. At this time sugar and PBLE were added as per the treatment combination (Table 1)where as CMC stabilizer was used @ 0.1 % of milk.

The contents were mixedproperly and heated for another 2 min fallowed by homogenizaton at 2000 psi. At last milk was fill in 200 ml transparent glass bottle which was then sealed by crown cap and placed in hot water bath maintained at 65°C for 45 min, subsequently when bottles get cooled it was transfered in refrigerator at 5ºC.

Process optimization

Selection of *Piper betel* variety

Piper betel leaves of Calcutta, Banaras and Maghai variety, were washed under running tap water. The extract from leaves were obtained as procedure given above. Flavoured milk was prepared by addition of extract of thse varieties @ 5 % of milk with addition of 10 % sugar and 0.1 % CMC in all samples. The treatment included control i.e. milk without PBLE (LF_0), flavoured milk with PBLE of Calcutta variety (LF_1), flavoured milk with PBLE of Maghai variety (LF_2) and flavoured milk with PBLE of Banaras variety (LF_3).Samples were evaluated for sensory qualities and one best was selected.

Selection of level of PBLE for preparation of flavoured milk

In priliminary experiment the leaf extract Calcutta varities of piper betel was mixed @ 0, 1, 3, 5, 7 and 11 % of milk (v/v),sweeented with 10 % sugar and evaluated for sensory qualities. Theflavoured milk added with PBLE @ 3, 5 and 7 % exerted score above 7 (like moderately) on 9-point hedonic scale. These levels of PBLE were considered for RSM experiment.

Optimization of level of piper betel leaves extract (PBLE) and sugar

Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM) used to optimize the level of PBLE and sugar for two factors as independent variables (www.statease.com).The level of ingredients of the design matrix for the experiment is presented in Table1.

The data generated was analyzed using Design-Expert Software and a generalized polynomial equation was obtained for each response. Adequacy of model was evaluated using F ratio and co-efficient of determination (R^2). The lack of fit was calculated. Model was considered adequate when F-calculated was more than Table F-value and R^2 will be more than 70 per cent (Henika, 1972). The effect of the variables at linear, quadratic and interactive levels on individual responses was described using 1 and 5 per cent levels of confidence. From the results obtained through sensory evaluation of flavored milk with different levels of PBLE and sugar, most desirable combination was selected by verification of their sensory qualities.

Analytical work

Physico-chemical analysis

The product was analysed for fat, protein, lactose, sucrose, TS content by AOAC (2000) method, whereas, specific gravity and viscosity were determined as per protocol given by Mann *et al.,* (2012) and Anandh *et al.,* (2014). pH was known estimated by using Oroion 3 star pH benchtop pH meter according to the mentioned standard procedure.

Sensory evaluation

Sensory evaluation of PBLE added flavoured milk samples were carried out by a semi-trained panel of judges from the staff of the Division of Animal Husbandry and Dairy Science College of Agriculture, Kolhapur, by using 9- point Hedonic scale (Appendix -I) as described by (Hue, 1993).

For colour and appearance, flavour, consistency, sweetness and overall acceptability. Samples were served in coded number flask. Water was given between two samples to cleanse the mouth.

Consumer acceptability study

The experimental flavoured milk was subjected to consumer acceptance. The test was carried out with 200 respondents from local area. The responses were collected on pre designed questionnaire.

Statistical analysis

Data generated during selection of variety of PBLE were analyzed by employing CRD technique (Snedecor and Cochran, 1967). Whereas the level of PBLE and sugar was optimized using Stat-Ease Design Expert 7.0.0 package procured from stat ease Inc., USA (www.statease.com).

Results and Discussion

Selection of varieties of PBLE in the preparation of flavored milk

The flavoured milk prepared from extract of Calcutta variety had significant ($p<0.05$) highest score for colour and appearance (score = 8.40±0.02), flavour (score = 8.38±0.05) and overall acceptability (score = 7.36±0.02) over the rest of the two verities under study (Table 2).

The variation in color and appearance score might be because of the natural variation in colour of *Piper betel* leaves of different verities. Rai *et al.,* (2011) studied that *Piper betel* contain a wide variety of biological active compound whose concentration depends on the variety of plants, season and climate. The effect of PBLE of different varieties of leaves was merely affected on sweetness score of flavoured milk due to presence of sugar at equal level in the flavoured milk.

Optimization of level of PBLE and sugar through RSM

Effect of level of PBLE and sugar on sensory qualities of flavored milk

The colour and appearance score of *Piper betel* flavoured milk ranged from 7.5 to 8.6. The maximum score was obtained for formulation, which had 5 per cent PBLE and 10 per cent sugar. PBLE shown significant ($P< 0.05$) effect on colour and appearance score of flavoured milk at linear (positively) and quadratic (negatively) term. The regression analysis of a data presented in Table 3 reveals that the coefficient of determination (R^2) for colour and appearance score was 0.9437. The adequate precision was found to be 12.83, appreciably higher than the minimum desirable (4) for high prediction

ability. Further, the statistical analysis indicated that the model fitted the data well (model 'F' value 23.48). It can be seen from fig. 2, that increasing sugar level slowly increase in the colour and appearance score whereas, it increased rapidly as the PBLE level increases up to a certain point, beyond that the colour and appearance was decreased. Similar observations were recorded by Natisti *et al.,* (2014) during reported that colour and appearance score of flavoured soy ice cream with addition of lemongrass leaf extract.

Flavour score of *Piper betel* flavoured milk ranged from 7.5 to 8.5. The regression analysis of data for flavour score reveals that the coefficient of determination (R^2) was 0.9568.It is cleared from fig 3 that flavour score increased with increasing level of PBLE and sugar level at certain point but beyond that flavour score declined slowly. In addition, the interaction effect of PBLE and sugar was exerted in same manner. Sudha *et al.,* (2015) mentioned the decline in flavour score at higher concentration of sugar in the preparation flavour milk. Pugazhenthi and Jothylingam, (2010) recorded the maximum score for low calorie herbal flavoured milk, by incorporating Aloe-vera pulp extract at 5%.

The response surface uncoded equation derived for predicting flavour score could be given as:

Flavour = -16.99511 + 1.85876 * PBLE + 4.00970 * sugar - 0.056250 * PBLE * sugar - 0.12629 * $PBLE^2$ - 0.18017 * $sugar^2$

From table 4, it is clear that linear and quadratic interaction term sugar formed significantly changes in the consistency score indicating its importance in mouth feeling of products. Sugar in dairy products is not only responsible for sweetness; it also contributes to thickness. Oliveira *et al.,* (2015) reported creaminess. The interaction effect of PBLE and sugar levels on consistency score had significant. The sweetness score of flavored milk was ranged from 7.50 to 8.50. From the fig 4, it is seen that as the sugar percentage increased the overall acceptability score increased, however it was largely affected due to PBLE level. From fig 5, it is clear that maximum overall acceptability score was recorded for the product containing 5 and 10 per cent PBLE and sugar, respectively. The minimum overall acceptability score was recorded for product containing 3 and 9 per cent PBLE and sugar respectively.

Effect of level of PBLE and sugar on physico-chemical qualities of flavored milk

It is observed from table 5 that the pH, specific gravity, fat, sucrose and Total solids were decreased with increase in the level of PBLE and sugar. The adequate precision was found to be appreciably higher than the minimum desirable (4) for high prediction ability and the model 'F' value implies the model was significant for all physicochemical properties (Table 6).

The interaction effect of PBLE and sugar level on the pH, specific gravity, Total solids was in positive terms and for viscosity, fat, lactose had significantly affected but negatively. The coefficient for viscosity of *Piper betel* flavored milk model shows that the level of different variables had significant effect. The viscosity of flavored milk was also affected (Fig.6) by the level of sugar at linear, quadratic and interactive terms.

The quadratic term sugar also showed the significant effect in negative terms. At linear term the sucrose content and total solids were significant. Poul *et al.,* (2009) reported that with increase in the content of custard apple milk shake the total solid content of the milk shake was also increased.

Table.1 Experimental level of Independent variables in RSM

Std	PBLE (X_1) %	Sugar (X_2) %
1	5	11
2	3	9
3	3	11
4	5	10
5	5	10
6	5	10
7	3	10
8	7	11
9	5	9
10	5	10
11	7	9
12	5	10
13	7	10

Table.2 Effect of varieties PBLE on sensory qualities (score*) of flavoured milk

Treatments	Scores for sensory attributes				
	Colour and appearance	Flavour	Consistency	Sweetness	Overall acceptability
LF_0	6.42^{a}±0.04	6.35^{a}±0.04	7.34±0.04	7.34±0.12	6.86^{a}±0.02
LF_1	8.40^{d}±0.02	8.38^{d}±0.05	7.38±0.03	7.47±0.09	7.93^{d}±0.03
LF_2	7.57^{b}±0.05	7.14^{b}±0.04	7.37±0.03	7.34±0.09	7.36^{b}±0.02
LF_3	7.82^{c}±0.02	7.74^{c}±0.04	7.29±0.03	7.47±0.13	7.58^{c}±0.03
CD (P<0.05)	0.11	0.13	NS	NS	0.09

* Means ± SE of five replications within column fallowed by same letter are non significantly different at p< 0.05 NS= Non significant

Table.3 Effect of ingredient levels on sensory attributes (score *) of flavored milk

	Ingredients		Sensory Score				
Run	**PBLE (%)**	**Sugar (%)**	**Colour & appearance**	**Flavor**	**Consistency**	**Sweetness**	**Overall acceptability**
1	5	9	8.20	8.00	7.20	7.60	7.75
2	5	10	8.60	8.40	7.40	8.50	8.23
3	5	11	8.30	8.40	7.55	8.00	8.06
4	7	9	8.00	7.80	7.20	7.70	7.68
5	3	10	7.60	7.75	7.40	8.10	7.71
6	5	10	8.30	8.50	7.45	8.20	8.11
7	3	9	7.50	7.50	7.30	7.50	7.45
8	5	10	8.40	8.40	7.47	8.35	8.16
9	5	10	8.50	8.50	7.48	8.25	8.18
10	5	10	8.40	8.30	7.45	8.40	8.14
11	7	11	7.80	7.75	7.60	7.90	7.76
12	3	11	7.80	7.90	7.50	8.00	7.80
13	7	10	7.90	8.00	7.45	8.30	7.91

Table.4 Coefficients of quadratic polynomial model for coded sensory score attributes

	Sensory Score				
Factor	**Colour & appearance**	**Flavors**	**Consistency**	**Sweetness**	**Overall acceptability**
Intercept	8.41	8.41	7.44	8.32	8.15
PBLE(A)	0.13*	0.067	8.333E-003	0.050	0.065*
Sugar (B)	0.033	0.12*	0.16*	0.18*	0.12*
AB	-0.12	-0.11*	0.050*	-0.075	-0.067
A^2	-0.59*	-0.51*	-8.621-004	-0.084	-0.30*
B^2	-0.086	-0.18*	-0.051*	-0.48*	-0.20*
R^2	0.9437	0.9568	0.9593	0.9377	0.9652
F-value	23.48*	31.02*	32.96*	21.08*	38.78*
Mean	8.10	8.09	7.42	8.06	7.92
SD	0.11	0.092	0.032	0.10	0.060
Adequate Precision	12.83	15.76	19.13	12.52	18.51

(*Significant at 5%)

Table.5 Effect of ingredient levels on physico-chemical qualities of flavoured milk

Std	**Run**	**PBLE (%)**	**Sugar (%)**	**pH**	**Acidity (%LA)**	**Specific gravity**	**Viscosity (cP)**	**Fat (%)**	**Protein (%)**	**Lactose (%)**	**Sucrose (%)**	**Total solid (%)**
8	1	5	9	6.51	0.168	1.067	8.21	1.69	3.62	4.68	8.96	19.83
9	2	5	10	6.40	0.183	1.071	12.31	1.64	3.45	4.59	9.91	20.54
5	3	5	11	6.33	0.206	1.074	17.11	1.57	3.36	4.40	10.94	21.29
2	4	7	9	6.47	0.169	1.066	8.20	1.68	3.65	4.67	8.88	19.76
11	5	3	10	6.41	0.181	1.072	12.46	1.65	3.43	4.62	9.97	20.63
12	6	5	10	6.40	0.182	1.070	12.40	1.63	3.44	4.57	9.94	20.53
1	7	3	9	6.55	0.165	1.068	8.24	1.70	3.61	4.71	8.98	19.9
6	8	5	10	6.43	0.183	1.071	12.43	1.64	3.45	4.59	9.96	20.58
3	9	5	10	6.42	0.184	1.071	12.37	1.63	3.47	4.59	9.95	20.58
4	10	5	10	6.41	0.182	1.070	12.41	1.62	3.46	4.58	9.93	20.54
13	11	7	11	6.27	0.207	1.073	16.49	1.56	3.37	4.38	10.9	21.26
7	12	3	11	6.34	0.202	1.075	17.14	1.57	3.34	4.41	10.96	21.27
10	13	7	10	6.38	0.186	1.069	12.35	1.61	3.49	4.56	9.9	20.48

Table.6 Coefficients of quadratic polynomial model for coded physico-chemical attributes

Factor	pH	Acidity (%LA)	Specific gravity	Viscosity (cP)	Fat (%)	Protein (%)	Lactose (%)	Sucrose (%)	Total Solid (%)
Intercept	6.41	0.18	1.07	12.41	1.63	3.45	4.58	10.08	20.44
PBLE (A)	-0.030*	2.333E-003*	-1.167E-003*	-0.13*	-0.012*	0.022*	-0.022*	-0.053	-0.065
Sugar (B)	-0.098*	0.019*	3.500E-003*	4.35*	-0.062*	-0.14*	-0.15*	0.82*	0.54*
AB	2.500E-003	2.500E-004	0.000	-0.15*	2500E-003	-2.500E-003	2.500E-003	5.000E-003	0.028
A^2	-0.015	-2.414E-004	-5.172E-005	-0.058	-2.241E-003	4.310E-003	4.310E-003	-5.54	-0.53
B^2	0.010	3.259-003*	-5.172E-005	0.20*	-2.241E-003	0.034*	-0.046*	0.062	0.052
R^2	0.9760	0.9975	0.9815	0.9993	0.9795	0.9931	0.9955	0.8114	0.7046
F-value	56.91*	549.48*	74.25*	2079.83*	66.79*	200.78*	309.65*	6.02*	3.36*
Mean	6.41	0.18	1.07	12.47	1.63	3.47	4.57	10.60	21.22
SD	0.015	8.936E-004	4.691E-004	0.10	8.425E-003	0.011	9.349E-003	0.40	0.40
Adeq Precision	25.16	69.73	29.28	126.68	25.62	42.82	52.48	8.14	6.31

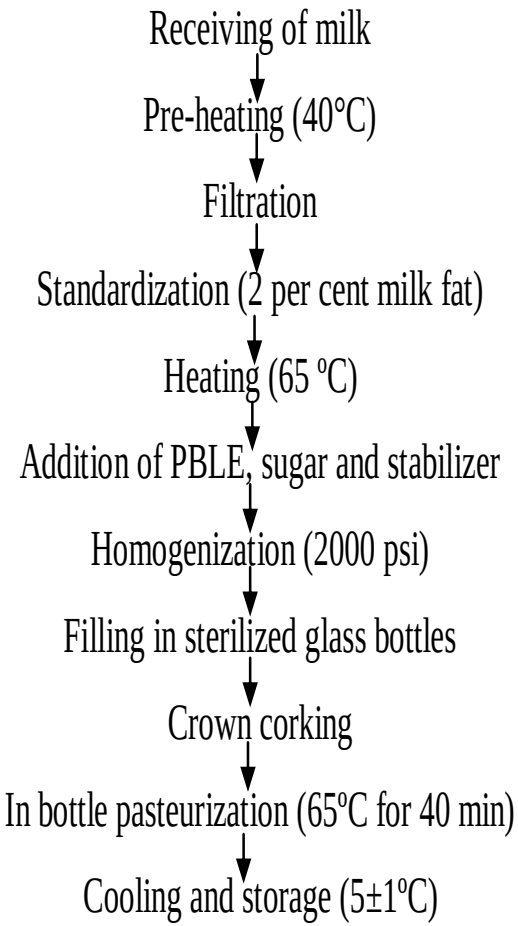
Table.7 Predicted and actual sensory score of suggested formulation by design expert 7.0.0 package

Sr. no	Ingredients (%)	Score	Sensory parameters				
			Colour & appearance	Flavour	Consistency	Sweetness	Overall acceptability
1	PBLE 5.15	Predicted	8.40	8.42	7.49	8.34	8.17
2	Sugar 10.30	Actual	8.49 ±0.05	8.50 ±0.06	7.40 ± 0.04	8.30 ±0.08	8.15 ± 0.01
T test			0.09 ±0.05	0.08 ±0.06	0.09 ±0.04	0.04 ±0.08	0.02 ±0.01

Table.8 Distribution of consumers based on acceptance of *Piper betel* leaves flavoured milk

Preference	Score	No. of respondent	Percentage
Fair	1	15	**7.5**
Good	2	35	**17.5**
Very good	3	47	**23.5**
Excellent	**4**	**113**	**56.5**

Fig.1 Flow diagram for manufacture of *Piper betel* leaves flavoured milk



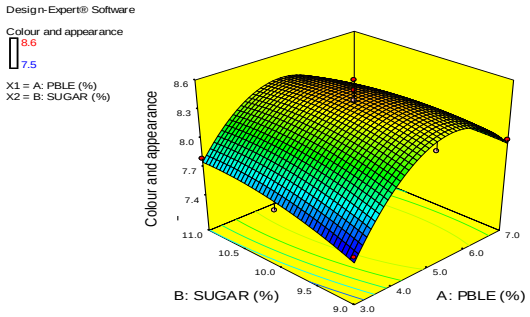


Fig.2 Effect of level of PBLE and sugar on color and appearance score of flavored milk

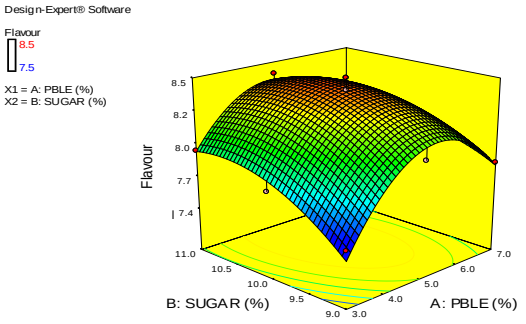


Fig.3 Effect of level of PBLE and sugar on flavour score of flavored milk

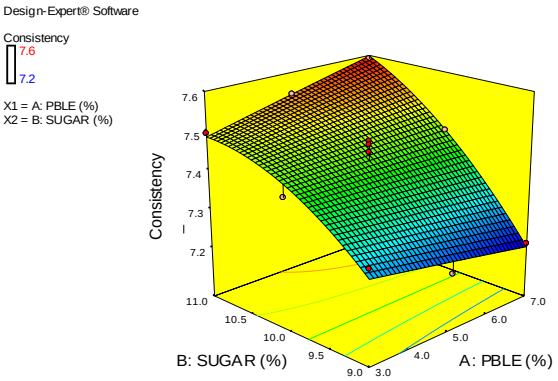


Fig.4 Effect of PBLE and sugar on consistency score

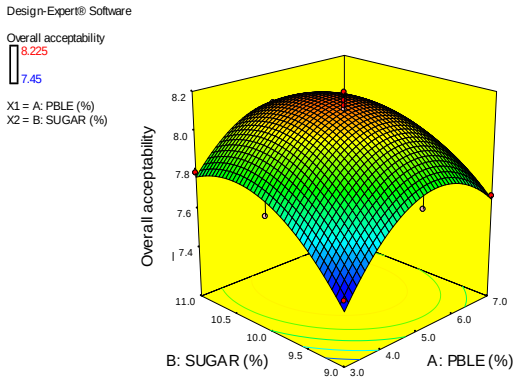


Fig.5 Effect of level of PBLE and sugar on overall acceptability score of flavored milk

Optimization of independent variables

The optimization of the variable levels was achieved by desirable maximization of the sensory response along the fitted polynomial model by numerical optimization procedure of design expert software. The best solution exerted through the software and their predicted score is presented in Table 5.The optimized levels of PBLE and sugar were 5.15 and 10.30 per cent, respectively.The validation of the prediction was done by actual observations recorded for sensory score of flavored milk prepared by addition of PBLE and sugar at 5.15 and 10.30 per cent, respectively. The predicted and actual response values (obtained after making the product using the optimum level of ingredients) were almost similar. Hence, 5.15% PBLE and 10.30% sugar levelwas recommended. Optimized flavoured milk had 8.49, 8.50, 7.40 8.30 and 8.17 score for colour & appearance, flavour, consistency, sweetness and overall acceptability, respectively(Table 7).

Consumer acceptance of piper betel flavored milk

It was found that 56.5 per cent of the consumers reported excellent quality of *Piper betel* flavored milk and 23.5 per cent reported very good remark about quality of *Piper betel* flavored milk.(Table 8) Ritu *et al.*, (2007) concluded that flavoured sweetened whey drink could be prepared by 4, 5 and 6 per cent mango pulp with highest consumer acceptability as compared to that of control

In conclusion use of Piper betel leaf extract of Calcutta variety in the preparation of flavored milk was significantly superior over the other two varieties under study. The most sensorial acceptable quality of *Piper betel* flavored milk can be prepared by using 5.15 per cent *Piper betel* leaves extract and 10.30 per cent sugar. The optimized formulated (5.15 % PBLE and 10.30 % sugar) product had 6.40 pH, 1.071 specific gravity, 18.50 (cP) viscosity and contained 01.64, 3.50, 4.55, 10.15, 0.95 and 20.79 per cent fat, protein, lactose, sucrose, ash, and total solid, respectively.

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