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OPTIMIZATION OF INGREDIENT LEVELS FOR THE FORMULATION OF LEGUMES MILK CHOCOLATE ENRICHED WITH PLANT SOURCES

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ABSTRACT

The present study was carried out to standardize the process for development of legumes based chocolate using peanut milk (PNM) and double bean milk (DBM). Response surface methodology (RSM) was employed to optimize the ingredients of PN: DB milk chocolate. The optimized concentrations of PNM, DBM, Jaggery (JG), Butter (BT) and Cocoa Powder (CP) were found to be 2.671 ml, 8.318 ml, 17.010g, 2.606g and 1.111g respectively. The nutritional parameters such as Total Phenolic Content (TPC), Folic acid and Protein were found to be 40.369 mg GAE/g, 4.623 µg & 5.463 g respectively and shows optimum in the chocolate. Addition of plant sources to chocolate increases the TPC, TFC and antioxidant activity in chocolate up to 20 to 30% and excellent in quality.

KEYWORDS: Bioactive compounds, Chocolate, Plant sources, Ingredients, Legumes, Optimization, Sensory.

1. INTRODUCTION

The ingredients for making confectionary products includes chocolate bars, fruits and nuts (Potter & Hotchkiss 1995) are the cow's milk and milk products and majority of the consumers accepts these food products all over the world (Manpreet *et al*., 2017). The products gained from cow's milk is more nutritious with passable sources of proteins of high biological value, lipids, vitamins and minerals and going all the way by consumer taste (Bolenz et al., 2003). However, inadequacy in supply of cow's milk and exclusion of milk in human diet due to metabolic diseases such as lactose intolerance (Szilagyi & Ishayek, 2018, Kundu et al., 2018), high cholesterol (Aydar et al., 2020), saturated fat content, cow's milk protein allergy, etc. (Aline *et al*., 2020) has directed to the development of alternate sources of plant milk (Falade et al., 2015; Swathi et al., 2016). As the substitute for cow's milk in the food processing to produce value added products such as beverages, yogurt, cheese, kefir, butter and ice cream are irreplaceble, with the high nutritional benefits (Krupa & Atanu, 2011; Falade et al., 2015; Swathi et al., 2016) in the food world. Legumes such as cowpeas, double bean, soybeans, peanuts and red kidney bean provides variable amounts of nutritional components including protein, carbohydrates, dietary fibre, minerals, vitamins and bioactive compounds are known for the health promoting vital components of a healthy diet (Malaguti et al., 2014; Aidoo et al., 2010; Margier et al., 2018). In addition, protein (Kouris-Blazos & Belski, 2016) and protein derived bioactive peptides of legumes have significant roles in contributing to increase the nutrients in processed food (Chakrabarti et al., 2018; Lopez-Barrios et al., 2014; Lopez-Cortez et al., 2016; Ortiz-Martinez et al., 2014). It is good source of folates and intake of folic acid decreases the risks of preterm delivery, low birth weight, foetal growth retardation, and developmental neural tube defects (NTDs) (Jagadish 2018; Bakiya et al., 2019). It also helps to lower cholesterol, triglycerides, inflammation, blood pressure and cardiovascular disease (CVD) as leguminous fibers are hypoglycosuria due to their valuable content of more amylase than amylopectin. (Afshin et al., 2014; Flight & Clifton, 2006). Legumes play major role in processing foods such as bakery products, bread, pasta, snack foods, soups, cereal bar filing, tortillas, meat, etc, due to their huge nutrition content (Reddy et al., 2009). Some studies reporting that the presence of high saponin and phytosterol contents in legumes reduce the formation of low-density lipoprotein which is a contrary correspondence with coronary heart disease, osteoporosis and other degenerative diseases (Afshin et al., 2014; Flight, I., & Clifton, P. 2006; Nagura et al., 2009; Maphosa & Jideani 2017).

Peanut and double bean are the major sources of high-quality protein, starch, dietary fibre, minerals and vitamins, being the part of human diet with high nutrition, low cost and easy availability. In addition, peas contain a wide range of phytochemicals with known bioactivity and potential health effects. (Martens, L.G., *et al.,* 2017).

As a suitable alternative for the dairy milk, peanut milk provides nutritional benefits for young and old people (Yadav et al, 2010) and it is prepared by grading, soaking, grinding with water, filtering through muslin cloth (Siva Sakthi et al, 2020) and extracted milk appears as cow's milk. Due to adequate sources of protein value (Albuquerque et al., 2015) it provides wide varieties of products such as yoghurt, butter milk, ripened cheese and tofu etc. (Diarra et al., 2005; Isanga & Zhang 2007). Green and beany flavour limits the application of peanut milk.

As the greater attention has been increased towards the nonconventional legumes such as double beans, for enhancing food and nutrition security and also for good health benefits due to its pontential source of natural antioxidants (Araoye et al., 2014; Bonita et al., 2020). Author studied the inclusion of double bean protein hydrolyzates with bioactive properties into a pasta extruded product have good bioactive compounds and decreases in cooking (Drago et al., 2016; Campos et al., 2014).

In order to avoid the global nutrient deficiencies, direct attention is needed to the nutritional profiling of various legumes increases the utilisation of underutilised legumes, produce cheap, innovative value-added products finds the ways of encouraging the use of existing legumes (Maphosa & Jideani 2017). The recent studies focused on the use of nutrient rich plant in fortifying foods increases in food applications such as soups, stiff dough, plantain flour, herbal biscuits, bread, cake and yoghurt will meet the expectations rapidly (Adewumi & Samson, 2018). Utilization of plant sources such as citrus medica leaves, moringa oleifera leaves and senna auriculata flower could leads to improvements in bioactive components in the food applications. Further fortification of bread with moringa leaf powder have been improved the protein and iron content of bread in order to avoid the protein malnutrition (Udeozor, 2012; Rao et al., 2007; Yadav et al., 2010; Yohanne et al., 2020) and iron deficiency globally (Govender & Siwela, 2020). Hence the presence of major and minor components like protein, fat, carbohydrate, calcium, iron, sodium, vitamin E and with some minerals (Agunbiade et al., 2011) in legumes milk can be utilized for chocolate production along with the other ingredients like cocoa powder, sugar and butter etc. Objective of this study was to develop and formulate the legumes based milk chocolate and to enhance the bioactive components with medicinal plant source in legume based chocolate.

2. MATERIALS AND METHODS

2.1 Raw materials procurement and pretreatment

Peanut (*Arachis hypogaea*), Double bean (*Phaseolus lunatus*), Jaggery (JG), Butter (BT) and Cocoa powder(CP) were purchased from the local super market in Chidambaram, India as shown in Figure 1. The mold free, dehulled beans and nuts were soaked in 2% $NaHCO_3$ for 3 h and 18 h respectively, washed thoroughly in clean water to soften and ensure the removal of beany flavor in the final product [Gatade, et al., 2009]. Fresh Leaves of Citron (Citron medica, CML) and drumstick (Moringa olifera, MOL) and Flower of Tanner's Cassia (Senna auriculata, SAF) are collected from local place as shown in Fig. 1. Fresh plant sources were shade dried to <5% moisture, powdered to 0.002mm and stored in air tight container for future use.

Figure 1: Legumes and Plant sources.

2.2 Extraction of legumes milk

Soaked beans and nuts were grounded separately using blender in the ratio of 2:1 (Legumes: Water) to obtain a smooth, fine, homogenized liquid, and then filtered with muslin cloth to obtain legumes-milk. The homogenized milk from peas and nuts was pasteurized at 80^{o}C for 15 minutes and then cooled to room temperature for future use.

2.3 Preparation of chocolate

Legumes milk (peanut milk (PNM) and Double bean milk (DBM)) in definite proportions was heated below its boiling point and then specific quantities of ingredients were added with continuous stirring to get a smooth fine paste, poured in a mold and refrigerated. The experiments were repeated for different ratios (1:1, 1:2, 1:3 and 1:4) as desired and also control milk chocolate. The nutritional and bioactive components of chocolates was determined using standard methods.

2.4 Experimental design and Evaluation

Response surface methodology (RSM) was used to investigate the influence of concentration of legumes-milk, jaggery, butter and Cocoa powder on the overall nutrient content of chocolate production. A central composite design with five factors and five levels including six replicates at the center point was used for fitting a second order polynomial equation. The nutrient content of chocolate was analyzed by multiple regressions through the method of least square to fit. The fit quality of the polynomial model equation was expressed by coefficient of determination R^2.

2.5 Sensory Analysis

Presumption of the consumer is very important for the acceptability of chocolate. The descriptive scheme was to evaluate the sensory attributes of chocolate with legumes based milk and replacement of sugar with jaggery to develop a complete nutritious chocolate. Sensory test was undertaken after one week preparation of chocolate with non- expert panel of 20 members using 9 point hedonic scale (where 9- Like Extremely, 8-Like Very Much, 7-Like Moderately, 6-Like Slightly, 5-Neither Like nor Dislike, 4-Dislike Slightly, 3-Dislike Moderately, 2-Dislike Very Much, 1-Dislike Extremely). (Szydlowska, et al., (2021) and Stone and sidel 1985). Each sample was evaluated for colour, flavour, mouthfeel, taste, texture and overall acceptability. Evaluation was done in triplicate and average value was reported.

3. RESULTS AND DISCUSSION

Among the different ratios of milk, prepared chocolate is selected on the basis of their sensory characteristics. By sensory evaluation, 1:3 ratio of PNM: DBM chocolate was accepted in overall. Response surface methodology (RSM) was employed to optimize the concentration of ingredients used in legumes milk chocolate, and nutritional parameters such as Total Phenolic Content (TPC), Folic acid and Protein content were analysed. Further, the enrichment of optimized ingredients with the employment of dry powdered plant sources such as *Citron medica* leaves (CML), *Moringa olifera* leaves (MOL) and *Senna auriculata* flowers (SAF) as they are rich in bioactive components and nutraceutical properties in the chocolate formulation individually. All experiments were carried out in triplicate and their mean values are presented.

3.1 Statistical analysis

The CCD is chosen to develop the second order polynomial model due to reduced number of actual experiments without significant loss of information (Tirado-Kulieva et al., 2021). Also, it is a good statistical tool in response to surface methodology because it predicts the variables of interest in the estimation of the parameters of the quadratic model and determines lack of fit of the model. This second order polynomial model demonstrates the rapport between PNM, DBM, JG, BT and CP on TPC, Folic acid and Proteins. The number of experiments (n) required for the development of CCD is defined as $n = 2^k$, where k is the number of experimental variables and Co is the number of experiments repeated at the centre point (k = 5; Co = 6). As a result, a total of 32 sets of experiments have to be performed. All other experimental conditions are kept constant during the experiments, and the runs are randomised to exclude any bias.

PNM, DBM, JG, BT and CP are the independent variables studied in the experimentation of bioactive components such as TPC, Folic acid and Proteins. These five variables are tested at different levels by associated plus signs (+2) with high levels, zero (0) indicating centre value and minus signs (−2) with low levels. Table 1 shows the levels and coded values of independent variables used in the experimental design for bioactive components.

Table 1: Parameters levels and coded values used in the experimental design for bioactive components.

Independent variables	Symbol	Variables with their uncoded levels				
		-2	-1	0	1	2
Peanut milk (ml)	PNM	1.5	2	2.5	3	3.5
Double bean milk (ml)	DBM	6.5	7	7.5	8	8.5
Jaggery(g)	JG	16	17	18	19	20
Butter (g)	BT	2	2.5	3	3.5	4
Cocoa Powder (g)	CP	0.8	0.9	1	1.1	1.2

For statistical calculation, the variables are coded according to the following Equation (1), where X_j is the coded value of the independent variable, x_i is its real value, x_o is its real value at the centre point and Δx is the step change in the variable X_i. Table 2 shows the experimental design and response value for bioactive components:

$X_j = \frac{x_i - x_0}{\Delta x}$ i = 1, 2, 3, 4, 5 (1)

Table 2: Central Composite Design matrix for the production of PNM: DBM chocolate.

Runs	Uncoded Values					TPC (GAE mg/g)	Folic Acid (µg/g)	Protein (g/ g of chocolate)
	PNM	DBM	JG	BT	CP	Experimental	Experimental	Experimental
1	2	7	17	2.5	1.1	36.560	3.850	3.521
2	3	7	17	2.5	0.9	30.632	2.852	3.031
3	2	8	19	2.5	0.9	35.956	2.935	4.023
4	3	8	19	2.5	1.1	39.846	4.293	4.965
5	2	7	17	2.5	0.9	30.808	3.565	3.896
6	3	7	17	2.5	1.1	33.954	1.185	3.006
7	2	8	19	2.5	1.1	37.286	1.235	3.882
8	3	8	19	2.5	0.9	30.845	2.999	4.361
9	2	7	17	3.5	0.9	40.913	2.262	3.996

10	3	7	17	3.5	1.1	42.693	1.795	3.452
11	2	8	17	3.5	1.1	31.097	3.655	3.785
12	3	8	17	3.5	0.9	34.723	3.525	3.003
13	2	7	19	3.5	1.1	32.006	4.556	2.896
14	3	7	19	3.5	0.9	40.963	4.085	4.053
15	2	8	19	3.5	0.9	32.763	2.653	3.091
16	3	8	19	3.5	1.1	45.336	2.389	4.369
17	1.5	7.5	18	3	1	36.178	3.391	3.023
18	3.5	7.5	18	3	1	38.446	3.016	4.012
19	2.5	6.5	18	3	1	36.496	3.025	4.003
20	2.5	8.5	18	3	1	43.112	3.155	4.108
21	2.5	7.5	16	3	1	44.912	3.548	3.978
22	2.5	7.5	20	3	1	40.810	2.893	3.952
23	2.5	7.5	18	2	1	35.943	2.652	4.059
24	2.5	7.5	18	4	1	36.453	3.290	3.003
25	2.5	7.5	18	3	0.8	32.883	3.055	3.612
26	2.5	7.5	18	3	1.2	39.508	2.362	4.089
27	2.5	7.5	18	3	1	43.145	3.166	3.959
28	2.5	7.5	18	3	1	41.974	3.002	4.042
29	2.5	7.5	18	3	1	44.023	2.862	4.202
30	2.5	7.5	18	3	1	43.545	3.198	3.896
31	2.5	7.5	18	3	1	42.906	3.306	4.112
32	2.5	7.5	18	3	1	42.796	3.062	4.053

The second-order polynomial regression model is given as Equation (3) to express Y as a function of the independent variables as follows,

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=2}^{k} \beta_{ij} x_i x_j \qquad (3)$$

where β_0 is a constant, while β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients, respectively. Xi and Xj are the levels of the independent variables:

The accuracy and ability of the above polynomial model could be evaluated by the coefficient of determination R^2 and F-test. The significance of the regression coefficient is tested by Student's t-test.

The experimental values for TPC, Folic acid and Protein for different experimental trails of PNM: DBM chocolate ingredients are presented in Table 2. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms are presented in Table 3. The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R^2 for all responses. The R^2 values for TPC, Folic acid and Protein were 93.95, 99.01 and 92.34 respectively. The closer the value of R^2 to the unity, better the empirical model fits the actual data. The smaller the value of R^2 the less relevant the dependent variables in the model have to explain of the behavior variation.

The probability (p) values of all regression models were less than 0.000, with no lack-of-fit. The results for TPC, Folic acid and Protein are evaluated according to the Central Composite Design matrix of 32 experiments with coded and actual independent process variables. The graphical representation of the surface plots and their corresponding contour plots shows the interaction between variables at each level for TPC, Folic acid and Protein are shown in Figures.

The three-dimensional response surface plot and a two-dimensional contour plot were obtained by the second order polynomial model by the linear, quadratic and interaction effects. Table 3, indicates the estimated regression coefficients and the corresponding statistical t and P values for TPC, Folic acid and Protein. Among linear effect of TPC on PNM: DBM chocolate, PNM (0.018 < 0.05), Butter (0.020 < 0.05) and Cocoa Powder (0.004< 0.05) are significant. In squared effects, PNM (0.001 < 0.05), DBM (0.013 < 0.05), Butter (0.00 < 0.00) and Cocoa powder (0.00 < 0.05) shows their significance.

Table 3: Regression coefficient with *t* and p value for Bioactive components.

Term	Total Phenolic content			Folic acid			Protein		
	Coefficient	t-value	P-value	Coefficient	t-value	P-value	Coefficient	t-value	P-value
Constant	43.302	56.25	0.000	3.1055	62.41	0.000	4.0460	44.05	0.000
PNM	1.089	2.76	0.018	-0.0974	-3.83	0.003	0.1303	2.77	0.018
DBM	0.523	1.33	0.211	-0.0086	-0.34	0.742	0.1599	3.40	0.006
JG	-0.694	-1.76	0.106	-0.1588	-6.23	0.000	-0.0114	-0.24	0.813
BT	1.068	2.71	0.020	0.1368	5.37	0.000	-0.1730	-3.68	0.004
CP	1.434	3.64	0.004	-0.1377	-5.41	0.000	0.0573	1.22	0.248
PNM * PNM	-1.675	-4.70	0.001	0.0199	0.86	0.407	-0.1336	-3.14	0.009
DBM * DBM	-1.052	-2.95	0.013	-0.0085	-0.37	0.719	0.0009	0.02	0.983
JG * JG	-0.288	-0.81	0.436	0.0241	1.05	0.317	-0.0217	-0.51	0.620
BT * BT	-1.954	-5.48	0.000	-0.0382	-1.66	0.125	-0.1302	-3.06	0.011
CP * CP	-1.954	-5.48	0.000	-0.1039	-4.51	0.001	-0.0503	-1.18	0.262
PNM * DBM	0.356	0.74	0.476	0.4403	14.11	0.000	0.1678	2.91	0.014
PNM * JG	0.929	1.93	0.080	-0.0696	-2.23	0.047	0.1811	3.15	0.009
PNM * BT	2.017	4.18	0.002	-0.0672	-2.16	0.054	0.0668	1.16	0.271
PNM * CP	1.760	3.65	0.004	-0.3550	-11.38	0.000	0.1416	2.46	0.032
DBM * JG	1.105	2.29	0.043	-0.4853	-15.56	0.000	0.0048	0.08	0.936
DBM * BT	-1.540	-3.19	0.009	-0.0304	-0.97	0.351	-0.2454	-4.26	0.001
DBM * CP	1.086	2.25	0.046	0.0524	1.68	0.121	0.2890	5.02	0.000
JG * BT	0.734	1.52	0.156	0.4620	14.81	0.000	0.0355	0.62	0.550

JG * CP	0.327	0.68	0.512	-0.3723	-11.93	0.000	-0.1824	-3.17	0.009
BT * CP	-1.102	-2.28	0.043	0.1036	3.32	0.007	0.0185	0.32	0.754

For Folic acid, the linear effects are significant for PNM, JG, BT and CP, in square effects, CP and for interaction effects, PNM *DBM, PNM*JG, PNM*CP, DBM*JG, JG*BT, JG*CP and BT*CP. For Protein, the linear effects are significant for PNM, DBM, and BT in square effects PNM and BT and for interaction effects, PNM * DBM, PNM * JG, PNM *CP, DBM *BT, DBM*CP and JG*CP.

To examine the statistical significance of factors and model, analysis of variance (ANOVA and regression analysis were conducted in for TPC, Folic acid and Protein. For TPC, coefficients (R^2) values were 93.95% and the adjusted coefficients (Adj.R^2) having 82.95% proves the high correlation between the experimental and predicted values. The F-value for the model is 8.54. For Folic Acid, the determination coefficients (R^2) values were 99.01% and the adjusted coefficients (Adj.R^2) also 97.20% proves the high correlation between the experimental and predicted values whereas for protein, the coefficients (R^2) values were 92.34% and the adjusted coefficients (Adj.R^2) is 78.41 % proves the high correlation between the experimental and predicted values.

3.2 Regression Equation for TPC, Folic Acid and Protein

The multiple regression analysis of the experimental results was fitted with the polynomial equation for the estimation of

TPC = 43.302 +1.089 PNM +0.523 DBM -0.694 JG +1.068 BT +1.434 CP -1.675 PNM*PNM -1.052 DBM*DBM -0.288 JG*JG -1.954 BT*BT -1.954 CP*CP +0.356 PNM*DBM +0.929 PNM*JG +2.017 PNM*BT +1.760 PNM*CP +1.105 DBM*JG -1.540 DBM*BT + 1.086 DBM*CP +0.734 JG*BT +0.327 JG*CP -1.102 BT*CP

Folic Acid = 3.1055 -0.0974 PNM -0.0086 DBM -0.1588 JG +0.1368 BT -0.1377 CP +0.0199 PNM*PNM -0.0085 DBM*DBM +0.0241 JG*JG -0.0382 BT*BT - 0.1039 CP*CP +0.4403 PNM*DBM -0.0696 PNM*JG -0.0672 PNM*BT -0.3550 PNM*CP - 0.4853 DBM*JG -0.0304 DBM*BT +0.0524 DBM*CP +0.4620 JG*BT -0.3723 JG*CP +0.1036 BT*CP

Protein = 4.0460 +0.1303 PNM +0.1599 DBM -0.0114 JG -0.1730 BT +0.0573 CP - 0.1336 PNM*PNM +0.0009 DBM*DBM -0.0217 JG*JG -0.1302 BT*BT -0.0503 CP*CP

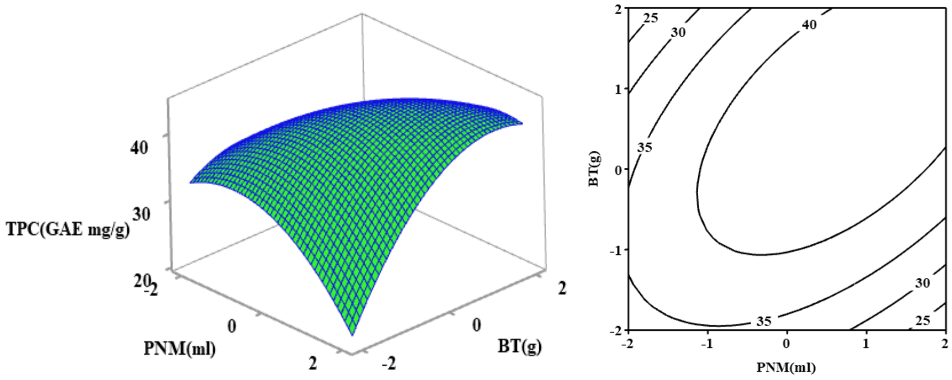
+0.1678 PNM*DB +0.1811 PNM*JG +0.0668 PNM*BT +0.1416 PNM*CP +0.0048 DBM*JG -0.2454 DBM*BT +0.2890 DBM*CP + 0.0355 JG*BT -0.1824 JG*CP +0.0185 BT*CP

3.3 Effect of Total Phenolic Content

The response surfaces for TPC are shown in Fig. 2a & 2b represents the surface plot and the contour plot for the interactive effect of PNM and BT, where DBM, JG, and CP were kept constant at the middle level. At the lower level of BT, the production of TPC goes on gradual decreasing till the middle value sudden declination was observed towards the higher value (1.5 ml) of PNM and reached minimum production. While at the maximum limit of BT, the production of TPC increases as the quantity of PNM increases (1.5ml to 3.5ml) similar effect was observed for BT when PNM is at higher limit. Initially the production of TPC is minimum a gradual elevation was noted as on BT increases from 2 - 4g. The highly elliptical nature of contour plot implies the interaction effect between PNM and BT are high significant.

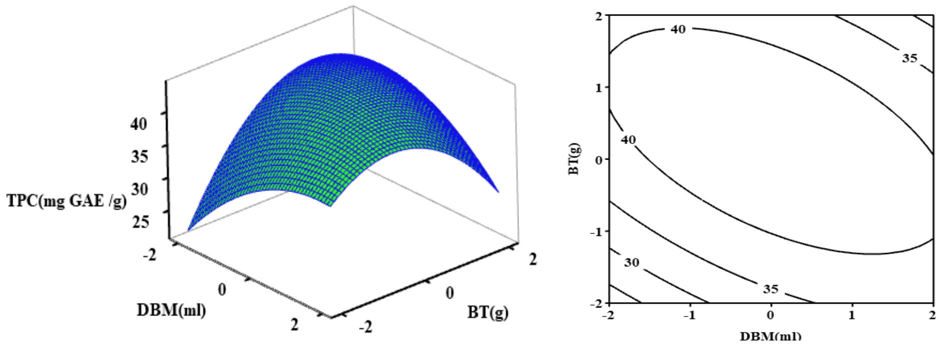
The response surfaces for TPC are shown in Fig. 3a represents the surface plot and Fig. 3b represents the contour plot for the interactive effect of DBM and BT, where PNM, JG and CP were kept constant at the middle level. At the lower level of BT, the production of TPC goes on increasing from the lower value (6.5 ml) to the higher level (8.5ml) of DBM and reaches maximum similar interaction effect was noted when BT varies from 2 - 4g at the lower level of DBM. But at the maximum level of BT, declined yield of TPC were obtained. The elliptical nature of the contour plot for the interactive effects DBM and BT on TPC ensures the significant nature of chocolate.

But there is a decreasing trend was found towards the upper limit (3.5ml) of PNM. At the higher value of DBM (8.5ml), as the PNM (1.5ml) increases the yield of TPC exhibits increasing trend and reaches maximum at the center point followed by gradual decreasing trend of TPC production was observed. The contour plot displays nearly circular at the center value, which infers that the interaction between the PNM and DBM are less significant on chocolate.



a) b)

Figure 2: (a) Surface and (b) Contour plot for PNM and BT on TPC.

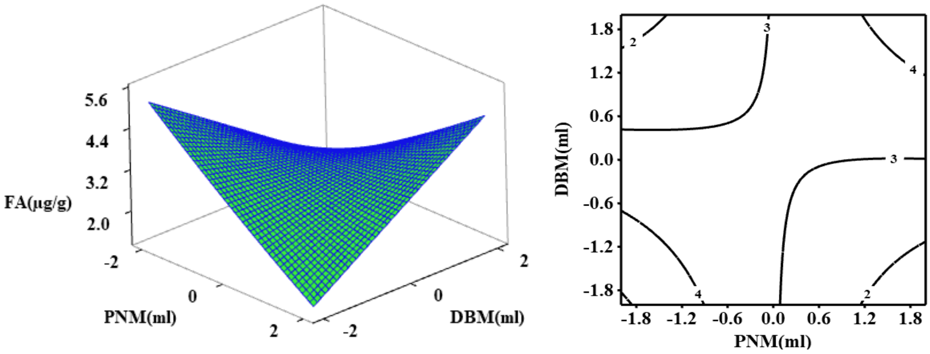


a) b)

Figure 3: (a) Surface and (b) Contour plot for DBM & BT on TPC.

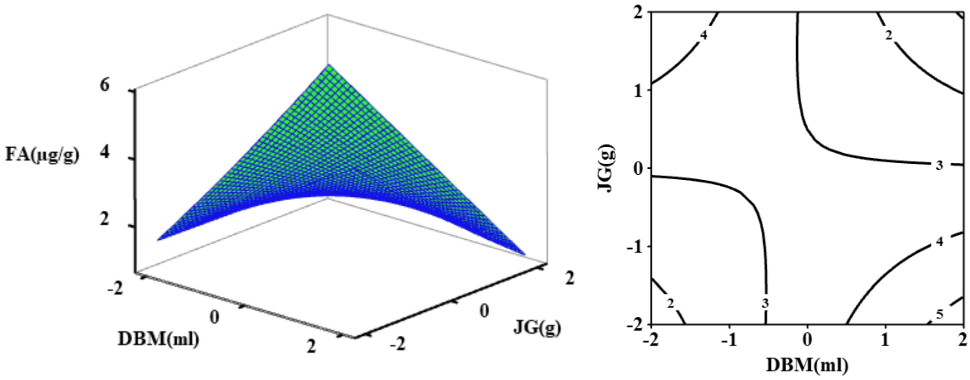
The surface plot and contour plot for the interactive effect of PNM and DBM, with JG, BT and CP were kept constant at the middle level. At the lower level of DBM, the production of TPC goes on increasing from the lower value (1.5 ml) with an increase in PNM and reached maximum production in the center value (2.5ml). But there is a decreasing trend was found towards the upper limit (3.5ml) of PNM. At the higher value of DBM (8.5ml), as the PNM(1.5ml) increases the yield of TPC exhibits increasing trend and reaches maximum at the centre point followed by gradual decreasing trend of TPC production was observed. The contour plot displays nearly circular at the centre value, which infers that the interaction between the PNM and DBM are less significant on chocolate.

3.4 Effect of Folic Acid



a) **b)**

Figure 4: (a) Surface and (b) Contour plot for PNM and DBM on Folic acid.



a) **b)**

Figure 5: (a) Surface and (b) Contour plot for DBM and JG on Folic acid.

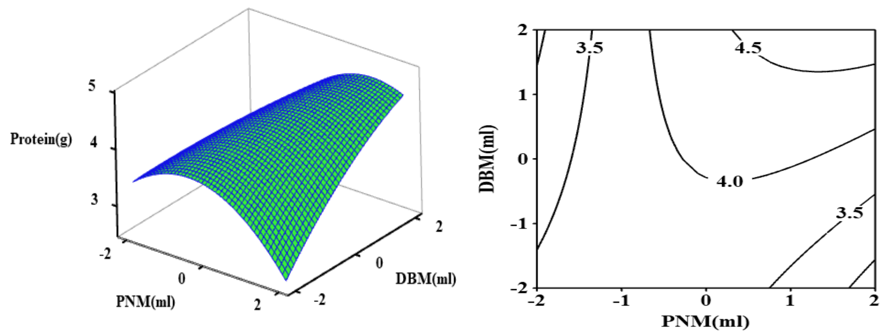
The response surfaces for Folic acid are shown in Fig. 4a. represents the surface plot and 4b represents the contour plot for the interactive effect of PNM and DBM where PNM, JG, BT and CP were kept constant at the middle level. In the lower value (6.5 ml) of DBM, decreasing in the production of Folic Acid was obtained when the quantity of PNM ranges from (1.5ml-3.5ml). The same effect was noted for the lower level of PNM, as DBM varies. At the higher value of PNM, the production of Folic Acid increases sharply and found elevated, towards the higher value of DBM. Similarly at the higher value of DBM, the production of Folic Acid increases till the upper value of PNM. This interaction shows that the lower value of PNM and the higher value of DBM had maximum production of Folic Acid. The contour plot clearly represents the high interaction at the diagonal side. P-value (0< 0.05) also ensure the significance of for this interaction effect.

The response surfaces for Folic acid are shown in Fig. 5a represents the surface plot and Fig. 5b represents the contour plot for the interactive effect of DBM and JG where PNM, BT and

CP were kept constant at the middle level. The interaction effect of DBM and JG found similar to the interaction effect of PNM and DBM. P-value (0< 0.05) for this interaction effect also implies the significant nature.

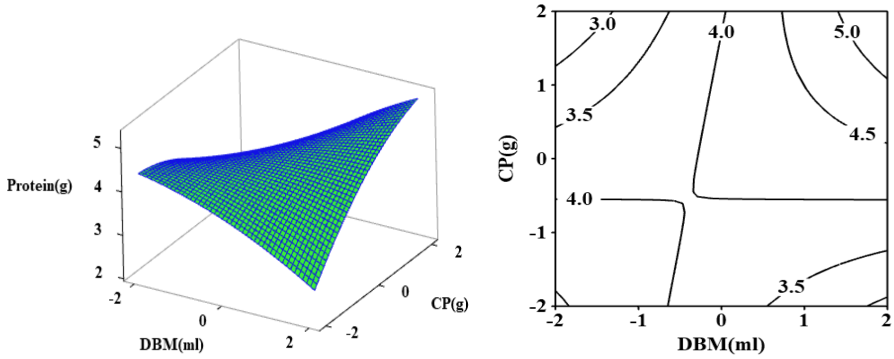
3.5 Effect of Protein

The response surfaces for Protein are shown in Fig. 7a represents the surface plot and 7b represents the contour plot for the interactive effect of PNM and DBM where JG, BT and CP were kept constant at the middle level. The production of Protein goes on increasing from the lower level DBM with an increase in DBM. At the middle value of PNM the production of Protein was found very high and gradual decrement was found towards the higher value of PNM. At the same time, the production of Protein was found elevated from the higher value (PNM) while DBM found increased. The response of Protein increases sharply with an increase in PNM, in the upper limit of DBM. The contour plot exhibits the semi elliptical nature of interaction effect among PNM and DBM. P-value (0.014< 0.05) also ensure the significance of for this interaction effect is high.



a) b)

Figure 7: (a) Surface and (b) Contour plot for effects PNM & DBM on Protein.



a) b)

Figure 8: (a) Surface and (b) Contour plot for DBM and CP on Protein.

The production of Protein from the interaction effect of DBM and CP are significant and are similar to the interaction effect of PNM and DBM. Low significance effect was observed for DBM and JG. The circular nature of contour plot found near the higher value of DBM shown in Fig. 9 and P-Value (0.936>0.05) confirms the insignificant nature for this interaction.

The polynomial equations were solved using MINITLAB version 19 and the optimal values of the variables in the coded values were found to be PNM = 0.3434, DBM = 1.6363, JG = -0.9898, BT = -0.7878 and CP = 1.1111, and the corresponding uncoded values were PNM = 2.671ml, DBM = 8.318ml, JG = 17.010g, BT = 2.606g and CP = 1.111g. To confirm the predicted response by the polynomial equation, validation experiment was carried out under optimum conditions. It was found that the experimental values of TPC = 40.369 mg GAE/g, Folic Acid = 4.623µg/g & Protein = 5.463g/g respectively, was near to the predicted values of TPC (mg GAE/g) = 42.4080 Folic Acid (µg) = 4.5589, Protein (g) = 5.5011 respectively. Bioactive components such as TFC and antioxidant activity obtained for the validation experiment are 34.564 mg QE/g and 28.753 µg/ml, respectively.

3.6 Enrichment with Traditional Plant Sources

The optimized PN: DB milk was enhanced with traditional plant source such as CML, MOL and SAF to increase the bioactive content. The values of TPC, TFC and Antioxidant activity are 43.65 mg GAE/g, 43.62 mg QE/g, 37.26 µg/ml for CML; 45.23 mg GAE/g, 37.30 mg QE/ml, 40.15 µg/ml for MOL; and 44.02 mg GAE/g, 42.35 mg QE/g, 38.039 µg/ml for SAF respectively. Extracts of citrus leaves shows the activity of antioxidant and enzymatic browning inhibition (Khettal et al., 2017). Plant sources added to the optimized ingredients for chocolate production shows the increase in nutritional components and bioactive components in chocolate upto 20 to 30% (Fig. 5). CML added to the chocolate have flavors associated with it is important to match the food. MOL based chocolate does not show any flavor as in CML and does not have significant bitter taste. SAF added chocolate have similar trend as in MOL chocolate. Incorporation of plant sources were inhibiting the natural microbes to food. But addition of plant sources has to be optimized to enhance the desirable characteristics in food applications.

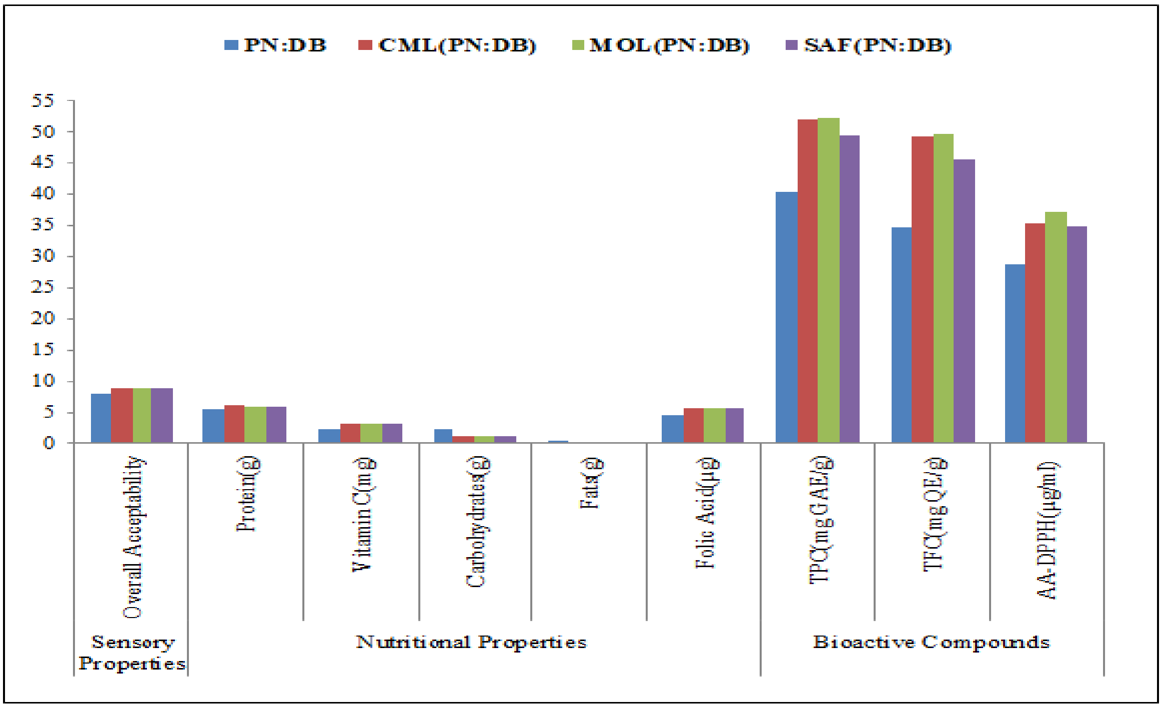


Figure 9: Plot of Sensory, Nutritional & Bioactive constituents of chocolate with plant sources.

4. CONCLUSION

From this study it could be concluded that underutilized legumes have great potential in substituting the dairy milk. Utilization of traditional plant sources in food applications increases the dietary bioactive components in food. The combined peanut milk and double bean milk chocolate with traditional plant sources was excellent in quality with increase in nutritional and bioactive components. Hence the production of legume-based chocolate can increase the utilization of double beans and peanut.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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