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Formulation and Optimization of Constituent in Legumes-Based Milk Chocolate Fortified with Citrus Peel Powder

Preethini Selvaraj, Anhuradha Shanmugam*, Arrivukkarasan Sanjeevirayar

Biochemistry Laboratory, Department of Chemical Engineering, Annamalai University, Annamalai Nagar, India
Email: *anhuradha@yahoo.co.in

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Abstract

The present study was employed to optimize the process for development of legumes-milk based chocolate using peanut (PN) and yellow-pea (YP) milk using Response surface methodology (RSM). Different combinations of legumes-milk chocolate at various ratios of PN:YP milk with fixed concentration of other regular ingredients were prepared, and the finest combination (1:1) was selected on the basis of their sensory and nutritional properties. PN and YP milk, Jaggery (JG), Butter (BT), and Citrus Peel Powder (CPP) served as independent variables while the dependent variables were allocated to the regression equation to determine folic acid ($R^2 = 93.15$) along with protein content ($R^2 = 93.11$) and vitamin C ($R^2 = 90.57$). The nutritional parameters such as Folic acid, Protein and Vitamin C content were found to be optimum in the milk chocolate. The optimized concentrations of PNM, YPM, JG, BT and CPP were found to be 5.0 ml, 5.5 ml, 18.1 g, 2.9 g and 0.53 g respectively. Addition of JG had an interactive effect on folic acid in YP milk ($p < 0.05$) and CPP shows a significant. Enhancement of vitamin C was perceived in legume based milk chocolate than the control chocolate due to supplement of CPP.

Keywords

Bioactive Compounds, Chocolate, Citrus Peel, Ingredients, Legumes, Optimization, Sensory

1. Introduction

The cow's milk and milk products, starches, fats, flavours, nuts and fruits are used as the ingredients for making confectionary products which includes chocolate-covered confectionaries, bars, fruits and nuts [1]. These food products are widely spread all over the world for the majority of the consumers due to its witty flavour and taste [2]. Milk chocolate and food products forged from cow's milk is more nutritious with adequate sources of macronutrients distinct by measurable properties and unfalteringly by consumer liking [3]. However, insufficiency in supply of cow's milk and some limitations in human health such as lactose intolerance [4], high cholesterol, saturated fat content, allerginicity, etc. Some researchers [5] has directed to the development of alternate sources of plant milk [6]. In recent development, plant based legumes milk are used as the substitute for cow's milk in the food processing to produce value added products with the high nutritional benefits [5] [6] [7]. Legumes and cereals such as cowpeas, lentils, soybeans, peanuts and other podded vegetables provides wide range of essential nutrients including protein, low glycemic index carbohydrates, dietary fibre, minerals, vitamins and folate, which are known for the health promoting vital components of a healthy diet [8] [9]. In addition, protein [10] and protein derived bioactive peptides of legumes have significant roles as health-enhancing compounds and it may contribute to increase the nutrients in processed food [11] [12] [13] [14]. 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) [15] [16]. 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 [17] [18]. Legumes provide vast opportunities to be utilized in the processed foods such as bakery products, bread, pasta, snack foods, soups, cereal bar filing, tortillas, meat, etc., due to their huge nutrition content [19]. 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 [17] [18] [20].

PN and YP are the major sources of high-quality protein, starch, dietary fibers, 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 [21].

PN milk is the good substitute for the dairy milk with underlying qualities and it is obtained by soaking, grinding and filtering the raw PN with water. With high nutritional value it provides wide varieties of milk-based products such as yoghurt, butter milk, ripened cheese and tofu etc. [22] [23]. Green and beany flavour limits the application of PN milk and YP milk.

Pasteurization at 85˚C or below will extend the application limits of PN milk and YP milk and reported that milk from tiger-nut and soybean contains high nutrient contents so as to crack the problem of protein-calorie malnutrition [19] [24] [25] [26] [27]. The consumption of dry legumes plays a key role on the global nutrient deficiencies and farming the structure of the developing world. In order to work out on global food shortage problem and improving the consumption of legume grains, recent studies focused on fortification of the legume milk with fruit peels will meet the expectations rapidly. Hence the presence of major and minor food constituents like protein, fat, carbohydrate, calcium, iron, sodium, vitamin E and with some other minerals [28] in legumes milk can be utilized for chocolate production along with the other ingredients like cocoa powder, sugar and butter etc. The rheological behaviour of chocolate influenced by the addition of cocoa butter and it shows impact on the quality of milk chocolate [29] [30]. The objective of this study was to develop and formulate the legumes-based milk chocolate instead of utilizing standard milk and also to analyze the sensory qualities of chocolate.

2. Materials and Methods

2.1. Ingredients Procurement and Legumes Pretreatment

Butter, Citrus fruits, Jaggery, Peanut (Arachishypogaea), Yellow-pea (Pisumsativum) and Dairy-milk powder for control chocolate were purchased from the local super market in Chidambaram, Tamilnadu, India. Extensive care was taken during screening, to ensure the quality of raw materials and mold free legumes. The dehulled peas and nuts were soaked in 2% $NaHCO_3$ for 3 h and 18 h respectively, and washed thoroughly in clean water to soften and ensure the removal of beany flavor in the final product [31].

2.2. Extraction of Legumes Milk

Soaked peas and nuts were grounded separately using blender in the ratio of 2:1 (Legumes: Water). It was blended 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 60˚C for 15 minutes and then cooled to room temperature for future use.

2.3. Preparation of Citrus Peel Powder

The fresh orange variety *Citrus indica* were collected from the local market Chidambaram, India. All the fruits were of good quality, washed immediately and peeled. The peels were tray dried at 60˚C, till it reaches constant weight. Then grinded and stored in air tight containers until use. Preliminary studies were conducted to optimize the CPP in the range of 0.5% to 3% by sensory analysis, and 1.5% was found to be ideal for chocolate preparation.

2.4. Preparation of Chocolate and Chemical Analysis

Chocolate mass was prepared by the double boiling method at 50˚C to 60˚C for 10 min with proper stirring of legumes-milk along with JG, BT and CPP in definite proportions. Conching process was carried out in pestle and mortar for 1hr with addition of butter and citrus peel powder as an emulsifier and flavouring agent at this stage respectively. The chocolate mass was tempered and poured in molds and were placed in a refrigerator at 4˚C for 2 h. The finished chocolates were wrapped in aluminum foil and stored at refrigerated condition until further analysis. The experiments were repeated for different ratios as desired and dairy milk chocolate as control. The proximate composition of chocolates was determined using standard methods. Protein content was analyzed using spectrophotometer by Lowry's method. Folic acid and Vitamin C content were determined using High Performance Liquid Chromatography (HPLC-SPD 20A C18) techniques.

2.5. Experimental Design and Evaluation

Response surface methodology (RSM) was used to investigate the influence of concentration of legumes-milk (PN:YP), JG, BT and CPP 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.

2.6. 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 JG 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) [32] [33]. 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

3.1. Experimentation

The production of chocolate using various ratios of peanut milk (PNM) and yellow-pea milk (YPM) milk (1:1), (1:2), (1:3) and (1:4)were carried out with fixed concentrations of other regular ingredients such as JG, BT and CPP. Among the different ratios of legume milk chocolate, the final product of chocolate prepared is selected on the basis of their sensory and nutritional properties. By sensory evaluation, 1:1 ratio of PN:YP milk chocolate was accepted in overall, and also the nutritive values were found to be sensible. Further, the enrichment of chocolates was carried out with the employment of citrus fruits peel powder, as the peels are rich in vitamin-C content. They also have good nutraceutical properties and functional food bioactive compounds in it.

In the selected ratio 1:1, Response surface methodology (RSM) was employed to optimize the concentration of ingredients used in legume (PN:YP) milk chocolate, and by the nutritional parameters such as Folic acid, Protein and Vitamin C content were analyzed, to determine the optimum concentration of ingredients.

All experiments were carried out in triplicate and their mean values are presented.

3.2. 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 [34]. Also, it is a good statistical tool in response to surface methodology because it predicts the variables of interest in 1) estimation of the parameters of the quadratic model, 2) building of sequential design, 3) determination of lack of fit of the model and 4) use of blocks. This second order polynomial model demonstrates the rapport between YPM, PNM, JG, BT and CPP on Folic acid, Vitamin C and Proteins. The number of experiments (n) required for the development of CCD is defined as n = 2k, 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 has to be performed. All other experimental conditions are kept constant during the experiments, and the runs are randomized to exclude any bias.

YPM, PNM, JG, BT and CPP are the independent variables studied in the experimentation of bioactive components such as Folic acid, Vitamin C 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.

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_0 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:

$$X_j = \frac{X_i - X_0}{\Delta x}, i = 1,2,3,4,5 \quad (1)$$

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

Factors	Symbol	Range and Level				
		−2	−1	0	+1	+2
Peanut milk	PNM	3	4	5	6	7
Yellow pea milk	YPM	3	4	5	6	7
Jaggery	JG	16	17	18	19	20
Butter	BT	2.0	2.5	3.0	3.5	4.0
Citrus Peel	CPP	0.3	0.4	0.5	0.6	0.7

Table 2. Formulation of chocolate using central composite design matrix.

Runs	Coded values					Uncoded Values				
	PNM	YPM	JG	BT	CPP	PNM	YPM	JG	BT	CPP
1	−1	−1	−1	−1	+1	4	4	17	2.5	0.6
2	+1	−1	−1	−1	−1	6	4	17	2.5	0.4
3	−1	+1	−1	−1	−1	4	6	17	2.5	0.4
4	+1	+1	−1	−1	+1	6	6	17	2.5	0.6
5	−1	−1	+1	−1	−1	4	4	19	2.5	0.4
6	+1	−1	+1	−1	+1	6	4	19	2.5	0.6
7	−1	+1	+1	−1	+1	4	6	19	2.5	0.6
8	+1	+1	+1	−1	−1	6	6	19	2.5	0.4
9	−1	−1	−1	+1	−1	4	4	17	3.5	0.4
10	+1	−1	−1	+1	+1	6	4	17	3.5	0.6
11	−1	+1	−1	+1	+1	4	6	17	3.5	0.6
12	+1	+1	−1	+1	−1	6	6	17	3.5	0.4
13	−1	−1	+1	+1	+1	4	4	19	3.5	0.6
14	+1	−1	+1	+1	−1	6	4	19	3.5	0.4
15	−1	+1	+1	+1	−1	4	6	19	3.5	0.4
16	+1	+1	+1	+1	+1	6	6	19	3.5	0.6
17	−2	0	0	0	0	3	5	18	3.0	0.5
18	+2	0	0	0	0	7	5	18	3.0	0.5
19	0	−2	0	0	0	5	3	18	3.0	0.5
20	0	+2	0	0	0	5	7	18	3.0	0.5
21	0	0	−2	0	0	5	5	16	3.0	0.5
22	0	0	+2	0	0	5	5	20	3.0	0.5
23	0	0	0	−2	0	5	5	18	2.0	0.5
24	0	0	0	+2	0	5	5	18	4.0	0.5
25	0	0	0	0	−2	5	5	18	3.0	0.3
26	0	0	0	0	+2	5	5	18	3.0	0.7
27	0	0	0	0	0	5	5	18	3.0	0.5
28	0	0	0	0	0	5	5	18	3.0	0.5
29	0	0	0	0	0	5	5	18	3.0	0.5
30	0	0	0	0	0	5	5	18	3.0	0.5
31	0	0	0	0	0	5	5	18	3.0	0.5
32	0	0	0	0	0	5	5	18	3.0	0.5

Note: YPM: Yellow pea milk, PNM: Peanut milk, JG: Jaggery, BT: Butter, CPP: Citrus peel powder.

The second-order polynomial regression model is given as Equation (2) to express Y as a function of the independent variables as follows, where β_0 is a constant, while β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables:

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

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 Folic acid, Protein and Vitamin C for different combinations of legumes-milk chocolate ingredients are presented in **Table 3**. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms are presented in **Table 4**. 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 folic acid, protein and vitamin C were 93.15, 93.11 and 90.57 respectively. The closer the value of R^2 to the unity, the 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 behaviour variation [35].

The probability (p) values of all regression models were less than 0.000, with no lack-of-fit. The results of the Folic acid, Protein and Vitamin C 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 Folic Acid, Protein and Vitamin C 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 4** indicates the estimated regression coefficients and the corresponding statistical t and P values for Folic Acid, Protein and Vitamin C. Among the linear effect of JG on Folic Acid, YPM on Protein and CPP on Vitamin C shows significant.

In squared effects, the P values on Folic acid, JG, BT and CPP are same ($0.000 < 0.05$) which shows their significance. On Protein, the P value for YPM ($0.003 < 0.05$), BT ($0.025 < 0.05$) and CPP ($0.008 < 0.05$) are significant. On Vitamin C, the P value of YPM ($0.001 < 0.05$) and JG ($0.000 < 0.05$) were found to be significant. Among the ten interaction effects on Folic acid, Protein and Vitamin C in PNM, YPM, JG, BT and CPP, the P value for YPM*JG ($0.005 < 0.05$) and JG*CPP ($0.003 < 0.05$) interactions on Folic Acid shows significance.

3.3. Folic Acid

The effect of the nutritive analysis on folic acid, protein and vitamin C are reported by the coefficient of the second order polynomials. To aid visualization,

Table 3. Response values and predicted values of folic acid, protein and vitamin C.

Runs	Folic acid		Protein		Vitamin C	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	0.010	0.01156	1.25	1.2439	0.400	0.4184
2	0.007	0.00815	1.27	1.2656	0.315	0.4059
3	0.014	0.01573	1.39	1.3956	0.395	0.3851
4	0.010	0.01665	1.40	1.4198	0.388	0.3859
5	0.012	0.01348	1.22	1.1889	0.352	0.3874
6	0.015	0.01440	1.17	1.1531	0.402	0.4201
7	0.013	0.01298	1.41	1.4031	0.410	0.4004
8	0.009	0.01556	1.41	1.4048	0.384	0.3698
9	0.011	0.01331	1.29	1.2673	0.369	0.3685
10	0.009	0.00923	1.27	1.2614	0.387	0.3943
11	0.012	0.01281	1.37	1.3714	0.407	0.4055
12	0.016	0.01640	1.40	1.4031	0.314	0.4159
13	0.014	0.01456	1.28	1.2448	0.411	0.4108
14	0.011	0.01115	1.25	1.2164	0.359	0.3542
15	0.008	0.00873	1.45	1.4264	0.373	0.3594
16	0.015	0.01565	1.42	1.4106	0.378	0.3722
17	0.013	0.00945	1.34	1.3775	0.397	0.4087
18	0.019	0.01045	1.37	1.3759	0.399	0.4044
19	0.016	0.01362	1.07	1.1275	0.321	0.4152
20	0.015	0.01829	1.49	1.4759	0.376	0.3989
21	0.007	0.01312	1.39	1.3742	0.305	0.3857
22	0.013	0.01379	1.27	1.3292	0.339	0.3594
23	0.010	0.01579	1.32	1.3209	0.399	0.4130
24	0.011	0.01412	1.31	1.3525	0.386	0.3900
25	0.012	0.01529	1.29	1.3242	0.345	0.3659
26	0.011	0.01662	1.30	1.3092	0.419	0.4062
27	0.018	0.01768	1.41	1.4144	0.421	0.4103
28	0.018	0.01768	1.44	1.4144	0.421	0.4103
29	0.018	0.01768	1.42	1.4144	0.421	0.4103
30	0.018	0.01768	1.42	1.4144	0.421	0.4103
31	0.018	0.01768	1.43	1.4144	0.421	0.4103
32	0.018	0.01768	1.41	1.4144	0.421	0.4103

Table 4. Estimated Regression coefficient with corresponding t and p value for Bioactive components.

Term	Folic acid			Protein			Vitamin C		
	Coefficient	t-value	P-value	Coefficient	t-value	P-value	Coefficient	t-value	P-value
Constant	0.017955	28.81	0.000	1.4144	87.21	0.000	0.41695	57.77	0.000
PNM	0.000417	1.31	0.218	−0.00042	−0.05	0.961	−0.00775	−2.10	0.060
YPM	0.000250	0.78	0.450	0.08708	10.49	0.000	0.00683	1.85	0.091
JG	0.000833	2.61	0.024	−0.01125	−1.36	0.202	0.00675	1.83	0.095
BT	0.000333	1.05	0.318	0.00792	0.95	0.361	−0.00308	−0.83	0.422
CPP	0.000333	1.05	0.318	−0.00375	−0.45	0.660	0.01958	5.30	0.000
PNM*PNM	−0.000455	−1.58	0.143	−0.00943	−1.26	0.235	−0.00170	−0.51	0.620
YPM*YPM	−0.000580	−2.01	0.070	−0.02818	−3.75	0.003	−0.01408	−4.21	0.001
JG*JG	−0.001955	−6.77	0.000	−0.01568	−2.09	0.061	−0.02070	−6.20	0.000
BT*BT	−0.001830	−6.34	0.000	−0.01943	−2.59	0.025	−0.00308	−0.92	0.376
CPP*CPP	−0.001580	−5.48	0.000	−0.02443	−3.25	0.008	−0.00570	−1.71	0.116
PNM*YPM	0.000500	1.28	0.227	0.0056	0.55	0.591	−0.00325	−0.72	0.488
PNM*JG	0.000500	1.28	0.227	−0.0094	−0.92	0.376	0.00900	1.99	0.072
PNM*BT	0.000875	2.24	0.047	−0.0019	−0.18	0.857	−0.00337	−0.75	0.471
PNM*CPP	0.000125	0.32	0.755	−0.0019	−0.18	0.857	0.00275	0.61	0.556
YPM*JG	−0.001375	−3.52	0.005	0.0181	1.78	0.102	−0.00075	−0.17	0.871
YPM*BT	0.000250	0.64	0.535	−0.0094	−0.92	0.376	−0.01013	−2.24	0.047
YPM*CPP	−0.000250	−0.64	0.535	0.0006	0.06	0.952	−0.00550	−1.22	0.250
JG*BT	−0.000500	−1.28	0.227	0.0106	1.05	0.318	−0.00037	−0.08	0.935
JG*CPP	0.001500	3.84	0.003	0.0006	0.06	0.952	−0.00350	−0.77	0.455
BT*CPP	−0.000125	−0.32	0.755	0.0006	0.06	0.952	0.00087	0.19	0.850

Note. YPM: Yellow pea milk, PNM: Peanut milk, JG: Jaggery, BT: Butter, CPP: Citrus peel powder.

the response surfaces for folic acid, protein and vitamin C are shown in Figure 1. Figure 1 represents the surface plot and contour plot for the interactive effect of YPM and JG, where PNM, BT and CPP were kept constant at the middle level. The production of Folic Acid goes on increasing from the lower value (3 ml) with an increase in YPM. Nearby the higher value (7 ml) of YPM, slight negligible decrease was noticed in production of Folic Acid. At the lower value of YPM, the production of Folic Acid increases sharply and found elevated, immediate decrement was noticed at higher value of JG. At the higher value of YPM, the production of Folic Acid increases till the middle value of JG (18 g) and decreased trend was observed towards the higher value of JG. At the same time, in the higher value of YPM, while we increase the JG from 3 ml to 7 ml gradually, there is an increased level of folic acid production was observed till the middle value and gradual decreased trend was noticed., i.e. Folic Acid production is increased sharply till 5 ml of PNM. Thereafter a gradual decreased trend was

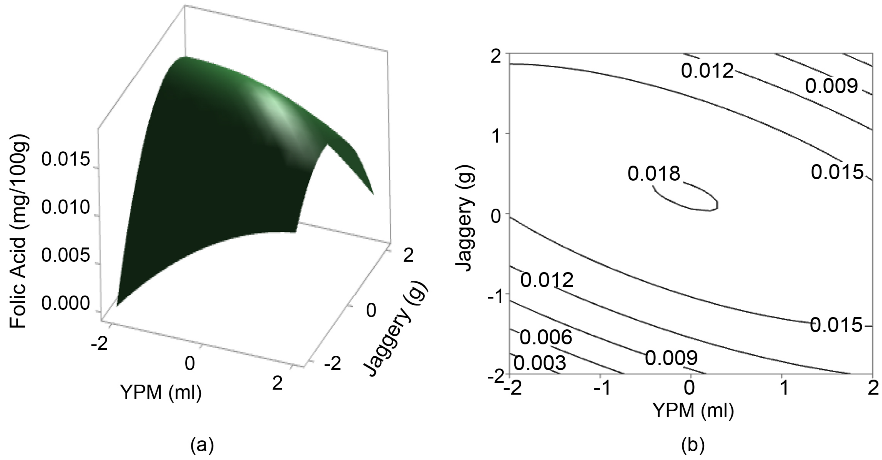

Figure 1. Response (a) Surface and (b) Contour plot of Yellow pea milk (YPM) and Jaggery (JG) on Folic acid.

observed till 7 ml of YPM. The elliptical nature of the contour plot for the interactive effects YPM and JG on Folic acid ensures the same. The Contour plot is shown in the **Figure 1**. **Figure 2** represents the surface plot and contour plot for the interactive effect of JG and CPP, where PNM, YPM and BT were kept constant at the middle level. The production of Folic Acid goes on increasing from the lower level (16 g) of JG with an increase in CPP. Thereafter a sharp decrease was noticed in the production of Folic Acid with an increase in CPP. At the higher value of JG (20 g) the production of Folic Acid was found very less. At the same time, the production of Folic Acid was found elevated from the lower value (0.3 g) to the higher value (0.7 g). At the higher level of CPP (0.7 g), the production of Folic Acid increases from 16 g to 18 g of JG, followed by gradual decrease till 20 g.

Contractually the Folic Acid production increases sharply with an increase in CPP in the throughout range of investigation. While we observe the effect of Folic Acid on the increase in the quantity of CPP, a sharp increased trend was observed at an elevated JG value (20 g).

The P-Value for the linear and square effects of Folic Acid ($0.024 < 0.05$ and $0.000 < 0.05$) with the square effects on JG, BT and CPP are found to be 0.000 which indicates the significance on the production on Folic Acid and it is presented in the **Table 4**. The elliptical nature of the contour plot in the **Figure 2(b)** also ensure the significance of JG.

Figure 3 represents the surface plot and contour plot for the interactive effect of PNM and BT, where YPM, JG and CPP were kept constant at the middle level. It is observed that the production of Folic Acid goes on decreasing from the lower level (3 ml) to the higher level (7 ml) of PNM. At the higher level of PNM, the production of Folic Acid has increased drastically and reaches maximum at the middle value (3 g) and gradual decrement was observed towards the higher value of BT. At the higher of BT, the production of Folic Acid found increasing from the lower level towards the higher level of PNM. The contour plot obtained

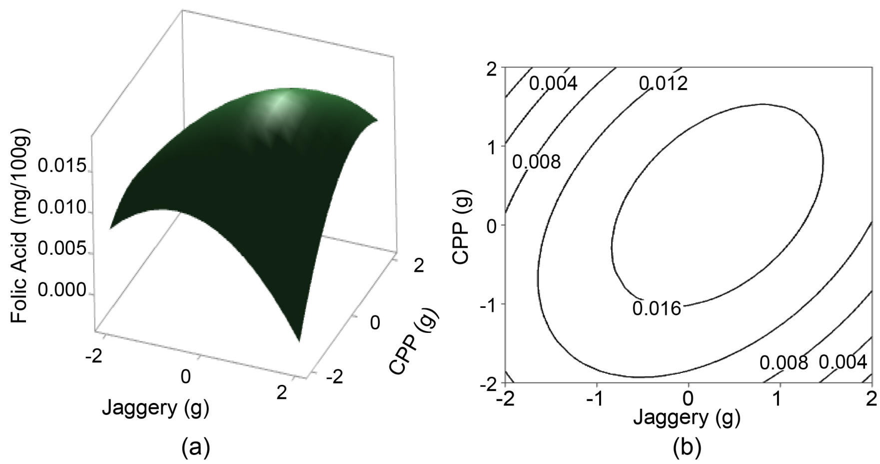

Figure 2. Response (a) Surface and (b) Contour plot of Jaggery (JG) and Citrus peel powder (CPP) on Folic acid.

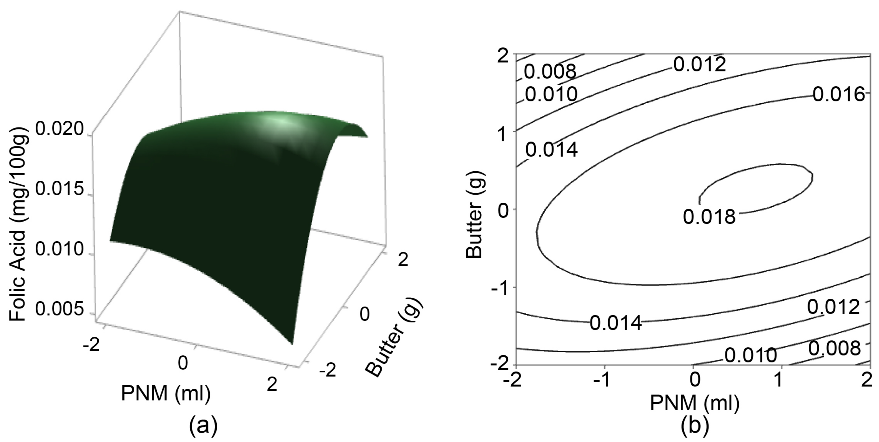

Figure 3. Response (a) Surface and (b) Contour plot of Peanut milk (PNM) and Butter (BT) on Folic acid.

in the elliptical shape. The p-value for the interaction of PNM and BT is 0.047 > 0.005, which implies below 10% of error.

3.4. Protein

The surface plot and the contour plot for an interactive effect of PNM with YPM on Protein is given in **Figure 4(a)** and **Figure 4(b)**, while JG, BT and CPP are kept constant at their middle level. On observing the **Figure 4** the production of protein shows on gradual increase from 3 ml with increase in PNM and observed high at their middle level (5 ml). Thereafter gradual decrease was found till 7 ml of PNM at the lower value of YPM. At the same time the effect of protein from the quantity of YPM shows a sharp increased trend from the lower value (3 ml) to the higher value of 7 ml. At the elevated value of PNM (7 ml), YPM shows high production of protein. The P-Value for the linear and square effects of YPM are found to be 0.000 and 0.003 which indicates the significance of protein production and the value is presented in the **Table 4**. The semi elliptical nature

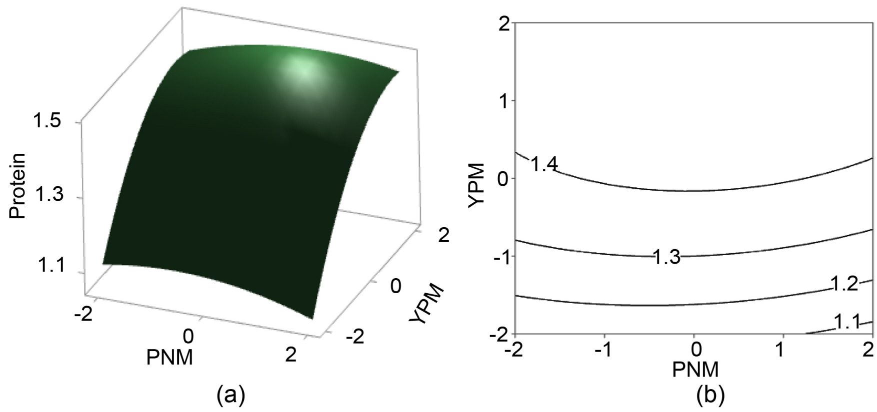

Figure 4. Response (a) Surface and (b) Contour plot of Peanut milk (PNM) and Yellow pea milk (YPM) on Protein.

of the contour plot in the **Figure 3(b)** also ensure the significance of YPM. The P-value for the square effect of Butter (0.025 < 0.05) and CPP (0.008 > 0.05) also shows their significance. From the **Table 4**, the P-Value for all the interaction effects on the production of protein are insignificant.

Figure 5 depicts the surface plot and contour plot for the interactive effect of YPM and JG, where PNM, BT and CPP were kept constant at the middle level in Protein. The production of Protein goes on increasing from the lower value (3 ml) with an increase in YPM. It is observed that the production protein shows on elevation with the quantity of YPM increases and reaches maximum within the middle value and higher value of YPM followed by a negligible decrease towards the higher value. The same effect was observed for JG, the production of Protein found near to 1.2 (g/100g) and goes on slight increasing towards the higher level of JG. At the lower level of YPM, production of Protein goes on decreasing as JG increases. At the higher level of JG, the production of protein found increasing from the lower level to the high level of PNM. The P value for this interaction is 0.102 > 0.005 which shows the error is 10.2%.

3.5. Vitamin C

Figure 6(a) and **Figure 6(b)** represents the surface plot and contour plot for the interactive effect of YPM and BT on Vitamin C, where PNM, JG and CPP were kept constant at the middle level. At the lower value of BT, the production of Vitamin C goes on increasing from the lower value (3 ml) with an increase in YPM and reaches maximum. Thereafter gradual decrease on Vitamin C production was observed near the higher value (7 ml) of YPM. At the lower level of BT, the higher production of Vitamin C enhanced by its soaking process [19], and gradual decrease was noticed.

Figure 7 depicts the graphical representation of Response surface and the contour plots of PNM & JG on Vitamin C. The surface plot represents the continuous decrement in the production of Vitamin C on the lower level of JG (16 g) as the quantity of PNM increases from 3 ml to 7 ml. At the higher level of JG, the

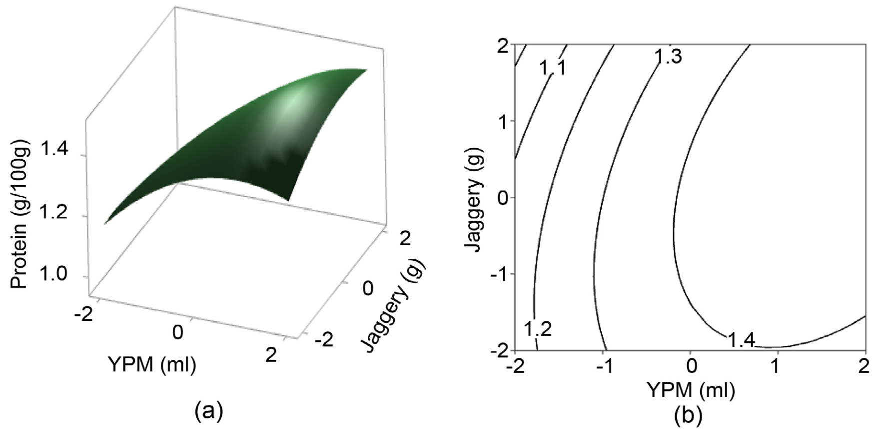

Figure 5. Response (a) Surface and (b) Contour plot of Yellow pea milk (YPM) and Jaggery (JG) on Protein.

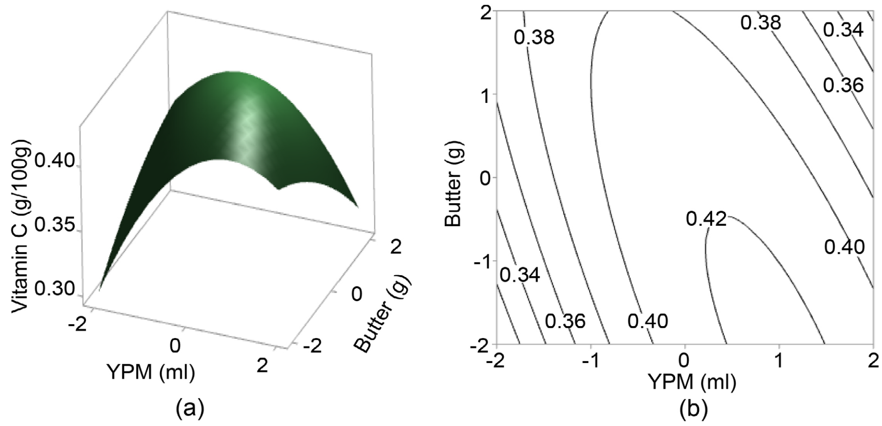

Figure 6. Response (a) Surface and (b) Contour plot of Yellow pea milk (YPM) and Butter (BT) on Vitamin C.

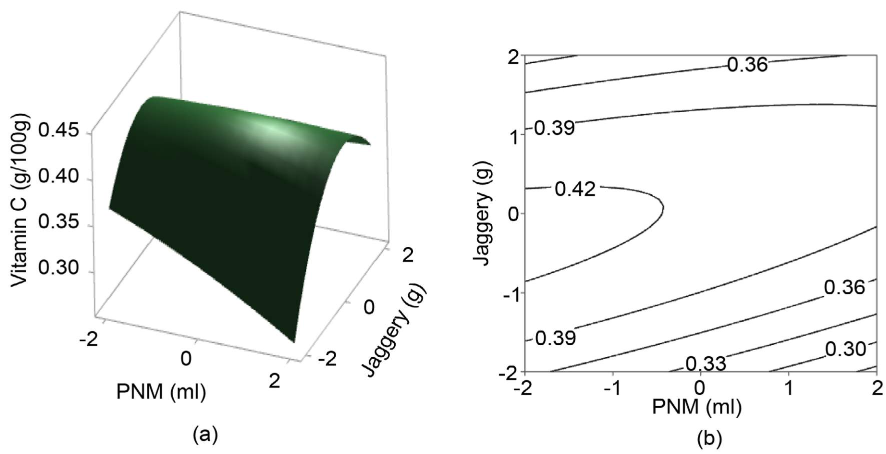

Figure 7. Response (a) Surface and (b) Contour plot of Peanut Milk (PNM) and Jaggery (JG) on Vitamin C.

production of Vitamin C shows increment as the PNM increases on the higher level of PNM, the yield of Vitamin C found increasing from the lower level and reaches maximum after the middle level (18 g) and slight negligible decrement towards the higher level of JG was noted. The contour plot also proves that it is the form of semi elliptical shape found at the lower level to the higher level of JG at low level of PNM. Also, the P value 0.072 > 0.005 shows the interaction effect is insignificant with 7.2% error. Hence this implies the less interaction effect between the PNM and JG.

3.6. Optimization of Legumes-Milk Chocolate Formulation and Validation

Optimization of legumes milk chocolate formulation was based on the maximum values of bioactive components such as folic acid, vitamin C and protein content in the chocolates, their respective polynomial equations are shown in Equations (3a), (3b) and (3c).

$$\begin{aligned}\text{Folic Acid} = {} & 0.017682 + 0.000250\text{PNM} + 0.001167\text{YPM} + 0.000167\text{JG} \\ & - 0.000417\text{BT} + 0.000333\text{CPP} - 0.001932\text{PNM} * \text{PNM} \\ & - 0.000432\text{YPM} * \text{YPM} - 0.001057\text{JG} * \text{JG} - 0.000682\text{BT} * \text{BT} \\ & - 0.000432\text{CPP} * \text{CPP} + 0.001500\text{PNM} * \text{YPM} \\ & + 0.000625\text{PNM} * \text{JG} + 0.000125\text{PNM} * \text{BT} \\ & + 0.000250\text{PNM} * \text{CPP} - 0.001250\text{YPM} * \text{JG} \\ & - 0.000500\text{YPM} * \text{BT} - 0.000125\text{YPM} * \text{CPP} \\ & - 0.000375\text{JG} * \text{BT} + 0.000750\text{JG} * \text{CPP} - 0.000000\text{BT} * \text{CPP}\end{aligned} \tag{3a}$$

$$\begin{aligned}\text{Protein} = {} & 1.4144 - 0.00042\text{PNM} + 0.08708\text{YPM} - 0.01125\text{JG} + 0.00792\text{BT} \\ & - 0.00375\text{CPP} - 0.00943\text{PNM} * \text{PNM} - 0.02818\text{YPM} * \text{YPM} \\ & - 0.01568\text{JG} * \text{JG} - 0.01943\text{BT} * \text{BT} - 0.02443\text{CPP} * \text{CPP} \\ & + 0.0056\text{PNM} * \text{YPM} - 0.0094\text{PNM} * \text{JG} - 0.0019\text{PNM} * \text{BT} \\ & - 0.0019\text{PNM} * \text{CPP} + 0.0181\text{YPM} * \text{JG} - 0.0094\text{YPM} * \text{BT} \\ & + 0.0006\text{YPM} * \text{CPP} + 0.0106\text{JG} * \text{BT} + 0.0006\text{JG} * \text{CPP} \\ & + 0.0006\text{BT} * \text{CPP}\end{aligned} \tag{3b}$$

$$\begin{aligned}\text{Vitamin C} = {} & 0.41032 - 0.00108\text{PNM} - 0.00408\text{YPM} - 0.00658\text{JG} \\ & - 0.00575\text{BT} + 0.01008\text{CPP} - 0.00094\text{PNM} * \text{PNM} \\ & - 0.00082\text{YPM} * \text{YPM} - 0.00944\text{JG} * \text{JG} - 0.00219\text{BT} * \text{BT} \\ & - 0.00607\text{CPP} * \text{CPP} + 0.00025\text{PNM} * \text{YPM} \\ & - 0.00413\text{PNM} * \text{JG} + 0.00013\text{PNM} * \text{BT} \\ & - 0.00675\text{PNM} * \text{CPP} - 0.00475\text{YPM} * \text{JG} \\ & + 0.00725\text{YPM} * \text{BT} - 0.00588\text{YPM} * \text{CPP} \\ & - 0.00438\text{JG} * \text{BT} + 0.00650\text{JG} * \text{CPP} + 0.00050\text{BT} * \text{CPP}\end{aligned} \tag{3c}$$

The polynomial equations were solved using MINITAB and the optimal values of the variables were found to be PNM = 0.0606061, YPM = 0.585859, JG = 0.10101, BT = −0.0606 and CPP = 0.30303 and the corresponding uncoded values are PNM = 5.0606061, YPM = 5.585859, JG = 18.10101, BT = 2.9697 and CPP = 0.530303 giving a predicted values of Folic Acid = 0.0179, Protein = 1.4521 and

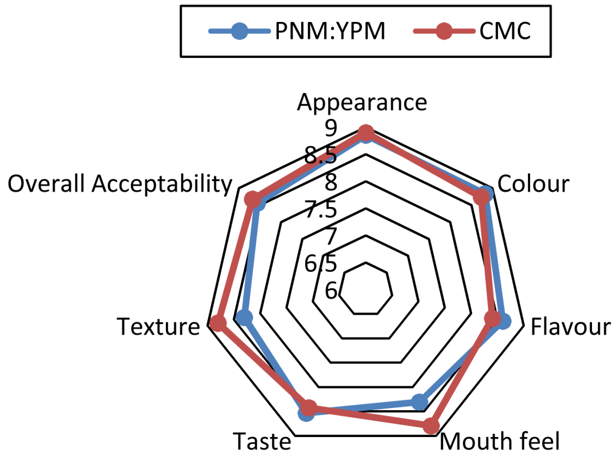

Figure 8. Rador chart representing the sensory attributes of formulated (PNM:YPM) and control milk chocolate (CMC).

Vitamin C = 0.4209. 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 Folic Acid = 0.0181, Protein = 1.552 and Vitamin C = 0.412 respectively, was near to the predicted values. Therefore, the predicted responses from the response surface methodology were significantly related to the experimental response that justifies the optimum conditions for nutritive value in legume milk chocolate and provides the better results.

3.7. Sensory Analysis

The optimized values of legume milk chocolate were evaluated using sensory attributes with the control chocolate. The overall score of sensory attributes ranges from 7.65 to 8.75. Color is similar to that of commercial chocolates, but texture is slightly in semi solid form. Radar chat was developed to represent the sensory attributes of formulated and control milk chocolate and it is shown in Figure 8. BT increases the mouthfeel whereas JG increases the firmness and hence, binding properties of chocolate also increases. PNM gives the pleasant flavor, enhances the taste and also significantly increases the physiochemical properties in legume milk chocolate [36]. Based on the sensorial reports, plant-based milk alternatives give the better nutritional value and sensorial profile.

4. Conclusion

From the study, it is feasible to obtain acceptable milk chocolates by substituting dairy milk with legumes milk. Optimum formulations were generated by the RSM model and the model was validated successfully. According to the results of optimization variations in legume milk combination enhances the either of the legumes flavour and CPP increases the bioactive components in the chocolate. The successful application and consumer acceptability of PNM:YPM has the potential to increase the utilization of these crops and enhance their market value.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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