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Research Article

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Development of Spirulina Rich Chocolate and its Optimization Using Response Surface Methodology

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Abstract

Chocolate is often a popular choice among children when it comes to sweets, but it should be enjoyed in moderation as part of a balanced diet, emphasizing nutritional variety. The objective of this study was to optimize the formulation of chocolate to develop a protein rich and low-fat chocolate with excellent sensorial attributes. In this study, an algal species, spirulina, was used as a protein source. To optimize the ingredients in the chocolate, the response surface methodology (RSM) Box-Behnken design was applied. The three independent variables were spirulina (%), olive oil (%), and coconut oil (%). Protein-rich chocolate has been successfully formulated using spirulina, olive oil, and coconut oil and protein content was 15.36% in comparison to control 10.09 ± 0.82%. The sensory and textural characteristics of the product were comparable to those of existing branded chocolates in market, including color, flavor, mouthfeel, body and texture, hardness, and fracture ability. The prepared chocolate was rich in functional properties which is good to overcome heart diseases, and malnutrition.

Keywords

Chocolate, Nutrition, Spirulina, Olive oil, Coconut oil

Introduction

Chocolate is often associated with positive experiences and emotions, such as celebrations, rewards, or treats. Among children, this emotional connection can further contribute to its popularity. There has been a steady increase in chocolate consumption in India over the past few decades. In India, the average chocolate consumption per person is 100 g to 200 g. Comparably, Japan, Asia's biggest chocolate consumer, consumes 2 kg per person, while Europe consumes 5 - 9 kg per person. It is no wonder that chocolate is being investigated as a functional food due to the health-promoting benefits it offers [1]. In a short research study (mainly 4 - 6 weeks), no significant differences were observed between the groups that consumed chocolate and those in the control group on skin, blood pressure, cholesterol profile, cognitive function, anthropometry, blood glucose, and quality of life irrespective of dose, form, and duration [2].

Chocolate contains cocoa, a source of flavonoids, mainly flavanols. Their intake reduces systolic and diastolic blood pressure and is beneficial to cardiovascular health. They are also potent antioxidants, antiradicals, and anti-inflammatory agents, these compounds improve blood pressure and endothelial function [3, 4].

Commercially, there are two major types of chocolate: white and dark. Dark chocolate increases verbal memory performance compared to white chocolate. Several studies shows that consuming dark chocolate can contribute to cognitive function and brain health in healthy people [5, 6]. Especially dark chocolate contains cocoa, which contains catechins, flavanols, procyanidins and theobromine

along with added sucrose and lipids [4]. Caffeine and theobromine, flavanols and methylxanthines of cocoa may enhance mood. Hence, flavanols, polyphenols and methylxanthines of cocoa might contribute to the popularity of chocolate due to their positive influence on human perception, mood, and behavior [3, 7].

The minor constituent salsolinol contributes to sensory properties [8, 9]. Therefore, in an experiment, chocolate paste was a medium used to administer drugs and counter the taste of bitter pharmacological additives [10]. In contrast to the positive health effects, adverse health effects like cardiovascular disease or dementia were also reported in persons consuming low-to-moderate chocolate intake (1 to 3 servings/week) [11]. Several researchers have reported potential health hazards associated with eating too much chocolate, such as obesity, dental problems, unhealthy lifestyle, and so on [12, 13]. Furthermore, chocolate was criticized for its fat content and consumption that was attributed to acne, caries, obesity, high blood pressure, coronary artery disease, and diabetes. Due to these health risks, many physicians warned patients against eating large amounts of chocolate. A longer duration clinical trials and larger population size-based study is needed to prove the health benefits of chocolate [14-16].

Individuals may have different views and preferences regarding chocolate consumption. Therefore, the trend of developing chocolate with nutraceutical properties is growing.

A healthy lifestyle and eating habits are becoming increasingly important to reduce chronic diseases among chocolate consumers. Due to this awareness, research is going on to reduce artificial additives in their products while promoting products with essential nutrients, ingredients that are beneficial to health, and ingredients that promote mental and physical well-being [17].

With progress in this direction, spirulina chocolate has been developed that provides the nutrition of spirulina with the indulgence of chocolate. Spirulina chocolate tastes different depending on the brand and the recipe used. Spirulina, a cyanobacterium, contains bioactive compounds, unsaturated fatty acids, and essential amino acids. These compounds contribute to basic human nutrition and can be used as a protein source. Additionally, they contain colorants and natural antioxidants such as chlorophylls, carotenoids, phycocyanins, and phenolic compounds [18]. Spirulina chocolate generally tastes earthy and slightly bitter due to its spirulina content. Chocolate adds sweetness and richness to balance the flavor. Spirulina's taste can be intensified or diminished depending on the concentration of the ingredient. Spirulina is used in some brands in a higher percentage, resulting in a stronger flavor, while others are milder.

Spirulina chocolate needs to be optimized for maximum health benefits and flavor acceptability. However, there is a lack of research in this direction. Therefore, in this study, chocolate was optimized using RSM for spirulina content to confer more benefits with better flavor.

Materials and Methods

Raw material and their sources

To develop spirulina-containing chocolate cocoa powder, cocoa butter, spirulina, olive oil, coconut oil, glucose, peanut, and milk powder were purchased from local market.

Ingredients of chocolate

Cocoa powder, cocoa butter, spirulina, olive oil, coconut oil, glucose, peanut and milk powder were mixed in the mentioned ratio (g/100 g of chocolate): Cocoa powder, cocoa butter, spirulina, olive oil, coconut oil, glucose, peanut and milk powder were mixed in the mentioned ratio (g/100 g of chocolate): cocoa powder - 8 g, cocoa butter - 24 g, olive oil - 2 g, coconut oil - 2 g, spirulina - 2 g, glucose - 20 g, and peanut - 42 g.

Preparation of chocolate

To produce rich dark chocolate using spirulina, cocoa butter was melted at 45 ˚C, then cocoa powder was homogeneously mixed in desired quantities. This was followed by adding spirulina, coconut oil, olive oil, and glucose which were mixed properly to obtain a homogeneous and smooth paste. Peanuts were also added to it for mass makeup. It was then manually tempered on a cool surface. The mixture was immediately poured into the mold and refrigerated at 3 ˚C. The prepared chocolate was then packaged properly in chocolate wrapping paper (Figure 1).

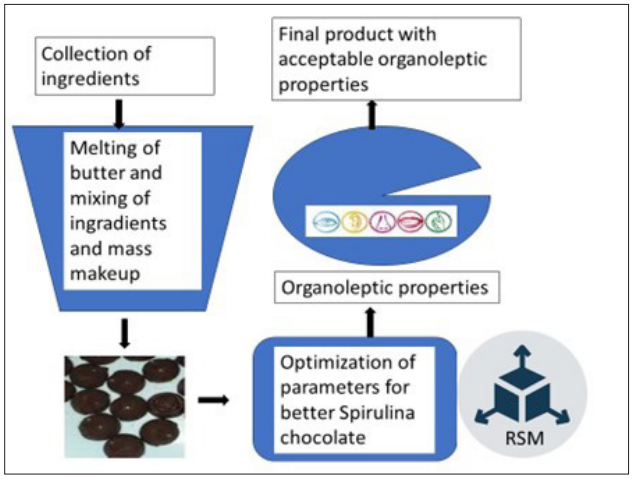

Figure 1: Flowchart showing optimization of chocolate.

Statistical analysis and optimization method

RSM was utilized for process optimization via Design Expert version 13 (Stat-Ease, USA) with Box-Behnken designs. The three independent variables were spirulina, olive oil, coconut oil. Twenty sets of experiments were carried out at different levels of independent variables (Table 1).

The design's experimental data was analyzed using the response surface regression approach and the following second-order polynomial equation 1.

$$Y = \alpha 0 + \Sigma \alpha i Xi + \Sigma \alpha ii Xi2 + \Sigma \alpha ij XiX \quad (1)$$

Table 1: Design of experiment and effect of independent variables on responses of high protein chocolate.

Std	Run	A: Spirulina (%)	B: Olive oil (%)	C: Coconut oil (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Overall acceptability (%)
13	1	4	2	2	4.3	11.2	40	1.83	7
1	2	2	1	2	4.42	12	38	2.31	6
9	3	4	1	1	4.52	12.8	30.51	1.98	7
12	4	4	3	3	4.21	11.85	45.75	1.86	8
7	5	2	2	3	4.25	11.21	43.24	1.95	6
5	6	2	2	1	4.55	11.25	40.25	1.85	8
2	7	6	1	2	4.89	14.75	37.54	1.94	8
20	8	4	2	2	4.36	12.98	40.35	1.85	7
11	9	4	1	3	4.12	12.35	40.25	1.95	7
6	10	6	2	1	4.8	15.45	40.54	1.89	7
8	11	6	2	3	4.45	12.85	44.52	1.95	7
18	12	4	2	2	4.35	12.98	38.15	1.89	7
16	13	4	2	2	4.35	11.21	38.12	1.56	7
15	14	4	2	2	4.21	13.27	38.56	1.83	7
3	15	2	3	2	4.53	11.23	41.59	1.48	8
17	16	4	2	2	4.1	12.45	38.52	1.85	7
14	17	4	2	2	4.2	12.23	39.85	1.87	7
4	18	6	3	2	4.6	15.52	43.23	1.98	7
19	19	4	2	2	4.19	12.48	40.12	1.85	7
20	20	4	3	1	4.12	11.25	37.52	1.68	9

Where Y represents the anticipated response value, 0 represents the intercept term, i represents the linear term, ii represents the squared term, ij represents the interaction term, and Xi and Xj represent the coded levels of independent variables.

The link between the expected response and the independent variables is expressed by the equation. The statistical parameters were estimated using analysis of variance (ANOVA). The experimental data was fitted using a second-order polynomial equation.

Moreover, the desirability function was performed to optimize the protein (%), fat (%), ash (%) and overall acceptability hedonic score. Myers et al. [19] and De-Paula et al. [20] reported the method that converts the response (Y_i) into desirability (d_i) ranges from 0 outside of the limits to 1 at the goal to obtain the overall desirability (D) using equation 2.

$$D = \sqrt[2]{d_1.d_2} \quad (2)$$

The individual desirability was calculated using equation 3.

$$d_i = \begin{cases} 0 \\ \left(\frac{Y_i - L_i}{T_i - L_i}\right)^{r_i} \end{cases}, Y_i < L_i, L_i \le Y_i \le T_i, Y_i > T_i \quad (3)$$

Where r_i is the weight of each response conferring to the desirability scale as follows: if $r_i = 1$, the desirability became linear, if $r_i > 1$ means it emphasizes on target value, and if $0 \le r_i \le 1$, the desirability is less critical. Y_i is the response value, L_i is the minimum response, and T_i is the maximum response [21].

Proximate analysis of optimized product

Total carbohydrate was estimated by phenol sulphuric method, total protein by Kjeldahl method, fat was estimated by Soxhlet method and ash content were estimated by Feldsine et al. [22]. The moisture and total phenolic content were also measured by Feldsine et al. [22]. The total phenolic content was determined by the Folin-ciocalteau method [23].

Sensory evaluation of spirulina chocolate

In this study, 9-point hedonic scale was used to collect the information on various sensory parameters such as flavor, color, mouthfeel, body texture, hardness, graininess [24]. Sensory analysis of spirulina-chocolate examines the product's sensory profile in more detail. It measures both qualitatively and quantitatively the intensities of each sensory attribute. To improve the final product, it is important to select the appropriate method to obtain sensory information.

The range of variables is spirulina 2 - 6%, olive oil 1 - 3%, and coconut oil 1 - 3%.

Results and Discussion

Design of experiment

Using RSM, it is possible to concurrently assess the impact of independent variables, both their main and interaction effects, which are evaluated in terms of linear, quadratic, and interaction terms. This evaluation is conducted on variables such as spirulina (A), olive oil (B), coconut oil (C) concerning each response (Yi). A Box-Behnken design was employed to derive the optimum process conditions for better protein content, lower fat, and better overall sensory scores for protein rich chocolate. Twenty sets of experiments were designed by Box-Behnken design as shown in table 1.

Model fitting and interactive effects of process variables on physicochemical properties of prepared chocolate

The coded design matrix has eight factorial points, six axial points, and five center points. Table 1 displayed the design matrix for the model's moisture, protein, fat, ash percentage, and overall acceptance score. All the responses' F-values revealed

that the model was significant. An F-value of 0.05 suggests that model terms are significant. All responses had a non-significant lack of fit, implying that the polynomial model satisfactorily suited all design points. The adjusted R^2/predicted R^2 value greater than 0.80 suggests that the design expert model for spirulina chocolate was statistically significant.

ANOVA table

The adjusted R^2/predicted R^2 values for R1, R2, R3, and R4 (0.9165/0.8218, 0.9540/0.8681, 0.9054/0.7843, 0.9134/0.7079, respectively) were found to be close to showing a correlation between adjusted and predicted values. Furthermore, while differences between squared and predicted R^2 values should be less than 0.2 for every dependent variable (indicating the fit for measuring whether the precisions are equal), the signal-to-noise ratio can be used, which should be greater than 4 to fit the model. In this experiment, the signal-to-noise ratio for R1, R2, R3, and R4 were 7.4695, 8.9156, 8.3776, and 15.8775, respectively; all the values were more than 4, indicating that the models were fitted correctly and suggesting that the proposed model can be used to navigate the design space. The ANOVA results for the quadratic models of R1, R2, R3, and R4 are listed.

Moisture

ANOVA table 2 showed a significant quadratic model. Maximum moisture content of 4.89% was observed in trial number 7 comprising of 6% spirulina, 1% olive oil, and 2% coconut oil. Similarly minimum 4.12% moisture was observed in trial number 20 comprising of 4% spirulina, 3% olive oil, and 1% coconut oil.

$$\text{Moisture \%} = 4.2575 + 0.12375 \times A + -0.06125 \times B + -0.12 \times C + -0.1 \times AB + -0.0125 \times AC + 0.1225 \times BC + 0.31125 \times A^2 + 0.04125 \times B^2 + -0.05625 \times C^2 \quad (4)$$

Where A is spirulina, B is olive oil, and C is coconut oil.

It is evident from equation 4 that an increase in moisture content on increasing the spirulina content in the chocolate. Which agreed with results obtained by Voloschenko et al. [25] on incorporation of spirulina in meat and vegetable pate it shows that spirulina has 117% moisture absorption capacity which is in contrast to results shown by Özbal et al. [26] in spirulina incorporated white chocolate which showed an increase in the amount of *Arthrospira platensis* did not cause any change in the moisture content of white chocolates.

The model F-value of 11.47 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In case A, C, BC, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 0.64 implies the lack of fit is not significant relative to the pure error. There is a 61.45% chance that a lack of fit F-value this large could occur due to noise.

Protein

ANOVA table 2 showed a significant quadratic model. Maximum protein content of 15.52% was observed in trial number 18 comprising of 6% spirulina, 3% olive oil, and 2% coconut oil. Similarly minimum 11.21% protein was observed in trial number 5 comprising of 2% spirulina, 2% olive oil, and 3% coconut oil.

The model F-value of 5.31 implies the model is significant. There is only a 0.77% chance that an F-value this large could occur due to noise. P-value less than 0.0500 indicates model terms are significant. In case A, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 0.91 implies the lack of fit is not significant relative to the pure error. There is a 48.33% chance that a lack of fit F-value this large could occur due to noise.

$$\text{Protein \%} = 12.35 + 1.61 \times A + -0.25625 \times B + -0.31125 \times C + 0.385 \times AB + -0.64 \times AC + 0.2625 \times BC + 0.82625 \times A^2 + 0.19875 \times B^2 + -0.48625 \times C^2 \quad (5)$$

Where A is spirulina, B is olive oil, and C is coconut oil.

Equation 5 depicts that protein increased with an increase in spirulina content while decreased on increasing both olive and coconut oil in the prepared chocolates. These results were similar to those reported by Silva et al. [27], Raczyk et al. [28], and Şahin [29] in pasta biscuits chocolates formulated with spirulina respectively due to high protein content in spirulina.

Fat

ANOVA table 2 showed a significant quadratic model. Maximum fat content of 45.75 % was observed in trial number 4 comprising of 4% spirulina 3% olive oil and 3% coconut oil similarly minimum fat content 30.51% was observed in trial number 5 comprising of 4% spirulina, 1% olive oil, and 1% coconut oil.

The model F-value of 11.81 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In case B, C, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The lack of fit F-value of 2.94 implies the lack of fit is not significant relative to the pure error. There is a 10.85% chance that a lack of fit F-value this large could occur due to noise.

$$\text{Fat (\%)} = 39.2087 + 0.34375 \times A + 2.34875 \times B + 2.7425 \times C + 0.525 \times AB + 0.2475 \times AC + -1.1275 \times BC + 2.63062 \times A^2 + -1.74938 \times B^2 + 0.298125 \times C^2 \quad (6)$$

Where A is spirulina, B is olive oil, and C is coconut oil.

Equation 6 depicts that fat % increased with increase in spirulina, olive oil, and coconut oil content. These results were similar to those reported by Dias et al. [30] and Elsayed et al. [31] on chocolate fillings.

Ash

The model F-value of 4.87 implies the model is significant. There is only a 1.06% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In case B, AB are significant model terms. Values greater than 0.1000 indicate the model terms

Table 2: ANOVA of quadratic model terms of variables on responses (F-value).

Source	Sum of squares	Df.	Mean square	F-value	p-value	Interference
Response 1: Moisture						
Model	0.5600	9	0.0622	4.52	0.0137	Significant
A: Spirulina	0.1275	1	0.1275	9.26	0.0124	-
B: Olive oil	0.0630	1	0.0630	4.57	0.0582	-
C: Coconut oil	0.0722	1	0.0722	5.24	0.0451	-
AB	0.0400	1	0.0400	2.90	0.1192	-
AC	0.0012	1	0.0012	0.0889	0.7717	-
BC	0.0182	1	0.0182	1.32	0.2768	-
A^2	0.2175	1	0.2175	15.79	0.0026	-
B^2	0.0000	1	0.0000	0.0012	0.9734	-
C^2	0.0407	1	0.0407	2.96	0.1163	-
Residual	0.1378	10	0.0138	-	-	-
Lack of fit	0.0580	3	0.0193	1.70	0.2541	Not significant
Pure error	0.0798	7	0.0114	-	-	-
Response 2: Protein						
Model	28.61	9	3.18	5.31	0.0077	Significant
A: Spirulina	20.74	1	20.74	34.67	0.0002	-
B: Olive oil	0.5253	1	0.5253	0.8782	0.3708	-
C: Coconut oil	0.7750	1	0.7750	1.30	0.2816	-
AB	0.5929	1	0.5929	0.9912	0.3429	-
AC	1.64	1	1.64	2.74	0.1289	-
BC	0.2756	1	0.2756	0.4608	0.5127	-
A^2	3.12	1	3.12	5.22	0.0455	-
B^2	0.1806	1	0.1806	0.3019	0.5948	-
C^2	1.08	1	1.08	1.81	0.2086	-
Residual	5.98	10	0.5982	-	-	-
Lack of fit	1.68	3	0.5594	0.9100	0.4833	Not significant
Pure error	4.30	7	0.6148	-	-	-
Response 3: Fat						
Model	153.07	9	17.01	11.81	0.0003	Significant
A: Spirulina	0.9453	1	0.9453	0.6566	0.4366	-
B: Olive oil	44.13	1	44.13	30.65	0.0002	-
C: Coconut oil	60.17	1	60.17	41.79	< 0.0001	-
AB	1.10	1	1.10	0.7658	0.4021	-
AC	0.2450	1	0.2450	0.1702	0.6886	-
BC	5.09	1	5.09	3.53	0.0896	-
A^2	31.64	1	31.64	21.97	0.0009	-
B^2	13.99	1	13.99	9.72	0.0109	-
C^2	0.4063	1	0.4063	0.2822	0.6069	-
Residual	14.40	10	1.44			-
Lack of fit	8.03	3	2.68	2.94	0.1085	Not significant
Pure error	6.37	7	0.9102	-	-	-
Cor total	167.47	19	-	-	-	-
Response 4: Ash						
Model	0.4330	9	0.0481	4.87	0.0106	Significant
A: Spirulina	0.0036	1	0.0036	0.3656	0.5589	-
B: Olive oil	0.1740	1	0.1740	17.61	0.0018	-
C: Coconut oil	0.0120	1	0.0120	1.22	0.2960	-
AB	0.1892	1	0.1892	19.15	0.0014	-
AC	0.0004	1	0.0004	0.0405	0.8446	-
BC	0.0110	1	0.0110	1.12	0.3157	-
A^2	0.0270	1	0.0270	2.73	0.1292	-
B^2	0.0054	1	0.0054	0.5467	0.4767	-
C^2	0.0013	1	0.0013	0.1317	0.7242	-
Residual	0.0988	10	0.0099			-
Lack of fit	0.0210	3	0.0070	0.6307	0.6181	Not significant
Pure error	0.0778	7	0.0111	-	-	-

Response 5: Overall acceptability						
Model	14.20	9	1.58	21.04	< 0.0001	Significant
A: Spirulina	10.12	1	10.12	135.00	< 0.0001	-
B: Olive oil	0.5000	1	0.5000	6.67	0.0273	-
C: Coconut oil	1.13	1	1.13	15.00	0.0031	-
AB	0.0000	1	0.0000	0.0000	1.0000	-
AC	0.2500	1	0.2500	3.33	0.0979	-
BC	1.0000	1	1.0000	13.33	0.0045	-
A^2	0.6429	1	0.6429	8.57	0.0151	-
B^2	0.6429	1	0.6429	8.57	0.0151	-
C^2	0.0714	1	0.0714	0.9524	0.3521	-
Residual	0.7500	10	0.0750	-	-	-
Lack of fit	0.7500	3	0.2500	-	-	-
Pure error	0.0000	7	0.0000	-	-	-

are not significant. The lack of fit F-value of 0.63 implies the lack of fit is not significant relative to the pure error. There is a 61.81% chance that a lack of fit F-value this large could occur due to noise.

$$\text{Ash (\%)} = 1.81625 + 0.02125 \times A + -0.1475 \times B + 0.03875 \times C + 0.2175 \times AB + -0.01 \times AC + 0.0525 \times BC + 0.076875 \times A^2 + 0.034375 \times B^2 + 0.016875 \times C^2 \quad (7)$$

Where A is spirulina, B is olive oil, and C is coconut oil.

Equation 7 depicts that ash % increased with increase in spirulina and olive oil content while decreased on increasing coconut oil. The change in ash % is generally due to mineral composition of spirulina. Similar results were reported by Agustini et al. [32] on addition of *Spirulina platensis* paste in dried noodles.

Overall acceptability

ANOVA table 2 showed a significant quadratic model. Maximum acceptability of 9 score in hedonic rating was observed in trial number 20 comprising of 4% spirulina, 3% olive oil, and 1% coconut oil. Similarly minimum overall acceptability 6 was observed in trial number 2 comprising of 2% spirulina, 1% olive oil, and 2% coconut oil.

The model F-value of 7.59 implies the model is significant. There is only a 0.20% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In case B, C, AB, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

$$\text{Overall acceptability} = 7 + 0.125 \times A + 0.5 \times B + -0.375 \times C + -0.75 \times AB + 0.5 \times AC + -0.25 \times BC + -0.25 \times A^2 + 0.5 \times B^2 + 0.25 \times C^2 \quad (8)$$

Equation 8 depicts that overall acceptability of hedonic rating increased with slight increase in spirulina content and olive oil content while decreased on increasing coconut oil. The change in ash % is generally due to mineral composition of spirulina (Table 3).

Optimization and model validation by desirability approach

A multi-criteria decision approach, such as a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot (Figure 2a and 2b), was used to optimize all responses with different targets.

The spirulina percentage positively correlates with protein and overall acceptability content, which is desirable, but a negative correlation was observed with increase in olive and coconut oil.

Desirability was determined by setting the protein content to maximum, fat to minimum and overall acceptability to maximum. Among the various runs suggested by the software for the set desirability, the run with maximum desirability of 0.744 (Figure 2a) at a combination of the factors as factor A (spirulina) at 5.96%, factor B (olive oil) at 1%, and factor C (coconut oil) at 1% was considered. As shown by the software, the predicted response variables were protein 15.520%, fat 33.922%, ash 1.920%. Overall acceptability 7.502 and desirability 0.730 as figure 2b which were closer to the experimental values of 4.775% moisture, protein 15.364%, fat 38.84%, and ash 15.592% respectively, indicating the suitability of the mod-

Table 3: Statistical parameters of protein rich spirulina chocolate.

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Overall acceptability (%)
Std. dev.	0.1174	0.7734	1.72	0.0994	0.2739
Mean	4.44	12.57	39.93	1.87	6.95
CV %	2.64	6.16	4.30	5.32	3.94
R^2	0.8026	0.8271	0.8518	0.8142	0.9498
Adjusted R^2	0.6249	0.6714	0.7184	0.6469	0.9047
Predicted R^2	-0.4787	0.0612	-0.4086	0.1763	0.1973
Adeq precision	8.9614	8.2282	11.1659	10.3856	18.7194

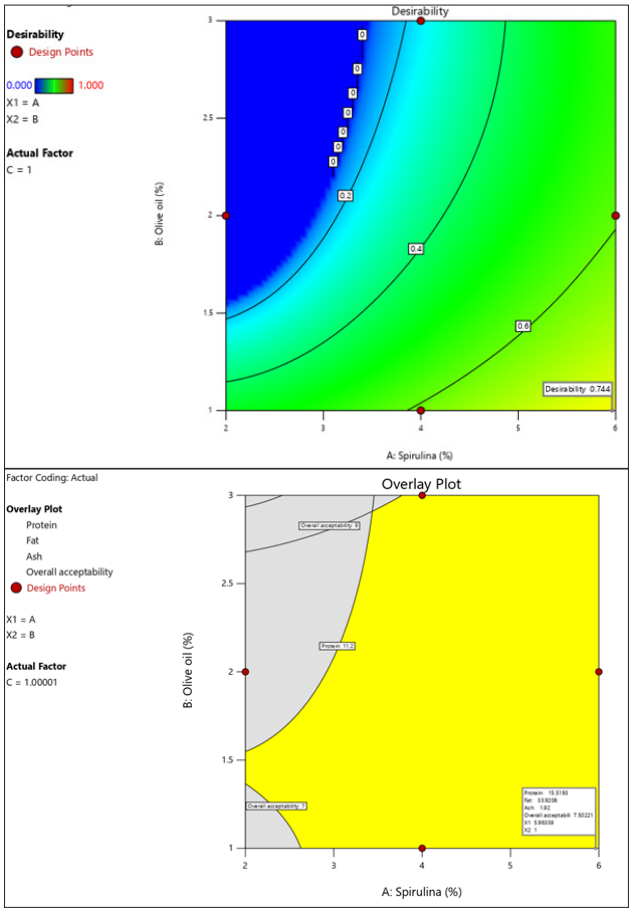

Figure 2: (a) Contour plot of desirability function and **(b)** Overlay plot showing the optimized values of the factors and predicted values of the responses.

el in optimizing the dark chocolate optimum amount for the production of quality dark chcocolate (Table 4 and table 5).

Conclusion

Spirulina, olive oil, and coconut oil were successfully incorporated to make protein rich spirulina chocolate. The sensory and textural characteristics like color, flavor, mouthfeel, body and texture, hardness and fracturability of the optimized product were better than control. Based on the results of sensory analysis of the spirulina chocolate formulated using different levels of spirulina, olive oil, and coconut oil a combination of the ingredients with very good, desired results was obtained. RSM was well suited for evaluating the individual and interaction effect of variables on sensory characteristics of chocolate. Spirulina chocolate was rich in protein, antioxidant and phenolic content, which is good to overcome heart disease, malnutrition. Due to the presence of functional and natural ingredients the market value of chocolate can also be increased.

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Table 4: Constraints, criteria for optimization, predicted and actual response values.

Constrains	Goal	Lower limit	Upper limit	Importance
A: Spirulina	Is in range	2	6	3
B: Olive oil	Is in range	1	3	3
C: Coconut oil	Is in range	1	3	3
Moisture	None	4.12	4.89	3
Protein	Maximize	11.2	15.52	3
Fat	Minimize	30.51	45.75	3
Ash	None	1.48	2.31	1
Overall acceptability	Maximize	6	9	3

Table 5: Actual value and predicted value of responses.

Responses	Actual value	Predicted value
Moisture (%)	4.775	4.998
Protein (%)	15.3648	15.520
Fat (%)	38.84	33.922
Overall acceptability	7	7.5

Conflict of Interest

None.

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