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Kumari Nisha

Department of Food Process Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India

K. Lakshmi Bala

Assistant Professor, Department of Food Process Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences., Prayagraj, Uttar Pradesh, India

Avanish Kumar

Assistant Professor, Department of Food Process Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences., Prayagraj, Uttar Pradesh, India

Deepak Kumar Sinha

Department of Food Process Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India

Corresponding Author:

Kumari Nisha

Department of Food Process Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India

Response surface methodology as an optimizing strategy in herbal latte mix formulation

Kumari Nisha, K. Lakshmi Bala, Avanish Kumar and Deepak Kumar Sinha

Abstract

Latte is a famous beverage of Italian origin. It is prepared using steamed milk. Textured milk is the base ingredient of Latte. The study aimed to develop an instant herbal Latte mix using Response Surface Methodology, one of the most exploited optimization techniques in food technology. Design and optimization has been assisted by a statistical program package "Design Expert (trial version 13, Stat-ease Inc, Minneapolis, MN, USA)". The outturn of three factors: Mulethi (*Glycyrrhiza glabra*) powder (A: 45-60%), Turmeric (*Curcuma longa*) powder (B: 8-18%) and Tulsi (*Ocimum sanctum*) powder (C: 4-10%) on proximate composition were investigated. All independent variables had statistical significance ($p < 0.05$) for each response variables. Optimum formulation of Latte mix consisted of 49.35% mulethi powder, 14.09% turmeric powder and 10% tulsi powder. Percentage moisture, ash, protein, fat and total soluble solids were 5.76%, 5.27%, 3.82%, 7.79% and 5.04% respectively.

Keywords: Response surface methodology, herbal latte mix, optimization, proximate composition, *Glycyrrhiza glabra*, *Ocimum sanctum*

Introduction

Latte or Cafe Latte is the Italian coffee drink that is prepared from steamed milk. Textured milk is the base ingredient of Latte. It is served both hot and cold (Roasty coffee: How to brew, 2020). Turmeric Latte is the new normal recipe that is being followed worldwide. Turmeric Latte, also known as "Golden milk" was 2016's drink of choice. The idea of turmeric Latte has its root from an Indian cuisine popularly called as 'Haldi Doodh'. This milk based beverage was bright yellow in color and was traditionally made by evaporating cow milk with spices like turmeric, cinnamon and ginger.

Glycyrrhiza glabra Linn, popularly known as 'Licorice' belongs to Leguminosae family (Sharma and Agrawal, 2013). *Glycyrrhiza* roots are useful to minister cough for the reason that it possess demulcent and expectorant effects. *Glycyrrhizin* (glycyrrhizic acid and glycyrrhizinate), the chief active ingredient and a saponin compound constituting 10% to 25% of mulethi root extract is almost 60 times sweeter than cane sugar (Pastorino *et al.*, 2018) [22]. Root extract of mulethi using hydro-methanol exhibits influential antibacterial activity. Secondary metabolites such as saponins, alkaloids and flavonoids present in mulethi exhibits such property. *In vitro* studies of aqueous extract and extracts of licorice using ethanol have demonstrated inhibitory activity on *S. aureus* and *S. pyogenes*.

Turmeric, scientifically called as *Curcuma longa* belongs to the Zingiberaceae family is one of the oldest herbal medicinal plant, popularly known for its use in curry ingredient and wound healing agent (Li *et al.*, 2011) [18]. India contribution world's turmeric production is 80% while it is 60% in world's total export. Indian turmeric is considered best because of its high curcumin content (Geethanjali *et al.*, 2016) [11]. Curcumin is the vital component of turmeric, which finds use in Ayurvedic medicines for centuries because of its strong anti-oxidant properties. Curcumin (3-4%) contributes to yellowish color of turmeric, and are classified as A: curcumin I (94%), B: curcumin II (6%) and C: curcumin III (0.3%) (Singh and Singh, 2018). It may also improve the function of blood vessel linings. It may also protect against heart diseases. Turmeric also improves the digestion of fat by increasing the production of bile by 62%. Mainly, Turmeric milk is touted for its immune boosting properties.

Ocimum sanctum Linn, popularly recognized as holy Basil or Tulsi belongs to Lamiaceae family. Tulsi is widely found aromatic perennial herb used in herbal tea and in treating cough, asthma gastritis, arthritis and many more. (Singh *et al.*, 2010) [27]. It is known as "queen of

herbs” or “mother of medicines”.

Tulsi issue 8% phytochemicals including Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and β -caryophyllene. Benefits of consuming tulsi has been attributed to its higher phenolic content. Anti-inflammatory action of tulsi has been attributed to eugenol and linoleic content. Tulsi has also been observed to spice up defenses against infective threats through enhancement of immune-response in stressed as well as non-stressed animals and healthy humans. Unique combination of antibacterial antioxidant, anti-inflammatory and analgesic properties of tulsi also makes it useful in wound healing.

Objective of this study was to find out an optimum formulation for Latte mix. Response Surface Methodology based CCRD design was used to find out an optimized ratio of mulethi, turmeric and tulsi in Latte mix. It is the need of an hour in current pandemic situation to have food which will fulfill the necessity of immune-booster as well as of delicacy. Turmeric-Tulsi Latte seems to fulfill the demand. The instant food mix provides convenience to consumers and helps in reducing the human efforts. Addition of tulsi to the same will improve the taste, health beneficial property and value.

Materials and Methods

Raw materials preparation

Raw materials were collected from local market of Prayagraj. Mulethi roots were cleaned using muslin clothes, reduced in size using pestle and mortar. It was then ground to powder using grinder. Tulsi leaves were sun dried at the rate of 6

hours per day up to 3 days and then ground to powder using grinder. Ginger, cardamom and black pepper powder were used at the constant rate of 8%, 2% and 2% respectively. Mulethi powder, turmeric powder and tulsi powder were sieved separately and finest particles were used in formulation.

Experimental Design

RSM was used to optimize the level of independent variables and their effect on proximate compositions. In this study, Central Composite Rotable Design was used. Maximum and minimum level of ingredients were based on early trials. 20 treatments were created using Response Surface methodology in which 6 treatments were replicates of center points. There were 8 factorial points as well as 6 axial (or star) points with $\alpha = 1.681$.

Sample formulation

Samples was formulated in laboratory according to randomized treatment design obtained by Central Composite Design of RSM. Amount of each sample (g/100g) lies on total weight of primary ingredient i.e. mulethi, turmeric and tulsi. Other ingredients used in preparation of Latte mix were 8.0 g ginger powder, 2.0 g cardamom powder and 2.0 g black pepper powder. Mulethi was mixed with turmeric and tulsi powder according to the experimental design as shown in *Table 1*. They were then primarily packed in LDPE sealable pouches and secondarily together in a CFB box.

Table 1: Response surface experimental design and response variables

Design	Space Type	Coded values			Actual values (%)		Responses (%)					
		A	B	C	Mulethi	Turmeric	Tulsi	Moisture	Ash	Protein	Fat	TSS
1	Central	0	0	0	52.5	13	7	7.5	5	2.8	8.4	6
2	Central	0	0	0	52.5	13	7	8	5.5	2.7	8.2	6
3	Factorial	+1	+1	-1	60	18	4	8.5	5.2	3.8	9	5.8
4	Central	0	0	0	52.5	13	7	7.5	5.3	1.7	7.6	5.5
5	Star	+ α	0	0	65.1	13	7	7.5	5.4	3.6	7.8	5.4
6	Star	0	0	- α	52.5	13	2	8	5	3.7	8.2	4.7
7	Factorial	-1	-1	+1	45	8	10	8	5.8	3.5	8.6	6.2
8	Star	0	+ α	0	52.5	21.4	7	8	5.1	3.2	6.8	5.9
9	Star	0	0	+ α	52.5	13	12	8	5.7	1.6	6.6	6
10	Factorial	+1	-1	+1	60	8	10	6	5	3.6	8	5.1
11	Central	0	0	0	52.5	13	7	5	5.8	3.6	7.4	5.4
12	Factorial	+1	-1	-1	60	8	4	5.5	5.7	3.6	7.4	5.4
13	Central	0	0	0	52.5	13	7	6	5.5	2.8	8.4	4.8
14	Star	- α	0	0	39.9	13	7	6	6	2	6.3	4.6
15	Factorial	+1	+1	+1	60	18	10	5	5.5	3.6	7.8	5.4
16	Central	0	0	0	52.5	13	7	4.5	5.5	3.6	7.6	5.4
17	Factorial	-1	+1	+1	45	18	10	4.5	5.7	3.2	6.5	4.8
18	Star	0	- α	0	52.5	4.6	7	5	5.5	1.4	6	5.8
19	Factorial	-1	-1	-1	45	8	4	5	5.7	3.1	7.4	4.2
20	Factorial	-1	+1	-1	45	18	4	4.5	5.8	3.6	7.7	5.4

Proximate composition

Moisture content, Ash content and Total Soluble Solid were determined in triplicate using methods described in AOAC, 2000. They were found using hot air oven, muffle furnace and hand refractometer respectively. Fat content was found using soxhlet apparatus in which petroleum ether was as solvent (5g sample, 65 °C for 6 hours). Protein content was estimated through micro-kjeldahl unit. All of them were expressed in % dry basis.

Statistical Analysis

A statistical program package “Design Expert (trial version 13, Stat-ease Inc, Minneapolis, MN, USA)” software was employed in designing, analysis and optimization. Statistical significance of independent variables over proximate composition of Latte mix was validated by ANOVA for each response at significance level of 5%. It gives an appropriate method capable of analyzing population variance. Significance of treatment was considered with the help of p-

value or 'probability' value. Model with p-value less than 0.05 were considered to have significant effect while larger values were considered insignificant.

Numerical optimization was carried out in order to determine the optimum formula for Latte mix in terms of proximate composition as shown in *Table 2*. Five physico-chemical attributes (moisture, ash, protein, fat and TSS) were considered as response variables for independent variables of mulethi, turmeric and tulsi powder. Quadratic models for all

the response attributes were used to generate contour plots using Design expert software. Second order quadratic equation fitted to the responses of experimental data where B_0 , X_i , X_i^2 and X_iX_j represents constant term, linear effects, quadratic effects and interaction effects respectively was as follows:

$$y = B_0 + 3\sum_{i=1} B_i X_i + 3\sum_{i=1} B_{ii} X_i^2 + 3\sum_{i=1} 2\sum_{j=i+1} B_{ij} X_i X_j \quad (1)$$

Table 2: Numerical optimization of independent and dependent variables

Factors	Goal	Lower limit (%)	Upper limit (%)	Importance	Optimized solution
Mulethi	Minimize	45	60	3	49.353
Turmeric	Maximize	8	18	3	14.096
Tulsi	Maximize	4	10	3	10
Ginger	Constant		8		8
Black pepper	Constant		2		2
Cardamom	Constant		2		2
Moisture	Minimize	4.5	8.5	3	5.764
Ash	Minimize	5	6	3	5.277
Protein	Maximize	1.4	3.8	3	3.823
Fat	Minimize	6	9	3	7.790
TSS	Maximize	4.2	6.2	3	5.041

Results and Discussion

Proximate composition of Latte mix includes analysis of moisture, ash, protein, fat and TSS of Latte mix. Average value of three replicates of responses are listed in *Table 1*. Moisture content in Latte mix ranges within 4.5% - 8.5%, ash content 5% - 6%, protein content 1.4% - 3.8%, fat content 6% - 9% and TSS 4.2% - 6.2%. ANOVA performed for quadratic regression analysis have shown p-value less than 0.050 for all the responses. It implies significant effect of independent variables over responses. Independent variables and responses were fitted to quadratic polynomial model equation and analyzed for goodness of fit. Correlation coefficient i.e. R^2 values for response surface were critically high conferring to the non-significant lack of fit. 3-D response surface plots were generated for each of the response variables. Optimum formulation for Latte mix has been listed in *Table 2*. Desirability of optimum solution was 0.661.

Moisture content

The effect of independent factors on moisture content of Latte mix are shown in *Fig. 1(a), 1(b) and 1(c)*. It can be seen that moisture content increased with increasing mulethi and turmeric content while decreased with increasing tulsi up to an extent. It has shown slight reduction at higher level of incorporation of tulsi. The results of ANOVA are listed in *Table 3*. Quadratic as well as linear model was suggested to describe the effects of ingredients. The obtained experimental data for moisture content i.e. 8.5% was relatively higher than 6.80% as reported by Al-Snafi (2018) [2] for mulethi. Quadratic model in terms of coded factors is shown in Equation 2.

$$\text{Moisture Content} = 5.32 + 0.8824 A + 0.6508 B - 0.1844 C - 0.5000 AB - 0.1250 AC + 0.3750 BC + 0.4644 A^2 + 0.5530 B^2 + 0.5626 C^2 \quad (2)$$

Table 3: Regression coefficients and ANOVA of response variables

Parameter	Estimated p and F values of different models									
	Moisture		Ash		Protein		Fat		TSS	
Coefficients	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value
Intercept	0.0444	3.14	0.0126	4.63	0.0009	9.25	0.0001	14.92	< 0.0001	65.30
A	0.0102	9.97	0.3036	1.18	0.5845	0.3193	0.0365	5.82	< 0.0001	539.20
B	0.0421	5.42	0.0141	8.81	0.0009	21.92	< 0.0001	123.36	0.5908	0.3086
C	0.5255	0.4328	0.0051	12.72	0.0003	29.44	0.3468	0.9746	0.0928	3.45
AB	0.2006	1.88	0.1123	3.03	0.5566	0.3700	0.4863	0.5225	1.0000	0.0000
AC	0.7390	0.1173	0.5745	0.3369	0.6936	0.1644	0.6402	0.2322	0.4848	0.5263
BC	0.3283	1.06	0.8505	0.0374	0.0980	3.33	0.6402	0.2322	0.4848	0.5263
A ²	0.1189	2.91	0.2815	1.30	0.2347	1.60	0.4462	0.6289	0.0062	11.93
B ²	0.0697	4.13	0.1017	3.25	0.0027	15.69	0.1656	2.24	0.0007	23.19
C ²	0.0686	4.16	0.0083	10.76	0.0036	14.30	0.8934	0.0189	0.0628	4.38
Lack of Fit	0.6575	0.6821	0.4059	1.25			0.0733	4.12		
R ²	0.7389		0.8065		0.8928		0.9307		0.9833	
Adj. R ²	0.5039		0.6323		0.7963		0.8683		0.9682	
Pred. R ²	-0.1075		0.0502		0.1572		0.5558		0.8680	

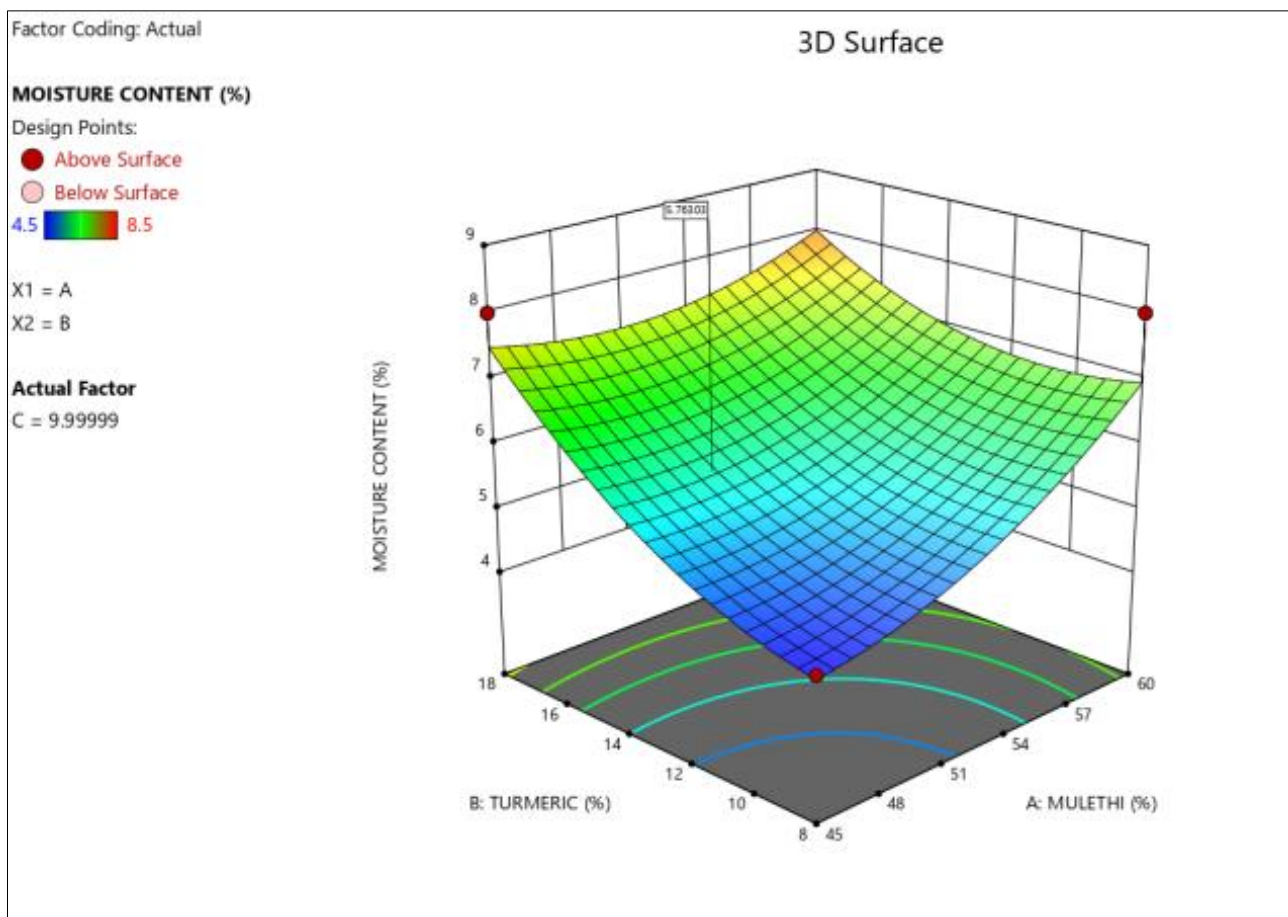


Fig 1(a): Effect of factors (A and B) on Moisture content (%)

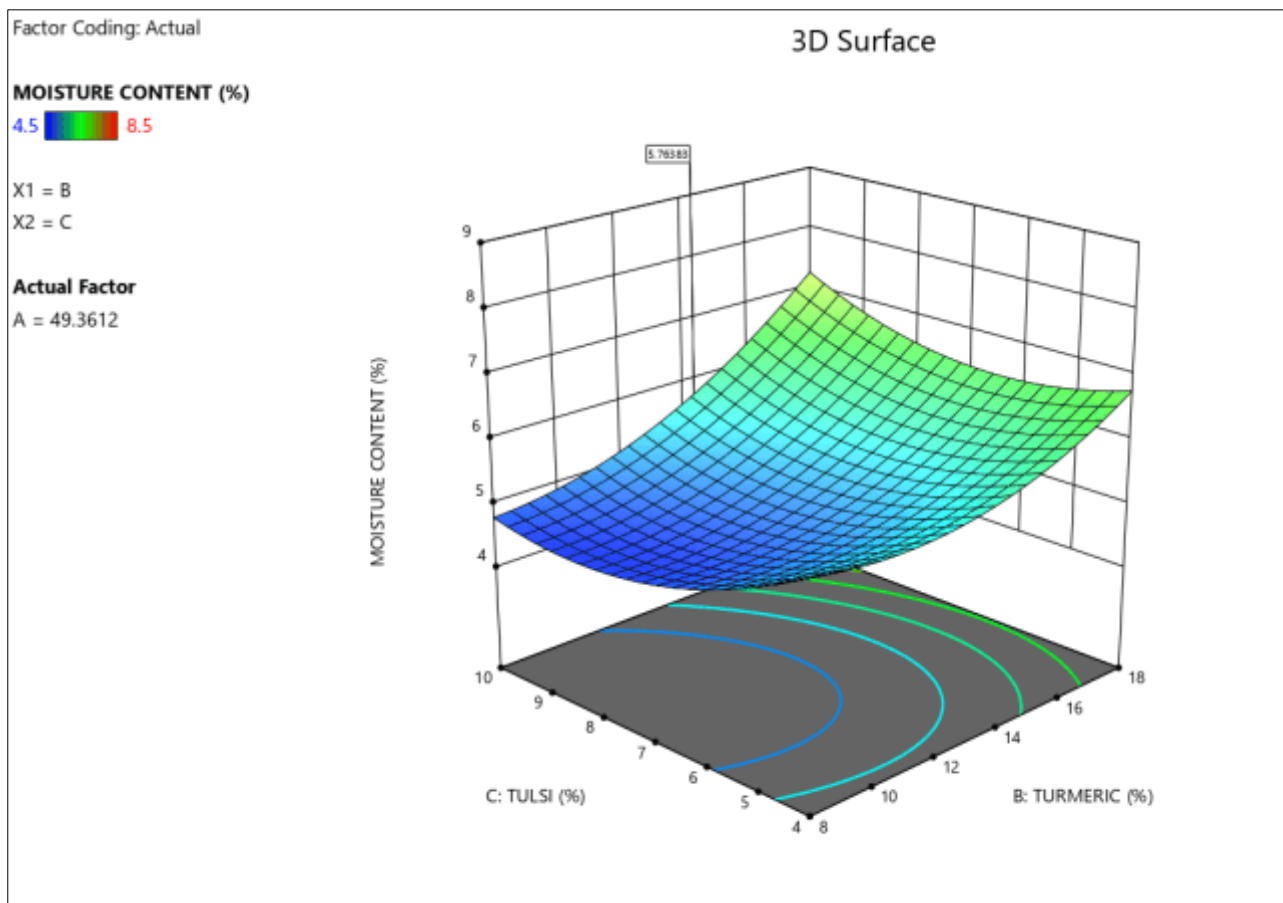


Fig 1(b): Effect of factors (B and C) on Moisture content (%)

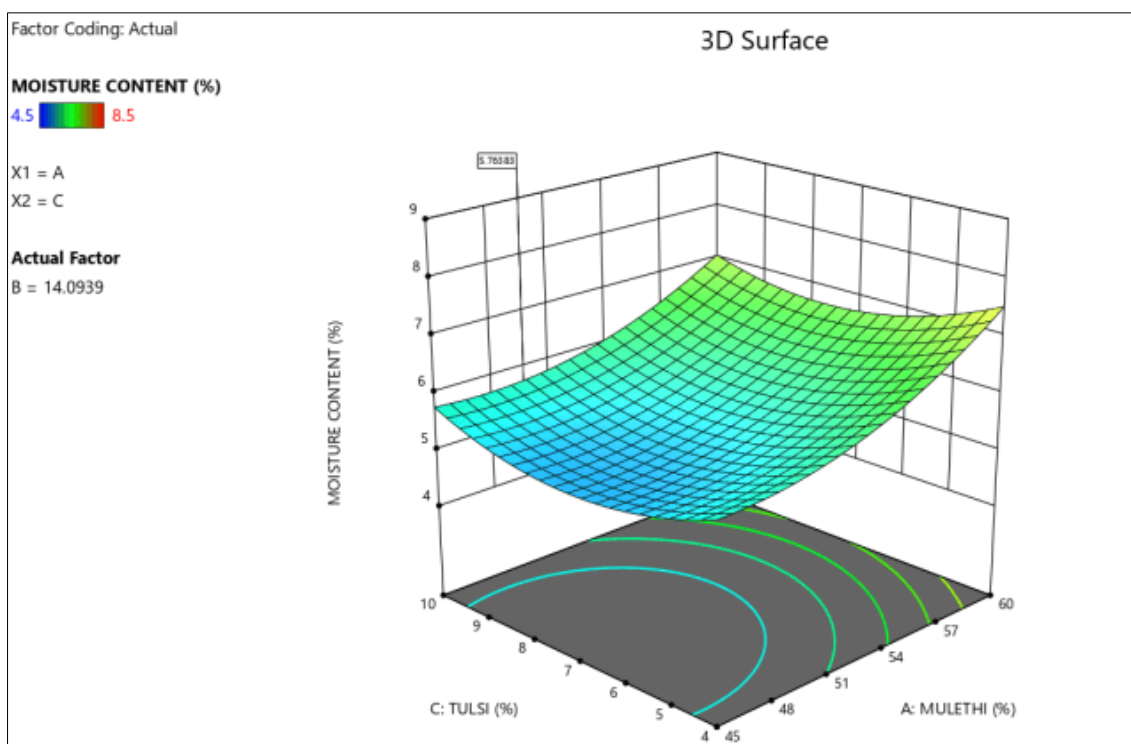


Fig 1(c): Effect of factors (A and C) on Moisture content (%)

Ash Content

Variation in ash content of latte mix as affected by ingredients are shown in Fig. 2(a), 2(b) and 2(c). Based on the results of fitted model to the experimental data, it was observed that turmeric and tulsi had significant effect on Ash Content of Latte mix. Significant decrement in values was observed ($p < 0.05$) as the level of embodiment of turmeric and tulsi inflated. Mulethi had insignificant effect on the same. Ash Content obtained 6% was lower than the value 7.70% as

reported by Al-Snafi (2018) [2] for raw mulethi. Reason can be attributed to the level of incorporation of turmeric and tulsi which shows reduction trend with an increasing amount. Model equation in terms of coded factors is shown in Equation 3.

$$\text{Ash Content} = 5.62 - 0.0536 A - 0.1469 B - 0.1770 C + 0.1125 AB - 0.0375 AC - 0.0125 BC + 0.0549 A^2 - 0.0869 B^2 - 0.1601 C^2 \quad (3)$$

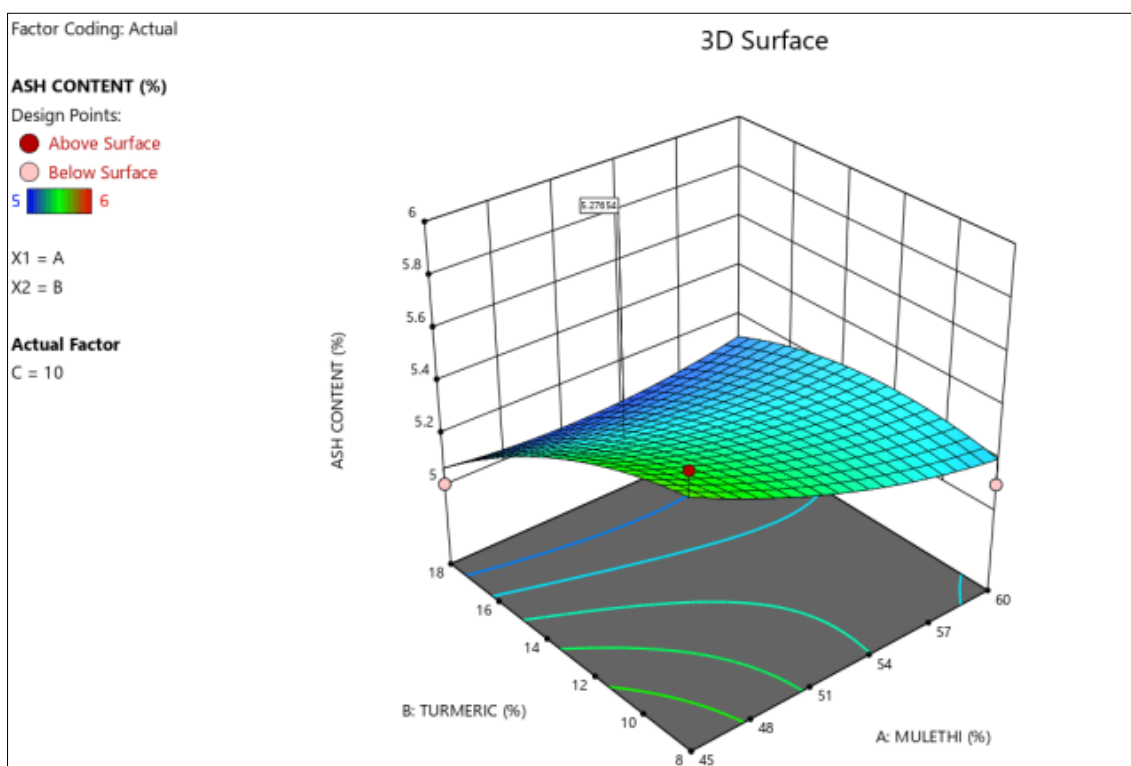


Fig 2(a): Effect of factors (A and B) on ash content (%)

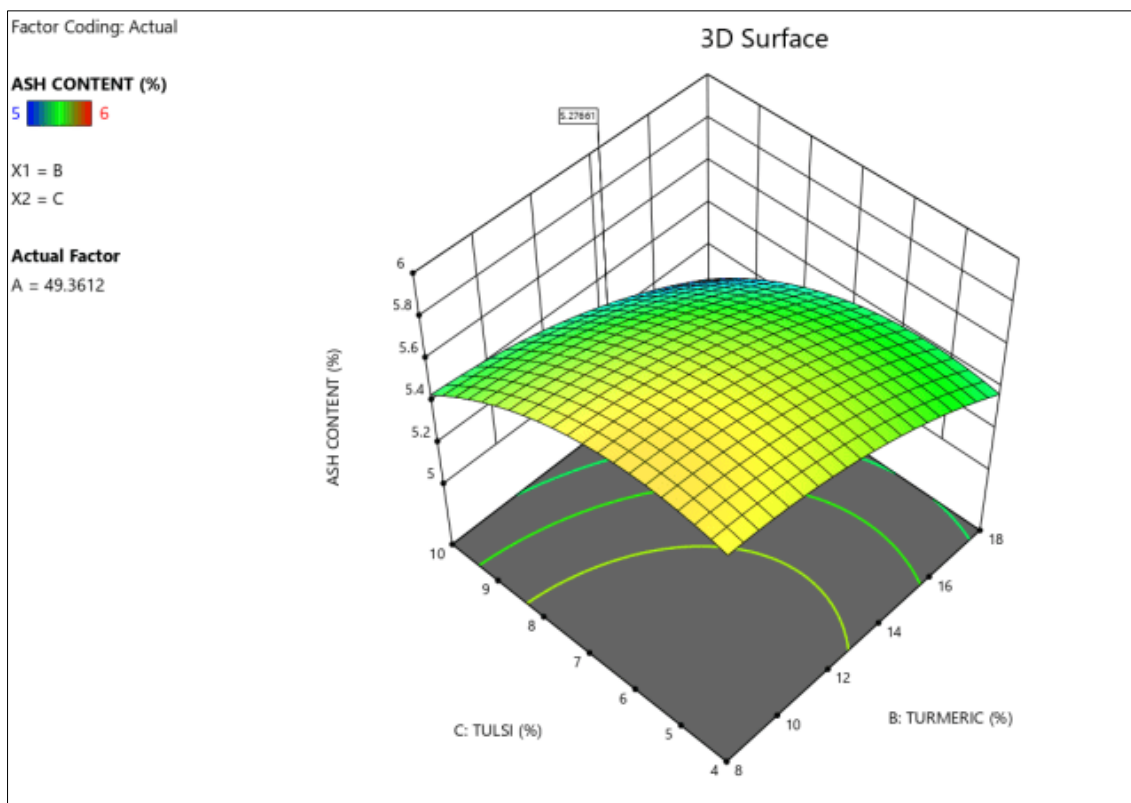


Fig 2(b): Effect of factors (B and C) on ash content (%)

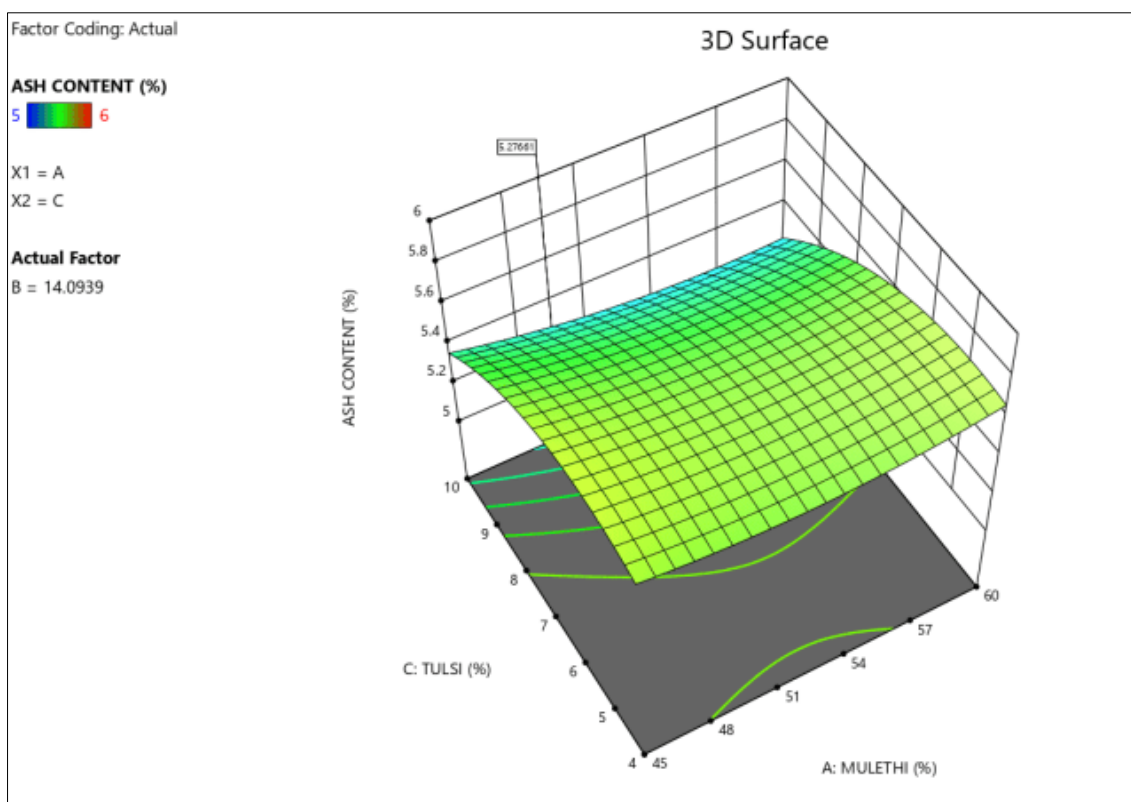


Fig 2(c): Effect of factors (A and C) on ash content (%)

Protein Content

As evident from Fig. 3(a), 3(b) and 3(c) protein content increased with increasing amount of turmeric and tulsi. Interaction of any two factors were non-significant. Experimental results of 3.8% were similar to 3.42% protein in turmeric as reported by Prematananda *et al.* (2021) and lower

than 4.2% as observed by Sethi and Bhadra (2020). It was much lower than 9.15% reported by Al-Snafi (2018) [2] for raw licorice. This inadequacy may be because of difference in sources and processing parameters. Higher Protein Content in some of the samples can be attributed to higher amount of turmeric as well as considerable amount of Tulsi. Linear as

well as Quadratic model was suggested for this response. Quadratic model in terms of coded factors has been shown in Equation 4.

$$\text{Protein Content} = 3.60 - 0.0534 A + 0.4421 B + 0.5139 C - 0.0750 AB - 0.0500 AC - 0.2250 BC - 0.1163 A^2 - 0.3643 B^2 - 0.3523 C^2 \quad (4)$$

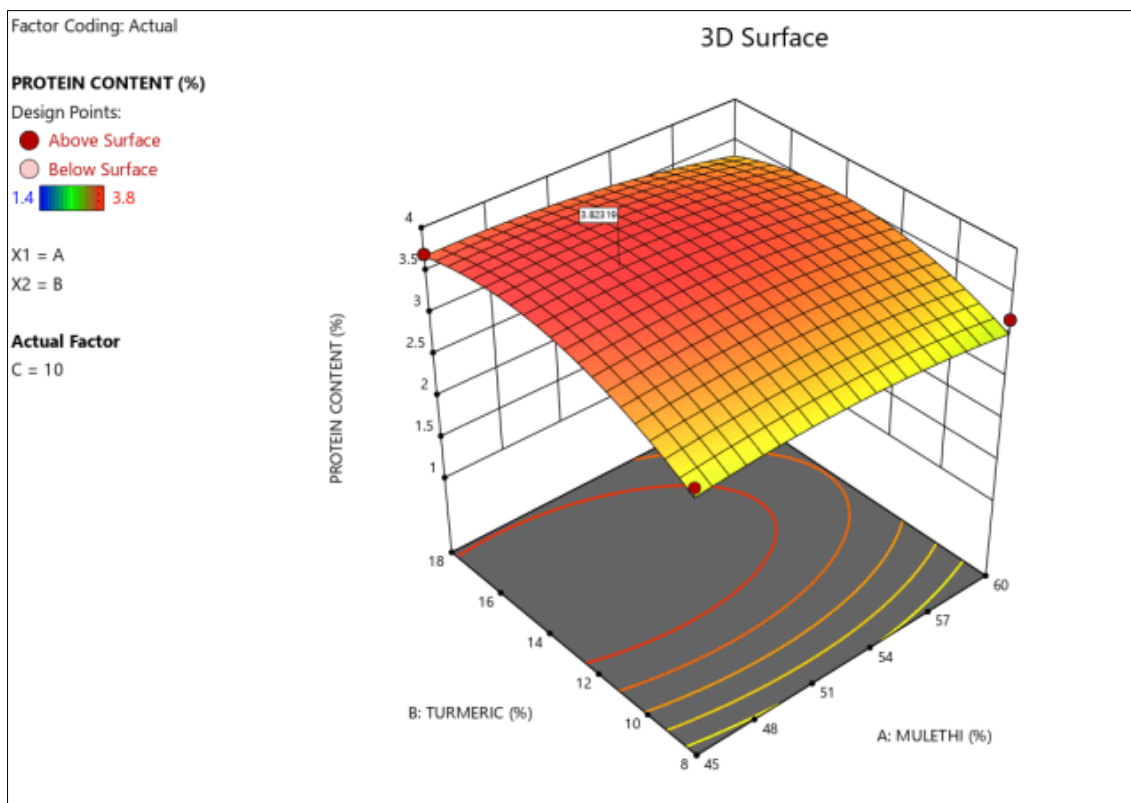


Fig 3(a): Effect of factors (A and B) on protein content (%)

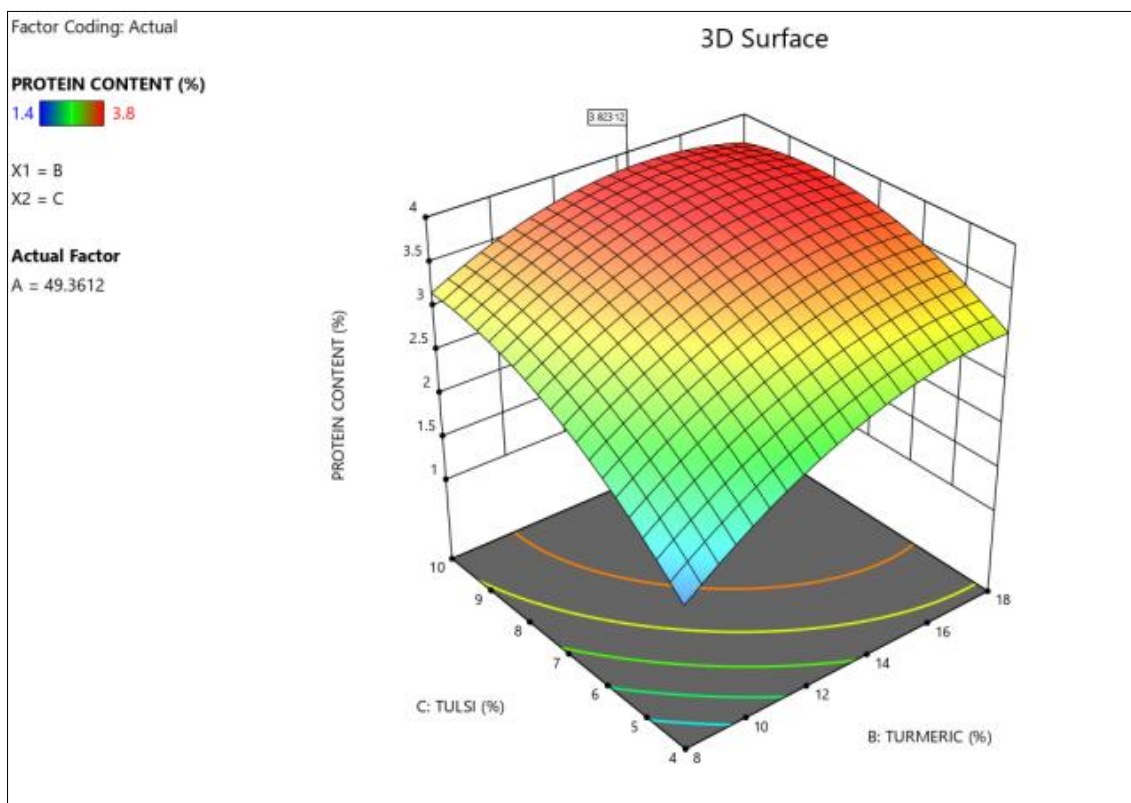


Fig 3(b): Effect of factors (B and C) on protein content (%)

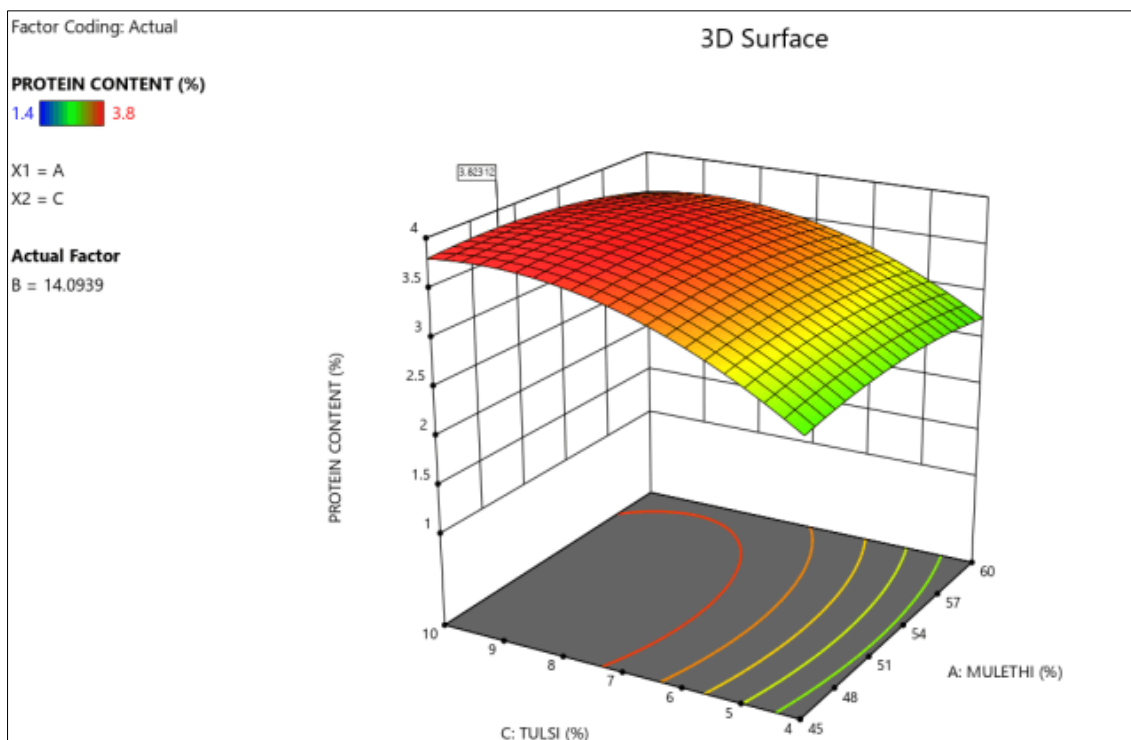


Fig 3(c): Effect of factors (A and C) on protein content (%)

Fat Content

Fat content in latte mix ranged from 6% to 9%. Effect of ingredients on fat content are shown in Fig. 4(a), 4(b) and 4(c). Fat Content increased significantly upon varying mulethi in higher proportions. There was insignificant difference in Fat Content when the amount of tulsi incorporated in the sample was varied. This could be due to addition of very small amount of tulsi in comparison to other ingredients. Values for fat content was much higher than that of tulsi as observed by Sethi and Bhadra (2020) while it was similar to

the values found by Singh and Singh (2008) [27]. Fat soluble extract of turmeric poses comparatively higher content of vitamin C & E, therefore poses strong antioxidant activity and it also exhibits hepato-protective effects due to antioxidant properties (Chanda & Ramchandra, 2019) [8]. Linear model was suggested to explain the variation of fat content as an effect of independent variables and it is shown in Equation 5.

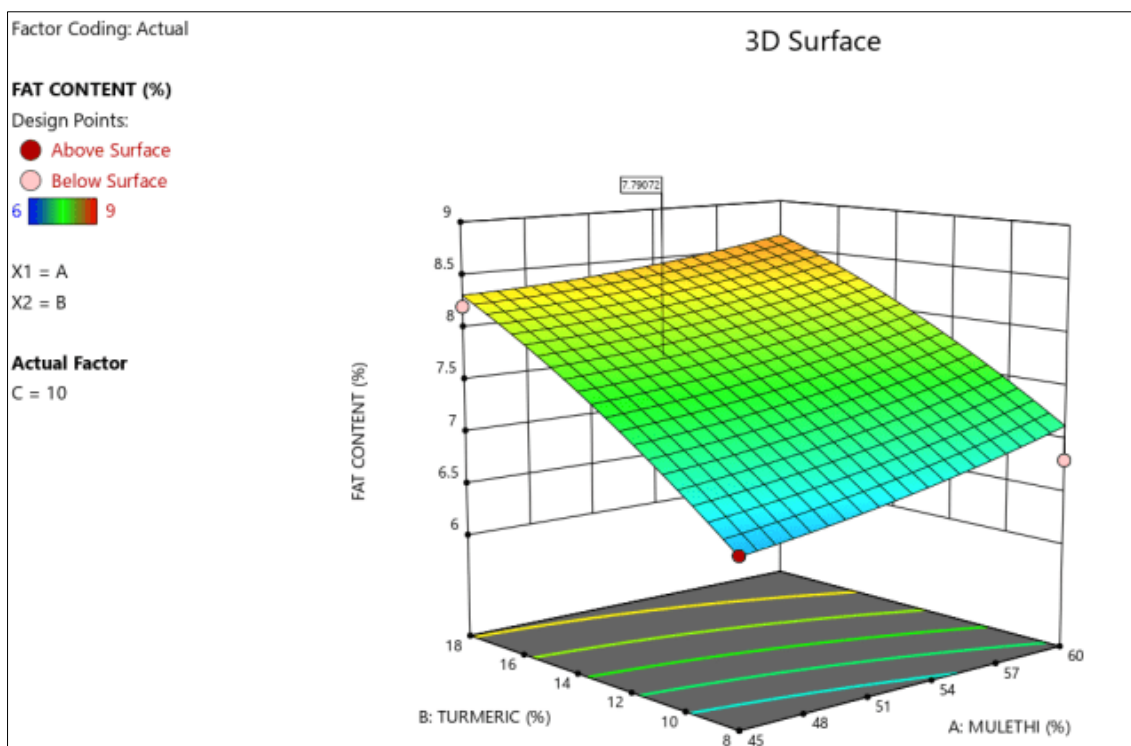
$$\text{FAT CONTENT} = 7.63 + 0.1917 A + 0.8824 B + 0.0787 C - 0.0750 AB + 0.0500 AC - 0.0500 BC + 0.0614 A^2 - 0.1158 B^2 - 0.0108 C^2 \text{ (5)}$$


Fig 4(a): Effect of factors (A and B) on fat content (%)

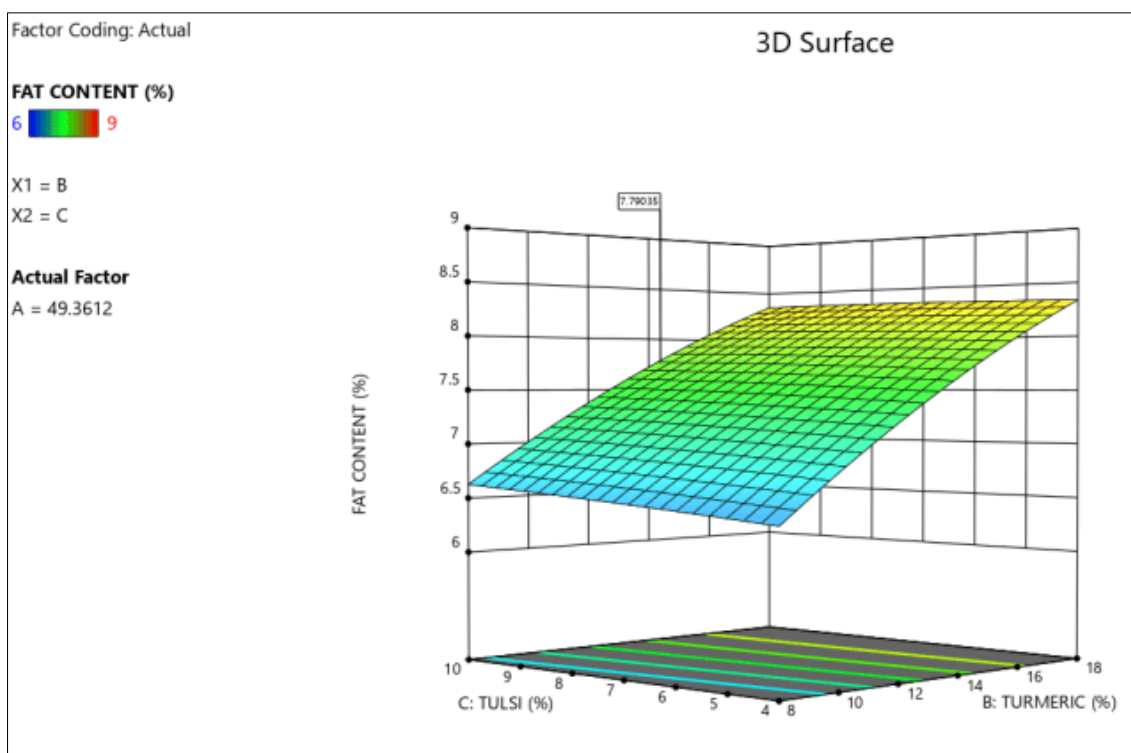


Fig 4(b): Effect of factors (B and C) on fat content (%)

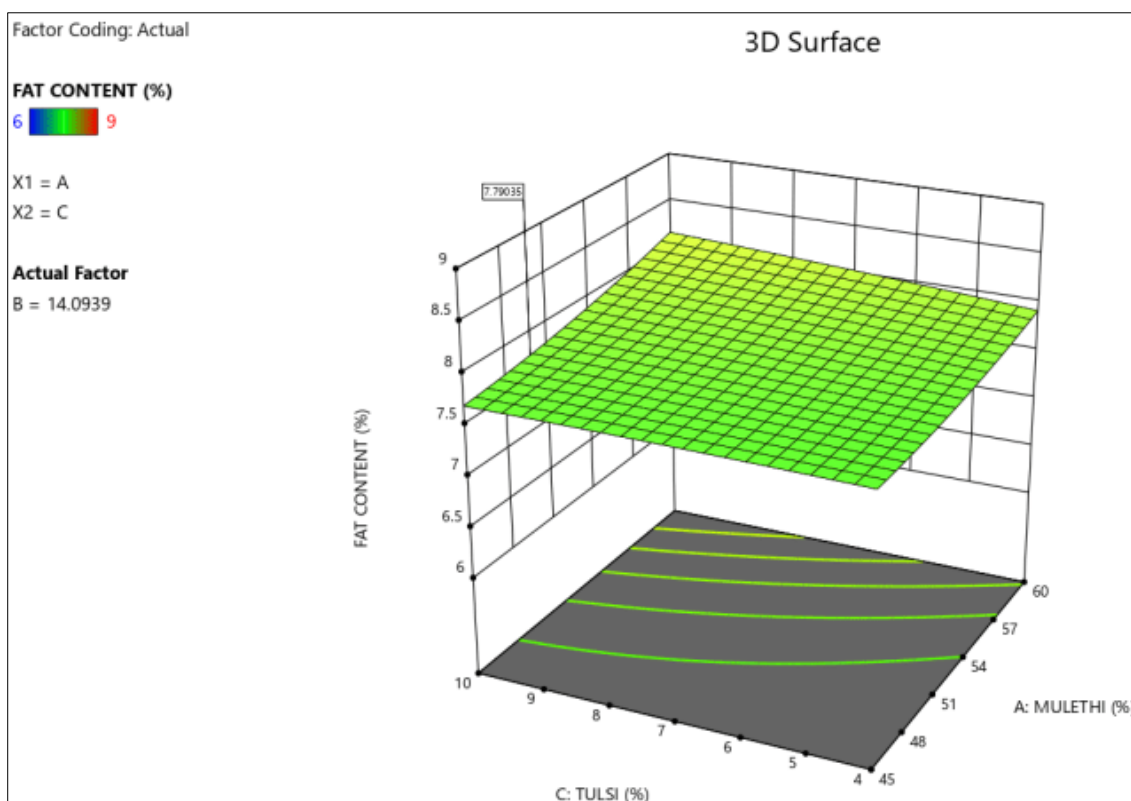


Fig 4(c): Effect of factors (A and C) on fat content (%)

Total Soluble Solids

Variations in TSS can be observed in *Fig. 5(a), 5(b) and 5(c)*. It was observed that the quadratic effect of mulethi ($p < 0.0001$) on amount of TSS in Latte mix was significant. As the percentage of mulethi increased, TSS value of Latte mix increased while effect of turmeric and tulsi was found to be not significant. This significant effect can be attributed to the higher ratio of mulethi in comparison with others. Also,

Glycyrrhizin, the primary active saponin compound present in the root extract of mulethi is 60 times sweeter than sucrose, therefore used as sweetening agent as discussed by Al-Snafi (2018) [2]. Quadratic model equation for this effect in terms of coded factors has been shown in Equation 6.

$$\text{Total Soluble Solids} = 5.40 + 0.6127 A + 0.0147 B - 0.0492 C + 0.0000 AB - 0.0250 AC - 0.0250 BC - 0.0888 A^2 + 0.1238 B^2 - 0.0545 C^2 \quad (6)$$

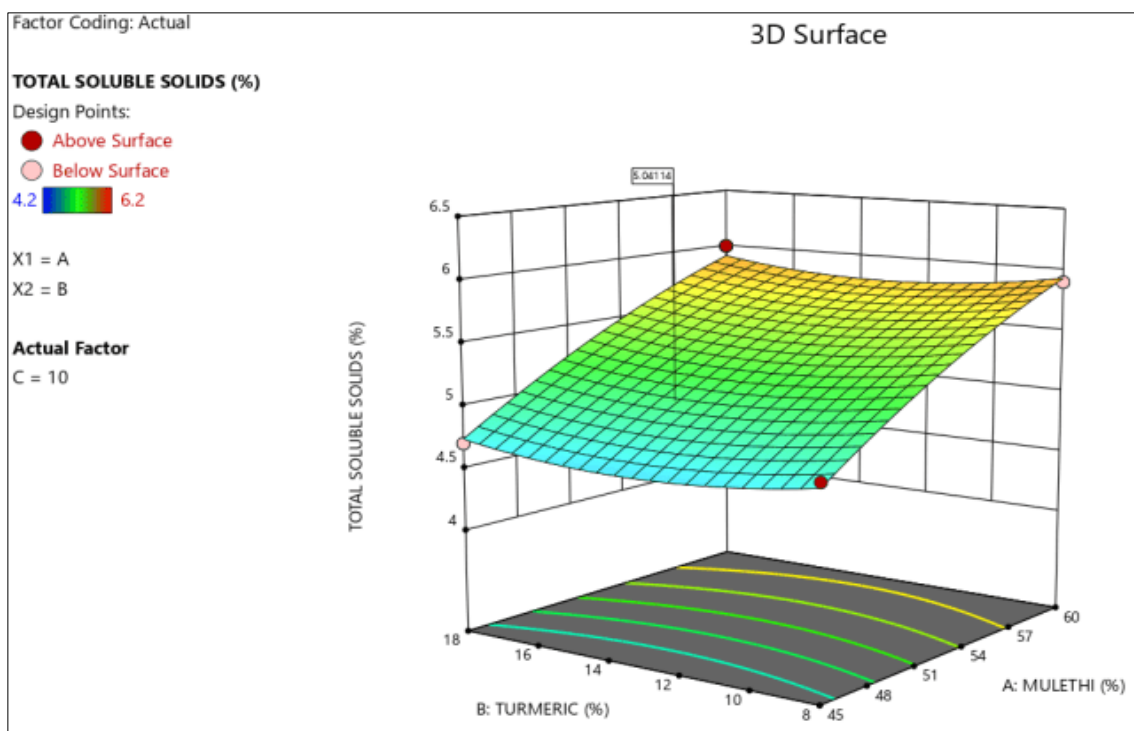


Fig 5(a): Effect of factors (A and B) on TSS content (%)

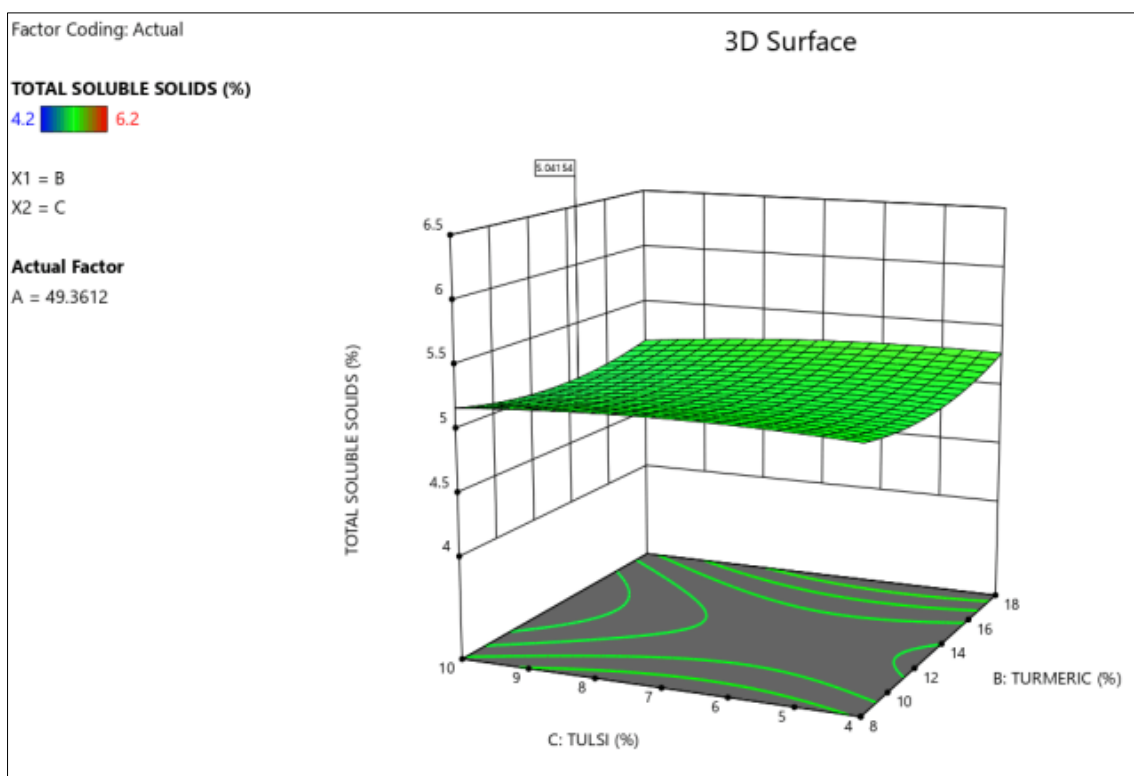


Fig 5(b): Effect of factors (B and C) on TSS content (%)

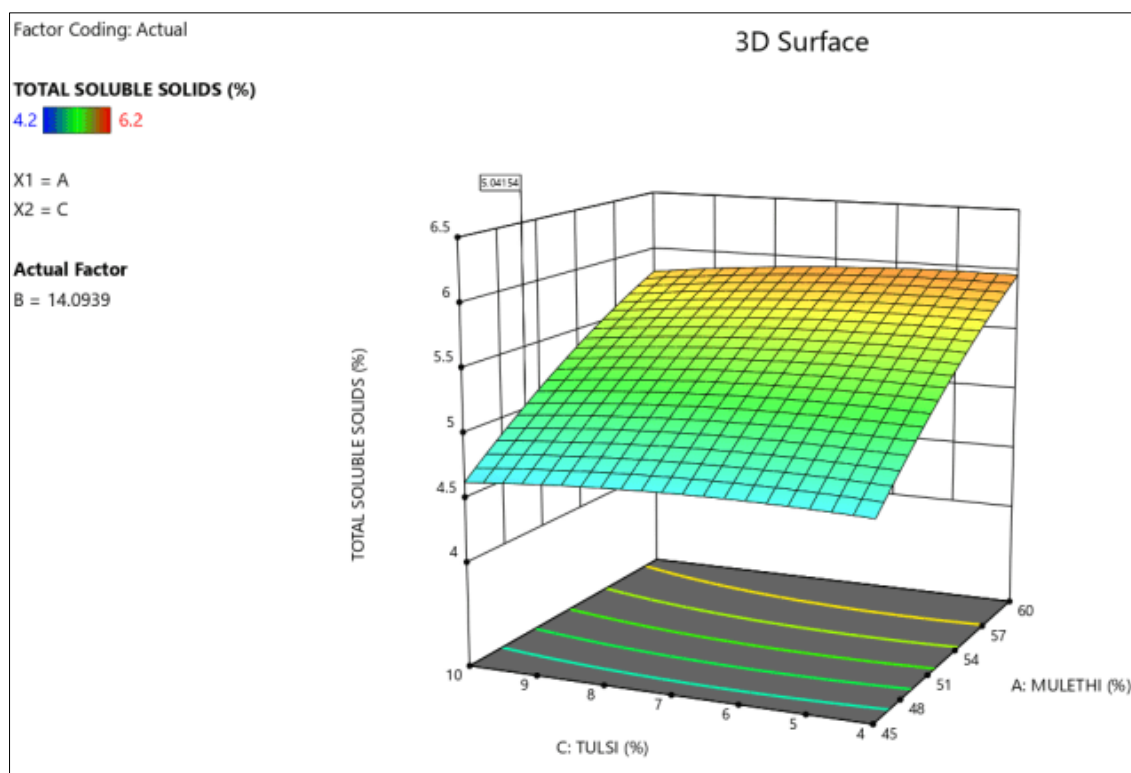


Fig 5(c): Effect of factors (A and C) on TSS content (%)



Fig 6: Herbal Latte Mix

Conclusion

Response Surface Methodology was successfully employed in identifying the standard combination of mulethi, turmeric and tulsi powder for Latte mix. Aim was to obtain an acceptable innovative product with high nutritional value. The optimum formula of Latte mix consisted of 49.35% mulethi powder, 14.09% turmeric powder and 10% tulsi powder, 8% ginger powder, 2% black pepper powder and 2% cardamom powder. It possessed 5.764% moisture, 5.277% ash, 3.823% protein, 7.790% fat and 5.041% TSS. Formulated Latte mix has been shown in Fig. 6.

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