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Applying response surface methodology for optimum formulation of flour blend

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Abstract
Blending is a process of carefully mixing one ingredient with another at a pre-defined ratio in order to obtain a mixture with desired characteristics. With the increase in health diseases, there is a need to alter food habits of society so as to eradicate micronutrient deficiencies and gain health through healthy food. Aim of this research was to blend wheat flour with barley flour and beetroot flour so as to balance its nutritional composition. Blended flour was prepared according to 20 treatments obtained through Central Composite Design of Design Expert (trial version 13, Stat-ease Inc., Minneapolis, MN, USA). Wheat, barley and beetroot flour ranged from 53.2% - 86.8%, 8% - 28.1% and 5.3% - 18.7% respectively relative to the treatment. Optimum formulation of flour blend consisted of 60% wheat, 24% barley and 16% beetroot flour. It possessed 8.72% moisture, 1.79% ash, 11.24% protein, 5.97% fat and 74.96% carbohydrate.

Keywords: Response Surface Methodology, Flour blend, Physico-chemical composition, *Beta vulgaris*, *Hordeum vulgare*, *Triticum aestivum*

1. Introduction
Blending is a process of carefully mixing one ingredient with another at a pre-defined ratio in order to obtain a mixture with desired characteristics. Blending of wheat flour is carried out in order to achieve consistency, uniqueness and balanced nutrition keeping cost control in mind. It can be done using two different varieties of wheat or mixing any other component with wheat flour. Composite flour puts forth better nutritional compositions concerning minerals, vitamins, fibers and proteins than flour obtained through one cereal alone (Hasmadi *et al.*, 2020). Blending of products also promotes better use of locally available and under-utilized crops. Alternative non-wheat cereals that can be substituted in wheat flour are *Zea mays*, Sorghum, Barley (*Hordeum vulgare*) and Rice. Legume flours are also mixed with wheat flour to enhance protein content. Processing and milling technique of barley is similar to that of wheat. Flour obtained by blending of wheat and barley possess enhanced protein and ash content. Though wheat flours are low in fat and good source of complex carbohydrates, they are not good sources of dietary fibers, especially soluble dietary fiber.

Wheat is one among the oldest and most vital cereal crops belonging to genus ‘Triticum’ and ‘Poaceae’ family. It is the foremost cereal crop in India. Physico-chemical characterization of wheat varies with differences in soil and climatic conditions. Wheat kernel encompasses 12% moisture, 70% carbohydrate, 12% protein, 2% fat, 1.8% minerals, and 2.2% crude fibre on an average. Barley or Jau, scientifically referred to as *Hordeum vulgare* L. is the foremost cereal crops within the world after rice, wheat and maize. It is a Rabi cereal crop of Poaceae family. It constitutes 11.5% protein, 74% carbohydrate, 1.3% fat, 3.9% crude fibre and 1.5% ash on an average. Barley is rich in carbohydrates and protein and therefore, are ideal source as livestock feed. Beetroot (*Beta vulgaris*) is a vegetable species of spermatophytes native to the goosefoot family. Beetroot encloses K, Mg, Fe, A, B_6 and C group of vitamins, folic acid, soluble fiber, antioxidants, protein and carbohydrates. Beetroot is abundant in soluble and insoluble dietary fiber, folate and antioxidants.

Aim of this research was to explore an optimum constitution of flour blend. RSM based experimental design was referred for finding out ratio of wheat, barley and beetroot flour in flour blend. Blending of barley with wheat flour to make chapatti was a tradition followed by our ancestors. With the increase in health diseases, there is a need to alter the food habits of society to eradicate micronutrient deficiencies and gain health through healthy food. Appropriate food in place of medicine will help combat the deficiency for long term.

2. Materials and Methods
2.1 Experimental design
CCRD based RSM was employed to optimize the level of independent parameters and their effect on response factors. Utmost and least level of independent factors were decided on the basis of earlier trials. There were 20 different designs including 8 factorial designs, 6 star designs and 6 repetitive central designs with $\alpha = 1.68179$.

2.2 Sample Preparation
Raw materials were collected from local market of Prayagraj and through e-commerce. Wheat flour, Barley flour and Beetroot flour obtained were sieved separately using sieve shaker and packed in LDPE sealable pouches. Finest particles were used in sample formulation. Samples were formulated in Food Process Engineering laboratory, VIAET, SHUATS. It was based on randomized treatment obtained by RSM. Samples were primarily packed in LDPE sealable pouches and secondarily together in a CFB box.

2.3 Proximate composition
Moisture content and ash content were determined by AOAC (2000) method using 2g sample at 130 °C for 2 hours using oven method and at 550 °C for 4 hours using muffle furnace respectively. Process was repeated until constant value was achieved. Fat % was found using Soxhlet apparatus (5g sample, 65°C for 6 hours). Protein % was estimated using micro-Kjeldahl apparatus. Carbohydrates were estimated using Phenol-Sulfuric acid method.

2.4 Statistical Analysis
Statistical scrutiny was brought off by adopting a completely randomized Central Composite Design in Response Surface Method through Stat-Ease's Design Expert software. Data recorded during work were analyzed by Analysis of Variance (ANOVA). This technique was developed by Dr. R. A. Fisher in 1923. Significance of test was analyzed by probability value or p-value at 5% level of significance. Values larger than 0.05 was considered as 'not significant". P-value is the estimate of goodness of fit in each case. Quadratic equation fitted to the model was explained below:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 \quad (1)$$

Where a represents the coefficients of polynomial as a_0 (constant), a_1, a_2 & a_3 (linear effect), a_{11}, a_{22} & a_{33} (quadratic effect) and a_{12}, a_{23} & a_{13} (interaction effect).
Numerical optimization was accomplished to find an optimum composition of blended flour. Barley flour and beetroot flour were maximized keeping wheat flour in range. Corresponding to it, moisture, ash and fat content were minimized while protein and carbohydrate content were maximized.

3. Results and Discussion
Flour blend components and their proportions given in Table 1 were used to produce flour blend. Wheat flour alone was used as control sample. Analysis of flour blend are listed in *Table 3*. Wheat flour, barley flour and beetroot flour ranged from $-\alpha$ to $+\alpha$ levels.

3.1 Moisture Content
Effect of ingredients on moisture content of flour blend are shown in *Fig. 2, 3 and 4*. Moisture content % in treatment ranged from 8.1% to 9.7%. ANOVA suggests significant effect of all the factors (*Table 5*). Interaction of wheat flour and barley flour had significant effect while others were not-significant. Increasing any of the constituents has shown rise in moisture content of flour blend, wheat flour being most significant. Moisture content 9.7% of treatment (70% wheat flour, 28.1% barley flour and 12% beetroot flour) was lower than moisture content 11.68%, as found by Bressiani *et al.* (2017) [6]. As noticed by Szostak *et al.* (2020), moisture content of wheat flour ranged between 7.6% and 14.3%. Low moisture content indicates better shelf life of flour blend. Model equation describing the effect is shown in Eq. 2
Moisture Content = $9.10 + 0.4585\,A + 0.3491\,B + 0.1275\,C + 0.1500\,AB + 0.0250\,AC - 0.1000\,BC - 0.0787\,A^2 - 0.0621\,B^2 + 0.0098\,C^2$ …. (2)

3.2 Ash Content
Effect of process variables on ash content of flour blend are shown in *Fig. 5, 6 and 7*. Highest ash content 2.7% was observed for treatment containing 86.8% wheat flour, 18% barley flour and 12% beetroot flour. Lowest 1.2% was observed for 53.2% wheat flour, 18% barley flour and 12% beetroot flour. Based on ANOVA, all the factors were found to have significant effect (*Table 5*). Interaction between any two factors were insignificant. With an increase in wheat flour, barley flour or beetroot flour, ash content increased significantly. Quadratic model was suggested for ash content of flour blend. Ash content of 2.7% was similar to that of 0.5% to 2.5% as found by Szostal *et al.* (2020). Model equation describing the effect is shown in Eq. 3.
Ash Content = $1.90 + 0.4045\,A + 0.2157\,B + 0.0933\,C + 0.0250\,AB + 0.0000\,AC - 0.0250\,BC + 0.0247\,A^2 + 0.0422\,B^2 - 0.0286\,C^2$ …. (3)

3.3 Protein Content
Effect of ingredients on Protein content are shown in *Fig. 8, 9 and 10*. Protein content in flour blend ranged between 10.7% and 11.9%. Highest protein content (11.9%) was observed in the treatment with highest wheat flour content of 86.8%. All the factors involved had significant effect on protein content of flour blend. Interaction between wheat flour and barley flour also depicted significance as observed from *Table 5*. Data was similar to the protein 10.55% as obtained by Sahoo *et al.* (2012) [24] for whole wheat flour. It was found to be in range of 10% to 15% as observed by Kumar *et al.* (2019) [25] for barley flour. Higher protein in flour due to wheat incorporation describes better binding ability during kneading and cooking. Model equation describing the effect is shown in Eq. 4.
Protein Content = $11.38 + 0.3553\,A + 0.2013\,B + 0.0417\,C + 0.0000\,AB - 0.0250\,AC - 0.0250\,BC - 0.0150\,A^2 + 0.0024\,B^2 - 0.0151\,C^2$ …. (4)

3.4 Fat content
As evident from *Fig. 11, 12 and 13*, quadratic effect of wheat and barley flour ($p < 0.05$) on amount of fat in flour blend was significant. As the value of wheat and barley flour increased, percentage of fat increased significantly and ranged from 5.1% to 6.5%. It was much higher than the fat content of 2.10% as reviewed by Kumar *et al.* (2011) [26]. Effect of beetroot and Interaction between any two factors was found to be insignificant. Model equation describing the effect is

shown in Eq. 5.

Fat Content = 6.02 + 0.2137 A + 0.2188 B + 0.0907 C - 0.0625 AB + 0.0875 AC + 0.0875 BC - 0.1267 A^2 - 0.0208 B^2 - 0.0563 C^2 …. (5)

3.5 Carbohydrate content

Effect of process variables on carbohydrate content of flour blend are shown in *Fig. 14, 15 and 16*. ANOVA for Second order polynomial equation of carbohydrate content was analyzed as not-significant ($p > 0.05$). Therefore, model reduction was performed. Modified backward model was further used to generate ANOVA Table which was found to have significance. Wheat flour and beetroot flour had shown significance on carbohydrate % of flour blend. With an increase in wheat flour content, carbohydrates increased while with an increase in beetroot flour content, carbohydrate content of flour blend decreased. Data found was similar to 78.10% as reviewed by Kumar *et al.* (2011) [26]. Model equation describing the effect is shown in Eq. 6.

Carbohydrate Content = 75.01 + 0.1785 A – 0.2087 C – 0.1625 AC + 0.1059 C^2. …. (6)

Table 1: Independent Variables for preliminary trials

Parameter	Level	Values (%)				
Variable		-α	-1	0	+1	+α
Wheat flour	5	53.20	60	70	80	86.80
Barley flour	5	8	12	18	24	28.10
Beetroot flour	5	5.30	8	12	16	18.70

Table 2: Experimental design in terms of coded and actual levels

Experiment	Space	Coded values			Actual values		
number	type	A	B	C	Wheat flour %	Barley flour %	Beetroot flour %
1	Center	0	0	0	70	18	12
2	Axial	0	+α	0	70	28.1	12
3	Axial	0	0	+α	70	18	18.7
4	Factorial	-1	+1	+1	60	24	16
5	Center	0	0	0	70	18	12
6	Axial	+α	0	0	86.8	18	12
7	Axial	0	0	-α	70	18	5.3
8	Factorial	-1	-1	-1	60	12	8
9	Factorial	+1	+1	+1	80	24	16
10	Center	0	0	0	70	18	12
11	Axial	0	-α	0	70	8	12
12	Factorial	-1	-1	+1	60	12	16
13	Factorial	+1	-1	+1	80	12	16
14	Axial	-α	0	0	53.2	18	12
15	Factorial	+1	-1	-1	80	12	8
16	Center	0	0	0	70	18	12
17	Factorial	+1	+1	-1	80	24	8
18	Factorial	-1	+1	-1	60	24	8
19	Center	0	0	0	70	18	12
20	Center	0	0	0	70	18	12

Table 3: Response parameters of Flour blend*

Sample	Moisture %	Ash %	Fat %	Protein %	Carbohydrate %
1	9	1.9	5.9	11.3	75.2
2	9.7	2.4	6.5	11.8	75.1
3	9.3	2	6	11.4	74.8
4	8.8	1.8	6.1	11.2	74.9
5	9	1.9	5.9	11.5	75
6	9.8	2.7	6.3	11.9	75
7	9.1	1.6	5.8	11.3	75.6
8	8.2	1.3	5.6	10.7	75.1
9	9.8	2.6	6.2	11.9	75
10	9.1	1.9	6.1	11.4	74.9
11	8.3	1.6	5.5	11	75
12	8.5	1.5	5.3	10.9	74.9
13	9.3	2.2	6	11.6	75.2
14	8.1	1.2	5.1	10.8	74.8
15	8.5	2	5.6	11.5	76
16	9.1	1.9	5.9	11.4	75.3
17	9.8	2.5	5.8	11.9	75.6
18	8.5	1.7	5.7	11.1	74.8
19	9.3	1.9	6.1	11.4	74.7
20	9.1	1.9	6.2	11.3	74.7

*Values are average of three replicates.

Table 4: Criteria for numerical optimization of independent and response variables

Factors	Goal	Lower limit (%)	Upper limit (%)	Importance
Wheat flour	In range	60	80	3
Barley flour	Maximize	12	24	3
Beetroot flour	Maximize	8	16	3
Moisture	Minimize	8.1	9.8	3
Ash	Minimize	1.2	2.7	3
Fat	Maximize	5.1	6.5	3
Protein	In range	10.7	11.9	3
Carbohydrate	Maximize	74.7	76	3

Table 5: Regression coefficients and ANOVA of dependent variables

Parameter	Estimated p and F values of different models									
	Moisure		Ash		Protein		Fat		Carbohydrate	
Coefficients	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Model	19.16	< 0.0001	107.06	< 0.0001	50.12	< 0.0001	4.61	0.0128	2.93	0.0545
A	96.11	< 0.0001	706.41	< 0.0001	335.77	< 0.0001	14.39	0.0035	7.49	0.0209
B	55.58	< 0.0001	200.29	< 0.0001	107.50	< 0.0001	15.04	0.0031	0.6918	0.4250
C	7.41	0.0215	37.48	0.0001	4.61	0.0573	2.59	0.1388	10.21	0.0096
AB	6.03	0.0339	1.58	0.2371	0.0000	1.0000	0.7214	0.4156	0.1939	0.6691
AC	0.1675	0.6909	0.0000	1.0000	0.9747	0.3468	1.41	0.2619	3.64	0.0855
BC	2.68	0.1326	1.58	0.2371	0.9747	0.3468	1.41	0.2619	0.5385	0.4799
A^2	2.98	0.1151	2.77	0.1269	0.6266	0.4470	5.33	0.0437	0.0044	0.9483
B^2	1.84	0.2051	8.02	0.0178	0.0158	0.9026	0.1419	0.7142	0.8403	0.3809
C^2	0.0462	0.8342	3.68	0.0841	0.6296	0.4459	1.04	0.3319	3.04	0.1118
Lack of Fit	3.97	0.0781			0.8106	0.5883	3.90	0.0806	0.8519	0.5676
R^2		0.9452		0.9897		0.9783		0.8059		0.7252
Adj. R^2		0.8959		0.9805		0.9588		0.6313		0.4780
Pred. R^2		0.6275		0.9221		0.9089		- 0.2776		-0.1708

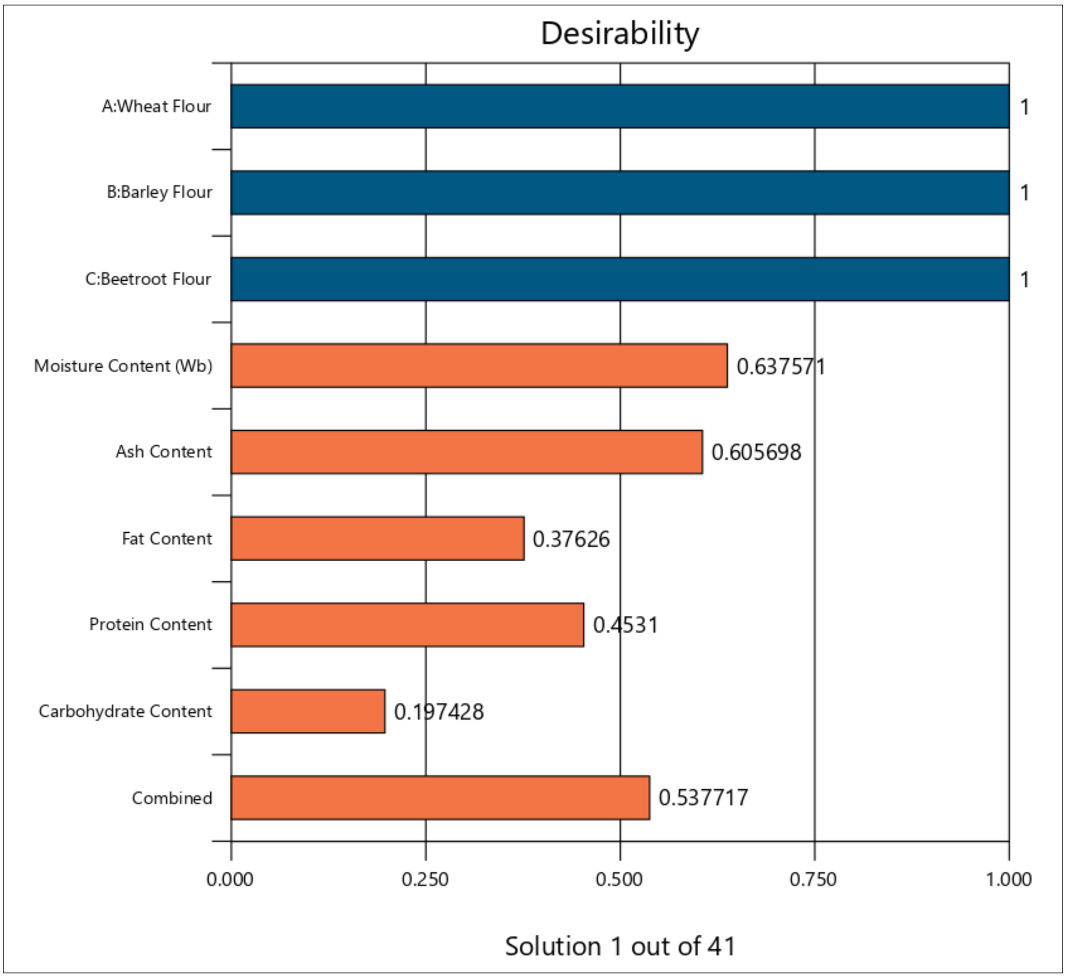

Fig 1: Optimized solution of flour blend

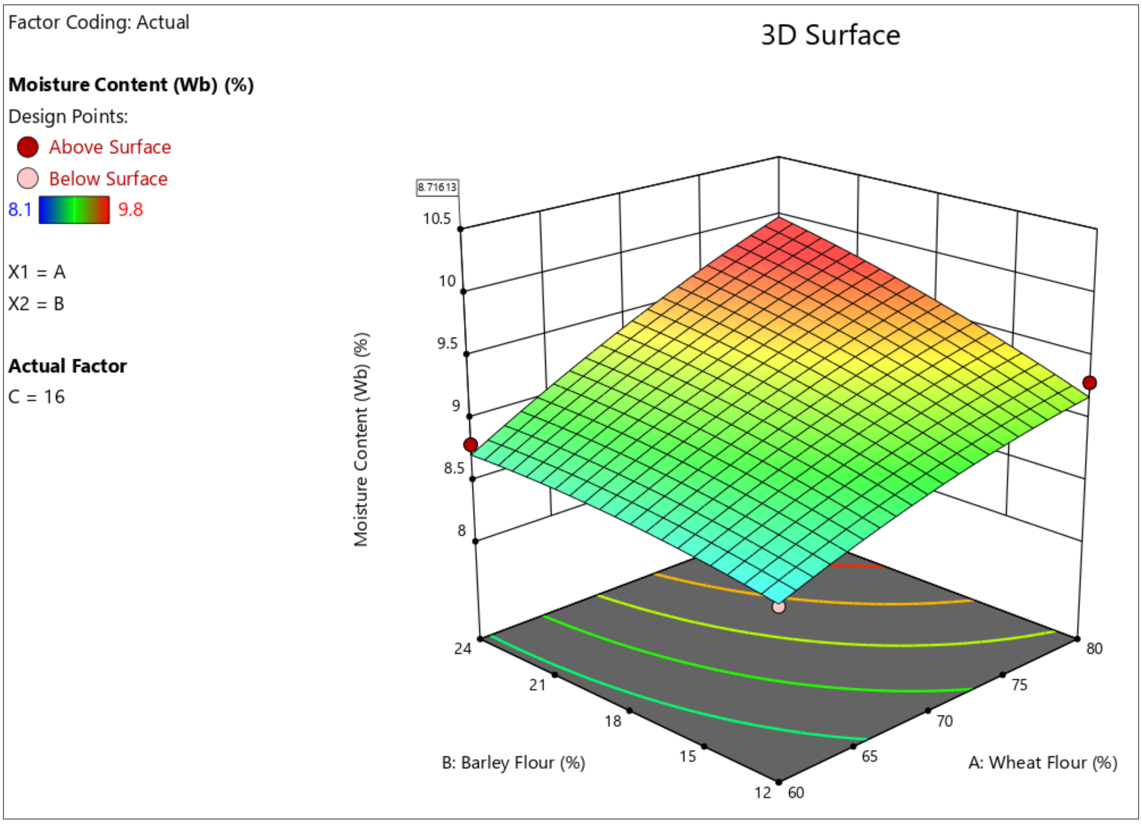

Fig 2: Effect of independent factors (A and B) on moisture content (%)

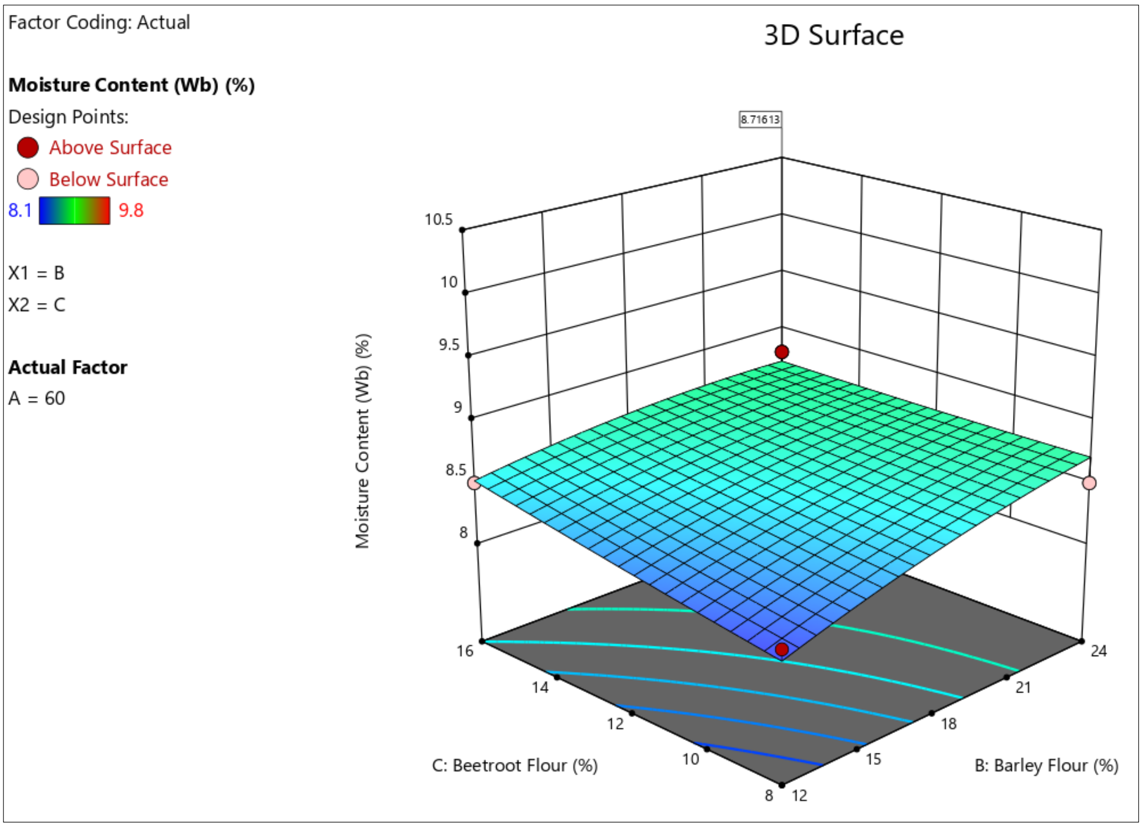

Fig 3: Effect of independent factors (B and C) on moisture content (%)

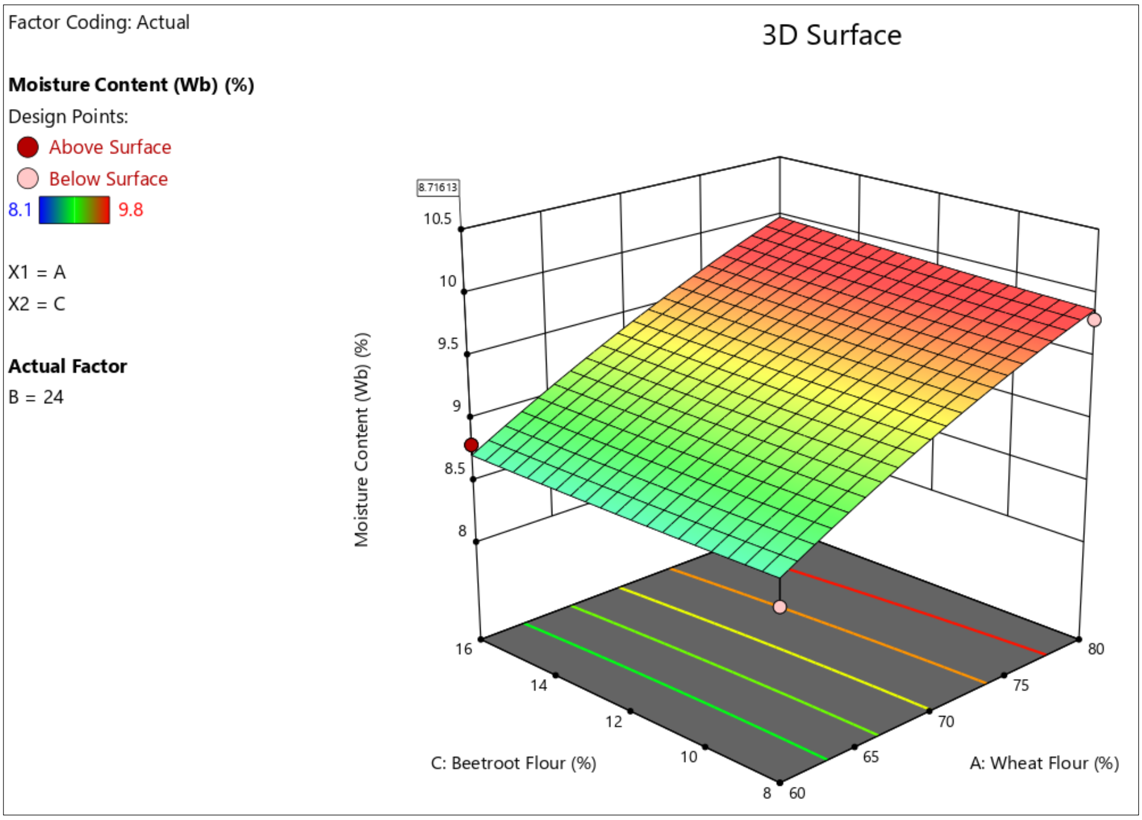

Fig 4: Effect of independent factors (A and C) on moisture content (%)

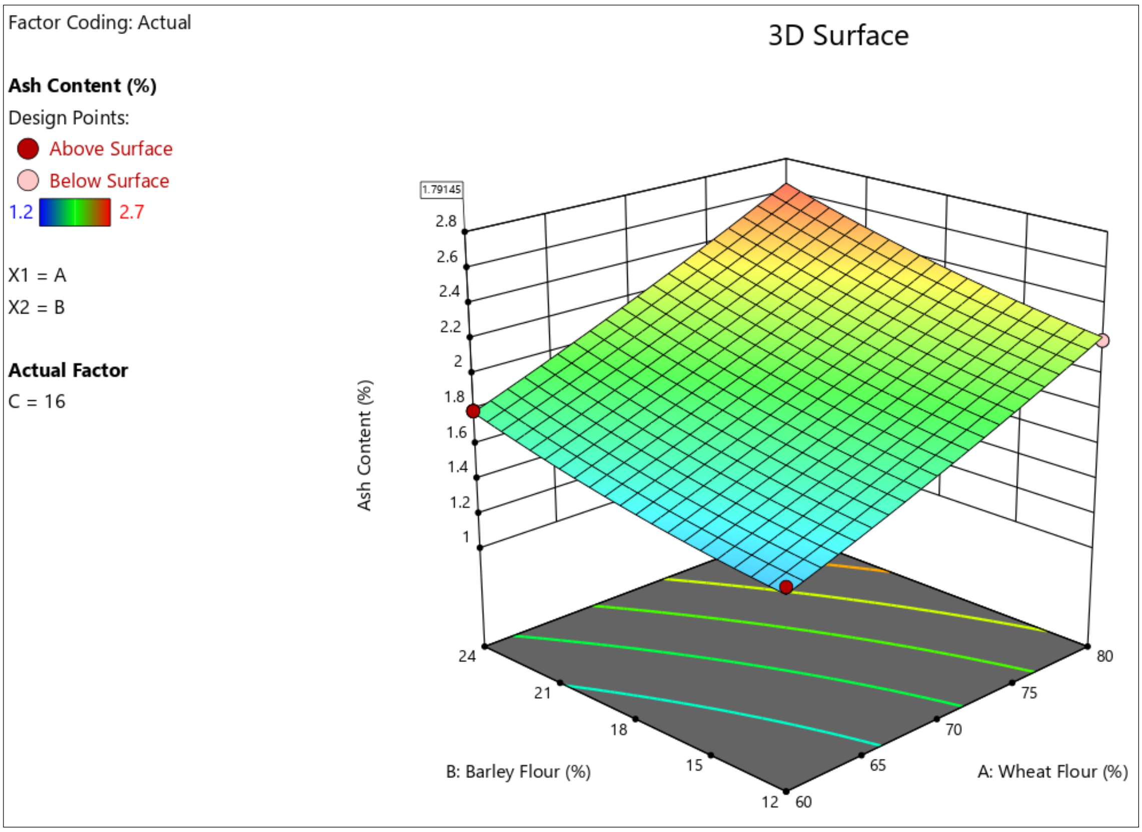

Fig 5: Effect of independent factors (A and B) on ash content (%)

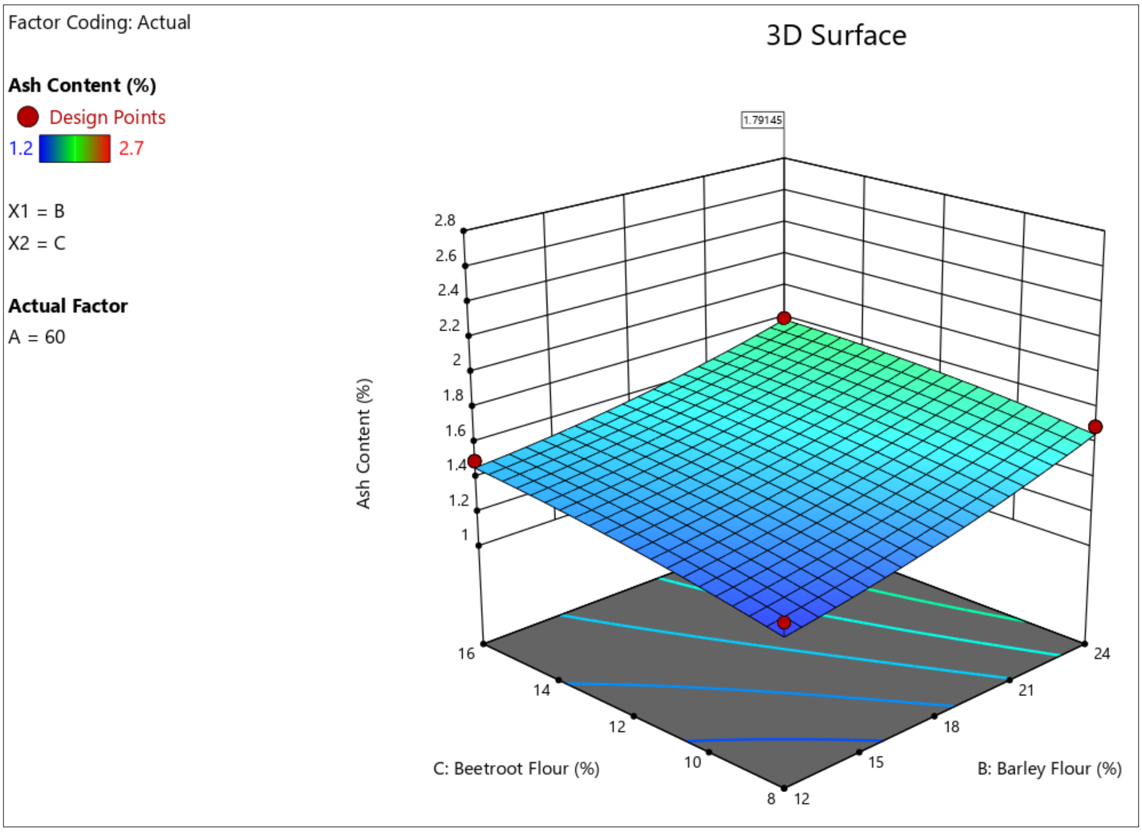

Fig 6: Effect of independent factors (B and C) on ash content (%)

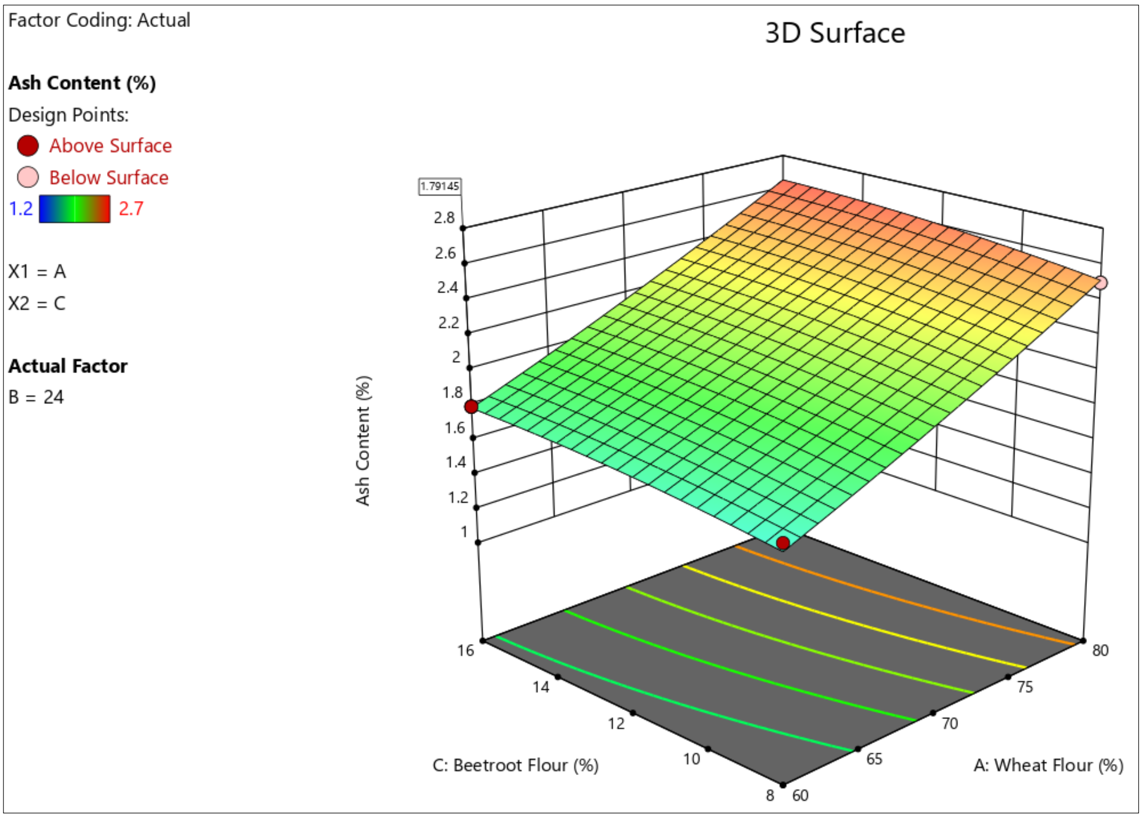

Fig 7: Effect of independent factors (A and C) on ash content (%)

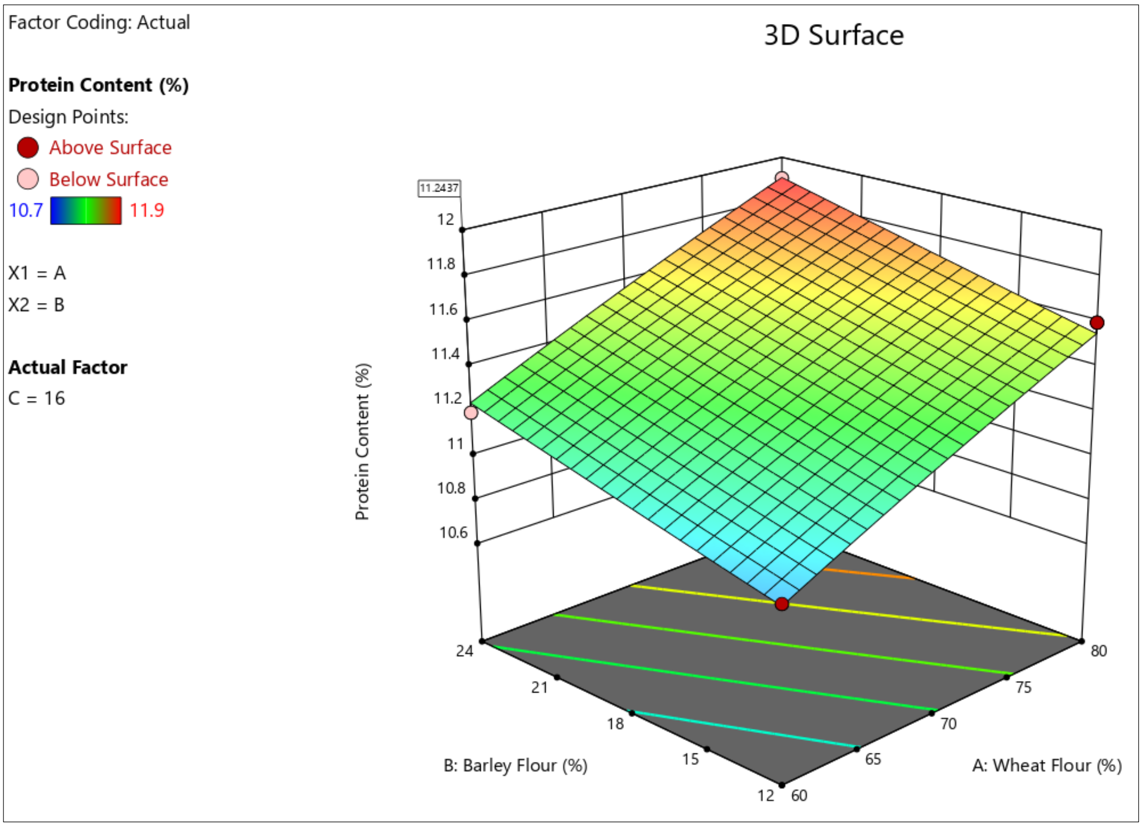

Fig 8: Effect of independent factors (A and B) on protein content (%)

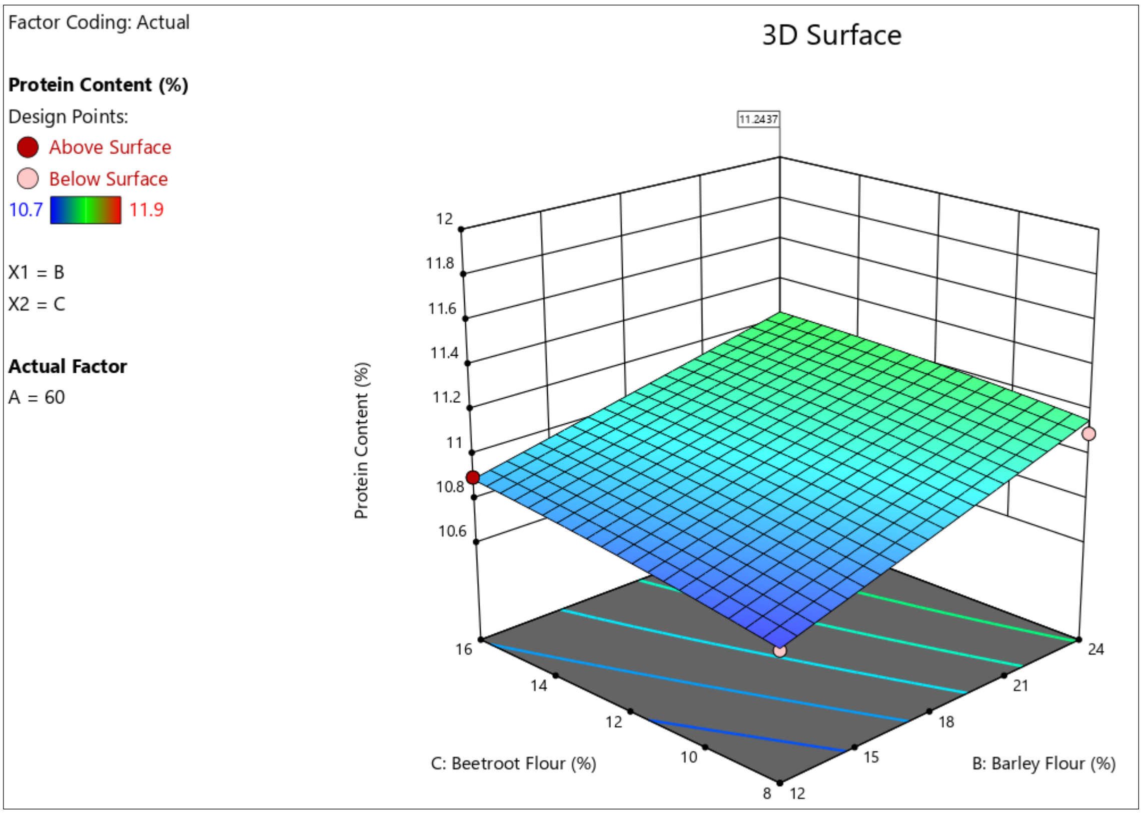

Fig 9: Effect of independent factors (B and C) on protein content (%)

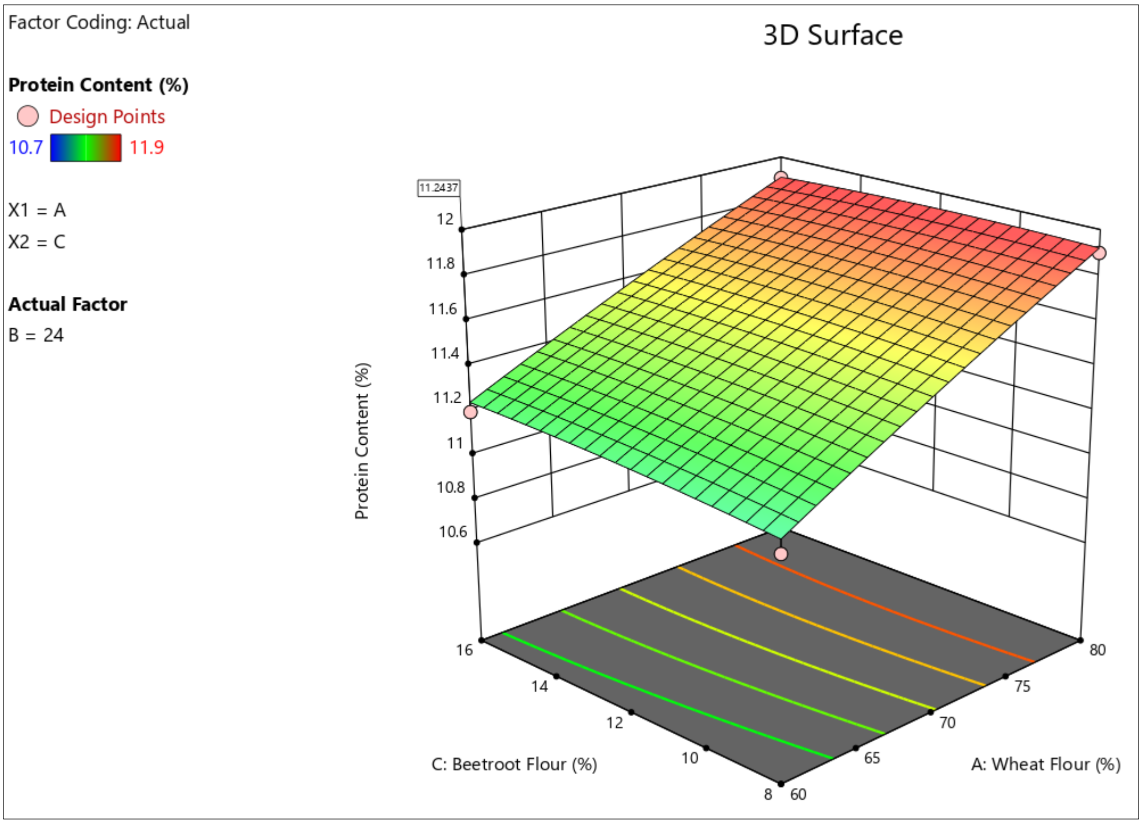

Fig 10: Effect of independent factors (A and C) on protein content (%)

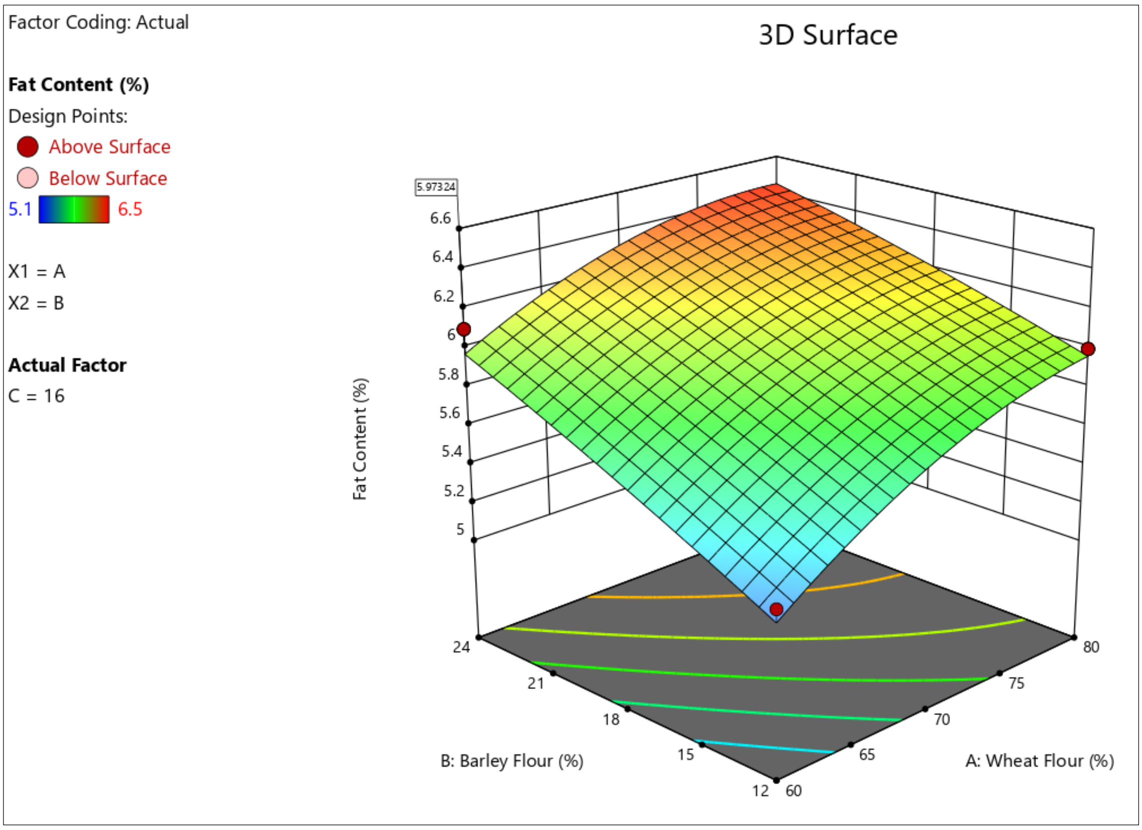

Fig 11: Effect of independent factors (A and B) on fat content (%)

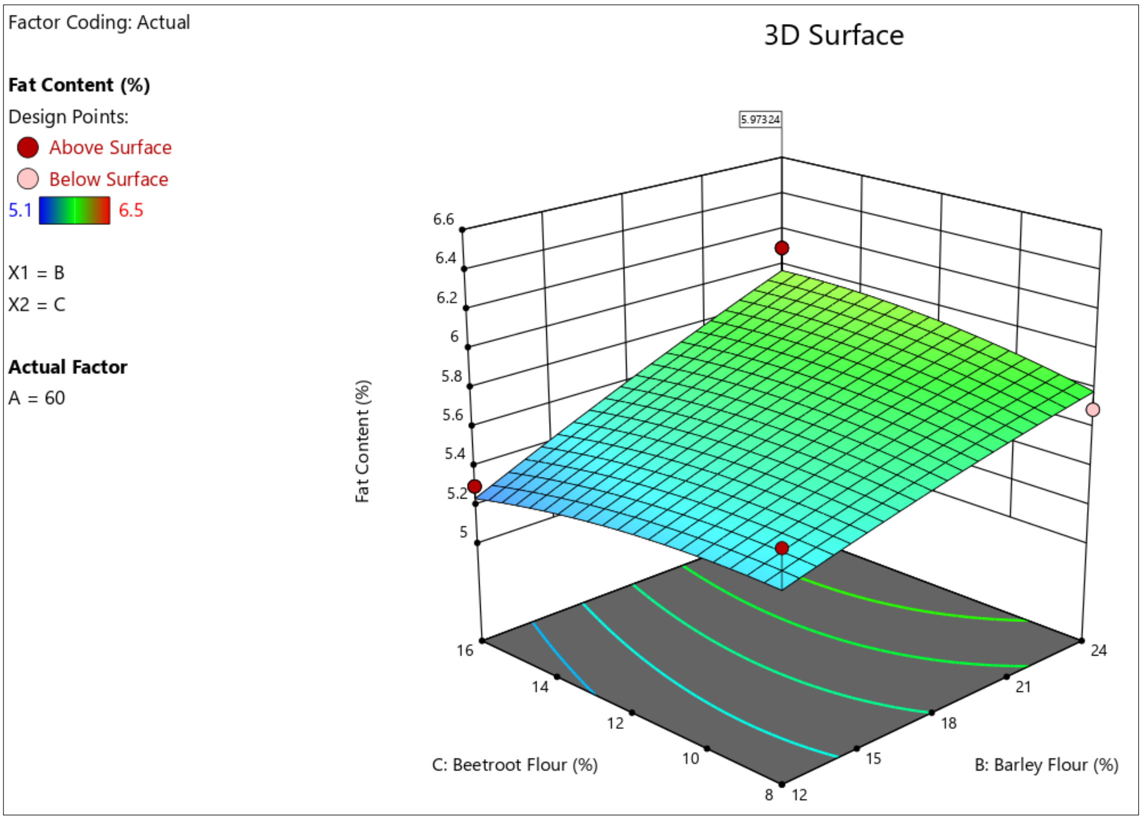

Fig 12: Effect of independent factors (B and C) on fat content (%)

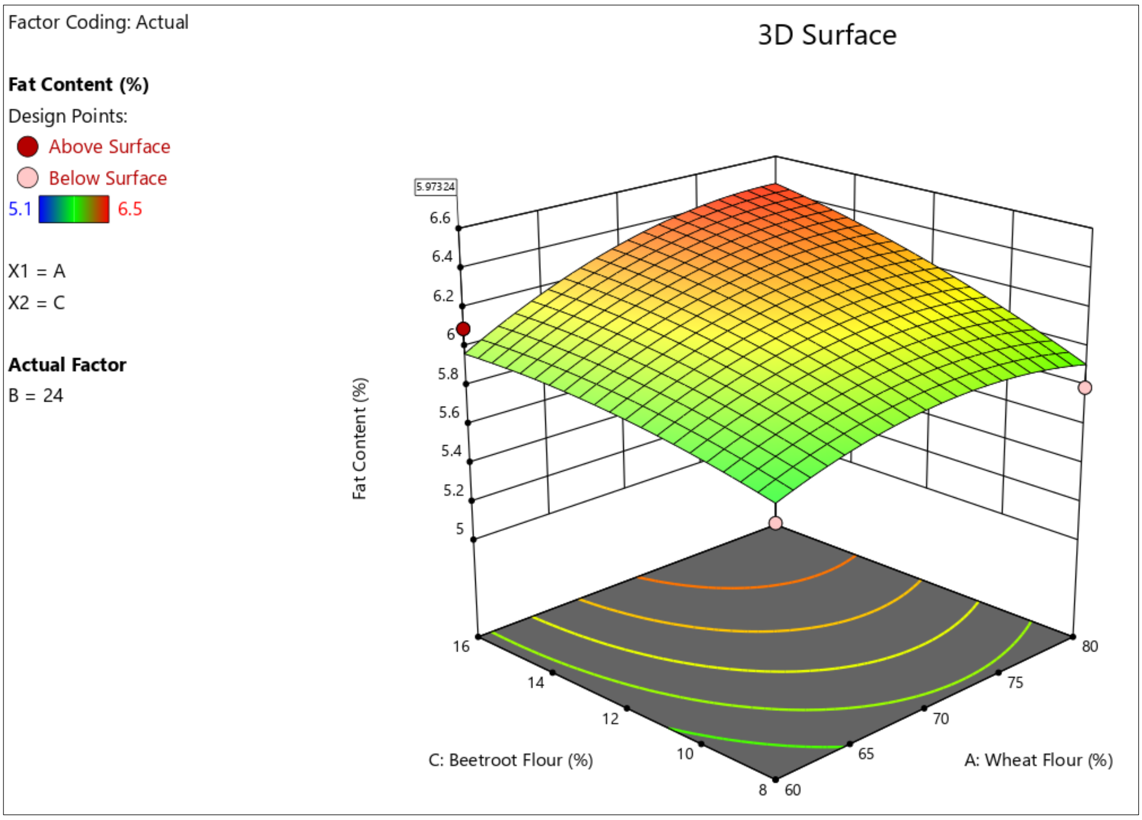

Fig 13: Effect of independent factors (A and C) on fat content (%)

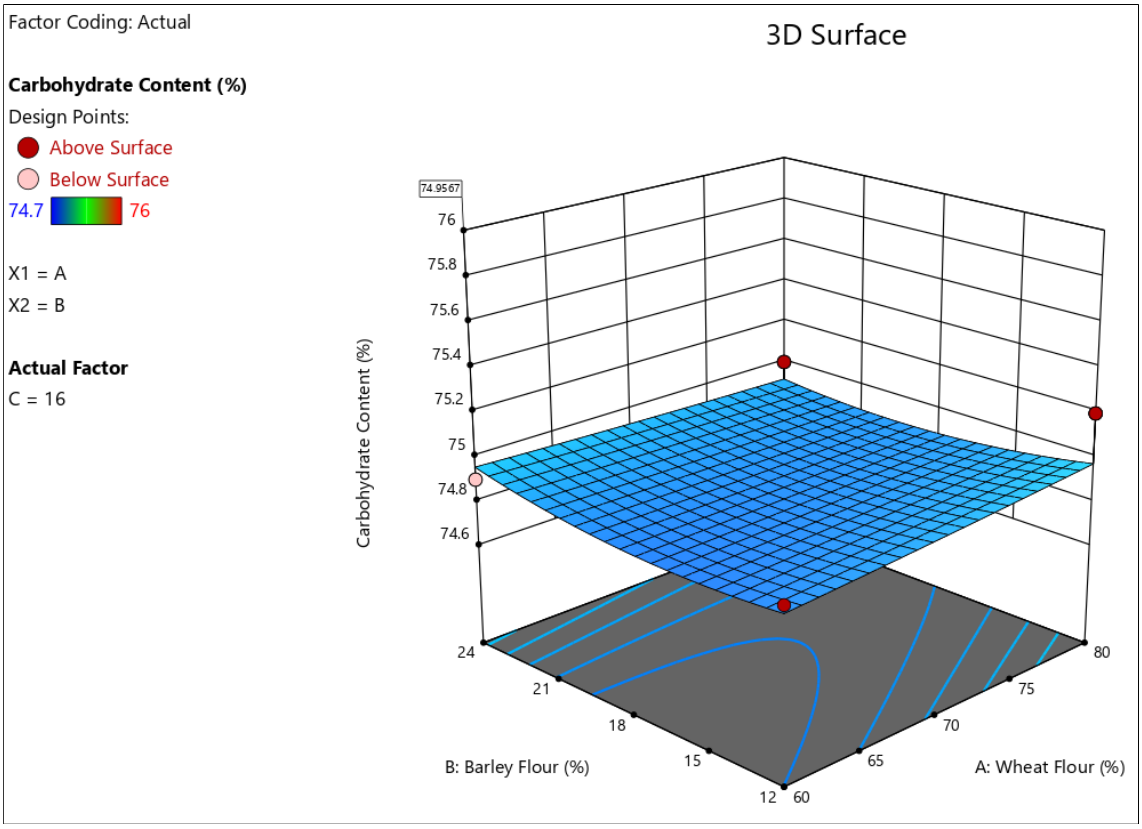

Fig 14: Effect of independent factors (A and B) on carbohydrate content (%)

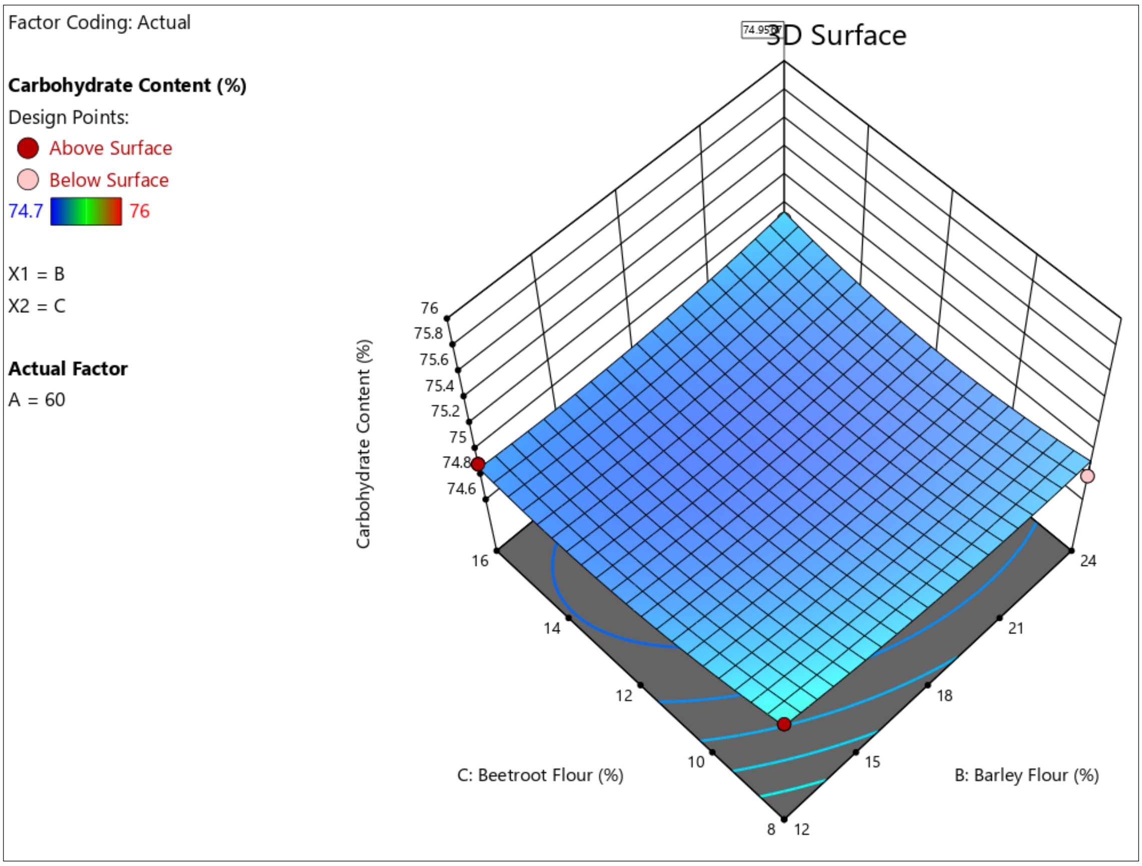

Fig 15: Effect of independent factors (B and C) on carbohydrate content (%)

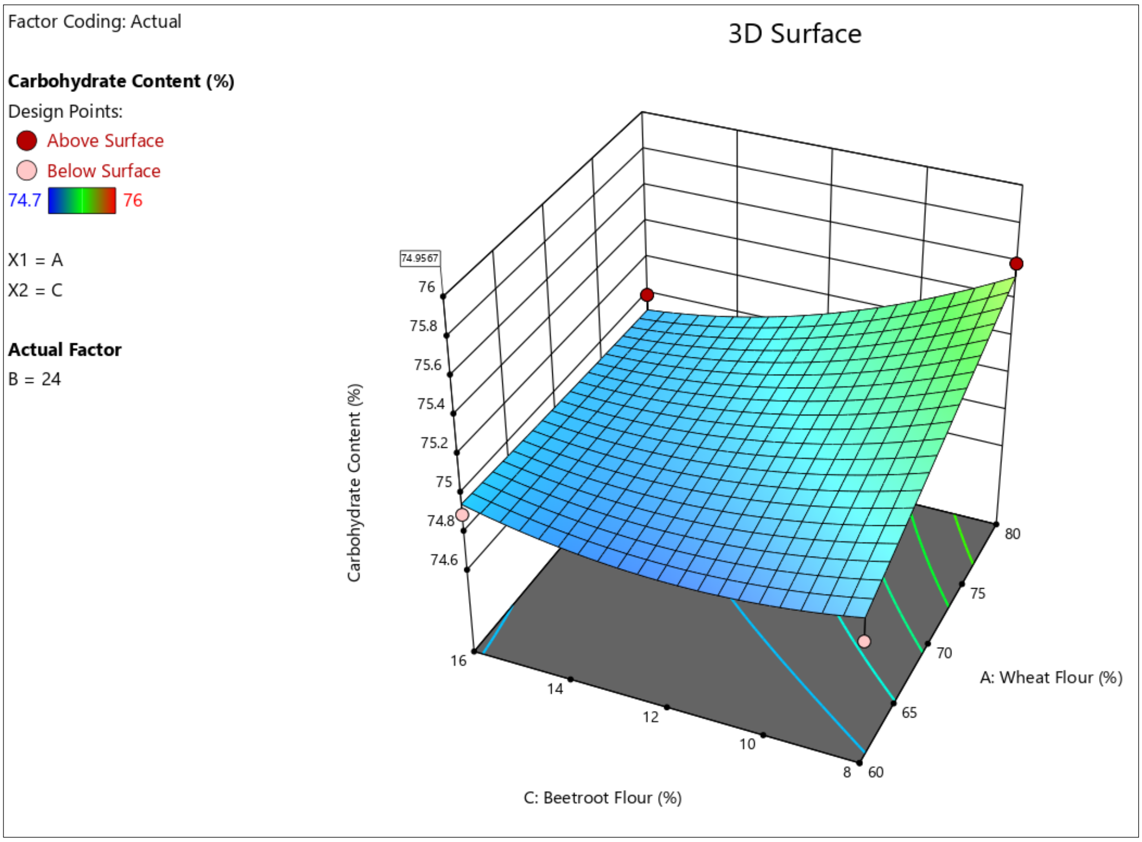

Fig 16: Effect of independent factors (B and C) on carbohydrate content (%)

4. Conclusion

It can be concluded that RSM can be pro-efficiently employed in optimizing the ingredients in formulation of Blended Flour. Goal of this research was to obtain an ingenious product which has enhanced nutritional composition. Optimum formulation consisted of 60% wheat flour, 24% barley flour and 16% beetroot flour. It possessed 8.72% moisture, 1.79% ash content, 5.97% fat content, 11.24% protein and 74.96% carbohydrate content.

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