

RESEARCH ARTICLE

Application of response surface methodology in preparation of spinach paneer

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Abstract: The objective of current study was to optimize the level of milk fat, rate of addition of whey protein concentrate -70 (WPC-70) and rate of addition of spinach paste to prepare spinach paneer using response surface methodology. Milk fat content, rate of addition of WPC-70 and spinach paste were used as variables while sensory attributes of paneer viz., flavour, body & texture, colour & appearance and textural attributes of paneer viz., hardness and chewiness as well as compositional parameters such as moisture content and fat content on dry matter basis are used as responses. On the basis of the results, RSM suggested the milk fat content, rate of addition of WPC-70 and spinach paste to be 3.08%, 0.96% and 10.11% respectively with desirability of 0.92. The experimental spinach paneer was prepared as per the suggestions from RSM and compared with control paneer prepared from standardized milk containing 4.5% milk fat and 8.5% MSNF. Moisture, fat and carbohydrate content of spinach paneer were significantly different from control paneer while protein and ash content were statistically similar. Textural and sensory characteristics were statistically similar. Hence, RSM can be a useful tool for optimization of spinach paneer from spinach paste.

Keywords: Spinach paneer; Whey protein concentrate-70 (WPC); response surface methodology; sensory parameters; textural characteristics

Introduction

Paneer, a popular heat and acid coagulated traditional Indian dairy product, is an unripened variety of soft cheese. Paneer is a rich source of animal fat and protein to the vegetarian population. It is also considered as an appreciable source of essential amino acids. The biological value (BV) of protein in paneer is in the range of 80 to 86. Ideally, paneer should have a marble white appearance with a firm, cohesive and spongy body and a close-knit texture. It should have a clean, pleasing, boiled milk flavour (Chaudhari et al. 2023).

Spinach (*Spinacia oleracea*) is a plant that is grown all over the world as a cool-season annual green leafy crop. It serves as an effective ingredient in a new product with excellent nutritional and biological benefits because it is a good source of protein, fibre and minerals. Major micronutrients including iron, manganese, zinc and magnesium are abundant in spinach, which also contains trace amounts of vitamin E, A, C, K, folate, thiamine (B1), pyridoxine (B6), and riboflavin (B2). Incorporation of processed spinach in food products enhances nutritive value and sensory profile of food products. Different forms of spinach have been added in yoghurt (Havaty et al. 2014), cheese (El-Sayed, 2020) and paneer (Pallavi et al. 2020) as well as extruded snack like product (Mangaraj et al. 2018), bread (Waseem et al. 2021) and biscuits (Galla et al. 2017).

Response Surface Methodology (RSM) has been widely used in recent years for the development of new products as well as improvement in existing products. RSM delineates the effect of the independent variables on responses of importance and is regarded as an effective method to optimize the new product formulations. It is a robust tool for data analysis that focuses on an adequate approximation relationship between input and output variables and determines the best operating circumstances for a system (Dean et al. 2017).

With a changing lifestyle and increasing awareness towards health and nutrition, consumers are now moving towards low-fat diet to reduce the risk of obesity, coronary heart disease, atherosclerosis and hypertension (Dharaiya et al. 2021). High fat diet is also linked with psychiatric disorders (Jeong et al. 2019). Fat, being a costliest constituent in milk, increases the cost of

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final product and make the product unaffordable by low-income group people. However, reduction in fat content of paneer influences sensory and textural characteristics of the product. Incorporation of spinach will make up for the deterioration taken place in the quality of paneer by reduction of fat along with improvement in the nutritional quality of the final product. Hence, in current investigation, spinach has been incorporated in low-fat paneer.

Materials and Methods

Fresh, raw mixed (cow and buffalo) milk was procured from Livestock Research Station (LRS) of the University and calculated quantity of whole milk was subjected to cream separation in order to obtain skimmed milk. Citric acid (edible grade), supplied by Loba-Chemical Pvt. Ltd., Mumbai was used as a coagulant. Whey protein concentrate-70 (WPC), containing 77.8 per cent protein, supplied by Saisukrithkar supplements Pvt. Ltd., Bengaluru was used as fat replacer. Spinach leaves were procured from local market and were packed in 12 µ polyester + 50 µ LDPE/LLDPE laminated pouches.

The fat and total solids content in milk, moisture, fat, protein and ash content in paneer as well as pH and acidity of paneer was estimated by methods described by FSSAI (2016). Lactose content was calculated by difference of all constituents in paneer.

Preparation of paneer

Paneer was prepared as per the method suggested by Paul et al. (2019) with minor modifications. The detailed method is as follows:

Milk was standardized to 3.0 per cent fat and 8.5 per cent SNF and utilized for spinach incorporated paneer. Standardized milk was heated up to 85°C for 10 minutes followed by addition of spinach paste at the rate of 8.0 per cent of the quantity of milk and cooled down rapidly to the coagulation temperature (75°C). Citric acid (1 per cent solution) was heated to 75°C and was added to the milk till stable coagulation was achieved. The coagulum formed was left as it is in the whey for 2 min to ensure the proper acidification. The whey was filtered and separated from the coagulum with the help of clean sterilized muslin cloth. The coagulum, collected within the muslin cloth, was immediately filled into paneer hoops and pressed for 30 minutes under pneumatic paneer pressed at a pressure of 2.5 kg/cm². After dehooping the paneer block was immersed into pasteurized chilled water (4 °C) for about 30 min to obtain desired firmness. Spinach paneer blocks were packed in metallized laminated pouch (12 µ high optical density metalized polyester + 50 µ LD/LLDPE) followed by refrigerated storage at 7±1°C.

Texture Profile Analysis: Compression testing of paneer samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962) using 5 KN probe which moved at a speed of 20 mm/min. The paneer samples were taken for texture measurement after tempering at 23±1°C for 1 h. All the textural measurements were

conducted in a room maintained at 23±1°C temperature and 65±1 per cent RH. Cubic samples of the experimental paneer, with edges of 20 mm, were placed in the compression support plate in uniform direction. The cubic samples were compressed up to 70% of their initial size. Five paneer samples were used for each experimental paneer under study and the average value of these readings was reported.

Sensory evaluation of paneer: Each block of paneer was cut into approximately 25 g rectangular pieces. The paneer samples were tempered to 15±2°C before judging. Sensory analysis of paneer samples was performed in isolated booths illuminated with incandescent light maintained at 23±2°C. The sensory panel (n=10) was composed of faculty members of the institute who have basic idea of the product. The paneer samples were evaluated using 100- point scale as described in Indian Standards (IS: 15346, 2003).

Statistical analysis: A Central Composite Rotatable Design (CCRD) of the Response Surface Methodology (RSM) technique was adopted for the optimization of milk fat, rate of addition of WPC-70 and spinach paste. The minimum and maximum levels of milk fat, WPC-70 and spinach paste were selected as 1.5 and 4.5 per cent, 0.5 and 1.0 per cent as well as 5 and 15 per cent respectively, on the basis of preliminary trials. The CCRD of three factors contained 20 combinations, including lower and upper limits, along with their responses for sensory attributes, textural characteristics and compositional parameters are displayed in Table 1. The data generated for different responses were analyzed using Design Expert® software (13.0.2 version) (Stat-Ease, Inc., 2021 E. Hennepin Avenue, Minneapolis, USA). A general polynomial equation given below was fitted for each response.

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_{12} + a_{22}x_{22} + a_{33}x_{32} + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + \text{Error term}$$

where Y represents the predicted response; a_0 the constant coefficient; a_{11} , a_{22} and a_{33} denote quadratic coefficients; a_{12} , a_{23} and a_{13} denote interaction coefficients; x_1 , x_2 and x_3 denote the level of milk fat, WPC-70 and spinach paste respectively.

Adequacy of the model was evaluated using coefficient of determination (R^2) and statistical significance was examined by F value. The effect of independent variables and individual responses was described at $P < 0.05$. t-test for two samples assuming equal variance was applied using Microsoft Excel for comparison of predicted values with the actual values of the responses. The variation between control sample prepared using standardized milk (4.5% fat and 8.5% SNF) and spinach paneer prepared from toned milk (3.0% fat and 8.5% SNF) was analyzed using independent t-test.

Results and Discussion

The optimization of the level of milk fat, rate of addition of WPC-70 and spinach paste was carried out on the basis of sensory attributes, textural characteristics and compositional parameters of spinach paneer. The paneer samples were prepared as per the suggestions of RSM and the responses were recorded (Table 1). The successive regression analysis of the responses produced the quadratic models for each response. The variation in the experimental data of fitted quadratic model was given by coefficient of determination (R^2) which ranged between 85 and 92 per cent (Table 2). The model F-value of the fitted quadratic model for all responses was found to be significant. The sufficient accuracy for predicting all response variables of spinach paneer prepared from any combinations of variables within the range was evaluated by non-significant lack of fit. These indicate that the obtained quadratic model fitted the data strongly. The signal to noise ratio called *Adequate precision value (APV)* for a well fitted model should be more than four. This measure also fulfilled for the obtained mode with APVs ranging between 7.83 and 9.89.

All these results firmly recommended that the model could be used to develop spinach paneer from spinach paste.

Influence of variables on colour and appearance of paneer: Colour and appearance is the first sensory parameter which is observed during sensory evaluation and the impression lasts through out during sensory evaluation. The colour and appearance score of the product ranged between 7.12 and 9.14. The minimum colour and appearance score was given to the product prepared with milk containing 3.0 per cent milk fat, 0.75 per cent WPC and 18.4 per cent spinach paste while the maximum score was obtained by paneer sample prepared with milk containing 3.0 per cent milk fat, 0.75 per cent WPC and 10.0 per cent spinach paste. Spinach paste significantly ($P < 0.05$) improved the colour and appearance of the product at linear level while it deteriorated colour and appearance at quadratic level. Milk fat content and rate of addition of WPC failed to exert impact on colour and appearance of the spinach paneer. The intense dark colour of spinach paneer, when spinach was added at higher rate, was disliked by the judges. Chaudhari et al. (2022) reported similar results for paneer incorporated with spinach powder.

Table 1: Design matrix showing factors and their responses for the development of spinach paneer

Milk fat content (%)	B: Rate of addition of WPC-70 (%)	C: Rate of addition of spinach paste (%)	Response 1: Flavour	Response 2: B&T	Response 3: C&A	Response 4: Hardness, N	Response 5: Chewiness, Nmm	Response 6: Moisture, %	Response 7: Fat on Dry Matter basis, %
5.0	1.00	15.00	35.57	30.24	8.05	18.89	43.45	61.38	15.23
5.00	0.75	01.59	38.14	30.41	7.39	17.89	43.21	58.13	20.08
5.50	1.00	15.00	35.24	31.05	8.14	18.85	44.23	59.53	19.81
6.00	0.75	10.00	41.56	32.11	9.06	17.56	43.48	58.65	18.59
6.50	0.50	05.00	36.25	27.54	7.19	18.53	43.18	56.27	17.51
7.00	1.00	05.00	37.41	32.16	7.51	18.41	41.24	60.19	22.56
7.50	0.50	15.00	36.85	28.61	8.31	16.48	44.12	56.98	21.89
8.00	0.75	10.00	41.26	32.14	9.14	17.85	43.18	57.92	18.60
8.50	0.50	15.00	36.87	28.68	8.09	18.78	43.13	57.48	16.28
9.47	0.75	10.00	34.52	30.26	9.04	19.02	43.85	56.02	3.76
10.00	0.75	10.00	40.63	32.45	8.19	17.45	42.38	58.81	18.42
10.00	1.17	10.00	40.71	31.54	8.22	17.46	40.26	61.64	17.62
10.00	0.75	10.00	41.05	31.24	8.17	17.69	42.26	58.55	18.58
10.00	0.75	10.00	41.81	32.35	8.25	17.85	43.18	58.71	18.64
10.00	0.75	18.40	37.45	29.31	7.12	18.10	43.34	59.91	13.36
10.00	0.75	10.00	41.64	32.01	7.98	17.56	43.48	58.86	18.71
10.50	0.50	05.00	41.23	28.14	7.45	17.86	43.21	56.99	23.61
10.52	0.75	10.00	38.21	31.62	8.81	18.53	42.86	56.45	21.96
10.00	0.32	10.00	40.29	32.19	8.71	17.56	41.12	55.41	20.32
10.50	1.00	05.00	36.54	32.27	7.23	18.42	41.28	59.98	16.64

Influence of variables on flavour of the paneer: Flavour is the most important sensory characteristics for paneer. The flavour score of spinach paneer varied from 34.52 to 41.81. The minimum flavour score was obtained when paneer was prepared from the milk containing 0.47 per cent fat, 0.75 per cent WPC and 10.0 per cent spinach paste while the maximum score was obtained when the paneer was prepared from milk containing 3.0 per cent fat, 0.75 per cent WPC and 10.0 per cent spinach paste. Milk fat and spinach paste significantly ($P<0.05$) improved flavour of the paneer at linear level. The interaction of milk fat and spinach paste also had significantly ($P<0.05$) positive impact on flavour of the experimental paneer. At quadratic level, milk fat significantly ($P<0.05$) improved flavour while WPC and spinach paste significantly ($P<0.05$) deteriorated the flavour. A typical rich and pleasant flavour provided by milk fat was appreciated by judges. A slight salty taste provided by spinach paste was liked by judges at lower level but was disliked at higher level. Addition of WPC resulted in more water binding and ultimately higher moisture in paneer which yielded flat flavour which was also disliked by judges. Al-Bedrani et al. (2021) reported a progressive increase in flavour scores of low-fat soft cheese with increase in level of WPC up to 2.0 per cent level. Paul et al. (2018) also observed that flavour score of herbal paneer samples (4.5% mint extract and 1.5 to 3.0% milk fat, 8.5 % SNF) increased with increase in fat per cent in milk.

Influence of variables on body and texture of paneer: Body and texture is an important sensory criterion for paneer. The body and texture score of spinach paneer ranged between 27.54 and 32.45. The minimum body and texture score was obtained when milk contained 1.5 per cent fat, WPC was added at the rate of 0.5 per cent and spinach paste was added at the rate of 5.0 per cent while the maximum score was obtained when milk contained 3.0

per cent fat, WPC was added at the rate of 0.75 per cent and spinach paste was added at the rate of 10.0 per cent. Addition of WPC significantly ($P<0.05$) improved body and texture characteristics at linear level while at quadratic level milk fat significantly ($P<0.05$) improved it but addition of WPC deteriorated it at quadratic level. Milk fat yielded soft velvety texture in paneer which was appreciated by the judges. WPC reduced chewiness when added at lower level but resulted in loose and weak body at quadratic level which was rejected by the experts. Pinto et al. (2014) also reported an improvement in body & texture of low-fat paneer with increase in fat content in milk.

Influence of variables on hardness of paneer: Hardness, the force necessary to attain a given deformation, is one of the important factors in determining paneer texture as well as acceptability of the paneer. The hardness of paneer varied between 16.48 and 19.02 N. The paneer sample prepared from milk containing 4.5 per cent milk fat as well as WPC and spinach paste were added at the rate of 0.5 per cent and 15.0 per cent respectively displayed minimum hardness while the maximum hardness was observed when milk fat content was 0.47 per cent as well as rate of addition of WPC and spinach paste were 0.75 per cent and 10.0 per cent respectively. Increasing milk fat content significantly ($P<0.05$) increased hardness at linear as well as quadratic level which could be attributed to higher melting point of milk fat. The interaction of milk fat and spinach paste significantly ($P<0.05$) reduced hardness of the experimental paneer while WPC-70 and spinach paste could not influence hardness of the paneer. Singh et al. (2015) also observed as increase in hardness with increase in fat content of paneer.

Table 2: Regression coefficients and ANOVA fitted quadratic model for the responses of spinach paneer

Partial Coefficients	Flavour	Body & texture	Colour & appearance	Hardness, N	Chewiness, Nmm	Moisture, %	FDM, %
Intercept	41.03	31.86	8.35	17.66	42.81	58.95	18.57
A-Milk fat	0.93*	0.26	0.37	0.82*	-0.98*	-0.29	1.81*
B-WPC-70	-0.43	0.91*	0.12	0.51	0.29	1.43*	-0.62*
C-Spinach paste	0.70*	-0.26	0.59*	-0.13	-0.25	0.18	-0.52*
AB	0.40	1.29	0.79	0.11	-0.03	0.42	0.56
AC	0.86*	1.78	1.07	-0.65*	0.22	0.12	0.61
BC	0.13	-0.50	0.74	0.06	0.33	0.51	0.16
A ²	1.53*	13.2*	0.49	1.44*	-1.56*	-0.46	2.52*
B ²	-1.22*	-1.52*	0.36	-0.26	0.89*	1.98*	-1.12*
C ²	-1.15*	0.57	-1.29*	-0.24	0.30	0.50	-1.18*
Model Fit Statistic							
Lack of fit	0.062	0.058	0.069	0.060	0.055	0.064	0.065
Model F value	15.41	12.96	14.46	17.81	15.56	14.84	15.61
R ²	0.89	0.86	0.91	0.90	0.85	0.92	0.91
APV	9.63	8.82	9.06	7.83	8.91	9.89	9.51

Influence of variables on chewiness of paneer: Chewiness is the energy required to chew a solid food product to a state where it is ready for swallowing (Berta et al. 2016). The chewiness of paneer ranged from 40.26 Nmm to 44.23 Nmm. The paneer samples with 3.0% milk fat, 1.17% WPC-70 and 10.0% spinach paste had minimum chewiness while the one with 4.5% milk fat, 1.0% WPC-70 and 15.0% spinach paste had maximum chewiness (Table 1). Milk fat significantly ($P<0.05$) reduced chewiness at linear and quadratic level. WPC-70 failed to exert impact on chewiness at linear level while significantly ($P<0.05$) increased it at quadratic level. Spinach paste had no significant influence on chewiness. Suthar et al. (2020) also reported reduction in chewiness of paneer with increase in fat content of milk.

Influence of variables on moisture content of paneer: Moisture content of low-fat paneer should not be more than 60% (w/w) (FSSAI, 2006). Moisture content of the experimental paneer varied between 55.41 per cent to 64.64 per cent. The paneer sample with 3.0 per cent fat in milk, 0.32 per cent addition of WPC-70 and 10.0 per cent addition of spinach paste contained minimum moisture while the sample with 3.0 per cent milk fat, 1.17 per cent WPC-70 and 10.0 per cent spinach paste showed maximum moisture content. WPC-70 significantly ($P<0.05$) increased moisture content of paneer which could be attributed to higher water holding capacity of whey protein. Milk fat, spinach paste as well as the interaction between all the variables failed impact moisture content of final product. Pinto et al. (2014) reported similar results for low-fat paneer.

Influence of variables on FDM content of paneer: Fat content on dry matter basis (FDM) should not be more than 20% (w/w) for low-fat paneer (FSSAI, 2006). The FDM content of paneer varied from 3.76 per cent to 23.61 per cent. The paneer sample prepared from milk containing 0.47 milk fat and added with 0.75 per cent WPC-70 and 10.0 per cent spinach paste had minimum FDM content while the one prepared from milk with 4.5 per cent fat and added with 0.5 per cent WPC-70 and 5.0 per cent spinach paste showed maximum fat content on dry matter basis. Fat content of milk significantly ($P<0.05$) increased FDM content of paneer at linear as well as quadratic level while rate of addition of WPC-70 and spinach paste significantly ($P<0.05$) reduced FDM content of paneer. Addition of WPC-70 significantly ($P<0.05$) increased moisture content of paneer resulting in reduction of fat content while addition of spinach paste increased yield of paneer without contributing fat and ultimately resulted in reduction of FDM content. Pinto et al. (2014) also observed an increase in FDM content of paneer with increase in fat content of milk. Addition of WPC resulted in reduction of fat content in paneer (Gawande et al. 2023).

Optimization of variables for preparation of spinach paneer

The optimization of different variables such as milk fat content, rate of addition of WPC-70 and rate of addition of spinach paste was carried out using numerical optimization technique. The criteria used for optimization are summarized in Table 3. Among the variables, spinach paste was maximized while milk fat and WPC-70 were kept in range. Among the responses, sensory

Table 3: Goal set for constraints to optimize spinach paneer

Constraint	Goal	Lower limit	Upper limit
Milk fat, %	In range	1.50	4.50
WPC-70, %	In range	0.50	1.50
Spinach paste, %	Maximize	5.00	15.00
Flavour	Maximize	34.52	41.81
Body & texture	Maximize	27.54	32.45
Colour & appearance	Maximize	7.12	9.14
Hardness, N	Range – 17 to 18	16.48	19.02
Chewiness, Nmm	Range – 42 to 44	40.26	44.23
Moisture, %	Range – 55 to 59	55.41	61.64
FDM, %	Range – 17 to 19	3.76	23.61

Table 4: Comparison for predicted values and observed values for spinach paneer

Attributes	Predicted value	Observed value	t-value
Flavour	41.04	41.10	NS
Body & texture	31.96	31.91	NS
Colour & appearance	8.53	8.57	NS
Hardness, N	17.64	17.57	NS
Chewiness, Nmm	42.76	42.70	NS
Moisture, %	58.75	58.78	NS
FDM, %	18.86	18.89	NS

Table 5: Comparison of spinach paneer with control paneer

Parameter	Control paneer	Spinach paneer	t-value
Chemical composition			
Moisture, %	53.26±0.71	58.78±0.36	21.98*
Fat, %	25.61±0.65	18.89±0.23	17.68*
FDM, %	58.55±0.79	32.13±0.27	20.66*
Protein, %	17.09±0.57	17.52±0.56	NS
Carbohydrates, %	2.32±0.17	4.90±0.25	8.98*
Ash, %	1.72±0.11	1.87±0.18	NS
Rheological characteristics			
Hardness, N	17.72±0.91	17.57±0.62	NS
Cohesiveness	0.39±0.05	0.37±0.09	NS
Springiness, mm	9.38±0.18	9.18±0.15	NS
Gumminess, N	6.91±0.15	6.50±0.12	NS
Chewiness, Nmm	64.81±1.27	59.67±1.07	NS
Adhesiveness, N	0.84±0.06	0.87±0.05	NS
Sensory characteristics			
Flavour	41.16±2.12	41.10±1.62	NS
Body & texture	31.51±1.71	31.91±1.53	NS
Colour & appearance	8.65±0.69	8.57±0.61	NS
Total score*	86.32±2.72	86.58±2.21	NS

Full score for Packaging (5) is added in Total score

parameters such as flavour, body & texture and colour & appearance were maximized while textural characteristics viz. hardness and chewiness as well as compositional parameters viz. moisture as FDM content were kept in range. The range for textural parameters was decided on the basis of textural characteristics of control paneer while the range for compositional parameters was decided as per the legal requirements. RSM suggested milk fat content, rate of addition of WPC-70 and spinach paste to be 3.08 per cent, 0.96 per cent and 10.11 per cent respectively with desirability of 0.92. Spinach paneer was prepared keeping milk fat content, WPC-70 and spinach paste as suggested by RSM. The predicted values for flavour, body & texture, colour & appearance, hardness, chewiness, moisture content and FDM content were 41.04, 31.96, 8.53, 17.64 N, 42.76 Nmm, 58.75 per cent and 18.86 per cent respectively. It is evident from table 4 that the observed values were not significantly ($P>0.05$) different from predicted values with respect to all attributes. Therefore, it was confirmed that the selected level of milk fat, rate of addition of WPC-70 and spinach paste is most suitable for the preparation of spinach paneer with optimum sensory, textural and compositional parameters.

Analysis of spinach paneer

Spinach paneer was analyzed and compared with control paneer for its compositional, textural and sensory attributes and analyzed statistically using t-test. Moisture and carbohydrate content of experimental paneer was significantly ($P<0.05$) higher due to addition of WPC-70 and spinach paste while fat and FDM content were significantly ($P<0.05$) lower than control. Protein and ash content of control and experimental paneer were statistically

similar. Similarly, no significant difference was observed between textural and sensory characteristics of control and experimental paneer (Table 5).

Conclusion

Spinach-based low-fat paneer was prepared using response surface methodology and the milk fat content as well as rate of addition of WPC-70 and spinach paste were optimized to obtain sensorially and legally acceptable product with similar textural characteristics to those of control. At linear level, increase in milk fat content improved flavour of the paneer, increase hardness and FDM content while reduced chewiness and moisture content. Increasing the rate of addition of WPC-70 improved body & texture, increased moisture and reduced FDM content. Increasing spinach paste improved flavour and colour & appearance and reduced FDM content of experimental paneer. At quadratic level, milk fat improved flavour and body & texture, increased hardness and FDM content and reduced chewiness while WPC-70 had negative impact on flavour and body & texture, increased chewiness and moisture content and reduced FDM content. Spinach paste also deteriorated flavour and colour & appearance and reduced FDM content. On the basis of the outcomes, RSM suggested to prepare low-fat spinach paneer with milk containing 3.08 per cent fat, 0.96 per cent WPC-70 and 10.11 per cent spinach paste. The final product was highly acceptable. Hence, an acceptable quality low-fat spinach paneer can be developed by using response surface methodology.

References

- Al-Bedrani DIJ, Hasan ST, Altaee AA, Alqotbi AA (2021) Improving Low-Fat Soft Cheese Quality Properties Made from Reconstituted Skim Milk by Using Whey Protein Concentrate as A fat Replacer. [In IOP Conference Series]: Earth and Environmental Science, 910 (1), 012040.
- Chaudhari BB, NS Kamble, Bhendegave RS, Bansode SS (2022) Studies on acceptability and microbial analysis of paneer incorporated with spinach powder. *The Pharma Innov J*, 11:144-148
- Chaudhari MP, Pinto SV, Dharaiya CN, Patel SM (2023) Application of response surface methodology in preparation of low-fat paneer from recombined milk. *Indian J Dairy Sci*, 76:231-237
- Dean A, Voss D, Draguljić D (2017) Response Surface Methodology. In: Design and Analysis of Experiments. Springer Texts in Statistics. Springer, Cham.
- Dharaiya CN, Jana A, Patel AM, Patel DH (2021) Comparison of natural Mozzarella cheese with acid casein-based Mozzarella cheese analogue. *Indian J Dairy Sci*, 74:301-308
- El-Sayed SM (2020) Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. *Heliyon*, 6:1-6
- FSSAI (2016) Notification issued by Ministry of Health and Family Welfare (Food safety and standards authority of India), New Delhi, dated the December 23, 2016.
- Galla NR, Pamidighantam PR, Karakala B, Gurusiddaiah MR, Akula S (2017) Nutritional, textural and sensory quality of biscuits supplemented with spinach (*Spinacia oleracea* L.). *Int J Gastron Food Sci*, 7:20-26
- Gawande H, Arora S, Mittan R, Lule V, Sharma V, Singh AK (2023) Effect of milk protein standardization using high milk protein ingredients on texture, composition and yield of paneer. *App Food Res*, 3: 1-6
- Hayaty NJ, Mohamadi SA, Hojjatoleslami M (2014) Sensory acceptability and quality of flavored yogurt enriched with *Spinacia oleracea* extract. *Nutr Food Sci*, 44:182-192
- Indian Standard: 15346 (2003). Method for Sensory Evaluation of Paneer/ Chhana. [Bureau of Indian Standards, Manak Bhavan, New Delhi.
- Jeong MY, Jang HM, Kim DH (2019) High-fat diet causes psychiatric disorders in mice by increasing Proteobacteria population. *Neurosci Lett*, 698:51-57
- Mangaraj S, Swain S, Deshpande SS (2018) Development of extruded functional snack foods from plants and dairy ingredients employing response surface methodology. *J Dairy Vet Sci*, 7:1-19
- Pallavi R, Rashmi HS, Devaki CS (2020) Development, Evaluation and Storage Studies of Flavour Enriched Herbs-Based Paneer. *Archives Nutrition and Public Health* 2: 141-146
- Paul P, Pinto S, Dharaiya C, Chaudhary ML, Tashvantha R, Vasava N (2019) Assessing suitability of different forms of coconut for usage in manufacturing of paneer-like soft cheese. *Indian J Dairy Sci*, 72:616-625
- Paul V, Kushwaha P, Paul A (2018) Enrichment of low-fat paneer by incorporating herbal extracts (Basil, Ginger and Mint). *The Pharma Innov J*, 7:472-476
- Pinto SV, Bhatt JD, Prajapati JP (2014) Evaluation of selected emulsifiers and buttermilk in the manufacture of reduced-fat paneer. *Basic Res J Food Sci Technol*, 1: 1-14
- Singh G, Kumar A, Kumbhar BK, Dar BN (2015) Optimization of processing parameters and ingredients for development of low-fat fibre-supplemented paneer. *J Food Sci Technol*, 52: 709-719
- Suthar J, Jana AH, Modha HM, Smitha B (2020) Effect of fat content and homogenization of milk on the recovery of milk constituents and quality characteristics of paneer. *Int J Chem Stud*, 8:3166-3173
- Waseem M, Akhtar S, Manzoor MF, Mirani AA, Ali Z, Ismail T, Karrar E (2021) Nutritional characterization and food value addition properties of dehydrated spinach powder. *Food Sci Nutr*, 9:1213-1221