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Development of Pumpkin Powder added Functional Lassi by using Response Surface Methodology (RSM)

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Abstract

The present study was conducted to optimize the level of ingredients (pumpkin powder and sugar) during the production of functional lassi using Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM). The antioxidant activity of pumpkin powder was determined. The results obtained revealed that total polyphenol and antioxidant activity (DPPH and ABTS method) of pumpkin powder were found to be 2148.25± 123.89 Gallic acid Equivalent/g and antioxidant activity (123.23± 10.45 and 145± 12.94ug Trolox equivalent/g) respectively. The product was optimized using different levels of pumpkin powder (0.5 to 1%w/v) and sugar(10-20%w/v) based on the sensorial attributes of the lassi using numerical optimization technique of Design Expert 12 and the result revealed that the functional lassi prepared with pumpkin powder (0.5%w/v) and sugar (13.92%w/v) resulted in sensory scores viz. colour and appearance8.08; sweetness7.27; flavour7.37; consistency 7.23; and overall acceptability 7.25. The applied quadratic model produced the desirability of 0.85. The flavor and consistency of optimized lassi was further enhanced using incorporation of vanilla essence (0.05%v/v) and pectin stabilizer (0.03%w/v). All the examined quadratic models had significant influence on the sensory quality attributes of lassi indicating that the statistical model designed for these attributes fitted well in all the aspects with regression coefficient of R2>0.85. Storage study of optimized lassi revealed that the product was stable up to 05 days and then its quality started declining significantly(P<0.05).

Keywords: Pumpkin powder; Sugar; RSM; Lassi; Sensory evaluation

Introduction

Vegetables are rich in dietary fiber, minerals as well as many bioactive compounds, such as antioxidants, e.g., carotenoids, ascorbic acid, tocopherols, phenolic compounds [1]. Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and may help to prevent chronic diseases, especially cancer and cardiovascular diseases [2]. Pumpkin is high in fiber which effectively prevents cardiovascular risks factor. Apart from that, contains wide range of phytochemicals i.e., phenols, flavonoids, Carotene (alpha & beta) and glycosides. It is used to maintain integrity of skin and mucus membrane. Pumpkin contains Zea-xanthin, a natural antioxidant which has V rays filtering action in the maculated in retina of the eyes. Thus, it helps to protect from age related macular disease in elderly.

Milk-based beverages are proving to be ideal vehicles for newly discovered bioactive food ingredients [3]. Dairy beverages are widely formulated as functional foods and the preferred carriers for several bioactive ingredients including plant extracts [4]. Supplementation of dairy beverages like lassi and flavoured milk with pumpkin will provide additional health properties, especially concerning antioxidant properties. When added directly to milk, the vegetable bioactive may adversely affect the organoleptic and physico-chemical characteristics which limit their application in the preparation of various dairy products. Therefore, it is necessary to subject pumpkin powder to pre-treatment so that undesirable effects on sensory and other quality attributes of dairy products will be minimized.

Lassi is a fermented milk beverage regularly consumed in different parts of India, regularly as a part of diet. The consumption of these products is linked to numerous health benefits. Lactic fermentation improves the digestibility of milk constituents, and these products can be consumed by lactose intolerant people. Its consumption is linked to reduced risk of heart ailments and increases the number of lymphocytes in the blood that fight back the diseases causing bacteria which is very useful in maintaining good health.

Lassi is an excellent medium to generate an array of products that fit into the consumer demand for health-based foods. It can serve as carrier for pumpkin powder supplementation to develop functional beverage. Also, incorporation of pumpkin powder in lassi can enhance its appeal and palatability and thereby, can have a positive impact on the consumption pattern. It will also contribute towards enhanced cultivation of pumpkin thus benefitting the farmers involved.

To our current knowledge, there is no literature available on optimization pumpkin powder incorporated functional lassi using RSM. In the view of above discussion, the present study carried out for development of functional lassi with least impact on sensory qualities.

Materials and Methods

Materials

Raw pumpkin was purchased from the local market of Pusad, Maharashtra, India. All the analytical grade chemicals used in this study were procured from reputed chemical manufacturers.

Procurement and preparation of pumpkin powder

The process for preparation of the pumpkin powder is given in Figure 1. The pumpkin powder thus obtained was stored in amber-coloured bottles at 5 °C until use.

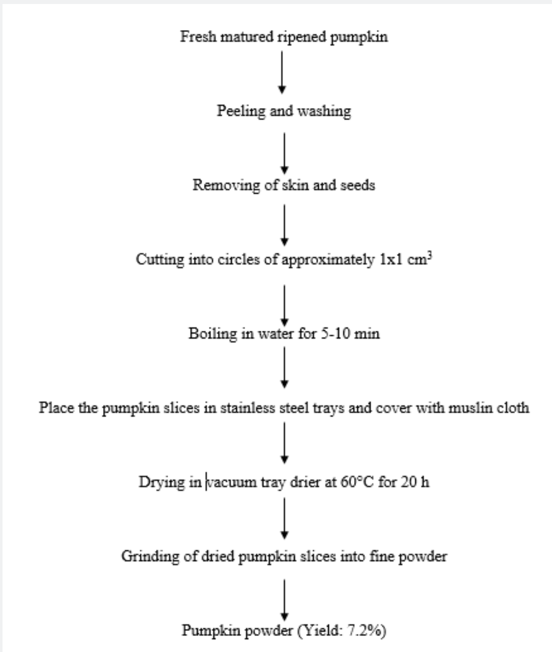

Figure 1: Preparation of pumpkin powder.

Physico-chemical properties of pumpkin powder

Polyphenol content: The polyphenol content of the pumpkin powder was evaluated using Folin-Ciocalteu reagent procedure described by Chaovanalikit and Wrolstad [5].

Antioxidant activity: The antioxidant capacity of the pumpkin powder was measured by using ABTS and DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Peng et. al. [6].

Preparation of Lassi: The process flow chart for the preparation of pumpkin powder incorporated lassi is described in Figure 2.

Selection and optimization of process variables (Experimental Design)

Central Composite Rotatable Design (CCRD) of the Response Surface Methodology (RSM) technique was adopted to optimize the functional formulations. Preliminary trials were conducted to select the range of independent variables viz. sugar and pumpkin powder. The responses viz. sensory evaluation was used to select the levels of independent variables for optimizing the functional lassi. A total of thirteen experiments were designed according to the experimental design of RSM. The data obtained from twenty experiments was analyzed using Design Expert® software version 12.00 (Stat-Ease, Inc., USA). A polynomial equation was fitted for response (Eq. 1).

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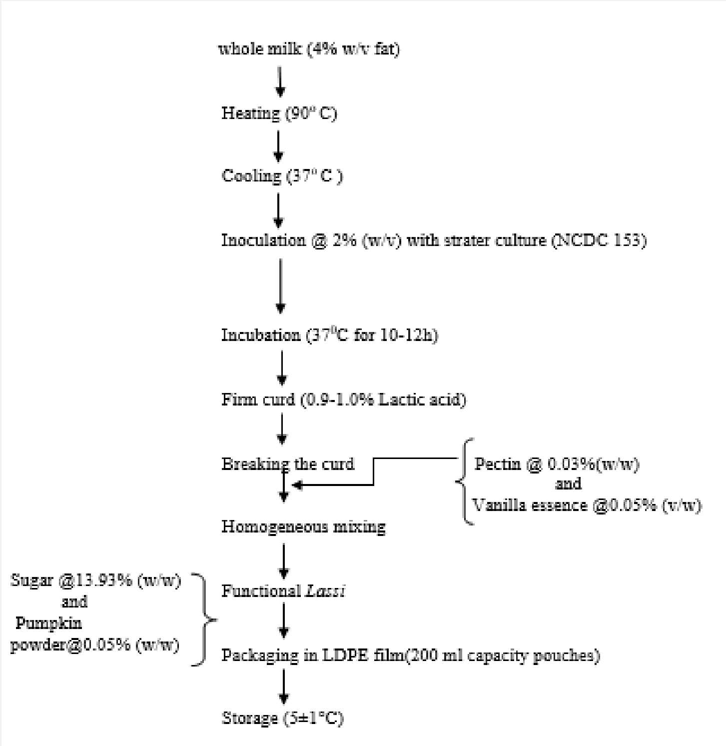

Figure 2: Flow diagram for preparation of pumpkin powder incorporated functional lassi.
Note: NCDC: National collection of dairy cultures, NDRI, Karnal, India

Where α_0 is constant, α_i, α_j and α_{ij} are linear, quadratic and interaction coefficients, respectively. X_i and X_j are independent variables and Y is the response. Model fit was determined using coefficient of determination (R^2) and F value was considered for statistical significance. The effect of independent variables on responses was taken at P<0.01 and P<0.05. T-test was used to statistically compare predicted values with the actual values. Afterwards, stabilizer and vanilla were added in optimized products for further enhancing the acceptability of functional lassi.

Sensory evaluation

The prepared functional lassi were evaluated by a panel of trained judges selected from the Institute for sensory characteristics. The panel of judges have adequate knowledge about dairy products. Their response for various attributes, namely flavour, colour and appearance, consistency, sweetness and overall acceptability were recorded using 9-point hedonic scale rating ranging from 'like extremely=9' to 'neither like nor dislike=5' to 'dislike extremely=1' and recorded their score on proforma for lassi.

pH

pH of drinks was determined by the method described in IS: SP-18 Part XI. A pH meter having combined glass electrode (pH Tutor, EUTECH Instruments, Malaysia) was used to measure the pH of lassi at 20°C. The pH meter was calibrated using standard buffers of pH 4.0 and 9.0 at 20°C before analyzing the samples.

Proximate analysis of the product

Total solids (TS) content was calculated by the gravimetric method as described in AOAC [7]. Fat content in lassi was measured by the Gerber method described in IS: SP [8]. Total protein content was estimated using Kjeldhal method [7]. The total ash content was determined as per the method described in AOAC [9].

Colour characteristics

Instrumental colour characteristics of functional lassi were measured by reflectance spectroscopy technique employing reflectance meter, Color flex (Hunter lab, Reston, Virginia, USA) along with the universal software (version 10). The instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data are received from the software in terms of L* [Lightness, ranges from 0 (black) to 100 (white)], a* [Redness, ranges from +60 (red) to -60 (green)] and b* [Yellowness, ranges from +60 (yellow) to -60 (blue)].

Storage study

The storage stability of functional lassi was conducted at refrigeration temperature (5±1ºC) along with control sample packaged in LDPE film pouch (200 ml) for a period of 5 days and

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the samples were analyzed atone day regular interval for sensory attributes and pH.

Results and Discussion

Polyphenol content and antioxidant activity

The total polyphenol content and the antioxidant activity (by ABTS and DPPH activity) of pumpkin powder were estimated to be 2148.25±123.89 ug Trolox/Gallic acid Equivalent /g and 123.23±10.45 ug Trolox/Gallic acid Equivalent /g and 145±12.94 ug Trolox/Gallic acid Equivalent /g, respectively (Table 1). The results were corroborated well with the study of Kulczyńskiet al. [10] who observed that methanol-water extracts of orange butternut cultivar of Cucurbita Moschata variety had highest antioxidant potential among the all variety of pumpkin (DPPH method: 122.57±0.89 148.83 mg and ABTS Method: 122.57±0.89 Trolox/100 g dm) with corresponding polyphenol content of 77.55±0.42. However, the aqueous extract had 137.16± 0.35 and 103.21±0.35 by DPPH and ABTS method respectively (polyphenol content: 73.8± 0.28). Zdunić et al., [11] was also found that the amount of total phenolics varied between 93.0 µg GAE/g of pumpkin juice and 905.9 µg GAE/g of fresh fruit.

Table 1: Physico-chemical characteristics and proximate composition of pumpkin powder.

S.No.	Parameters	
A.	**Antioxidant properties**	**ug Trolox/Gallic acid Equivalent /g**
1	ABTS method	123.23±10.45
2	DPPH method	145±12.94
3	Polyphenol content	2148.25± 123.89
B.	**Instrumental colour analysis**	
1	L*(Lightness)	69.95±0.25
2	a*(Redness)	4.05±0.40
3	b*(Yellowness)	27.67±0.27
C.	**Compositional analysis**	**Percentage (% w/w)**
1	Moisture	6.46±0.16
2	Fat	2.59±0.16
3	Protein	8.53±1.10
4	Ash	7.5 ±0.98
5	Total solids	93.54±4.67

Note: All the values expressed as Mean±SEM; n=3.

Peng et al. [6] reported that roasting (120, 160, and 200 °C for 10 min) raised the polyphenol content of pumpkin (*Cucurbita pepo* L.) seeds due to release of bound phenols and flavonoids. Furthermore, they observed the destruction of cell structure and production of many compounds during Maillard reaction which can react with Folin-Ciocalteu's reagent might be responsible increasing in polyphenol content. In addition, Ross et al. [12] observed the polyphenol gallo catechin and gallic acid content was increased during the heating (120 to 240 °C for 0 to 90 min) of grape seed flour due to liberation of phenolic compounds

In the present study, an increase in polyphenol content of the pumpkin powder may be attributed to release of bound polyphenol during the blanching of pumpkin slices in boiled water for 5-10 min and vacuum tray drying at 60 °C for 20 hrs.

Colour and compositional analysis of pumpkin powder

The moisture, fat, protein, ash and total solids content in pumpkin powder were found to be 6.46±0.16, 2.59±0.16, 8.53±1.10, 7.5±0.98 and 93.54±4.67 respectively. The pumpkin powder had 69.95±0.25 lightness (L*) value, 4.05±0.40 redness (a*) value and 27.67±0.27 yellowness (b*) value (Table 1).

The obtained compositional parameters were well supported with the study of Lim et al. [13] who reported that the moisture, protein, fat and ash content in oven drying pumpkin powder as7.00±0.17, 8.60±0.03, 3.68±0.15 and 8.62±0.07, respectively. In addition, the authors also reported colour values of47.13±0.06 L*, 17.70±0.30 a* and 58.67±0.35 b* value.

In the present study, the yellowness (L*) and yellowness (b*) values of pumpkin powder were higher than previous reports. However, redness (a*) value was lower. This might be due to inactivation of polyphenol oxidase enzyme during the heating treatment which was responsible for reduced browning [14].

Optimization of levels of pumpkin powder and sugar for the formulation of functional lassi

In order to optimize the levels of pumpkin powder and sugar during the production of functional lassi, a standard process mentioned in Figure 2 was used and experiments were conducted

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as per a CCRD design. The actual combinations of two levels of independent ingredient variables and five responses measured in term of subjective analysis of product recorded for each run are presented in Table 2. The regression coefficient, % p-value, adequate precision and model fit statistics of the fitted quadratic models (Eqn. 1) obtained for all the five responses are presented in Table 2. The adequacy of the models was tested using F-ratio, PRESS and co-efficient of determination (R^2).

Table 2: Sensory scores of functional lassi prepared using varying levels of pumpkin powder and sugar.

Run	Factors		*Sensory attribute scores (Responses)				
	Pumpkin powder (% w/w)	Sugar (% w/w)	C & A	Flavour	Consist.	Sweetness	OA
1	0.5	20	7.13	6.2	6.7	5.5	6.15
2	0.75	15	7.4	6.3	6.8	5.8	6
3	0.75	15	7.43	7.21	7.2	6.8	7.03
4	0.75	15	7.5	7	6	6.5	7
5	0.39	15	7.83	6.94	6.18	6.27	6.55
6	1	20	7.83	6.67	6.09	5.6	6.25
7	0.75	15	7.75	7.25	7.25	6.25	6.75
8	0.75	15	8	7	6.83	6.61	5.67
9	0.5	10	7.67	6.67	6.17	5.5	6.33
10	1	10	8	6.25	6.5	6	6.13
11	1.1	15	7.98	6.5	6.75	6.25	6.25
12	0.75	22.07	7.95	6.4	6.5	5.9	5.9
13	0.75	7.93	7.9	6.2	6	5.05	5.25

Note: *Scored on a 9-point hedonic scale

It can be seen from Table 3 that the coefficient of determination (R^2 value) for all five sensory parameters (colour & appearance, flavour, sweetness, consistency and overall acceptability) was > 0.85 indicating a good fit to the model. The "lack of fit" test, which inversely measures the fitness of the model, was not significant.

Effect of level of ingredient on colour and appearance of functional lassi

The results obtained from optimization trials consisting of the effect of level of ingredients on colour and appearance of functional lassi is presented on Table 2. Colour and appearance score found to vary between 7.13 to 8.00. The colour and appearance score is an important and foremost sensory attribute affecting the consumer acceptability of the product. The response surface plots depicting the effect of levels of ingredients on the colour and appearance score of functional lassi is presented in Figure 3a.

A close perusal of the ANOVA table (Table 3) and the response surface plots revealed the significant ($p \leq 0.05$) negative effect of pumpkin powder (A) on the colour and appearance of function lassi, indicating that as the level of pumpkin powder (A) increases the colour and appearance score of lassi decreased. Different levels of sugar concentration did not have any significant effect on the colour and appearance of functional lassi. However, a non-significant interactive effect on the colour and appearance of functional lassi was observed with varying levels of pumpkin powder and sugar (A x B). The square of factor is reflective of the quadratic effect of level of ingredients at the higher order used in the optimization process. A significant ($p \leq 0.01$) negative effect on the colour and appearance of functional lassi was observed with a higher level of pumpkin powder (A^2), while a non-significant positive effect on colour and appearance of functional lassi was observed with the higher level of sugar (B^2). Similar observations have been reported by Khan et al., [15] who observed that incorporation of more than 15%pumpkin powder adversely affected on the texture, colour and overall acceptability of biscuit. Pongjanta et al., [16] was also observed that a sensory score of bakery products at different levels of wheat flour substituted with pumpkin powder decreased as the concentration of pumpkin powder increased. Intense orange yellow colour of the pumpkin powder could be the reason for disliking by the panelists.

The coefficient of determination (R^2) value was 0.987, which is indicative of a good fit of the model to the experimental data. The "lack of fit" test, which inversely measures the fitness of the model, was not significant; indicating that the model was accurate for predicting the colour and appearance of functional lassi. Adequate precision was 28.66, which was appreciably greater than

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the minimum desirable value of 4; hence, it can be deduced that this response was a good candidate to navigate the experimental design for optimization. Further, the statistical analysis indicated that the model fitted the observed data well, the model F value (78.24) being significant (p≤ 0.01).

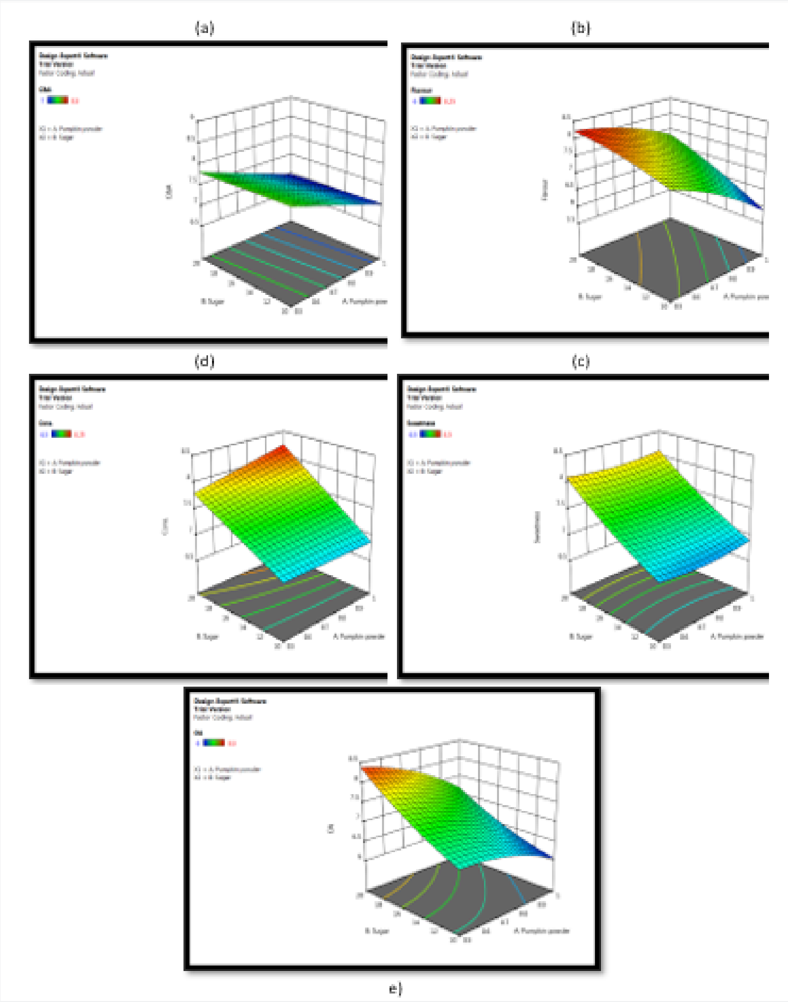

Figure 3: Response surface plots obtained for sensory score as influenced by the levels of pumpkin powder and sugar.

Effect of level of ingredient on flavour of functional lassi

The results obtained from optimization trials consisting of the effect of level of ingredients on flavor of functional lassi are presented on Table 2 and its sensory profile score measured in term of flavour attribute was found to be in the range of 6.20 to 7.25. The response surface plots depicting the effect of levels of ingredient on the flavour profile of functional lassi is presented in Figure 3b.

From the response surface plot (Figure 3b) and ANOVA data (Table 3), it was evident that the level of pumpkin powder (A) had significant (p≤ 0.05) negative effect while sugar level (B) had a significant (p≤ 0.05) positive effect on the flavor profile of functional lassi. This was an anticipated result, since a higher level of pumpkin powder may impart bitter taste to the lassi which might had decreased its flavor score. Thus, an increase in the level of pumpkin powder, reduced flavor while increase in the level of sugar increased the flavor scores of functional lassi. The obtained results were in well agreement with those of Rosidah et al. [17]. They postulated that the pumpkin flavor gives a bitter aftertaste which affected significantly on acceptability of cookies with fortification of pumpkin flour. Kulkarni and Joishi [18] also showed that as replacement level of pumpkin powder was increased from 2.5 to 10.0% in biscuit, the gradual increase in bitterness was observed.

However, a significant (p≤ 0.01) positive interactive effect on the flavor of functional lassi was observed with varying levels of pumpkin powder and sugar level (A x B). A significant (p≤ 0.01)

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negative effect on the flavor of functional lassi was observed with a higher level of pumpkin powder (A^2), while a non-significant positive effect on flavor of function lassi was observed with the higher level of sugar (B^2).

Effect of level of ingredients on sweetness of functional lassi

The effect of level of ingredients (sugar and pumpkin powder) on sweetness of functional lassi is given in Figure 3c. Sweetness score of functional lassi fortified pumpkin powder was ranged from 5.05 to 6.80.

From the response surface plot (Figure 3c) and ANOVA data (Table 3), it was indicating that the level of sugar (B) had significantly ($p \leq 0.05$) positive effect on the sweetness level of functional lassi. Thus, with an increase in the level of sugar, sweetness score of lassi increased which concurrently mask the off flavour produced due to addition of pumpkin powder at higher levels. However, interactive effect (A x B) and the effect of level of ingredient at higher level (A^2 and B^2) did not contribute any significant effect on the sweetness level of functional lassi. Sawale et al., [19] developed the vanila chocolate flavoured milk by masking a bitterness of herb arjuna by combining effect of encapsulation and addition of sugar.

Effect of level of ingredient on consistency of functional lassi

Consistency of the product indicates ability of material to resist deformation by an applied force. The results obtained from optimization trials pertaining the effect of level of ingredients on consistency of functional lassi are presented on Table 2 and its consistency score found to vary between 6.00 and 7.25. The response surface plot depicting the effect of levels of ingredient on the consistency of function lassi is presented in Figure 3d.

From the response surface plot (Figure 3d) and ANOVA data (Table 3), it is clearly indicative that only the level of sugar (B) had positive significant ($p \leq 0.05$) effect on the flavor profile of functional lassi. However, varying level of pumpkin powder (A), interactive effect (AxB) and the effect of level of ingredient at higher level (A^2 and B^2) did not contribute any significant effect on the constancy level of functional lassi. Chetana et al. [20] advocated that sugar concentration was contributed toward the consistency of the products.

Table 3: Regression coefficients and ANOVA of quadratic model for sensory characteristics of functional lassi.

Factors	Sensory score				
	Colour & Appearance	Flavour	Sweetness	Consistency	Overall Acceptability
Intercept	7.44	7.2	7.35	7.45	7.15
Pumpkin powder (A)	-0.515**	-0.541**	0	0.1	-0.478**
Sugar (B)	0.053	0.622**	0.603**	0.565**	0.684*
A^2	-0.142*	-0.178*	0.075	-0.003	-0.1844
B^2	-0.057	-0.0094	0.075	-0.031	0.128
AB	0	0.12^5*	0	0.112	0.125*
R^2	0.987	0.906	0.866	0.895	0.85
Adequate Precision	28.66	11.78	16.5	18.65	8.9
PRESS	0.22	3.23	4.94	0.72	6.25
Model F-value	78.24	13.57	3.63	25.98	8.17
Lack of fit	NS	NS	NS	NS	NS

Note: *significant at 5% level of significance, **significant at 1% level of significance, NS: Not significant.

Effect of level of ingredient on overall acceptability of functional lassi

The overall acceptability (OA) score of functional lassi ranged from 5.25 to 7.00. The effect of level of ingredients (sugar and pumpkin powder) on overall acceptability of functional lassi is illustrated in Figure 3c and is also presented in Table 2.

From the response surface plot (Figure 3e) and ANOVA data (Table 3), it is evident that the level of sugar (B) had significantly ($p \leq 0.01$) positive effect while pumpkin powder (A) had significant negative effect ($p \leq 0.05$) on the overall acceptability of functional lassi. However, interactive effect (A x B) contributes significantly ($p \leq 0.01$) with positive effect on the overall acceptability of functional lassi. The effect of level of ingredient at higher level (A^2 and B^2) did not contribute any significant effect.

Optimisation of Ingredient level for production of functional lassi

The level of ingredient (sugar and pumpkin powder) was optimized using the in-built numerical optimization technique of

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Design-Expert 12.0 for obtaining the best possible combinations of different levels of process ingredients. The target value for flavour is maximizing. The level of ingredients and other responses were set to be in range during the numerical optimization process.

Considering the parameters and their limits, the software suggested different solutions for the optimum combination of process variables with the most suited solution having a desirability of 0.85. The suggested solution from numerical optimization technique of RSM analysis is the level of pumpkin powder @ 0.5% (w/v) and sugar at level of 13.92% (w/v) was identified as the optimum combination for best quality production of pumpkin powder incorporated functional lassi.

Validation of the optimized process conditions

The level of ingredient optimized in the study were validated against experimental values obtained by preparing the functional lassi under the recommended process conditions and recording the real time values of all the responses. The obtained values were compared with the predicted values (generated by the software). Statistical analysis revealed that there was no significant difference (p> 0.05) between the predicted and actual value for all the responses which confirmed the adequacy of the developed quadratic models.

The formulated functional pumpkin powder incorporated lassi was further added with vanilla essence at 0.05% (v/v) and stabilizer pectin at 0.03% (w/v) to improve their sensory properties.

From Table 4, non-significant (P<0.05) difference was observed in composition of optimized lassi with fortified pumpkin powder as compared to its control.

Table 4: Compositional parameters of Control and pumpkin supplemented functional lassi.

Constituent (% w/w)	Control	Pumpkin supplemented functional lassi
Fat	2.10±0.08^{a}	2.07±0.0578^{a}
Protein	3.55±0.16^{a}	3.57±0.152^{a}
Ash	1.75±0.32^{a}	1.78±1.07^{a}
Total carbohydrate	18.52±2.45^{a}	17.98±1.66^{a}
Total solid	25.95±0.119^{a}	25.92±0.67^{a}

Note: Data are represented as mean±SEM (n=3). Means in each row with different superscripts (a, b, c, d) were significantly (P<0.05) different from each other. Statistical comparisons were made between samples using ANOVA single factor.

Storage study of functional lassi

Table 5: Changes in the sensory attributes# and pH of lassi during storage

		Storage period (days)					
		0	1	2	3	4	5
Sensory attributes							
Colour and Appearance	CL	7.37±1.18^{aA}	7.10±0.8^{aA}	7.20±1.1^{aA}	7.00±0.20^{bA}	6.95±0.8^{cA}	6.25±0.8^{dA}
	PL	7.90±0.080^{aB}	7.60±0.5^{aB}	7.47±1.0^{aB}	7.15±0.15^{bB}	7.01±0.6^{cB}	6.90±0.6^{dB}
Flavour	CL	8.05±0.56^{aA}	8.60±1.8^{aA}	7.95±0.7^{aA}	7.50±0.35^{bA}	7.35±0.6^{cA}	7.05±0.6^{dA}
	PL	8.98±0.16^{aB}	8.90±1.2^{aB}	7.97±0.8^{aA}	7.78±0.2^{bB}	7.57±0.4^{cB}	7.27±0.4^{dB}
Sweetness	CL	8.50±1.02^{aA}	8.50±0.50^{aA}	8.98±0.50^{aA}	8.35±0.12^{aA}	8.95±0.2^{aA}	8.55±0.2^{aA}
	PL	8.78±0.9^{aA}	8.70±1.7^{aA}	8.78±0.7^{aA}	8.60±0.72^{aA}	7.99±0.60^{aA}	7.96±0.60^{aA}
Consistency	CL	8.10±1.0^{aA}	7.90±1.30^{aA}	8..22±1.4^{aA}	8.0±0.45^{aA}	8.92±0.5^{aA}	8.52±0.5^{aA}
	PL	8.80±1.7^{aA}	8.75±1.6^{aA}	8.28±1.0^{aA}	8.08±1.0^{aA}	8.50±0.6^{aA}	8.60±0.6^{aA}
Overall acceptability	CL	8.55±0.90^{aA}	8.34±1.8^{aA}	8.90±1.0^{aA}	7.43±0.3^{bA}	7.05±0.8^{cA}	6.90±0.8^{dA}
	PL	8.99±1.0^{aB}	8.75±0.7^{aB}	8.92±0.7^{aA}	8.22±0.80^{bB}	8.01±0.67^{cB}	7.92±0.67^{dB}
pH	CL	4.34±0.08^{aA}	4.23±0.08^{aA}	4.02±0.08^{bA}	4.00±0.8^{bA}	3.59±0.4^{cA}	3.10±0.4^{dA}
	PL	4.45±0.058^{aA}	4.25±0.7^{aA}	4.30±0.8^{aB}	4.82±0.78^{aB}	4.68±0.05^{aB}	4.38±0.05^{aB}

Note: #On 9-point hedonic scale. CL: Control lassi, PL: Pumpkin powder supplemented lassi. Data are represented as means SEM (n=3). Means in each row with different superscripts (a, b, c, d) were significantly (P<0.05) different from each other. Means in each column with different superscripts (A, B) were significantly (P<0.05) different from each other. Statistical comparisons were made between samples using ANOVA two factors.

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The results pertaining to sensory evaluation of pumpkin incorporated lassi during storage at refrigeration temperature (5±1ºC) are presented Table 5. It was evident from the table that sensory scores for attributes such as sweetness and consistency score of control and pumpkin added lassi did not change significantly (p>0.05) during entire period of storage. However, the flavour, colour and appearance and overall acceptability scores decreased significantly (P<0.5) and gradually beyond 3 days of storage in both the samples. A similar trend was also found by Patel et al. [21] who reported that the flavour and overall acceptability scores decreased gradually during the storage period of pumpkin flavoured buffalo milk. The decrease in flavour score might be due to non-enzymatic browning reaction during storage which leads to burnt/ bitter-sweet aftertaste in milk drinks [22].

In fresh sample (0 days of storage), the pumpkin added lassi had significantly higher (P<0.5) sensory score as compared to control lassi. These results were well supported by Barakat et al. [23] who observed a significant increase in sensory parameters such as flavor, color, and overall acceptability except in consistency and acidity in fortifying yogurt with pumpkin pulp as compared to its control. This could be due to the reason that pumpkin contains an intense yellow orange colour obtained from carotenoids which was imparted to yogurt colour thus might resulted in higher colour and appearance scores [24]. pH of the pumpkin powder added lassi did not decline significantly(P<0.05) during the entire storage period, however, the pH of control lassi was reduced progressively and significantly beyond 2 days of storage. This finding was accordance with research by Bakirci et al. [25] who reported that yogurts with pumpkin fibre had no change in pH as compared to control lassi. It means that addition of pumpkin fibre (PF) did not suppress the action of yogurt bacteria and also development of pH. Espirito-Santo et al. [26] reported that no significant differences were observed between pH values of fibre-added or fibre-free yogurts which were co-fermented by probiotics.

Conclusion

RSM was used to optimize the formulation of pumpkin supplemented functional lassi. The optimized formulation suggested by the design expert package for functional lassi had 0.5% (w/v) pumpkin powder and 13.92% (w/v) sugar which was further added with vanilla essence (0.05% v/v) and stabilizer (0.03% w/v) for improvement of their sensory quality. Pumpkin powder was also contributed to improved sensory attributes during storage of the functional lassi. It can be concluded that RSM successfully employed to obtain optimized combination of process variables for manufacturing functional lassi with acceptable sensory quality. The pumpkin powder had contained bioactive compounds which can enhance the therapeutic properties of lassi.

Conflict of interest

Authors of the present manuscript have no conflict of interest.

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