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Optimization of casein based edible coating for paneer using Response Surface Methodology#

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Abstract

The perishability of paneer, a popular and nutritious dairy product, poses a serious threat to its marketability. This study is focused on applying an optimized edible coating based on Sodium caseinate (SC) and clove bud essential oil on paneer. The ingredients, SC, glycerol, pectin solution, and clove bud essential oil were optimized using the Central Composite Rotatable Design (CCRD) of response surface methodology and the results showed that the experimental data could be adequately fitted to a second-order polynomial model with a satisfactory Coefficient of determination (R^2 > 50%). The optimized formulation of the coating solution, obtained after validation and verification, was 13% SC, 3% glycerol, 1.25% pectin, and 0.3% clove bud essential oil.

Keywords: *Sodium caseinate, clove bud essential oil, response surface methodology*

Innovative packaging systems made of edible biopolymers are becoming increasingly popular since they are environmentally safe, biodegradable, require minimal or no disposal, and are a good substitute for petroleum-based materials that are frequently employed in the food business (Paidari *et al.*, 2021). Protein-based films and coatings are proven to have effective gas barrier properties due to their densely packed network structures (Schmid *et al.,* 2012). Milk proteins, such as casein and whey, are the effective ingredients for edible coatings (Shendurse *et al.,* 2018).

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To enhance the mechanical properties of films, plasticizers like glycerol and sorbitol are added to the protein-based coating solution. Plasticizers reduce intermolecular interactions between protein chains, improving flexibility (Fematt-Flores *et al.,* 2022). The addition of pectins improves the strength and rigidity of protein films. SC/methoxy pectin composite films have shown better properties and potential for extending the shelf life of food products (Eghbal *et al.*, 2017).

Essential oils contain bioactive components with antimicrobial and antioxidant properties, classified as GRAS by the Food and Drug Administration (FDA). This has led to their use as preservatives in the food industry (Wrona *et al.,* 2015; Atares and Chiralt*,* 2016). Carvacrol, thymol, cinnamaldehyde, eugenol, and citral are effective compounds in essential oils (Bassole and Juliani, 2012). Increasing public awareness of diet-health relationships has resulted in the production of food products enriched with bioactive ingredients. Biopolymer packaging is eco-friendly, prevents food deterioration, and can release bioactive compounds into the food product (John *et al.,* 2021).

The Response Surface Methodology (RSM) is a statistical and mathematical method used to optimize a process by analysing and modeling its variables (Montgomery, 2017). RSM helps to identify the optimal region for accurate responses by examining the topography of the response surface, including maximums, minimums, and ridgelines (Bradley, 2007). The primary benefit of RSM is the decreased number of experimental runs required to obtain assessed to fit a statistical model (Linear, Quadratic, 2FI (two-factor interaction or cubic). The 3D graphs are useful in providing information about the model but may not represent the true behavior of the system (Kidane, 2021).

Paneer, a popular dairy product made by heat-acid coagulation, has a limited shelf life. It can be stored at room temperature for one day and in the refrigerator for approximately six days without compromising its overall quality (Vyshak *et al.,* 2023). In the present study, a process was developed for the manufacture of casein-based edible coating incorporated with essential oil for paneer. The aim of this study was to optimize and validate the ideal formulation of edible coating solutions using the Response Surface Methodology and to analyze its effect on the quality of the paneer.

Materials and methods

Raw materials

Milk for the investigation was provided by the University Dairy Plant, KVASU, in Thrissur. Sagar brand (Amul) skimmed milk powder was used to prepare sodium caseinate. Glycerol and pectin (CKS Products, Ernakulam, Kerala) and essential oil (Synthite Private Ltd, Ernakulam) were procured locally. The chemicals were provided by Merck India Pvt. Ltd. and Sigma Aldrich, and the microbiological media were provided by Himedia, Mumbai.

Preparation of casein-based edible coating solution

The edible coating was prepared as per Bonnaillie *et al.* (2014) with minor modifications. Sodium caseinate was used as the base material for the development of the edible coating as per the procedure of Sarode *et al.* (2016). Glycerol (plasticizer), pectin, and clove bud essential oil were the other ingredients used in the coating solution. Paneer was prepared from milk as per the method described by Bhattacharya *et al.* (1971).

Experimental design and verification of results

To optimize using RSM, the minimum and maximum levels of each ingredient and their compatibility with the product must be known. So, a series of trials were conducted to standardize the basic formulation for the preparation of edible coating solution. The Central Composite Rotatable Design (CCRD) of response surface methodology (Design-Expert® Software Version 9.0.4.10 (Statease Inc., Minneapolis, USA) was used for the optimization of paneer coated with the casein-based solution. The minimum and maximum level of variables fed to the software ranged from 12% to 14% sodium caseinate, 2% to

4% glycerol, 1 to 1.5% pectin solution, and 0.25% to 0.35% clove bud essential oil. The response to variation in process parameters was measured in terms of water activity and sensory characteristics such as color and appearance, flavour, body and texture, and overall acceptability. Thirty experiments were performed according to second-order central composite rotatable design (CCRD) with four independent variables and five levels (Table 1). The factorial design comprised of eight axial points, sixteen factorial points and six centre points. All response data were fit with a second-order mathematical model.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i$$
$$\sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=iH}^{4} \beta_{ij} X_i X$$
$$\sum_{i=1}^{3} \sum_{j=iH}^{4} \beta_{ij} X_i X_j$$

Where, Y represents the response variables, β_0, β_i, β_{ii}, and β_{ij} represents regression coefficients, and X_i and X_j are the levels of the independent variables.

The desired goals for each factor and response were chosen. The goals may apply to either factors or responses. Desirability is an objective function that ranges from zero outside the limits to one at the goal (Wani *et al.,* 2017). For product preparation, the solution with the highest level of desirability is selected. For all standardized values of responses, analysis of variance (ANOVA) and multiple regression analysis were conducted using Design Expert – version 9 to examine the statistical significance of model terms. The adequacy of developed models was determined using F values, lack-of-fit test, R^2 (coefficient of determination), Coefficient of variation (CV), PRESS, and adequate precision ratio (APR). The surface plots showing the relationship between the independent variables and the responses can be depicted in as 3-dimensional graphs (3D). Scores obtained for its sensory analysis were recorded (observed value) and compared with the sensory scores predicted by RSM for final solutions (predicted value).

Compositional analysis

Moisture in paneer samples was determined by the method of Sachdeva (1983). The fat content was determined by Rose-Gottlieb's method described in IS 5162 (1980), total nitrogen content by the Micro Kjeldahl method (AOAC, 1990), and ash content by the method described for chhana described in IS: 5162 (1980). The pH of the paneer sample was determined by blending 10g of paneer with 10 ml of distilled water and dipping the pH electrode (Eutech, Model- EC510). The titratable acidity was determined by the method recommended by AOAC (1990) for cheese. The water activity of the sample was measured using a water activity meter (AQUA LAB).

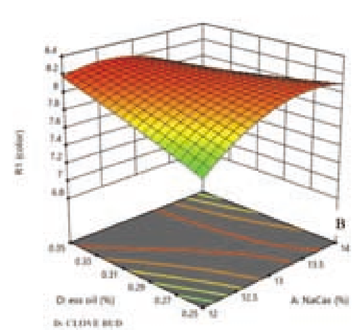
Sensory analysis

The paneer samples were evaluated organoleptically for different quality attributes like flavor, body and texture, color and appearance, and overall acceptability by a selected panel of judges comprising five members. The paneer was evaluated in raw form. A nine point hedonic scale scorecard was used for evaluation.

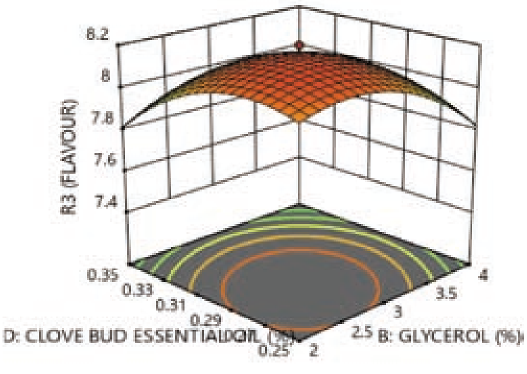
Results and discussion

Optimization of ingredients using Response Surface Methodology

The Central Composite Rotatable design matrix for the four factors and the sensory scores and water activity values for the different combinations obtained are summarized in Table 1. The partial coefficients of regression of linear, quadratic, and interaction terms for each model and their R^2 values are shown in Table 2. The response surface plot obtained as a 3-dimensional (3D) graph along with the 2-dimensional (2D) contour plot is given in Fig. 1(A to E).



A



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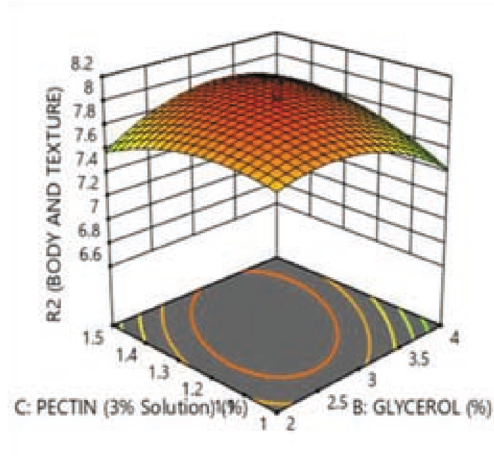

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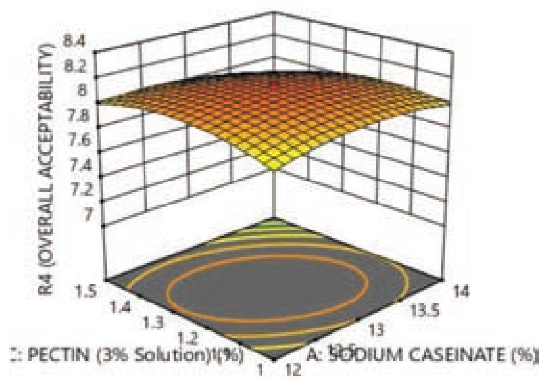

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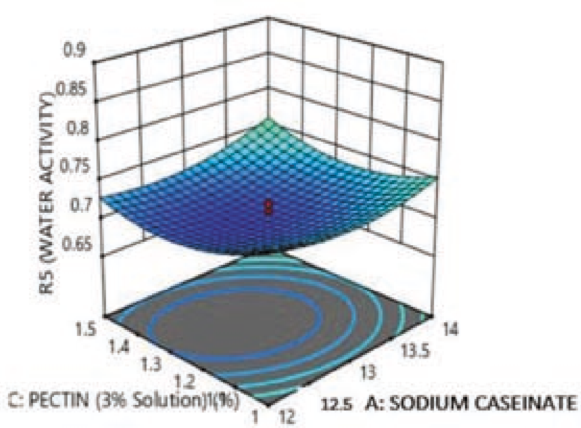

E

Fig. 1. (A – E) Response surface plot relating to sensory score as influenced by sodium caseinate, glycerol, pectin and clove bud essential oil

***A** – Response surface showing the effect of Clove bud essential oil and SC on Color and appearance of coated paneer; **B** - Response surface showing effect of Clove bud essential oil and glycerol on flavor of coated paneer; **C** - Response surface showing the effect of pectin and glycerol on body and texture of coated paneer; **D** - Response surface showing effect of pectin and SC on overall acceptability of coated paneer. **E** - Response surface showing the effect of pectin and SC on the water activity of coated paneer

Table 1. Central composite Rotatable design matrix for the four factors with their responses

Standard order	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3	Response 4	Response 5
	A: SC (%)	B: Glycerol (%)	C: Pectin (%)	D: Clove bud essential oil (%)	Colour and Appearance	Body and Texture	Flavour	Overall Acceptability	Water activity
1	12	4	1	0.25	6.9	7.05	7.75	7.75	0.82
2	14	4	1.5	0.35	6.9	6.95	7.6	7.5	0.8
3	14	4	1	0.25	7.8	7.25	7.56	7.56	0.85
4	13	3	1.25	0.3	8.12	7.95	8.2	8.15	0.71
5	13	3	1.25	0.3	8.15	8	8.2	8.12	0.7
6	12	2	1	0.35	7.84	7.69	7.75	7.5	0.75
7	14	4	1	0.35	7.65	7	7.56	7.43	0.81
8	13	3	1.25	0.3	8.2	7.95	8.15	8.15	0.69
9	13	3	1.75	0.3	8.02	7.5	7.9	7.63	0.78
10	13	1	1.25	0.3	7.69	7.21	7.75	7.98	0.75
11	13	3	1.25	0.3	8.12	8.04	8.15	8.15	0.69
12	13	3	1.25	0.2	7.99	7.68	7.75	7.56	0.77
13	12	4	1.5	0.25	7.8	7.95	7.75	7.95	0.84
14	13	3	1.25	0.3	8.24	6.9	7.45	7.15	0.71
15	14	4	1.5	0.25	7.69	7.85	7.65	7.63	0.84
16	12	2	1.5	0.25	7.95	7.51	7.95	7.65	0.75
17	13	3	1.25	0.3	8.2	8.06	8.15	8.15	0.72
18	12	2	1.5	0.35	8.05	7.34	7.65	7.45	0.73
19	13	3	1.25	0.4	8.19	8	8.15	8.13	0.72
20	14	2	1.5	0.35	7.52	6.63	7.65	7.49	0.75
21	15	3	1.25	0.3	7.24	6.9	7.75	7.75	0.81
22	14	2	1.5	0.25	8.15	7.32	7.95	7.85	0.82
23	12	4	1	0.35	7.85	6.95	7.75	7.65	0.85
24	14	2	1	0.25	7.91	7.21	7.85	8.25	0.79
25	13	5	1.25	0.3	7.3	7.05	7.45	7.95	0.89
26	14	2	1	0.35	8.11	7.32	7.6	7.75	0.73
27	13	3	0.75	0.3	7.98	7.35	7.95	7.75	0.81
28	12	2	1	0.25	6.85	7.35	8	7.85	0.8
29	12	4	1.5	0.35	7.95	7.05	7.65	7.98	0.82
30	11	3	1.25	0.3	7.3	7.35	7.95	7.88	0.72

Effect on overall acceptability

The average overall acceptability score ranged from 7.15 to 8.25. The 3-D graph obtained for overall acceptability (Fig.1-D) reveals that the overall acceptability was least affected by the levels of incorporation of pectin and sodium caseinate. The following response surface equation was generated to forecast the change in overall acceptability with different levels of sodium caseinate (A), glycerol (B), pectin (C), and clove bud essential oil (D):

Overall acceptability = 8.14167 - 0.0241667 * A - 0.0166667 * B - 0.02 * C - 0.106667 * D - 0.13125 * AB - 0.05 * AC - 0.03125 * AD + 0.09875 * BC + 0.0675 * BD + 0.02625 * CD - 0.0822917 * A2 - 0.0447917 * B2 - 0.113542 * C2 - 0.197292 * D2

The F value of the model for flavour was significant ($p < 0.05$) and the lack of fit was non-significant. The coefficient of determination (R^2) was found to be 0.99 with an adequate precision of 63.35 which strongly recommends the use of this response, i.e., overall acceptability to navigate the design.

Effect on Water Activity

The average water activity scores ranged from 0.89 to 0.69. Fig. 1 –E depicts that the increasing levels of sodium caseinate and pectin tend to reduce the water activity of the coated paneer. This might be because sodium caseinate can form stable films and has strong water activity, as suggested by Semwal *et al.* (2022). The following response surface equation was generated to forecast the change in water activity with different levels of sodium caseinate (A), glycerol (B), pectin (C), and clove bud essential oil (D):

$$\text{Water activity} = 0.70 + 0.008{*}A + 0.032{*}B - 0.004{*}C + 0.016{*}D - 0.005{*}AB + 0.006{*}AC - 0.009{*}AD - 0.008{*}BD - 0.001{*}CD + 0.017{*}A^2 + 0.031{*}B^2 + 0.025{*}C^2 + 0.011{*}D^2$$

The F value of the model for water activity was significant ($p < 0.05$) and the lack of fit was non-significant. The coefficient of determination (R^2) was found to be 0.92 with an adequate precision of 12.71 which strongly recommends the use of this response, i.e., water activity to navigate the design.

Optimized solutions and their validation

Numerical optimization was carried out to attain the best possible combination of sodium caseinate, glycerol, pectin, and essential oil to be added to the casein-based edible coating for paneer. The criteria for optimization are summarized in Table 3. The levels of sodium caseinate, glycerol, pectin solution, and clove bud essential oil were kept within range. The sensory scores were kept maximum while the water activity was kept minimum during the optimization process. Table 4 shows the suggested solution for the preparation of casein-based edible coating incorporated with clove bud essential oil. The predicted values for all the responses suggested for both formulations are tabulated in Table 5. It was also noted that the solution had a high desirability value of 0.99. The optimum formulation obtained from the software was verified and found that the observed values were not significantly different ($p>0.05$) from the predicted values concerning all attributes.

The method of preparation of the coating solution is given below

Acid Casein

↓

Addition of 2.5M Sodium hydroxide solution until neutral pH 7 is attained.

↓

Preparation of 13% SC solution using distilled water

↓

Addition of 3% glycerol

↓

Addition of 1.25% of 3 per cent Pectin solution

↓

Addition of 0.3% Clove bud essential oil (CEO)

↓

Magnetic stirring for 1 hour

↓

Dipping of paneer cubes ($1 cm^3$) in the coating solution

↓

Drying of samples at room temperature for an hour

↓

Packaging in sterilised LLDPE pouches

↓

Refrigerated storage (7 ± 1^0C)

Proximate composition and water activity

The proximate analysis results (Table 6) showed no significant difference ($p<0.01$) in the composition of the casein-based edible coating and the control sample. Similar findings were reported in studies on paneer with edible film for protein, fat, and ash content (Raju and Sasikala, 2016; Jotarkar *et al.,* 2018). Moisture and total solids content also did not differ significantly between the control and paneer with casein-based edible coating (Labuza and Hyman, 1998). Acidity showed a non-significant difference ($p<0.01$) on the first day of storage, like other studies with essential oil-treated edible films (Karunamay *et al.,* 2020). However, there was a significant difference ($p>0.01$) in water activity between the control and paneer with casein-based edible coating, suggesting that the protective barrier property of the coating

Table 2. Regression coefficients and ANOVA of fitted quadratic model for sensory characteristics as well as water activity of coated paneer samples

Partial Coefficients	Sensory characteristics				
	Color and Appearance	Body and Texture	Flavour	Overall Acceptability	Water activity
Intercept	8.16	8	8.163	8.14	0.70
A- SC	0.017^{ns}	-0.094**	-0.051**	-0.024**	0.008^{ns}
B- Glycerol	-0.109**	-0.026^{ns}	-0.072**	-0.016**	0.032**
C- Pectin	0.049**	0.045**	-0.002^{ns}	-0.02**	-0.004^{ns}
D- Clove bud essential oil	0.055**	-0.171**	-0.077**	-0.106**	0.016**
AB	-0.091**	0.091**	-0.014*	-0.0131**	-0.005^{ns}
AC	-0.22**	-0.052**	0.033**	-0.05**	0.006^{ns}
AD	-0.222**	-0.056**	0.003^{ns}	-0.031**	-0.009^{ns}
BC	-0.051*	0.145**	0.001^{ns}	0.098**	-0.000^{ns}
BD	-0.031^{ns}	-0.108**	0.059**	0.067**	0.008^{ns}
CD	-0.197**	-0.1725**	-0.015**	0.026**	-0.001^{ns}
A^2	-0.229**	-0.213**	-0.081**	-0.082**	0.017**
B^2	-0.173**	-0.211**	-0.144**	-0.044**	0.031**
C^2	-0.047**	-0.138**	-0.062**	-0.113**	0.025**
D^2	-0.018^{ns}	-0.171**	-0.144**	-0.197**	0.011*
Lack of fit	0.05^{ns}	0.13^{ns}	0.83^{ns}	0.05^{ns}	0.11^{ns}
Model F value	67.98*	78.97*	238.87*	266.08*	12.76*
R^2	0.98	0.98	0.99	0.99	0.92
Press	0.40	0.34	0.02	0.04	0.03
Adeq. Press	26.41	28.32	49.09	63.35	12.71

*-Significant at five per cent level (p<0.05), **- Significant at one per cent level (p<0.01), ns- non significant (p >0.05)

Table 3. Constraints and criteria for optimization of paneer with casein-based edible coating

Constraint	Goal	Lower limit	Upper limit
SC (%)	In range	12	14
Glycerol (%)	In range	2	4
Pectin (3%)	In range	1	1.5
Clove bud essential oil (%)	In range	0.25	0.35
Color and appearance	Maximize	6.85	8.24
Body and texture	Maximize	6.63	8.06
Flavour	Maximize	7.45	8.20
Overall acceptability	Maximize	7.15	8.25
Water activity	Minimize	0.89	0.69

Table 4. Solutions obtained after response surface analysis

Ingredients	Sodium Caseinate (%)	Glycerol (%)	Pectin (3%)	Clove bud essential oil (%)	Desirability
Levels	13	3	1.25	0.3	0.99

Table 5. Verification of the optimum formulation

Attributes	Predicted value	Observed value	t value
Colour and appearance	8.16	8.12 ± 0.02	0.81 ns
Body and texture	8	7.95 ± 0.06	0.92ns
Flavour	8.16	8.15 ± 0.02	0.53ns
Overall acceptability	8.14	8.09 ± 0.09	0.51ns
Water activity	0.80	0.82 ± 0.005	0.63ns

*Figures are mean ± standard error of six replications, ns-non significant (p>0.05)

Table 6. Proximate compositional analysis of coated paneer and control

Parameter	Coated paneer	Control sample	t value
Moisture (%)	55.83±0.232	55.93 ±0.168	-0.77ns
Total Solids (%)	44.06 ±0.232	44.18±0.168	0.404ns
Fat (%)	20.52±0.272	20.81±0.219	0.83 ns
Protein (%)	19.425±0.203	19.29±0.175	-0.50 ns
Ash (%)	2.2 ±0.112	2.105±0.096	-0.65 ns
Acidity (% lactic acid)	0.23 ±0.008	0.22±0.006	-0.52 ns
Water activity	0.88 ±0.003	0.985±0.002	26.37**

*Figures are mean ± standard error of four replications, ns-non significant (p>0.05)

may have contributed to the lower water activity in the coated paneer compared to the control.

Conclusion

The quadratic models acquired by RSM made a satisfactory fit to data concerning color and appearance, body and texture, flavor, overall acceptability, and water activity. The optimum formulation of edible coating given by RSM to achieve the predicted maximum response values was 13.00% sodium caseinate, 3.00% glycerol, 1.25%pectin solution, and 0.30% clove bud essential oil. The compositional analysis of the samples advocated that there was no significant difference between the paneer coated with casein-based edible coating and control sample. The water activity of the paneer with the edible coating (0.88) was much lower than that of the control sample (0.98). The application of edible coating also induced a color change in the product which did not deleteriously affect the appearance of the product during sensory evaluation. Hence it can be concluded that RSM can be successfully applied for optimizing the level of ingredients for obtaining a product with desired sensorial attributes.

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Conflict of interest

The authors report no conflict of interest

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