Study of sensory and textural properties of protein based edible coated paneer using Response surface methodology

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Abstract

The important of milk and milk product has been recognized since Vedic time. Paneer is one of the traditional dairy products of India which is analogous to the western cottage cheese. Paneer is a heat and acid coagulated with permitted organic acid and subsequent drainage of whey followed by pressing. Short life of paneer is one of the most serious problems faced in marketing. Use of edible films on the food products could also be utilized as a protective coating to extend the shelf life. Whey protein based films and coatings are generally flavorless, tasteless, and flexible and source of protein. In preservation of paneer edible coating with antimicrobial agents could be best alternative. The application of edible coatings and films can reduce particle clustering and improve physical strength, visual and tactile feature on product surface. Mechanical strength increased as the protein plasticizer ratio increase. Edible films and coatings may control adhesion, cohesion and durability, and improve the appearance of coated foods. They could enhance the organoleptic, mechanical or nutritional properties of food. . To prepare edible coating using whey protein concentrate (WPC), glycerol, potassium sorbate and nisin used as anti microbial agent. To optimization of level of edible coating ingredients were designed using Response Surface Methodology (RSM) based on hypothesis that the response of the product. Design of experiment was carried out by the software: DESIGN EXPERT, Central Composite Rotatable Design (CCRD) was chosen

Keywords: Paneer, edible coating, Response Surface Methodology (RSM)

Introduction

Milk and its products are an excellent source of vital nutrients and functional constituents. It is described as nature's nearly perfect food and some leading experts recognize milk and its product as important constituents of a well balanced, nutritionally adequate diet. Indigenous dairy products have played an important role in the socioeconomic life of our people since time immemorial. India is emerging as the largest milk producing country in the world with the milk production of about 94.60

million tones in 2008 (Bhasin, 2008). Paneer is one of the popular indigenous dairy products in which the technique of acid coagulation of milk at high temperature is used to conserve wholesome and unique nutrients of milk. Paneer is a heat and acid coagulated traditional milk product, mainly consisting of milk solids obtained from the coagulation of hot milk with permitted organic acid and subsequent drainage of whey followed by pressing The phenomenon of precipitation involves the formation of large structural aggregates of protein in which milk fat and other colloidal and soluble solid are entrained with whey. It is estimated that about five percent of the total milk produced in India is converted into paneer (Makhal and Sen, 2001) which accounts for about 3, 00,000 tones of paneer annually valued at rupees 1050 crores. It is estimated that Annual production of paneer was 4,496 MT in year 2003-04 (Dairy India, 2007). Paneer has a fairly high level of fat and protein, low level of sugar and contains some important minerals (e.g. calcium) and vitamins. In general paneer contains 55-70 percent moisture, 22-27 percent fat , 17-18 percent protein , 2.0-2.5 percent lactose , and 1.5- 2.0 percent minerals (Kanawjia *et al*, 1990). According to Prevention of Food Adulteration Act (PFA) (1976), paneer should contain not more than 70 percent moisture and its fat content should not be less than 50 percent of dry matter where as skim milk *paneer* should contain not more than 70 percent moisture and the fat content should not be more than 13 percent of dry matter (Kanawjia *et al.*, 1990). Paneer should contain not more than 50,000 total bacterial counts, not more than 90 coliform counts and 250 yeast and mould count per gram of paneer (ISO: 1084, 1983). Paneer is recommended for diabetic patients, dental carries, growing children and pregnant women due to high calcium and protein (Chopra and Mamtani, 1995). However, short shelf life is one of the most serious problems faced in marketing and distribution of paneer to the restaurants and fast food centers, which consume a huge quantity of this product as a base material for patties, sandwiches, culinary dishes, etc. Paneer has a good market value, but it is not able to find its rightful place in Indian market due to its short life of about a week under refrigeration and one day at room temperature. Various chemical preservatives were tried by different scientists singly or in combination for the preservation of paneer and they succeeded in extending the shelf life to some extent. Apart from various chemical preservatives natural preservatives are also having promising results in improvement of shelf life of various dairy products e.g. nisin has been successfully used in a variety of dairy products. On the other hand use of edible films on the food products could also be utilized as a protective coating to extend the shelf life. An edible coating/film simply is defined as a thin continuous layer of edible material formed on or placed between food and food components (Torres, 1994). The most important functionalities of an edible film or coating include control of mass transfer, mechanical protection and better sensory appeal. Whey protein based films and coatings are generally flavorless,

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tasteless and flexible materials, water based, and the films varies from transparent to translucent depending on formulation, purity of protein sources and composition. In the preservation of paneer edible coating with antimicrobial agents could be best alternative because this technique is successfully applied in some dairy products (e.g. Cheese) and other food products (like fruits, confectionary products, and chocolate etc). The application of edible films and coatings can readily improve the physical strength of food products, reduce particle clustering and improve visual and tactile feature on product surface (Cuq *et al.*, 1995). It can protect food products from moisture migration, microbial growth on the surface, light induced chemical changes, oxidation of nutrients, etc. (Kester and Fennema, 1986).

Objective

1. To improve sensory parameter of paneer
2. To improve textural properties of paneer

Material and methods

Materials

Commercially cow milk was (Livestock Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Distt. Udham Singh Nagar, Uttarakhand) used for preparation of paneer. Coating material (Whey protein concentrate (DARS specific, Tansen Road, Gwalior, M.P) Glycerol and potassium sorbate (Himedia Laboraties. Private Limited, 23, Vadhani Mumbai India. Estate) were used in preparatuion of edibe coating and nisin (Shree Additives Pharma and Food Ltd, Ahemdabad, Gujrat) was used as a antimicrobial agent.

Preparation of paneer

Milk was first filtered through double layered muslin cloth in order to remove dust and dirt particles and then separated by centrifugal cream separator (Modern Cream Separator, Dairy Appliances Corporation, India) for standardization purpose. Buffalo milk was standardized to 6 percent fat and 8.5 percent solid-not-fat (SNF) using buffalo skim milk and cream. The standardized buffalo milk was heated up to 90^0C followed by cooling to 70^0C for coagulation. Heat treatment aids in co-precipitating casein and whey proteins and also proved to increase the yield of paneer to some extent. Milk samples were coagulated at $70^0C \pm 1^0C$ by adding 1 percent citric acid solution slowly to the milk with continuous and gentle stirring till a complete coagulation occurred and transparent greenish yellow whey separated out from coagulated mass. After coagulation of milk, the contents were left undisturbed for about five

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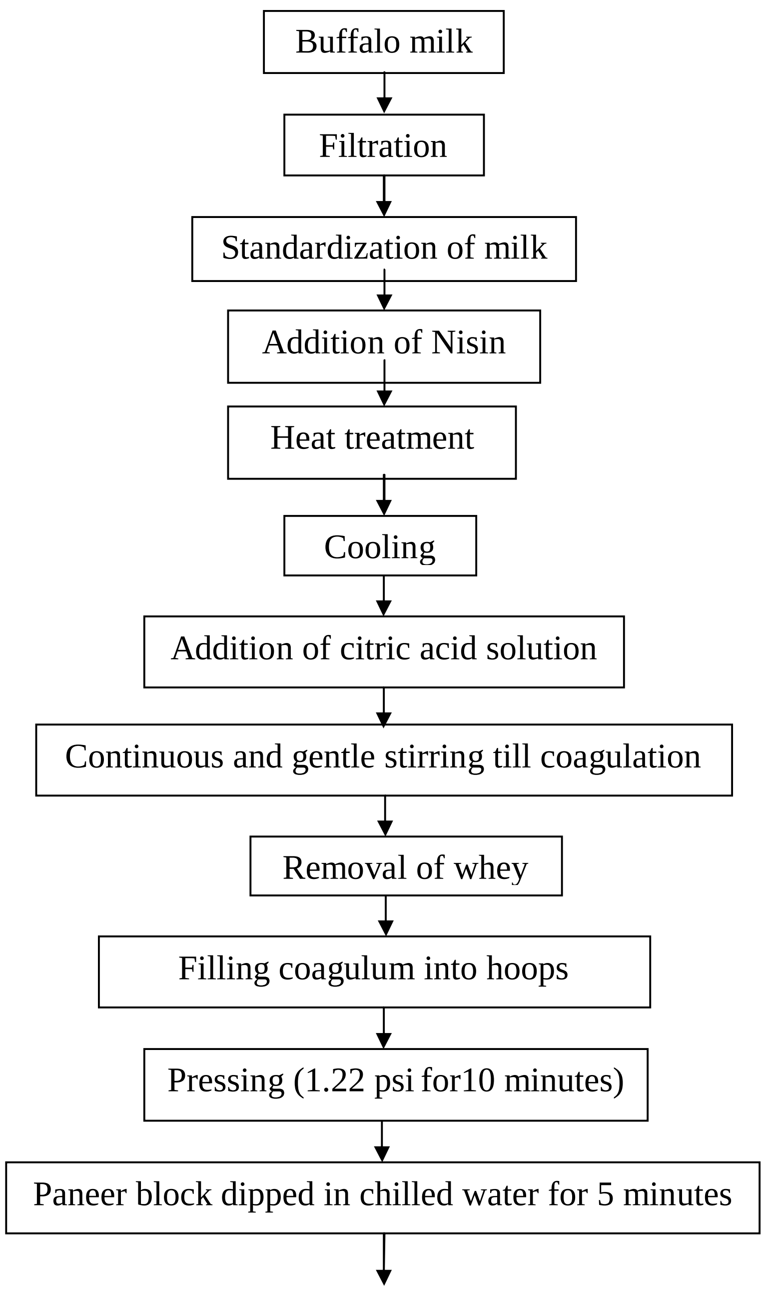
minutes. The whey then removed by filtration through a double layered muslin cloth. The hot coagulum was transferred into a circular stainless steel hoop and pressed for 10 minutes by applying weight of 1.5 kg /cm ^{2} for texturization of coagulated mass. Coagulated paneer was dipped in chilled water for 5 minutes for good texture.

PROXIMATE COMPOSITION

Proximate composition of paneer was 58.6 % moisture (w/w), 16.45 % fat (w/w), 19.94 % protein (w/w), 2.51 % carbohydrates (w/w), 2.5 % ash content (w/w), 0.228 (% LA) titratable acidity and 6.09 pH. The mentioned values may vary depending on the type of milk used and also on the method of manufacture.



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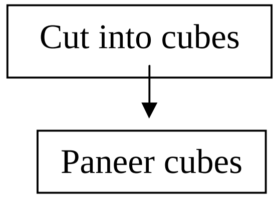

Figure 1: Preparation of Paneer

Edible Film and Coating Formation

There are many methods to produce whey protein -based edible films, such as heating and irradiation. A process of protein cross linking is necessary to obtain a flexible, easy to handle film. The resulting film properties are affected by the amino acid composition, distribution and polarity, conditions affecting formation of ionic cross linking between amino and carboxyl groups, presence of hydrogen bonding, intramolecular and intermolecular disulfide bonds. However, the formation of whey protein-based films mainly involved heat denaturation in aqueous solution at 75-100°C, which produces intermolecular disulfide bonds, which might be partly responsible for film structure (www.enwikipedia.org.).

Heat treatment promotes water insolubility, which may be beneficial to maintain film and food integrity. Bi-tyrosine bridges between protein chains can be produced by γ-irradiation. This process seems to modify the conformation of proteins, which become more ordered and stable and films can be obtained from the inclusion of these proteins to a matrix such as cellulose. WPI films have also been formed by using enzymatic methods, such as transglutaminase (γ-glutamyltransferase, E.C. 2.3.2.13) as a cross-linking agent. Compression molding of WPI films has also been conducted by using a carver press at 0.8-2.2 MPa, at 104-140°C, for up to 2 min. This is the first step towards a continuous extrusion process. There are reports where WPI films plasticized with sorbitol (S) or glycerol (G) was slightly sweet and adhesive. On the other hand, the textural impact of glycerol plasticized whey protein isolate (WPI) films could be reduced if film thickness was reduced to about 23 µm, as tested on crackers and melted cheese. Texture perception of WPI films may also be reduced by making them more soluble, so that they dissolve readily in the mouth during mastication (www.enwikipedia.org).

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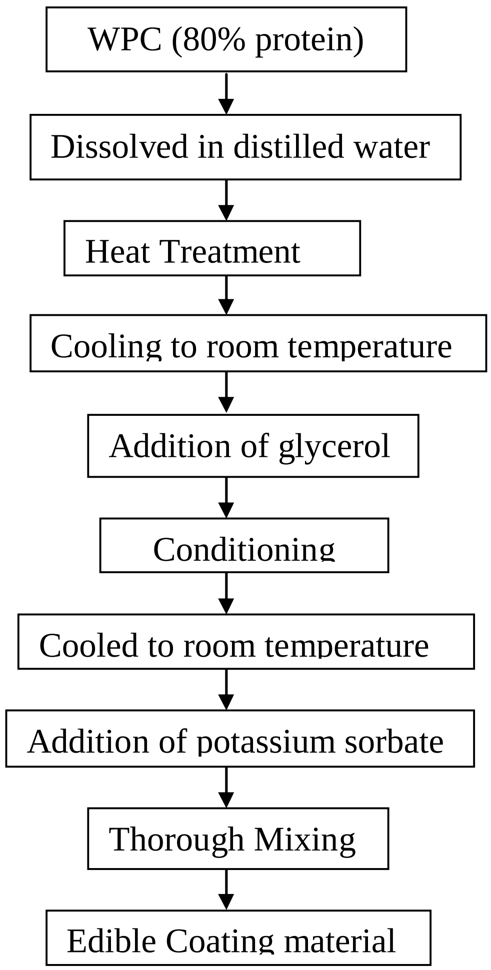

Figure 2: Preparation of Edible Coating Solution

Application of edible coating

Since most of the work on the application of edible films and coating was done fruits and vegetables and no work done in dairy products so related review is presented here, according to **Martins**

***et al., (*2003),** alginate and gelatin coating at different concentrations with plasticizers such as glycerol and carboxymethyl-cellulose (CMC) and sucroesters coating plasticized with mono/diglycerides were tested. The effects of those coating on the storage stability were followed by measurements of peel and pulp firmness. The 2 percent alginate and 5 percent gelatin coating significantly reduced weight loss, thus maintaining fruit firmness and thereby preserving fruit freshness. The effect of these coatings includes the improving appearance and imparting an attractive natural looking sheen to the fruit.

Dangaran *et al*., (2006) worked on whey protein-sucrose coating and reported that whey protein coating protects foods from deterioration and can extend product shelf life. Whey protein coatings may also undergo change over a period of time if not properly formulated. Whey protein isolates sucrose, high gloss coatings, with and without crystallization inhibitors, were formed on chocolate covered peanuts. WPI coating containing raffinose had significantly higher gloss values. Edible coating of WPI can be used to improve the effectiveness of water based high gloss edible coatings.

Han and Krochta (2007) studied physical properties of whey protein coating solution and films containing antioxidants. Antioxidants (ascorbyl palmitate and alfa- tocopherol) were incorporated into 10 percent of whey protein isolate (WPI) (w/w) and coating solution containing 6.67 percent (w/w) glycerol (WPI:glycerol= 6:4) were studied. The antioxidants mixture was incorporated into heat denatured whey protein solution and viscosity and turbidity to determine transparency and oxygen barrier properties (permeability, diffusivity, and solubility). WPI film and antioxidant containing WPI film had very low oxygen permeability and solubility.

According to **Kim (2002)** the shelf life of grape by edible coating material such as methyl cellulose (MC) which antimicrobial substances such as n- capric acid isopropyl ester (Ci) and sodium nitrate (sn) added and coating applied on grapes by spraying method. The quality changes of packaged grapes with wrapping PE film on EPS tray were investigated for 16 days at 30^{o}C. The reduction in rate of firmness of grape, MCsn and MCCi after 16 days at 30^{o}C were 42.2, 26.5 and 23.2 percent respectively. The addition of Ci and Sn had much affected the reduction of bacteria and yeast counts especially at early stage of storage.

According to **Bravin *et al.*, (2006),** edible coating of polysaccharide- lipid film composed of corn starch, methylcellulose (MC) and soybean oil had effective moisture transfer control in moisture sensitive products. It was evaluated by coating crackers, a low water activity type cereal food. Spread film gave better water vapour barrier and mechanical properties than sprayed film. Coated crackers had longer shelf life and higher reference at all storage conditions.

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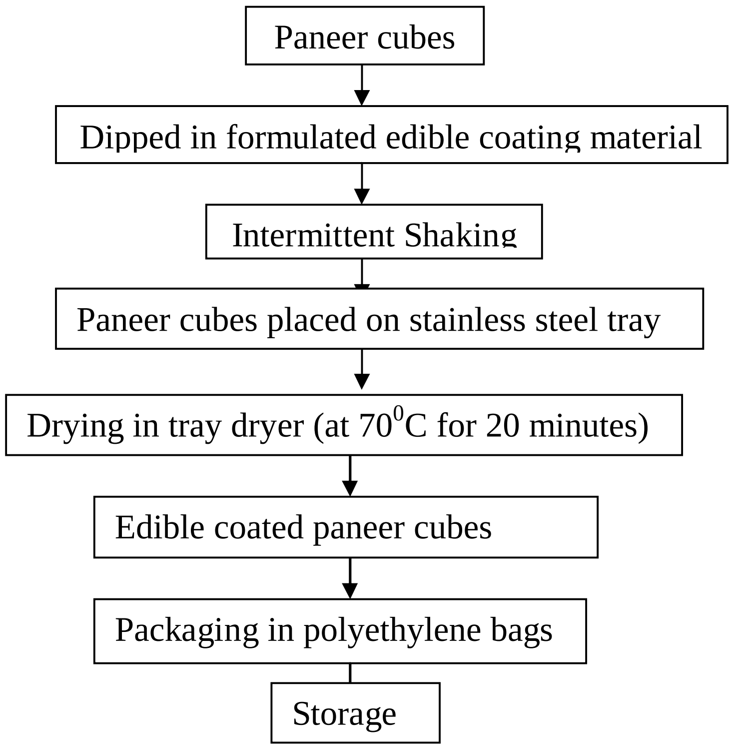

Figure 3: Application of Edible Coating on Paneer

Optimization of level of edible coating ingredients

To optimization of level of edible coating were using Response Surface Methodology. (RSM) based on the hypothesis that the response of the products were functionally related to specific composition and attempts were made to fit the multiple regression equation correcting response to obtain an optimized quality competition. Design of experiments was carried out by the software: DESIGN EXPERT. Central Composite Rotatable Design (CCRD) was chosen because it is the most popular response surface design, and it gives very good predictions in the middle of design space. The alpha value was chosen so as to make the design rotatable. The number of experiments in half factorial CCRD was found out as follows:

$$N_0 = 2^{K-1} + 2K + n_0$$

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where,

K = Number of independent variables

N_0= Number of experiment design

n_0 = Number of center point replicates, which in this design is 6

The experimental plan consists of 30 experiments (Table 3.1). The first sixteen experiments were in first order. The next eight experiments were in second order, and the last 6 experiments were centre point replicates. All the 30 experiments were carried out in a random order.

Analysis of paneer

Sensory evaluation

The paneer samples with edible coating were evaluated for its acceptability, during the process of optimization and storage studies. For sensory evaluation paneer samples were served to a panel of five semi-trained panelists consisting of faculty from the department of Food Science and Technology in the University. The panelists were asked to evaluate the sensory quality of paneer samples as per sensory score card (Appendix-I). Panel members were directed to judge each samples on the basis appearance, flavor, body and texture and overall acceptability, and indicate their degree of liking on a 9-point Hedonic Scale (Lawless and Hayman, 1998).

Evaluation of texture

Textural profile analysis was conducted for paneer samples for all experiments to obtain textural responses viz, hardness, springiness, adhesiveness, cohesiveness, gumminess and chewiness.

Statistical analysis

Results obtained were statistically analyzed in completely randomized design (CRD) and ANOVA technique on four factors simple CRD according to methods described by Cochran (1980) and Snedecor and Cochran (1967), respectively.

Data Analysis

RSM was used to determine the effect of the independent variables statistically. A full second order mathematical model of the following form was fitted to all the response data.

For statistical analysis of the experiments designed by the RSM , it was assumed that n mathematical functions, f_k (k= 1,2,3,…………..n), existed for each response variable, Y_k in terms of m independents processing factors X_i (i= 1,2,3…………m).

$$Y_k = f_k(X_1, X_2, X_3 \ldots\ldots X_m)$$

In this case n= 15 and m = 4. The function was assumed to be approximated by a second degree polynomial equation.

$$Y_k = \beta_{k0} + \sum_{i=1}^{K} \beta_1 X_1 + \sum_{i=1}^{K} \beta_{ii} X_i^2 + \sum_{i=1}^{K-1} \sum_{j=i+1}^{K} \beta_{ij} X_i X_j \ldots\ldots$$

Where, b_{k0} is the value of the fitted response at the center point of the design, i.e. point (0,0,0,0) and $b_{k0,}$ $b_{kii,}$ and $b_{kij,}$ are the linear, quadratic and cross product regression terms, respectively. Data were analyzed using the statistical package DESIGN EXPERT 7.0.

Results and discussion

The fat: solid not fat content of the buffalo milk used for preparing paneer (1:1.65). The levels of variable were optimization of paneer with edible coating on the basis of coefficient of determination (R^2). Optimization of ingredient levels for pH, acidity, and water activity, sensory and textural parameters of paneer. The software "DESIGN EXPERT used for getting the optimum combination of ingredients. Optimization of ingredient levels on cost of product, characteristics, legal obligation and shelf life of the product. The parameter optimized using the response surface methodology were pH, acidity water activity, appearance, flavour, body and texture and overall acceptability and textural parameter (hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness).

Sensory analysis

Appearance

The color and appearance score of paneer range from 6.8 to 8.2 which had levels of 8 percent to 6 percent whey protein concentrate. 6 percent whey protein concentrate showed better sensory appearance score than at 8 percent levels of whey protein concentrate. The coefficient of determination (R^2) for the regression modal for appearance was 59.2 percent. The representation of the response surface is given in Fig 1. The expression of the model permitted the evaluation of the factors. The increase in nisin level decrease appearance. Similarly the increase in WPC level decrease appearance. The increase in WPC level with decrease appearance resulted yellowish appearance. Interactive effect of nisin and glycerol level increase in glycerol level increase appearance.

Flavour

The average flavour score of paneer varied from 6.9 to 8. The level of WPC, glycerol, and potassium sorbate and nice were 6, 7, 1.3 percent and 10 ppm respectively. The coefficient of determination (R^2) for the regression modal for flavour was 41.3 percent.

Body & texture

Body and texture score varied from 6.5 to 8. Data was showing that whey protein concentrates not much affect the body and texture. But level of glycerol affects the body and texture. 5 percent and 7 percent level of glycerol give better sensory score than 1 percent level of glycerol. The coefficient of determination (R^2) for regression model for body and texture was 55.27 percent. The representation of the response surface is given in Fig 2. The increase in nisin increase body and texture first and then decrease and most significant. The decrease body and texture with increase level of nisin resulted more hard texture. Similarly the increase in glycerol level increase body and texture first and then decrease.

Overall acceptability

The overall acceptability score for paneer ranged between 6.7 to 8 percent. Decrease level of nisin and potassium sorbate give better sensory score than the upper level. The coefficient of determination (R^2) for regression model for overall acceptability was 48.14. Fig: 3 shows that the response surface of nisin and glycerol on overall acceptability. An addition of nisin affects OA significantly and increase nisin level increase OA first then decreases. Similarly the increase in glycerol level increase OA.

Textural Profile Analysis (TPA)

Adhesiveness

The adhesiveness of paneer was range between -0.048 to -0.38.05). The combination of ingredients level of whey protein concentrate, glycerol, potassium sorbate and nisin are 10, 3, 0.7% and 5 ppm respectively. The coefficient of determination (R^2) for the regression model for adhesiveness was 36.93%, which implies that the model could account for 36.93%, data. Lack of fit was non-significant for the adhesiveness. Interaction of whey protein concentrate with glycerol was significant at 10% level of significance. Fig 4 show that the response surface of nisin and glycerol on adhesiveness. The maximum adhesiveness occurred with increased level of glycerol and similarly effect, increase WPC level increase adhesiveness first then decrease. Increase level of WPC made high concentrated coating solution.

Springiness

The springiness of paneer varied from 0.615 to 0.868. The coefficient of determination (R^2) for the regression model for springiness was 48.81%, which implies that the model could account for 48.81%, data. Lack of fit was non-significant for the sprinsiness. Interaction of potassium sorbate was significant at 5% level of significance. Nisin and glycerol level studies indicate that high springiness could be obtained by employing nisin level. Similarly decrease in glycerol level with increase springiness (Fig 5).

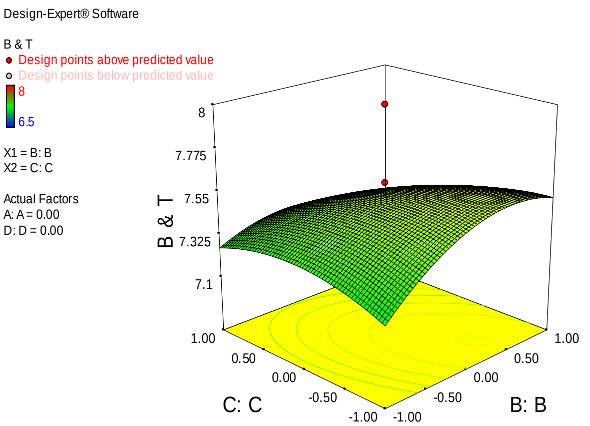


Fig 1 effect of interaction between nisin and WPC and nisin and glycerol on appearance

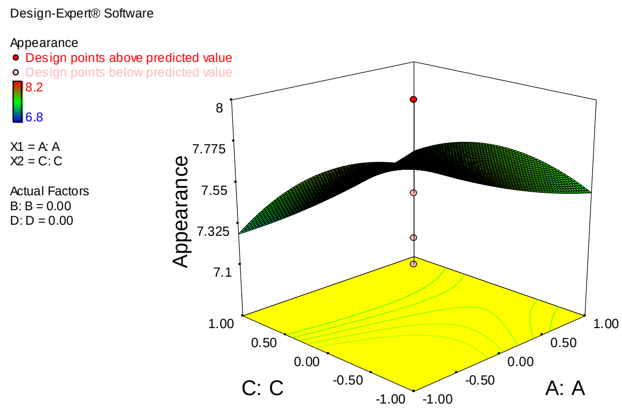


Fig 2 Effect of interaction between nisin and glycerol on body and texture

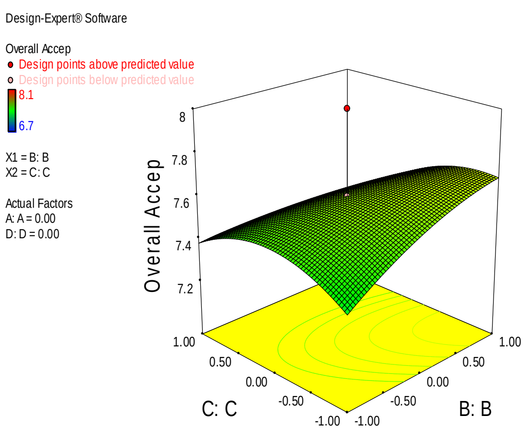


Fig 3 effect of interaction between nisin and glycerol on overall acceptability

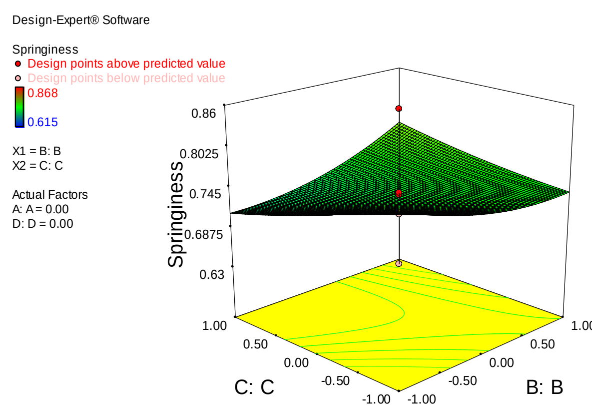
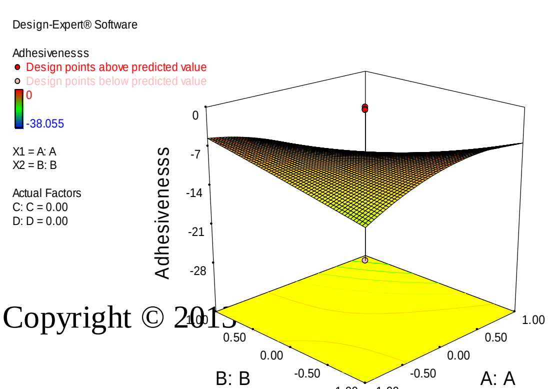


Fig 4 effect of interaction between nisin and glycerol on springiness



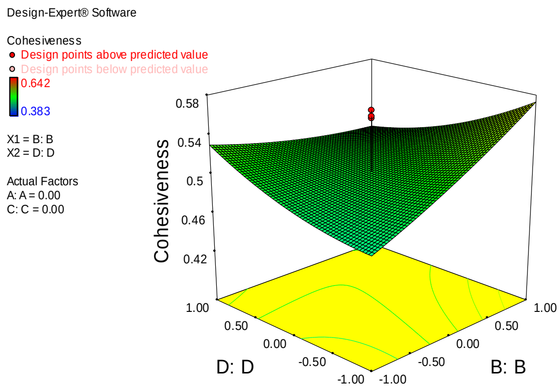


Fig 5 Effect of interaction between glycerol and WPC on adhesiveness

Fig 6 effect of interaction between glycerol and potassium sorbate on cohesiveness

Cohesiveness

The cohesiveness ranged from 15 to 24 The coefficient of determination (R^2) for the regression model for cohesiveness was 59.65 %, which implies that the model could account for 59.65 %, data. Lack of fit was non-significant for the cohesiveness. Interaction of glycerol with potassium sorbate was significant at 5% level of significance. Fig 6 showing the interaction between glycerol and potassium sorbate on cohesiveness maximum cohesiveness obtained with increase level of potassium sorbate and similarly in increase glycerol level increase cohesiveness.

Gumminess

The gumminess varied from 10.41 to 2339.20 The coefficient of determination (R^2) for the regression model for gumminess was 49.66%, which implies that the model could account for 49.66%, data. Lack of fit was non-significant for the gumminess. Fig.7. showing the interaction between nisin and potassium sorbate on gummines. It’s representing the increase level of potassium sorbate level increase gumminess and most significant. Similarly the increase in nisin level increase gumminess.

Chewiness

The chewiness varied from 7.238 to 2018.66. The coefficient of determination (R^2) for the regression model for chewiness was 50.19%, which implies that the model could account for 50.19%, data. Lack of fit was non-significant for the chewiness. Glycerol significant at 5% level of significance while interaction of nisin with potassium sorbate was significant at 10% level of significance. Representation of response surface given in fig 8. The increase level of potassium sorbate level increase chewiness and most significant. Similarly the increase in nisin level increase chewiness.

Optimization of process parameters

Numerical optimization carried out using EXPERT VISION 7.1.3 (State Ease Inc.) Statistical software. The goal fixed for x_1, x_2, x_3, and x_4 were range (-2 to 2). The response of OA and Shelf life were given a goal to maximize. The optimized levels of X_1, X_2, X_3, and X_4 were 6%, 7 %, 5ppm and 1.3

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percent respectively. The predicted value corresponding to the above optimized condition for OA WAS 8 and shelf life at 30 ^{0}C was 4 days. so that the optimized levels of x_1, x_2, x_3, and x_4 were selected and recognized.

Table 5: Optimum level of ingredients for optimized product

Variables	Coded levels
WPC	-1
GLYCEROL	1
NISIN	-1
POTASIUM SORBATE	1

Conclusion

The present investigation is directed to improve the sensory and textural properties of paneer by edible coating of whey protein concentrate and glycerol. Considering the above facts the study was planed with the aim of improvement of sensory and texture of paneer using edible coating.

Table 1: CCRD Design of experiment for optimization of paneer with edible Coating

	Levels of independent variables (coded)			
	X_1	X_2	X_3	X_4
First order experiments				
1	-1(6)	-1(3)	-1(5)	-1(0.7)
2	1(10)	-1(3)	-1(5)	-1(0.7)
3	-1(6)	1(7)	-1(5)	-1(0.7)
4	1(10)	1(7)	-1(5)	-1(0.7)
5	-1(6)	-1(3)	1(10)	-1(0.7)
6	1(10)	-1(3)	1(10)	-1(0.7)
7	-1(6)	1(7)	1(10)	-1(0.7)
8	1(10)	1(7)	1(10)	-1(0.7)
9	-1(6)	-1(3)	-1(5)	1(1.3)
10	1(10)	-1(3)	-1(5)	1(1.3)
11	-1(6)	1(7)	-1(5)	1(1.3)
12	1(10)	1(7)	-1(5)	1(1.3)

13	**-1(6)**	**-1(3)**	**1(10)**	**1(1.3)**
14	**1(10)**	**-1(3)**	**1(10)**	**1(1.3)**
15	**-1(6)**	**1(7)**	**1(10)**	**1(1.3)**
16	**1(10)**	**1(7)**	**1(10)**	**1(1.3)**
Second order experiments				
17	**-2(4)**	**0(5)**	**0(7.5)**	**0(1)**
18	**2(12)**	**0(5)**	**0(7.5)**	**0(1)**
19	**0(8)**	**-2(1)**	**0(7.5)**	**0(1)**
20	**0(8)**	**2(9)**	**0(7.5)**	**0(1)**
21	**0(8)**	**0(5)**	**-2(2.5)**	**0(1)**
22	**0(8)**	**0(5)**	**2(12.5)**	**0(1)**
23	**0(8)**	**0(5)**	**0(7.5)**	**-2(0.4)**
24	**0(8)**	**0(5)**	**0(7.5)**	**2(1.6)**
Center point experiments				
25	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**
26	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**
27	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**
28	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**
29	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**
30	**0(8)**	**0(5)**	**0(7.5)**	**0(1)**

Where, X_1- whey protein concentrate, X_2- glycerol, X_3 – potassium sorbate X_4-Nisin

Table 2: Sensory responses obtained in the experiments

APPERENCE	FLAVOUR	BODY &TEXTURE	OVERALL ACCEPTABILTY
7.2 ± 0.404	6.9 ± 0.288	6.6 ± 0.50	6.7 ± 0.25
8 ± 0.0577	7.5 ± 0.25	7.3 ± 0.25	7.6 ± 0.626
7.41 ± 0.252	7.37 ± 0.25	7.33 ± 0.25	7.41 ± 0.409
7.1 ± 0.132	7.5 ± 0.464	7.1 ± 0.419	7.25 ± 0.500
7.58 ± 0.381	8 ± 0.125	7.5 ± 0.152	7.75 ± 0.264
7.41 ± 0.180	7.2 ± 0.152	7.4 ± 0.404	7.4 ± 0.30
7.25 ± 0.388	7.15 ± 0.25	7.2 ± 0.144	7.2 ± 0.125
7.2 ± 0.10	7.6 ± 0.10	7.2 ± 0.25	7.3 ± 0.25
7.78 ± 0.435	7.87 ± 0.381	8 ± 0.160	7.78 ± 0.381
7.85 ± 0.225	7.75 ± 0.10	7.6 ± 0.10	7.5 ± 0.257
7.91 ± 0.25	7.91 ± 0.520	7.75 ± 0.50	7.51 ± 0.629
7.5 ± 0.25	7.9 ± 0.321	7.5 ± 0.25	7.55 ± 0.427
7± 0.132	7 ± 0,208	7 ± 0 .2	7 ± 0.1
7.1 ± 0.305	7.1 ± 0.4	7.5 ± 0.1	7.50 ± 0.152
6.8 ± 0 .10	7.2 ± 0.1	7 ± 0.435	7 ± 0.381
6.9 ± 0.125	7.2 ± 0.25	7.3 ± 0.208	7.4 ± 0.20
7.8 ± 0.230	7.6 ± 0.205	7.6 ± 0.208	7.6 ± 0.152
7.8 ± 0.381	7.4 ± 0.102	7.6 ± 0.381	7.8 ± 0.25
8.2 ± 0.251	7.8 ± 0.5	7.7 ± 0.2	8.1 ± 0.152

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7.25 ± 0.381	7.6 ± 0.264	7 ±. 0404	7.6 ± 0.264
7.75 ± 0.50	7.7 ± 0.5	7.72 ± 0. 585	7.7 ± 0.305
7.25 ± 0.152	7.25 ± 0.208	7.6 ± 0. 208	7.5 ± 0.251
7.5 ± 0.25	7.6 ± 0.305	7.6 ± 0.50	7.6 ±. 0.327
7.5 ± 0.189	7.4 ± 0.20	7.6 ± 0.5	7.6 ± 0.302
7 ± 0.25	7.2 ± 0.360	7.4 ± 0.360	7.3 ± 0.381
7.2 ± 0.803	7.4 ± 0.381	7.5 ± 0.629	7.5 ± 0.629
7 ± 0.25	7 ± 0.25	6.5 ± 0.208	7 ± 0.381
8 ± 0.152	8 ± 0.563	8 ± 0.152	8 ± 0.381
8 ± 0.25	8 ± 0.20	8 ± 0.315	8 ± 0.321
8 ± 0.25	8 ± 0.25	8 ± 0.25	8 ± 0.25

Table 3: Estimate significant coefficient of the fitted equation for the different responses

FI	AP	B &T	OA	AD	SP	CH	GM	CW
B_0	0.201	0.297	0.493	0.802	0.481	0.193	0.456	0.4404
β_1	0.056***	0.863	0.712	0.298	0.957	0.011*	0.064***	0.0826***
β_3	0.835	0.531	0.427	0.379	0.789	0.135	0.528	0.5989
β 3	0.029**	0.231	0.153	0.703	0.450	0.283	0.267	0.2944
β_4	0.458	0.731	0.921	0.556	0.048**	0.735	0.261	0.1813
β_{12}	0.821	0.401	0.951	0.096***	0.350	0.591	0.666	0.5029
β_{13}	0.098***	0.101***	0.102***	0.669	0.614	0.634	0.825	0.8161
β_{14}	0.865	0.944	0.598	0.914	0.614	0.263	0.654	0.5944
β_{23}	0.082***	0.228	0.206	0.464	0.101***	0.334	0.877	0.8439
β_{24}	0.966	0.726	0.763	0.689	0.393	0.043**	0.236	0.2414
β_{34}	0.293	0.921	0.821	0.560	0.562	0.692	0.080***	0.0856***
β_{11}	0.521	0.430	0.466	0.235	0.988	0.678	0.777	0.7559
β_{22}	0.208	0.031**	0.402	0.958	0.211	0.619	0.456	0.4158
B_{33}	0.047**	0.172	0.056***	0.933	0.715	0.281	0.515	0.5465
β_{44}	0.754	0.255	0.918	0.704	0.152	0.317	0.275	0.2384
R^2 (%)	59.29	55.27	48.4	36.93	48.81	59.65	49.66	50.19
F Value	1.56	1.32	1.01	0.63	1.02	1.58	1.06	1.08
LOF	ns	ns	ns	ns	ns	ns	ns	ns

***,** ,* are significant at 1, 5 and 10% level of significance

β 1 whey protein concentrate, β 2 glycerol, β 3 nisin, β 4 potassium sorbate
FI- Factor Interpretation, Ap- Appearance, B&T - Body &Texture, OA- Overall Acceptability, AD- Adhesiveness, SP- Springiness, CH- Cohesiveness, GM- Gumminess, CW- Chewiness

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