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RESEARCH PAPER

Development of Compressed Meat based Bar using Response Surface Methodology

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ABSTRACT

Studies were carried out to optimize the percentage of ingredients for the development of ready to eat mutton bar. Central composite rotatable design of response surface methodology was used for designing the experimental combinations. Matrix for compression was designed by selecting factors like mutton powder, binders and applied pressure. Protein percentage, hardness and over all acceptability (OAA) were taken as responses. OAA and hardness showed highly significant and fitted with quadratic model whereas other response i.e. protein levels found to be significant and fitted with linear model. From the design of experiments 45 g / 100 g of mutton powder with 5 g /100 g binders having an applied pressure of 142 kg/cm2 yielded a bar having a protein percentage of 35 g /100 g with a hardness of 20.9 N with an overall acceptability score of 8.6 ±0.2 on a 9 point hedonic scale. The product gives energy of 393 kcal per 100 g.

Keywords: RSM; OAA; Protein; Mutton powder; Hardness; Responses

NOMENCLATURE

RSM	Response surface methodology
OAA	Over all acceptability
CCD	Central composite designs
CCRD	Central composite rotatable design
g	Gram
kCal	Kilo calorie
N	Newton
Kg:	Kilo gram
Cm^2	Centimetre per square
R^2	The coefficient of determination
AOAC	Association of official analytical chemist
ANOVA	Analysis of variance

1. INTRODUCTION

Convenient and functional foods play an important role in human health promotion and have taken a distinct change in the recent past. Due to the changing lifestyle habits amongst consumers, the consideration of quality and safety becomes the important yardstick in the case of meat and meat products. Ensuring longer shelf stability by protecting physico-chemical, microbiological and sensory alterations have become very crucial in meat industry to develop quality convenient products[1-2]. Nowadays, consumers prefer food products with more healthier and functional components with modified composition of nutrition that can cater to the demanding requirements[3]. Development of compact energy bars which are quick source of high energy nutrition that can replace usual meals in a convenient form is gaining importance in food industry. These bars can be prepared by balancing the macro nutrient supplements through cereals, meat, vegetables and targeted for people who require quick energy in a convenient form. Energy bars are handy, portable and refrigerator-free snack with no preparation needed. Bar is meant to give soldiers in training or combat an energy boost while on the move. Energy bars give soldier in combat a boost at critical times. The bar is often packaged with field rations. Soldier fuel often named as ‘Hooah’ is the first energy bar which was introduced to the U.S military in 1996. Energy bars provide more energy and calories to hungry soldier who need to eat while working and in physically demanding situations.

There are few studies related to development of cereal and protein bars from vegetarian sources[4-5]. As such literature is scanty on non-vegetarian based protein supplementary food bars. Meat is a good source of protein, vitamins and minerals which can supplement good energy. Bars are usually multi component heterogeneous systems and can undergo several changes in terms of physico-chemical, microbiological and sensory attributes during transportation and storage[6]. Hence standardization of the ingredients for getting better quality attributes is crucial in the development of compressed bars. Applied pressure plays an important role in establishing the textural properties and it has to be in accordance with the ingredients and binders. The greatest difficulty in obtaining a good mutton bar is combination of several ingredients with which it can deliver macro and micro nutrients and also functionality, using binders which can be turned in to a bar with flavour, texture and decent appearance. The problem in the development of a product is essentially optimization of the ingredients. So in the present study for the search for the best formulation determination of optimum levels of key

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ingredients were taken as variables and the dependent factors as responses.

The RSM approach was first tried by Box and Wilson[7] in 1951 and it deals with the relationships between several explanatory variables and one or more response variables. In this methodology, the optimal response is obtained by using a sequence of experiments. In this approach, data on mathematical and statistical techniques are employed for building an empirical model. Here the experiments are designed to optimize a response (output variable) that is influenced by various independent variables (input variables). Experiment is usually conducted in the form of series of tests which are called 'runs' in which the input variables are usually changed to interpret the reasons for the output response changes.

Initially, RSM had been introduced for the modelling of experimental responses[8], further and it was extended to numerical experiments. The introduction of RSM was mainly aimed to replace the other expensive analytical methods (e.g. finite element method of central factorial design analysis) and their associated numerical noise. The advantages of using RSM for design optimization applications have been discussed[9]. With the application of central composite designs (CCD) a second-order model has been efficiently constructed[10]. CCD is first-order (2N) design augmented by additional centre and axial points to allow estimation of the tuning parameters of a second-order model.

Sensory analysis plays an important role in designing and formulation of a product with good organoleptic attributes. Here physiological and psychological perceptions are employed for the evaluation of the products. Evaluation is based on the Hedonic scales usually carried out by trained and untrained panelists and the best results are usually achieved by untrained panelists[11]. RSM technique gives the effects of an individual parameter as well as interactive effect[12]. This statistical tool has been employed for the standardization and optimization of processing variables[13]. So studies have been carried out to formulate the ingredient combination and binders for the development of meat based bar having good quality attributes.

2. MATERIAL AND METHODS

2.1 Raw materials

The meat employed in the study was procured from Mysuru local market. Boneless meat within 1-2 hours slaughter and after rigor mortis was cut into uniform pieces. The meat was washed thoroughly in running water and used in the study.

2.2 Chemicals and Reagents

Analar grade reagents and chemicals were employed in the study and procured from M/s BDH Company.

2.3 Experimental design

Central composite rotatable design (CCRD) was applied to determine the best combination of mutton powder , binder and applied pressure to optimize the ingredients combination for the development of protein enriched mutton bar using software State–Ease (Design Expert version 6.0.10). Mutton powder, binder and applied pressure were taken as independent variables OAA, hardness and protein as responses. Design of experiments for the development of protein enriched mutton bar has been shown in Table 1. The factorial design with 17 sets of experiments consisted of seven factorial points, six central points and seven axial points[14]. The variables with significant level $p < 0.05$ of the polynomial regression have been included in the model and to establish the accuracy, coefficient of determination (R^2) was generated. Using the values of each independent variable to the maximum quadratic response the response surfaces were generated from the equation of the second order polynomial[15].

First order Linear Eqn (1)

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i \tag{1}$$

Second-order polynomial Eqn (2)

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} {x_i}^2 + \sum_{i=j=1}^{n} \beta_{ii} x_i x_{ij} \tag{2}$$

where, 0 was the value of the fitted response at the center point of the design, i.e., point (0,0,0) in case of mutton powder, binder and applied pressure ; *i*, *ii* and *ij* were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables.

2.4 Texture Profile Analysis

The textural characteristics of the product in terms of hardness were established using Texture Analyser, (TA Plus, Lloyd Instruments, Hampshire, UK) as per Kim[16], *et al*.

2.5 Sensory Evaluation

The samples have been evaluated for Sensory characteristics by 13 semi-trained panellists using a 9 point hedonic scale (9- like extremely, 1- dislike extremely) as per Murray[17], *et al.* . The overall acceptability of the product can be estimated from these studies.

2.6 Proximate Analysis of the Product

Proximate composition of optimised mutton bar was established as per AOAC[18] for moisture, protein, fat, carbohydrate and total ash.

2.7 Statistical Analysis

The generated data from the analysis were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to establish the statistical significance of the treatments and significance was established at $p<0.05$. Response surfaces were generated using the Design Expert version 6.0.10 software (Stat Ease Inc., Minneapolis, MN).

3. RESULTS AND DISCUSSION

The designing of the experimental combinations for mutton bar development was carried out by the application of central composite rotatable design (CCRD) of RSM. The independent variables were chosen as Mutton powder, binder and applied pressure. The responses selected in this design approach were OAA, hardness and protein percentage. Sensory attributes of the food product was the criteria for the determination of overall acceptability of the product.

Experimental ranges and levels of independent variable terms of actual and coded factors and design of experiment with variables and responses were shown in Table 1. The second order polynomial equation was fitted using the results obtained from Central composite design. Regression analysis of all the three responses such as OAA, Hardness and Protein were conducted by fitting the quadratic (OAA & Hardness) and linear model (Protein). Analysis of variance was calculated and model statistics for all the responses were shown in Table 2. Responses like OAA, Hardness showed highly significant ($p<0.05$) and fitted with quadratic model whereas other response i.e. protein levels found to be significant and fitted with linear model. The statistical significance was established at $p < 0.05$.

Table 1. Design of experiment for the development of protein enriched mutton bar

Run	Factors			Responses		
	Mutton powder (g/100g)	Binder (g/100g)	Applied pressure (Kg/cm^2)	Hardness (N)	OAA	Protein (g/100g)
1	45.00	5	142.00	20.9	8.6	35
2	65.00	3	124.00	18.6	7.2	53.6
3	45.00	5	111.73	16.6	7.6	34.4
4	65.00	3	160.00	26.0	7.2	52.7
5	78.64	5	142.00	20.5	6.8	56.6
6	25.00	7	160.00	23	7.2	19.2
7	45.00	5	142.00	20.9	8.5	35
8	25.00	3	124.00	19.2	6.3	19.2
9	65.00	7	160.00	23	6.9	52.7
10	45.00	5	172.27	24.5	8.0	36.1
11	25.00	7	124.00	18	6.9	19.2
12	45.00	5	142.00	20.9	8.6	35
13	11.36	5	142.00	23	7.0	9.3
14	65.00	7	124.00	20.1	7.2	53.6
15	45.00	1.64	142.00	19.5	7.1	34.8
16	25.00	3	160.00	22	6.8	19.2
17	45.00	8.36	142.00	21.5	6.8	34.6

Table 2. ANOVA and model statistics for protein enriched mutton bar

Parameters	Hardness	OAA	Protein
SD	1.08	0.20	1.74
Mean	21.05	7.52	35.26
C.V	5.11	2.59	4.95
Press	34.18	2.84	89.18
R-squared	0.7928	0.9675	0.9859
Adjusted R Squared	0.7540	0.9382	0.9832
Predicted R Squared	0.6179	0.7575	0.9741
Adequate precision	15.89	15.16	67.98
Model	**Linear**	**Quadratic**	**Linear**

Dressed meat
⇩
Washing & Deboning
⇩
Cutting meat into uniform pieces
⇩
Marination (lemon juice, salt and spices at 5°C for 1hr)
⇩
Cooking (open pan cooking at 90-95°C for 30 min)
⇩
Mincing (Hobart, Model 4812-CE, Offenburg, Germany)
⇩
Hot air dehydration (75°C for 8 hrs, Model 4596, HEMCO Madras, India)
⇩
Coarse powdering of dried mince

Figure 1. Process flow chart for the preparation of mutton powder for mutton bar.

The effect of variations in the levels of independent variables on three responses has been depicted as 3D response plots as hardness, OAA, and protein levels in Figs. 2, 3, and 4, respectively. From these figures it was observed that levels of addition of mutton powder found to have more impact followed by binder and applied pressure levels on desirability and OAA, whereas addition of binder and applied pressure levels have shown higher impact on hardness and protein levels in mutton bar as seen from the Figs. 2, 3, and 4.

Responses were optimised using Design Expert version-6 software. Optimization of the independent variable levels (mutton powder, binders and applied pressure) were achieved based on the maximization of the responses (OAA, Hardness and Protein) and suitable desirability was taken as optimised ingredient levels. From this design best among the suitable desirability was taken as optimised ingredient levels. Optimised levels of mutton bar variables are mutton powder (45 g/100 g) binder (5 g/100 g) and applied pressure (142 kg/cm^2) and responses found to be OAA (8.6), hardness (20.9 N) and protein (35 g/100 g). Optimised levels of variables were employed in the development of the product and the responses generated were evaluated and examined with the predicted values. The results indicated similarities in predicted and actual values and hence, the optimised levels of ingredients were recommended for the development of the product.

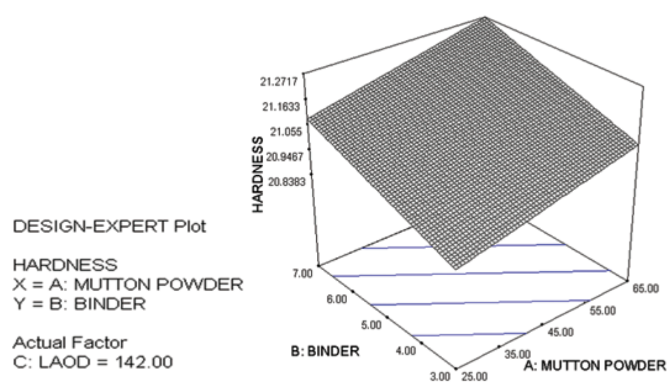

Figure 2. 3D plot depicting effect of independent variables binder and mutton powder on hardness.

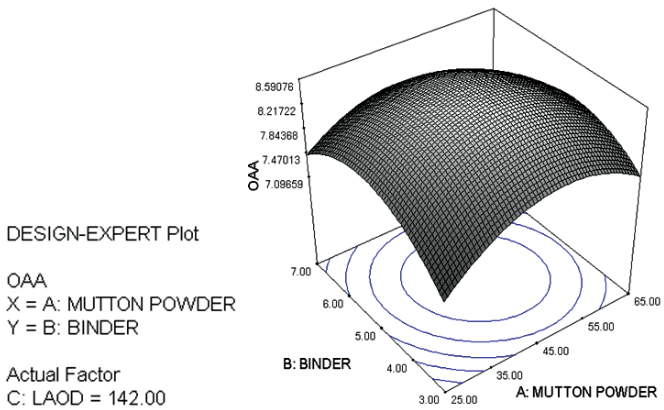

Figure 3. 3D plot depicting effect of independent variables binder and mutton powder on OAA.

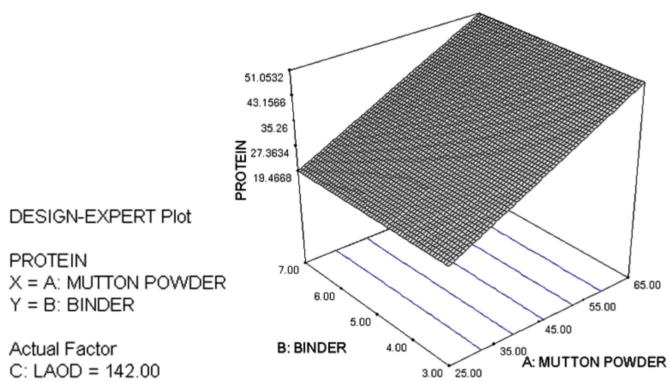

Figure 4. 3D plot depicting effect of independent variables binder and mutton powder on Protein.

Multiple regression equations (in terms of coded factors) generated for all three responses are represented as follows:

Final Equation

Sensory score of protein enriched mutton bar (OAA)

(OAA)=+8.59+0.71*a+0.014*b+0.086*c-0.63*a^2-0.61*b^2-0.31*c^2-0.16*a*b-0.14*a*c-0.062*b*c

Hardness levels in protein enriched mutton bar

Hardness = +21.06+0.095*a+0.12*b+2.27*c

Protein percentage in protein enriched mutton bar

Protein = +35.26+15.77*a-0.025*b+0.078*c

3.1 Proximate Composition

The mutton bar prepared as per the designed formulation has been subjected for proximate evaluation and the values are reflected in Table 3. It has a good percentage of protein 35.13 g/100 g and a moderate percentage of fat 10.14 g/100 g. It also provides 36.98 g/100 g of carbohydrate. These macronutrients provide nearly 393 kCal of energy per 100 g. This is convenient combat ready to eat bar which exhibit good textural characteristics.

4. CONCLUSION

Studies revealed the feasibility of employing mutton powder and binder as major ingredients for the preparation of mutton bar. Optimisation of applied pressure is very important to get optimum textural characteristics and sensory attributes. RSM can be successfully employed for the optimisation of ingredients to give better quality characteristics of the product. 45 g/100 g of mutton powder, 5 g/100 g of binder and applied pressure of 142 kg/cm^2 were found to be ideal in delivering a product with good physical attributes and a better protein percentage in addition to good sensory attributes. These bars are handy, portable, combat and refrigerator-free snack with no preparation needed. It is meant to give soldiers in training or combat, an energy boost while on the move and also at critical times. These convenient ready to eat protein rich bars will have great potential in civilian and Armed forces.

Table 3. Proximate composition of ready to eat protein enriched mutton bar

Parameter (g/100g)	Protein enriched mutton bar
Moisture	9.83± 1.30
Fat	10.14 ± 0.89
Protein	35.31 ± 0.36
Total carbohydrate	36.98 ± 0.15
Total ash	5.74 ± 0.13
Energy (kCal)	393

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Mrs Khudsia Sultana is working as a Technical Officer ‘A’ at Defence Food Research Laboratory, Mysuru. Her current research interests include development of light weight meat products and freeze dried probiotic juice powders. She has contributed significantly towards the development and evaluation of convenient meat and poultry products and beverage powders with functional attributes by various processing techniques like irradiation, freeze dehydration, compression technology and freezing.
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He contributed towards experimental planning, execution, data analysis of the manuscript.