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RESEARCH ARTICLE

Development of mushroom and wheat gluten based meat analogue by using response surface methodology

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Abstract

The central composite rotatable design of response surface methodology (RSM) was used for designing the experimental combinations for the development of meat analogue. Ingredient combination like Mushroom, Gluten and Soya chunk were taken as independent variables and overall acceptability (OAA), hardness and protein levels as responses. Responses like OAA, 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. 40 % mushroom and 30 % wheat gluten resulted in a product with good sensory and textural attributes in addition to a good source of protein (36%). Optimised levels of ingredients after mixing and subjected for steaming for 15 psi for 20 min delivered a product with good binding characteristics and exhibited OAA score of 7.6±0.3. The product gives energy of 224 kcal per 100 g.
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INTRODUCTION

Meat analogue is a vegetarian and meat substitute product. Foods high in protein are known to satiate more fully than foods high in other constituents. Development of new food products that are attractive to consumers is a challenge to food technologists (Van Trijp et *al.*, 2008). However, it is even more complex when these new foods are meant as a substitute for products that are highly appreciated, like meat (Wansink *et al.*, 2005). This challenge is faced by researchers and developers of new sustainable meat substitutes that need to reduce the negative environmental impact of industrial-scale meat production for human consumption (Aiking *et al.*, 2004). These new meat substitutes are thus not intended for vegetarians but need to attract new consumers, namely current meat consumers, and ought to facilitate meat avoiders to decrease their consumption of meat even further. Meat substitute products are currently primarily aimed and used by vegetarians and semi-vegetarians and have a strong emphasis on health and ethical quality aspects (Hoek *et al.*, 2004). Studies have shown that a premium for organic alternatives (organic meat) was a critical limiting factor in consumer acceptance (Grunert *et al*., 2004; Van Loo *et al.*, 2010). Soy protein, mixed with gluten and starch, was extruded into fibrous meat analogues under high moisture and high-temperature conditions (Keshun Liu and Fu-Hung Hsieh, 2008). Soy protein and wheat gluten have been the dominant raw materials for meat surrogates for a long time (Li *et al.*, 2007). Over recent decades, protein products from other plant based raw materials such as peas, chickpeas, lupins, rice, maize and canola have been developed in food grade quality (Boye *et al*., 2010). Additionally, protein products from animal-based sources such as milk and eggs and from new sources such as mushroom (fungi) and bacteria are available nowadays (Florian wild and Michael czerny *et al.*, 2014).

RSM explores the relationships between several explanatory variables and one or more response variables. RSM is a collection of mathematical and statistical techniques for empirical model building. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. This statistical tool has been employed

for the standardisation and optimizing processing variables for different products like mutton Manchurian (Jalarama reddy *et al.*, 2013) and food-processing operations (Madamba, 2002).

As such most of the meat substitutes are soya based and not matching with the micronutrients formulation of actual meat. So studies have been carried out to formulate the ingredients combination and binders for the development of a mushroom based meat analogue which can supplement better nutritional profile than actual meat. In this regard for the optimization of the ingredients RSM has been employed.

Material and method

Raw material selection

Fresh button mushroom (*Agaricus biosporus*) and soya chunk were procured from the local market, cleaned and washed thoroughly in running water. The other ingredients used for the preparation include wheat gluten, Garlic & Ginger paste, Turmeric powder, lemon juice, Salt, Garam masala and Pectin.

Chemicals and Reagents

All the reagents and chemicals used for the study were of Analar grade and procured from M/s Sigma Chemicals, Corporation, USA and M/s BDH Company.

Experimental design

Central composite rotatable design (CCRD) was applied to determine the best combination of Mushroom, Gluten and Soya chunk to optimize the ingredients combination for the development of meat analogue using software State–Ease (Design Expert version 6.0.10). Mushroom, Gluten and Soya chunk were taken as independent variables and overall acceptability (OAA), hardness and Protein as responses. Design of experiments for the development of meat analogue was shown in Table 1. The factorial design with 20 sets of experiments consisted of seven factorial points, six central points and seven axial points (Myers and Montgomery, 2002). All variables of the polynomial regression at a significance level of $p < 0.05$ were included in the model, and the coefficient of determination (R^2) was generated in order to assess the accuracy of the model. The response surfaces were generated from the equation of the second order polynomial, using the values of each independent variable to the maximum quadratic response (Sin *et al.*, 2006).

First order Linear Equation (1)

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

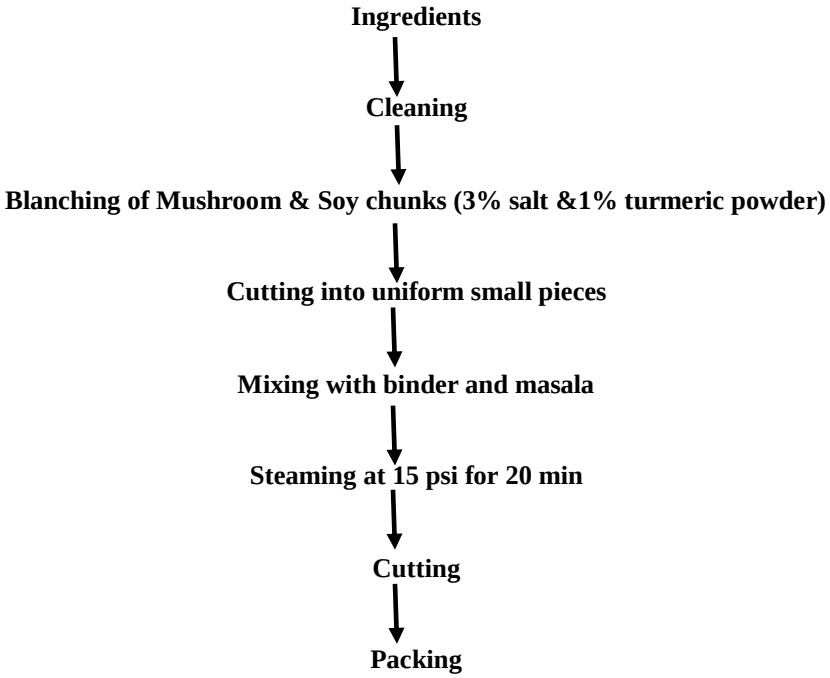
Second-order polynomial Equation (2)

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

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 Mushroom, Gluten and Soya chunk ; *i, ii* and *ij* were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables.

Preparation of product

The product was prepared as per the standardized formulation of the ingredients and the process flow chart has been depicted below.



Texture Profile Analysis (TPA)

The textural characteristics of the product in terms of hardness was estblished using Texture Analyser, (TA Plus, Lloyd Instruments, Hampshire, UK).

Sensory Evaluation :

Sensory evaluation of meat analogue samples were carried out by 13 semi-trained panellists on a 9 point hedonic scale (9- like extremely, 1- dislike extremely) as per Murray *et al.*, (2001) to establish the overall acceptability of the product.

Proximate analysis of the product

Proximate composition of optimised meat analogue was established as per AOAC methods (2000) for moisture, protein, fat, carbohydrate and total ash.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to evaluate 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).

Results and discussion

The central composite rotatable design (CCRD) of RSM was used for designing the experimental combinations for the development of meat analogue. Ingredient combination like Mushroom, Gluten and Soya chunk were taken as independent variables and OAA, hardness and protein as responses. 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. Central composite design results were used to fit the second order polynomial equation. Regression analysis of all the three responses such as OAA, Hardness and Protein was conducted by fitting the quadratic (OAA & Hardness) and linear model (Protein). Analysis of variance was calculated and model statistcs for all the responses were shown in Table 2. Responses like OAA, 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. The p-value showed less than 0.05 for model to be significant.

The effect of variations in the levels of independent variables on three responses has been depicted as 3D response plots as desirability, OAA, Hardness and protein levels in Figs 1, 2, 3 & 4 respectively. From these Figs it was observed that levels of addition of mushroom found to have more impact followed by gluten and soy chunk levels on desirability and OAA, whereas addition of gluten and soy chunk levels have shown higher impact on hardness and protein levels in meat analogue.

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

Table 1: Design of experiment for the development of meat analogue

Run	Factors			Responses		
	Mushroom (%)	Gluten (%)	Soya chunk (%)	OAA	Hardness	Protein (%)
1	37.50	32.50	6.95	7.40	2.9	28.14
2	40.00	30.00	9.00	7.40	3.4	33.11
3	37.50	32.50	12.00	6.0	2.02	31.45
4	37.50	32.50	17.05	6.2	3.12	39.33
5	33.30	32.50	12.00	6.0	2.5	31.62
6	35.00	35.00	9.00	7.90	2.67	32.14
7	35.00	30.00	15.00	6.80	2.55	34.36
8	35.00	30.00	9.00	6.30	2.64	33.2
9	40.00	30.00	15.00	7.60	2.5	36.03
10	37.50	32.50	12.00	6.40	2.3	34.60
11	37.50	32.50	12.00	6.20	2.3	33.60
12	37.50	32.50	12.00	6.00	2.35	34.20
13	35.00	35.00	15.00	7.00	3.25	32.43
14	37.50	36.70	12.00	7.20	2.3	35.20
15	37.50	32.50	12.00	6.50	2.35	34.19
16	40.00	35.00	9.00	7.50	2.5	34.04
17	37.50	28.30	12.00	6.80	2.58	31.14
18	37.50	32.50	12.00	6.40	2.1	31.81
19	40.00	35.00	15.00	7.00	2.3	39.04
20	41.70	32.50	12.00	7.20	2.2	33.31

Table: 2 ANOVA and model statistics for meat analogue

Parameters	OAA	Hardness	Protein
S.D	0.32	0.14	1.77
Mean	6.79	2.54	33.40
C.V	4.75	5.70	5.31
Press	6.52	0.98	86.97
R squared	0.8464	0.9200	0.6157
Adjusted *R* Squared	0.7081	0.8481	0.5437
Predicted *R* Squared	0.0357	0.6261	0.3355
Adequate Precision	6.390	10.369	9.771
Model	**Quadratic**	**Quadratic**	**Linear**

Table 3: Proximate composition of ready to eat meat analogue

Parameter (g/100g)	Meat analogue
Moisture	**47.89 ± 0.96**
Fat	**5.15 ± 0.21**
Protein	**36.38 ± 0.85**
Total carbohydrate	**8.15 ± 0.28**
Total ash	**2.43 ± 0.15**
Fibre	**4.51 ± 0.54**
Energy (k/cal)	**224**

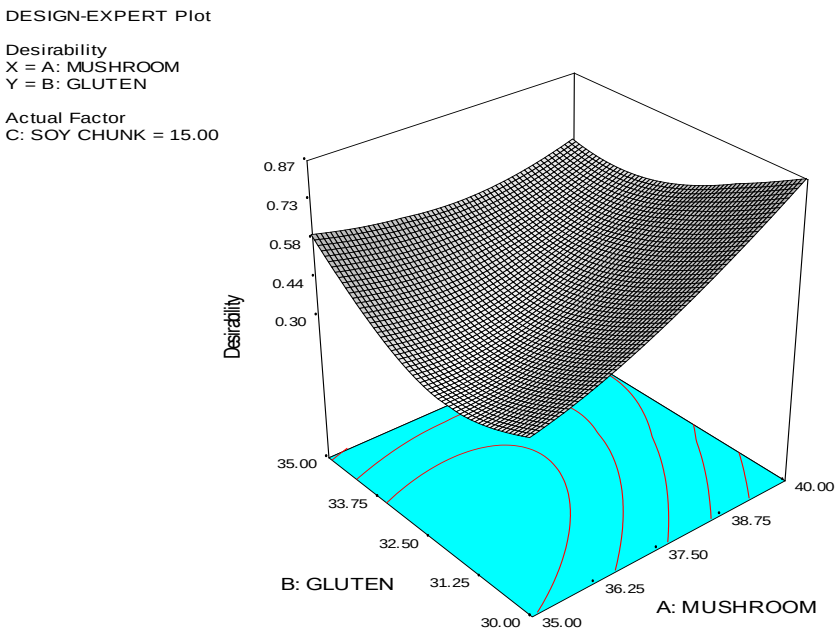


Fig 1: 3D plot depicting effect of independent variables Gluten and Mushroom on Desirability

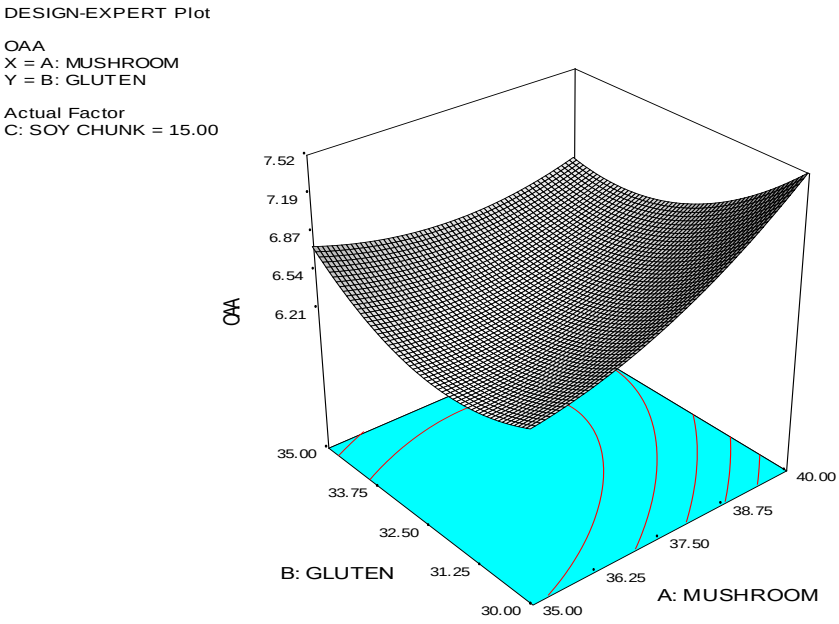


Fig 2: 3D plot depicting effect of independent variables Gluten and Mushroom on Overall acceptability

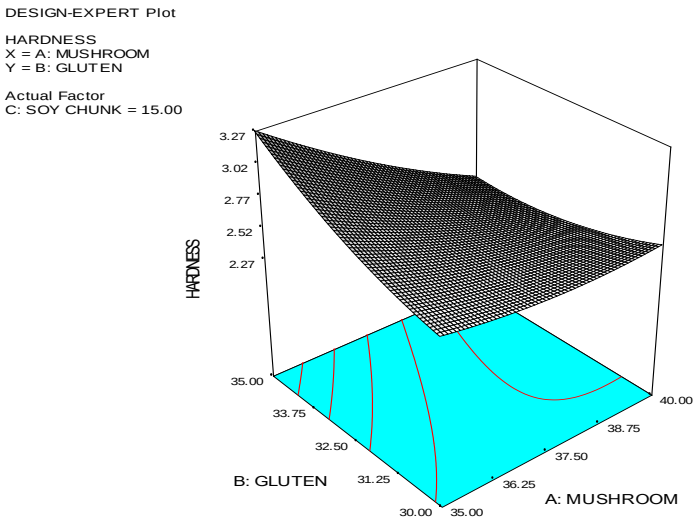


Fig 3: 3D plot depicting effect of independent variables Gluten and Mushroom on Hardness

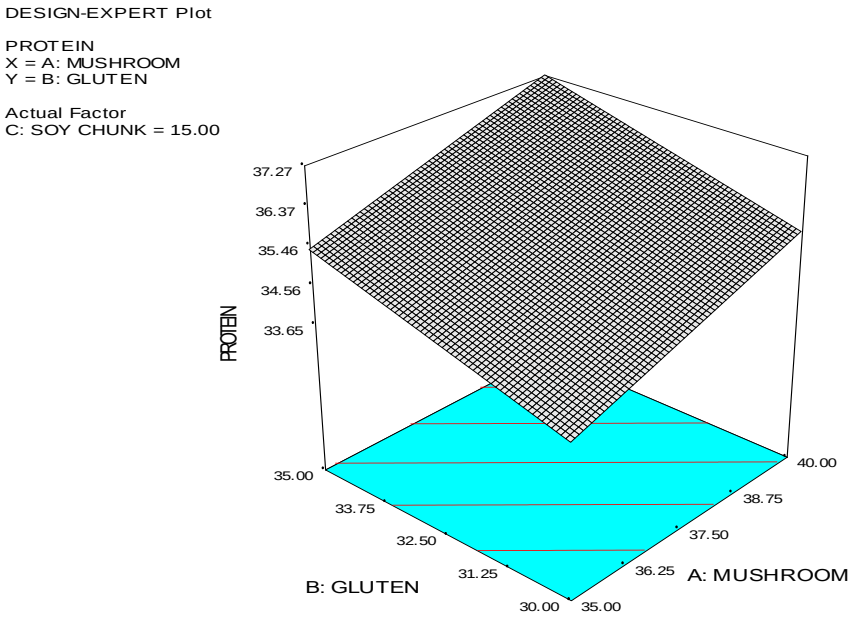


Fig 4: 3D plot depicting effect of independent variables Gluten and Mushroom on Protein (%)

Sensory score of meat analogue (OAA)

OAA =+6.24+0.26*A+0.14*B-0.20*C+0.20*A2+0.34*B2+0.27*C2-0.29*A* B+0.012*A*-0.26 * B * C

Hardness levels in meat analogue

HARDNESS=+2.23-0.067*A-0.062*B-0.018*C+0.062*A2+0.094*B2+0.30*C2-0.23*A*B-0.20*A*C+0.17*B*C

Protein percentage in meat analogue

PROTEIN = +33.40+0.95*A+0.86* B+2.06*C

Responses were optimized using Design Expert version-6 software. Optimization of the independent variable levels (Mushroom, Gluten and Soya chunk) was achieved based on the maximization of the responses (OAA, Hardness and Protein) and suitable desirability was taken as optimized ingredients levels. From this design best among the suitable desirability was taken as optimised ingredients level. Optimised levels of meat analogue variables are mushroom (40%) gluten (30.10%) and soy chunks (15 %) and responses found to be OAA (7.61), hardness (2.50 N) and protein (36.11%). Product was developed with optimised levels of variables and responses were analysed and verified with the predicted values. It showed that predicted and actual values were almost similar hence, the above levels of ingredients were recommended for the optimization of the product.

Proximate composition

The ready-to-eat (RTE) meat analogue is having a good percentage of quality protein (more than meat) from vegetable and cereal sources and a moderate percentage of fat. In addition to these macro nutrients the product also provides carbohydrates and important minerals. As such meat lacks in fibre content but the substitute made from vegetable and cereal sources (meat analogue) contains about 4-5 % fibre. It provides 224 kcal of energy per 100 g and a convenient product which exhibits good textural characteristics. Proximate composition of RTE meat analogue has been shown in Table 3.

Conclusion

Studies revealed the feasibility of employing mushroom and gluten as major ingredients for the preparation of meat analogue. RSM can be successfully employed for the optimisation of ingredients to give better quality characteristics of the product. 40 % of mushroom and 30 % of wheat gluten was found to be ideal in delivering a product with good physical attributes and a better protein percentage than meat in addition to good sensory attributes. The product is an ideal substitute to meat in terms of its nutrient formulation and a boon to vegetarians as well as non vegetarians who wants to change their lifestyle.

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