Agriculture and Food

http://www.aimspress.com/journal/agriculture

AIMS Agriculture and Food, 1(4): 409-418
DOI: 10.3934/agrfood.2016.4.409
Received 14 August 2016,
Accepted 18 October 2016,
Published 24 October 2016

Research article

Modelling and optimization of texture profile of fermented soybean using response surface methodology

Birabrata Nayak[1], **Bibhu Prasad Panda**[2,*]

[1] Department of Food Technology, Faculty of Engineering and Interdisciplinary Sciences, Jamia Hamdard, New Delhi, India
[2] Microbial and Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India

* **Correspondence:** Email: bibhu_panda31@rediffmail.com; Tel: + 91-9990335013.

Abstract: In the present research, a meaty textured soybean was prepared by solid-state fermentation using *Rhizopus oligosporus* and dried *Agaricus* mushroom. The textural profile of the fermented soybean was optimized, modelled and validated by comparing the product with poultry meat. Under the optimum condition; thickness of solid substrate, inoculums volume and quantity of *Agaricus* mushroom powder were measured to be 1.12 cm, 5.92% (v/w) and 4.84 % (w/w), respectively. The final product is found to possess hardness 538.11 *g*, cohesiveness 0.41, springiness 0.39, gumminess 314.85 *g*, chewiness 79.43 *g* and resilience 0.45. There is an increase in absorbable isoflavone (daidzein) and antioxidant activity with lower carbohydrate and saturated fat content due to fermentation of soybean with *R. oligosporus*. The developed product possesses good nutritional (17.4% protein and 15.12% total fiber) and functional (3.9 g/100 g diadzein; antioxidant activity 3.9 mMTR) quality with low calorific value of 212.10 kCal/100 g, and it can be considered as a good "meat analogue" having the nutritional and nutraceutical richness of fermented soybeans and mushroom.

Keywords: meaty texture; texture profile analysis; soybean; *Rhizopus oligosporus*; *Agaricus* mushroom

1. Introduction

Fermented soybean (*temphe*) produced by solid-state fermentation of soybeans with *Rhizopus oligosporus* is popularly known for its attractive flavor, texture and superior digestibility [1].

Fermented soybean provides health benefits of isoflavone and secondary metabolites of fungus *R. oligosporus* [2]. In recent years, fermented soya foods are in great demand, mainly due to its therapeutic benefits instead of nutritional quality [3,4]

Traditional fermentation of soybeans involves the use of boiled and dehulled soybean as the substrate followed by inoculation with fungal starter culture. However, this fermentation process is labour intensive, nutritionally poor. Research on soybean fermentation were tried by various groups in order to produce a meaty textured soybean by employing distinctive strategies such as mixing soybeans with other substrates [5,6], changing the fermentation parameters [7,8] and by changing microorganisms [9].

Solid-state fermentation process, functional quality, and texture of fermented soybean are greatly influenced by the different process parameters like inoculum volume, thickness of substrate, fermentation temperature and substrate mixture. Fermentation process and quality fermented products can be produced by utilizing statistical optimization techniques like response surface methodology (RSM) coupled with good experimental design such as Box-Behnken design [10]. Predicting and analyzing the actual food texture responses of the fermentation process can easily be modified by optimizing the fermentation process variables [11].

RSM is a statistical optimization method applied widely in drug formulation, bioprocess, extraction of natural molecules, analytical method development. Various experimental designs like central composite design (CCD), Box-Behnken design (BBD), D-optimal design, Full factorial, partial factorial are used for optimization by RSM. Box-Behnken designs have three design points i.e. high, low and initial search level however CCD have five design points which include high, low, initial search level, + alpha and – alpha. Therefore CCD requires higher experimental runs than BBD [11].

In the present research, solid-state fermentation of soybean along with *Agaricus* mushroom was carried out, and the fermentation process conditions were optimized in order to produce a meaty textured soybean with excellent functional and nutritional quality.

2. Materials and Method

2.1. Microorganisms, substrate and chemicals

The fungal strain *Rhizopus oligosporus* NCIM 1215 was collected from National Collection of Industrial Microorganisms, national chemical laboratory, Pune, India. It was maintained on slants of potato dextrose agar medium at 32 °C and sub-cultured at every 30 days interval. Soybean seeds DS-9814 were collected from IARI, Pusa, New Delhi. Microbiological media were procured from Hi media Mumbai India. All other chemicals were procured from Merck, Mumbai, India. Daidzin and trolox standard were procured from Sigma-Aldrich, Bangalore*,* India.

2.2. Bio-processing of soybean

2.2.1. Preparation of substrate

For fermentation substrate preparation, *Agaricus* mushroom was dried at different temperatures between 40 to 90 °C for 30 min. The mushroom dried at 70 °C for 30 min resulted in good quality (nutritional and sensory) powder. Soybean variety PUSA DS-9814 was ground to mesh size of 20

(U.S Mesh). Ground soybean seeds were suspended in deionized water and allowed to soak for three hours at room temperature. Excess water was drained out. Soggy soybeans were mixed with different concentration (3, 5, 7, 10, 20 and 30% w/w) of dried *Agaricus* mushroom powder. Mushroom in the powdered form provides a gentle meaty flavour to the final product besides increasing its nutritional value [12].

The pH of the mixture was adjusted to 5.7 and sterilized at 121 °C for 15 min.

2.2.2. Preparation of fungal inoculum

Actively growing fungal mycelium was dislodged from the agar surface by distilled sterile water (0.9% NaCl). The fungal cell count was adjusted to 4×10^6 cells/mL. A total of 5 mL of fungal mycelium suspension was applied uniformly throughout the surface of the cooled sterilized soybean and *Agaricus* mushroom powder.

2.2.3. Fermentation process

Solid-state fermentation of soybean was carried out by incubating the soybean substrate at the different temperatures, with different solid substrate height, inoculums volume and quantity of *Agaricus* mushroom powder. The fermentation process was optimized by response surface methodology. Optimization of key factors influencing the texture of fermented soybeans, i.e. thickness of solid substrate (soybean), inoculum volume and quantity of *Agaricus* mushroom powder was carried out at three levels (+1, 0, −1) with total seventeen experimental runs. The individual and interactive effects of all the factors were studied by conducting the fermentation runs according to Box-Behnken Design. The texture profile data such as hardness, gumminess and chewiness expressed in g; and adhesiveness, springiness, cohesiveness and resilience of fermented sample in each run were analysed using the Design Expert 7.1.6 Software (statease inc. USA). The optimum values of the fermentation parameters were calculated by using the point predition tools of the software.

2.3. Texture profile analysis

Different fermented soybean samples consisting of small, pre-cut, cylindrical-shaped specimens (1 cm thickness and 3 cm diameter) were used for texture profile analysis (TPA). Poultry breast meat of the same dimensions as that of the fermented soybean samples were taken in triplicate as standard. The texture parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience) tests were performed on a texture analyser (TA.XTplus Texture Analyser, Stable Micro Systems Ltd., UK) equipped with a 50 N load cell and TEE 32 software with the TPA calculation module. A 7.5 cm in diameter compression platen was positioned at the crosshead. The specimen was placed at the centre of the lower platen, and the top platen was lowered to position just above the surface of the specimen. The TEE software was programmed with speed 300 mm/min under 50% strain for time 0.01 min. The test consisted of two successive compression ramps to a value of 70% of the unloaded specimen height. After the test is run, the texture analysis data was calculated by the TPA calculation module.

The TPA of poultry breast meat was compared with that of the fermented soybean and fermented product with identical TPA was selected for further optimization. The optimized TPA was evaluated for theoretical and practical value for validation of the model.

2.4. Functional analysis

2.4.1. Soya isoflavone estimation

The fermented soybean was analyzed for its glycosidic isoflavone (daidzin) content. To each fermented soybean product (2 g), 15 mL of 80% Acetonitrile-0.1N HCl was added. It was agitated for 15 hours at 30 ℃ and 200 rpm followed by centrifugation. Organic layer was separated and concentrated to 1 mL. Final volume was made to 2.0 mL with 80% methanol. Extract was filtered through 0.45 µm membrane before HPLC analysis [13]. The extracted daidzin was analyzed by HPLC system (Schimadzu Japan). The system category was class VP, using Lichrospher® 100 with RP-18 (5 µm) column, using mobile phase as water and acetonitrile with a flow rate of 1.0 mL/min under gradient elution (acetonitrile: 25 %, 0–3 min; 45 %, 3–7 min; 60%, 7–10 min). The retention time of daidzin was measured at 260 nm.

2.4.2. Antioxidant activity

The antioxidant activities of the fermented soybean products were estimated by cupric reducing antioxidant capacity (CUPRAC) assay method using trolox as the standard antioxidant [14].

2.4.3. Nutritional analysis

The final fermented soybean was analysed for total carbohydrate, fat (saturated and unsaturated), fiber (crude & dietary), protein, calcium, and energy value. All the tests were performed according to the Association of Analytical Communities (AOAC) standard procedure.

3. Results and Discussion

3.1. Optimization of texture parameters

Fermentation parameters such as thickness of solid substrate (TS), inoculum volume (IV), quantity of *Agaricus* mushroom powder (MP), fermentation time (FT), incubation temperature (IT) were optimized. The fermentation parameters such as FT and IT were optimized by one factor at a time method. The fermentation factors like TS and IV were optimized by response surface methodology (RSM). Factor like MP was first optimized by one factor at a time for zero value calculation. Further optimum value was evaluated by response surface methodology (RSM).

The texture profile (TPA) of different fermented soybean made from dissimilar combination was shown in the Table 1. Comparing to the TPA of poultry meat, sample B1 has the highest resemblance with it. B1 has the least mushroom concentration (3%) among all the samples and hence facilitates fluent growth of *R. oligosporus*. As the mushroom concentration increases, the binding network becomes weak, which leads to lose texture properties, i.e. increase in chewiness and decrease in resilience. As a result, samples B5 and B6 are too fragile to be comparable to poultry meat texture [15].

Table 1. Texture Profile Analysis results of different fermented soybean samples.

Sample	Hardness (g)	Adhesiveness	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Poultry meat	306.80	−9.71	0.26	0.66	204.64	54.74	0.61
A1	691.79	−0.08	0.34	0.53	368.02	126.33	0.36
A2	1021.67	ND	0.43	0.51	524.70	227.11	0.30
A3	1574.37	−1.81	0.52	0.65	1029.81	536.29	0.48
B1	352.93	−0.21	0.31	0.58	207.8	64.51	0.41
B2	364.10	−1.36	0.46	0.53	196.04	90.12	0.33
B3	925.65	−0.23	0.43	0.53	490.15	212.97	0.37
B4	1429.04	−0.87	0.43	0.44	641.84	276.83	0.25
B5	518.67	ND	0.28	0.63	330.95	92.91	0.41
B6	874.37	ND	0.54	0.46	408.56	221.79	0.26
C1	71.31	−0.46	0.21	0.40	28.80	6.14	0.36
C2	100.62	ND	0.32	0.37	37.31	12.19	0.33

A1, A2 and A3 represents texture profile of fermented soybeans where fermentation was carried out at 30, 35 and 40 °C, respectively; B1, B2, B3, B4, B5, B6 represents texture profile fermented soybeans with 3, 5, 7, 10, 20 and 30% *Agaricus* mushroom powder, respectively, C1 represents texture profile unfermented soybeans; C2 represents texture profile unfermented soybeans with 3% *Agaricus* mushroom powder.

The individual and interactive effects of these parameters on texture profile of the fermented product were analysed by the Design Expert 7.1.6 Software. The predicted and measured TPA of each run is showed in Table 2. The quadratic models created from the software are as follows.

Hardness (g) = $124.546 + 154.629\ TS - 245.402\ IV + 11.518\ MP - 278.79\ TS \times IV - 1185.34\ TS \times MP + 227.868\ IV \times MP + 810.131\ TS^2 + 54.681\ IV^2 + 486.564\ MP^2$

Cohesiveness = $0.475 - 0.06338\ TS + 0.006\ IV + 0.0356\ MP + 0.0385\ TS \times IV + 0.0527\ TS \times MP + 0.0515\ TS \times IV + 0.0833\ TS^2 - 0.0398\ IV^2 + 0.0423\ MP^2$

Springiness = $0.492 - 0.090\ TS + 0.047\ IV + 0.0368\ MP - 0.123\ TS \times IV + 0.112\ TS \times MP + 0.041\ TS \times IV + 0.180\ TS^2 - 0.038\ IV^2 - 0.170\ MP^2$

Gumminess (g) = $59.188 - 55.057\ TS - 111.549\ IV + 89.249\ MP - 95.899\ TS \times IV - 678.531\ TS \times MP + 128.565\ TS \times IV + 453.262\ TS^2 - 54.861\ IV^2 + 314.426\ MP^2$

Chewiness (g) = $29.134 - 54.485\ TS - 48.866\ IV + 73.633\ MP - 65.501\ TS \times IV - 278.265\ TS \times MP + 49.952\ IV \times MP + 248.615\ TS^2 - 28.715\ IV^2 + 92.929\ MP^2$

Resilience = $0.216 - 0.094\ TS + 0.0046\ IV + 0.0636\ MP + 0.045\ TS \times IV - 0.006\ TS \times MP + 0.017\ IV \times MP + 0.131\ TS^2 - 0.021\ IV^2 + 0.133\ MP^2$

These multiple nonlinear quadratic models resulted in three response surface graphs for each TPA parameter. A few representative response surface plots of the calculated model for TPA are shown in Figure 1. The analysis of variance of the models for different TPA is represented in Table 3.

Table 2. The measured and predicted values of different texture parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience) under different experimental conditions.

Run	TS (cm)	IV (mL)	MP (g)	Hardness (*g*)		Springiness		Cohesiveness		Gumminess (*g*)		Chewiness (*g*)		Resilience	
				Measured value	Predicted value	Measured value	Predicted value	Measured value	Predicted value	Measured value	Predicted value	Measured value	Predicted value	Measured value	Predicted value
1	0.5	3	3	394.80	801.34	0.52	0.55	0.66	0.61	262.66	528.29	138.01	286.88	0.47	0.46
2	1.5	3	3	2315.9	1668.18	0.51	0.61	0.42	0.41	989.62	609.97	512.21	308.91	0.23	0.18
3	0.5	7	3	220.30	868.11	0.99	0.89	0.53	0.54	117.35	496.99	116.85	320.15	0.32	0.38
4	1.5	7	3	1026.33	619.79	0.49	0.46	0.44	0.49	460.71	195.08	229.05	80.18	0.27	0.28
5	0.5	5	1	390.46	69.75	0.62	0.66	0.61	0.68	240.51	114.15	151.05	73.26	0.47	0.50
6	1.5	5	1	2016.06	2749.69	0.29	0.26	0.41	0.44	842.19	1361.1	246.43	520.82	0.25	0.33
7	0.5	5	5	3197.10	2463.47	0.48	0.51	0.67	0.64	2168.62	1649.71	1051.45	777.06	0.71	0.64
8	1.5	5	5	81.33	402.05	0.60	0.56	0.69	0.62	56.17	182.53	33.77	111.56	0.47	0.44
9	1.0	3	1	1213.36	1127.54	0.31	0.24	0.50	0.48	608.88	469.61	189.62	118.53	0.29	0.27
10	1.0	7	1	508.09	181.00	0.19	0.25	0.47	0.39	242.66	−10.61	46.41	−79.10	0.33	0.25
11	1.0	3	5	367.74	694.84	0.29	0.23	0.37	0.45	137.70	390.98	40.38	165.89	0.28	0.37
12	1.0	7	5	573.95	659.77	0.33	0.40	0.55	0.57	285.75	425.01	96.97	168.06	0.39	0.41
13	1.0	5	3	124.23	124.54	0.46	0.49	0.44	0.47	87.10	59.18	39.11	29.13	0.31	0.21
14	1.0	5	3	122.44	124.54	0.44	0.49	0.44	0.47	79.18	59.18	38.18	29.13	0.32	0.21
15	1.0	5	3	124.24	124.54	0.49	0.49	0.47	0.47	81.11	59.18	39.10	29.13	0.29	0.21
16	1.0	5	3	128.57	124.54	0.49	0.49	0.47	0.47	89.34	59.18	42.13	29.13	0.33	0.21
17	1.0	5	3	126.23	124.54	0.47	0.49	0.47	0.47	89.16	59.18	41.97	29.13	0.32	0.21

Table 3. Analysis of variance of the models for different TPA parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience).

Variance	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Regression						
Sum of square	10826722	0.46	0.113	3480876	731203.9	0.269
Df	9	9	9	9	9	9
Mean squares	1202969	0.05	0.012	386764	81244.87	0.029
F value	3.14	8.067	2.952	2.320	1.716	6.380
P value	0.0727	0.0059	0.0837	0.1400	0.2443	0.0116
Residual						
Sum of square	2680717	0.044	0.029	1166930	331282.2	0.032
Df	7	7	7	7	7	7
Mean squares	382959.6	0.006	0.004	166704.3	47326.03	0.004
Correlation coefficient (R^2)	0.801	0.912	0.791	0.748	0.688	0.891
Coefficient of variance (CV %)	81.373	16.708	12.670	103.461	123.361	20.695
Adequate Precision	5.646	10.805	5.688	5.302	5.131	8.809

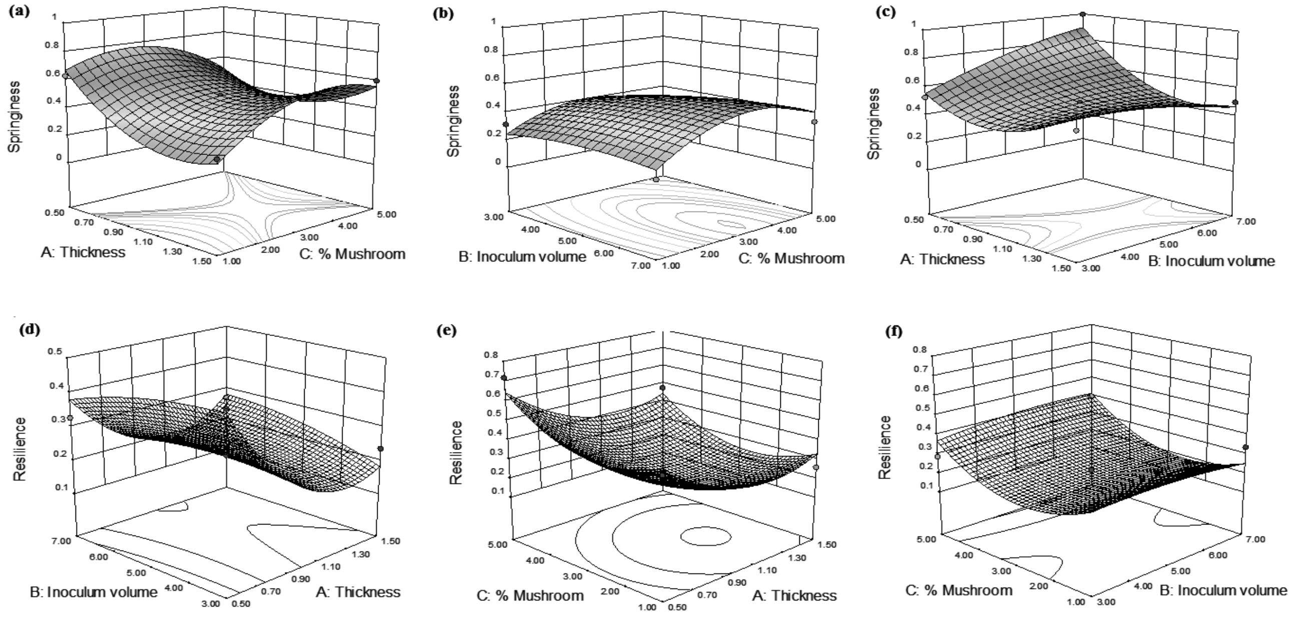

Figure 1. Response surface plots (a, b, c, d, e and f) of the calculated model for texture profile parameters.

Point prediction of the design expert software was used to determine the optimum values of the fermentation parameters for matching textural profile with that of poultry meat. Values of "Prob > F" less than 0.0500 indicate model/model terms are significant. Therefore, for "Springiness" ("Prob > F" = 0.0059) and "Resilience" ("Prob > F" = 0.0116); Model F-values of 8.07 and 6.38, respectively (Table 3), imply the models are significant. There are only 0.59 and 1.16% chances, respectively, that "Model F-Values" this large could occur due to noise. In case of "springiness"; TS, TS × IV, TS × MP, TS^2, MP ^{2} and for "Resilience"; TS, MP, TS^2, MP ^{2} are critical design terms as their corresponding "Prob > F" values are less than 0.0500. The "adequate precision" measures the signal to noise ratio. A ratio greater than 4 is desirable for a model to be considered suitable. As shown in Table 3, all the model TPA parameters have "Adequate Precision" greater than 4 especially springiness (10.80) and Resilience (8.80), indicating adequate signal that all the models can be used to navigate the design space. The optimum values for thickness of solid substrate (TS), inoculum volume (IV), quantity of *Agaricus* mushroom powder (MP) are 1.12 cm, 5.92% v/w, 4.84 % w/w, respectively. These values predict different TPA results, which are validated by performing experiments. Predicted process parameters (predicted values) are suggested by the software to achieve target textural characteristics similar to poultry meat. The validated TPA values which are actually the experimental values of the optimized final product bears close resemblance with the TPA results of poultry meat. The TPA curve of final product and poultry meat was shown in Figure 2. The predicted data and validated result of TPA are shown in the Table 4.

3.2. Evaluation of functional properties

The functional property of the fermented products was evaluated by daidzin content and antioxidant activity. The least amount of daidzin (1.909 mg/100g) and maximum antioxidant activity

(3.9 mMTR) were found in the fermented soybean sample having mushroom powder (3%). As the mushroom concentration increased in the fermented soybean samples, daidzin content gradually increased whereas antioxidant activity decreased, hence, the fermented sample having 30% mushroom powder in it was found to be having diadzin content of 3.726 mg/g and antioxidant activity 0.56 mMTR. As the concentration of mushroom powder increased in the fermented sample, the growth or penetration of fungal growth into the sample became more difficult. This trend of result implies that the more fluent the growth of *Rhizopus* is, higher is the glucosidase enzyme produced. It breaks down the glycosidic linkages and converts daidzin into its aglycone form, i.e. diadzein [16]. A higher concentration of diadzein in final product is desirable since it is more absorbable isoflavone than diadzin. A similar effect was also observed with antioxidant activity, higher the growth of *Rhizopus,* more is the antioxidant activity. This may be due to increase concentration of aglycone soya isoflavones in the fermented soybean.

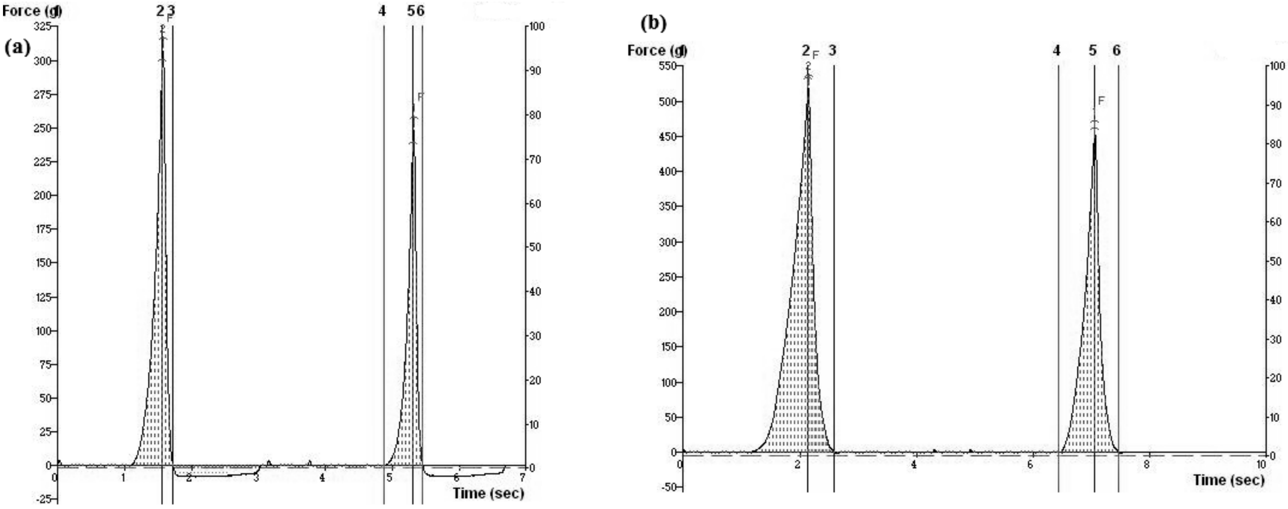

Figure 2. The texture profile analysis curve of (a) poultry meat and (b) optimized meaty textured fermented soybean blended with *Agaricus* mushroom.

Table 4. Predicted and validated result of TPA parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience).

Response	Prediction Values	Validated Values	% validation
Hardness	329.02	538.11	163.54
Cohesiveness	0.56	0.41	72.74
Springiness	0.41	0.39	95.88
Gumminess	247.34	314.85	127.29
Chewiness	98.53	79.43	80.61
Resilience	0.38	0.45	119.16

3.3. Evaluation of nutritional properties

The nutritional properties of fermented product were evaluated by the total carbohydrate, fat (saturated and unsaturated), fiber (crude and dietary), protein, calcium, and energy value as shown in Table 5. The result shows that fermented food is a good source for diadzein with high antioxidant activity and dietary fiber with lower saturated fat. The energy value of the fermented food is found to be 212.10 kCal per 100 g, which is half of the energy value of the un-fermented soybean. This may be due to lowering of total fat after the fermentation.

Table 5. The nutritional analysis of the validated fermented product.

Components	Quantity (% w/w)
Ash	2.46
Moisture	55.09
Total Fat	8.46
Saturated fat	0.54
Protein	17.40
Carbohydrate	16.59
Energy (kCal/100 g)	212.10
Crude fibre	2.58
Dietary fibre	12.54
Calcium	0.14
Diadzein (g/100 g)	8.9
Antioxidant activity(MmTR)	3.9

4. Conclusions

In the present research, a meaty texture fermented soybean mixed with *Agaricus bisporus* is developed by solid state fermentation using *Rhizopus oligosporus*. The final product has been validated for poultry meat like textural properties, i.e. springiness, chewiness and resilience with poultry meat. It can be considered as a good “meat analogue” having the nutritional and functional richness of fermented soya beans and mushroom with low calorific value.

Acknowledgments

We acknowledge Dr. S. K. Lal, IARI, PUSA, New Delhi for providing soybean samples.

Conflict of interest

There is no conflict of interest in publishing the research findings and in publishing the results.

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