Fermentation Technology
ISSN: 2167-7972

Singleton et al., Ferment Technol 2017, 6:2
DOI: 10.4172/2167-7972.1000146

Research Article Open Access

# Fermentation Optimization of Macro-Fungus Pleurotus Sajor-Caju on Soymeal

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**Received date:** September 07, 2017; **Accepted date:** September 26, 2017; **Published date:** October 03, 2017



**Abstract**

An investigation was undertaken to evaluate the impact of edible mushroom Pleurotus sajor-caju fermentation on the nutritional profile of soymeal under high solid submerged fermentation. Eight day fermentation resulted in changes in the nutritional profile of soymeal. A statistically significant enhancement in fermented sample was seen for crude protein, crude fiber and total amino acid. Except for hydroxyproline and hydroxylysine all amino acids showed significant increase in fermented samples. Specifically, amino acids such as glutamic acid and lysine which are important in animal feed showed significant increase in fermented samples. The overall increase of amino acids ranged from 10% to 200%. Response Surface Methodology (RSM) is an effective tool to optimize submerged fermentation of macro-fungi like Pleurotus. The results of the current study can serve as a basis of optimization of other macro-fugal submerged fermentation.

**Keywords:** Optimization; Macro-fungi; Mushrooms; Profiling; Nutritional; Animal feed; Soymeal

## Introduction

Mushrooms have been an integral part of various culinary cultures all over the world since several centuries. There has been a reported documents that it utilization started in oriental medicine and has it origin in Chinese culture. The large scale production of shiitake mushrooms on logs have been initiated in China, which is popular in many western countries [1-4]. In many countries traditional foods contained mushroom as an ingredient. When people immigrated from one country to another they brought the culture of mushroom with their food preparation. Though over 2000 different types of mushrooms have identified as edible, most of them are wild and only a few are commercially produced. Example, portobello mushroom (*Agaricus bisporus*), Shiitake (*Lentinula edodes*) and Oyster mushroom (*P. ostreatus*) to name a few, which are commercially cultivated for consumption [5]. Generally mushrooms are saprophytic macro-fungi which grow on dead and decaying biomass under abundant moisture and relative humidity. Mushrooms are classified under class Basidiomycete [6,7]. Naturally growing mushrooms are a part of ecosystem that are involved in vital nutrient recycling [8].

Mushrooms are well documented to provide a suite of biochemicals that are important in human and animal nutrition and health. Mushrooms are rich source of dietary fiber (DF). Mushroom cell walls are rich in chitin and polysaccharides such as glucans and mannans [9]. Further a review by Cheung [10] lists a series documented health benefits of mushroom DF. Mushrooms are rich source of high quality protein that can be produced relatively inexpensively in a short span of time using agricultural wastes such as crop residues [10-13]. Mushrooms are also rich in unsaturated fatty acids, vitamin C, thiamine, riboflavin and do not contain cholesterol [1]. Mushrooms are also known to produce variety of pharmacological, and immunomodulating compounds [2,14-17]. Mushrooms are known to induce special flavor to food ranging from fruity flavor to meaty flavor [4,18,19]. A 2015 Zion Market Research report indicated the world over mushroom market was valued at US $35.08 billion and projected to reach $59.48 billion by 2021 at an annual rate of 9.2% (https://www.zionmarketresearch.com/news/global-mushroom-market). Due to the antioxidant and immunomodulatory effects of Mushrooms (Oyster mushrooms) they are becoming most popular in modern western diet. Exhaustive reports indicate the use of mushroom spent as animal feed which provide easily digestible carbohydrates, proteins and minerals [20-24].

Soybean is the second most important commercial crop after corn in the United States [25]. Extensive reviews [26,27] dealing with various fermented products from soybean co-products have been reported. Some of the soy co-products used in fermentation process are soymeal, soy hull and soy-isolate apart from whole soy flour. Soymeal is extensively used in bacterial and fungal fermentation for production of enzymes to reduce anti-nutritional factors in soymeal [26,27].

Though large scale production of mushrooms are produced on solid state fermentation (SSF) of cheap agricultural wasted such as wheat and rice straw, it leaves a lot of post-harvest waste such as spent in commercial production that leads to a lot of landfill induced environmental problems [24,28]. Also SSF comes with set of banes such as contamination, fluctuation in quality and high labor. Submerged fermentation (SmF) is often an alternative to SSF which bears some of the salient features such as quick turnaround time, low contamination, consistency in quality and excellent space utilization [29]. Pleurotus has been studied under SmF for production of bioactives by various researches [6,29-31]. Since various agricultural coproducts/wastes such as wheat straw, rice straw, wheat bran and soybean products are primarily used as growth medium along with $CaSO_4$, the present investigation choose soymeal and $CaSO_4$ as primary ingredients to optimize submerged fermentation. $CaSO_4$ is primarily used to adjust the pH that favors macro-fungal growth. The

overall objective of the study was to develop a SmF of Pleurotus sajor-caju using soymeal and $CaSO_4$. Response Surface Methodology (RSM) statistical method was used to optimize the media ingredients. RMS design especially important in process optimization where interaction of parameters influence the growth of microbes [32-34]. The optimization process will serve as a blueprint for any macro-fungal growth optimization using soymeal as primary substrate.

## Materials and Methods

### Mushroom culture

Pleurotus sajor-caju (707) was obtained from the Penn State Mushroom culture collection and was maintained on potato glucose agar (PGA) medium at room temperature.

### Soymeal and other chemicals

Soybean meal was obtained from National Oil Seed Processors Association (NOPA). All the other chemicals used were AR grade and obtained from VWR Scientific.

### Experimental design for optimization

Media optimization was carried out using optimal design (OD) where two independent variable used were soymeal and $CaSO_4$. Design expert 7.1.6 (Stat-Ease Inc., Minneapolis, MN, USA) was used to generate experimental designs, estimate the responses of dependent variables and also generate the contour and/or response surface plots.

The two independent variables and their levels for Optimal Design was given in Table 1. The OD consisted of six central points and 14 non-central points. The experiment consisted of 16 runs with no blocking. The upper and lower limits of the variables are given in the Table 2.

The relation between coded and actual values is according to the following equation

$$xi = \frac{X_i - X_0}{\Delta X} \rightarrow (1)$$

where $x_i$ is the coded value of the independent variable ($X_1$=Soymeal, $X_2$=$CaSO_4$), $x_i$ is the real value of the independent variable, $X_0$ is the real value of the independent variable at the center point and $\Delta X$ is the step change value.

The relationship between independent variables and dependent variables was obtained as the sum of the contributions of the three factors through first order, second order and interaction terms according to the quadratic polynomial function in equation 2. Where $Y$ is the predicted response, $\beta_i$ is the linear coefficient, $\beta_{ii}$ the squared coefficient and $\beta_{ij}$ the interaction coefficient and k the number of factors.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta iXi + \sum_{i=1}^{k} \beta iiXiXj + \sum i \sum_{i<k} j\beta ijXiXj \rightarrow (2)$$

All RSM experiments were conducted in 250 mL Erlenmeyer flasks. The total volume in each flask was maintained at 50 mL. The flasks were sterilized at 120°C for 30 min and cooled to room temperature. About 2.5 mm block of actively growing culture was added to the flask and incubated for 8 days at 200 rpm and 30°C.

### Validation of RSM

The fermentation medium contained 60.50 g of soymeal in a 2 L flask with 200 mL de-ionized water along with 7.9 g of $CaSO_4$. The flasks were sterilized at 120°C for 30 min and cooled to room temperature. About 5 mm block of actively growing culture was added to the flask and incubated for 8 days at 200 rpm and 30°C. Four replicates were maintained. Samples from two flasks were pooled and finally two replicates were used for nutritional profiling. All samples were freeze dried before analysis.

| Run# | Soymeal % | $CaSO_4$ | CFU |
|---|---|---|---|
| 12 | 35.00 | 7.08 | 3000 |
| 4 | 35.00 | 1.00 | 180000 |
| 3 | 23.38 | 1.00 | 500000 |
| 2 | 10.00 | 1.00 | 300000 |
| 9 | 20.88 | 4.87 | 6.00E+06 |
| 8 | 20.88 | 4.87 | 5.80E+06 |
| 5 | 14.75 | 3.18 | 1.20E+06 |
| 16 | 27.88 | 10.00 | 2100 |
| 13 | 18.90 | 8.47 | 2500 |
| 7 | 20.88 | 4.87 | 380000 |
| 1 | 10.00 | 1.00 | 210000 |
| 15 | 27.88 | 10.00 | 12000 |
| 14 | 10.00 | 10.00 | 3200 |
| 6 | 30.13 | 3.97 | 4.00E+07 |
| 10 | 10.00 | 6.36 | 26000 |
| 11 | 35.00 | 7.08 | 2100 |

**Table 1:** Experimental design matrix with soymeal and $CaSO_4$.

| Factor | Name | Units | Type | Subtype | Minimum | Maximum |
|---|---|---|---|---|---|---|
| A | Soymeal | % | Numeric | Continuous | 10 | 35 |
| B | CaSO4 | % | Numeric | Continuous | 1 | 10 |
| Response | Name | Units | Obs | Analysis | Minimum | Maximum |
| R1 | CFU | CFU/G | 16 | Polynomial | 2100 | 4.00E+07 |

**Table 2:** Experimental design with upper limit and lower limit for soymeal and $CaSO_4$.

### Determination of colony forming units (CFU) of pleurotus

Serial dilution technique was used to determine CFU which represents viable cells per gram of the final fermented samples. In brief, a serial dilution method was used and spread plate method of culturing yeast cells on PDA medium was carried out. Sample dilutions up to 10-12 were carried out. After incubation of plates at 30°C for 4 days, colonies from plates with highest dilutions (where the colony numbers varied between 50-100) were counted and CFU/g of fermented samples was expressed.

### Nutritional profiling

Known quantity of freeze-dried samples were weighed and sent to the Agricultural Experiment Station Chemical Laboratories (AESCL) at the University of Missouri-Columbia for biochemical compositional analyses using the ASOS methods as described in Nanjundaswamy and Vadlani [35]. Nutrition composition ana1yses included total amino acid profile, crude fat and protein, crude fiber, % NDF and % ADF.

| Source | Sum of squares | df | Mean Square | F Value | p-value (prob>F) | Significance |
|---|---|---|---|---|---|---|
| Model | $1.26 \times 10^{15}$ | 9 | $1.40 \times 10^{14}$ | 3.69 | 0.0629 | not significant |
| A-Soymeal | $7.18 \times 10^{14}$ | 1 | $7.18 \times 10^{14}$ | 19 | 0.0048 | |
| B-$CaSO_4$ | $5.37 \times 10^{13}$ | 1 | $5.37 \times 10^{13}$ | 1.42 | 0.2787 | |
| AB | $2.36 \times 10^{14}$ | 1 | $2.36 \times 10^{14}$ | 6.24 | 0.0466 | |
| $A^2$ | $5.19 \times 10^{13}$ | 1 | $5.19 \times 10^{13}$ | 1.37 | 0.2861 | |
| $B^2$ | $4.67 \times 10^{14}$ | 1 | $4.67 \times 10^{14}$ | 12.4 | 0.0126 | |
| $A^2B$ | $9.65 \times 10^{13}$ | 1 | $9.65 \times 10^{13}$ | 2.55 | 0.1614 | |
| $AB^2$ | $2.37 \times 10^{14}$ | 1 | $2.37 \times 10^{14}$ | 6.26 | 0.0463 | |
| $A^3$ | $4.61 \times 10^{14}$ | 1 | $4.61 \times 10^{14}$ | 12.2 | 0.013 | |
| $B^3$ | $5.98 \times 10^{13}$ | 1 | $5.98 \times 10^{13}$ | 1.58 | 0.2555 | |
| Residual | $2.27 \times 10^{14}$ | 6 | $3.79 \times 10^{13}$ | | | |
| Lack of Fit | $2.07 \times 10^{14}$ | 1 | $2.07 \times 10^{14}$ | 50.8 | 0.0008 | significant |
| Pure Error | $2.03 \times 10^{13}$ | 5 | $4.07 \times 10^{12}$ | | | |
| Cor Total | $1.49 \times 10^{15}$ | 15 | | | | |

**Table 3:** ANNOVA for Response Surface Cubic model for Soymeal and $CaSO_4$.

## Results and Discussion

Optimization experiments revealed that both soymeal and $CaSO_4$ influenced the CFU of Pleurotus. From the RSM plots (Figures 1 and 2) it is clear that as the soymeal concentration increased the growth of mycelia increased while the $CaSO_4$ had the opposite effect. The desirability (Figure 2) for the optimal production of Pleurotus was about 0.89 when the concentration of Soymeal and $CaSO_4$ were 30.27% and 3.89% respectively. The analysis of variance (Table 3) indicate that the model is non-significant and the lack of fit is significant. The concentration of soymeal and $CaSO_4$ influenced to increase the mycelial growth of Pleurotus. There was a positive correlation (0.220) between soymeal and CFU of Pleurotus, while the $CaSO_4$ had a negative correlation (-0.133) with CFU. The overall interaction of both substrates had a net positive correlation (0.252) with CFU. The predicted CFU from the model was $1.8 \times 10^7$ CFU/g. From the optimization results the ideal percentage of soymeal and $CaSO_4$ were 30.27% and 3.99% respectively. Very little information is available from the submerged fermentation of mushrooms with respect to media optimization. The validation experiment conducted with 30.27% and 3.99% of soymeal and $CaSO_4$ respectively resulted in a $2 \times 10^7$ CFU/g of Pleurotus. The percentage of $CaSO_4$ predicted in the present optimization is in range with the $CaSO_4$ levels used in solid state fermentation of Pleurotus [36].

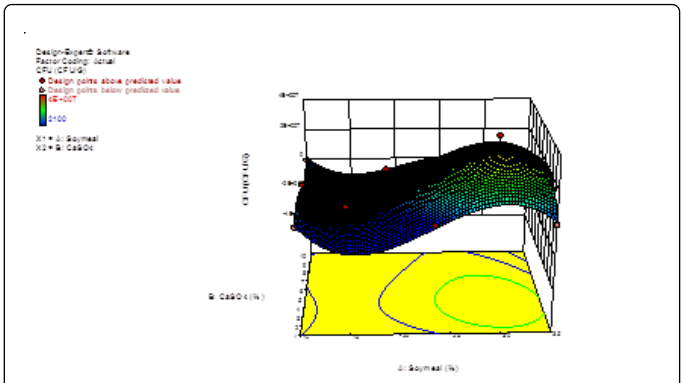

**Figure 1:** Response Surface plot for CFU as influenced by interaction of Soymeal and $CaSO_4$.

We predict that increased soymeal concentration increased the nutrient availability for the fungus and it resulted in enhanced mycelial growth. Many scientific studies as reported in the recent review by

Soccol et al. [6] that high solid fermentation is ideal for fungi that predominantly grow in mycelial form. High solid SmF is beneficial for production of mushrooms enriched products used in animal feed as it can reduce cost significantly by preventing excessive downstream processing.

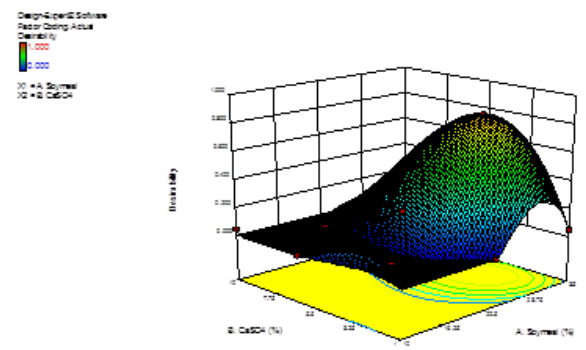

**Figure 2:** Desirability RSM plot for CFU as influenced by interaction of Soymeal and $CaSO_4$.

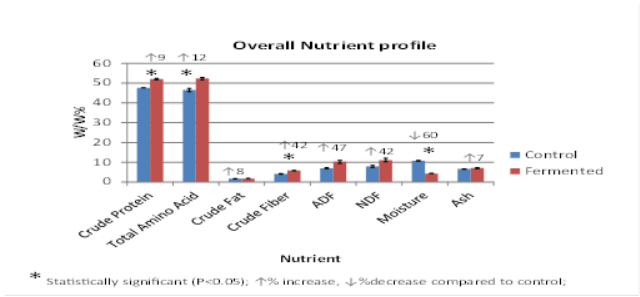


**Figure 3:** Nutrition profile of fermented and control soymeal. Means and standard errors provided (values are w/w expressed in %).

## Nutritional profiling

Figure 3 describes the overall nutritional profile, except for moisture, all other nutrients were enhanced in the fermented sample than the control. The enhancement varied from 7% to 47%. A statistically significant enhancement in fermented sample (compared to control) was seen only for crude protein, crude fiber and total amino acid. Moisture in control was significantly greater (60%) than that in the fermented sample. A similar line of enhancement of crude protein was noticed when soymeal was fermented with Apergillus oryzae [27].

## Amino acid profile of fermented and un-fermented soymeal

Figure 4 describes the nutritional profile of fermented an unfermented soymeal samples. Except for hydroxyproline and hydroxylysine, all other amino acids were enhanced in the fermented sample compared to the control. The enhancement varied from 10% to 200%. The enhancement was not statistically significant for taurine, alanine, cystine, phenylalanine and tryptophan. The amino acid profile for fermented soymeal by *Aspergillus* and combination of *Aspergillus* and *Lactobacillus* had a similar profile compared to Pleurotus fermented soymeal. A similar enhancement in Threonine, Serine, Proline, Glycine, Alanine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Lysine, Histidine, Arginine and crude protein. Enhanced amino acid and protein content in fermented samples are attributed to microbial growth. It is interesting to note that some of the amino acids like Glutamic acid and lysine have showed at least 11% and 10% increase [37]. It is important note that the fungi did not decrease any essential amino acids during fermentation of soymeal. Similar trends were also reported by Hong et al. [38].

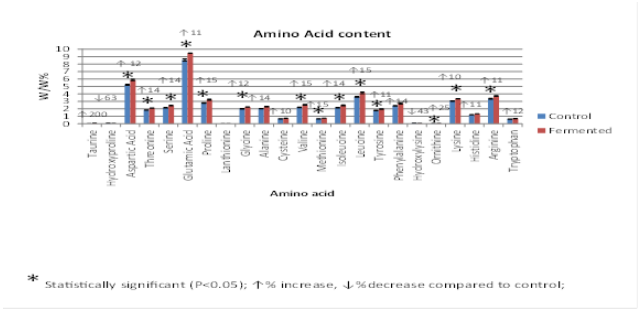


**Figure 4:** Amino acid profile of fermented and control soybean. Means and Standard errors provided (values are w/w expressed in %).

## Conclusion

Fermentation of soymeal with marcro-fungus Pleurotus resulted in Fermented Soymeal (FSBM) with positive nutritional attributes. In our study all of the nutrients were enhanced in the fermented sample than the control except for moisture. A statistically significant enhancement in the fermented sample was only seen in crude protein, crude fiber and total amino acid, the enhancement varied from 7% to 47%. All the amino acids were enhanced in the fermented sample compared to the unfermented soymeal. Optimization of media to enhance yield of macro-fungal mycelia by response surface methodology (RSM) provides a blue print for future mushroom submerged fermentation.

## Acknowledgement

The authors thank USDA Evans-Allen funding support to conduct this research.

## References

1. Alam N, Khan A, Hossain MS, Amin SMR, Khan LA (2007) Nutritional Analysis of dietary Mushroom-Pleurotus florida Eger and Pleurotus sajur-caju (Fr) Singer. Bangladesh J Mushroom 1: 1-7.
2. Kitzberger CSG, Smânia Jr. AS, Pedrosa RC, Ferreira SRS (2007) Antioxidant and antimicrobial activities of shiitake (Lentinula edodes) extracts obtained by organic solvents and supercritical fluids. J Food Eng 80: 631-638.
3. da Fonseca TRB, de Amorim Silva T, Alecrim MM, da Cruz Filho RF, Teixeira MFS (2015) Cultivation and nutritional studies of an edible mushroom from North Brazil. Afr J Microbiol Res 9: 1814-1822.
4. Buller AHR (1915) The fungus lore of the Greeks and Romans. Trans Br Mycol Soc 5: 21-26.
5. Bononi VLR, Capelari M, Maziero R, Trufem SFB (1995) Cultivo de cogumelos comestíveis. Ícone, São Paulo.

6. Soccol CR, da Costa ESF, Letti LAJ, Karp SG, Woiciechowski AL, et al. (2017) Recent developments and innovations in solid state fermentation. Biotechnol Res Innovat.
7. Subramanian CV (1995) Mushrooms: Beauty, diversity, relevance. Curr Sci 69: 986-997.
8. Dighton J, Poskitt JM, Howard DM (1986) Changes in occurrence of basidiomycete fruit bodies during forest stand development with specific reference to mycorrhizal species. Trans Br Mycol Soc 87: 163-171.
9. Bartnicki-Garcia S (1970) Cell wall composition and other biochemical markers in fungal phylogeny, in: J.B. Harborne (Ed.), Phytochemical Phylogeny, Academic Press, London.
10. Cheung PCK (2013) Mini-review on edible mushrooms as source of dietary fiber: Preparation and health benefits. Food Sci Hum Wellness 2: 162-166.
11. Chang R (1996) Functional properties of mushrooms. Nutri Rev 54: 91-93.
12. Gupta RS (1986) Mushroom cultivation. Indian Hort 31: 1-7.
13. Vishwanatha KS, Rao AA, Singh SA (2010) Acid protease production by solid-state fermentation using Aspergillus oryzae MTCC 5341: optimization of process parameters. J Ind Microbiol Biotechnol 37: 129-138.
14. Elmastas M, Isildak O, Turkekul I, Temur N (2007) Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Composition Anal; 20: 337-345.
15. Moradali MF, Mostafavi H, Ghods S, Hedjaroude GA (2007) Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). Int Immunopharmacol 7: 701-724.
16. Shamtsyan M, Konusova V, Maksimova Y, Goloshchev A, Panchenko A, et al. (2004) Immunomodulating and anti-tumor action of extracts of several mushrooms. J Biotechnol 113: 77-83.
17. Egli S (2011) Mycorrhizal mushroom diversity and productivity-an indicator of forest health? Annal Forest Sci 68: 81-88.
18. Singer R (1961). Mushrooms and Truffles, Leonard Hill Books Ltd. p. 272.
19. Zasler ND, McNeny R, Heywood PG (1992) Rehabilitative management of olfactory and gustatory dysfunction following brain injury. J Head Trauma Rehab 7: 66-75.
20. Durrant AJ, Wood DA, Cain RB (1991) Lignocellulose biodegradation by Agaricus bisporus during solid substrate fermentation. J Gen Microbiol 137: 751-755.
21. Fazaeli H, Mahmodzadeh H, Azizi A, Jelan ZA, Liang JB, et al. (2004) Nutritive value of wheat straw treated with Pleurotus fungi. Asian-Aust J Anim Sci 17: 1681-1688.
22. Fazaeli H, Jelan ZA, Azizi A, Liang JB, Mahmodzadeh H, Osman A (2002) Effects of fungal treatment on the nutritive value of wheat straw. Malaysian J Anim Sci 7: 61-71.
23. Giovannozzi-Sermanii G, Bertoni G, Porri A (1989) Biotransformation of straw to commodity chemicals and animal feeds. In: Enzyme Systems for Lignocellulose Degradation (Ed. W. Coughlan). Elsevier science, Amsterdam.
24. Langar PN, Sehgal JP, Garcha HS (1980) Chemical changes in wheat and paddy straws after fungal cultivation. Indian J Anim Sci 50: 942-946.
25. http://www.soystats.com/2012
26. Garg P (2014) Mushroom Production: Cost Effective Use of Soyabean Meal (De-Oiled Cake) During Composting. IOSR J Environ Sci Toxicol Food Technol 8: 113-114.
27. Chen CC, Shih YC, Chiou WS, Yu B (2010) Evaluating nutritional quality of single stage-and two stage-fermented soybean meal. Asian-Aust J Anim Sci 23: 598-606.
28. Kleyn JG, Wetzler TF (1981) The microbiology of spent mushroom compost and its dust. Can J Microbiol 27: 748-753.
29. Tang YZ, Zhu LW, Li HM, Li DS (2007) Submerged culture of mushrooms in bioreactors challenges current-state-of-the-art, and future. Food Technol Biotech 45: 221-229.
30. Papaspyridi LM, Katapodis P, Gonou-Zagou Z, Kapsanaki-Gotsi E, Christakopoulos P (2010) Optimization of biomass production with enhanced glucan and dietary fibres content by Pleurotus ostreatus ATHUM 4438 under submerged culture. Biochem Eng J 50: 131-138.
31. Téllez-Téllez M, Fernández F J, Montiel-González A M, Sánchez C, Díaz-Godínez G (2008) Growth and laccase production by Pleurotus ostreatus in submerged and solid-state fermentation. Appl Microbiol Biotechnol 81: 675-679.
32. Kar S, Ray RC, Mohapatra UB (2008) Alpha-amylase production by Streptomyces erumpens MTCC 7317 in solid state fermentation using response surface methodology (RSM). Pol J Microbiol 57: 289-296.
33. Shaktimay K, Datta TK, Ray RC (2010) Optimization of thermostable a-amylase production by Streptomyces erumpens MTCC 7317 in solid-state fermentation using cassava fibrous residue. Braz Arch Biol Technol 53: 301-309.
34. Sharma P, Goel R, Capalash N (2007) Bacterial laccases. World J Microbiol Biotechnol 2007; 23: 823-832.
35. Nanjundaswamy A, Vadlani PV (2011) Fiber reduction and lipid enrichment in carotenoid-enriched distillers dried grain with solubles produced by secondary fermentation of Phaffia rhodozyma and Sporobolomyces roseus. J Agri Food Chem 58: 12744-12748.
36. Thongsook T, Kongbangkerd T (2011) Influence of calcium and silicon supplementation into Pleurotus ostreatus substrates on quality of fresh and canned mushrooms. Food Sci Technol Int 17: 351-365.
37. El-Shemy HA (2013) Soybean Bio-Active Compounds. InTech, Croatia.
38. Hong KJ, Lee CH, Kim SW (2004) Aspergillus oryzae 3.042GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. J Med Food 7: 430-434.