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Optimization of microwave-convective drying of Oyster mushrooms (*Pleurotus ostreatus*) using response-surface methodology

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Abstract

Ergothioneine, a potent antioxidant, has been found in highest concentration in oyster mushroom (*Pleurotus ostreatus*) among other mushroom species. The purpose of the present study was to optimize the drying air temperature and residual moisture content (wb) for the maximization of ergothioneine content, total phenolic content (TPC), 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity, total reductive power activity (potassium ferricyanide), and the reduction of colour difference (ΔE) using response surface methodology (RSM). Oyster mushrooms were dried in a microwave-convective dryer, the temperature range was varied from 60 to 80°C and residual moisture content (wb) was varied from 5 to 20%, each at 5 levels. A rotatable central composite design (RCCD) consisting of 13 experimental runs with five replicates at the central points were applied and second-order polynomial models were used to describe the experimental data regarding the responses. Ergothioneine content was found to be highest at 70°C and 12.5% residual moisture content (wb). Total phenolic content was found to decrease with increase in temperature, 11.8 to 4.6 mg GAE/gm dw going from temperature 60 to 80°C. The IC_{50} for DPPH and total reductive power activity showed a gradual increase, showing decline in the antioxidant activity with rise in temperature, 9.9 to 0.1 mg/ml and 0.0033 to 0.0012 mg/ml, respectively. A rise in drying temperature caused darkening. Thus, the optimized values of responses can be obtained at drying temperature of 69°C and 9% (wb) residual moisture content, yielding an ergothioneine content of 1.73 mg/gm dw, total polyphenols of 8.9 mg GAE/gm dw, IC_{50} for DPPH activity of 0.09 mg/ml, IC_{50}^{*} (absorbance at 0.5 AU) for total reductive power of 0.0027 mg/ml and colour difference (ΔE) of 18.

Introduction

Reactive oxygen/nitrogen species are responsible for oxidative stress which may ultimately play a major factor in many diseases, such as cancer (Ames *et al.*, 1995), Alzheimer's (Christen, 2000), atherosclerosis (Diaz *et al.*, 1997), and the entire aging process (Yu, 1996). Secondary plant metabolites such as polyphenolic compounds can act as strong antioxidants. Polyphenols are multifunctional as they act as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (Rice-Evans *et al*., 1996). Folin-Ciocalteu reagent method is employed for the determination of total polyphenols, the reduction of the reagent by polyphenols results in colour development which in turn is analysed with the help of a spectrophotometer. Apart from polyphenols, mushrooms contain a unique antioxidant, ergothioneine that has been identified and quantified in various genera of mushrooms utilizing HPLC-MS (Dubost *et al*., 2007). Ergothioneine has been shown to possess numerous antioxidant and cytoprotective effects *in vitro* and a few *in vivo*, including free radical scavenger activity (Hartman, 1990; Akanmu *et al*., 1991; Asmus *et al*., 1996; Aruoma *et al.*, 1997), radioprotective properties (Motohashi *et al*., 1977; Hartman *et al.*, 1988; Laurenza *et al*., 2008) and protection against UV radiation (Decome *et al*., 2005; Botta *et al.*, 2008; Damaghi *et al*., 2008; Markova *et al*., 2009) or neuronal injury (Jang *et al*., 2004; Song, 2010). The antioxidative properties of ergothioneine are based on its ability to scavenge and quench most reactive oxygen species (Aruoma *et al*., 1997), to chelate various divalent metallic cations (Motohashi *et al.*, 1976; Akanmu *et al.*, 1991), and to suppress the oxidation of hemoproteins (Arduini *et al.*, 1990). Beelman *et al*. (2007) showed that ergothioneine content in the button mushroom (*Agaricus bisporus*), Shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*), king oyster (*Pleurotus eryngii*) and maitake mushroom (*Grifola frondosa*) varied from 0.4-2.0 mg/g dry weight (dw). Shiitake and oyster mushrooms contained the highest level of ergothioneine at approximately 2.0 mg/g dw. Ergothioneine rich extract prepared from the fruiting body and the solid cultivating media of *F. velutipes* has been successfully used to control the discolouration and lipid oxidation of fish meats and

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the development of melanosis in crustaceans during postmortem storage (Ashida *et al*., 2005; Bao *et al.*, 2009; Encarnacion *et al.*, 2011).

Polyphenols and related antioxidants are one of the most important bioactive components in mushrooms. They play an important role in prevention against food oxidation (Bandoniene *et al*., 2002; Shan *et al.*, 2009). Moreover, phenolic compounds and ergothioneine, isolated from mushrooms, might have wide application in nutraceuticals, as they can serve as dietary supplements. Unfortunately, conventionally dried mushrooms exhibit low antioxidant retention. Therefore, for higher retention of biologically active components, better colour preservation, reduction of the structural damage after drying and minimizing of process costs, scientists have advocated the use of novel hybrid drying technologies, like microwave-convective. Microwave drying has been shown to reduce loss of active compounds, e.g. in mint (Arslan *et al*., 2010) and oregano (Jałoszyński *et al.*, 2008).

The aim of the present work was to investigate the effect of microwave-convective drying on oyster mushrooms with respect to ergothioneine content, total polyphenols, antioxidant activity and colour changes of the dried product.

Materials and Methods

Experimental material and apparatus

Fresh oyster mushrooms (*Pleurotus ostreatus*) were obtained from Rural Development Centre, Indian Institute of Technology Kharagpur. Initial moisture content of the mushroom was 90.5 ± 1%, obtained using hot air oven (Relco-DTC96S1, Kolkata, West Bengal, India) at 105°C until the mushroom weight reached a fixed value. Each experiment was performed in triplicate. The microwave assisted hot air drying system (Enerzi Microwave Systems Pvt. Ltd., Bangalore, India) was used to dry mushroom slices. The instrument comprised of two microwave sources of 1.5 kW capacity each, with a microwave power range of 250-3000W and a working frequency of 2450 MHz. For hot-air circulation, heater of 6 kW power was fitted, air temperature could vary from 25-200°C. The weighed mushroom samples (250 g) were spread uniformly in a single layer over the tray. Experimental runs were recorded at a constant air velocity of 1.5 ± 0.05 m/s and power density of 1 W/gm (Funebo and Ohlsson, 1998). Drying was performed at different levels of temperature and residual moisture content, which was calculated by measuring the weight loss of sample using an analytical balance (Sartorius TE 153S, Sartorius Weighing India Pvt. Ltd., Bangalore, India). Samples were withdrawn as soon as they reached the desired residual moisture content (wb). Relative humidity of the ambient air changed between 21% and 23%. Dry matter present in the mushroom sample was calculated by using moisture content (wet basis) and amount of the sample subjected to drying. Each experiment was performed in triplicate.

Ergothioneine analysis

Quantification of ergothioneine was carried out according to the method of Dubost *et al*. (2007). HPLC (Dionex UltiMate 3000, Sunnyvale, USA) separation was carried out using two C18 columns (Dionex Acclaim 120) with each column being 4.6 x 250 mm, 5 µm particle size connected in tandem. The isocratic mobile phase was 3% acetonitrile, 0.1% triethylamine and 50 mM sodium phosphate, pH 7.3. Detection was at a wavelength of 254 nm. The injection volume was 20 µl; column temperature was ambient. Quantification of ergothioneine in mushroom samples was calculated by plotting a calibration curve obtained from different concentrations of the authentic standard (Sigma Aldrich, Kolkata, India). Amount of ergothioneine was expressed as mg/g dw. Each experiment was performed in triplicate.

Determination of total phenols

Total polyphenol (TP) concentration was measured using Folin Ciocalteu Reagent (FCR) by a modified method of Fu *et al.* (2002). Dried mushroom powder (5 gram) was added to 60 ml of 80% ethanol and heated to 60°C for one hour using a water bath (Reico Equipments and Instruments, Kolkata, India). The final volume was made upto 100 ml by adding 80% ethanol. Ethanolic extract (1 ml) was added to FCR (4 ml), which was diluted with distilled water (1:10). After few minutes, five milliliter of 7.5% aqueous sodium carbonate solution was added. Absorbance was measured at a wavelength of 765 nm by an UV–Visible spectrophotometer (Varian, Cary 50 Bio, UV-Vis spectrophotometer, Australia). TP content was standardized against gallic acid and the quantification of TP in samples was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g dw). Each sample was extracted in triplicate.

Antioxidant activity

One gram of dried mushroom powder was homogenized in 10 ml boiling water for 2 minutes. The homogenate was centrifuged at 4000 rpm for 15 minutes and the supernatant was collected. The collected supernatant was evaporated under vacuum and the residue was dissolved in 5 ml of distilled

water.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Different volumes (2 to 80 µl) of the mushroom extracts were mixed with 0.5 ml of 0.4 mM DPPH ethanol solution and made up to a final volume of 2 ml with distilled water. The mixture was mixed thoroughly and then placed at 25°C for 30 min in the dark. Absorbance was measured at a wavelength of 517 nm by an UV–VIS spectrophotometer.

Reducing power assay (Ferric reducing assay. Different volumes of the extract ranging from 2 to 80 µl was mixed with 0.5 ml of 1% potassium ferricyanide and final volume was made up to 1.5 ml with 0.2 M sodium phosphate buffer (pH 6.6). The reaction mixture was incubated at a temperature of 50°C for 20 min. 0.5 ml of 10% trichloroacetic acid was added, followed by the addition of 2 ml of distilled water and 400 µl of 0.1% ferric chloride. Absorbance was measured at a wavelength of 700 nm by an UV–VIS spectrophotometer.

Colour analysis

The chromaticity of the dried mushroom powder was measured using a Konica Minolta colorimeter. Colour was measured in terms of L^* the (degree of the lightness), a^* (degree of redness) and b^* (degree of yellowness) values. The calibration of colorimeter was done using a standard calibration plate of a white surface. The measurements of color were replicated three times after shaking the dried samples and the average values of L, a, and b were reported. Color change (ΔE) was calculated was calculated according to Equation (1).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Where, $\Delta L = L_{sample} - L_{standard}$, $\Delta a = a_{sample} - a_{standard}$ and $\Delta b = b_{sample} - b_{standard}$

Experimental design

The software Design Expert (Trial Version 7.0.3, Stat-Ease Inc., Minneapolis, USA) was used. Variables chosen for microwave assisted hot-air drying experiments were drying temperature (X_1) and residual moisture-content (wb) (X_2). The relative contribution of above two variables on ergothioneine, total polyphenols, antioxidant activity and colour was determined using response surface methodology. Thirteen experiments were performed according to central composite rotatable design with two variables, each at five levels. The limits of each variable were set on the basis of preliminary drying experiments. Table 1 shows the experimental variables in coded and actual levels used in experimental design.

Table 1. Independent variables and their coded and actual values used for optimization

Symbol	Name (units)	Coded level				
		-1.414	-1	0	1	1.414
X_1	Temperature (°C)	60	63	70	77	80
X_2	Residual Moisture Content (wb)	5	7.2	12.5	17.8	20

Experiments were randomized, so as to reduce the unexplained variability in observed responses due to extraneous factors. Five replicates at central points were performed, to minimize sum of squares of pure error. Experimental data were fitted to a second-order polynomial equation, as shown in Equation (2).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} b_{ji} X_i X_j \quad (2)$$

Where b_0, b_i, b_{ii} and b_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, whereas, X_i and X_j are the independent variables.

Results and Discussion

Effect of temperature and residual moisture content on ergothioneine content

Figure 1 (a) and (b) shows the chromatogram of ergothioneine in authentic standard and in dried mushroom sample, ergothioneine yield was observed to vary from 1-1.8 mg/g dw in different dried samples (Table 2). LC-MS was used confirm the identity of analyte, mass spectral product for the ion-scan spectra of ergothioneine in authentic standard and dried mushroom sample was obtained at m/z 229. It was observed that linear term of drying temperature (X_1) and quadratic terms of both drying temperature $(X_1)^2$ and residual moisture content (wb) $(X_2)_2$ significantly affected yield of ergothioneine ($p < 0.05$). Ergothioneine yield increased with the rise in drying temperature up to 70°C and then gradually decreased with further rise in drying temperature. Microwave heating causes the product temperature to rise drastically, creating flux to facilitate rapid evaporation of water vapor. Figure 2 show a positive correlation of ergothioneine yield and residual moisture content up to 12.5% (wb) and then a gradual decline was observed in ergothioneine yield with further rise in residual moisture content, though the effect was not remarkably significant. Thus, as the drying temperature and residual moisture content rises above 70°C and 12.5% (wb) respectively, it leads to generation of excessive heat which might be responsible for denaturation of ergothioneine (a betaine of 2-thio-L-histidine amino acid). Equation relating actual levels of drying parameters to ergothioneine content is given by Equation 3, and had a high R^2 of 0.9819 (Table 3):

Table 2. Central composite design with the observed responses for antioxidant content, antioxidant activity and colour of dried oyster mushrooms

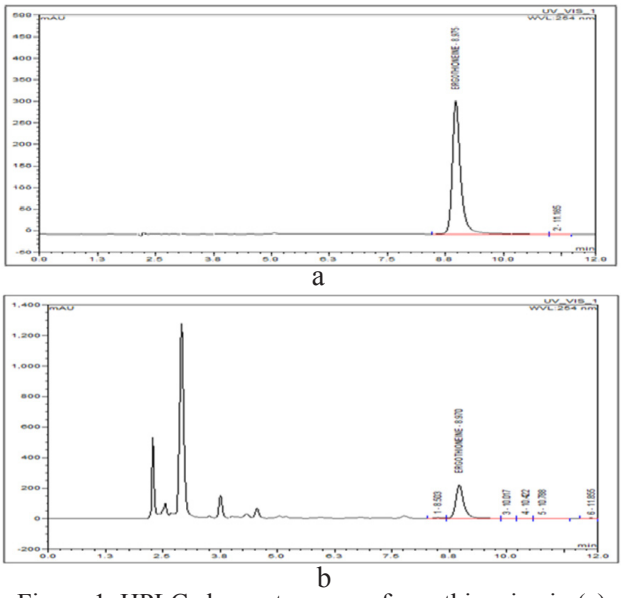
Run	Factor 1 X1: DT[a]	Factor 2 X2: MC[b]	Response 1 Ergo Content[c]	Response 2 TPC[d]	Response 3 RSA[e]	Response 4 RPA[f]	Response 5 Colour[g]
1	77 (+1)	7.2 (-1)	1.49	5.76	5.90	0.0030	20.1
2	77 (+1)	17.8 (+1)	1.41	6.30	6.05	0.0032	21.0
3	63 (-1)	7.2 (-1)	1.23	11.00	0.21	0.0016	16.0
4	60 (-1.414)	12.5 (0)	1.00	11.80	0.10	0.0012	14.7
5	70 (0)	5 (-1.414)	1.72	8.39	0.41	0.0022	17.9
6	70 (0)	12.5 (0)	1.76	8.99	0.48	0.0028	19.4
7	70 (0)	12.5 (0)	1.77	8.00	0.70	0.0023	19.4
8	70 (0)	12.5 (0)	1.81	8.01	0.86	0.0025	19.0
9	63 (-1)	17.8 (+1)	1.22	10.93	0.39	0.0021	16.4
10	70 (0)	12.5 (0)	1.71	8.02	0.50	0.0028	19.2
11	70 (0)	12.5 (0)	1.78	8.89	0.99	0.0028	19.0
12	80 (+1.414)	12.5 (0)	1.16	4.60	9.90	0.0033	21.6
13	70 (0)	20 (+1.414)	1.64	10.00	1.09	0.0030	19.9

[a]Drying Temperature (°C), [b]Moisture Content (wb)
[c]Ergothioneine Content(mg per g dw), [d]Total Phenol Content (mg GAE/ g dw), [e]DPPH scavenging assay (IC_{50}), [f]Reducing power assay (IC_{50}^{*}) (conc. at 0.5 Abs), g Colour (ΔE)

Table 3. Coded values of regression coefficients of predicted quadratic polynomial models for the responses

Coefficients	Response 1 Ergo Content[a]	Response 2 TPC[b]	Response 3 RSA[c]	Response 4 RPA[d]	Response 5 Colour[e]
b_0	1.77 [c]	8.38 [c]	0.71[c]	$2.64*10^{-3}$ [c]	19.21 [c]
Linear					
b_1	0.09 [b]	-2.52 [c]	3.17[c]	$6.97*10^{-4}$ [c]	2.32 [c]
b_2	-0.03 [ns]	0.34 [ns]	0.16[ns]	$2.10*10^{-4}$ [a]	0.53 [b]
Cross product					
b_{12}	-0.02 [ns]	0.15 [ns]	-0.006[ns]	$-7.43*10^{-5}$ [ns]	0.13 [ns]
Quadratic					
b_{11}	-0.35 [c]	-0.14 [ns]	2.22[c]	$-1.79*10^{-4}$ [a]	-0.57 [b]
b_{22}	-0.06 [a]	0.36 [ns]	0.1[ns]	$-1.86*10^{-5}$ [ns]	-0.18 [ns]
R^2	0.98	0.97	0.99	0.94	0.98

[a]Significant at 5%, [b]Significant at 1%, [c]Significant at 0.1%, [ns] Not significant
[a]Ergothioneine Content(mg per g dw), [b]Total Phenol Content (mg GAE/ g dw), [c]DPPH scavenging assay (IC_{50}), [d]Reducing power assay (IC_{50}^{*}) (conc. at 0.5 Abs), [e]Colour (ΔE)



a

b

Figure 1. HPLC chromatograms of ergothioneine in (a) authentic standard and (b) dried mushroom sample

$$\text{Ergothioneine Content} = -34.51 + 1.01X_1 + 0.075X_2 - 4.39^{*}10^{-4}X_1X_2 - 7.09^{*}10^{-3}X_1^2 - 1.95^{*}10^{-3}X_2^2 \quad (3)$$

Effect of temperature and residual moisture content on total phenolics

Yang *et al.* (2002) analyzed shiitake and oyster mushrooms for total phenol and found between 6 and 15 mg/g dw of total phenol depending on the species of mushroom chosen. Figure 3 show the effect of drying temperature and residual moisture content on the total phenolic content. The quadratic regression model showed the value of the determination coefficient

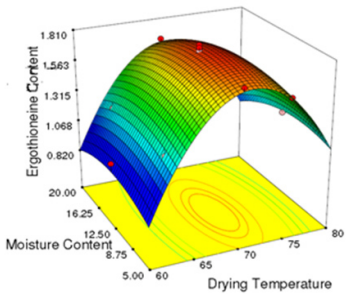


Figure 2. Response surface plot of drying temperature (°C) and residual moisture content (wb) and their mutual interactions on ergothioneine yield

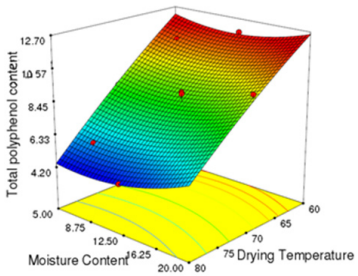


Figure 3. Response surface plot of drying temperature (°C) and residual moisture content (wb) and their mutual interactions on total phenolic content

(R^2) was 0.9715, which implied only that 97.15% of the variations could be explained by the fitted model. Figure 3 show that the rise in temperature from 60 – 80°C led to the decline of total phenolic content from 11.8 - 5.7 mg GAE/g dw. Residual moisture content did not show any significant effect on the total phenols. Absorption of microwave energy by water molecules results into generation of heat which leads to inactivation of degradative enzymes at a faster rate as compared to oven heating. Along with degradation of enzymes, it may also lead to degradation of phytochemicals such as total phenols.

The independent variable X_1 was the only significant factor on experimental yield of total phenols ($p < 0.0001$). The regression equation of total phenolic content relating to actual levels of drying parameters was found as (Equation 4):

$$\text{Total polyphenol content} = 24.26 - 0.013^{*}X_1 - 0.54^{*}X_2 + 4.07^{*}10^{-3}X_1X_2 - 2.81^{*}10^{-3}X_1^2 + 0.013^{*}X_2^2 \quad (4)$$

Effect of temperature and residual moisture content (wb) on antioxidant activity

DPPH radical scavenging activity. Independent variable X_1 and its quadratic term X_1^2 significantly affected the DPPH scavenging activity of the samples. Residual moisture content did not have significant effect on IC_{50} value. IC_{50} value for DPPH scavenging activity ranged from 0.1 to 9.9 mg/ml. Ergothioneine did not show any significant effect on antioxidant activity upto 70°C. Figure 4 show as the temperature

Table 4. Predicted and experimental values of response at the optimum conditions

	Factor 1 X1: DT[a]	Factor 2 X2: MC[b]	Response 1 Ergo Content[c]	Response 2 TPC[d]	Response 3 RSA[e]	Response 4 RPA[f]	Response 5 Colour[g]
Predicted values	68.58	9.43	1.73	8.82	0.1	0.0024	18.4
Experimental values	69	9	1.7	8.93	0.09	0.0027	18.1

[a]Drying Temperature (°C), [b]Moisture Content (wb)
[c]Ergothioneine Content (mg per g dw), [d]Total Phenol Content (mg GAE/ g dw), [e]DPPH scavenging assay (IC_{50}), [f]Reducing power assay (IC_{50}^*) (conc. at 0.5 Abs), g Colour (ΔE)

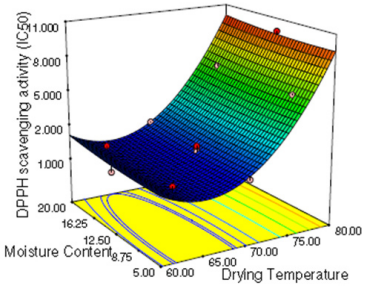


Figure 4. Response surface of drying temperature (°C) and residual moisture content (wb) and their mutual interactions on DPPH scavenging activity (IC_{50})

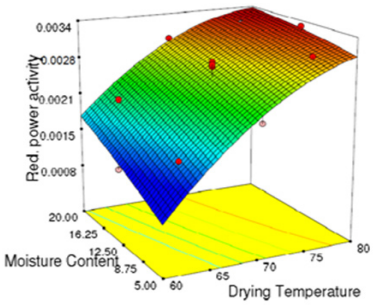


Figure 5. Response surface plot of drying temperature (°C) and residual moisture content (wb) and their mutual interactions on reducing power assay

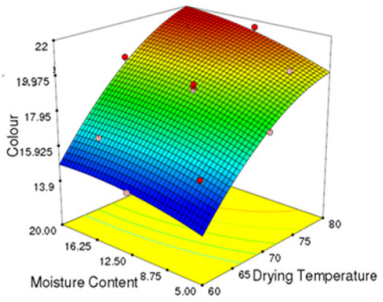


Figure 6. Response surface plot of drying temperature (°C) and residual moisture content (wb) and their mutual interactions on colour

increased from 70 to 80°C, antioxidant activity decresed as the ergothioneine content decreased from 1.81 to 1.16 mg/g dw and the IC_{50} increased from 0.8 to 9.9 mg/ml. Whereas, as the temperature increased, total phenolic content decreased which led to gradual decrease in antioxidant activity. Thus it shows that polyphenols masks the effect of ergothioneine on the total antioxidant activity. The regression equation of DPPH scavenging activity (IC_{50}) relating to actual levels of drying parameters was found as (Equation 5):

$$\text{DPPH scavenging activity } (IC_{50}) = 187.1 - 5.77X_1 - 0.04X_2 - 1.8^{*}10^{-4}X_1{}^{*}X_2 + 0.04X_1{}^2 + 3.46^{*}10^{-3}X_2{}^2 \quad (5)$$

Reducing Power Activity (Ferric reducing assay). Figure 5 show that drying temperature and residual moisture content (wb) had significant effect on reducing power activity. The IC_{50} value ranged from 0.0012 to 0.0033 mg/ml at 60 and 80°C, respectively. The reducing power activity showed a strong correlation with total phenols, whereas effect of ergothioneine on reducing power activity was masked by total phenols. In this case, linear terms X_1 and X_2 as well as quadratic term $X_1{}^2$ were found to be significant ($p < 0.05$). The regression equation of reducing power activity was expressed as:

$$\text{Reducing power activity } (IC_{50})^{*} = - 0.024 + 6.24^{*}10^{-4}X_1 + 1.95^{*}10^{-4}X_2 - 1.98^{*}10^{-6}X_1X_2 - 3.58^{*}10^{-6}(X_1)^2 - 6.63^{*}10^{-7}(X_2)^2 \quad (6)$$

Effect of temperature and residual moisture content (wb) on colour

The color change of the dried mushroom samples was determined in terms of ΔE, which ranged from 14.7 to 21.6 for different drying conditions. The values of color difference ΔE are given in the Table 1. Drying air temperature and residual moisture content (wb) had significant effect ($p < 0.05$) on color change. Figure 6 shows as the residual moisture content (wb) and drying temperature increased, darkening was observed among the samples which resulted to significant increase in color change.

Among all process variables, the linear term for air temperature (X_1) and residual moisture content (wb) (X_2) has maximum effect ($p < 0.001$) and quadratic term for air temperature $(X_1)^2$ ($p < 0.01$) on color change. R^2 value for this model was found as 0.98, indicating a good model fit. The regression equation of color change (ΔE) relating to actual levels of drying parameters was found as (Equation 7):

$$\text{Colour } = - 59.24 + 1.89X_1 + 0.013X_2 + 3.58^{*}10^{-3}X_1X_2 - 0.011(X_1)^2 - 6.57^{*}10^{-3}(X_2)^2 \quad (7)$$

Optimization of drying parameters and validation of model

By the computation, the optimal drying conditions were determined as follows: a drying temperature of 68.58°C and residual moisture content 9.43% (wb). Table 4 shows that under predicted optimized conditions, yield of ergothioneine was 1.73 mg/g dw, total phenol content was 8.82 mg GAE/g dw, IC_{50} for DPPH scavenging activity was 0.1 mg/ml, IC_{50}^* for reducing power activity was 0.0024 mg/ml and ΔE for colour was 18.36. However, considering the operatability in actual production,

the optimal conditions can be modified as follows: drying temperature of 69°C and residual moisture content 9% (wb). Under these optimal conditions, the experimental values agreed with the predicted values, using analysis of variance, indicating a high goodness of fit of the model used.

Conclusion

The high correlation of the mathematical model indicated that a quadratic polynomial model could be employed to optimize the ergothioneine, total polyphenol yield, antioxidant activity and colour of the dried mushroom powder using a microwave-convective dryer. From response surface plots, it was evident that when the temperature reached 70°C, ergothioneine content increased, whereas total phenol and antioxidant activity decreased. This shows that total phenols masks the effect of ergothioneine on the total antioxidant activity. Thus with the decrease in the total phenols, the antioxidant activity also declines with the rise in temperature. Whereas, at the temperature above 70°C, due to intense heat generated by microwave-convective drying, ergothioneine along with total phenols degrade drastically owing to fall in total antioxidant activity. The residual moisture content showed to have less influence on responses as compared to drying temperature. The optimal conditions to obtain the highest ergothioneine and total phenol yield, highest antioxidant activity and colour of *P. ostreatus* were determined to be 68°C and 9% (wb) of residual moisture content. Under optimal conditions, the experimental values agreed with the predicted value. Thus, this methodology could provide a basis for quantitative prediction of responses on the basis of developed regression models in a short-term experiment.

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