ANTIOXIDANT ACTIVITY STABILITY TEST OF CULTURING MEDIA FROM *Pleurotus ostreatus* VAR. BHUTAN IN COSMETIC PRODUCTS

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Abstract

Free radicals and reactive oxygen species are relevant to many diseases and aging process. Consuming and utilizing of antioxidants are widely used to reduce these molecules. Synthetic antioxidants are usually applied in cosmetics though many are toxic. Natural antioxidants are much interested and widely studied from fruit, vegetables, herbs and edible mushroom. *Pleurotus ostreatus* var. Bhutan is widely cultivated in Thailand. Measurement of antioxidant activities from extracts of culturing media of *Pleurotus ostreatus* var. Bhutan, i.e. Glucose and potato dextrose broth (PDB) media, showed high activities indicating that they could be very good sources of antioxidants used in cosmetic products. This project was aimed to study the antioxidant activity stability of these 2 extracts mixing with 3 cosmetic product types, e.g. cream, serum and gel in order to evaluate the tendency for their applications. After homogenizing of either Glucose or PDB extracts with cream, serum or gel bases without any active ingredients including dyes and fragrance compounds at the concentration of 0.2, 0.4, 0.6, 0.8 and 1.0%, all products were treated with accelerated test method by placing them at 45°C for 24 hr and -10°C for 24 hr alternatively for 5 cycles.

Then the DPPH radical scavenging activity, ferrous ion chelating ability and pH measurements including noticing of color change were done before and after treatment comparing to all 3 control products (without any extracts). The results showed that the tendency of both antioxidant activities measurements of these 2 extracts, Glucose and PDB, were similar. All extracts at any concentrations reduced their activities at cycle no. 5 about 11-12% comparing to cycle no. 0. This indicates that the antioxidant activities of either Glucose or PDB extracts are stable. The results of pH measurement and noticing the color change of products showed no difference comparing between before or after treatment except for serum mixing with PDB extracts at 0.8 and 1.0% in which the pH was changed from 5.5 at start to 5.0 at the end. Though the pH changed, it was around 5.0-5.5 which was near the pH of skin. This result indicates that either Glucose or PDB extracts may not irritate the skin after applying. Comparison of antioxidant activities of products mixing with extracts at different concentrations showed that the more concentration of extracts used, the less antioxidant activities obtained (in terms of µg BHT equivalent / mg crude extract and µg EDTA equivalent / mg crude extract). This result indicates that the optimal concentration in cosmetic products should be 0.2%.

Keywords: antioxidant, *Pleurotus sp*., cosmetic product, stability

Introduction

Free radicals and reactive oxygen species are relevant to many diseases and aging process.

Consuming and utilizing of antioxidants are widely used to reduce these molecules. Synthetic antioxidants, e.g. butylated hydroxyl toluene (BHT) or butylate hydroxyanisole (BHA), are usually applied in cosmetics though many are toxic. Natural antioxidants are much interested and widely studied from fruit, vegetables, herbs and edible mushroom.

Mushrooms are used both in industry, e.g. for production of lignin-degrading enzymes, or in food. It has low content of protein and lipid but high of fiber. There are many types of secondary metabolites in mushrooms such as phenol, polyketides, terpenes, steroids etc. which may be relevant to their medicinal activity (Turkoglu et al, 2007). Fruiting bodies of many types of mushroom were studied their antioxidant activity. This includes Shiitake, abalone, oyster, Maitake, Morel, Termite, Bunashimeji, Hon-shimeji mushrooms (Yang et al 2002, Cheung et al 2003, Mau et al 2004, Lee et al 2007)

Pleurotus ostreatus var. Bhutan is widely cultivated in Thailand. Measurement of antioxidant activities from extracts of culturing media of *Pleurotus ostreatus* var. Bhutan, i.e. Glucose and potato dextrose broth (PDB) media, showed high activities indicating that they could be very good sources of antioxidants used in cosmetic products. This project was aimed to study the antioxidant activity stability of the 2 extracts mixing with 3 cosmetic product types, e.g. cream, serum and gel in order to evaluate the tendency for their applications.

Methodology

Production of crude extracts of antioxidants from culturing media of *Pleurotus ostreatus* var.Bhutan The mycelia of *Pleurotus ostreatus* var. Bhutan were inoculated in 2 culturing media, i.e. Glucose medium, which was composed of glucose, yeast extract, polypeptone, MgSO • 7H2O, K2HPO4 and MnSO4 • 5H2O, and potato dextrose broth (PDB). Both inoculated media were incubated at 28°C and shaken at 100 rpm but for different incubation time in which the inoculated Glucose medium was incubated for 14 days while the inoculated PDBwas for 21 days. Then the filtrate was collected and freeze-dried. Stability test of antioxidant activities of crude extracts from culturing media of *Pleurotus ostreatus* var.

Bhutan in cosmetic products

Crude extracts of antioxidants from culturing media of *Pleurotus ostreatus* var. Bhutan at the concentration of 0.2, 0.4, 0.6, 0.8, 1.0 % w/w were homogenized with cream base, gel base or serum base without adding any active ingredients, dyes, fragrance compounds or essential oils. Then the products were tested with accelerated test by alternatively placing at 45°C for 24 hrs and at -10°C for 24 hrs for 5 cycles. The antioxidant activities were measured before and after accelerated test with comparison to the control (cosmetic products without crude extract).

Determination of antioxidant activity

The products with/without crude extracts were determined their antioxidant activities by 2 methods, i.e. DPPH radical scavenging activity and ferrous ion chelating ability. For DPPH radical scavenging activity, briefly, the products were diluted to be clear, incubated in the dark at room temperature for 30 min with 1,1-diphenyl;-2-picryl-hydrazyl (DPPH (and measured A517 using 2,6-ditert-butyl 4-methyl phenol (BHT) as standards. For ferrous ion chelating ability assay, the products were diluted to be clear, added FeCl2 and ferrozine, incubated in the dark for 20 min and measured A562 before and after adding ferrozine. EDTA was used as standard for this assay.

Results

Production of crude extracts of antioxidants from culturing media of Pleurotus ostreatus var. Bhutan After freeze-drying of culturing media of Pleurotus ostreatus var. Bhutan, crude extracts were obtained at 35.5 and 35.8 g for Glucose medium and PDB, respectively. Stability test of antioxidant activities of crude extracts from culturing media of Pleurotus ostreatus var. Bhutan in cosmetic products The results of antioxidant activities, DPPH radical scavenging activity and ferrous ion chelating ability, of cream, gel or serum with/without crude extracts from culturing media of Pleurotus ostreatus var. Bhutan before and after accelerated test were shown in Table 1 and 2 for Glucose medium, respectively, and Table 3 and 4 for PDB, respectively. pH and color of cosmetic products blended with crude extracts from culturing media of *Pleurotus ostreatus* var. Bhutan The pH of all products was measured and their color was also noticed for the products homogenized with/without crude extracts from Glucose medium or PDB. It was found that the pH of all cream homogenized with crude extracts, all serum homogenized with crude extracts and all gel homogenized with crude extracts was 5.0, 5.5 and 5.5, respectively, which was similar to the control of cream base, serum base and gel base, except for the serum blended with PDB 0.8 and 1.0% in which their pH was 5.0 comparing to the serum control (pH 5.5). The color of all products was light yellow, different from the control of cream base, gel base and serum base in which the color was white, no color and no color, respectively.

Discussion and Conclusion

Stability test of antioxidant activities of crude extracts from culturing media of *Pleurotus ostreatus* var. Bhutan in cosmetic products Comparison of DPPH radical scavenging activity and chelating ability of crude extracts from PDB blended in cosmetic products before and after accelerated test shows that they have similar tendency (Table 1 and 3) in that the antioxidant activities of crude extracts of every concentration used at cycle no. 5 reduce about 11-12% comparing to cycle no. 0. The results of DPPH radical scavenging activity and chelating ability of crude extracts from Glucose medium blended in cosmetic products before and after accelerated test also give similar tendency (Table 2 and 4) in that the antioxidant activities of crude extracts of every concentration used at cycle no. 5 reduce about 10-12% comparing to cycle no. 0, which is similar to the tendency of products added with PDB crude extracts. This reveals that both PDB and Glucose crude extracts have stable antioxidant activities.

Table 1 DPPH radical scavenging activity of cosmetic products homogenizing with crude extracts from inoculated PDB.

Product type / concentration (%)	DPPH radical scavenging activity at cycle no. 0 *,**			DPPH radical scavenging activity at cycle no. 5 *,**			% Recovery - Cycle♥
	Mean ξ	SD ξ	% Relative €	Mean ξ	SD ξ	% Relative €	
Cream base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	32.59	0.71	100.00	28.736	0.96	100.00	88.16
0.4	17.62	0.37	54.06	15.644	0.24	54.44	88.79
0.6	12.19	0.21	37.39	10.881	0.29	37.87	89.29
0.8	9.74	0.13	29.89	8.641	0.29	30.07	88.69
1.0	8.03	0.16	24.62	7.173	0.18	24.96	89.37
Serum base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	27.17	2.65	100.00	23.93	0.65	100.00	88.07
0.4	14.18	1.76	52.20	12.62	0.32	52.72	88.96
0.6	10.03	1.10	36.90	8.91	0.24	37.25	88.90
0.8	7.86	0.97	28.92	6.98	0.13	29.17	88.84
1.0	6.43	0.81	23.65	5.69	0.13	23.77	88.54
Gel base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	29.43	1.38	100.00	26.19	0.77	100.00	89.00
0.4	15.46	1.05	52.56	13.68	0.37	52.25	88.48
0.6	11.16	0.52	37.93	9.95	0.21	37.99	89.14
0.8	8.64	0.54	29.35	7.72	0.27	29.49	89.44
1.0	7.18	0.40	24.40	6.37	0.10	24.30	88.67

Table 2 DPPH radical scavenging activity of cosmetic products homogenizing with crude extracts from inoculated Glucose medium.

Product type / concentration (%)	DPPH radical scavenging activity at cycle no. 0 *,**			DPPH radical scavenging activity at cycle no. 5 *,**			% Recovery - Cycle♥
	Mean ξ	SD ξ	% Relative €	Mean ξ	SD ξ	% Relative €	
Cream base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	34.62	0.94	100.00	30.76	0.59	100.00	88.86
0.4	18.51	0.36	53.47	16.37	0.64	53.22	88.43
0.6	12.86	0.20	37.16	11.38	0.35	36.99	88.45
0.8	10.02	0.29	28.93	8.90	0.32	28.95	88.91
1.0	8.28	0.11	23.91	7.37	0.25	23.97	89.09
Serum base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	28.70	2.86	100.00	25.35	0.57	100.00	88.33
0.4	14.99	1.78	52.23	13.37	0.36	52.72	89.17
0.6	10.40	1.29	36.24	9.26	0.22	36.53	89.04
0.8	8.14	0.94	28.34	7.27	0.11	28.68	89.38
1.0	6.71	0.81	23.39	5.99	0.13	23.61	89.17
Gel base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	31.52	1.35	100.00	28.06	0.79	100.00	89.02
0.4	16.69	0.86	52.93	14.95	0.28	53.29	89.61
0.6	11.53	0.64	36.57	10.29	0.39	36.66	89.23
0.8	9.22	0.38	29.26	8.25	0.18	29.39	89.43
1.0	7.66	0.30	24.31	6.78	0.20	24.16	88.48

*µg BHT equivalent / mg crude extract.
**tested with 2 lots of cosmetic products homogenized with crude extracts.
ξ n = 6.
€ compared to the mean of the highest value of each data.
♥ compared to the mean of cycle no. 5 with the mean of cycle no. 0.

Table 3 Chelating ability of cosmetic products homogenizing with crude extracts from inoculated PDB.

Product type / concentration (%)	DPPH radical scavenging activity at cycle no. 0*,**			DPPH radical scavenging activity at cycle no. 5*,**			% Recovery - Cycle▼
	Mean$^{\xi}$	SD$^{\xi}$	% Relative$^{\epsilon}$	Mean$^{\xi}$	SD$^{\xi}$	% Relative$^{\epsilon}$	
Cream base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	196.59	3.00	100.00	174.58	2.27	100.00	88.80
0.4	102.30	2.16	52.04	91.48	1.65	52.40	89.42
0.6	71.24	0.60	36.24	64.35	0.92	36.86	90.32
0.8	55.17	0.44	28.06	49.81	0.83	28.53	90.28
1.0	44.98	1.08	22.88	40.68	0.71	23.30	90.44
Serum base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	27.93	1.58	100.00	24.92	1.05	100.00	89.22
0.4	18.68	0.87	66.87	16.68	0.64	66.93	89.30
0.6	14.36	0.50	51.41	12.81	0.90	51.41	89.22
0.8	11.66	0.31	41.72	10.35	0.35	41.55	88.84
1.0	10.04	0.26	35.94	8.94	0.20	35.88	89.05
Gel base							
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	32.30	0.46	100.00	28.76	0.88	100.00	89.02
0.4	19.86	0.29	61.48	17.59	0.32	61.19	88.59
0.6	15.13	0.20	46.84	13.50	0.25	46.96	89.23
0.8	12.56	0.12	38.90	11.14	0.21	38.74	88.66
1.0	11.07	0.08	34.26	9.78	0.11	34.02	88.39

Table 4 Chelating ability of cosmetic products homogenizing with crude extracts from inoculated Glucose medium.

Product type / concentration (%)	DPPH radical scavenging activity at cycle no. 0*,**			DPPH radical scavenging activity at cycle no. 5*,**				% Recovery -Cycle▼
	Mean$^{\xi}$	SD$^{\xi}$	% Relative$^{\epsilon}$	Mean$^{\xi}$	SD$^{\xi}$	% Relative$^{\epsilon}$		
Cream base								
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	202.02	3.11	100.00	180.46	3.77	100.00	202.02	3.11
0.4	106.18	0.98	52.56	95.99	1.21	53.19	106.18	0.98
0.6	73.60	0.86	36.43	66.33	0.92	36.76	73.60	0.86
0.8	56.48	0.60	27.96	51.21	0.84	28.38	56.48	0.60
1.0	46.35	0.37	22.94	41.89	0.50	23.22	46.35	0.37
Serum base								
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	30.98	3.42	100.00	27.44	1.06	100.00	30.98	3.42
0.4	22.05	1.08	71.18	19.61	0.59	71.48	22.05	1.08
0.6	16.77	0.41	54.13	14.89	0.49	54.27	16.77	0.41
0.8	13.36	0.35	43.14	11.94	0.29	43.52	13.36	0.35
1.0	11.67	0.22	37.67	10.45	0.31	38.08	11.67	0.22
Gel base								
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	34.75	1.20	100.00	30.97	0.47	100.00	34.75	1.20
0.4	23.75	0.37	68.33	21.08	0.44	68.08	23.75	0.37
0.6	17.70	0.32	50.93	15.78	0.30	50.94	17.70	0.32
0.8	14.03	0.13	40.38	12.39	0.16	40.01	14.03	0.13
1.0	12.87	0.15	37.04	11.44	0.15	36.95	12.87	0.15

*µg EDTA equivalent / mg crude extract.
**tested with 2 lots of cosmetic products homogenized with crude extracts.
$^{\xi}$n = 6.
$^{\epsilon}$compared to the mean of the highest value of each data.
▼compared to the mean of cycle no. 5 with the mean of cycle no. 0.|

Consideration of various concentrations of either PDB or Glucose crude extracts used in cosmetic products and their antioxidant activities (µg BHT equivalent / mg crude extract and µg EDTA equivalent / mg crude extract) reveals that the more crude extracts used, the less antioxidant activities obtained (% Relative at cycle no. 0 and % Relative at cycle no. 5). The optimal concentration of either PDB or Glucose crude extracts used in cosmetic products should be 0.2%. pH and color of cosmetic products blended with crude extracts from culturing media of Pleurotus ostreatus var. Bhutan The unchange of the pH of all products (pH 5.5), except for the pH of the serum homogenized with PDB 0.8 and 1.0% (pH 5.0) reveals that the extracts may not affect any ingredients of cosmetic products tested and have

tendency not to irritate the skin since the pH of the end products blended was similar to the pH of the human skin. Comparison of the color of all products with the color of control reveals that the color occurred in the products is the color of the extracts. However, comparison of the color of all products before and after accelerated test showing no color change reveals that the extracts may not affect any ingredients of cosmetic products tested.

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