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Optimal Extraction Conditions of Anti-obesity Lipase Inhibitor from *Phellinus linteus* and Nutritional Characteristics of the Extracts

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In an effort to develop novel mushroom-derived anti-obesity nutraceuticals, water and ethanol extracts containing the lipase-inhibitory compound from *Phellinus linteus* were prepared, and their nutritional components were determined. The optimal conditions for the extraction of *P. linteus* lipase inhibitor involved the treatment of the fruiting bodies with distilled water at 80°C for 72 hr and 80% ethanol at 100°C for 60 hr, respectively. The distilled water extract and ethanol extract contained 10.9% and 6.11% of crude protein, and 0.96% and 15.86% of crude fat, respectively. Additionally, the distilled water extract contained a large quantity of minerals, including 239.5 mg of K, 39.3 mg of Mg, and 39.3 mg of Na. The free amino acid content of the distilled water extracts was also higher than that of the ethanol extracts, and in particular, the distilled water extracts contained 5,139 mg of asparagine, 3,891 mg of tryptophan, 2,598 mg of alanine, and 2,066 mg of serine in 100 g of the distilled water extracts. 100 g of the distilled water and ethanol extracts were found to contain 12.31 g and 8.16 g of malic acid, respectively.

KEYWORDS : Anti-obesity, Lipase inhibition, Nutritional characteristic, *Phellinus linteus*

Phellinus linteus is classified as a member of the genus *phellinus* of the family *Hymenochaetaceae*. This mushroom is perennial and thrives in broad-leaved trees, particularly dead trees of *Morus* and *Prunus sargentii Rehder*. It has previously been evaluated for its immuno-stimulatory effects, as well as its polysaccharides and putative anti-cancer compounds [1-4]. Recently, its anti-obesity lipase inhibitor was also identified [5].

Obesity has been shown to induce serious cardiovascular diseases, cancers, and other conditions. Several anti-obesity agents have been developed thus far, including amylase and lipase inhibitors as well as commercial anti-obesity products such as orlistat (Xenical). Among them, it is generally believed that a potent lipase inhibitor could prove extremely useful as an anti-obesity compound, because lipase (triacylglycerol hydrolase, EC 3.1.1.3) is a key enzyme for dietary fat adsorption, hydrolyzing triacylglycerols to 2-monoacylglycerols and fatty acids [6].

Many studies have been conducted involving lipase inhibitors derived from natural sources such as tannin [7], algae [8], pumpkin and Job's tear [9], saponins [10], *Rhei Rhizoma* and chunghyuldan [11] and *Monascus* pigment [12], etc. The orlistat from *Streptomyces toxylricim*, a potent lipase inhibitor, has proven useful in the treatment of obesity [13]. However, commercial lipase inhibitors have some troubling side effects, including fecal incontinence and low efficiency, among others. Therefore, it is clearly necessary to develop a potent anti-obesity lipase inhibitor with no side effects.

As part of a broader effort to develop new anti-obesity functional foods, we have already reported on the screening of a potent anti-obesity lipase inhibitor-containing mushroom, and assessed its characteristics to some degree [5]. In this paper, we attempted to determine the optimal water and ethanol extraction conditions of the lipase inhibitor from fruiting bodies of *Phellinus linteus* in order to develop new anti-obesity functional foods, and its nutritional characteristics were assessed.

Materials and Methods

Mushroom and chemicals. *P. linteus* was acquired from the Chungnam Agricultural Research and Extension Service of Yesan, in Chungnam province. Unless specified otherwise, all chemicals and solvents utilized herein were of analytical grade. Lipase (porcine pancreatic lipase, Type II), triolein as substrate, TES (N-Tris [hydroxymethyl] methyl-2-aminoethane-sulfonic acid), taurocholic acid, and gum arabic were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extract preparation. The lyophilized powder of fruiting bodies of *P. linteus* was added to distilled water and 60% ethanol (1 : 20, w/v), then shaken for 18 hr at 40°C. These extracts were centrifuged for 20 min at 8,000 rpm, concentrated with a rotary vacuum evaporator, and lyo-

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Table 1. Analysis conditions of HPLC for determination of free sugars, organic acids, and amino acids

	Organic acid	Free sugars	Free amino acids
Instrument	Waters 2695	Waters 2690	Waters 2965
Column	YMC C_{18} column (3.9 × 150 mm, 5 μm)	Polaris NH_2 column (3.9 × 250 mm, 5 μm)	AccQ-Tag column (3.9 × 150 mm, 4 μm)
Column temp.	35	35	37
Detector	UV (214 nm)	RI	FLD (Ex. 250 nm, Em. 395 nm)
Mobile phase	0.02 M KH_2PO_4 (pH 2.8)	Acetonitrile : Water = 75 : 25	0.14 M sodium acetate, 60% acetonitrile gadient
Flow rate	0.7 mL/min	1.0 mL/min	1 mL/min
Injection vol.	10 μL	10 μL	10 μL

philized.

Assay of lipase inhibitory activity. Lipase inhibitory activity was assessed by determining the rate of release of oleic acid from triolein, via a modified version of the method developed by Bitou *et al.* [8]. A suspension of 120 mg of triolein, 90 mg of gum arabic, 10.16 mg of taurocholic acid in 9 mL 0.1 N TES buffer (pH 7.0), containing 0.1 M NaCl was sonicated for 5 min. A mixture of 50 μL of pancreatic lipase (500 U/mL, 50 μL of mushroom extracts (4 mg/mL), and 300 μL of substrate mixture was incubated for 30 min at 37°C, and the quantity of oleic acid generated was determined via the method developed by Zapf *et al.* [14] with some slight modifications. The 400 μL incubation mixtures were added to 3 mL of chloroform/hexane (1 : 1) containing 2% (v/v) ethanol, then extracted by 10 min of shaking in a shaker. The mixture was then centrifuged (2,000 × g) for 10 min and copper reagent was added to the lower organic layer, and then shaken for 10 min. The mixture was centrifuged (2,000 × g, 10 min), and 1 mL of the upper organic layer, which contained the copper salts of the extracted oleic acid, was allowed to react for 10 min with 0.5 mL of 0.1% (w/v) bathocuproine-chloroform solution containing 0.05% (w/v) 3-tert-butyl-4-hydroxyanisol, and the absorbance was determined at 480 nm. The inhibition (%) was calculated via the following equation:

$$\text{Inhibitory activity (\%)} = (A - B)/A \times 100,$$

in which A is the lipase activity in the reaction solution without the sample and B is the lipase activity in the reaction solution containing the sample.

Determination of nutritional components. The general components of distilled water extract and ethanol extract from the fruiting bodies of *P. linteus* were determined via the methods of Helrich [15]. The mineral contents were analyzed via ICP (Varian, Nederland) after wet degradation. The free sugar and organic acid contents of the distilled water extract and ethanol extract were determined by HPLC (2695; Waters Corp., Milford, MA, USA), and the amino acid contents were also determined via HPLC (2965; Waters Corp., FLD) after pretreatment with the Accq-tag method (Table 1).

Results and Discussion

Optimal conditions for extraction of lipase inhibitor. To collect data regarding the application of *P. linteus* lipase inhibitor for use in the functional food industry, the effects of temperature and time on the extraction of the lipase inhibitor from *P. linteus* by distilled water and ethanol were determined. As is shown in Figs 1 and 2, the lipase inhibitory activities of the fruiting bodies of *P. linteus* were higher in the ethanol extracts than in the distilled water extracts, and the inhibitor extraction yield increased with increasing extraction temperature and time. Furthermore, the optimal ethanol concentration for lipase inhibitor extraction was 80% (data not shown).

The lipase inhibitor was optimally extracted when *P. linteus* was treated with distilled water at 80°C for 72 hr (52.7%) and 80% ethanol at 100°C for 60 hr (73.2%), respectively. It has been determined that even when the same inhibitor is used, the optimal extraction temperature

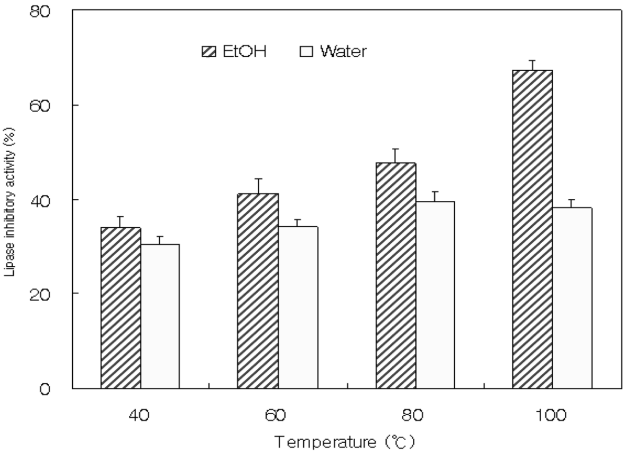

Fig. 1. Effects of extraction temperature on the extraction of lipase inhibitor from *Phellinus linteus*.

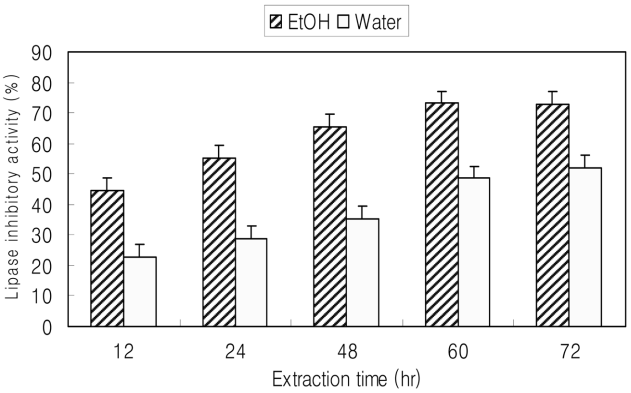

Fig. 2. Effects of extraction time on the extraction of the lipase inhibitor from *Phellinus linteus*.

Table 2. General component contents of lipase inhibitor-containing extracts from *Phellinus linteus* (unit: %)

Extracts	Carbohydrates	Crude protein	Crude fat	Crude ash
Ethanol extract	75.04	6.11	15.86	2.98
Distilled water extract	83.82	10.9	0.96	4.32

between plants and microorganisms differs. For example, the acetylcholinesterase (AChE) inhibitor from *Umbilicaria esculenta* [16] and Job's tears [7] was optimally extracted at 40°C; however, the AChE inhibitors from some plants were most efficiently extracted with hot water [17, 18].

Nutritional characteristics of lipase inhibitor-containing extracts. The lipase inhibitor-containing distilled water extract and ethanol extract from *P. linteus* contained crude protein contents of 10.9% and 6.11%, and crude fat contents of 0.96% and 15.86%, respectively (Table 2). The mineral contents of the distilled water extract were higher than those of the ethanol extract--specifically, the distilled water extract contained 239.5 mg of K, 36.3 mg of Mg and 39.3 mg of Na (Table 3).

The free amino acid contents of the distilled water extract and ethanol extract were determined (Table 4) and included tryptophan (3,891 mg/100 g solid), alanine (2,598 mg/100 g solid), serine (2,066 mg/100 g solid) and interestingly, asparagines (5,139 mg/100 g solid). These amino acid contents were higher than those of the ethanol extract, with the notable exception of the asparagine contents (6,137 mg/100 g solid). However, these amino acid contents were 2.0~6.0 times higher than those of the corresponding tryptophan (60 mg/100 g solid) and serine (1,400 mg/100 g solid) contents in the methanol extracts from *Umbilicaria esculenta* [16], and were also similar to the alanine contents (3,400 mg/100 g solid) and lower than the glutamic acid contents (44,400 mg/100 g solid). The glutamic acid (17.19 mg/g solid) and histidine contents (6.42 mg/g solid) of the distilled water extract from *P. linteus* were similar to those of *Lentinus edodes* (15.84 mg/g solid and 7.95 mg/g solid, respectively).

Table 4. Free amino acid contents of lipase inhibition-containing extracts from *Phellinus linteus* (unit: mg/100 g, dry basis)

Amino acids	Ethanol extract	Distilled water extract
Alanine	1,329	2,598
Arginine	479	1,384
Asparagine	6,137	5,139
Aspartic acid	778	1,762
Glutamic acid	802	1,719
Histidine	471	642
Proline	627	520
Leucine	561	979
Lysine	n.d[a]	865
Methionine	511	600
Phenylalanine	662	1,368
H-Proline	615	997
Serine	479	2,066
Theronine	448	972
Tryptophan	540	3,891
Tyrosine	482	892
Valine	553	1,098

[a]n.d, not detected.

The principal organic acid detected in the distilled water extract and ethanol extract of *P. linteus* was malic acid (12,312 mg/100 g solid, 8,160 mg/100 g solid, respectively). The distilled water extract also contained 590 mg/100 g solid of oxalic acid, and the ethanol extracts contained tartaric acid (620 mg/100 g solid); no other organic acids were detected (data not shown).

References

1. Lee JH, Cho SM, Kim HM, Hong ND, Yoo ID. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. J Microbiol Biotechnol 1997;7:52-5.
2. Lim JS, Kim SH, Park JS, Choi JY, Park SJ, Shin KS. Antitumor activity of the fruitbody extract of *Basidiomycete*, *Phellius linteus*. Kor J Microbiol 2001;39:121-5.
3. Park IH, Jeon SY, Lee HJ, Kim SI, Song KS. A beta-secre-

Table 3. Mineral contents of lipase inhibitor-containing extracts from *Phellinus linteus* (unit: mg/100 g, dry basis)

Extracts	Mg	Ca	K	Si	Al	P	Fe	B	Mn	Cu	Na	Ni	Zn
Ethanol extract	5.7	1.5	90.6	0.5	0.31	57.3	1.0	0.11	0.09	0.01	5.1	0.08	0.07
Distilled water extract	26.3	23.5	239.5	3.8	0.02	137.1	1.8	1.37	1.88	1.85	39.3	0.28	2.08

tase (BACE1) inhibitor hispidin from the mycelial cultures of *Phellinus linteus*. Planta Med 2004;70:143-6.
4. Shon YH, Lee JS, Lee HW, Kim JW, Lim JK, Kim CH, et al. Antimutagenicity of *Phellinus linteus* in *Salmonella typhimurium*. J Microbiol 1999;37:136-40.
5. Lee JK, Jang JH, Lee JT, Lee JS. Extraction and characteristics of anti-obesity lipase inhibitor from *Phellinus linteus*. Mycobiology 2010 (in press)
6. Bray GA, Blackburn GL, Ferguson JM, Greenway FL, Jain AK, Mendel CM, et al. Sibutramine produces dose-related weight loss. Obes Res 1999;7:189-98.
7. Seo DS, Jang JH, Kim NM, Lee JS. Optimal extraction condition and characterization of antidementia acetylcholinesterase inhibitor from Job's tear (*Coix lachrymajobi* L.). Korean J Medicinal Crop Sci 2009;17:434-8.
8. Bitou N, Ninomiya M, Tsujita T, Okuda H. Screening of lipase inhibitors from marine algae. Lipids 1999;34:441-5.
9. Kim SG, An GH, Yoon SW, Lee YC, Ha SD. A study on dietary supplement to reduce obesity by the mechanism of decreasing lipid and carbohydrate absorption. Korean J Food Sci Technol 2003;35:519-26.
10. Yoshizmi K, Hirano H, Ando H, Hirai Y, Ida Y, Tsuji T, et al. Lupane-type saponins from leaves of *Acanthopanax sessiliflorus* and their inhibitory activity on pancreatic lipase. J Agric Food Chem 2006;54: 335-41.
11. Yang HK, Kim YS, Bae HS, Cho KH, Shin JE, Kim NJ, et al. Rhei rhizoma and chunghyuldan inhibit pancreatic lipase. Nat Product Sci 2003;9:38-43.
12. Kim JH, Kim HJ, Kim C, Jung H, Kim YO, Ju JY, et al. Development of lipase inhibitors from various derivatives of *monascus* pigment produced by *Monascus* fermentation. Food Chem 2007;101:357-64.
13. Bray GA, Greenway FL. Current and potential drugs for treatment of obesity. Endocr Rev 1999;20:805-75.
14. Zapf J, Schoenle E, Waldvogel M, Sand I, Froesch ER. Effect of trypsin treatment of rat adipocytes on biological effects and binding of insulin and insulin-like growth factors: further evidence for the action of insulin-like growth factors through the insulin receptor. Eur J Biochem 1981; 133:605-9.
15. Helrich K. Official methods analysis of the Association of the Official Analytical Chemists. chapter 3.1.01-05. 15th ed. Washington, DC: Association of the Official Analytical Chemists; 1990.
16. Lee JS, Min GH, Lee JS. Nutritional and physicochemical characteristics of the antidementia acetylcholinesterase-inhibiting methanol extracts from *Umbilicaria esculenta*. Mycobiology 2009;37:203-6.
17. Jeong CH, Kang ST, Joo OS, Lee SC, Shin YH, Shin KH, et al. Phenolic content, antioxidant effect and acetylcholinesterase inhibitory activity of Korean commercial green, puer, oolong and black teas. Korean J Food Preserv 2009;16:230-7.
18. Kwak JH, Jeong CH, Kim JH, Choi GN, Shin YH, Cho SH, et al. Acetylcholinesterase inhibitory effect of green tea extracts according to storage condition. Korean J Food Sci Technol 2009;41:435-40.