



Literatur Review: The New Super Antioxidant, Ergothioneine In *Pleurotus ostreatus*

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Abstract

Pleurotus ostreatus is one of the mushrooms that is widely consumed by Indonesian people. The easy cultivation of *Pleurotus ostreatus* is one of the reasons why Indonesian people choose *Pleurotus ostreatus* as a cultivated mushroom and it is also used as a source of income. The main reason why people consume *Pleurotus ostreatus* is because it tastes good. On the other hand, *Pleurotus ostreatus* contains many chemical compounds that are beneficial to health, one of which is a rare amino acid compound, ergothioneine. Ergothioneine (EGT; 2-mercapto histidine trimethyl betaine) is an amino acid betaine thio-histidine with a

sulfur group that has the potential to be a stable, safe, and strong antioxidant (super antioxidant). In the health sector, ergothioneine antioxidants have the potential to prevent diseases such as cardiovascular, kidney, cancer, and inhibit the growth of tumor cells. In addition, ergothioneine also has the potential to be used as a supplement and medicine. The method used to compile this review article is a literature study of indexed and leading research journals conducted on the Google Scholar database, Science Direct, and PubMed. This review article discusses the ergothioneine content in *Pleurotus ostreatus* and the health benefits of ergothioneine

Keywords: *Pleurotus ostreatus*, antioxidants, ergothioneine, content and benefits ergothioneine



Introduction

White oyster mushroom (*Pleurotus ostreatus*) is a type of mushroom that is widely consumed by Indonesian people besides edible mushrooms, ear mushrooms and shitake mushrooms. This is due to the easy way of cultivation that has the potential to improve the economy (Rahmawati, 2015). The import demand for white oyster mushrooms in major cities in Indonesia reaches 25,000 kg / day, and it is estimated that in 2015 the market will increase by 5% per year (Priyadi & Alviantoro, 2013). One of the locations for *Pleurotus ostreatus* cultivation is Banyumeneng Village, Mranggen District, Demak Regency. The people of Banyumeneng Village cultivate a lot of *Pleurotus ostreatus* and become a source of income for their economy. *Pleurotus ostreatus* cultivation is carried out in a conventional manner and is only marketed in raw conditions without any processed products. Product diversification is an effort to increase its selling value. For that we need an understanding of the advantages of *Pleurotus ostreatus*.

Pleurotus ostreatus is a type of wood fungus that has a higher nutrient content compared to other wood fungi. Oyster mushrooms contain up to 35% protein, 9 kinds of amino acids, 2.2% fat consisting of 72% unsaturated fatty acids and carbohydrates in every 100gr of white oyster mushrooms (Egra et al., 2018).. The content of *Pleurotus ostreatus* which has a major role in the health sector is the antioxidant ergothioneine.

The highest ergothioneine (EGT) antioxidants content is 25% using common water extract (Egra et al., 2018). According to research by Ito, *Pleurotus ostreatus* contains EGT antioxidant as much as 1.98 mg / g using hot water extract (Ito et al., 2011). Mushrooms that contain antioxidants or having the ability to increase the activity of antioxidant enzymes can be used to reduce oxidative damage in humans. *Pleurotus ostreatus* can also potentially be a hepatoprotector (Mira Yustika Susilo, 2019).

However, small amount of research on the characteristics and benefits of EGT on processed *Pleurotus ostreatus* have been done. Based on a study conducted by the Agency for the Assessment of the Application of Technology (BPPT), it is explained that so far *Pleurotus ostreatus* has been used in the development of healthy drinking products technology, flavorings and ready-to-eat food, and beta-glucan extraction technology (BPPT, 2011). Whereas in today's society, *Pleurotus ostreatus* is widely used to be sold raw or as a food ingredient that is processed into several types of food such as nuggets, crispy mushrooms, and so on. Almost on average, *Pleurotus ostreatus* in Indonesia has not been widely used as a medicinal ingredient or health supplement (Rahmawati, 2015). *Pleurotus ostreatus* is easy to find because the number of cultivated products of the Banyumeneng people is quite large (40-100kg / day).

Based on the existing potentials, the purpose of this research review is to determine the ergothioneine content in *Pleurotus ostreatus* and its benefits for health. So that with this article, it is hoped that the public will know more about the ergothioneine content in *Pleurotus ostreatus* along with its benefits.



Methods

The research method was carried out by using a literature review. Literature review was done by collecting and summarizing data from previous studies. In searching for published articles in the portal using keywords, namely white oyster mushrooms, *pleurotus ostreatus*, ergothioneine, antioxidants in mushrooms, benefits of Ergothioneine, benefits of antioxidants, types of mushrooms and their benefits, and the content of ergothioneine in mushrooms. Journals or articles that match the inclusion and exclusion criteria are taken for further analysis. This Narrative Review uses literary articles that have been published in 2010-2020 which can be accessed in 250 national and international journals, then 29 articles are obtained which has close results to the research theme. The duration of the study was 3 months, namely August - October 2020. The inclusion and exclusion criteria included are as follow:

Article Inclusion Criteria	- Article or books corresponding to the white oyster mushroom, <i>Pleurotus ostreatus</i>, ergothioneine, antioxidants on mushroom, ergothioneine benefits, antioxidant benefits, mushroom types and its benefits, ergothioneine in mushroom contents. - Articles published in 2010-2020 period - Articles that have possessed DOI (Digital Object Identifier) - Articles are national and international journal derived from publications portals. - Full text article
Article Exclusion Criteria	- Articles derived from un-accredited journal portal both nationally and internationally. - Article published before 2010 - Articles that haven't possess DOI (Digital Object Identifier) - Proceeding Article, Scientific article (Thesis)

Research Stages

1. Problem formulation

The background of this research review is the existence of natural potential, namely white oyster mushrooms which are widely consumed by the public, but the benefits of white oyster mushrooms have not been optimized yet, especially in the health sector. This study aims to determine the ergothioneine content in mushrooms and its benefits for human health. The object of our research is white oyster mushroom. The problem formulation of this research is how is the content of ergothioneine in white oyster mushrooms and what is its benefit for human health.

2. Collecting Data

Search for published articles based on keywords was carried out on the Google Scholar page, SINTA Journal Portal, scopus, Science direct, PubMed. The criteria for the selected articles were articles related to research topics, especially articles that discussed the content and benefits of ergothioneine in white oyster mushrooms. After searching the journal portal database using a key



word, 1512 articles were found. Several articles that did not fit the research theme were excluded. So that there were 29 articles, consisting of 6 articles on Google Scholar and SINTA Journal Portal, 8 articles on Scopus, 8 articles on Sciencedirect, and 7 articles on PubMed.

3. Data Evaluation

The articles identification and deletion was carried out in accordance with the specified inclusion criteria, namely articles that match the keywords White oyster mushrooms, *Pleurotus ostreatus*, Ergothioneine, Antioxidants in mushrooms, Benefits of Ergothioneine, Benefits of Antioxidants, Types of Mushrooms and their benefits, Ergothioneine Content in Mushrooms; Articles published in 2010-2020 period; Articles that have possessed DOI (Digital Object Identifier); Articles are national and international journals that was derived from the publication portal

4. Discussion and Public Presentation

Data analysis and interpretation were carried out based on the selected articles. The articles that fit the inclusion criteria are presented in the form of narrative text with its data presented in the form of descriptive sentences and tables.

5. Conclusion

Combination of several results and discussion of articles adjusted to the purpose of the research review.

Results and Discussion

White Oyster Mushroom (*Pleurotus Ostreatus*)

Mushrooms are one of the organisms without chlorophyll that live like plants. Mushrooms do not carry out photosynthesis because they have organic components as a source of energy and carbon. Fungi reproduce by sexual and asexual reproduction. The majority of fungi produce large spores which are then spread out through the wind. Fungi that reproduce through spores that produce fine threads are called mycelia, it will then grow to form their bodies and fruits. In the ecosystem, fungi play an important role as a decomposer and participate in breaking down organic material into simpler forms (Rahmawati, 2015). Mushrooms have many types. Currently identified by scientists there are about 1.5 million species of fungi, which is an abundance of fungi from around the world. In Indonesia, the number of mushroom species reaches 200,000 species of the 1.5 million species found in the world including both mushrooms that can be consumed by the community and mushrooms that are used for antibiotic production. However, until now there is no definite data regarding the number of types of fungi that have been identified, used, or extinct due to human activity. In Indonesia, the diversity of fungi in the Tricholomataceae family is the second highest after the polyporaceae family, namely 6 types of fungi (Annisa et al., 2017).

White oyster mushroom is a type of mushroom from the Tricholomataceae family. White oyster mushroom is included in the food category because it is safe and non-toxic when consumed and has high nutrition, one of which is fiber. White oyster mushrooms are widely consumed by dieters because of their fiber content which is good for digestion (7.4 - 24.6%) (Alex, 2011). In addition to the delicious taste of white oyster mushrooms like meat, white oyster mushrooms are also preferred because of their effects and benefits for health.



White oyster mushrooms are useful as exogenous antioxidants. Oyster mushroom extract was able to prevent the increase in blood levels of Malondialdehyde (MDA) (0.4667 ± 0.22295 nm / mL). Where the ethanol extract of white oyster mushrooms with a dose of 250 mg / kg BW has a large antioxidant effect with an average of 62.2% (Rahimah et al., 2009). This is because mushrooms have substances that contain antioxidants such as ergotien, vitamin C, selenium, beta carotene, and phenols. Viewing from the phenol content in white oyster mushrooms, mushrooms are classified as strong antioxidants that are useful as free radical scavengers. The effects of free radicals arising from exposure to cigarette smoke can be balanced with the provision of ethanol extract of white oyster mushrooms which has strong antioxidant properties that can balance between oxidants and prooxidants (Rahimah et al., 2009). Based on this statement, it can be seen the potential of white oyster mushrooms, their extracts can be used as drugs or supplements to prevent oxidative damage due to exposure to cigarette smoke, both for children, teenagers and adults.

White oyster mushroom micelia extract can be used as a natural antioxidant product for the food and pharmaceutical industries. This is because the oyster mushroom micellia contains biologically active components. So that the oyster mushroom extract contains very high antioxidant activity (> 100 ppm) which is useful for inhibiting free radicals and inhibiting pathogenic microbes for humans, but this content also depends on the concentration of the sample and additional solution at the time of sample extraction. Existing oyster mushroom extracts include the addition of hot water, plain water and ethanol with significant statistical values (Vamanu, 2012). The total polyphenol content also has a positive correlation with antioxidant activity, which gets additional drying treatment using freeze drying so that the highest quantity of antioxidant components are obtained (Dai and Mumper, 2010).

Ergothioneine Contents

Ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine) is an amino acid betaine thio-histidine with a sulfur group in the imidazole ring which has strong antioxidant activity, namely 50 ppm - 100 ppm based on the IC₅₀ value (Stampfli, Blankenfeldt and Seebeck, 2020). This is because the unique ergothioneine structure binds sulfur and contains a carbonyl group. Ergothioneine is a histidine amino acid derivative that undergoes sulfur substitution such as the amino acid cysteine (Stampfli et al., 2020).

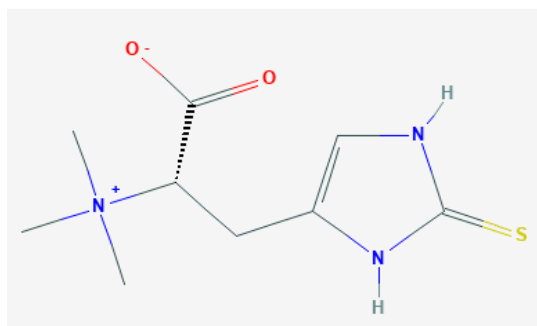


Figure1. Ergothioneine Structure (PubChem, 2019)

The only organisms known to synthesize ergothioneine are bacteria belonging to the order Actinomycetales (eg microbacteria) and fungi such as non-yeasts which belong to the Basidiomycota and Ascomycota division (Pfeiffer et al., 2011). This microbe synthesizes EGT from L-histidine via an intermediate hercynine, betaine histidine. 4,5 Sulfur groups are added to hercynine to form EGT. 4. Mushrooms are the main source of ergothioneine containing 0.4 to 2.0 mg / g (dry weight) (Weigand-Heller et al., 2012). The ergothioneine content of various types of mushrooms can be described in table 1.

Table.1 Ergothioneine Content From Various Type of Mushrooms

Mushroom Type	Ergothioneine (mg/gr)	Reference
Agaricus bisporus (white)	0. 4-2.0	Weigand, 2012
Agrocybe cylindracea	0.279	Chen et al., 2012
Volvariella volvacea	0.0537	Lin et al., 2013
Boletus edulis	0.528	Bao et al., 2010
Herichium erinaceus	0.063	Cohen et al., 2014
Termitomyces clypeatus	0.122	Woldegiorgis et al., 2014
Lentinula edodes	0.198	Pahila et al., 2017
Agrocybe cylindracea	0.025	Lin et al., 2013
Pleurotus ostreatus	1.98 ± 0.04	Ito et al., 2011
Pleurotus cornucopiae	2.082 ± 0.12	Bao et al., 2010
Pleurotus citrinopileatus	3. 94	Kalaras et al., 2017

Table 1 shows that the highest ergothioneine content in fungi is in the pleurotus genus. This is because in the pleurotus genus fungi, the content of ergothioneine is found in the mycelium and stems (Chen et al., 2012). The ergothioneine content of various types of pleurotus genus fungi can be explained in table 2.



Table.2 Ergothioneine Contents In Various Types of Mushroom In The Pleurotus Genus

Types of Mushrooms	Ergothioneine (mg/gr)	Reference
Pleurotus cystidiosus	0.26	Chen et al., 2012
Pleurotus salmoneostramineus	1.25	Chen et al., 2012
Pleurotus eringii	1.41 ± 0.12	Ito et al., 2011
Pleurotus ostreatus	1.98 ± 0.04	Ito et al., 2011
Pleurotus cornucopiae	2.08 ± 0.12	Bao et al., 2010
Pleurotus citrinopileatus	3.94	Kalaras et al., 2017

Table 2 shows that the highest ergothioneine content in pleurotus citrinopileatus is 3.94 mg / gr. However, Pleurotus citrinopileatus is still very rarely cultivated in Indonesia. Pleurotus citrinopileatus is a fungus that grows in Russia, Japan and China. In Indonesia, the pleurotus mushroom genus is mostly white oyster mushroom (Pleurotus ostreatus) which contains ergothioneine 1.98 mg / gr (Ito et al., 2011). The ergothioneine content in the pleurotus ostreatus is also strongly influenced by the country where the fungus grows which is described in table 3.

Table.3 Ergothioneine Content In Pleurotus Ostreatus In Various Countries

Mushroom Type	Ergothioneine (mg/gr)	Country
Pleurotus ostreatus	0.944	Japan
Pleurotus ostreatus	1.458	Taiwan
Pleurotus ostreatus	1.829	Korea

(Chen et al., 2012)

Analysis of the ergothioneine content in mushrooms can be carried out by extracting the mushrooms continued with testing them using HPLC instruments as was done in Kalaras (2017) research which was modified from Dubost's research (2006). A total of 0.5 grams of sample was added to 7 ml of ethanol extraction (10 mM dithiothreitol, 100 mM betaine, 100 mM 2-mercapto-1-methyl imidazole) and 3 ml of distilled water in a centrifuge tube. Then mixed until homogeneous with vortex for 10 seconds. Next, 2 ml of 1% SDS solution in ethanol were added and mixed with vortex for 10 seconds. Centrifugation for 20 minutes at 4000 rpm and 25 ° C. A total of 1 ml of supernatant was then added to the microcentrifuge tube and placed on the speedVac for 24 hours. The dry residue was dissolved in 0.5 ml of ultrapure water. The samples were then placed in a microcentrifuge for 1 minute at 10,000 rpm. The supernatant was collected and filtered into glass HPLC bottles for analysis using instrumental HPLC. The results obtained were that the ergothioneine content in Pleurotus ostreatus was 1.21 ± 0.25 mg / gr. Whereas in the study by Ito et al., (2011), which refers to the dubost research that also refers to modification using a water solvent with extraction using hot water, the ergothioneine content of Pleurotus ostreatus was found to be 1.98 ± 0.04 mg / g (Ito et al., 2011).



Ergothioneine Benefits

a. Antioxidant and anti-inflammation

Ergothioneine is an antioxidant and anti-inflammatory substance that cannot be synthesized by humans. Ergothioneine is a naturally occurring sulfur-containing amino acid, this causes ergothioneine to only be synthesized by fungi and bacterial microbes in the soil (Mira Yustika Susilo, 2019). Ergothioneine is usually found in the diet by humans and other animals through transporters and then accumulates to high levels in certain tissues that experience relatively high levels of oxidative stress, such as erythrocytes, airway epithelium, liver, and kidneys (Kerley et al., 2018; Mira Yustika Susilo, 2019).

EGT is found throughout the human body, especially in red blood cells, liver, kidney and urine (Kalaras et al., 2017). EGT is a stable, safe, and strong antioxidant (Cheung, Bernice., Kwan, Macy., Chan, Ruth., Sea, Mandy & Woo, 2016) In addition, EGT is an antioxidant that has safety without toxicity (Kalaras et al., 2017). There are various benefits generated by EGT. EGT has a role as a biological agent that acts with other antioxidants to be able to protect against oxidative stress in mitochondria (Kerley et al., 2018; Krakowska et al., 2020). EGT is able to protect humans from liver damage, increase survival, increase HSP70, reduce MDA and lipid peroxidation (Kerley et al., 2018).

EGT is an antioxidant that can increase serum levels of SOD and GSH-Px and reduce MDA (Cheung, Bernice., Kwan, Macy., Chan, Ruth., Sea, Mandy & Woo, 2016). EGT acts as a protector of the body from UV and gamma radiation. EGT is a physiological protector against ultraviolet-induced damage and formation of ROS. The addition of EGT was able to significantly increase cell viability and reduce caspase-9. In addition, EGT can inhibit tumor cell growth (Cheah & Halliwell, 2012). EGT is a safe antioxidant, paying more attention with drug interactions especially metformin, gabapentin, and chemotherapy (Cremades et al., 2020).

b. Neurodegenerative, Cardiovascular, Kidney, and Cancer Disease Inhibitors

EGT is useful as a human therapeutic supplement (Cheah & Halliwell, 2012). The potential of EGT is proven as a therapeutic agent to treat and prevent various diseases including neurodegenerative, cardiovascular and kidney diseases and cancer (Aruoma et al., 2012). EGT is able to play as an agent that can maintain overall health and reduce the risk and progression of disease. EGT is able to help prevent various oxidative diseases associated with stress (Pahila et al., 2017). Also EGT is able to restore learning and memory deficits caused by cisplatin injury (Kerley et al., 2018).

c. Anti Diabetes

Another perceived benefit of EGT is that it can reduce pain and improve functionality in people who suffer from mild to moderate chronic pain and those that affect joints (Aruoma et al., 2012). EGT with strong hydroxyl radicals, superoxide anion, hypochlorous acid and peroxynitrite, protects blood vessel function from oxidative damage and prevents endothelial dysfunction (Merone & McDermott, 2017). EGT is used to protect the heart against oxidation of myoglobin by ROS / RNS to cytotoxic myoglobin. EGT helps in patients with Chronic



Kidney Disease (CKD), that is, it can help reduce oxidative damage due to CKD accumulating in the kidneys (Cheah & Halliwell, 2012). In addition, EGT protects endothelial cells during hGluc treatment as well as a form of defense against ROS production in diabetes (Servillo et al., 2017).

White oyster mushroom contains bioactive metabolites used as a source that has not been widely used, but in recent years it has been used as traditional medicine by the public because of its bioactive properties (Papaspayridi et al., 2012; Widyastuti, 2019). The benefits that are in mushrooms will be lost when the mushroom processing process is not good. The processing of oyster mushrooms by boiling and frying can reduce the nutritional content, this is because most of the nutrients are dissolved in cooking water and cooking oil. Heat treatment can reduce the amount of antioxidants and destroy some amino acids, changing the quality of the antioxidant composition in foods. This is related to the EGT content in mushrooms. The content will be destroyed, if processed at a temperature of 1750 °C (CJK, 1998). In *Pleurotus* mushrooms, the activity of decreasing antioxidant content was specifically detected after processing by boiling or frying, but for processing by roasting or heating in a microwave, the resulting antioxidant value was 80% higher (Imenez-Zamora A, Delgado-Andrade C, 2016). This occurs due to the microwaving process to maintain the nutritional profile in the fungus (Roncero-Ramos et al., 2017).

There are several methods used in the utilization of oyster mushrooms. For consumption, oyster mushrooms are processed by boiling, stirring, steaming or frying. Meanwhile, as medicine, people traditionally use it in dry form or boil it with water which is then drunk as medicine (Rahmawati, 2015). Therefore, it is necessary to have further research related to the processing of white oyster mushrooms which can be used as food or medicine for health. The HPLC test is used to check the EGT content. DPPH test to check for antioxidant content in mushrooms. Other tests that are needed are nutritional and beta-glucogan tests.

Conclusion and Recommendations

Ergothioneine (EGT; 2-mercaptop histidine trimethyl betaine) is a betaine thio-histidine amino acid with a sulfur group that has strong antioxidant activity. EGT is found throughout the human body, which is a stable, safe and powerful antioxidant and has safety without toxicity. EGT is useful as a human therapeutic supplement, and helps prevent neurodegenerative, cardiovascular, kidney, cancer, and possess the ability to inhibit tumor cell growth. Therefore, it is necessary to have further research related to the processing of white oyster mushrooms which can be used as food or medicine for health. The HPLC test is used to check the EGT content. DPPH test to check for antioxidant content in mushrooms. Other tests that are needed are nutritional and beta-glucogan tests.



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