CHAPTER 2

LITERATURE REVIEW

2.1 Oxidative stress

The exposure of living organisms to reactive oxygen species, notably oxygen free radicals and hydrogen peroxide, is associated with the varied aerobic lives. Oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage. Every cell in the living organism can generate reactive oxygen species as well, and some cell-types are even specialized to do so continually or in the form of an 'oxidative burst' (Sies, 1991). This oxidative burst leads to the production of reactive oxygen species (ROS) which is catalyzed by NADPH oxidase, one of the membrane-associated enzymes. ROS alone or in combination with lysosomal enzymes can act as killing agents and is important in the elimination of viruses, bacteria, yeast, fungi, and protozoa. The initial metabolite, superoxide anion (O^{2-}) is dismutated to hydrogen peroxide (H_2O_2), which may then be converted to other toxic ROS, such as hydroxyl radical (OH^{-}) and singlet oxygen ($^1O_2$). Hydrogen peroxide also interacts with myeloperoxidase (MPO) and halide to produce hypochlorous acid (HOCl). All of these metabolites play an important role in phagocyte-mediated killing of microorganisms (Adema *et al*., 1991).

Free radicals may be defined as any molecules with one or more unpaired electrons that react with biological compounds in the body, causing damage to living tissues (Parker and Ong, 1992). They are extremely reactive and generally highly unstable (Martinez, 1995). However, the imbalance between reactive oxygen species generating and

scavenging systems (oxidative stress) has been implicated in the number of pathology disorders, such as artherosclerosis, ischemia-reperfusion injury, cancer, malaria, diabetes, inflammatory joint, asthma, cardiovascular diseases, cataracts, immune system decline and can play a role in neurodegenerative diseases and ageing processes (Begell, 2000). This is supported by a study carried by Naik *et al*. (2005) in which he found that antioxidants are substances that can be supplemented either endogenously or exogenously to combat the state of oxidative stress. Endogenous antioxidants can be obtained from several natural products such as vitamin E, vitamin C, α-carotene, curcumin which show excellent antioxidant activity (Naik *et al*., 2005).

2.2 Oxidation of lipid-rich foods

Food lipids contribute in producing fatty acids that can add flavours and also work as solvents for carrying hydrophobic flavours and aromas (nutrients). Even small quantities of free fatty acids can give flavors and texture to many foods like cheese and milk chocolate but they also have harmful effect by causing foaming and off-flavors in milk, fruits and vegetables. The other type of lipids is polyunsaturated fatty acid (PUFA), which acts as a precursor to potent bioactive compounds such as prostaglandins, platelet anti-aggregate and thromboxanes (platelet aggregate). However, PUFA intake requirement should make up to 3% of fatty acid and deficiency of PUFA can cause serious diseases like growth retardation, skin lesions, neurological and visual abnormalities. Lipid peroxidation is the process of oxidative degradation of lipids whereby free radicals take electrons from the lipids in cell membranes, resulting in cell damage. This free radical chain reaction mechanism process often affects polyunsaturated fatty acids, because they contain multiple double bonds in between methylene -CH_2- groups that possess especially reactive hydrogens. The radical reaction

consists of three major steps which are initiation, propagation and termination. In the initiation stage, combination of reactive oxygen species (ROS), such as OH initiators with hydrogen atom produces a fatty acid radical and water. The unstable molecule of fatty acid radical reacts readily with molecular oxygen and creating a peroxyl-fatty acid radical in the propagation stage. This, which is also an unstable species reacts with another free fatty acid producing a different fatty acid radical and a lipid peroxide or a cyclic peroxide if it reacts with itself. This cycle continues as the new fatty acid radical reacts in the same way. The termination stage appears when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of the two radicals to actually collide. The cell membrane is protected if different molecules in living organisms have evolved and speed up termination by catching free radicals. If they are not terminated fast enough, it causes damage to the cell membrane, which consists mainly of lipids. In addition, the end products of the lipid peroxidation may be mutagenic and carcinogenic (Marnett, 1999).

Lipid oxidation in food has caused major deterioration in the quality of food products such as loss of flavor, color, texture and nutritive value, and also health risks. Rapid development of rancid flavors during storage is a major problem faced by our food industry, especially with the increasing demand for precooked food items. Ma (2004) defined oxidative rancidity as a chemical change that results in unpleasant odors and taste in a fat. They showed that, there is a rapid onset of rancidity in cooked meats during refrigerated storage and oxidized flavors are readily detectable just after 48 hours the meat is cooked. The production of rancid flavors, which may be caused by the oxidation products such as hydroperoxide can lead to the formation of aldehyde (alkanals and hexanal) which then damage other compounds including vitamins,

proteins and finally cause rancidity to food. Processing or packaging method used in food industry are based on temperature, water activity, metal ions and light affects oxidation in food. It is believed that if a breakdown of lipid hydroperoxides and non-hydrated metal with ion occurred readily, they will become more effective to induce rancidity in dehydrated foods. However, in the presence of chelators which restrict the amount of free metal, the rate of lipid oxidation will be slower. Other than that, the use of light as a source of energy in food packaging processes also leads to the formation of radical initiators and become harmful when consumed (Ma *et al*., 2004).

In daily cooking preparation of most foods involves the use of cooking oil, and this cooking oil are rich in polyunsaturated lipids, which is easily oxidised when subjected to such high heat. The presence of lipid free radicals may oxidise flavours, pigments and vitamins. Hydroperoxides, the primary products of autoxidation, may form dark-coloured, possibly toxic products or decompose to yield rancid off-flavour compounds. These autoxidation was initiated by exposure to the enzyme lipoxygenase, heat, ionising radiation, light, metal ions and metallo-protein catalysts. The food industries are well aware of these reactions and are concerned with rancidity and the oxidative spoilage of foodstuff (Shahidi and Wanasundara, 1992).

In the food industry, the rate of autoxidation is reduced by freezing, refrigeration, packaging under inert gas in the absence of oxygen and vacuum packaging. A study done by Alonso *et al.* (2007) on a plant extract showed that minimizing or retarding lipid oxidation in lipid-based food products improves the quality of food. He also found that some natural phenolic compounds mostly flavonoids, have beneficial effects on lipid metabolism. The use of antioxidants is an effective way to minimize or prevent lipid oxidation in food products, retard the formation of toxic oxidation products,

maintain nutritional quality and prolong the shelf life of food. Examples of synthetic antioxidants that are widely used as additives to protect food against deterioration are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) and propylgallate (PG), even though the use is increasingly restricted, due to their potential health risks and toxicity (Kosar *et al.*, 2007 ; Yingming *et al.*, 2007).

2.3 Natural antioxidants

An antioxidant can be defined as a chemical that reduces the rate of particular oxidation reactions or we can say it reduces the reactions that are involved in the transfer of electrons from a substance to an oxidizing agent. Antioxidants are very important in all living organisms or complex system as they prevent chemical damage to the cells' components by oxidation (Sies, 1991). Antioxidants are found naturally in varying amounts, in vegetables such as spinach, fruits like berries and peppers, grain cereals like hops, maize, barley, legumes, nuts and fungi like mushrooms (Choi *et al.*, 2007). Research done by Kanjana *et al.* (2000) showed the beneficial roles of fruits and vegetables in the human diet, providing protection against cellular damage caused by the exposure to high levels of free radicals. Numerous studies have shown that consuming foods which are high in antioxidants may reduce the risk of developing chronic diseases. For example, ergothioneine, a unique metabolite produced by fungi, has been shown to have strong antioxidant properties and to provide cellular protection within the human body (Thompson, 2005).

Many studies have shown that antioxidants from natural sources are widely used as ingredients in dietary supplements for health purposes such as preventing cancer and

heart disease. These supplements may include specific antioxidant chemicals, like resveratrol (from grape seeds), combinations of antioxidants, like the "ACES" products that contain beta carotene (provitamin A), vitamin C, vitamin E and Selenium, or specialty herbs that are known to contain antioxidants such as green tea and jiaogulan (Alonso *et al.,* 2007). Not only for health and medical purposes, antioxidants are also an important subject of extensive research in industrial processes such as the corrosion of metals, explosives and the vulcanization of rubber; food additives like ascorbic acid, tocopherol, BHA, citric acid and acetic acid which can be found in vinegar. Oxygen radical absorbance capacity (ORAC) has become the current industry standard for assessing antioxidant strength of whole foods, juices and food additives. However, there are other measurement tests including reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating that are cheaper and used widely by researchers in identifying antioxidant activities (William *et al.*, 1995).

2.4 Mushrooms as a source of antioxidants

Values of antioxidants in various edible, medicinal and wild mushrooms have a long history and have been used worldwide in the Orient, especially in China for medicinal purposes. In China, mushrooms are traditionally used as food. For instance, oyster mushrooms, *Agaricus bisporus,* paddy straw mushrooms (*Volvariella volvacea*), shiitake (fragrant mushrooms; *Lentinula edodes*) and golden mushrooms (*Flammulina velutipes).* These mushrooms are not only highly valued as a centrepiece of Taiwanese cooking but also stated to have health enhancing properties (Joan-Hwa *et al.,* 2002). Research done by Mau *et al.* (2005) showed, the antioxidants activity of methanolic extracts from Lingzi fruiting body showed high DPPH radical scavenging abilities of

88.4% and 93.8% at only 5.0mg/ml respectively and the mycelia by 85.7% at 10.0mg/ml. Shiitake has also been stated to show some effects on bowel cancer, liver cancer, stomach cancer, ovarian cancer and lung cancer when tested on rats. Shiitake is also rich in several antioxidants (Selenium, Uric acid and Vitamin A, E, & C) as well as Vitamin D that may lower blood pressure in those with hypertension, lower serum cholesterol levels, increase libido, stimulate the production of interferon which has anti-viral effects, and has proven effective against hepatitis in some cases (Raymond and Robert, 2002).

Huang *et al*. (2002) also found that, the methanolic extract from *Agrocybe cylindracea* fruiting body, has excellent antioxidant properties with DPPH radicals scavenge abilities 89.0% at 1.0mg/ml, the mycelia and filtrate 91.4% and 94.9% at 20.0mg/ml respectively. In addition, Yang *et al*. (2002) also showed, black and red ears mushrooms methanolic extracts scavenged DPPH radicals by 94.5% at 0.4mg/ml and 95.4% at 3.0mg/ml respectively and this followed by silver ears 71.5% at 5.0mg/ml. Daker *et al.* (2008) showed the antioxidant activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50}; unit = mg extract/ml methanol) and in her study the unfermented and fermented maize of *Marasmiellus* sp. exhibited IC_{50} values of 3.9mg/ml and 1.9mg/ml, respectively. Thus, the lower the IC_{50} is, the higher the antioxidant power being produced by the mushroom extracts (Daker *et al.*, 2008).

In 2002b, Mau *et al*. showed four commercially Taiwan mushrooms *Dictyophora indusiata* (basket stinkhorn), *Grifola frondosa* (maitake), *Hericium erinaceus* (lion's mane) and *Tricholoma giganteum* (white matsutake) were excellent sources of antioxidants at 6.4mg/ml with scavenging effects 92.1% (basket stinkhorn) and 63.2–

67.8% for the rest of the mushrooms. On the other hand, at the same concentration of 40.0mg/ml, oyster mushrooms were only 54.3%. The antioxidant properties of *Ganoderma tsugae* studied by Tseng *et al.* (2008) showed the hot water and hot alkali extracted polysaccharides were good in antioxidant activity as evidenced by their particularly low IC_{50} values (<0.1mg/ml). With regard to scavenging ability on DPPH radicals, IC_{50} values of polysaccharides from mature and baby Lingzi were less than 7.0mg/ml whereas mycelia were 10.5–11.2mg/ml. However, for filtrate, IC_{50} value of hot water and hot alkali extracted polysaccharides were 12.9mg/ml and 4.8mg/ml, respectively. Both polysaccharides extracts showed scavenging ability on hydroxyl radicals but IC_{50} values were higher than 20.0mg/ml, except for that of hot alkali extracted polysaccharide from mature Lingzi with 17.9mg/ml. Overall, both polysaccharide extracts possessed good antioxidant properties, except for scavenging ability on hydroxyl radicals and can be developed as a new dietary supplement and functional food (Tseng *et al.*, 2008).

Barros *et al.* (2007) used several biochemical assays to screen antioxidant properties: reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, inhibition of erythrocytes hemolysis mediated by peroxyl radicals and inhibition of lipid peroxidation using the β-carotene linoleate model system. In the study, the effects of fruiting body maturity on antioxidant activity and production of the wild mushroom *Lactarius piperatus* was evaluated at different stages of fruiting body maturity. The stages used were at immature spores (stage I and II) which revealed a higher content in phenol and flavonoid compounds, stages III (with mature spores) and IV (degraded). It can be concluded that the scavenging effects of fruiting body extracts on DPPH radicals increased with the increased concentration and was very good for stage II (84.9% at 20.0mg/ml), but lower than the scavenging effects of the BHA (96% at 3.6mg/ml) and

a-tocopherol (95% at 8.6mg/ml). The radical scavenging activity values at 20.0mg/ml were moderate for the other stages (stage I – 64.2%, stage III – 49.4%, stage IV – 43.4%) and, particularly stage III and IV revealed a very similar scavenging activity (Barros *et al*, 2007). Overall, the stage II mushroom exhibited better antioxidant properties (significantly lower; $p < 0.05$) than other stages. As for reducing power ability, hemolysis inhibition and lipid peroxidation inhibition the absorbance values were 0.946 (phenols) and 0.986 (flavonoids) ($p < 0.001$), 0.792 (phenols) and 0.798 (flavonoids) ($p < 0.001$) and 0.940 (phenols) and 0.914 (flavonoids) ($p < 0.001$), respectively (Barros *et al.*, 2007). These negative linear correlations prove that the samples with lower IC50 values contain the highest antioxidant properties (stage II, mature with immature spores), while the sample with lowest antioxidant properties presents higher IC_{50} values (stage IV, degraded) (Barros *et al.*, 2007). The study concluded that, the antioxidant activity mechanism for the extracts may be identical and related with the phenols and flavonoids contents.

2.5 Principles of methods for antioxidants determination

2.5.1 DPPH radical scavenging ability

The first and popular method to detect antioxidant activity is by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. In this method, the antioxidant potential is assessed based on the ability of the extract to scavenge the DPPH free radical. Mechanisms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by Molyneux (2004) are based on Blois (1958).

The original Blois method has been adopted by several recent researchers like Williams *et al*. (1995) and was used in this study. The study showed that delocalisation of spare electron in the molecule of DPPH is the main reason why the reaction mixture is deep violet in color. DPPH molecule is also characterized as a stable free radical, so that the molecules do not dimerise (Figure 2.1). However, when a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the loss of this violet color to pale yellow color exhibits the reduction of free radical to non-radical form (Figure 2.2).

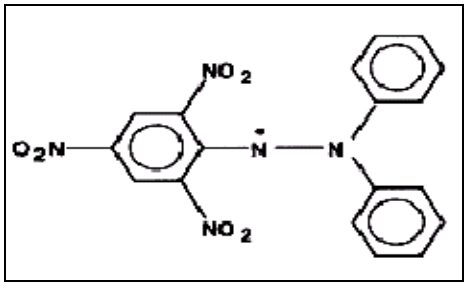


Figure 2.1: Diphenylpicrylhydrazyl (free radical) chemical structure

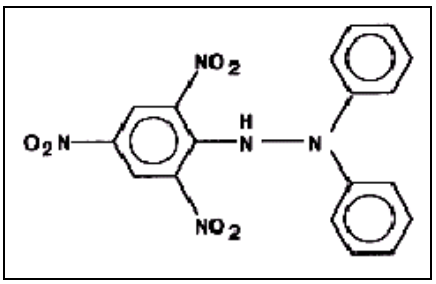


Figure 2.2: Diphenylpicrylhydrazine (non-radical) chemical structure

For example, equation [1] representing the DPPH radical by Z* and the donor molecule by RSH, the primary reaction is where ZH is the reduced form and RS* is the free radical produced.

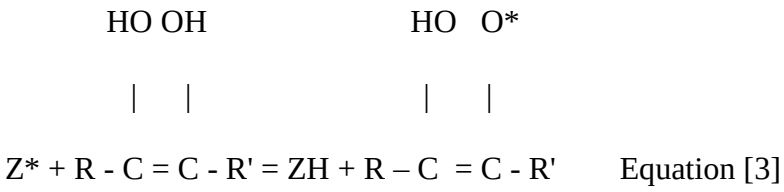
$$Z^* + RSH = ZH + RS^* \qquad \text{Equation [1]}$$

This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z* is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance

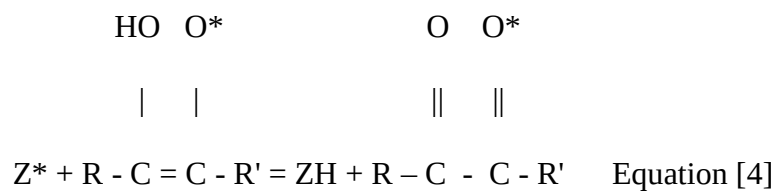
ZH. The free radical RS* will then react with another molecule of the same kind that is produced by a parallel reaction to equation [2].

$$RS^* + RS^* = RS - SR \qquad \text{Equation [2]}$$

This then leads to the observed reduction of two molecules of DPPH by two molecules of RS*, that is, a 1:1 stoichiometry. If, however, the molecule has two adjacent sites for hydrogen abstraction which are internally connected, as in the case of ascorbic acid (Vitamin C), then there may be a further hydrogen abstraction reaction after the first one. This leads to a 2:1 stoichiometry, that is, two molecules of DPPH reduced by one molecule of ascorbic acid (Equation 3 and 4).



Equation [3]



Equation [4]

2.5.2 Lipid peroxidation

Lipid peroxidation is probably the most extensively investigated free radical induced process (Sodergren, 2000). Peroxidation of lipids can be assessed by measurement of the loss of unsaturated fatty acids, generation of primary peroxidation products or

secondary degradation products (Sodergren, 2000) such as malondialdehyde (MDA). MDA is a natural product formed in lipid peroxidation which produced highly reactive three-carbon dialdehyde as a by-product of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. MDA readily combines with several functional groups on molecules including proteins, lipoproteins and DNA. MDA modified proteins may show altered physico-chemical behaviour and antigenicity (Mancuso, 2005). Certain diagnostic tests are available for the quantification of the end products of lipid peroxidation, specifically malondialdehyde. The most commonly used test is called a TBARS (Thiobarbituric Acid Reactive Substances) assay for lipid peroxidation by using a colorimetric reaction between TBA and MDA, a secondary product of lipid peroxidation (Frankel, 1993).

The lipid peroxidation refers to the oxidative degradation of lipids which leads to the extraction of electrons from the lipids in cell membranes and resulting in cell damage. Reaction of free radicals with cellular membranes may lead to the formation of lipid hydroperoxides, which are degraded to various products, including aldehydes. The production of aldehyde are based on the rate of peroxide breakdown, which may be enhanced by the present of transition metal ions. Lipid autoxidation is a free radical process involving induction, propagation and termination processes (Ohkawa *et al.*, 1979). In a peroxide-free lipid system, the initiation of a peroxidation sequence refers to the attack of a ROS with highly reactive hydroxyl radicals (OH·) for example hydroxide radical and hydrogen peroxide. The iron catalyzed hydrogen peroxide has been discovered by Fenton in 1894 and being called as Fenton's reaction. This Fenton's reaction involves hydrogen peroxide and a ferrous iron catalyst. The peroxide is broken down into a hydroxide ion and a hydroxyl free radical. The hydroxyl free radical is the primary oxidizing species.

$$[Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-]$$

The hydroxyl radical, is a powerful oxidant which can damage cells by causing changes in DNA and lead to the mutations. This hydroxyl radicals (·OH) can easily cross cell membrane and attack unsaturated lipid membrane to form malondialdehyde (Czapski, 1984). Hydroxyl radicals present can be detected by its ability to react with thiobarbituric acid in the form of pink chromogen (Decker, 2002).

2.6 Antioxidant activity of phenolic compounds

Phenolic acids are a group of natural products commonly found in many cereal, grains, herbs, fruits and vegetables. These phenolic acids may vary in structure due to the difference in number and position of the hydroxyl groups on the aromatic ring. As a group, these naturally occurring compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species (ROS), the major cause of many chronic human diseases such as cancer and cardiovascular diseases. The health benefits of cereal grains have significant implications for the improvement of food quality, particularly in functional foods and nutraceuticals industries. There are mainly two groups of phenolic acids in cereal bran: benzoic and cinnamic acid derivatives. Ferulic acid and other hydroxycinnamic acids have been found to have good antioxidant activities (Kyung *et al.*, 2006).

A study by Othman *et al.* (2007) showed that the Malaysian cocoa beans had the highest phenolic content compared to Sulawesi, Ghana and Ivory Coast, and she also mentioned that there were several studies showing a correlation between antioxidant activity and

phenolic content. Ma (2004) showed a strong correlation between phenolics and antioxidant activity in his study for mushrooms. They used four fresh mushrooms commonly found in Asian cuisine which are *Agaricus bisporus*, *Lentinus edodes*, *Volvariella volvacea* and *Agrocybe aegerita*. The mushrooms were screened for the antioxidant activity of their alcoholic and water extracts. They found that alcoholic extracts of *A. aegerita*, *A. bisporus*, and *V. volvacea* had the highest antioxidant activity against DPPH free radical with IC_{50} below 1 mg/ml. The Trolox equivalent antioxidant activity (TEAC) at 10mg/ml of *L. edodes* extracts were significantly lower than the others ($p<0.05$). Significant correlation was found, between the total phenolic content and the DPPH antioxidant activity ($p<0.01$) in most extracts of all the tested mushrooms. This study showed that the processing conditions can be optimized to preserve the antioxidant and phenolics potency of the mushrooms (Ma, 2004).

Phenolic compounds are one of the most widely distributed plant secondary products and the antioxidants ability of these compounds has been well established. For example, polyphenols, are multifunctional antioxidants that act as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers. Mushroom phenolic compounds have been found to be excellent antioxidants and synergists that are not mutagenic. Some common consumable edible mushrooms in Asian culture have recently been found to possess antioxidant activity, which was well correlated with their total phenolic content. Thus, mushrooms may become a potential source of natural antioxidants for many applications especially in the food industry (Wong *et al*., 2009). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. In addition, they have metal-chelating potential. Moreover, phenolic compounds show different biological activities such as anti-bacterial, anti-carcinogenic,

anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating agents (Tawaha *et al.*, 2007).

Wong *et al*. (2009) in her study, showed a weak positive correlation between the antioxidant activity determined by ferric reducing antioxidant power (FRAP) assay and total phenolic content of the extracts from fruiting body and mycelium (R^2=0.4084), indicating the possible role of the phenolic compounds in the antioxidant activity of *Hericeum erinaceus*. Moreover, a study between the scavenging ability on DPPH radical and total phenolic content in the extracts of fruiting body and mycelium (R^2=0.5629) also gave a strong negative correlation which indicates that high scavenging ability on DPPH radical is not due to phenolic compounds in *H. erinaceus* extracts. The study concluded that prolonged heating time or temperature significantly increased the amount of antioxidant compounds and enhanced the overall antioxidant activity especially free polyphenolic compounds. The formation of phenolic compounds during the heating process might be due to the availability of precursors of phenolic molecules by non-enzymatic interconversion between phenolic molecules subjected to the effects of external factors, such as temperature. Thus, the mushroom composition and the degree of heating could be important factors contributing to high total polyphenol content (Wong *et al*., 2009).

2.7 *Lentinus squarrosulus* (Mont.)

Lentinus squarrosulus (Mont.) belongs to the kingdom of Fungi, phylum Basidiomycota, class Basidiomycetes, subclass Agaricomycetidae, order Polyporales and family Polyporaceae (Kirk *et al.*, 2001). Polyporales is a large pore fungus order (division Mycota) within the class of Basidiomycetes. Species in this class are often

coriaceous, leathery to woody, but may also be fleshy and they have sporophores (fruiting bodies) in which sometimes the spore bearing layer (hymenium) appearing either tube-shaped, gill-like, rough, smooth, or convoluted (Britannica Encyclopedia, 2007). Basically, *L. squarrosulus* have numerous basidia which are the source of sexual spores in asexual reproduction (Cambell *et al.*, 1999) and the basidiocarps which is either sessile to its substratum or attached by a short stalk. Basidiocarps may be thin and crust-like, gelatinous, cartilaginous, papery, fleshly, spongy, corky, woody or indeed of almost any texture. Most Basidiomycetes bear their basidia in basidiocarps. Basidiocarps may open from the beginning, exposing their basidia, or open at a later stage, or even remained closed. In species which basidiocarps remained closed, the spores are liberated only upon the disintegration of the basidiocarp or upon its accidental fracture by external forces (Alexopoulos *et al*., 1996). The basidia of *Lentinus* sp. are typically short, narrow and cylindricoclavate, usually within the range of 14-20 x 3.5-5.5 um (Figure 2.3).

The *L. squarrosulus* spores are cylindrical, hyaline, inamyloid, non-dextrinoid, thin-walled and smooth, characteristic of Polyporaceae. Spores of *L. squarrosulus* are white, small and length within the range of 5-8µm (Pegler, 1983). The hymenophore of *L. squarrosulus* is lamellate, with a decurrent attachment to the stipe. The stipe is eccentric, lateral or absent and the gills are thin, membranous and serrate to lacerate or dentate on the edges of maturity (Pegler, 1983). These fungi have typically tough or leathery fruiting bodies and this character is correlated with their ability to revive when moistened (Smith, 1964). Gross morphology in *L. squarrosulus* can be extremely variable, with basidiomes either slow growing, long-lived or both (Pegler, 1983).

In an investigation carried out by Nwanze *et al.* (2006), many Nigerians are turning to mushrooms like *Lentinus squarrosulus* as an alternative source of protein due to the high cost of meat and fish and it has been documented as highly nutritious and one of the favorable mushroom compared with other foreign edible species (Nwanze *et al.*, 2006). In Malaysia, the *L. squarrosulus* mushrooms can be found in abundance throughout the tropical forest and occasionally encountered in the temperate regions. This is where, the basidiomes represent a dominant element and are xeromorphic with a tough, firm texture and are long-lived. However, some *Lentinus* spp. are said to have the most complicated structure of all fungi and have remained exceptionally complex owing to the combination of a high degree of polymorphism. A number of species are economically important as agents of timber decay, whilst others are widely used for their esculent and supposedly therapeutic properties (Pegler, 1983).

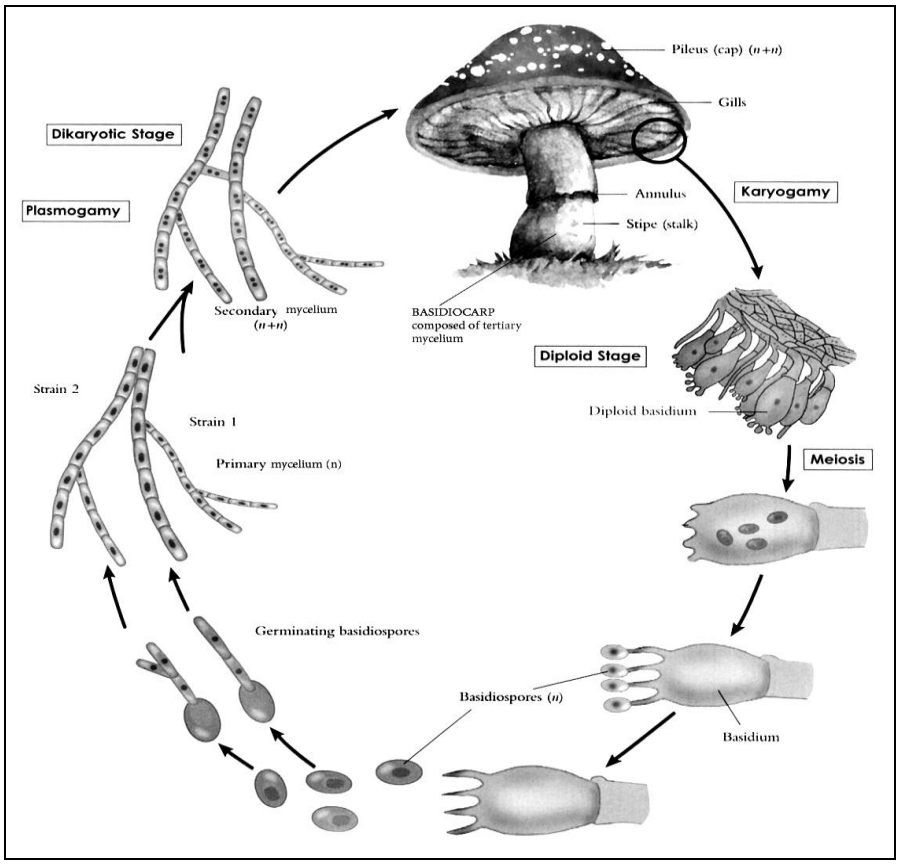


Figure 2.3: Life cycle of a typical Basidiomycota (Alexopoulos *et al*., 1996)

2.8 Comparison of antioxidants production from mushroom mycelia and fruiting body

The used of mushroom mycelia in nutritious food production was said to have a simple, mechanized and inexpensive method (Block, 2004). It is also capable of producing foods with good nutritive value, high protein supplement for human food and animal feed (Falanghe, 1962). The mycelium is the vegetative stage in the life cycle of the mushroom, it secretes enzymes to digest organic matter for the hyphal cells to absorb nutrients through their cell walls. The mycelium will generate new hypha within several weeks and will develop into a highly organized structure that we call a mushroom fruiting body (Pegler, 1983). Kurbanoglu *et al.* (2004) showed the protein content of *Agaricus bisporus* mycelium was higher with 47.1% of dry matter compared with the fruiting body (45.9%). The *A. bisporus* mycelium contains all of the essential amino acids such as leucine, phenylalanine, histidine, lysine and sulphur containing amino acids (methionine, cysteine) and was also recorded to be better than that of fruiting body (Table 2.1). Kurbanoglu *et al.* (2004) also stated in the study the essential and sulphur containing amino acid composition for a protein determines the mycelium potential for nutritional value. Tsai (2002) found that, the antioxidant activities from hot water extracts of *Agrocybe cylindracea* fruiting bodies and mycelia at 20.0mg/ml were 63.6% and 81.6% respectively. Also the methanolic extract from Lingzhi mycelia showed high chelating abilities of 80.2–85.9% at 5.0–10.0mg/ml. The activity is better than methanolic extract from Lingzhi fruiting body which gave 70.4% at 20.0mg/ml (Mau *et. al.*, 2005). At 1.0mg/ml of methanolic extract from *G. tsugae* fruiting body and mycelia exhibited 4.54% and 20.1% inhibition of radical scavenging activity respectively (Mau *et al.*, 2005).

Table 2.1: Comparison of amino acid composition of mycelium and fruiting bodies of *A. bisporus* (Kurbanoglu *et al.*, 2004).

Amino acids	Mycelium (mg g^{-1})	Fruiting bodies (mg g^{-1})
Aspartic acid	29.8	22.6
Threonine	23.6	26.6
Serine	16.8	14.8
Glutamic acid	30.1	28.4
Glycine	18.4	16.6
Alanine	18.6	13.6
Cysteine	23.4	18.4
Valine	28.5	33.5
Methionine	17.6	10.6
Isoleucine	24.8	26.8
Leucine	8.4	7.1
Tyrosine	14.4	12.2
Phenylalanine	22.6	16.6
Histidine	38.1	33.3
Lysine	36.2	32.2
Arginine	23.4	26.4
Proline	16.4	19.4

In other comparisons, the antioxidants extracted from mycelia were high in reducing powers and chelating abilities compared to fruiting body with IC_{50} values 0.9mg/ml and 5.0mg/ml, whereas 1.1mg/ml and 4.8mg/ml for chelating activity respectively (Mau *et al.*, 2005). With regard to antioxidant activity, it seemed that the mycelial extracts were more effective than the fruiting body's extracts. Therefore, mushroom in the form of mycelia is the best form to be used as food or a food ingredient because of its therapeutic effects and high content of total phenols which serve as possible protective agents to help humans reduce oxidative damage. That is why in this study, antioxidant activity from mycelium of *L. squarrosulus* was assayed in two different conditions, the solid substrate fermentation and liquid fermentation in order to determined which form of this mushroom mycelium showed a better antioxidant activity as the study has never been done before.