NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPERTIES AND SENSORY ACCEPTABILITY OF CARBOHYDRATE BASED PRODUCTS FORMULATED WITH *Pleurotus sajor-caju*

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by

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❖ MUSHROOM POEM

- ‘Without leaves,
- without buds,
- without flowers,
- yet, they form fruit;
- As a food, as a tonic,
- as a medicine,
- the entire creation is precious’
- - Chang & Miles 1989

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

AA	Antioxidant activity
ANOVA	Analysis of Varians
AOAC	Association of Official Agricultural Chemists,
a_w	Water activity
BCB	β-carotene bleaching
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene
BGEM	β-glucan-enriched materials
BN	Bubur nasi
CC	Creaming cake
CBP	Carbohydrate-based products
CTK	Cendawan tiram kelabu
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyil-picrylhydrazyl
EC_{50}	Median effective concentration (required to induce a 50% effect)
ET	Electron transfer
FRAP	Ferric ion reducing antioxidant power
FAO	Food and Agriculture Organization
FC	Folin-Ciocalte
GAEs	Gallic acid equivalents
GI	Glycaemic index
GO	Gas laboratory oven
HAT	Hydrogen atom transfer
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical

KK	Kek krim
LDLs	Low-density lipoprotein
NCCFN	National Coordinating Committee on Food and Nutrition
NO•	Nitric oxide
NST	New Straits Times
$O_2^{\bullet-}$	Superoxide anion
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite
PB	Paratha bread
PSC	*Pleurotus sajor-caju*
PPM	Parts per million
RNI	Recommended Nutrient Intakes
ROS	Reactive oxygen species
ROS	Reactive oxygen species
$ROO^{\bullet}$	Peroxyl radicals
RP	Rice-porridge
RP	Roti paratha
SD	Sun drying
SDF	Soluble dietary fibre
SOD	Superoxide dismutase
SSC	Superoxide scavenging capacity
TBHQ	*Tert*-butylated hydroxyquinine
TDF	Total dietary fibre
TPC	Total phenolic content
WHO	World Health Organization

KOMPOSISI PEMAKANAN, CIRI ANTIOKSIDAN DAN PENERIMAAN SENSORI KE ATAS PRODUK BERASASKAN KARBOHIDRAT YANG DIFORMULASI DENGAN *Pleurotus sajor-caju*

ABSTRAK

Bahagian utama cendawan tiram kelabu (CTK) telah dikeringkan menggunakan kaedah pengeringan matahari, ketuhar gas dan Anjaad^{TM} untuk membandingkan beberapa kualiti pemakanan selepas pengeringan. Serbuk yang dihasilkan dianalisis kandungan proksimat, kandungan β-glukan, jumlah serat diet dan keupayaan anti-pengoksidanya. Serbuk CTK digunakan dalam formulasi bubur nasi (BN), roti paratha (RP) dan kek krim (KK). Kajian awal menunjukkan CTK yang dikeringkan dengan kaedah Anjaad^{TM} menunjukkan nilai aktiviti air (a_w) yang lebih rendah dan kandungan jumlah fenol yang lebih tinggi berbanding dengan dua kaedah yang lain. Untuk analisis tekstur, atribut kekerasan RP berkadar songsang dengan tahap serbuk CTK yang digunakan. Manakala untuk KK yang ditambah dengan 6% serbuk CTK menunjukkan nilai kekerasan, kelikatan dan kekenyalan yang lebih rendah berbanding dengan sampel kawalan. Dalam analisis proksimat, kajian ini mendedahkan bahawa BN yang berasaskan CTK mempunyai peratus abu yang lebih tinggi berbanding dengan sampel kawalan. Manakala RP dan KK yang disediakan dengan serbuk CTK mempunyai nilai peratus lemak yang lebih rendah berbanding dengan sampel kawalan. Kek yang ditambah dengan 6% serbuk CTK pula menunjukkan nilai peratus protein yang lebih tinggi berbanding dengan sampel kawalan. Dalam keputusan lain, jumlah serat diet dan kandungan β-glukan meningkat seiring dengan tahap penggunaan serbuk CTK dalam semua formulasi produk. Kandungan jumlah fenol juga meningkat seiring dengan tahap penggunaan serbuk CTK dalam semua formulasi produk. Bubur nasi dan RP yang ditambah

dengan 6% serbuk CTK mempunyai kuasa penurunan yang lebih tinggi berbanding dengan sampel kawalan. Krim kek yang ditambah dengan 4% serbuk CTK juga menunjukkan keupayaan perencatan yang lebih tinggi berbanding dengan sampel kawalan. Dalam analisis pelunturan β-karotin, aktiviti anti-pengoksida bagi ketiga-tiga produk kawalan didapati lebih rendah berbanding dengan sampel yang ditambah dengan 6% serbuk CTK. RP yang ditambah dengan 4% serbuk CTK mempunyai nilai keupayaan merencat superoksida yang lebih tinggi berbanding dengan sampel kawalan. Sebagai tambahan, BN yang ditambah dengan serbuk CTK mempunyai nilai yang lebih tinggi bagi atribut aroma berbanding dengan sampel kawalan dalam analisis sensori. Oleh itu, CTK boleh digunakan secara meluas sebagai satu ramuan makanan yang mengandungi pelbagai komponen berfungsi yang bermanfaat. Cendawan ini boleh dipertimbangkan untuk digunakan dalam makanan lain dengan tujuan untuk meningkatkan komposisi nutrien tanpa memberi kesan kepada ciri sensorinya.

NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPERTIES AND SENSORY ACCEPTABILITY OF CARBOHYDRATE BASED PRODUCTS FORMULATED WITH *Pleurotus sajor-caju*

ABSTRACT

This study was carried out to determine proximate analysis, β-glucan content, dietary fibre and antioxidant properties in PSC powder and some selected carbohydrate-based products formulated with PSC powder at different levels. The textural and sensory acceptabilities of rice-porridge (RP), paratha bread (PB) and creaming cake (CC) incorporated with different levels of mushroom PSC powder were also studied. Fruiting bodies of grey oyster mushroom (*Pleurotus sajor-caju, PSC*) were dried by using either sun drying, gas oven or Anjaad™ methods to compare some nutritional qualities after drying. The study showed PSC dried with Anjaad™ method showed significantly lower water activity (a_w) value and significantly higher total phenolic content compared to the other two methods. For texture analysis, the firmness attribute of PB decreased proportionally with the level of PSC powder used. As for CC added with 6% of PSC powder showed lower value of firmness, gumminess and chewiness compared to control sample. In proximate analysis, the present study found that the percentage of ash in PSC-based RP was significantly higher than control sample. Meanwhile the percentage of fat in PB and CC prepared with PSC powder up to 6% had significantly lower values than control. The percentage of protein in CC added with 6% of PSC powder was significantly higher than control sample. In another result, total dietary fibre and β-glucan content increased in line with the level of PSC powder used in all product formulations. The content of total phenolic increased proportionally with the level of PSC powder used in all three products. Rice-porridge and PB added with 6% of PSC powder had

significantly higher reducing power than the control sample. Creaming cake (CC) added with 4% PSC powder recorded higher scavenging ability than the control sample. In β-carotene bleaching assay, the antioxidant activity of all three control products were found to be significantly lower than the samples added with 6% of PSC powder. PB added with 4% PSC powder had significantly higher superoxide scavenging capacity compared to control sample. In addition, RP added with PSC powder had significantly higher value for odour attribute as compared to the control sample, in the sensory analysis. Thus, PSC could be widely used as a food ingredient which contains many essential functional components. Grey oyster mushroom can be considered to be applied in other food items too with the purpose of enhancing nutrient compositions without affecting it sensorial characteristics.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Mushrooms along with other fungi are something special in the living world, being neither plant nor animal. It is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand (Chang and Miles, 1992). Mushrooms need not be Basidiomycetes or aerial or fleshy or edible. It can be Ascomycetes, grow underground, have a nonfleshy texture and need not be edible. In fact there is a countless variety of forms (Chang, 2008).

Humans have been eating mushrooms for thousands of years. Presently, mushrooms are widely cultivated on every continent of the world. These fungi have currently been consumed as food and medicine in many cultures (Yang *et al.,* 2001, Chocksaisawasdee *et al.,* 2010, Bobek *et al.,* 1997). The Egyptians for example believed that they were a gift from the god Osiris, while the ancient Romans called them a 'devine food'. The latter thought that mushrooms resulted from the lightning thrown to earth by Jupiter during storms (Manzi *et al.,* 1999).

Mushrooms are actually not a true vegetable in the sense that it does not have any leaves, roots or seeds and really does not need any light to grow. It is a fungus which grows in the dark and propogates through its spores. It seems to grow like magic,

springing up overnight from nowhere and some contain enough phosphorus to glow in the dark. It appears after the first month and continues to yield crops for three or four months. They are versatile and can be eaten raw or cooked whole, sliced or chopped. Today, mushrooms are eaten and appreciated by people for their flavour, texture, economic, ecological values as well as for the health benefits that they accord (Sanchez, 2004).

Mushrooms are basically have fleshy and spore-bearing fruiting body. Apart from being used for dietary consumption purposes they are also used for medicinal purposes since time immemorial. The ancient Greeks believed them to be a source of strength and Chinese regarded them as health food, an 'elixir of life' (Chang and Buswell, 1996). The Pharaohs, who were considered gods as well as kings during their time, declared mushrooms sacred and reserved them for their own consumption, as a delicacy. The Romans called them food of the gods too but allowed anyone to eat them on holy days and holidays (Sherman and Rolland, 2004).

Mushrooms come in literally thousands of varieties. Some experts estimate their number in tens of thousands with less than 10% of the edible species and roughly equal proportion of them is considered to be poisonous (Mattila *et al.,* 2000). There is no home test that can distinguish between edible and poisonous varieties. Some mushrooms are edible when fresh and young and become poisonous when old or hit by frost. Some are poisonous in one part of the country and some are not in another country. Some mushrooms can be poisonous to animals and not to humans and vice versa. Only a

trained mycologist can identify whether wild mushrooms are edible or not (Sherman and Rolland, 2004).

Some species of wild mushrooms are sold in the local markets as a good source of income. Many researchers focused on therapeutic effects and cultivation methods of wild mushrooms (Beluhan and Ranogajec 2011, Tan and Wahab 1997, Rashad *et al.*, 2009, Pathmashini *et al.*, 2008). In recent times, amounts consumed have risen greatly, involving a larger number of species. Because of increasing mushroom consumption, data on their nutritional value are needed.

The information regarding the nutritional values of wild growing mushrooms are limited when compared with other vegetables. There is limited information about the chemical composition and antioxidant properties of mushrooms after being added into food products. Very few studies are performed especially in Malaysia. Hence, the aims of the present study are to determine the nutritional value and the anti-oxidative properties of oyster mushroom and carbohydrate-based products formulated with dried oyster mushrooms.

1.2 Objectives

The objectives of this study are:

1.2.1 General Objectives

To investigate the nutritional composition, antioxidant properties and sensory evaluation of carbohydrate based products (CBP) formulated with *Pleurotus sajor-caju* (PSC).

1.2.2 Specific objectives

1. To determine the physical properties of PSC powder produced by three different techniques of drying.
2. To determine texture properties, nutritional composition, anti-oxidative properties and evaluate sensory of paratha bread (PB).
3. To determine texture properties, nutritional composition, anti-oxidative properties and evaluate sensory of rice porridge (RP).
4. To determine texture properties, nutritional composition, anti-oxidative properties and evaluate sensory of creaming cake (CC).

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom

Mushrooms bearing a common scientific name *Agaricus*, having different names for different species. The cultivated mushrooms mostly belong to the family *Agaricaceae* of class *Basidiomycetes* (Asghar *et al.*, 2007). A mushroom is the fleshy, spore-bearing fruiting body of a fungus which is also called carpophores or mycocarp. It is typically produced above ground on soil or on its food source. The fruiting body is formed from spacious underground mycelia (hypae) by the process of fruitification. The lifetime of the bulk of fruiting body is only about 10-14 days (Kalac, 2009).

The most common type of mushroom is umbrella shaped with a pileus and a stipe. Other species additionally have a volva (cup) for example *Volvariella volvacea* or an annulus (ring) that is *Agaricus campestris* or both that is *Amanita muscaria*. Furthermore, some mushrooms are in the form of pliable cups, others are round like golf balls. Some are in the shape of small clubs, some resemble coral. Others are yellow or orange jellylike globs and some even very much resemble the human ear (Chang, 2008).

Mushrooms are a group of macroscopic fungi, lacking in chlorophyll and requiring a substrate for their own absorptive nutrition. They produce enzymes which degrade complex organic matter and absorb the soluble substances (Walde *et al.*, 2006). Edible mushroom can be saprophytes, symbiontes and parasites of different plants. All

need organic matter to grow (heterotrophic organisms). The most commonly for used controlled production are the saprophytes (Manzi *et al.,* 1999). Due to saprophytic, they grow on dead organic matter of vegetative origin and can utilize almost all agricultural waste as substrates (Rashad *et al.,* 2009).

Recently mushroom production has increased rapidly. The annual world production of cultivated mushrooms was reported at 6 million metric tons in 1997, increased more than 1 million compared in 1994 (Chang, 2008). The countries producing the largest amounts of cultivated mushrooms were China, the United States, Japan, France, Holland, the United Kingdom and Italy in that order in 1993 (Mattila *et al.*, 2002). In 2007, Malaysians consumed 8100 tonnes per year of mushrooms but the total mushroom production in the country only 5500 tonnes per year (Akmal, 2011).

China was found to be the biggest producers for mushrooms, as they produced more than 1.5 million metric tons in the year 2007. This showed an increment of about 65% in 10 years times. This was followed by United States and Canada. Israel and India showed drastic increased in the number of metric tons produced in 10 years, while Singapore and Kazakhstan can be regarded as new beginners as they are producing the least mushrooms since 1997. Production of mushrooms seems to continuously increase over time. This might due to high consumer demand and increase in consumer awareness on the health benefits of mushrooms (Aida *et al.,* 2009).

Some mushrooms are very palatable due to their exotic taste and some are deadly poisonous. The several species of Amanita are extremely poisonous but obvious symptoms do not appear until 8-12 hours after ingestion. The poisonous compound,

Amatoxin, is not destroyed by boiling or processing. It cannot be denied that some mushrooms even though they represent less than about 1% of the world's known mushrooms, are dangerous if eaten (Chang, 2008). Poisonous mushrooms used to be called 'toadstools'. Whether a mushroom is easy or hard to peel has nothing to do whether or not it's poisonous. Wild mushroom picking should be done only under the supervision of an expert (mycologist) who is absolutely certain they have an edible mushroom.

2.2 Oyster mushroom

Among higher fungi, *pleurotus* is well acknowledged as an economically important genus (Hassan *et al.*, 2010). *Pleurotus* sp. commonly known as oyster fungus, 'hiratake', 'shimeji', or 'houbitake' (Moda *et al.*, 2005). The best well known species of *Pleurotus* are *P. ostreatus, P sajor-caju, P. eringii, P. florida, P. cornucopie, P. cystidiosis* and *P. flabellatus* (Asghar *et al.*, 2007). Roy *et al.*, (2008) reported that among different types of *Pleurotus* genes of mushrooms, namely *P. ostreatus, P sajor-caju P. florida P and P. citrinopileatus,* are reported as commonly available edible mushrooms having components with immunostimulating and antitumor materials. These are also known as oyster mushrooms or abalone mushrooms (Pramanik *et al.*, 2005).

Sanchez (2010) reported that *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* which is 25% of total world production of cultivated mushrooms. *Pleurotus* mushrooms are produced world-wide with China is the major producer. China has become a leading producer and consumer of cultivated edible mushrooms (Chang, 2008).

Oyster mushrooms have a shape that is reminiscent of a trumpet or even a strangely shaped ear atop a short and off-centered stem. Oyster mushrooms from around the world can come in a wide array of colours. Normally they are white to light grey when they are fresh and light brown to golden when dried. These mushrooms can grow to a size of more than a half foot across and are found in overlapping clusters, mostly on hardwoods which are decaying. There are about 40 species of *pleurotus* mushrooms (Chirinang and Intarapichet, 2009).

Pleurotus ostreatus was first cultivated in the United State of America in 1900 (Sanchez, 2004). During the last few decades, oyster mushroom cultivation has increased tremendously throughout the world. In 1997, Asia contributed 74.4% of the total world mushroom tonnage and oyster mushroom accounted for 14.2% of the total world production of edible mushroom (Chang, 2008). These mushrooms are uniquely distinctive and do look like oysters. In the nature, it grows on trunks and stumps of deciduous trees but nowadays it is also cultivated on modified lignocelluloses substrates in farms (Strmiskova *et al*., 1992).

Pleurotus ostreatus is an edible and are among the easiest mushroom to cultivate compared to other varieties (Rashad, *et al*., 2007, 2009). It gave the maximum yield and biological efficiency on sawdust. Of the sawdust types, softwood sawdust like mango and cashew are known to be more suitable than hardwood sawdust (Pathmashini *et al*., 2008). Oyster mushroom cultivation can play an important role in managing organic wastes. Several agricultural residues have been used to produce this edible mushroom such as sugarcane bagasse, sawdust, cereal straw, fruit straw as the carbon source (Moda *et al.,* 2005, Lal and Panda, 1995, Pathmashini *et al*., 2008, Asghar *et al.,* 2007, Bonatti

et al., 2004). It is a biotechnological process for lignocellulosic organic waste recycling with the reduction of environmental pollution (Sanchez, 2010).

The major components of lignocellulosic waste used for mushroom cultivation are cellulose, hemicelluloses and lignin. In Malaysia, the PSC cultivation has now been commercialized by utilizing agro-industrial wastes which contain a high quantity of lignocelluloses (Tan and Wahab, 1997). Mushroom production is light dependent. Some growers operate a 12 hour light cycle using fluorescent lamps.

For scientific classification of PSC species, it is from Pleurotus genus, Pleurotaceae family, Agaricales order, Hymenomycetes class, Basidiomycota phylum and Myceteae/Fungi kingdom (Cheung, 2008). These mushrooms grow in clusters and range in colour from off-white to shades of brown. Pacioni and Lincoff (1981) descript that *Pleurotus ostreatus* have cap size 6-14 cm, often imbricate, superposed, violet black to brownish gray in colour, fading with age, eccentric and asymmetrical, shell or spatula shaped, slightly depressed at attachment to stipe, smooth shiny and glabrous.

Manzi *et al.*, (2004) reported that *Pleurotus* sp. naturally found in tropical and subtropical rainforest and can be artificially cultivated. Varieties of oyster mushrooms can be found growing in temperate forests throughout the world, mostly in the fall but occasionally during any of the warmer months of the year. Grey oyster mushroom or PSC was found growing naturally on succulent tissues of *Euphorbia royleans*, in the foothills of the Himalayas and reported to produced sporophores by artificial culture on banana pseudostems and chopped paddy straw (Chang *et al.*, 1981).

It is comparable to the high-temperature species for fructification. It cultivation is easy with relatively less complicated procedures (Chang, 2008). It is a delicious edible fungus, which was first found by an Indian, Yan Dai ke, at the foot of Himalayas and then distributed to many other countries throughout the world (Pramanik *et al.,* 2005).

2.3 Nutritional benefits of mushrooms

Edible mushrooms have been used to maintain health and increase longevity for centuries. Mushrooms are consumed as a source of protein throughout the world. It has been identified as an excellent food source to alleviate malnutrition in developing countries (Pathmashini *et al.,* 2008). Dunkwal *et al.,* (2007) reported that among main three species of mushrooms in India, PSC posses unique nutritional and medicinal values, characteristic aroma and taste.

Rashad *et al*., (2009) and Hassan *et al.,* (2010) stated that mushrooms serve as delicacies for human consumption and as nutriceuticals food that also cures. Mushrooms, the fruiting body of basidomycetous fungi, contain substances of various kind that are highly valued as medicines, flavouring and perfumes. This mushroom is now considered as a delicacy and treasured for its flavour and taste (Chang *et al.,* 1981). Mushrooms are eaten as meat substitutes and flavouring (Wan Rosli *et al.,* 2011a, Pathmashini *et al*. 2008, Chocksaisawasdee *et al.,* 2010). In general, edible mushrooms are healthy foods due to the fact that it has low calories and fat and rich in vegetable proteins, chitin, vitamins and minerals (Manzi *et al*., 1999, Pathmashini *et al*., 2008, Bonatti *et al*., 2004).

Mushrooms have potential source of dietary fibre due to the presents of such nonstarch polysaccharides (Cheung, 1996). Dietary fibre consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants (Queenan *et al.*, 2007). According to Recommended Nutrient Intakes (RNI) for Malaysia, the recommended dietary fibre intake is 20-30 grams per day (NCCFN, 2005).

Fibre which has numerous health benefits, is a polysaccharide that may be soluble or insoluble. Soluble fibre dissolves in water and forms a gel. It's primarily found in fruits, vegetables, oats, legumes and the grains psyllium. Soluble fibre binds bile acids so they cannot be reabsorbed in the colon, which aids in their excretion. This reduces serum cholesterol, risked factor for cardiovascular disease. Soluble fibre also helps delay blood glucose, concentration in diabetic patients by slowing glucose absorption in the small intestine. Contradictory, insoluble fibre doesn't dissolve in water. It's found primarily in the bran layers of cereal grains. Insoluble fibres, in addition increase fecal bulk and decreases free radical in the Glycaemic Index track (McCann, 2002).

Polysaccharides called β-glucans as well as β-1,3-D-glucans or β-1,4-D-glucans are presents in the cell walls of higher plants and also in the seeds of some cereals such as oats and barley. The β-1,3-D-glucans is also known as lichenins. Related polymers which are also called β-glucans and/or β-1,3-D-glucans and β—1,6-D-glucans, are synthesized by fungi, molds and yeast (Rop *et al.*, 2009).

Glucans are polysaccharides that contain glucose as the only monomer unit. This group of polysaccharides involves glycogen, cellulose and dextran. The general formula

for glucan is $C_6H_{12}O_5$ (Rop *et al*., 2009). Glucans bound to protein or to chitin are usually insoluble in water (Synytsya *et al.,* 2008). However, β-glucan can be said represent partly soluble and partly insoluble food ingredients. The solubility of β-glucan in water is depend on their structure and associated with their origin. Their solubility increases with temperature. Some molecules can produce gels after partial hydrolysis but native molecules lack of this capability (Rop *et al.*, 2009).

The (1→3, 1→4) β-D-glucan are commonly referred to as β-glucan (Brennan and Cleary, 2005). Beta-glucans are particularly effective in lowering blood cholesterol levels and glycemic response *in vivo* and clinical intervention studies (Manzi *et al.*, 2004, Queenan *et al.,* 2007). Chen and Seviour (2007) reported that fungal beta-glucan appear to act by stimulating the whole immune system so they may have an advantage in treating diseases. Beta-glucans are not synthesized by humans, so these compounds are recognized by our immune systems as non-self molecules, inducing both innate and adaptive immune responses.

Cereals contain essentially beta-glucans with β(1→3)(1→4) mixed links, while mushrooms present larger amounts of β(1→4)(1→6) than β(1→3)(1→4) mixed links and different single linkages such as β(1→3)(1→4) and (1→6) (Manzi and Pizzoferrato, 2000). Jayakumar *et al.,* (2009) and Yim *et al*., (2010) reported that mushrooms contain relatively large amounts of vitamin A and C and of β-carotene, all of which have their antioxidant properties.

Oyster mushrooms are loaded with nutrition, thus good for health. They provide carbohydrates, protein, fibre as well as vitamins A, C, B, D and K. They also contain

potassium, sodium, copper, phosphorus, calcium, iron and are low in fat, making them suitable for low calorie diets (Kim *et al*., 2008, Manzi *et al*., 1999, Puttaraju *et al*., 2006). That have made mushrooms attractive as nutritionally beneficial foods and a source of drugs development (Yim *et al*., 2010).

In food composition tables, the carbohydrate content is usually given as total carbohydrate by difference, that is, the percentage of water, protein, fat and ash subtracted from 100 (Alam *et al.*, 2008). Carbohydrates are, as their name imply, hydrates of carbon. Whether the compound is the smallest (monosaccharide) or the largest (polysaccharide) carbohydrate, the ratio of hydrogen to oxygen in the molecule is essentially two to one, just as in water. Complex carbohydrates exhibit different characteristics, which vary with the specific type of compound. Starch is valued for its thickening ability. Cellulose and hemicelluloses modify textures when they are incorporated into food products, contributing a somewhat harsh mouthfeel. Gums and pectins serve as thickening agents that are valued for their limited calorie contribution (McWilliams, 2008).

Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. It is important in many industrial problems, for example, in the evaluation of material's balance or of processing losses (Pomeranz and Meloan, 2000). Water is such a common constituent of foods and is used so frequently in food preparation that it is easy to take it for granted. It can be found in solid, gaseous or liquid form in foods. The state in which it is found reflects varying energy states. The crystalline or solid form (ice) represents the lowest energy state and steam (gaseous state) represents the high energy (McWilliams, 2008).

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. There are two major types of ashing which are dry and wet ashing (oxidation). The former is primarily for proximate composition and the latter as a preparation for the analysis of certain minerals. Ash content represents the total mineral content in foods. Ashing is the first step in the preparation of a food sample for specific elemental analysis. Usually, a constant elemental content from the ash of animal products can be expected, but from plant sources is variable (Harbers and Nielsen, 2003).

Ash is the inorganic residue from the incineration of organic matter. The ash of baked products depends mainly on their salt content. Ash in foods is determined by weighing the dry mineral residue of organic materials heated at elevated temperature (around 550°C). Total ash content is a useful parameter of the nutritional value of some foods and feeds. In dry ashing, the organic matter is burned off without flaming for a fixed period of time and the residue must be free from carbon. Porcelain crucibles were used for the dishes. They are widely used because of their good weight constancy and relatively low price. Platinum is the best widely used crucible material but they are too expensive for routine ashing of large numbers of samples. Porcelain crucibles retain their smooth surface and are easy to clean with diluted hydrochloric acid. In fact, unglazed crucibles can withstand up to 1200°C for routine work (Pomeranz and Meloan, 2000).

Proteins are molecules composed of many amino acids joined together by peptide linkages. Proteins are complex in their composition and in their behaviour in food products. Careful attention needs to be paid to the preparation of protein rich foods

because the treatment of the protein greatly influences the quality of the final product. Heating for too long or to too high a temperature can cause some highly detrimental changes in the protein of a food (McWilliams, 2008).

Protein content in food varies widely. Foods of animal origin and legumes are excellent sources of proteins. Proteins are composed of elements including hydrogen, carbon, nitrogen, oxygen and sulphur. Twenty α–amino acids are the building blocks of proteins. Nitrogen is the most distinguishing element present in proteins. However, nitrogen content in various food proteins ranges from 13.4% to 19.1% due to the variation in the specific amino acid composition of proteins. Generally, proteins rich in basic amino acids contain more nitrogen (Chang, 2003).

A nitrogen determination is the most commonly used protein assay. It is generally assumed that a mixture of pure proteins will contain 16% nitrogen. Thus the protein content of a sample is obtained by multiplying the determined nitrogen by the factor 6.25 = (100/16). The general conversion factor of 6.25 is used for most foods. Basically, the sample is heated in sulphuric acid and digested until the carbon and hydrogen are oxidized and the protein nitrogen is reduced and transformed into ammonium sulphate. Then concentrated sodium hydroxide is added and the digest heated to drive off the liberated ammonia into a known volume of a standard acid solution. The unreacted acid is determined and the results are transformed by calculation, into a percentage of protein in the organic sample (Pomeranz and Meloan, 2000).

Lipids, proteins and carbohydrates constitute the principal structural components of foods. Lipids are a group of substances that, in general, are soluble in ether, chloroform or other organic solvents but are sparingly soluble in water (Min and Boff, 2003). Lipids, like carbohydrates, are organic compounds composed of carbon, hydrogen and oxygen. However, oxygen is present in a much smaller proportion and hydrogen in a larger proportion in lipids than in carbohydrates. This difference in composition accounts for the large differences in the energy value of lipids and carbohydrates which are 9 and 4 kilocalories per gram respectively (McWilliams, 2008).

Successful extraction requires that bonds between lipids and other compounds be broken so that the lipids are freed and solubilised. Generally, such solubility is attained when polarities of the lipid and the solvent are similar. Ethyl and petroleum ether are the common extraction solvents. There is a growing tendency to use petroleum ether because it is more selective toward true lipids. Ethyl ether is a better solvent for fat than petroleum ether and will dissolve oxidised lipids. However, it is more expensive, danger of explosion, picks up water during extraction of a sample and dissolves non lipid materials (Pomeranz and Meloan, 2000).

Fats and oils are lipids. At room temperature, fats are solid and oils are liquid. Lipids are chemical compounds that are soluble in organic solvents but not soluble in water. Lipids can be broken down in foods by hydrolysis and oxidation. Hydrolysis involves the separation of a fatty acid from the glycerol backbone of a triacylglycerol or phospholipid. Lipid oxidation is a reaction of lipids with molecular oxygen that proceeds through a free-radical mechanism. It can result in severe off-odours and flavours or oxidative rancidity (Shewfelt, 2009).

2.4 Processing of mushroom

Preservation of food can be accomplished by chemical, biological or physical means. Chemical preservation involved the addition to food of such substances as sugars, salts or acid or exposure to food chemicals, such as smoke or fumigants. Biological preservation involves alcoholic or acidic fermentations. Physical approaches to preserving food include temporary increases in the product's energy level (heating, irradiation), controlled reduction of the product temperature (chilling, freezing), controlled reduction in the product's water content (concentration, air dehydration, freeze drying) and the use of protective package. Physical method of preservation are used extensively in developed countries of the world and they are likely more common worldwide (Fennema, 1975). Spoilage is also a major cause of food wastage. Much of the fresh food supply is perishable because of its moderate to high water content and its nutritious nature.

Mushrooms are highly perishable commodities. Drying these mushrooms confer a stabilizing property. Thus they can be stored for a longer period. Food preservation processes have in common their goal of extending the shelf life of foods to allow storage and convenient distribution. The first and most dangerous limitation of shelf life is the activity of microorganisms. Hence the food preservation processes are designed to eliminate the danger of spoilage due to microbes and to avoid the health threatening activities. Several food preservation processes achieve this in by lowering the availability of water to microorganism. These processes include concentration, dehydration and freezing dry (Karel, 1975).

Mushrooms are easy to depreciate within a day after harvest (Walde *et al.*, 2006). Due to drying, oyster mushrooms are now available the year round. Drying and deep freezing processes have been used to increase storage stability and facilitate mushroom consumption without seasonal constraints (Barros *et al.*, 2007a). The aim of commercial food preservation is to prevent undesirable changes in the wholesomeness, nutritive value or sensory quality of food by economical methods which control growth of microorganism: reduce chemical, physical and physiological changes of undesirable nature and obviate contamination.

Drying mushrooms under the sun yields unhygienic and poor quality product (Gothandapani *et al.*, 1997). Furthermore, the authors mentioned that scientific method of drying and storing will help in preserving mushroom for a long period of time. In addition, Gothandapani *et al.* (1997) stressed the fact that to commercialize mushroom, application of the best post-harvest techniques to enhance the shelf life and to maintain the quality of mushrooms plays a vital role.

2.5 Functional foods

Functional food products are a new category of food products that are marketed as having health benefits (Urala and Lahteenmaki, 2007). The science of nutrition has moved to understanding the physiological and genetic mechanisms by which the diet and individual food components influence health and disease (Young, 1996). A number of different terms are used to describe many natural products currently being developed for health benefit. These include nutraceutical, functional food, pharmafood,

phytochemical, designer food, vitafood and foodaceutical. In the modern diet, consumers are demonstrating an increasing preference for healthy and functional foods (Jang and Lee, 2012).

Functional foods are not medicines. The term functional food is commonly used for food-based products whereas the term nutraceutical is used for a supplemented form. Both terms can be considered to be natural products which provide health benefits (Stephen, 1988). In the United Kingdom, the Ministry of Agriculture, Fisheries and Food (MAFF) has developed a definition of functional food as: A food that has a component incorporated into it to give a specific medical or physiological benefit, other than a purely nutritional benefit (Cockbill, 1994).

2.5.1 Oyster mushroom as food ingredient

Mushrooms are the fungi that have been used as food since time immemorial. Nutritionally they are a valuable source of health food which is low in calories and rich in carbohydrates, essential amino acids, fibre, important vitamins and minerals (Lakhanpal and Rana, 2005). *Pleurotus* genuses have high gastronomic value. Appreciated because of its delicious taste, this fungus has high quantities of proteins, carbohydrates, minerals and vitamins as well as low in fat content (Bonatti *et al.*, 2004).

Oyster mushrooms are highly desirable due to the pleasant flavour and texture. They have been described most often as oyster-like, hence their generic name. The flavour changes depending on the growing substrate used. They had a definite anise-like odour when grown on aspen wood (Cuppet *et al.*, 1998). It is best when picked young.

As the mushroom ages, the flesh becomes tough and the flavour becomes acrid and unpleasant.

Mushrooms are consumed as a delicacy and liked particularly for their specific aroma and texture. They are a food item of low energy value (Kalac, 2009). It poses highly umami taste. Umami taste, also called the palatable taste or the perception of satisfaction, is a good taste commonly induced or enhanced by monosodium glutamate (MSG) and 5'-nucleotides (Beluhan and Ranogajec, 2011).

Cheung (1997) reported that the fruiting body of edible mushrooms are commonly used in human diets as a source of protein. They are high in protein and have a good balance of vitamins and minerals compared to vegetables. They are suitable for low calorie diets because of low in fat and digestible carbohydrate (Mattila *et al.*, 2002). It can be made into soup or cooked with a variety of foods.

Oyster mushroom powder rich in protein and low in fat contents can be incorporated into various recipes for improving the nutritional status of vulnerable population in developing countries (Dunkwal *et al.,* 2007). Recently oyster mushrooms (*Pleurotus ostreatus*) were used as a substitute for pork meat in the development of the Thai glutinous fermented sausage (Chockchaisawasdee *et al*., 2010). In Korea, Kim *et al.,* (2011) produced β–glucan-enriched materials (BGEMs) as a high-fibre and low-calorie substitute for wheat flour for baked foods from mushrooms.

Human have been eating different food groups such as meat and plant-based including fungi or edible mushrooms for thousands of years. Edible mushrooms have been consumed as food and medicine in many cultures (Wan Rosli *et al.,* 2011, Yang *et al.,* 2001, Chocksaisawasdee *et al.,* 2010, Bobek *et al.,* 1997). Mushrooms have been recorded as a source of vegetable and medicines for human throughout the world. It has been used for traditional foods and medicines in Asia (Choi *et al.,* 2006).

Pleurotus sajor-caju is edible and an important ingredient of pizza and many other popular bakery dishes (Asghar *et al*., 2007). Today, mushrooms are eaten by people for their unique flavour, texture as well as for the health benefits they accord. Oyster mushrooms are delicious addition to many dishes. The oyster's stems do not need be discarded; they can either be finely chopped and incorporated into recipes, or saved for making a delicious mushroom stock.

Woody in flavour quality oyster mushrooms are intense in flavour and are a delicious addition to ravioli, soups and even risotto. They are also wonderful in cream sauces and pair extremely well with chicken, veal, sausage, pork and even seafood. It is important to cook the rehydrated oyster mushroom. Eating them raw is not recommended. Presently, there is a trend to cultivate *Pleurotus sajor-caju* globally especially in India, Malaysia and other parts of the world. This species is commonly grown on the lignocellulosic wastes from rubber tree, cotton seed, rice straw and other cheap agricultural waste. However, the application of mushroom in enhancing nutritional composition in other food especially in bakery based products is limited. Thus, this present study is ventured.

2.5.2 Oyster mushroom as functional food item

Mushrooms are excellent source of nutrition. It has long been used as food or food-flavouring material due to their unique and subtle flavour including aroma and taste components, which add functionality to foods (Beluhan and Ranogajec, 2011). For millennia, mushrooms have been valued as flavourful foods and as medicinal substances. They are widely sold as nutritional supplements and touted as beneficial for health (Borchers *et al*., 2008).

Since ancient times mushrooms have been consumed by humans not only as a part of the normal diet but also as a delicacy because they have a highly desirable taste and aroma. In addition, the nutritional, tonic and medicinal properties of mushrooms have been recognized for a long time. Certain ancient religious scriptures such as the Vedas have mentioned their medicinal importance. Romans considered mushrooms to be the Foods of the Gods (Mattila *et al.*, 2000).

The low total fat content and the high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fatty acids, are considered as a significant contributor to the health value of mushrooms (Chang and Buswell, 1996). Mushrooms also appear to be a good source of vitamins, including thiamine, riboflavin, niacin, biotin and ascorbic acid, and minerals. Strmiskova *et al*., (1992) reported oyster mushroom contains little, insignificant sodium amount. This fact could be well utilized in diet of persons who need low sodium content in their diet due to particular diseases.

Edible mushrooms are becoming more important in our diet for their nutritional, organoleptic and pharmacological characteristics (Kavishree *et al*., 2008). Mushrooms are claimed to exhibit antiviral, antibiotic, anti-inflammatory, hypoglycemic, hypocholesterolemic, and hypotensive activities (Borchers *et al*., 2008). Mushrooms and mushroom extracts have been shown to possess anticarcinogenic properties, antimutagenic activity and to stimulate immune responsiveness in experimental models, both *in-vitro* and *in-vivo* (Zhang *et al*., 2009).

Currently mushroom-derived substances were shown to exhibit anti-platelet aggregation, hypocholesterolaemic, cardioprotective, antibacterial, antiviral, antifungal and antiparasitic effects (Nada *et al*., 2010). In view of developing new antitumor compounds with low toxic potential, numerous polysaccharides from different biological origins like yeast, algae, bacteria, higher plants and especially fungi have been investigated for antitumor and immunomodulating activities. Antitumor activity of polysaccharide was mediated by a stimulation of the host's immune systems (Kim *et al*., 1996).

Mushrooms have become attractive as functional foods and a source of physiologically beneficial substances. Some edible mushrooms have also been reported as therapeutic foods which are useful in preventing diseases such as hypertension, hypercholesterolaemia and cancer (Manzi *et al*., 2001, Tsai *et al*., 2009). Mushrooms are used for chronic catarrh diseases of the breast and hinges, lower the cholesterol level of blood, improves circulation, remedy for night sweating in tuberculosis, rheumatism,

gout, jaundice, dropsy, intestinal worms and have anti-tumor, anti-viral and anti-cancer agents (Asghar *et al*., 2007).

Mushrooms have also been associated with many medicinal and pharmacological properties by both eastern and western medicine. These functional characteristics are mainly due to the presence of dietary fibre, in particular, chitin and β-glucans. Chitin is a structural polysaccharide of cellular walls while beta glucans are homo- and hetero-glucans with β(1-3), β(1-4) and β(1-6) glucosidic linkages (Manzi *et al*., 2001). Dietary fibre is a mixture of polysaccharides, lignin and other plant cell wall constituents resistant to hydrolysis by human enzymes. It protects the body from colon cancer and against coronary disease (Manzi and Pizzoferrato, 2000).

Medicinal mushrooms have an established history of use in traditional oriental therapies. Medicinal effects have been demonstrated for many traditionally used mushrooms including extracts of species from genera *Pleurotus* (Zaidman *et al*., 2005). The most thoroughly researched medicinal effect of mushrooms, however, is their antitumor activity in mice as well as in humans, especially in the Shiitake mushroom *(Lentinus edodes),* the Maitake mushroom *(Grifola frondosa), Sclerotinia sclerotiorum,* and *Schizophyllum commune* (Borchers *et al*., 2008).

The immune response is a mechanism that destroys and eliminates antigens from genetically nonidentical organisms as well as other foreign bodies. Its specific immunity mainly involved in lymphocytes and the most common type of lymphocytes are B and T cells (Kodama *et al*., 2003). It is well established that many mushroom-extracted