EVALUATION OF NUTRITIONAL QUALITY AND HEALTH BENEFITS OF MILKY MUSHROOM (*Calocybe indica* P&C)

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2011-16-103

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Department of Home Science

COLLEGE OF AGRICULTURE

THIRUVANANTHAPURAM-695 522

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation of nutritional quality and health benefits of milky mushroom (*Calocybe indica* P&C)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title of any other university or society.

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Date: 7-11-2013

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CERTIFICATE

Certified that this thesis entitled **"Evaluation of nutritional quality and health benefits of milky mushroom (*Calocybe indica* P&C)"** is a record of research work done independently by Anju. R. P (2011-16-103) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVATIONS

C	-	*Calocybe*
AAS	-	Amino acid score
EAA	-	Essential amino acid
NI	-	Nutritional index
IF	-	Inflammation factor
Fig	-	Figure
G	-	Gram
Mg	-	milligram
Et al	-	and others
g/100g	-	gram per 100 gram
Kcal/100g	-	Kilo calories per 100 gram
Min	-	minutes
T	-	Treatments
Ppm	-	Parts per million
d/w	-	Dried weight
BMI	-	Body mass index
WHR	-	Waist hip ratio

Introduction

INTRODUCTON

Mushrooms are the edible fungi prized for their delicacy and distinctive flavor. Because of their unique nutritional status they are known as "The ultimate health food". The Greeks and Romans described mushrooms as "Gift from God" and were served only on festive occasions. Reference to mushrooms is found in Vedas (Adhikari, 2010). The prominence of fungi can now be seen increasingly evidenced by their use as a major source of pharmaceuticals and medicinal foods (Law, 2001).

More than 140,000 species of mushrooms exists in nature, but less than 25 species are widely accepted as food and only a few have attained the level of an item of commerce (Lindequist *et al.*, 2002). Edible mushrooms have long been considered to have medicinal value and devoid of undesirable effects. Due to their high content of protein, vitamins and minerals mushrooms are considered as "poor man's protein" (Pandey, 2004). According to Fukushima, (2000) mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quality.

According to Whitcomb (2008), major medicinal properties attributed to mushrooms include anti cancerous, antibiotic, anti viral activities and lipid lowering effects and enhanced immunity. Mushrooms are considered as a pro biotic food and fight off illness by maintaining physiological homeostasis and thus increase our body's adaptive abilities and vitality (Oatman, 2000). Mushrooms are being increasingly realized as they are low in carbohydrate, cholesterol, fat and high in B complex vitamins and minerals (Dunkwal *et al.*, 2006).

Calocybe indica, popularly known as Milky mushroom or summer mushroom, is a relatively new introduction from India to the world of mushroom growers. *Calocybe indica* P&C is one of the promising mushrooms cultivated in summer introduced by Purkayastha *et al.*, in 1974. The name is derived from the ancient Greek terms kalos "pretty" and cubos "head". In Orissa it is known as *dudha chhatu* and in some places they are called *kuduk*. Around nine species of *Calocybe* are found in neotropical regions (Nilson *et al.*, 1997)

Krishnamoorthy (1997), identified a potential strain of *Calocybe indica* occurring in a sugarcane field near Coimbatore, later it was released as a new variety called APK2 from Tamil Nadu Agricultural University. Natural occurrence of this mushroom *Calocybe* in the plains of Tamil Nadu and Rajasthan has also been reported. Geetha (2011), reported a high yielding strain of *Calocybe gambosa* from westernghat region of Kerala.

Among eighty edible mushrooms are considered for commercial exploitation, milky mushroom*(Calocybe indica*) has become the focal point of exploitation in India as it grows in hot humid climate and suitable for cultivation almost throughout the year. Yadav (2005), reported that milky mushroom is considered as a better proxy for oyster mushroom notably in tropical regions with longer shelf life of 3-4 days and offers wide export potential. Cultivation of milky mushroom has become popular in Tamilnadu, Kerala, Karnataka and Andrapradesh.

Recently *Calocybe indica* have become an attractive functional food mainly because of their chemical composition. Chang *et al.* 2004 reported that milky mushroom known for its delicacy, flavor, and aroma. Nutritionally it is considered as a valuable vegetable, consisting of protein (10-40 per cent) carbohydrate (13-70 per cent) fat less than (1-8 per cent) minerals and significant amount of essential amino acids. Rahul *et al.* (2010) reported that *Calocybe indica* is rich in protein, carbohydrates, and vitamins and contain abundant amount of essential amino acids and fibre.

Since milky mushroom gained spectacular growth in the commercial front, its potential to boost health is to be explored. In this context the present study is taken up to investigate the nutritional and medicinal value of milky mushroom, so that the commercial cultivation of milky mushroom could be promoted and popularized further.

Review of Literature

2. REVIEW OF LITERATURE

Mushrooms have been valued throughout the world both as food and medicine for thousands of years. They are effective functional foods with wide spectrum of pharmacological potential (Shah *et al*., 2007).

Literature pertaining to the study is reviewed under the following sub titles.

2.1. Production and cultivation of milky mushroom
2.2. Nutritional value of mushrooms
2.3. Medicinal importance of mushrooms
2.4. Health potential of mushrooms
2.5. Post harvest Processing and value addition of mushrooms

2.1. PRODUCTION AND CULTIVATION OF MILKY MUSHROOM

Mushroom farming today is being practiced in more than 100 countries and its production is increasing at an annual rate of 6 to7 per cent. Mushroom production is an eco- friendly activity where agricultural or industrial wastes are utilized and recycled. During the last four decades, mushrooms have attained the status of commercial crop (Ramkumar *et al*., 2011).

Arumuganathan (2004), reported that the production and consumption of mushrooms are increasing at faster pace throughout the world, mainly due to greater awareness of their nutritive and medicinal attributes, besides unique flavor and texture.

China alone is reported to grow more than 20 different types of mushrooms at commercial scale and mushroom cultivation has become China's sixth largest industry (Singh, 2010). Total world production of mushrooms is estimated to be five million tones.

India is blessed with varied agro climate, abundance of agricultural wastes and manpower making it most suitable for the cultivation of all types of temperate, subtropical and tropical mushrooms (FAO, 1997). Milky mushroom grows well in a temperature range of 25-35° C and relative humidity of 80 per cent. Thus milky mushroom can be cultivated throughout the year in the entire plains of India (Saranya *et al.*, 2011).

Arumuganathan (2009), stated that milky mushroom (*Calocybe indica*) has become the third commercially grown mushroom in India after Button and Oyster mushroom. First attempt on the induction of fruit bodies of *Calocybe indica* in culture was made almost two decades ago (Purkayastha and Nayak, 1982). Kerala has an ideal environment for the cultivation of milky mushroom and the average daily demand of mushroom in Kerala is approximately 2,500 Kg (Govinder, 2005).

In modern mushroom production panorama, button mushroom (*Agaricus bisporus*) ranks first, nevertheless recent figures found a decreasing trend. This is mainly due to incredible acceptability of "specialty mushrooms" viz. (*Calocybe indica*) among US and European consumers. Ever increasing production of specialty mushroom like *Calocybe indica* pose a serious challenge to the current supremacy of button mushroom in the world market. Thus milky mushroom cultivation in India has witnessed a tremendous revolution in recent years. Serious attempts were made to exploit this mushroom commercially (Miller, 1994).

2.2. NUTRITIONAL VALUE OF MUSHROOMS

Stamets (2000), reported that the use of mushrooms as food is probably as old as civilization and mushrooms currently have greater importance in the diet of mankind. Mushrooms contain reasonable amounts of proteins, carbohydrates, minerals, vitamins and fiber. Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity (Fukushima, 2000).

Chang *et al*. (2004) reported that milky mushroom known for its delicacy, flavor, and aroma. *Calocybe indica* is considered as a valuable vegetable, consisting of protein (10-40 per cent) carbohydrate (13-70 per cent) fat less than (1-8 per cent)

minerals and significant amount of essential amino acids. Rahul *et al*. (2010) reported that *Calocybe indica* is rich in protein, lipids, fiber, carbohydrates, and vitamins and contain abundant amount of essential amino acids and low in fat.

Carbohydrate

The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 50 to 65 per cent on dry weight basis. Free sugars amounts to about 11 per cent (Florezak *et al*., 2004). The calorific value of mushrooms is quite low compared to other foods. When compared to oyster mushroom milky mushroom had more carbohydrate protein, and fat. (Krishnamoorthy, 1997). Milky mushroom consists of 13-70 per cent of carbohydrate. There for milky mushroom can be recommended for "slimming diet"

According to Mauand Beelan (1999), the mannitol, also called mushroom sugar constitutes about 80 per cent of the total free sugars. A fresh mushroom contains 0.9 per cent mannitol, 0.28 per cent reducing sugar, 0.59 per cent glycogen and 0.9 per cent hemicelluloses. According to Dikeman (2005), mushrooms contain polysaccharides glycogen and chitin, occurring in animals, not starch and cellulose as plants. Chitin is a water- insoluble structural N- containing polysaccharide accounting for up to 80-90 per cent of dry matter in mushroom cell walls.

Protein

Mushrooms are excellent source of high quality proteins as compared to most of the vegetables; they are in easily digestible form. Quality of protein is comparable with meat, egg and milk. (Aletor, 1995). The protein value of mushrooms is twice as that of asparagus and potatoes four times as that of tomatoes and carrots, six times as that of oranges. *Calocybe indica* consists of about 15- 40 per cent protein (Tapasya and Rashmi, 2011).

Verma *et al*. (1987) reported that mushrooms are very useful for vegetarian because they contain some essential amino acids which are found in animal proteins. Mushrooms contain all the essential amino acid required by an adult (Hayes and Haddad, 1976). Friedman (1996), reported that the total nitrogen content of dry

mushrooms is contributed by protein amino acids and also revealed that crude protein is 79 per cent compared with 100 per cent for an ideal protein.

According to Rai (1995), mushrooms are a good source of good quality protein especially rich in lysine and thus supplement well in the cereal based Indian diet. FAO recognizes mushrooms as the right source of protein to fight off protein malnutrition in the cereal dependent developing countries like India.

Pushpa and Purushothama (2010), reported that *C.indica* is rich in protein and fiber with low fat content, which make the mushroom as a low energy, healthy food stuff. They were also of the opinion that milky mushroom can also be used as protein supplementing diet. Digestibility of milky mushroom protein is 72-83 per cent.

Purkayastha and Chandra (1976), found that *C. indica* contains 14-27 per cent crude protein on dry weight basis. According to Bano and Rajarathnam (1988), normally mushrooms contain 19-35 per cent proteins compared to 7.3 per cent in rice, 12.7 per cent in wheat, 38.1 per cent in soybean and 9.4 per cent in corn.

Purkayastha and Nayak (1982), reported that mature fruit bodies of *Calocybe indica* contain more amounts of proteins than button mushroom. Purkayastha and Chandra and Purkayastha (1976), reported that leucine, threonine, tyrosine and alanine are the amino acids predominant in *Calocybe indica*.

Fat

Mushrooms are considered good source of fats and minerals (Jiskani, 2001). Yilmaz *et al*. (2006) and Pedneault *et al*. (2006) reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids. According to Usha (2007), mushrooms are low calorie food with very little fat which is free of cholesterol and rich in linoleic acid. Crude fat in mushrooms contains all classes of lipid compounds including free fatty acids, triglycerides, sterols, sterol esters and phospholipids.

Mushroom is a low fat food containing 1.1 to 8.3 per cent fat on dry weight basis, however it contain all the classes of lipids including free fatty acids, glycerides, sterols and phospholipids (Kumari and Murthy, 2000). The fat content of *Calocybe*

indica is 1 to 8 per cent. When compared to oyster mushroom, milky mushroom contain more protein, carbohydrate and fat (Krishnamoorthy *et al*., 1997).

Vitamins

Mushrooms are excellent source of vitamins especially B complex vitamins viz. thiamin, riboflavin, niacin, panthothenic acid, biotin, folic acid and vitamin B12, vitamin D and ascorbic acid. Mushrooms are excellent source thiamine riboflavin, nicotinic acid and ascorbic acid.

Mushrooms also provide vitamin D which helps to keep bones stronger and healthy and helps to regulate the growth of the cells. Rai (1995), pointed out that vitamin B_{12} and folic acid, which are normally not found in vegetarian items are present in mushrooms and along with availability of iron and protein, are reported to maintain hemoglobin level as single source in diet. Selvi *et al*. (2007), reported that *Calocybe indica* consists of vitamin A, C, E and B complex vitamins viz. thiamin, riboflavin, niacin, panthothenic acid.

Fibre

Fresh mushrooms contain both soluble and insoluble fibers. The soluble fiber is mainly beta-glucans, polysaccharides and chitosans which are components of the cell walls (Sadler, 2003). According to Chandalia et al. (2000) soluble fiber present in mushrooms prevents and helps in the management of cardiovascular diseases.

Jacob (2010), reported that one type of fibre found in mushrooms called beta- glucan is similar to that of main oat products, which is beneficial for sugar and blood cholesterol management. According to Kalac and Svoboda (2009), cooked mushrooms contain more fibre because they are more concentrated. Mushrooms contains about 4-9 per cent and 22-30 per cent soluble and insoluble fibre respectively. An average serving of mushrooms (100g) guarantees 9-40 per cent of the daily recommendations of dietary fiber (Manzi, 2001). According to Doshi *et al*. (1988) due to its alkaline ash and high fiber content, milky mushroom is highly suitable for people with hyperacidity and constipation.

Volatile flavor compounds

The characteristic flavor of mushroom species, mainly, dried is highly valued by consumers. Tsai *et al*. (2008) viewed that according to their chemical structure flavor components in mushrooms are classified as derivatives of octane and octanes, lower terpenes, aldehydes, sulfur and heterocyclic compounds.

Chandravadana *et al*. (2005), found that a total of twenty volatile flavor components were identified in *C. indica*. Drying significantly increases the concentrations of these components. The volatile flavor compounds present in *Calocybe indica* are 1- octon-3-ol, n-octanol, 3-octanol (Venkateshwarlu *et al.*,1999).

Minerals

Mineral content viz. calcium, phosphorous, sodium, potassium, magnesium, and other trace elements present in fresh mushroom is higher than many fresh fruits and vegetables. Nita (2009), reported that mushrooms are a source of some minerals including selenium, potassium and phosphorus. Potassium is a mineral that helps lower elevated blood pressure and reduces the risk of stroke (Mussala, 2009). The fruiting bodies of mushrooms are characterized by a high level of well assimilated mineral elements (Ahmad *et al*., 2010).

Mushrooms are rich in copper, a mineral that has cardio-protective properties. A single serving of mushroom is said to provide 20-40 per cent of the daily needs of copper. Mushrooms are also excellent source of selenium, an antioxidant that works with vitamin E to protect cells from the damaging effects of free radicals. (Bano and Singh, 1981). Doshi (1988), reported that *Calocybe indica* have most of the mineral salts required by the human body such as potassium, sodium, phosphorus, iron, and calcium.

2.3. MEDICINAL IMPORTANCE OF MUSHROOMS

Mushrooms are now increasingly recognized as they correct diet, controls and modulate many functions of human body and consequently participate in the maintenance of state of good health and reduce the risk of many diseases (Mirunalini *et al.*, 2012). Modern pharmacological research confirms large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and antiviral properties, besides used as functional foods. (Bilal, 2010)

Hawksworth (2001), pointed out that out of the 41,000 to 15,000 species of mushrooms in the world; around 700 have vast prospects as source of medicinal value. Mushrooms have been used in health care for treating simple and age old common diseases like skin diseases to present day complex and pandemic disease like AIDS. They are reputed to possess anti-allergic, anti cholesterol, anti-tumor and anti-cancer properties (Jiskani, 2001). Edible and medicinal mushrooms not only convert the huge lignocelluloses biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals, myconutraceuticals and myocosmeceuticals for many years (Selvi *et al.*, 2007).

Medicinal mushrooms also behave as adaptogens performing broad- based actions, supporting the function of all major systems including the nervous, hormonal and immune systems. Adaptogens bolster the body's resistance to toxic environment influences, stress and pathogens like bacteria and viruses. They are especially noted for their ability to build endurance and reduce fatigue (Harry, 2010).

Mushrooms as functional foods are used as nutrient supplements to enhance immunity in the form of tablets. Due to low starch content and low cholesterol, they suit diabetic and heart patients. Their polysaccharide content is used as anticancer drug. Even, they have been used to combat HIV effectively (Nanba, 1993; King, 1993).

Bhal (1983), reported that mushrooms cure epilepsy, wounds, skin diseases, heart ailments, rheumatoid arthritis, cholera besides intermittent fevers, diaphoretic,

diarrhea, dysentery, cold, anesthesia, liver disease, gall bladder diseases and used as vermicides. Most of the mushroom drugs are now available in tablet form in China (Yang *et al*., 1993). Mushroom medicines are without side effects (Sagakami *et al*., 1991). Mushrooms have low sodium, carbohydrate and fat content which made them good for weight loss. Mushrooms are excellent source of potassium, since it helps lower blood pressure and diminishes the risk of stroke. Mushrooms are recommended for people suffering from hypertension also (Pamela, 2010).

Biologically active polysaccharides are the best known mushroom- derived substances, which are particularly effective in retarding the progress of various cancers and in alleviating the side effects of chemotherapy and radiation treatment through cell-level regenerative effects (Hobbs, 1995). According to Beetz (2004), *Calocybe indica* is an indigenous popular edible mushroom, possessing a variety of secondary metabolites such as phenolic compounds, terpenes and steroids possibly involved in their medicinal effects and nutritive value.

2.4 HEALTH POTENTIAL OF MUSHROOMS

Dietary mushrooms are considered as valuable health foods since they are known for proteinacious food, consisting of about 75 per cent proteins and are low in calories and fat. (Murugkar and Subbalakshmi, 2005). Mushrooms have been valued throughout the world both as food and medicine for thousands of years. They are effective functional foods with wide spectrum of pharmacological potentials. They are good source of high quality protein comprising all the amino acids (Tapasya and Rashmi 2011).

Mushrooms have low purine level which is beneficial in the diet of persons suffering from metabolic diseases. Yang, (1993) reported that mushrooms act as biological response modifiers by promoting the positive factors and eliminating the negative factors from the human body and thus regarded as the fourth principal form of the conventional cancer treatment.

Being low in fat and devoid of cholesterol, mushrooms make an ideal diet for the heart patients. Mushrooms are low in calorie: high in protein, with no starch and

sugars, and are called the diabetics delight (Rai, 1995). According to Usha (2007), mushrooms are low calorie food with very little fat which is free of cholesterol and rich in linoleic acid.

Edible mushrooms contain interesting functional components, particularly beta glucans, homo and hetero glucans with glucosidic linkages that are responsible for some health properties of mushrooms (Pamela, 2010). Usha (2007), reported that *Calocybe indica* on different substrates shows the presence of altogether eighteen fatty acids especially eicosapentaenoic acid and docasohexaenoic acid. These two omega3 PUFAs known to decrease the incidence of coronary heart diseases, stroke and rheumatoid arthritis. The bioactive substances with immunomodulating effects of mushrooms include polysaccharides, glycoprotein, terpenoids, and fungal immunomodulatory proteins. The biologically active polysaccharides have antitumor and immunostimulating properties (Chang, 2004).

2.4.1 Antibacterial activity

Mushrooms are considered to be natural nutraceuticals and are cultivated for both edible and medicinal purposes. Many edible mushrooms possess enriched proteins and medicinal properties including antibacterial, antiviral, and anti-AIDS. Periyasamy (2005), found that mushroom substances called terpenoids help to kill bacteria and viruses and exert anti- inflammatory effects. A compound extracted from fruiting bodies of *Calocybe indica* shows antibacterial activity against some human pathogenic bacteria, such as Bacillus spp., E-coli, vibrio cholera and salmonella typhy.

2.4.2 Immune Activation

Mushrooms are an excellent source of iron, selenium, potassium, phosphorus, riboflavin, panthothenic acid, copper and zinc in addition to providing antioxidant value; these nutrients play a role in enhancing immunity and preventing diseases (Lelly and Vetter, 2005). Shah *et al. (*2007) reported that mushroom polysaccharides are being investigated for their immune modulatory as well as anticancer activities.

Mandala et al. (2012) reported that an immune stimulating water soluble glucan was isolated from hot extract of fruit bodies of edible mushroom C. *indica*. An immune enhancing cytotoxic hetero glycan from aquous extract of C.indica var. APK2 was also reported. Two new water soluble (1->6)-(1->4) alpha, beta glucan and water insoluble (1->3)-(1->4) beta glucan were isolated from the alkaline extract of *Calocybe indica* .

Maity *et al.* (2008) found that the antiproliferative and immune modulatory properties of a protein designated as Cibacron blue affinity eluted protein (CBAEP) was isolated from *C. indica*. This protein fraction mediated the anti proliferative activity of several tumour cell lines, showing stimulating effect of splenocytes, thymocytes and bone marrow cells. According to Shanker (2009), *Calocybe indica* contains vitamins and minerals, volatile compounds which showed anti proliferative and immune stimulating activities.

2.4.3 Enzymatic Antioxidant activity

Antioxidants are chemical compounds that protect cells from the damage caused by unstable molecules known as free radicals. They are capable of damaging all components of the body viz. lipids, proteins, DNA, sugars and are involved in mutations and cancers (Ahamad, 2010). The antioxidant activity of mushrooms is of significant importance in exploring their therapeutic potential (Lakshmi *et al.*, 2005). Phenolic compounds present in mushrooms have been found to be an excellent antioxidant and synergist that is not mutagenic. (Miyashita *et al.*, 2005).

According to Mirunalini *et al.* (2012) *C. indica,* a common edible mushroom possesses polyphenols such as flavonoids, alkaloids and triterpenoids. These active compounds may be involved in scavenging processess and thereby enhancing antioxidant capacity. The potent antioxidant activity of C. indica could overcome free radicals mediated diseases; hence intake of *C. indica* may prevent oxidative stress diseases. Selvi *et al.* (2012) reported that alkaline extract of C. *indica* shows good dose dependent free radical scavenging property.

Suganya (2012), reported that enzymatic antioxidant content specifically GPX and catalase is high in *C. indica* mushroom variety. *C. indica* have a higher amount of ascorbic acid and alpha tocopherol. (Suganya, 2012). Ramkumar *et al.* (2010) found

that *C. indica* possess antioxidant properties, 1- diphenyl-2- picrylhydrazyl (DPPH), free radical scavenging activity and reducing sugar. *C. indica* have a highest level of antioxidant enzymes, ascorbate oxidase, glucose-6-phosphate dehydrogenase, reduced glutathione and vitamin C. (Tapasya, 2011)

Maity *et al.* (2011) reported that a water soluble glucan isolated from an alkaline extract of fruit bodies of *C.indica* var. APK2 strains showed antioxidant properties with immune activation of macrophage splenocyte and thymocyte.

2.4.4 Non enzymatic antioxidant property

According to Selvi (2012), Calocybe *indica* consists of non enzymatic antioxidant include Vitamin A, vitamin C, vitamin E and reduced glutathione which scavenge a wide variety of free radicals. Gopalan *et al.* (2000) reported that both fresh and powdered samples of milky mushroom possess non enzymatic property.

Vitamin C is regarded as the first line natural antioxidant defense in plasma and a powerful inhibitor of lipid peroxidation (LPO) and act as a free radical scavenger (Maxwell, 1995).

2.4.5 Anti cancerous activity

The antitumor components of mushrooms vary in their chemical nature and include polysaccharides such as hetero-β-glucan, heteroglycane, β-glucan-protein and hetero glycan protein complexes (Ooi, 1999). Many species of mushrooms have been found to be highly potent immune enhancers against cancer (Feng *et al.*, 2001). According to Yoshika *et al.* (1975) water soluble polysaccharides of mushrooms exhibit antitumor activities. Miyashita *et al.* (2005) reported that the antigenotoxic factors in the mushrooms include polysaccharides such as beta and alpha glucan which act against cancer.

Zhang *et al.* (2009) reported that mushroom extracts have been shown to possess anti carcinogenic properties and to stimulate immune response. Selvi *et al.* (2012) reported that alkaline extract of C. *indica* shows good dose dependent free radical scavenging property. According to Selvi, (2012) mushroom varieties of *C. indica* can

be employed as potential anti cancer drugs against bladder carcinoma, which is due to the presence of rich anti oxidant content, flavonoids and phenols in them.

2.5. POST HARVEST PROSESSING AND VALUE ADDITION OF MUSHROOMS.

Increased productivity of mushrooms demands proper post harvest infrastructure in order to enhance shelf life and marketability. Mushrooms are marketed as fresh, dried and preserved forms. Specific aroma and texture of mushrooms are appreciated for culinary use (David *et al*., 2007).

According to Behari (2011), two most common post harvest practices and aspects of mushrooms are proper packaging and storage of fresh mushrooms and processing for long term storage as well as value addition. Effective processing techniques will not only diminish the post harvest losses but also result in greater remuneration to the growers as well as processors. Krishnamoorthy *et al*. (1997), reported that the shelf life of milky mushroom is more than three days.

According to Sohliya *et al*. (2010), LDPE bags with no ventilation prolonged the shelf life of *Calocybe indica* to a maximum level of nine days with good textural qualities. According to Pandey (2002), mushrooms have low shelf life hence preserve them by adopting standardized methods of sun drying or oven drying. Studies have shown that dried products give about 10-20 per cent of the original weight.

Hema (2002), pointed out that mushrooms preserved by drying have a good flavor prevents microbial deterioration and enhances the appearance. The moisture content of dried mushrooms is in the range of 10-15 per cent and has superior quality of protein content varying from 25-35 per cent.

Long term preservation methods such as canning, pickling and drying are most commonly used methods of preservation of mushrooms to make the product available throughout the year (Arumuganathan, 2009). Canning is an established process of preserving mushroom pieces in brine, butter, oil, vinegar etc. It involves six basic

operations like cleaning, blanching, can filling, sterilization, cooling and labeling. Canned mushrooms form major share of world market (Gothandapani *et al.*, 1997).

Kapoor (1989), found that preservation with preservatives like potassium metabisulphite, sodium benzoate; citric acid and sodium chloride extend the shelf life and also maintain the qualities of mushroom. Edible mushrooms are used extensively in cooking, in many cuisines (notably Korean, European, Chinese and Japanese). Their flavor normally intensifies during cooking and their texture holds up well to usual cooking methods (Craig, 2003).

Mushrooms are gaining immense popularity and the consumers demand for variety has led to the development of readymade or value added processed foods from mushrooms (Kumar and Barmanray, 2007). Besides canning, drying, steeping and pickling currently resorted to for the long term storage and trade, it is the production and consumption of the readymade or ready- to-make value added mushroom products (Rai, 1995).

According to Arumuganathan (2004), real value added product in the Indian market is the mushroom soup powder. Attractive packaging of the value added products is yet another area which may be called the secondary value addition. Technologies for production of some variety products like mushroom based biscuits, nuggets, preserve, noodles, pappad, candies and readymade mushroom curry in retort pouches were also developed.

Mane *et al.* (2000) reported that dried mushroom powder made either from *Pleurotus spp* , *Agaricus bisporus*, *Calocybe indica* can be incorporated to various conventional recipes to increase their nutritional quality . Mushroom powder can be incorporated with wheat flour, maize and millet flour to make rotis and bread for daily consumption. Mushroom powder act as a good supplementary food item to cereal and millet preparations.

Mushrooms not only have their own flavor which intensifies during cooking, but can absorb the flavor of other ingredients. They can be used as appetizers, added to salads, soups, stews, and sandwiches (Das and Padnayak, 2003). Pickles and chutneys

prepared from mushroom powder have a good shelf life of more than one year with sensory qualities (Rai, 1995).

Dehydrated mushrooms are used as an important ingredient in several food formulations including instant soup mixes, pasta, stuffing, meat and rice dishes (Tuley, 1996). Khader and Padmavathi (1991), reported that mushroom can also be utilized in the preparation of various weaning foods.

Mushroom cultivation is a good hobby and also a good income opportunity. As a livelihood diversification option, mushroom cultivation has enormous potential to improve food security and income generation, which in turn can help to boost rural and peri- urban economic growth (Govinder, 2005).

Mushroom cultivation activities can play an important role in supporting the local economy by contributing to subsistence food security, nutrition, and medicine; generating additional employment and income through local, regional and national trade; and offering opportunities for processing enterprises (Ramkumar, 2010). Value added products will not only cater to the protein and micronutrient requirement but at the same time will enable the population to live a healthy life.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Evaluation of nutritional quality and health benefits of milky mushroom (*Calocybe indica* P&C)" was conducted in a systematic manner and the methodology adopted is discussed under the following subtitles.

3.1 Selection of mushroom
3.2 Conduct of the study
3.3 Quality evaluation of milky mushroom
3.4 Processing treatments applied to assess nutrient loss in milky mushroom
3.5 Assessment of health benefits of milky mushroom
3.6 Statistical analysis

3.1 SELECTION OF MUSHROOM

Milky mushroom (*Calocybe indica*) cultivated on paddy straw is selected for the study. Milky mushroom has become an attractive functional food mainly because of their high nutritive value and also due to its delicacy, flavor and aroma (Chang *et al.*, 2004). Commercially cultivated milky mushroom was collected from the College of Agriculture, Vellayani and also from the local mushroom growers in Thiruvananthapuram.

3.2 CONDUCT OF STUDY

The study was carried out in two experiments. Under the first experiment, quality evaluation of milky mushroom and the effect of processing on the nutrient composition and chemical constituents were ascertained. The health benefits of milky mushroom were investigated in the second experiment through case studies conducted among selected human volunteers.

Plate- 1 Fresh milky mushroom

Plate- 2 Mushroom supplement

3.3 QUALITY EVALUATION OF MILKY MUSHROOM

Chemical and nutritional composition of the milky mushroom is one of the major parameters influencing the quality of mushroom. Alam *et al*. (2007) reported that milky mushroom (*Calocybe indica*) is rich in protein (20-25 per cent) and fiber (13-24 per cent) with less amount of lipid (4-5 per cent) and hence considered as a functional food.

3.3.1 Assessment of chemical and nutritional composition

In the present study macro and micro nutrients present in the fresh and processed milky mushroom were estimated. Nutrients such as calorie, total carbohydrate, protein, fat, vitamins viz. vitamin C, B complex vitamins viz. thiamin, riboflavin were the estimated nutrients. Minerals and trace elements viz. calcium, phosphorus, sodium, potassium and iron were also ascertained in the mushroom under study. Apart from the above nutrients moisture, fiber, tannin and total poly phenols were estimated in the fresh and processed mushroom.

3.3.1.1 Estimation of macro nutrients in milky mushroom

Milky mushroom is considered as a rich source of protein and carbohydrate and contain less amount of fat (Pushpa, 2010). Calorie content of the fresh mushroom was computed based on the Atwater equation (Energy (Kcal) = (CHO (g) ×4) + (fat (g) ×9) + (protein (g) ×4). Calorie content of processed milky mushroom was analyzed by the method suggested by Gopalan *et al*. (1991) while carbohydrate and fat were estimated by the method suggested by Sadashivam and Manikkam (1992). Protein was determined by Bradford's method (1976).

3.3.1.2 Evaluation of mushroom protein

According to Ghosh and Chakravarthy (1990), the quality of protenacious food depends on its amino acid composition in relation to the protein content and digestibility. Amino acid composition may also serve as a good relative measure to compare mushroom with other food stuffs of established nutritive value. In the

present study, the quality of mushroom was analyzed in detail by estimating amino acid content of the test protein through HPLC method. Total of eighteen amino acid including eight essential amino acids were determined. Based on the amino acid content, Amino acid score (AAS), essential amino acid index (EAA index), nutritional index (NI) were also computed.

Essential amino acid index is the ratio of essential amino acid contained in a food to the essential amino acid content in reference protein (Ghosh and Chakravarthy, 1990). In the present investigation, EAA computation was done following the method suggested by Oser (1995). Amino acid score also coined as chemical score is considered as second alternative to the animal feeding studies for the determination of nutritional value. It is based on the amount of limiting amino acid present in the test protein in relation to its presence in reference protein. Amino acid score was calculated using the following formula.

$$\text{Amino acid score} = \frac{\text{mg of amino acid/ g test protein}}{\text{mg of amino acid/ g reference protein}} \times 100$$

In the present investigation the EAA index was computed using the formula given below

$$\text{EAA index} = \frac{\text{Geometric mean of amino acid in the mushroom}}{\text{Geometric mean of amino acid in the reference protein}} \times 100$$

Nutritional index (NI) based on the protein quality was computed using the formula presented by Crisan and Sanda (1978).

$$\text{Nutritional index (NI)} = \frac{\text{EAA index} \times \% \text{ protein}}{100}$$

3.3.1.3. Estimation of vitamins in Milky mushroom

Selvi *et al.* (2007), reported that milky mushroom (*Calocybe indica)* consists of vitamin A, C, E and B complex vitamins like thiamin, riboflavin, niacin, panthothenic acid. In the present experiment, B complex vitamins viz. thiamine, riboflavin and niacin were estimated by the method suggested by Sadashivam and Manikkam (1992). While vitamin C was determined by the method suggested by Ranganna (2001).

Table: 1 Methods adopted for the determination of nutrients and other chemical constituents in milky mushroom

Nutrients/chemical constituents	**Methods adopted**
Calorie (Kcal)	Gopalan *et al* (1991)
Total carbohydrate (g)	Sadashivam and Manikkam (1992)
Protein (g)	Bradford (1976)
Total fat (%)	Ranganna (2001)
Vitamin C (mg)	Ranganna (2001)
Thiamin (mg)	Sadashivam and Manikkam (1992)
Riboflavin (mg)	Sadashivam and Manikkam (1992)
Niacin (mg)	Sadashivam and Manikkam (1992)
Total ash (g)	Sadashivam and Manikkam (1992)
Calcium (mg)	Sadashivam and Manikkam (1992)
Phosphorus (mg)	Jackson (1973)
Sodium (mg)	Jackson (1973)
Potassium (mg)	Jackson (1973)
Iron (mg)	Jackson (1973)
Zinc (mg)	Jackson (1973)
Copper (mg)	Jackson (1973)
Moisture (%)	AOAC method (1990)
Fiber (g)	AOAC method (1990)
Tannin (mg)	Sadashivam and Manikkam (1992)
Total poly phenols (mg)	Sadashivam and Manikkam (1992)

3.3.1.4. Estimation of minerals and trace elements in milky mushroom

Mineral content of fresh mushroom is higher than that of fruits and vegetables with respect to calcium, phosphorous, sodium, potassium, magnesium and other trace elements. Doshi (1988), reported that milky mushroom (*Calocybe indica*) have most of the mineral salts required by the human body such as potassium, sodium, phosphorus, iron and calcium. In the present study, minerals such as phosphorus, sodium, potassium, iron, zinc and copper were determined by the method suggested by Jackson (1973).

3.3.1.5. Estimation of other constituents in milky mushroom

Moisture content of milky mushroom was recorded as it is an indicator of freshness of the mushroom. Fresh mushroom contains both soluble and insoluble fibers, including beta- glucan, polysaccharides and chitosans which are components of the cell wall (Sadler, 2003). Moisture and fiber content was determined by AOAC method, (1990) and other chemical constituent's viz. tannin and poly phenols were determined by method suggested by Sadashivam and Manikkam (1992).

3.3.2. IF positives and anti-oxidant property of milky mushroom

IF (Inflammation factor) positives or IF rating provides an estimation of the particular foods effect on inflammation process in the body. IF positives are denoted by the presence of known anti- inflammatory nutrients including vitamin C, folate, zinc and selenium. Among the anti-inflammatory nutrients vitamin C and zinc was determined in the mushroom under study.

Antioxidants are chemical compounds that protect cells from the damage caused by unstable molecules known as free radicals, which are capable of damaging all components of the body viz. lipids, proteins, DNA, sugars and are involved in mutations and cancers (Ahmad, 2010). The antioxidant activity of mushrooms is of significant importance in exploring its therapeutic potential (Lakshmi *et al.*, 2005).

According to Suganya (2012), milky mushroom has higher amount of enzymatic antioxidant specifically GPX and catalase. Selvi (2012), reported that milky mushroom consists of non enzymatic antioxidant including Vitamin A, C, E and reduced glutathione which scavenge a wide variety of free radicals. Vitamin C is regard as the first line natural antioxidant defense in plasma and a powerful inhibitor of lipid peroxidation (LPO).

In the present study tannin and poly phenols present in the milky mushroom were determined apart from vitamin C.

3.4 PROCESSING TREATMENTS APPLIED TO ACCOUNT NUTRIENT LOSS IN MILKY MUSHROOM

Mushrooms are generally cooked within no time. Simple cooking method viz. boiling, steaming frying and drying are followed for cooking mushrooms. In the present study nutritional quality of the mushroom after processing and the nutrient loss were ascertained.

3.4.1 BOILING

Boiling is the simplest method in which foods are cooked in a liquid at boiling point. While adopting boiling method, water soluble vitamins will be practically lost (Dake, 2009). In the present study mushrooms were cut in to small pieces and boiled for 10 minutes and nutrients were estimated in order to account the nutrient loss.

3.4.2 STEAMING

Steaming is the cooking of food by the application of steam (Vallejo, 2003). In this cooking process, the food is put in to a steamer which is a cooking utensil that consists of a vessel with a perforated bottom, placed over a vessel containing water. As the water boils, steam rises and cooks the food in the upper or perforated vessel. Steaming is preferable to boiling because there is no loss of mineral salts. The flavor retention is also more in steamed foods. In the present experiment, cleaned mushrooms were cut in to pieces and steamed for five minutes.

3.4.4 FRYING

Frying differs from other methods of heat processing. In this method, the cooking medium is hot oil. Because of the difference between the temperature of the oil and the food, as well as the small size of the food pieces, cooking is completed in a relatively short time. Fried foods are known for their characteristic crispy outer surface as well as high fat content. The fat that is absorbed by the food varies from 10 to 40 per cent depending on the time the food is immersed in the oil (Granda *et al*., 2004).

3.4.4 DRYING

Sun drying is the traditional method for reducing the moisture content of food by spreading the food in the sun (Flickety, 2011). Drying removes the moisture from the food so bacteria, yeast and mold cannot grow and spoil food. Drying can also be done in an oven or in a food dehydrator by using the right combination of warm temperature, low humidity and air current.

In the present study, cleaned mushrooms were cut in to pieces and dried in the open sun spreading over a tray till it is crisp. Care was taken to avoid dust particles to settle over the mushrooms by covering a thin sheet.

3.5 ASSESSMENT OF HEALTH BENEFITS OF MILKY MUSHROOM

To assess the health benefits of milky mushroom, supplementation study was carried out in which dried mushroom supplement was prepared in the laboratory and distributed for consumption to the selected human volunteers with specific disease condition. Health benefits of mushroom supplement were ascertained in the subjects through monitoring blood pressure, blood sugar, lipid profile, general health and morbidity.

3.5.1 Formulation of mushroom supplement

Milky mushroom was sun dried, powdered in a grinder and sieved using a fine mesh of about 10x size. Either the chemicals or preservatives were added to the

mushroom powder. One kilogram of fresh milky mushroom on drying gives fifty gram powder and that is in the ratio of 20:1. Dried mushroom powder was packed in five gram sachet for supplementation to the respondents selected for the case study. Impact of the mushroom supplement on the subjects was monitored initially and after supplementation.

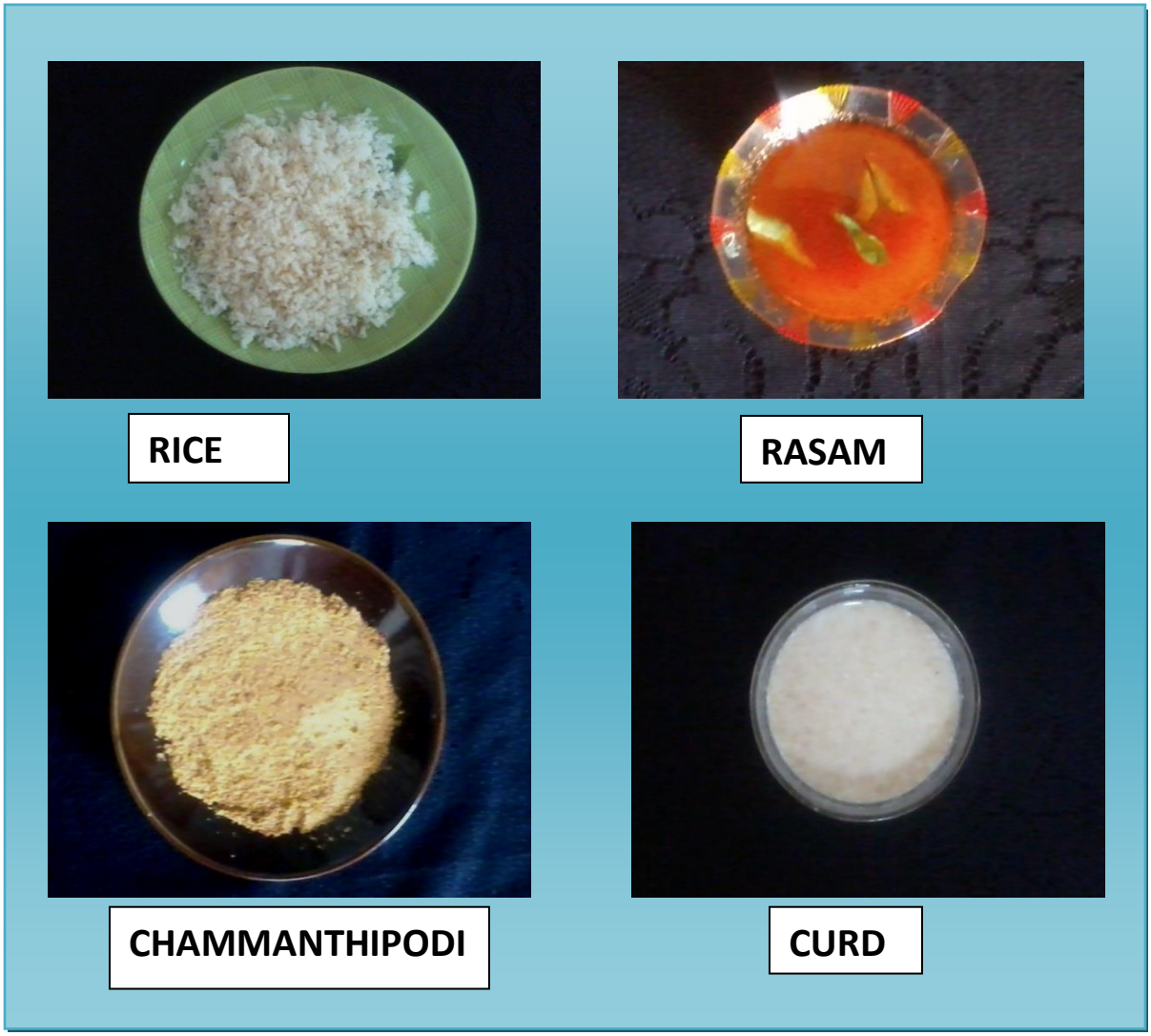
Due to its alkaline ash and high fiber content milky mushroom is highly suitable for people with hyperacidity, constipation and also for managing diabetes and cardiovascular diseases (Doshi *et al*., 1988).

3.5.2 Standardization of recipes with mushroom supplement

Various recipes were standardized in the laboratory incorporating mushroom supplement, in order to promote the prompt inclusion of the supplement in the diet of the respondents. Recipes standardized with mushroom powder were commonly consumed popular breakfast dishes like dosa, idly, chapathy etc. Apart from the above, other dishes like chutney powder, rice, curd and black tea were also standardized in the laboratory incorporating mushroom powder. In all the recipes five gram of mushroom powder was incorporated which neither alter the texture nor the acceptability of the preparation. Earlier studies proved that five gram of mushroom powder is enough to control the blood profile to a desirable level.

Plate- 3 Standardized recipes with mushroom supplement

Plate-4 Standardized recipes with mushroom supplement



3.5.3 CONDUCT OF CASE STUDIES

3.5.3.1. Selection of subjects for case study

In the present experiment subjects were selected for investigating the effect of mushroom supplement on three disease condition viz. hyperglycemia, hyperlipidemia and hypertension. For each disease condition, two subjects with similar clinical parameters were selected. Subjects were identified through personal interview based on the following criteria.

i) Willingness to co -operate in the study

ii) Age between 40-50 years

iii) Subjects with similar sex for each disease condition

iv) Blood profile of the subjects

Disease condition	**Blood profile**
Hyperglycemia	Fasting blood sugar 140 mm/dl & above
Hyperlipidemia	Total blood cholesterol 200 mm/dl & above
Hypertention	120/80 mm Hg & above

v) Persons who are not under medication for the hyperglycemia, hyperlipidemia and hypertension

More than 100 respondents were interviewed for the preliminary screening among the faculty members of the College of Agriculture, Vellayani and also from outside campus. Thus a list of hyperglycemic, hyperlipidemic and hypertensive subjects were prepared. From among the above list, person under medication and subjects with co-morbidities were deleted. Following the criteria suggested for selection of subjects, two subjects for each disease condition were identified for the case studies. After selection, preliminary information's regarding their medical history, socio economic background, dietary and life style pattern were collected through a suitably structured pre tested questionnaire.

Plate-5
Subjects under case study

3.5.3.2. Socio economic profile

The socio economic profile of the subjects such as socioeconomic status, religion and family background in general has a very distinct part to play in determining attitude and food consumption, health and behavioral pattern of the individual (Arrora, 1991).

The socio economic profile collected from the subjects were family size, type of family, educational status, occupation of family members, total monthly income, income spent on food and health care etc. Questionnaire used is appended (Appendix I).

3.5.3.5. Medical and Health status

Details on the medical history of the subjects, food consumption pattern, use of medicines, other personal habits, blood profile and blood pressure of the respondents were estimated. Nutritional status of the respondents was estimated through anthropometry. Anthropometric measurements relevant to the study include height, weight, waist and hip circumference. Measurements were recorded using standard technique as detailed below.

3.5.3.3.1 Measurement of Height

The height is a measure of long standing nutritional status. To determine height, an anthropometric rod was fixed vertically on a smooth wall, perpendicular to the ground taking care to see that the floor area was clean and smooth.

The subjects were asked to remove their slippers and to stand with the centre of the back touching the wall with feet paralleled and heels, buttocks, shoulders and back of head touching the wall. The head was held comfortably erect, the arms hanging closely by the side.

A smooth, thin ruler was held on the top of the head in the centre crushing the hair at angles to the wall and the ruler to the nearest 0.5 cm. Each reading was taken twice to ensure the correctness of the measurement.

3.5.3.3. Measurement of Weight

Weight is the measurement of body mass. (Gopalan, 1988) Body weight is the most widely used and simplest reproducible anthropometric measurement for the evaluation of nutritional status of individuals. Weight was measured using a platform balance.

3.5.3.3.3 Waist and hip measurement

According to Lean *et al*. (1995) waist circumference is used as a measurement that indicates the need for weight measurement. In the present study, the circumference of the waist was measured by passing a fiber glass tape around the waist and for hip measurements; the circumference of hip at the maximum point of proleons was measured using fiber glass tape as per the technique suggested by Bray (1991).

3.5.3.3.4 Waist hip ratio

After recording waist and hip measurement of the subjects, waist hip ratio was calculated by dividing the circumference of hip by waist as suggested by Chanda *et al*. (1995).

3.5.3.3.5 Body Mass Index

Body mass index is used as a good indicator of nutritional status. Nutritional status refers to the state of health of an individual as it is affected by the intake and utilization of nutrients. From the recorded height and weight, body mass index was computed. It is expressed as the weight to height in meter square. (Weight (Kg)/ height (m^2)) (Delpeuch, 1992).

3.5.3.4 Current Dietary Pattern

Dietary pattern have been used to identify typical combinations of food which is associated with disease risk. According to Swaminathan (1993), through diet surveys, information on nutrient intake level, source of nutrients, food consumption pattern and preferences of the subjects could be collected. Food habits of the respondents were collected in order to understand whether diet has any influence on their disease condition.

3.5.3.5 Life Style Pattern

Life styles are group specific forms of how individuals live and interpret their lives in a social context. Life style pattern include the personnel habits, stress and strain in the daily life, type of food they consume. Life style pattern has its own effect on the health of an individual. Data regarding the habit of doing exercise as well as the stress and strain faced by the subjects were also recorded.

3.5.4 Diet Counseling

Nutrition education is a process by which beliefs, attitudes, environmental influences and understandings about food lead to practices that are scientifically sound, practical and consistent with individual needs. Individual counseling was imparted to the selected six respondents under case study regarding the dietary regime to be followed for the specific disease condition. Need for special diet, foods to be restricted for disease condition, importance of mushroom supplement in the diet, how to incorporate the supplement in the diet. During the counseling session, incorporation of mushroom supplement in the diet was also demonstrated to the subjects.

3.5.5. Conduct of Feeding trial

Mushroom supplement was distributed to the subjects for consumption for a period of three months. Subjects were given five gram sachet of mushroom supplement distributed on a weekly basis. Investigator made a good rapport among the respondents and ensured the incorporation of supplement daily in their diet.

Investigator also helped to tackle any problem if arise during the course of incorporation of mushroom supplement. Investigator has made interaction with the respondents personally and through telephone to know whether the subjects were consuming the supplement regularly. The supplementation study continued for three months. Milky mushroom supplement have an intense flavor while incorporated in the dishes, so that two of the subjects discontinued the trial.

3.5.6. Assessing the efficacy of the supplement on the Blood profile of the selected subjects

Feeding trial over a given period of time is considered as the most reliable method to determine the impact of the food. The feeding experiment was conducted for a period of three months to assess the efficacy of mushroom powder on hyperglycemia, hyperlipidemic and hypertension. Blood profile of the subjects recorded before the introduction of the supplement and after 45^{th} and 90^{th} day of supplementation.

3.6. Statistical Analysis

In order to obtain meaningful interpretation, the generated data was subjected to suitable statistical analysis- ANOVA.

Results

4. RESULT

Results of the present study entitled "Evaluation of nutritional quality and health benefits of milky mushroom (*Calocybe indica* P&C)" were discussed under the following headings.

4.1. Quality evaluation of milky mushroom

4.2. Effect of processing on the nutritional value of milky mushroom

4.3. Assessment of health benefits of milky mushroom

4.1. QUALITY EVALUATION OF MILKY MUSHROOM

In the present study, quality evaluation of milky mushroom was ascertained by assessing the chemical and nutritional composition of fresh and processed milky mushroom.

4.1.1. Assessment of chemical and nutritional composition of fresh milky mushroom

Nutritional composition and selected chemical constituents present in the fresh milky mushroom were assessed and results are depicted in table (2).

Table 2. Chemical and nutritional composition of fresh milky mushroom

NUTRIENTS/100 g	MEAN VALUE
MACRONUTRIENTS	
Energy (Kcal)	46.56 (d/w-338)
Carbohydrate (g)	6.70
Protein (g)	4.26
Total fat (%)	0.68
VITAMINS	
Vitamin C (mg)	12.82
Thiamin (mg)	6.20
Riboflavin (mg)	7.50
Niacin (mg)	10.0
MINERALS	
Calcium (mg)	10.40
Sodium (mg)	111.8
Potassium (mg)	415.6
Phosphorus (mg)	622.6
Iron (mg)	10.86
Copper (mg)	11.2
Zinc (mg)	3.8
OTHER CONSTITUENTS	
Moisture (%)	90.73
Fiber (g)	3.24
Tannin (mg)	1.80
Polyphenols (mg)	3.70
Ash	1.60

4.1.1.1. Estimation of macro nutrients

The macro nutrients present in mushrooms are energy, carbohydrate, protein and fat. The calorific value of mushrooms is quite low compared to other foods. The calorie content of milky mushroom on fresh weight basis was found to be 46.56 Kcal and on dry weight basis it was 338 Kcal/100g. Carbohydrate content of fresh milky mushroom under study was found to be 6.70 g/100g which is comparatively higher than those reported in other mushrooms. The fat content of fresh milky mushroom was found to be negligible and recorded as 0.68 per cent.

4.1.1.2. Estimation of vitamins

Mushrooms are excellent source of B complex vitamins including thiamine, riboflavin, niacin, panthothenic acid and vitamin C. Vitamin C content of fresh milky mushroom studied was 12.82 mg/100g where as thiamin, riboflavin and niacin content was estimated as 6.20 mg, 7.50 mg and 10.0 mg respectively per 100 g mushroom.

4.1.1.3. Estimation of minerals and trace elements

Mushrooms are a valuable part of the diet, being a good source of B vitamins and essential minerals like calcium, iron, potassium, and selenium, copper and phosphorus as well as being low in calories, fat and sodium (Whanger, 2004). Apart from such elements the presence of iodine, zinc, and manganese were also recorded in mushrooms.

Calcium content of milky mushroom under study was estimated to be 10.40 mg, while phosphorus content was recorded as 622.6 mg/100g. Iron content was estimated to be 10.86 mg whereas sodium and potassium content were 111.8 mg and 415.6 mg respectively. Trace elements estimated were zinc and copper and the values obtained were 3.8 mg and 11.2 mg respectively.

4.1.1.4. Estimation of other constituents

Other constituents such as moisture, fiber, tannin and polyphenols were also estimated in milky mushroom. Mushrooms are highly perishable commodity and it contains more than 90 per cent moisture. Moisture content of milky mushroom under study was determined as 90.73 per cent.

Tannin content was found to be 1.80 mg while polyphenols estimated to be 3.70 mg/100g. Apart from the above, fiber and ash content of milky mushroom was found to be 3.24g, 1.60 g/100g respectively.

4.1.2. Quality evaluation of mushroom protein

Mushrooms are good source of high quality protein ranging from 30 to 40 per cent on dry weight basis comprising all the amino acids. According to Satish (2011), mushrooms contain high quality proteins compared to fruits and vegetables and rich in lysine and tryptophan, which are deficient in cereals.

In the present investigation, eighteen amino acids including eight essential amino acids were estimated and the results are depicted in table (3) comparing with reference protein value.

Table 3. Essential amino acid content of milky mushroom in comparison with reference protein

Sl.no.	Essential amino acids	Amount of essential amino acid content in milky mushroom (g/100g)	Amount of essential amino acids in reference protein egg (g/100g)
1	Isoleucine	9.8	4.10
2	Methionine	3.22	2.10
3	Phenylalanine	2.58	3.60
4	Threonine	10.4	2.50
5	Tryptophan	-	0.90
6	Valine	9.86	4.50
7	Leucine	-	5.20
8	Lysine	-	4.40

As indicated in the table milky mushroom contains appreciable amount of all the essential amino acids. Because of the deprotenization of mushroom powder essential amino acids viz. lysine, leucine, and tryptophan get denatured. Content of essential amino acids viz. isoleucine, methionine, phenylalanine, threonine, and valine were found to be 9.8g, 3.22g, 2.52g, 10.4g, and 9.86g respectively.

A striking feature is that, the amount of essential amino acids viz. isoleucine, methionine, threonine and valine was exceptionally higher than that of the reference protein. Apart from the essential amino acids, other amino acid content determined is given in table (4). Non essential amino acids present are serine (3.66g), glutamic acid (6.56g), glycine (17.7g), alanine (28.56g) cystine (18.18g) and proline (0.86g), histidine and arginine were not found in the milky mushroom studied.

Table4. Non essential amino acids present in milky mushroom

Sl. No.	Amino acids	Amount of amino acid content in milky mushroom (g/100gm)
1	Aspartic acid	0.86
2	Serine	3.66
3	Glutamic acid	6.56
4	Proline	0.86
5	Glycine	17.7
6	Alanine	28.56
7	Cystine	18.1

Highest amount of amino acids present in the milky mushroom was alanine (28.56g) followed by cystine (18.1g) and glycine (17.78g) and threonine (10.4g) which is an essential amino acid.

Amino acid composition of milky mushroom was compared with other three species *Pleurotus florida* , *P. sajor caju and P.citrinopeleatus* . It was noticed that amino acids content of alanine, glycine, cystine, valine. threonine, methionine and isoleucine were found to be higher than *P.florida* , *P. sajor caju* and *P.citrinopeleatus* (Table 5).

Table 5. Comparison of amino acid composition of milky mushroom with *Pleurotus florida, P. sajor caju and P.citrinopeleatus*

Amino acids	Amount of amino acids g/100gm			
	C. indica	*P.florida*	*P. sajor caju*	*P.citrinopeleatus*
Isoleucine	9.8	3.6	2.04	1.84
Leucine	-	5.9	3.49	3.34
Lysine	-	4.9	3.35	3.19
Methionine	3.22	1.75	1.19	1.00
Phenylalanine	2.58	10.2	3.63	4.60
Threonine	10.4	5.2	2.8	2.8
Tryptophan	-	0.81	-	-
Valine	9.86	3.8	2.78	2.39
Glutamic acid	6.56	17.2	12.31	11.13
Aspartic acid	0.86	10.5	6.17	4.56
Serine	3.66	4.9	3.10	2.6
Proline	0.86	3.72	2.99	2.4
Glycine	17.7	3.6	2.68	2.75
Alanine	28.56	4.4	3.66	3.90
Cystine	18.1	-	7.1	6.5
Tyrosine	-	7.3	2.08	1.78
Histidine	-	3.2	1.20	1.26
Arginine	-	-	3.03	3.00

Quality of mushroom protein was analyzed through applying the indices such as AAS, EAA Index and NI. Based on the essential amino acid content of milky mushroom, amino acid score (AAS) was computed as per the method suggested by Oser (1995).

Table 6. Comparison of Amino acid score of milky mushroom with *Pleurotus florida, P. sajor caju* and *P.citrinopeleatus*

Amino acids	Amino acid score			
	C. indica	*P. florida*	*P. sajor caju.*	*P.citrinopeleatus*
Isoleucine	239.02	87.8	60.0	54.12
Leucine	-	113.46	64.63	61.85
Lysine	-	11.36	76.14	72.50
Methionine	153.33	83.33	52.78	45.83
Phenylalanine	71.66	283.33	98.45	100.0
Threonine	416.0	208.0	97.25	98.62
Tryptophan	-	90.0	-	-
Valine	219.11	84.4	67.80	58.29
Limiting aminoacid sequence	**Phenylalanine, methionine**	**Methionine, isoleucine, valine**	**Methionine, isoleucine, leucine**	**Methionine, isoleucine, valine**

Highest amino acid score of *C. indica* under study was observed for threonine (416.0) and lowest was for phenylalanine (71.66). Next to threonine highest score was observed in isoleucine (239.02) followed by valine (219.11), methionine (153.33), phenylalanine (71.66).

Limiting amino acid sequence in *C. indica* is phenylalanine and methionine. The limiting amino acid sequence in other three species is methionine, isoleucine and valine, methionine, isoleucine and leucine respectively.

EAA index of milky mushroom was computed as mentioned in methodology and found to be 188 while the nutritional index based on the protein quality was determined as 8.0 for the studied milky mushroom.

4.1.3. Anti inflammatory Factors (if positives) and Anti oxidant property of milky mushroom

IF positives present in the milky mushroom are vitamin C, zinc and selenium. Anti inflammatory nutrients possess anti effects on inflammation process of a food in the body.

The results revealed that the milky mushroom contain 12.82 mg vitamin C on fresh weight basis. IF positive quality of milky mushroom contribute to its positive impact on health.

Antioxidants prevent the oxidative damage caused to our body. Antioxidants present in the milky mushroom other than vitamin C are polyphenols, tannin. The poly phenol content of fresh milky mushroom estimated to be 3.70 mg while tannin content was found to be 1.80 g on fresh weight basis.

4.2. EFFECT OF PROCESSING ON THE NUTRITIONAL VALUE OF MILKY MUSHROOM

Milky mushroom was subjected to different methods viz. boiling, steaming, frying and drying which are commonly adopted methods for cooking and processing mushrooms.

4.2.1. Effect of processing on the macro nutrients of milky mushroom

The milky mushroom subjected to different processing treatments was assessed with regard to its nutrient content. The result of the changes in macro nutrients with processing are given in the table (7).

Table 7. Effect of processing on the macro nutrients of milky mushroom

Nutrients	T_1	T_2	T_3	T_4	CD (0.05)
Calories (Kcal)	40.45 [a]	39.25 [b]	35.11 [c]	338.0 [d]	1.761
Carbohydrate (g)	2.12 [a]	3.5 [b]	2.94 [b]	40.0 [d]	.594
Protein (g)	2.27 [a]	2.95 [a]	2.45 [a]	19.61 [d]	.751
Fat (g)	0.22 [a]	0.42 [b]	3.25 [b]	2.58 [d]	.293

T_1-Boiling, T_2- Steaming, T_3- Frying, T_4- Drying

Treatment means with the same alphabets are on par with each other. Treatments without same alphabets are significantly different from each other

Data revealed that the calorie content of the milky mushroom was significantly different when subjected to different processing methods. Calorie content of the mushroom treated with different processing methods ranged between 35.11 to 338 Kcal. It was found that the dried mushrooms depicted highest calorific value with 338.80 Kcal per 100 g and was significantly superior to all other treatments. However, all the other processing treatments T_1 (40.45 Kcal), T_2 (39.25 Kcal), T_3 (35.11 Kcal) were found to be on par with each other though all of them are significantly inferior to T_4. The calorific value was found lowest in T_3 (frying).

Carbohydrate content of milky mushroom subjected to different processing methods indicated that the mean values of carbohydrate content was significantly different from each other and the values ranged between 2.9 to 40.0 g. According to the result, the highest carbohydrate content was noticed in dried mushroom with a mean value of 40.0 g and was significantly superior to all the other treatments. A close observation on the various treatments on the carbohydrate content revealed that T_1 (2.12 g), T_2 (3.5 g), T_3 (2.94 g) were on par with each other. The lowest carbohydrate content was observed in T_3 (fried mushroom) with the value of 2.94 g.

Percentage loss of carbohydrate was observed to be highest (68.35 per cent) in boiling treatment. The minimum loss was observed in steaming (47.76 per cent) Table (9). Drying enhanced the carbohydrate content of the sample by moisture loss, there by concentrated the carbohydrate content.

Changes in the protein content of milky mushroom treated with different processing techniques revealed that there was significant difference between all the treatments. Total protein content of mushroom varies from 2.27 to 19.61 g. The highest protein content was noticed in T_4 (dried mushroom) with a mean value of 19.61 g and lowest in T_1 (boiled mushroom) with a mean value of 2.27 g. A close observation on the various treatments on the protein content revealed that T_1 (2.27 g), T2 (2.95 g), T_3 (2.45 g) were found to be on par with each other. The data on the loss of protein during processing indicated that the highest loss was observed in boiling (46.71 per cent) followed by frying (31.92 per cent) and steaming (30.75 per cent) (Table 8). Drying enhanced the protein content.

Changes in the fat content of the mushroom treated with different processing techniques indicated that fat content fall between 0.22 to 3.25 per cent. When milky mushroom was processed significant difference was observed in the mean value of fat content. T_3 (fried mushroom) was found to recorded higher fat content (3.75 g) when compared with others, and found to be significantly superior to all the other treatments. Among the four processing treatments, T_1 (boiling) had the lowest value (0.22 per cent) of fat, while values for T_2 and T_4 were 0.42 per cent and 2.58 per cent respectively. The loss of fat content was noticed to be highest in boiling method (67.64 per cent) and lowest in steaming (38.23 per cent). T_3 depicted enhanced fat value up to 377.94 per cent.

Table 8. Percentage Loss and gain of nutrients during processing

Nutrients	Boiling (%)	Steaming (%)	Frying (%)	Drying (%)
Carbohydrate	68.35	47.76	56.71	497.01
Protein	46.71	30.75	31.92	360.32
Fat	67.64	38.23	377.94	279.41
Vitamin C	71.76	62.94	85.51	58.11
Thiamin	51.61	39.51	51.61	183.38
Riboflavin	42.0	30.66	50.0	124.0
Niacin	70.0	50.0	70.0	130.0
Calcium	60.57	51.44	32.01	73.65
Sodium	41.86	66.50	58.64	155.20
Potassium	50.81	49.84	50.28	72.69
Phosphorous	26.56	26.82	34.45	69.80
Iron	49.53	27.53	37.01	90.14
Copper	53.12	25.89	33.48	79.46
Zinc	53.94	61.84	50.0	87.63
Moisture	88.09	88.55	88.70	92.11
Fiber	53.70	41.35	62.96	275.30
Tannin	22.22	38.88	44.44	66.66
Polyphenol	34.59	25.67	28.37	139.18

4.2.2. Effect of processing on the vitamin content of milky mushroom

Effect of processing on the vitamin content of milky mushroom is depicted in table (9).

As indicated in the table, the mean values of vitamin C content of milky mushroom treated with different processing technique were found to vary significantly and the values ranged between 1.84 to 20.27 mg. The highest vitamin C content was noticed in T_4 (20.27mg). Among four processing treatments, dried mushroom recorded higher vitamin C and that of boiled mushroom showed the

lowest. Close observation on the various treatments on the vitamin C content revealed that T_2 (4.75 mg), T_3 (3.62 mg) were on par with each other.

Boiling decreased the vitamin C content of milky mushroom to maximum (71.7 per cent) and the loss was minimum in steaming (62.394 per cent). Drying process enhanced the vitamin C in milky mushroom by 58.11 per cent.

Table 9. Effect of processing on the vitamin content of milky mushroom

Nutrients	T_1	T_2	T_3	T_4	CD (0.05)
Vitamin C (mg)	1.84 [a]	4.75 [b]	3.62 [b]	20.27 [d]	1.440
Thiamin (mg)	3.0 [a]	3.75 [b]	3.0 [a]	17.57 [d]	.741
Riboflavin (mg)	4.35 [ab]	5.2 [a]	3.75 [b]	16.8 [d]	1.301
Niacin (mg)	3.0 [a]	5.0 [a]	3.0 [a]	23.0 [d]	3.328

Thiamin content of milky mushroom subjected to different processing methods varied from 3.0 to 17.57mg. Significant difference was noted between the treatments. The highest thiamin content was found to in T_4 (17.57 mg) and lowest in T_1 and T_3 (3.0 mg). It was observed that among the four processing treatments, dried mushroom recorded highest thiamin content while boiled and fried mushroom depicted the lowest thiamin value. Treatments T_1 (3.0 mg) and T_3 (3.0 mg) were on par as thiamin content was concerned.

On taking in to account of the percentage loss of thiamin in milky mushroom under study, highest loss was recorded in boiling and frying (51.61 per cent) and lowest in steaming (39.51 per cent). Drying increases the thiamin content in milky mushroom by 183.38 per cent.

Changes in the riboflavin content of milky mushroom subjected to different processing treatments were significantly different. Riboflavin content of T_1, T_2 and T_3 were 4.35 mg, 5.2 mg and 3.75 mg respectively and were on par with each other. The highest riboflavin content after treatment was noticed in T_4 (drying) with mean value of 16.8 mg and lowest in T_3 (frying) with a mean value of 3.75mg. The highest percentage loss of riboflavin was noted in frying (50.0 per cent) and lowest in steaming (30.66 per cent).

The highest niacin content after treatment was noticed in T_4 (drying) with a mean value of 23 mg and lowest value in T_1 (boiling) and T_3 (frying) with a mean value of 3.0 mg. Niacin content of milky mushroom after processing treatments showed 3.0 mg, 5.0 mg, 3.0 mg respectively in T_1, T_2 and T_3 respectively.

Maximum loss of niacin during processing was reported in boiling and frying (70.0 per cent) followed by steaming (50.0 per cent). The percentage loss of niacin in boiling and frying were same (70 per cent) followed by steaming (50 per cent) while niacin was increased up to 130.0 per cent during drying process.

4.2.3. Effect of processing on the mineral content of milky mushroom

In the present investigation, the mineral content of milky mushroom subjected to different processing techniques was also analyzed and results are depicted in Table (10).

Table 10. Effect of processing on the mineral content of milky mushroom

Nutrients	T_1	T_2	T_3	T_4	CD (0.05)
Calcium (mg)	4.1 [a]	5.05 [a]	7.07 [b]	18.06 [d]	1.410
Sodium (mg)	65.00 [a]	37.45 [b]	46.24 [c]	285.32 [d]	3.490
Potassium (mg)	204.42 [a]	208.45[a]	206.6 [a]	717.72 [d]	5.384
Phosphorus (mg)	360.4 [a]	359.15 [a]	321.7 [c]	833.4 [d]	55.903
Iron (mg)	5.48 [a]	7.87 [b]	6.84 [ab]	20.65 [d]	1.483
Copper (mg)	5.25 [a]	8.3 [a]	7.45 [a]	20.1 [d]	5.04
Zinc (mg)	1.75 [a]	1.45 [a]	1.30 [c]	7.13 [d]	1.24

As indicated in table, the mean value of calcium content of milky mushroom subjected to different processing treatments was found to be significantly different from each other.

The highest calcium content was noticed in T_4 (drying) with a mean value of 18.06 mg. A close observation of the various treatments on the calcium content of milky mushroom revealed that T_1 (4.1 mg) and T_2 (5.05 mg) were on par. The lowest calcium content was observed in boiled mushroom.

Highest amount of calcium was lost during boiling (60.57 per cent). Loss of calcium during steaming and frying were 51.44 per cent and 32.01 per cent respectively. Whereas an increase in calcium to 73.65 per cent is noticed during drying.

Changes in the sodium content of milky mushroom treated with different processing technique indicated that the mean value of sodium content of milky mushroom differ significantly. Sodium content was 65.0 mg, 37.45 mg and 46.24 mg and 285.32 mg respectively in T_1, T_2, T3, and T_4.

The highest sodium content was noticed in T_4 with a mean value of 285.32 mg. It was observed that the dried mushroom was significantly superior to all the other treatments in the sodium content. A close observation on the various treatments on the sodium content revealed that T_2 (37.45 mg) and T_3 (46.24 mg) were found to be significantly different from each other.

The per cent loss of sodium content was noticed to be highest in steaming (66.50 per cent) and lowest in boiling 41.86 per cent. Drying on the other hand enhanced the sodium content up to 155.20 per cent.

Changes in the potassium content of milky mushroom treated with different processing techniques showed a significant difference between processing methods. The highest potassium content was noticed in T_4 with a mean value of 717.72 mg and lowest in T_1 (204.42mg). On comparing the four processing treatments it was observed that potassium content of T_1 (204.42 mg), T_2 (208.45 mg) and T_3 (206.6 mg) were on par.

Table (8) depicted the potassium loss during processing of mushrooms. It was revealed that highest per cent of potassium was lost during steaming (49.84 per cent) followed by frying (50.28 per cent) and boiling (50.81 per cent). Boiling steaming and frying resulted in potassium loss while potassium was enhanced during drying (72.69 per cent).

Phosphorus content of milky mushroom treated with different processing technique indicated significant difference between the treatments. Phosphorus content of T_1, T_2, T_3 and T_4 are 360.4 mg, 359.15 mg, 321.7 mg and 833.4 mg respectively.

The highest phosphorus content after treatment was noticed in T_4 (dried) with a mean value of 833.4 mg. Phosphorous content in T_1 (360.4 mg) and T_2 (359.15 mg) were found to be on par.

It was revealed that phosphorus content of milky mushroom during drying increased to (69.80 per cent). While highest per cent of loss was found in boiling

(26.56 per cent) followed by steaming (26.82 per cent) compared with T_3 (34.45 per cent).

Iron content of milky mushroom when processed was also found to be varying significantly between the treatments. The highest iron content was noticed in T4 with a mean value of (11.66 mg). Boiled mushroom depicted the lowest value (5.48 mg). A close observation of iron content of various treatments revealed that T_2 (6.84 mg) and T_3 (7.87 mg) were on par. The highest loss of iron was recorded in boiling (49.53 per cent) and lowest in steaming (27.53 per cent).

The copper content of milky mushroom treated with different processing methods revealed that the highest value of copper was observed in T_4 (20.1 mg) and lowest in T_1 (5.25 mg). Analysis of the data revealed that significant difference was observed between treatments.

The highest percentage loss of copper was seen in boiling (53.12 per cent) followed by frying (33.48 per cent). The lowest per cent of loss was reported in steaming (25.89 per cent). While drying copper content was increased up to (79.46 per cent).

As indicated in the above table, the mean value of zinc content of milky mushroom treated with different processing technique was found to differ significantly. On taking in to account of the recorded values, highest zinc content was noticed in T_4 with a mean value of 7.13 mg. It was observed that the dried mushroom was significantly superior to all the other treatments in the zinc content.

A close observation on the various treatments on the zinc content revealed that T_1 (1.75 mg), T_2 (1.45 mg) and T_3 (1.30 mg) were on par with each other. The lowest content of zinc (1.30 mg) was noticed in T_3 (frying).

Percentage loss of zinc observed to be on par with the treatments of boiling, steaming and frying. The per cent loss of zinc was highest in steaming 61.84 per cent. Where as in boiling and frying the loss was 53.94 per cent and 50.0 per cent respectively. But it was observed that drying increases 87.63 per cent of zinc.

4.2.4. Effect of processing on other constituents in milky mushroom

Other chemical constituents such as moisture, fiber, tannin and polyphenol content of milky mushroom subjected to different processing methods were also analyzed in detail. Results of which are given in table (11).

Table 11. Effect of processing on other chemical constituents in milky mushroom

Nutrients	T_1	T_2	T_3	T_4	CD (0.05)
Moisture (mg)	10.80 [a]	10.38 [a]	10.25 [a]	7.15 [d]	1.20
Fiber (mg)	1.5 [a]	1.9 [a]	1.2 [b]	23.50[d]	0.4
Tannin (mg)	1.4 [a]	1.1 [ab]	1.0 [b]	3.0 [d]	0.3
Polyphenol (mg)	2.42 [a]	2.75 [b]	2.65 [b]	8.85 [d]	0.1

Because of the high moisture content mushrooms are highly perishable commodity, and it contains more than 90 per cent moisture. Moisture content of milky mushroom under study determined as 90.73 per cent.

As indicated in table (11) moisture content of milky mushroom subjected to different processing techniques was on par with the other treatments than drying (T_4).

Moisture content of milky mushroom was reported to be highest in T_1 followed by T_2 (10.38 per cent), T_3 (10.25 per cent) and was on par with each other. Drying (T_4) reduces the moisture content to the maximum extent (7.15 per cent) and was significantly different from other processing treatments. Moisture loss was minimum and almost same in boiling, steaming and frying, while drying reduces the moisture level to the maximum 92.11 per cent.

On comparing the mean values of fiber content it was found that the highest fiber content was obtained in T_4 with a mean value of 23.50 g. A close observation on the various treatments showed that T_1 (1.5 g), and T_3 (1.2 g) were found to be on par with each other. The percentage loss of fiber during boiling (53.70 g), steaming (41.35 g) and frying (62.96 g) were on par. Drying (T_4) increases the fiber content to 275.30 per cent.

Changes in the tannin content of milky mushroom treated with different processing techniques were found to be significantly different. On comparing the mean value of tannin content, it was observed that the highest tannin content was obtained in T_4 with a mean value of 2.25 mg.

Tannin content of milky mushroom after processing varies between 0.1 to 2.0 mg. A close observation on the various treatments applied revealed that T1 (1.4 mg) and T2 (1.1 mg) were found to be on par with each other. Highest amount of tannin content was lost during frying (44.44 per cent) while in boiling and steaming the losses were 22.22 per cent and 38.80 per cent respectively.

Data on the polyphenol content of milky mushroom treated with different processing technique also found to vary significantly with different processing technique. According to the result, T4 (drying) had the highest polyphenol content with a mean value of 8.85 mg and it was significantly higher to all the other treatments.

On comparing the treatment mean values, T_1 (2.42 mg), T_2 (2.75 mg), T_3 (2.65 mg) were significantly different from each other. Boiling treatment (T_1) showed the lowest poly phenol content when compared with other treatments. On taking in to account of the percentage loss of poly phenols in milky mushroom during processing, highest loss was recorded in boiling (34.59 per cent) and the lowest was recorded in steaming (25.67 per cent).

On assessing the various treatments like boiling, steaming and frying it was revealed that nutrients loss was minimum in steaming followed by boiling and frying.

Results also depicts that on drying because of the moisture loss, the nutrient content is in the more concentrated form.

When analyzing the results, it may be concluded that steaming is the best method for cooking mushroom. In steaming moist heat is the cooking medium and nutrients may not leach out. While in boiling the nutrients especially water soluble vitamins may leach out.

4.3. ASSESSMENT OF HEALTH BENEFITS OF MILKY MUSHROOM

Mushrooms have been used as food and medicine in many parts of the world since time immemorial. They are potent exemplary sources of natural medicines with anti-diabetic activity (Tapasya, 2010). According to Bobek (1999), mushrooms are not only source of nutrients but also have been reported as therapeutic foods, useful in preventing diseases such as hypertension, diabetes, hypercholesterolemia and cancer.

In the present investigation, health benefits of milky mushroom were assessed by supplementing milky mushroom powder to selected subjects with specific disease condition through case studies.

Mushroom supplement was prepared in the laboratory as detailed in the methodology. Supplementation study was carried out by selecting human volunteers with specific disease conditions.

4.3.1. Formulation of the mushroom supplement and standardization of recipes

Milky mushroom was sun dried and powdered using a grinder and sieved using a fine mesh of about 10x size. Dried mushroom powder was packed in five gram sachet for distribution to the respondents selected for the case studies. No chemicals or preservatives were added to the mushroom powder.

Various recipes were standardized in the laboratory with the incorporation of mushroom supplement, in order to suggest different ways to incorporate supplement in the daily diet which will enable the prompt inclusion of the supplement in the daily

diet. Recipes were standardized with mushroom supplementation were breakfast dishes such as dosa, idly, chapatti etc. Other recipes standardized were rice, rasam, chammanthipodi and curd. Plates (3 & 4) depict the mushroom supplemented preparations.

In all the preparations five gram of mushroom powder was incorporated. In the case of dosa and chapatti, incorporation of mushroom powder does not rendered any color change or off flavor. While as in idly slight dull colour was imparted. Chutney powder prepared was very much acceptable, since the flavor of mushroom powder blended well with the chutney flavor. Similarly mixing the supplement along with curd also gave encouraging results. Adding supplement to the black tea was less acceptable. Subjects were asked to incorporate the powder which way they prefer.

However, all the subjects were of the opinion that, the supplement was easy to incorporate in the diet in different forms, and through different dishes. Mixing the supplement along with rice and curry was the easiest way of incorporation. However, powdered milky mushroom rendered fishy odor than the powdered oyster mushroom. Unlike the Oyster mushroom supplement some of the subjects expressed difficulties in consuming the supplement especially vegetarians due to its long lingering flavor. Two of the subjects discontinued the trial. Incorporation of mushroom supplement does not hinder the acceptability of the dishes also.

4.3.2. Result of case study on the hyper glycemic subjects

Two hyperglycemic subjects selected for the case study were two female subjects aged 50 (A) and 47 years (B). Two of them belong to Hindu community. Both of them were from nuclear family and follow small family norm. Respondent A was holding government job while respondent B was a house wife. Both of them were well educated and belonged to middle income family. They had an income ranged between Rs 35,000/- Rs 10,000/- per month

Considering the monthly expenditure pattern on food, it was noticed that both of the subjects spend Rs 1000/- to Rs 5000/- per month. They spend Rs 500/- to Rs 1000/- per month for meeting health needs.

Dietary pattern of the two subjects indicated that both of them were non vegetarians and generally followed three meals a day and do not follow any special diets for their disease condition.

Frequency of consumption of various food groups in the diet, revealed that subject A includes cereals, vegetables, fish, milk and milk products, tea daily; pulses on alternative days and meat, fruits, bakery items occasionally while the respondent B include cereals, pulses, vegetables, fish, milk and milk products, tea daily; meat, fruits and bakery items occasionally.

The subject A expressed that she is having some stress and strain in her daily life which was either related to health and financial problems while the subject B does not reported any stress and strain in her daily life.

Health profile of the subject

Table 12. Anthropometric parameters of the two hyperglycemic subjects

Body measurements	Subject A	Subject B
Height(cm)	154	160
Weight(Kg)	79	76
Waist circumference(cm)	45	46
Hip circumference(cm)	47	49
BMI	25.64	23.87
Waist hip ratio	0.95	0.93

On assessing the anthropometric measurements of two respondents, which is a direct indicator of health status, it was found that subject A recorded body height of 154 cm with body weight of 79 kg while subject B was having body height of 160 cm with body weight of 76 kg. Body mass index of subject A and B were found to be 25.64 and 23.75 respectively, which indicate subject A is not under the normal BMI, while subject B fall under the normal BMI.

Waist hip ratio indicates that fat deposition is mainly around the abdomen and the viscera; produces a large waist in relation to hip size associated with both diabetic and cardiovascular mortality (Ogawa, 1993).

The waist hip ratio (waist circumference divided by hip circumference) is a simple method for distinguishing between fatness in the lower trunk (hip and buttocks) and fatness in the upper trunk (waist and abdomen area). WHR of >1.0 for men and >0.85 for women is an indicator of abdominal obesity. Lower trunk fatness (lower waist to hip ratio) is often referred to as "gynoid obesity" upper trunk or central fatness (higher waist to hip ratio) is called "android obesity".

Waist hip ratio of both the subjects A and B were found to be 0.95 which indicate that both the subjects were at risk and prone to develop life style diseases in further if not attended. Both of them come under the gynoid obesity.

Morbidity pattern of the two subjects revealed that both of them are not often susceptible to fever and other infections and seemed to be healthy. Subject A having diabetes for the past two years and subject B identified as diabetic only before six months. Biochemical profile of the subjects revealed that subject A was having fasting blood glucose level of 135 mg/dl and subject B having 165 mg/dl and confirms both are diabetic.

Impact of the mushroom supplement on the blood sugar levels

Both the subjects were willingly participated in the mushroom supplementation study and were not taking any oral hypoglycemic agents for controlling the disease. Mushroom supplement prepared in the laboratory was distributed to the subjects for a period of three months. Close observation was made by the investigator and ensured that the subjects were consuming the supplement promptly.

Both of them incorporated the mushroom supplement in the breakfast preparations viz. dosa, idly, chapatti. They also used to mix the supplement with milk and consumed along with rice. Better acceptance is noticed while incorporating the supplement with rice.

The efficacy of the mushroom supplement was assessed by monitoring blood sugar levels at different intervals. The blood sample of both of the subjects were collected and blood profile was analyzed, details of which are given in the table (13).

Table 13. Fasting blood sugar level of respondents

Monitoring intervals	Fasting blood sugar level of subjects	
	Subject A	Subject B
Initial	135 mg/dl	165 mg/dl
45 th day	128 mg/dl	128 mg/dl
90 th day	112 mg/dl	98 mg/dl
Reduction of sugar level (%)	17.03 %	40.60 %

The result revealed that initial value obtained for fasting blood glucose of subject A and B were 135 mg/dl and 165 mg/dl respectively. After supplementation for 45 days, blood profile was monitored and found that the level has reduced to 128 mg/dl in both subjects. At the end of the 90 th day, fating blood sugar level was again monitored and the value were 112 mg/dl and 98 mg/dl respectively. A steady decline was observed in the two subjects studied with regard to fasting blood sugar levels.

4.3.3. Result of case study on the hyper cholesterolemic subjects

Two hyper cholesterolemic subjects were selected for the study in which subject A was 48 year old and subject B 50 years old. Both of them were females and subject A belong to Muslim community and subject B from Hindu community. Respondents belonged to nuclear family and follow small family norm. Subject A was a government employee belongs to high income family and subject B was a house wife, belonging to middle income family.

Considering the expenditure pattern on food, it was noticed that subject A spends Rs 15,000/- and subject B spend Rs 1000 /-per month for food and others. They also spend Rs 500/- to 1000/- per month for meeting health needs.

Dietary pattern of the two subjects indicated that both of them were non vegetarians and generally follow four meals a day pattern. Subject A do not resort to any special diets for reducing the cholesterol level, while subject B generally avoid fatty foods and bakery items due to increased level of cholesterol.

Frequency of consumption of various food groups in the diet, it was noticed that subject A include cereals, vegetables, fish, fruits, milk and milk products daily, pulses and meat once in a week and bakery items on alternative days while the respondent B include cereals, pulses, vegetables, fish, milk and milk products daily, meat and fruits occasionally in the diet.

Subject A reported to have some stress and strain in daily life which was either related to family problems while subject B does not specify any stress and strain in daily life.

Health condition of the two selected hyper cholesterolemic subjects was ascertained and details were given in table (14).

Table 14. Anthropometric parameters of the two hyper cholesterolemic subjects

Body measurements	Subject A	Subject B
Height(cm)	155	155
Weight(Kg)	65	65
Waist circumference(cm)	42	43
Hip circumference(cm)	45	47
BMI	20.96	20.96
Waist hip ratio	0.93	0.91

Anthropometric measurements indicated that both of the subjects were of same height of 155 cm and body weight of 65 kg each. Body mass index of the two subjects were found to be same as 20.96 indicating that both of them are under normal BMI.

Waist hip ratio of the subjects was computed as 0.93 and 0.91 in subject A and subject B respectively. The result indicated that both the subjects were at risk, and depicts gynoid obesity.

Morbidity pattern of the two subjects revealed that both the respondents do not fall sick frequently. However subject A having elevated blood cholesterol level for the past two years and subject B confirmed as hyperlipidemic for the last one year and was not taking any medicines for controlling the condition

Biochemical profile of the subjects revealed that subject A was having fasting blood cholesterol level of 231 mg/dl and subject B having 240 mg/dl.

Impact of the mushroom supplement on the cholesterol levels

Both the hyper cholesterolemic subjects were interestingly participated in the feeding experiment and were not on medication. Mushroom supplement was distributed for a period of three months. Direct monitoring was done by the investigator.

Both of the subjects were incorporated the mushroom supplement mainly in breakfast dishes like dosa, idly and chapatti and consumed it along with rice and curd. Both of the subjects do not express any difficulty in the incorporation of supplement in the daily diet.

The impact of the mushroom supplement was assessed by determining the blood cholesterol level periodically. The blood sample of both of the subjects was collected and blood profile was analyzed which is depicted in table (15).

Table 15. Blood cholesterol level of respondents

Monitoring intervals	Fasting blood cholesterol level of subjects	
	Subject A	Subject B
Initial 45 th day 90 th day	231 mg/dl 231 mg/dl 223mg/dl	240 mg/dl 225 mg/dl 178mg/dl
Reduction in cholesterol level (%)	3.46 %	25.83 %

The result showed that value obtained for fasting blood cholesterol level in subject A and subject B was 231 mg/dl and 240 mg/dl respectively in the initial stage. After 45 days of supplementation, there is no decrease in the cholesterol level in subject A while in subject B cholesterol level decreased to 225 mg/dl. At the end of 90 th day of supplementation, the cholesterol level reduced to 223 mg/dl and 178 mg/dl for subject A and B respectively. Remarkable decline in the cholesterol level in the subjects was noticed when milky mushroom powder was incorporated in the diet.

4.3.4. Result of case study on the hypertension subjects

Two hypertension subjects selected for the case study were 49 and 46 years old. One of them was male (subject A) and the other one is female (subject B) belongs to Hindu community. Both of them belonged to nuclear family and follow small family norm. Subject A was a farmer and subject B was holding government job. Both of them belonged to middle income family. They had an income ranged between Rs 10,000/- to Rs 20,000/- per month.

Both of the subjects incurred an amount of Rs 1000/- per month for meeting food expenditure and Rs 500/- to 1000/- per month for meeting health needs.

Dietary pattern of the subjects indicated that both of them were non vegetarians and subject A follow four meals a day while subject B follow three meals a day pattern. They do not follow any therapeutic diets for controlling hypertension.

Frequency of consumption of various food groups in the diet, it was noticed that subject A include cereals, pulses, vegetables, milk and milk products daily, fish and fruits on alternative days and meat once in a week while subject B include cereals, pulses, vegetables, milk and milk products daily, fruits on alternative days and meat, fish, bakery items occasionally.

Subject B have the habit of doing exercise, and go for morning walk regularly for one hour. Subject A reported to have stress and strain in daily life which was related to health and financial problems.

Health profile of the subjects.

Health condition of the two selected hypertension subjects was ascertained and details were given in table (16).

Table 16. Anthropometric parameters of the hypertension patients

Body measurements	Subject A	Subject B
Height(cm)	175	155
Weight(Kg)	78	60
Waist circumference(cm)	40	46
Hip circumference(cm)	41	48
BMI	22.28	25
Waist hip ratio	0.97	0.95

On assessing the anthropometric measurements of the two respondents, it was found that subject A and B were having 175 cm and 155 cm body height respectively. Body weight of subject A was found to be 78 kg and for subject B 60 kg.

Body mass index of subject A and B were found to be 22.28 and 25 respectively, indicating that subject A fall under normal BMI while subject B not under the normal BMI (25).

On assessing waist hip ratio of the subjects, it was found to be 0.97 and 0.95 respectively, which is above the recommended standards and both of them were at risk and depict gynoid obesity. Morbidity pattern of the two subjects revealed that both the respondents do not fall sick frequently. Both of the respondents recorded elevated blood pressure for the past two years. Both of them having blood pressure above the normal levels.

Impact of mushroom supplement on the blood pressure levels

Both of the subjects were willingly participated in the experimental case study. Both the subjects were not under any medication for controlling hypertension.

Both the subjects incorporated the mushroom supplement along with rice, chutney powder and milk. They also consume the supplement along with curd and sambar. Both of them reported little difficulty when consuming the mushroom supplement because of its lingering taste and flavor.

The impact of the mushroom supplement was monitored by determining the blood pressure at different intervals given in table (17).

Blood pressure recorded in the subject A and B was 170/100 mm Hg and 140/100 mm Hg respectively before the onset of supplementation. After 45 th days of supplementation subject A showed reduction in blood pressure level to 150/90 mm Hg but in subject B slight increase in the level was seen to 150/100 mm Hg. When monitored after 90 days of supplementation there was steady decline in the case of subject A to 100/80 mm Hg and 120/80 mm Hg in subject B.

Table 17. Blood pressure level of respondents

Monitoring intervals	Blood pressure level of respondents			
	Subject A		Subject B	
	Systolic	Diastolic	Systolic	Diastolic
Initial	170 mm Hg	100 mm Hg	140 mm Hg	100 mm Hg
45 th day	150 mm Hg	90 mm Hg	150 mm Hg	100 mm Hg
90 th day	100 mm Hg	80 mm Hg	120 mm Hg	80 mm Hg

Discussion

5. DISCUSSION

Mushrooms are part of the human diet for thousands of year, involving a large number of edible species. In most countries there is a well established consumer's acceptance for cultivated mushrooms, probably not only due to their unique flavor and texture but also for their physic chemical properties and nutritional characteristics (Manjunathan and Kaviyarasan, 2011).

According to Murugar *et al*. (2005) mushrooms are considered as a valuable health food because they known for rich protinacious food, it consists of about 75 per cent proteins and are low in calories, fat, fatty acids, vitamins and minerals.

Milky mushroom (*Calocybe indica*) a common edible mushroom possesses many nutrients such as vitamins, proteins, minerals, and essential amino acids thereby assuming increasing popularity and acceptance in the daily diet. The result obtained from the present investigation throw light on the above aspects which are discussed below.

5.1. Quality evaluation of Milky mushroom

Chemical and nutritional composition of the milky mushroom is a major parameter influencing the quality of mushrooms. According to Fukushima (2000) mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quality. The quality of milky mushroom was assessed in order to understand the amount of nutrients and chemical constituents present in the milky mushroom and how best it could be utilized for health promotion.

5.1.1. Assessment of chemical and nutritional composition of fresh milky mushroom

The major nutrients assessed in the present investigation were calories, protein, fat, vitamins viz. vitamin C, B complex vitamins. Mineral elements such as iron, calcium, sodium, potassium, phosphorus, zinc, and copper were also assessed in the milky mushroom.

5.1.1.1. Estimation of macro nutrients

In the present study, the macro nutrients present in the milky mushroom were estimated with regard to the calories, total carbohydrate, protein and fat. According to Rahul *et al*. (2010) milky mushroom consists of more carbohydrate, protein and fat than oyster mushroom. Chang et al. (2004) reported that milky mushroom consists of protein (10-40 per cent), carbohydrate (13-70 per cent), fat (1-8 per cent) and significant amount of essential amino acids.

The calorie content of milky mushroom on fresh weight basis was 46.56 Kcal/100g while on dry weight basis it was found to be 338 Kcal. A study conducted by Jaziya, (2011) reported that in oyster mushroom (*Pleurotous florida*) the calorie content on dry weight basis is 453 Kcal/100g. In this context it can be stated that calorific value of *Calocybe indica* is less than oyster mushroom on dry weight basis. Mussala (2009), pointed out that mushrooms are low calorie foods approximately 20 calories in an ounce of mushroom. According to Usha (2007), mushrooms are low calorie food with very little fat which is free of cholesterol and rich in linoleic acid.

The carbohydrates are pivotal nutrients required for a balanced diet. Carbohydrate content of fresh milky mushroom was 6.3 g/100g, which is on par with the findings of Mirunalini (2011), who reported 6.3 to 7.3 g of carbohydrate in milky mushroom. According to Krishnamoorthy (1997), milky mushroom consists of 13-70 per cent of carbohydrate on fresh weight basis. Alam *et al*. (2008) reported that milky mushroom contains 7.3 g of carbohydrate. Carbohydrate content of mushroom was found to vary depending on the variety of species and substrates to be used. Carbohydrate content of oyster mushroom is reported as 4.70 g which is less than milky mushroom (Jaziya, 2011).

Mushroom polysaccharides support some or all of the major systems of the body, including nervous, hormonal and immune systems as well as regulatory function (Wasser, 2002). Dunkwal *et al*. (2006) pointed out that the food value of mushroom is being increasingly realized as they are low in carbohydrate, cholesterol and fat and high in vitamins. Demirbas (2001), stated that mushrooms are good source of high

quality protein which is higher than vegetables and fruits and is of superior quality also called "white vegetables" or "boneless vegetarian meat".

Milky mushroom under study recorded 4.26 g /100g of protein. Dembitsky (2010), opined that in mushrooms the protein content vary from 1.75 to 5.9 per cent on fresh weight basis. According to Satish *et al*. (2011) mushrooms contain high quality proteins rich in lysine and tryptophan which are deficient in cereals. According to Yehia (2011), protein content of edible mushrooms is twice as that of onion and cabbage (1.4 per cent), four times that of oranges and twelve times higher than that of apples (0.3 per cent)

Glucin *et al*. (2002) reported 5.6 g protein in fresh mushrooms. Alam *et al*. (2007) reported that 100 g of *Calocybe indica* contain 2.6 to 2.9 g of proteins, whereas in *Pleurotus florida* the protein content is reported to be 2.5 to 2.75g. Alam *et al* (2008) reported that fresh mushroom *Pleurotus florida, P.ostreatus and P. sajor caju* found to have 2.6 g, 3.4 g and 3.2 g of protein respectively. In this respect milky mushroom contain protein to much higher level. Chang and Buswell (1996), reported that the digestibility of mushroom protein to be high as 72 to 83 per cent. Dunkwal (2009), opined that methionine is an essential amino acid present in mushroom which improves the quality of mushroom protein as this amino acid is lacking in pulses.

The fat content of milky mushroom was recorded as 0.68 per cent. This is similar to those reported by Alam *et al*. (2007) he found that fat content of fresh *Calocybe indica* contains 0.6 to 0.7 g/100g of fat. Yilmaz *et al*. (2006) reported that fat fraction in mushroom is mainly composed of unsaturated fatty acids. According to Pedneault *et al*. (2006) in mushrooms, the fat content is very low as compared to carbohydrates and protein.

Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (75 to 85 per cent) mainly due to linoleic acid. The high content of linoleic acid is one of the reasons why mushrooms are considered a healthy food (Mshigeni, 2001). According to Florezak *et al*. (2004) the content of fatty acid compounds in 100 g fresh matter is 0.4 g and in dry matter it is 1.8 g.

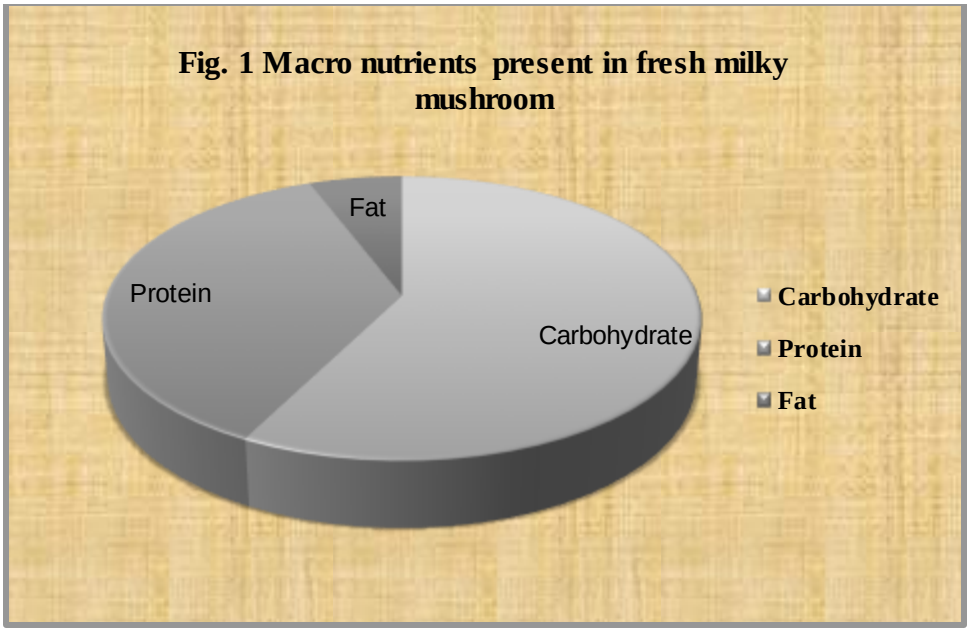


Fig. 1 Macro nutrients present in fresh milky mushroom
Fat
Protein
Carbohydrate
Carbohydrate
Protein
Fat

5.1.1.2. Quality evaluation of mushroom protein

Mushrooms are excellent source of high quality proteins as compared to most of the vegetables and they are in easily digestible form (Radde *et al.*, 1999). According to Sadler (2003), mushrooms are rich in protein and they contain abundant amount of essential amino acids.

In the present investigation eighteen amino acids including eight essential amino acids were estimated in the milky mushroom which yields more precise data on the quality of protein.

The studied variety of mushroom (*Calocybe indica*) contains almost all the essential aminoacids needed for the body in fairly good amounts. The essential amino acid viz. isoleucine, methionine, phenylalanine, threonine and valine in milky mushroom under study were found to be 9.8g, 3.22g, 2.55g, 10.4g, and 9.86g respectively.

Among the essential amino acids threonine was exceptionally high when compared to reference protein in the milky mushroom studied. Similarly isoleucine, valine, methionine content were also higher than the reference protein. Essential amino acids viz. lysine, leucine and tryptophan were not found in the sample and may be due to the deproteinization of mushroom powder.

Among the eighteen amino acids presents in milky mushroom, alanine (28.56) was found to be very high followed by cystine (18.1g) glycine (17.7g) and threonine (10.4g). Study conducted by Jaziya (2011), reported that highest amount of amino acid present in oyster mushroom (*Pleurotous florida*) was glutamic acid (17.2g) followed by aspartic acid (10.5g) and phenylalanine (10.2g) which is an essential amino acid.

Purkayastha and Chandra (1976), reported that leucine, threonine, tyrosine and alanine are the aminoacids predominant in *calocybe indica*.

Amino acid content of the milky mushroom when compared to *Pleurotus florida, P. sajor caju* and *P.citrinopeleatus* observed that alanine was the highest and it was even higher than the other three species. Similarly cystine, isoleucine, valine are also higher than the other three species of mushrooms.

Data clearly indicated that milky mushroom is superior to *Pleurotus florida, P. sajor caju* and *P.citrinopeleatus* with respect to aminoacid composition and also with essential amino acid content.

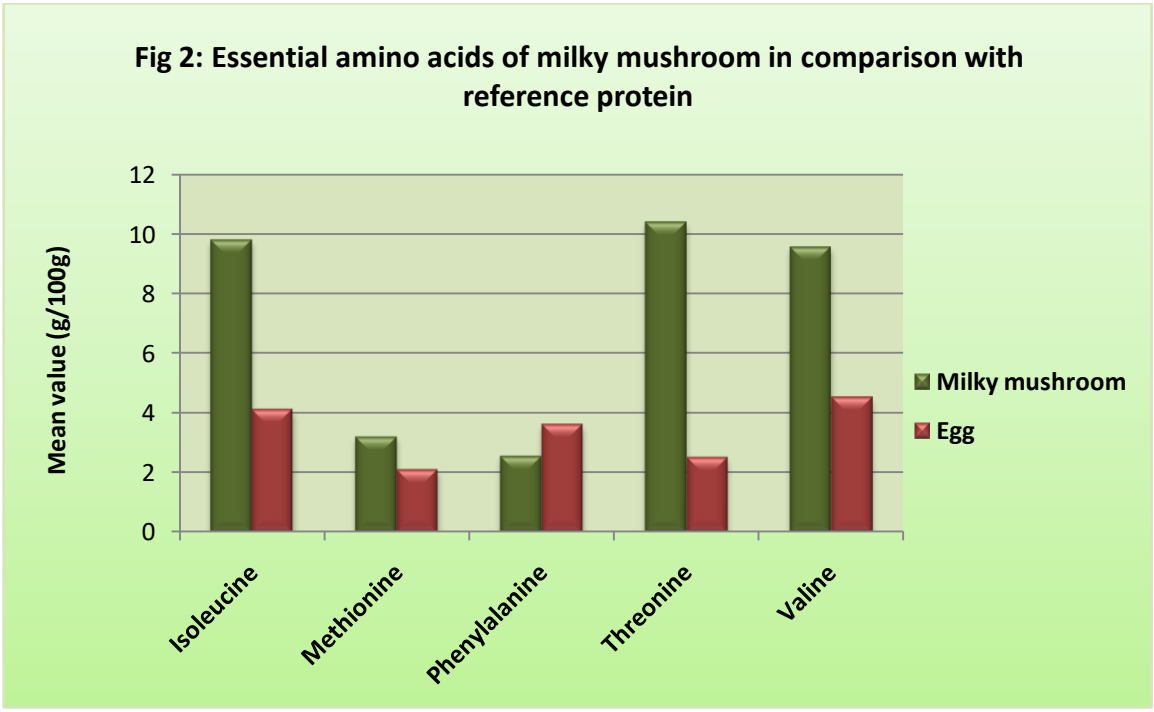
Determination of amino acid score (AAS) is another index to evaluate protein quality. Threonine recorded the highest amino acid score (416.0) followed by isoleucine (239.02) and valine (219.11) and all the three are essential amino acids. Where as in *Pleurotus florida* phenylalanine recorded the highest score (283.33) followed by threonine (208.0) and leucine (113.46). In *Pleurotus sajor caju* and *P.citrinopeleatus* phenylalanine recorded the highest score (100.0, 98.45) followed by threonine (98.62, 97.24) and lysine (72.50, 76.14).

The above data confirmed that AAS also in favour and better in milky mushroom, and superior in protein quality when compared to *Pleurotus florida, P. sajor caju* and *P.citrinopeleatus*.

Sequence of limiting aminoacid in *C. indica* was phenylalanineand methionine. In *Pleurotus florida* sequence of limiting amino acid is methionine, isoleucine and valine. While in *Pleurotus sajor caju* and *P.citrinopeleatus* limiting amino acid sequence is methionine, isoleucine, leucine and methionine, isoleucine, valine respectively.

EAA index of milky mushroom computed was found to be 130.20 while the nutritional index based on protein quality was determined as 2.60. EAA index of oyster mushroom computed was found to be 119 while nutritional index based on protein quality was determined as 6.42 (Jazia, 2011). Ghosh and Chakrabarty (1990), reported that EAA index of *Pleurotus sajor caju* as 72.04 and nutritional index as 12.78.

Fig 2: Essential amino acids of milky mushroom in comparison with reference protein



5.1.1.3. Estimation of vitamins

In the present study, the vitamins assessed were vitamin C, thiamin, riboflavin, and niacin. Singh (2002), reported that mushrooms contain significant quantities of B vitamins, including thiamin (B1), riboflavin (B2), pyridoxine (B6), niacin and folate and also vitamin C.

Vitamin C content of milky mushroom was recorded as 12.82 mg/ 100 g. Sies (1999), pointed out that vitamin C is regarded as the first line natural antioxidant defense in plasma and a powerful inhibitor of lipid peroxidation. According to Kuroyangi *et al.* (2002) vitamin C readily undergoes reversible oxidation and reduction and plays an important role as a redox agent in biological systems.

The thiamin content of milky mushroom studied was observed to be 6.20 mg/ 100 g which was much higher than the value reported by Ranote *et al.* (2007) in *Pleurotus* species 1.16 to 4.8 mg/ 100g. Riboflavin content of milky mushroom was recorded as 10.0 g/100g. According to Goyal (2002), 100 g of mushroom seems to take care of daily requirement of thiamin of an adult, whereas, 25 to 40 g of mushroom can meet the daily requirement of riboflavin of an adult. Kakon (2012), reported that mushrooms contain a large amount of vitamins such as thiamin 1.4 to 2.2 mg, riboflavin 6.7 to 9.0 mg and niacin 60.6 to 73.3 mg /100 g.

According to Rai (1995), mushroom contains 0.4 mg riboflavin and forms 24 percent of a person's daily requirement. It is an essential vitamin required for metabolism of carbohydrate, lipids and amino acids and also supports antioxidant properties. Jaziya (2011), reported that 100 g of *Pleurotus florida* contain 8.70 mg riboflavin which is much higher than the milky mushroom. Mushrooms contain 3.6 mg niacin, which contribute to 18 per cent of the body's niacin value. Niacin is essential for energy metabolism and act as a coenzyme in fatty acids and carbohydrate metabolism (Latifah *et al.*, 1996). B complex vitamins present in the milky mushroom are depicted in fig (3).

5.1.1.4. Estimation of minerals and trace elements

In the present investigation, the minerals and trace elements present in the milky mushroom were assessed with regard to the phosphorus, calcium, sodium, potassium, iron and zinc. The presence of various mineral elements enriches food value of mushrooms. Mineral content of milky mushroom is depicted in Fig (4).

Calcium content of the milky mushroom studied was 10.40 mg/100g. Nutrition data (2007) reported 9.4 mg /100g of calcium content in fresh mushroom. According to Kalac (2000), in general mushrooms had good amount of minerals including trace minerals, calcium, phosphorus, potassium and sodium constitute about 56 to 76 per cent of the total ash content of the mushroom. Alam *et al.* (2007) reported that 100 g of mushrooms contain 2 mg of calcium.

Sodium content of fresh mushroom observed to be 11.8 mg/100g. Kurtzman (2005), pointed out that mushrooms have the double benefit of low sodium and more potassium and iron than most foods. According to Manzi (2000), the low sodium concentration and the presence of a greater amount of potassium suggest the utilization of mushrooms in an anti-hypertensive diet. According to Obodai *et al.* (2003) potassium, phosphorus, sodium, magnesium constitutes about 56 to 70 per cent of the total ash content of the mushroom while potassium alone forms 45 per cent of the total ash.

Potassium content of milky mushroom under study was observed to be 415.6 mg/100g. Nutrition data (2007) reported 555 mg of potassium in fresh mushroom. Garcia *et al.* (2009) pointed out that mushrooms have 98 to 376 mg of potassium per 100 g serving which is 3 to 11 per cent of the daily value. Also potassium is an important mineral helps in the maintenance of normal fluid and mineral balance, which helps to control blood pressure. According to Harsha (2004), mushrooms contain 318 mg of potassium which helps in regulation of fluid balance and forms 9 per cent of the daily value.

According to Bobek *et al.* (1996) amount of phosphorous present in mushrooms are 86.0 mg which is major component of teeth and bones. It forms 9 per cent of the daily value. Phosphorous content of milky mushroom under study was recorded as 622.6 mg/100g. Phosphorus content of *Pleurotus florida* was reported as 477 mg/100g (Jaziya, 2011).

Iron content of milky mushroom under study was estimated to be 10.86 mg/100g which is same as those reported in *Pleurotus florida* by Jaziya (2011). Beelan *et al*. (2004), reported that one third of the iron in the mushrooms is in available form. According to Clark (1996), mushrooms provide small, but nutritionally important amounts of iron, about 1 mg per 100g. Unlike many vegetable sources of iron, mushrooms do not contain phytates, which reduce the body's ability to absorb iron. The bioavailability of the iron in mushrooms is therefore high and up to 90 per cent of the iron present can be absorbed. In mushrooms iron content usually ranges from 6.1 to 12.7 mg/100g in *Pleurotus spp*. (Gruen *et al.*, 1982).

Copper content of milky mushroom under study was found to be 11.2 mg/100 g. According to Zhu *et al*. (2011) copper helps to make red blood cells, which carry oxygen throughout the body. Copper also helps to keep bones and nerves healthy. Copper content ranged from 8.0 to 21.5 mg/100g in *Pleurotus spp.* and *A. bisporus* (Isildak *et al.*, 2004). Copper has properties that help to protect our cardiovascular system, and one small serving of mushroom contains more than 20 per cent of the copper we need daily (Feng *et al*., 2001).

Zinc content of the milky mushroom in the present study was found to be 3.8 mg/100g. Nutrition data (2007) reported that the mushrooms contain 1.4 mg of zinc on fresh weight basis.

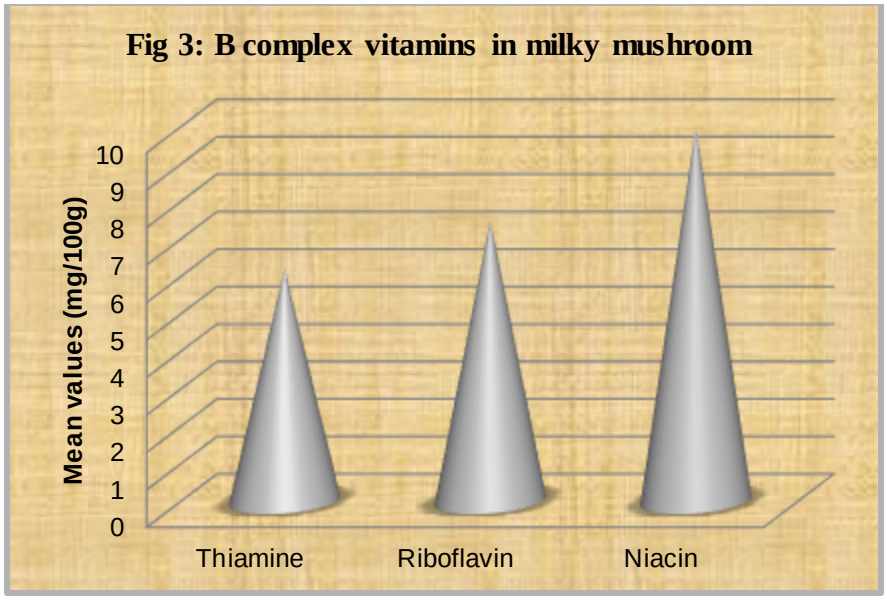


Fig 3: B complex vitamins in milky mushroom
Mean values (mg/100g)
10
9
8
7
6
5
4
3
2
1
0
Thiamine
Riboflavin
Niacin

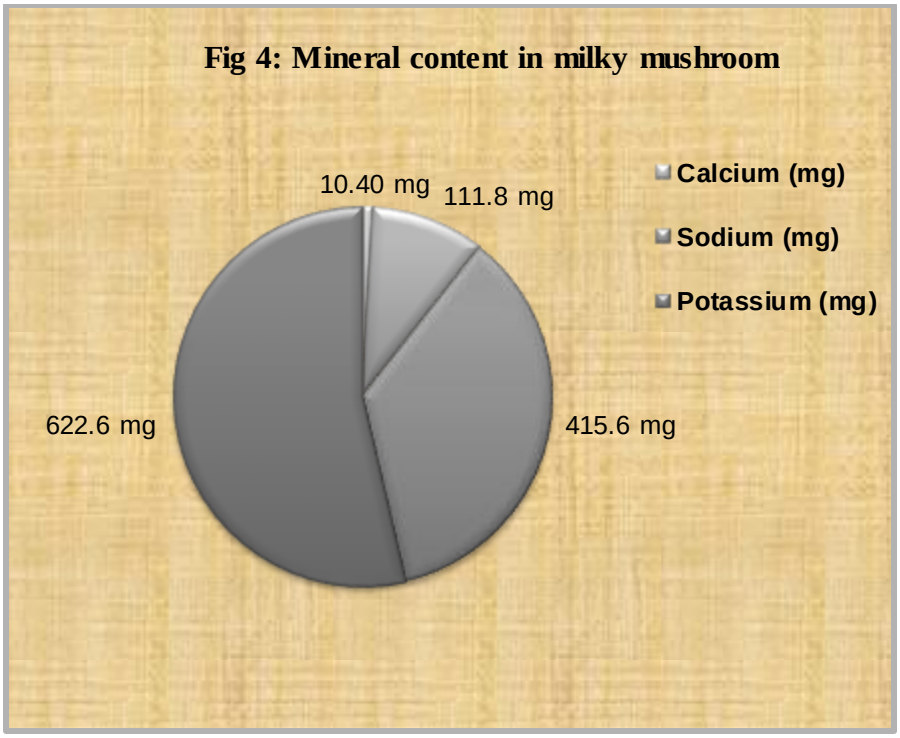


Fig 4: Mineral content in milky mushroom
10.40 mg
111.8 mg
622.6 mg
415.6 mg
Calcium (mg)
Sodium (mg)
Potassium (mg)

5.1.1.5. Estimation of other constituents

Moisture content of the milky mushroom studied was determined as 90.73 per cent. High perishability of the mushroom is due to its moisture content and it varies with species. According to Beelan (2005), high water content in mushrooms means that fresh mushroom contain very little fat or carbohydrate and low in energy.

Borchers *et al.* (2004) pointed out that high water content (90 per cent) in mushrooms can contribute to a feeling of fullness, and low energy density can help to promote weight maintenance. According to Mirunalini (2012), fresh mushroom has 90.67 per cent of moisture. According to Nutrition data (2007) 90 per cent of moisture is present in edible mushrooms on fresh weight basis.

A study conducted by Alam *et al.* (2008) reported that the moisture content of *Calocybe indica, Pleurotus florida. P. sajor caju, P. ostreatus* were found to be 86, 87.5, 87 and 87.4 per cent respectively. The value obtained in the studied milky mushroom was much higher than the reported value.

Fiber content of fresh milky mushroom under study was found to be 3.24 g/100g. According to Sadler (2003), fresh mushroom is known to contain both soluble and insoluble fibers; the soluble fiber is mainly beta-glucans, polysaccharides and chitosans which are components of the cell walls. The fiber content reported in *Pleurotus florida* by Jaziya (2011), was 3.20 g/100g in fresh weight basis which is much less than the studied milky mushroom.

Tannin content of milky mushroom under study was found to be 1.80 mg/100g. Randhava and Ranote (2004), reported *Pleurotus florida* and *P. sajor caju* contains 0.02 mg and 0.018 mg tannin respectively which was much lower than those found in *calocybe indica*.

Polyphenol content of fresh milky mushroom under study was found to be 3.70 mg/100g. According to Manzi *et al.* (2001) polyphenol content of fresh mushrooms was found to be 51.4 to 403.8 mg/100g. Jaziya (2011), reported that 100 g *Pleurotus florida* contains 4.30 mg which is much higher than the studied milky mushroom.

5.1.2. Anti inflammatory Factors (IF Positives) and Anti oxidant property of milky mushroom

Inflammation plays an important role in various diseases such as rheumatoid arthritis, atherosclerosis and asthma. The anti-inflammatory factor is the constituent in the food that helps to prevent inflammation (Jegtvig, 2010). Mushrooms have anti inflammatory factors like vitamin C, folate, zinc and selenium (Nutrition data, 2007).

Milky mushroom under study was found to have 12.82 mg of vitamin C on fresh weight basis. It also contains the anti inflammatory nutrient zinc as 3.8 mg on fresh weight basis. The presence of vitamin C and zinc contribute to the positive effect of mushroom on health. Kuroyanagi *et al.*, (2002) opined that vitamin C readily undergoes reversible oxidation and reduction and plays an important role as a redox agent in biological systems.

Mushrooms are the leading source of the antioxidant selenium and help to maintain a healthy immune system and also help to protect body cells from damage that might lead to chronic diseases. Beneficial health effects of dietary antioxidants have been associated with the inhibition of atherosclerosis, cancer, prevention of chronic inflammation etc. The bioactivity of antioxidants is released to their ability to chelate metals, inhibit lipoxygenases and scavenge free radicals (Tapasya and Kapoor, 2011).

According to Mirunalini (2012), *Calocybe indica* is an indigenous popular edible mushroom, possessing a variety of secondary metabolites such as phenolic compounds, terpenes and steroids possibly involved in their medicinal effects and nutritive value. The study also stated that, ethanolic extract of *C. indica* exhibited increased antioxidant activity which may overcome free radical mediated diseases.

The polyphenol content of fresh and dry milky mushroom under study was recorded as 3.70 mg and 8.85 mg respectively. While tannin and fiber content found to be 1.80 mg and 3.24 g respectively on fresh weight basis. Tapasya (2011), reported

that the levels of antioxidant enzymes and reduced glutathione were found to be highest in *calocybe indica* in comparison to various herbs. A study conducted by Selvi *et al.* (2007) reported that *C. indica* posse' non enzymatic antioxidants which include vitamin A, C, E and reduced glutathione, vitamin A in the fresh and powdered samples of milky mushrooms was found to be 2.51 mg/100g and 1.52 mg/100g respectively.

5.2. Effect of processing on the nutritional value of milky mushroom

The way the food is cooked is absolutely essential for avoiding nutrient loss. As mushrooms are very precious with abundant nutrients and medicinal values, it is essential to understand the nutrient loss during cooking of mushrooms. Schultz (2010), pointed out that cooking causes the loss of some nutrients in vegetables and fruits.

Selvi *et al.* (2007) reported that mushrooms preserved by drying have a good flavor, prevents microbial deterioration and also loss of moisture, increase concentration of nutrients in the remaining mass. The study also revealed that moisture content of dried mushrooms is in the range of 10 to 15 per cent and has superior quality of protein content varying from 25 to 35 per cent.

In the present study, changes in the nutrient content of milky mushroom subjected to different processing treatments were ascertained.

5.2.1. Effect of processing on the Macro nutrients of milky mushroom

Energy value of milky mushroom ranged from 35.11 to 338.0 Kcal respectively when treated with different processing methods. On assessing the data it was found that the dried mushroom was significantly superior to all the other treatments. However, the other processing treatments T_1 (40.45 Kcal), T_2 (39.25 Kcal), T_3 (35.11 Kcal) were found to be on par with each other though all of them are significantly inferior to T_4 (338 Kcal). According to Dikeman *et al.* (2005) mushrooms are low in energy and low in fat, 80 g serving provides 10 Kcal and 0.4 g of fat.

The carbohydrate content of milky mushroom get concentrated after drying and it was found to be reduced when subjected to boiling, steaming and frying. Comparison of treatment means depicted an increasing trend of carbohydrate on drying. Highest carbohydrate was noticed in T4 (40.0 g) and was significantly superior to all the other treatments in the carbohydrate content. Almost similar results were reported in *Pleurotus florida* (40.3 g), *P. sajorcaju* (39.4 g), and *P. ostreatus* (37.2 g) on dry weight basis (Khan *et al*., 2008).

According to Kalac (2012), carbohydrate constitute the prevailing component of mushroom dry matter, usually about 50 to 60 per cent, constitute monosaccharide's, their derivatives and oligosaccharides and both reserve and construction polysaccharides glycans. Of the dry matter constituents of mushrooms, carbohydrate was found in the greatest amounts, constituting 16 to 85 g/100g (Muzzarelli, 1999).

Significant difference was noted in carbohydrate content of milky mushroom under study in all processing treatments. Dried mushroom sample depicted higher carbohydrate content. Data regarding processing treatments depicted a gradual reduction in carbohydrate during boiling, frying and steaming. The carbohydrate value recorded in boiling was 2.12 g, where in steaming and frying the value were 3.5 g and 2.94 g respectively. Highest amount of carbohydrate was lost during boiling when compared with other treatments.

On account of percentage loss of carbohydrate, it was noticed that highest loss was recorded in boiling treatment (68.35 per cent) and lowest in steaming (47.76 per cent). Lowe (2009), pointed out that the starch from vegetables is insoluble when the cell wall is broken by cutting, disintegration of starch during cooking or from abrasion which will occur in violent boiling.

Protein content of milky mushroom treated with different processing treatments ranged from 2.27 to 19.61 g. The highest protein content was noticed in T_4 with a mean value of 19.61 g. According to Yehia (2011), on dry weight basis, mushrooms normally contain 19 to 35 per cent protein, as compared to rice (7.3 per cent), wheat (12.7 per cent), soybean (38.11 per cent) and corn (9.4 per cent). Furthermore mushroom protein contains all nine essential amino acids required by humans.

Alam *et al*. (2007) reported that 100 g dried *Calocybe indica* contain 20 to 23 g of protein. Similar values were reported by Ozdemir (2000), in edible mushrooms. Tapasya (2011), reported that mushrooms are good source of high quality protein ranging from 30 to 40 per cent on dry weight basis. Protein content of milky mushroom showed a decreasing trend while processing. The protein content of milky mushroom after various treatments were T_1 (2.27 g), T_2 (2.95 g), T_3 (2.45 g) and was significantly different from T_4 (drying). The per cent loss of protein was observed to be highest in boiling 46.71 per cent and lowest in steaming 30.75 per cent. Gopalan *et al*. (2010) stated that some proteins may be lost if vegetables are cooked in water and the cooked water is discarded.

Fat content of milky mushroom ranged from 0.22 to 3.25 g respectively when treated with different processing methods. It was observed that fat content increased during frying to 3.75 g because of using fat (oil) as medium for frying. According to Ranote *et al*. (2007) fat content of milky mushroom on dry weight basis ranged between 1.0 to 8.3 per cent. In the present study, fried mushroom (T_3) was found to have higher fat content when compared with others. On assessing the percentage loss steaming (T_2) depicted the lowest value (38.23 per cent), while boiling (T_1) depicted highest (67.64 per cent).

5.2.2. Effect of processing on the vitamin content of milky mushroom

Mushrooms contain significant quantities of several B vitamins including thiamin (B1), riboflavin (B2), pyridoxin (B6), niacin and folate (Borchers *et al*., 2004). According to Mattila *et al*. (2000) mushroom is an important source of vitamins especially B group vitamins like thiamin, riboflavin, niacin, folic acid, nicotinic acid as well as other vitamins such as ergosterol, biotin and tocopherols.

Vitamins including vitamin C and B complex like thiamin, riboflavin and niacin present in milky mushroom treated with different processing methods ranged from 3.62 to 20.27 mg, 3.0 to 17.57 mg, 3.75 to 16.8 mg and 3.0 to 23.0 mg respectively. In the present study, it was observed that all the vitamins are higher in dried mushrooms.

In drying (T_4) vitamin C, thiamin, riboflavin and niacin content was found to be 20.27 mg, 17.57 mg, 16.8 mg and 20.0 mg respectively.

Boiling is the cooking method that causes the greatest loss of nutrients in vegetables (Schultz, 2010). Many of the water soluble vitamins are transferred in to the water and lost if the vegetables are drained and the water is thrown away. When compared to boiled milky mushroom steamed mushroom recorded vitamin C content of 4.75 mg, thiamin 3.0 mg, riboflavin 3.75 mg and niacin 5.0 mg respectively.

Steaming is generally considered to be best method to cook vegetables in order to preserve the nutrients. Steaming is relatively quick method of cooking so the vegetables are only exposed to heat for a short time (Schultz, 2010). The nutrient loss was comparatively less in the steamed milky mushroom compared to boiled and fried mushroom.

5.2.3. Effect of processing on the mineral content of milky mushroom

Changes in the mineral content of milky mushroom subjected to different processing method were also determined in the study. As the moisture is removed from the mushroom, on drying the concentration of nutrients is high. According to Lepsova (1988), trace element concentration in mushrooms is considerably higher than those in agricultural crop plants, vegetables and fruits.

Results of the present study indicated an increase in calcium content in dried mushroom sample. Calcium content was high as 18.06 mg in T_4 (dried). The result is similar as Jandaik (1989), who found that the calcium content of mushroom on dry weight basis is 20 mg/100g. In a study, Alam *et al*. (2008) found that the calcium content of dried *Calocybe indica* was found to be 33.7 mg/100g.

Losato (1988), observed that calcium content of mushroom ranged between 11.8 to 162.2 mg/100g on dry weight basis. The lowest value of calcium content was seen in T_1 (4.1 mg) followed by T_2 (5.05 mg) and T_3 (7.07 mg). As indicated in table, the mean value of calcium content of milky mushroom subjected to different processing method was found to be statistically different from each other. The per cent loss of

calcium during processing was observed to be highest in boiling (60.57 per cent) and lowest in frying (32.01 per cent).

The principal minerals in foods that may be lost during cooking or processing are the salts of calcium, sodium and potassium. Calcium salts are not as soluble as the other salts found in vegetables (Lowe, 2009).

Sodium content of milky mushroom treated with different processing treatment indicated that the mean value of sodium content of milky mushroom differs significantly.

The highest sodium content was noticed in T_4 with a mean value of 285.32 mg. It was noticed that the dried mushroom was superior to all the other processing treatments viz. boiling, steaming and frying and their values determined as T_1 (65.0 mg) T_2 (37.45 mg) T_3 (46.24 mg) respectively. The percentage loss of sodium was noticed to be highest in steaming (66.50 per cent) and lowest in boiling (41.86 per cent).

Potassium content of the milky mushroom treated with different processing method ranged between 204.42 to 717.72 mg. The highest value of potassium was recorded in dried milky mushroom (717.72 mg). The study revealed that potassium content was higher in dried sample as the concentration of nutrients is more when moisture is lost.

Highest percentage loss of potassium was noticed in boiling (50.81 per cent) and lowest in steaming (49.84 per cent) followed by frying (50.28 per cent). In support of the above findings Schultz, (2010) noticed that 70 per cent of potassium is lost during boiling of vegetables.

Phosphorus content of the milky mushroom treated with different processing method revealed that highest phosphorus content was observed in dried milky mushroom 833.4 mg. The lowest value was noticed in frying (321.7 mg) followed by steaming (359.15 mg) and boiling (360.4 mg). Phosphorus content of milky

mushroom treated with different processing methods indicated significant difference between the treatments.

Study conducted by Agarwala and Jandaik (1989), found that phosphorous content of *Pleurotus sajor caju* was 760 mg on dry weight basis. Highest per cent loss of Phosphorus was noticed in frying (34.45 per cent) followed by steaming (26.82 per cent) and boiling (26.56 per cent).

With respect to iron content in processed mushroom dried mushroom depicted highest iron content (20.65 mg). Alam *et al*. (2008) studied the iron content of *P. sajor caju* and reported to contains 33.45 mg of iron on dry weight basis. Jaziya (2011), studied the iron content of pleurotus *florida* and reported to contain 20.76 mg of iron on dry weight basis.

Results showed that as with other nutrients iron was also highest in boiling (49.53 per cent) and lowest in steaming (27.53 per cent). Loss of iron content during cooking vegetables was reported to be 51.5 per cent (wh.food.com, 2010).

Copper content of dried mushroom was found to be 20.1 mg/100g. Highest copper content was noticed in dried sample and lowest in boiled mushroom. Paraskevi *et al.* (2009) observed in his study that dried mushroom contains 7.83 to 75.06 mg of copper.

The highest per cent of copper was lost in boiling (53.12 per cent) followed by frying (33.48 per cent). The lowest percent loss was found in steaming (25.89 per cent). Shcultz (2010), observed that boiling and draining vegetables results loss of 45 to 59 per cent loss of copper.

Drying of milky mushroom enhances the zinc content. Maximum zinc content was noticed in T_4 (7.13 mg). Sanmee *et al*. (2003) reported in his study that the highest amount of zinc was noticed in drying and ranged between 37.8 to 253 mg/100g. The per cent loss of zinc was highest in steaming 61.84 per cent followed by boiling 53.94 per cent and frying 50.0 per cent respectively.

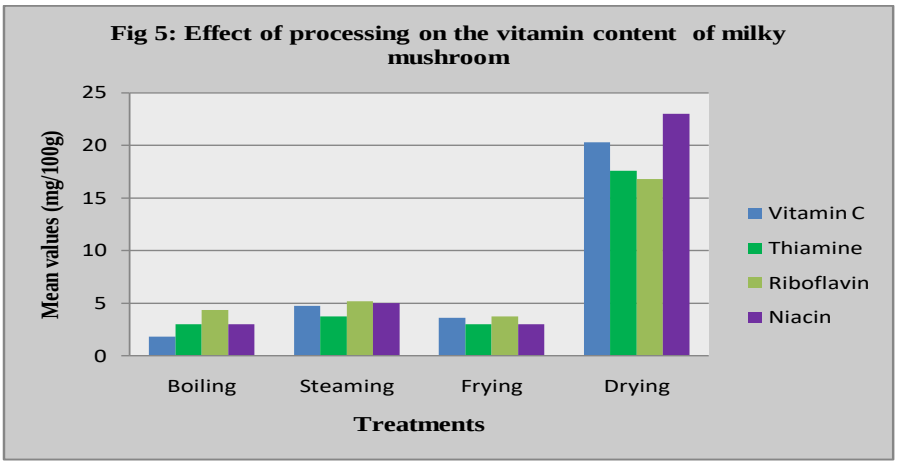


Fig 5: Effect of processing on the vitamin content of milky mushroom
Mean values (mg/100g)
25
20
15
10
5
0
Boiling
Steaming
Frying
Drying
Treatments
Vitamin C
Thiamine
Riboflavin
Niacin

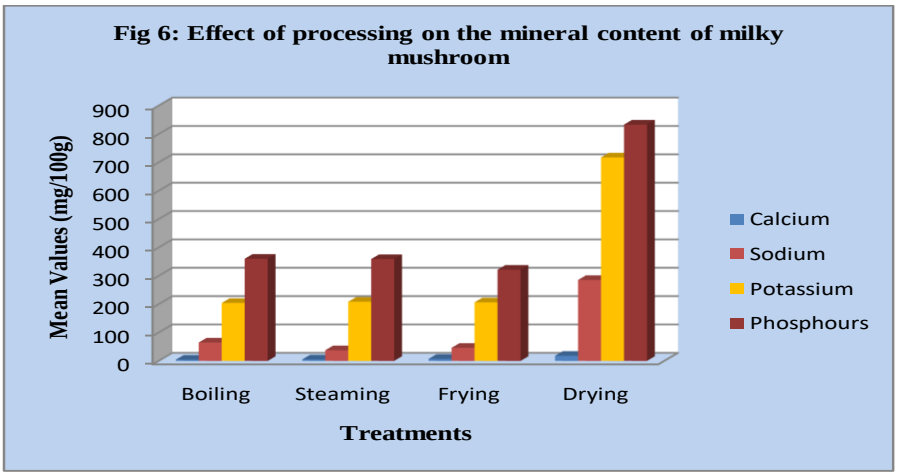


Fig 6: Effect of processing on the mineral content of milky mushroom
Mean Values (mg/100g)
900
800
700
600
500
400
300
200
100
0
Boiling
Steaming
Frying
Drying
Treatments
Calcium
Sodium
Potassium
Phosphours

5.2.4. Effect of processing on other constituents in milky mushroom

Moisture content was higher in T_1 (10.80 per cent) and was on par with T_2 (10.38 per cent) and T_3 (10.25 per cent). Drying reduces the moisture content and T_4 depicted the lowest value 7.15 per cent. Drying reduces the moisture content to the maximum 92.11 per cent. All the other processing treatments enhance the moisture level. The per cent of moisture gain during boiling were 88.09 per cent, steaming 88.55 per cent, frying 88.70 per cent respectively.

Fiber content of dried mushroom was as high in mushroom treated with different processing methods and ranged from 2.3 to 23.50 mg/100g. Alam *et al.* (2008) reported that fiber content of fresh and dried *C. indica* was found to be 3.1 g and 23.6 g respectively. The percentage loss of fiber during processing was observed to be 53.70 per cent in boiling, 41.35 per cent in steaming and 62.76 per cent in frying.

On assessing the changes in the tannin content of milky mushroom during processing, it was observed that an increase during drying (66.66 per cent) and decrease when subjected to different processing methods. It was noted that 44.44 per cent of tannin was lost during frying mushroom. The dried mushroom had exhibited higher phenol content (8.85 mg) when compared with other processed mushrooms. According to Moss (2004), phenolic antioxidants are much more sensitive and amount of phenol increase or decrease during cooking or processing. The author reported that 20 to 30 per cent loss of phenol antioxidant in many vegetables.

On the whole, loss of nutrients in different processing treatments varies significantly; steaming method retains nutrients to the maximum, while nutrient loss was maximum in boiling. By drying, moisture content is less, nutrient concentration will be more. As a preservative method, drying can be used, and incorporation of dried mushroom powder in the daily dietaries is also much easier.

5.3. ASSESSMENT OF HEALTH BENEFITS OF MILKY MUSHROOM

For assessing the health benefits of milky mushroom human volunteers having diabetes, cholesterol and hypertension with more or less clinical background and that not on medication were selected.

According to Bobek (1999), mushrooms are not only source of nutrients but also have been reported as therapeutic foods, useful in preventing diseases such as hypertension, diabetes, hypercholesterolemia and cancer. Kakon *et al*. (2012) pointed out that mushrooms, a nutrient dense versatile food can share some of the benefits of fruits and vegetables and complement almost any everyday meal. Due to culinary, nutritional and health benefits, the mushroom market is expected to grow as "a food, a tonic and a medicine".

5.3.1. Formulation of mushroom supplement and standardization of recipes

Mushroom supplement was formulated in the laboratory without adding any chemicals or preservatives. Dried mushroom powder was packed in five gram sachet for distribution to the respondents. Five gram supplement per day was incorporated in the daily diet of selected subjects. Incorporation of mushroom supplement was accomplished without any difficulty in the subjects. They incorporated the supplement either in the breakfast preparation or along with lunch.

Various breakfast recipes were standardized in the laboratory incorporating mushroom supplement, in order to ensure the prompt inclusion of the supplement by the respondents. According to Gupta (1986), mushrooms is an alternative rich source of meat, fish, vegetables, fruits etc. mushrooms is the source of extra ordinary power and virility and is used in the preparation of many essential dishes.

In case of dosa and chapatti, incorporation of mushroom powder does not render any colour change or off flavor. While as in idly slight cream colour was imparted. Chutney powder prepared was very much acceptable, since the flavor of mushroom powder blended well with the chutney flavor. Mixing mushroom powder along with the rice and milk gave much acceptance among the subjects. Since mixing

the supplement with curd, rasam and other dishes yielded encouraging result. However adding supplement to the black tea was less acceptable to the subjects.

However all the subjects were of the opinion that, the supplement was easy to incorporate in the diet in different form, and through different dishes. Some of the vegetarian subjects expressed some difficulty for consumption of the supplement during case study because of its lingering flavor. Incorporation of mushroom supplement does not hinder the acceptability of the dish.

5.3.2. Case study on hyper glycemic subjects

Two female middle aged subjects selected for the case study on diabetes belonged to middle income family. Subject A was a Government employee residing in the campus while subject B was a house wife. Both of them belong to nuclear family and were non vegetarians.

On account of the body measurements both of them were having normal BMI while on account of the waist hip ratio, two of them were at risk and exhibited gynoid obesity. While checking the food consumption pattern, it was revealed that consumption of meat products found to be less while fish and vegetable consumption was found to be adequate. Both of them follow three meals a day pattern.

Impact of mushroom supplement on the blood sugar levels.

The figure (7) shows the changes in the blood sugar level of hyperglycemic subjects. The result indicated that the fasting blood glucose level of subject A and subject B initially was 135 mg/dl and 165 mg/dl respectively. After 45th day of supplementation the sugar level was reduced to 128 mg/dl in both subjects. After 90 th days of supplementation a steady decline was seen in both subjects. The percentage decline in fasting blood sugar level was 17.03 per cent in subject A and 40.60 per cent in subject B. The result confirmed a remarkable decline in blood sugar level of the subjects after administration of dried *Calocybe indica* at five gram/day. The observation is on track with earlier findings.

According to Perera (2011), mushrooms appear to be effective for both the control of blood glucose and the modification of the course of diabetic complications

without side effects. Tapasya (2012), opined that mushrooms are potent exemplary sources of natural medicines with anti diabetic activity. The high protein, fiber, low fat and energy content of edible mushrooms make them excellent food for diabetics.

A study conducted by Tapasya and Kapoor (2012), reported that the feeding trial with incorporation of milky mushroom powder (instant upma) with other ingredients viz. semolina, whole Bengal gram, soya nuggets and specie mix (cumin seeds, onions, green chilies, coriander leaves) results glucose lowering effects in non insulin dependent diabetic and healthy subjects.

Jeong *et al.* (2010) opined that, mushrooms are recently being recognized to elicit low postprandial responses, due to the presence of high content of polysaccharides and soluble fiber. A study conducted by Jaziya (2011), reported that incorporation of mushroom supplement (*pleurotus florida*) shows significant decrease in the blood glucose and blood cholesterol levels in the human subjects.

5.3.3. Case study on hyper cholesterolemic subjects

Two subjects selected for the case study were middle aged females, of which subject A belongs to Muslim community holding government job, while subject B was from Hindu community. Both of them were from nuclear family and follow small family norm. Both respondents were non vegetarians. On assessing the dietary habits of the subjects it was found that subject A do not follow any dietary restrictions while subject B avoid fatty foods in her daily diet.

Considering the body measurements, both of the subjects fall under normal BMI. On account of the waist hip ratio subject A and B were at risk and depicted gynoid obesity. Morbidity pattern of the two subjects revealed that both of them do not fall sick frequently.

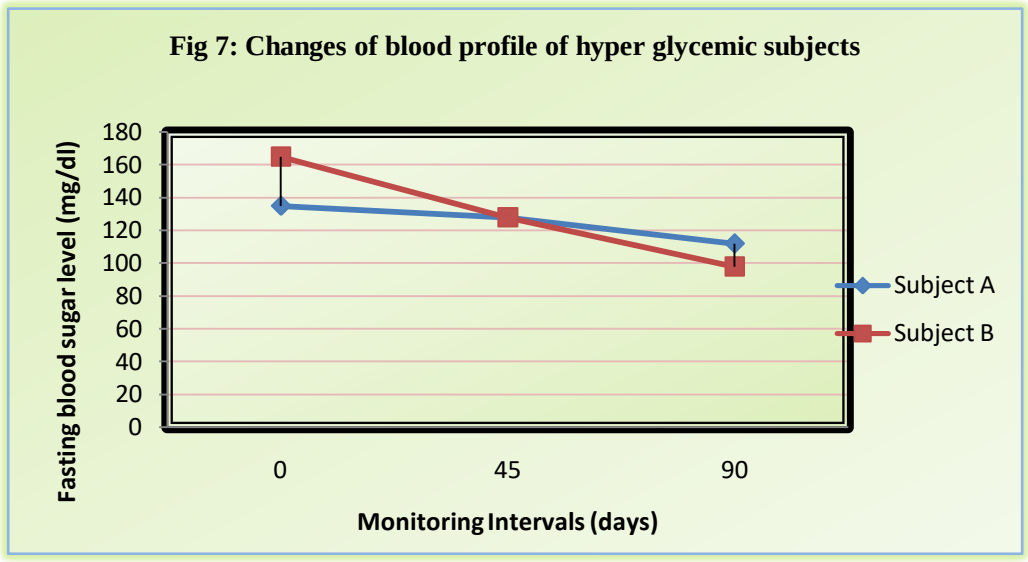


Fig 7: Changes of blood profile of hyper glycemic subjects
Fasting blood sugar level (mg/dl)
180
160
140
120
100
80
60
40
20
0
0
45
90
Monitoring Intervals (days)
Subject A
Subject B

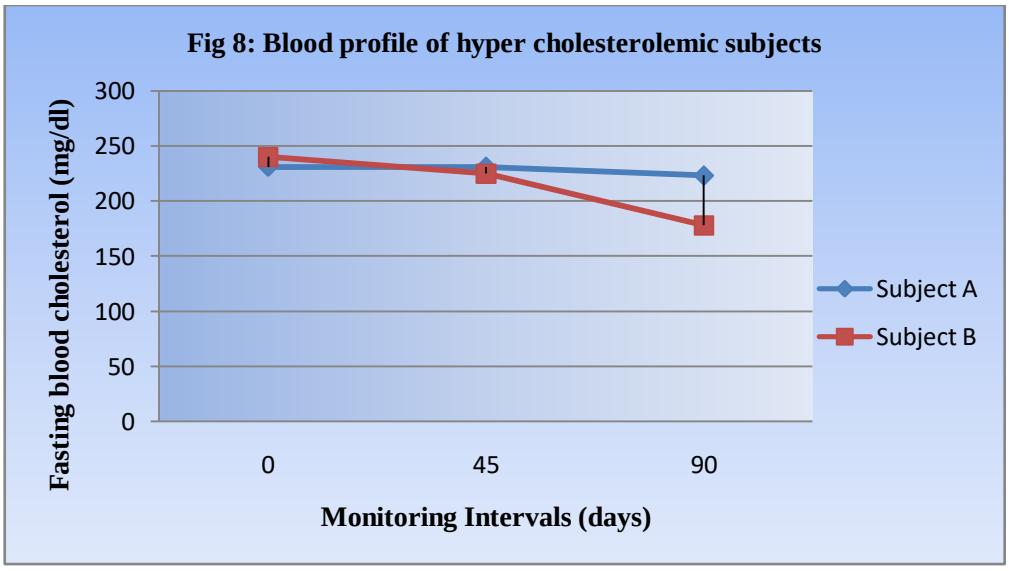


Fig 8: Blood profile of hyper cholesterolemic subjects
Fasting blood cholesterol (mg/dl)
300
250
200
150
100
50
0
0
45
90
Monitoring Intervals (days)
Subject A
Subject B

Impact of mushroom supplement on the cholesterol levels

Two of the subjects were interestingly participated in the feeding experiment, and they incorporated the mushroom supplement through different dishes in the daily diet. Both of them do not express any difficulty while incorporating the mushroom supplement in the daily diet. The result of the feeding trial depicted gradual decrease in the blood cholesterol level in subject A and B after supplementation.

The initial blood cholesterol level of the respondents was 231 mg/dl and 240 mg/dl for subject A and subject B respectively. After 45 th day of supplementation the initial level was reduced to 225 mg/dl in subject B, while there was not much change in the cholesterol level of subject A (231 mg/dl) but the level of triglycerides (TG) has decreased to 170 mg/dl from the initial level of 205 mg/dl. After 90 days of supplementation the cholesterol level was reduced to 223 mg/dl and 178 mg/dl in subject A and B respectively (Fig.8).

The result obtained in the supplementation trial indicated that dried powder of *Calocybe indica* act as an effective functional food for the control of elevated cholesterol level.

Khan *et al*. (2010) pointed out that mushrooms have been regarded as important medicinal food item, which have preventive and protective role against many disorders, including hyper cholesterolemia. Ahmed *et al*. (2004) reported that the lean protein present in mushrooms helps to burn cholesterol in the body, He has also opined that mushrooms are rich in dietary fiber, nutrients and some particular compounds which are known to bring physiological benefit to humans, including lowering of plasma total cholesterol level.

According to Cheung (1996), mushrooms are a valuable source of lovastatin, which suppresses the activity of the main cholesterol synthesis enzyme, hydroxymethylglutaryl CoA reductase (HMG CoA reductase), and thus has a hypocholesterolaemic effect. Chihara (1987), reported that mushrooms have a positive impact on cardiovascular disease through their ability to reduce blood cholesterol levels.

Endo (1988), pointed out that, consumption of mushrooms has the potential to reduce cholesterol uptake from the diet, leading to a fall in the total blood cholesterol concentration and in the low density lipoprotein (LDL) fraction (often known as 'bad' cholesterol), without any change to concentration in the high-density lipoprotein (HDL) fraction (often known a 'good' cholesterol). Result of the present study also supports the fact that, mushroom powder has the ability to reducing cholesterol level.

5.3.4. Case study on hypertension subjects

Two subjects selected for the case study belonged to Hindu community. Subject A was male while subject B was female. Both of them belonged to nuclear family and were non vegetarians. Subject A was a farmer and subject B was holding government job. Both of them were from middle income family. No special diet was followed for hypertensive disease condition.

Subject B have the habit of doing exercise like morning walk regularly for one hour. Subject B stated to have some stress and strain in the daily life related to health and financial problems.

Based on the anthropometric measurements both of the respondents fall under normal BMI. Assessment of waist hip ratio revealed that both of them were at risk and were prone to develop degenerative diseases. Both of them were hypertensive.

Impact of mushroom supplement on the blood pressure levels.

Blood pressure level recorded in the subjects at the time of supplementation was 170/100 mm Hg in subject A and 140/100 mm Hg in subject B. After 45 days of supplementation there was a slight decline in the subject A to 150/90 mm Hg. However in subject B the blood pressure was found to elevate to 150/90 mm Hg. After 90 th day of supplementation remarkable decline is seen in the blood pressure level of both the subjects to 100/80 mm Hg in subject A and 120/80 mmHg in subject B .

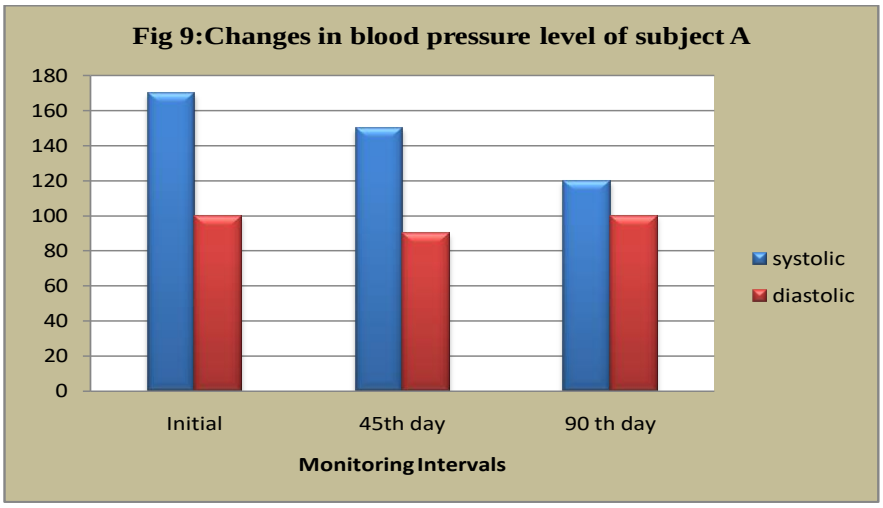


Fig 9:Changes in blood pressure level of subject A
180
160
140
120
100
80
60
40
20
0
Initial
45th day
90 th day
Monitoring Intervals
systolic
diastolic

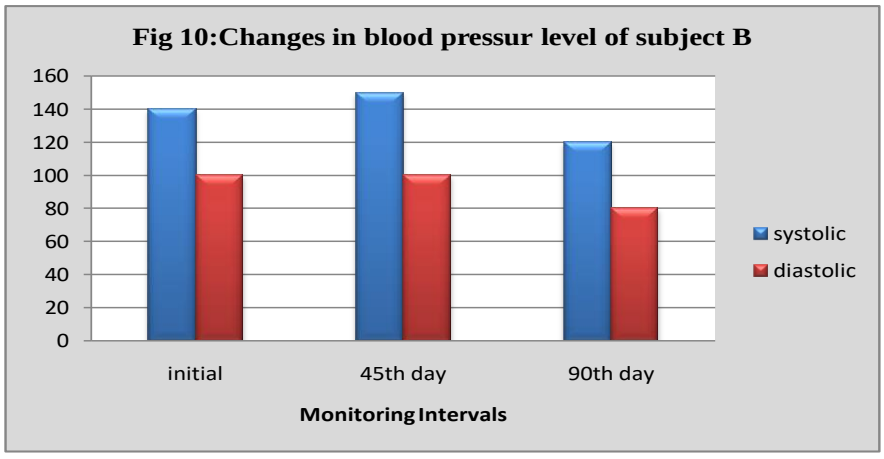


Fig 10:Changes in blood pressur level of subject B
160
140
120
100
80
60
40
20
0
initial
45th day
90th day
Monitoring Intervals
systolic
diastolic

According to Mattila *et al.* (2000) mushrooms are frequently regarded as a therapeutic food having anticarcinogenic, anticholesterolemic and antiviral properties and also prophylactic properties with regard to coronary heart disease and hypertension.

Mowsurni (2010), reported that mushrooms of the species *Pleurotus florida* are outstanding in decreasing the harmful plasma lipids and in reducing blood pressure there by reducing the risk of cardiovascular diseases. Obarzanek *et al.* (2001) opined that mushrooms out rank bananas on the potassium level. Potassium helps the body to process sodium and lower blood pressure. Milky mushroom contain exceptionally high potassium level.

According to Miller (1994), the minimal sodium content makes mushrooms suitable for suffering from high blood pressure.

The result of the supplementation study on the human subjects as case studies with *Calocybe indica* indicate a positive trend in the blood sugar, blood cholesterol and hypertension in the subjects.

Summary

6. SUMMARY

The present study entitled "Evaluation of nutritional quality and health benefits of milky mushroom" (*Calocybe indica* P&C)" was a comprehensive study carried out with an objective to determine the nutritional quality, nutrient loss during processing and also to investigate the health impact of the mushroom supplement on the blood profile of the subjects with specific disease condition.

The popular and most versatile commercially cultivated and consumer preferred milky mushroom (*Calocybe indica*) was selected for the study. Chemical and nutritional composition of the fresh and processed milky mushrooms was assessed with regard to macro nutrients, vitamins, minerals and other chemical constituents.
The energy value of milky mushroom was 46.56 g and the carbohydrate, protein and fat content were 6.70 g, 4.26 g and 0.68 per cent respectively in fresh weight basis. As reported by earlier researchers milky mushrooms are low in calories with low fat and carbohydrate.

Quality of protein in the milky mushroom was determined in detail. Eighteen amino acids including eight essential amino acids were analyzed, which yielded more precise data on the quality aspects of mushroom protein. Milky mushroom contains all the essential amino acids and the content of isoleucin, valine, threonine were exceptionally high when compared to reference protein. Because of the deprotenization of the mushroom sample essential amino acids viz leucine and lysine get denatured. Methionine content was also found to be higher than reference protein. Among the other amino acids present, glycine and cystine content was remarkably high followed by alanine and valine. Amino acid content of *C.indica* when compared to *P. florida, P. sajor caju and P. citrinopelealtus* was observed to be better, and alanine, glycine, cystine were also higher than the other three species.

Determination of amino acid score is an index to evaluate protein quality, indicated that the essential amino acids viz. threonine recorded the highest amino acid score (416.0) followed by isoleucine (239.02) and valine (219.11). Limiting amino acid in sequence *calocybe indica* was phenylalanine and methionine.

On computing the amino acid content and amino acid score (AAS) it was clear that, milky mushroom is superior in protein quality with respect to amino acid composition and essential amino acid content when compared to *Pleurotus florida, P. sajor caju and P. citrinopelealtus*. Essential amino acid (EAA) index of milky mushroom determined and was found to be 188 while the nutritional index based on protein quality was estimated 8.0

Milky mushroom contains B complex vitamins viz. thiamin, riboflavin and niacin as 6.20 mg, 7.50 mg and 10.0 mg/100 respectively, which is fairly very high when compared to other plant foods. However vitamin C content of milky mushroom was found to be comparatively low (12.82 mg/100 g).

In this study it was clear that milky mushrooms are rich source of various minerals. Calcium content was recorded as 10.40 mg/100g. While sodium, potassium and phosphorus were found to be 11.8 mg, 415.6mg, 622.6 mg/100g respectively. Milky mushroom also contains fairly good amount of iron (10.86mg/100g). Trace elements viz. copper and zinc also analyzed and estimated as 1.9 mg and 1.47 mg/100 g respectively.

Milky mushroom contain 90.73 per cent moisture which account for easy perishability. Compared to other mushrooms milky mushroom contain 3.24 g/100 g fiber which is slightly higher than the other varieties. Tannin and poly phenol which are considered as antioxidants were determined as 1.80 mg and 3.70 mg/100 g respectively.

Vitamin C and zinc are considered as IF positives are also present in milky mushroom. Antioxidants such as polyphenols, tannins and fiber were also present in milky mushroom studied which account for their medicinal value.

On assessing the loss of nutrients in milky mushroom when subjected to different processing treatments revealed that all the macro nutrients decreased during processing such as boiling, steaming and frying while drying enhanced the nutrients due to moisture loss. It was noticed that the drying (T4) was superior to all the other processing treatments, with respect to calories (338 Kcal), carbohydrate (40.0g),

protein (19.61g) and fat (2.60 per cent). All the nutrients were found to depict significant variation when subjected to different processing methods.

The present study indicated that the nutrient loss was maximum, while steaming the nutrient loss was minimum so steaming is the best method of cooking. It was also observed that all the nutrients were high in T4 treatment (drying).

The important minerals in food that may be lost during cooking or processing are the salts of calcium and potassium. The mineral content of milky mushroom treated with different processing treatments indicated that the mean value for minerals significantly varies with each treatment. It was noticed that the dried mushroom (T4) depicted higher values with regard to all minerals. As the moisture is lost from the mushroom, nutrient concentration increases and hence dried mushroom recorded higher values for all the nutrients.

Health impact of milky mushroom was ascertained through supplementation study on the selected human volunteers, with three specific disease condition viz. hyperglycemia, hypercholesterolemia and hypertension. Dried mushroom powder formulated without adding any chemicals was distributed to the subjects under case study on the basis of 5g/person/day for a period up to 3 months. Incorporation of mushroom supplement in the dietaries of subjects was accomplished without much difficulty.

Various recipes were standardized in laboratory with incorporation of mushroom supplement, in order to ensure proper inclusion of the in the dietary. Diet counseling was imparted to the subjects along with incorporation of supplement in the diet. The impact of mushroom supplement on the disease condition was assessed by monitoring blood profile of the subjects at periodical intervals.

On account of the result obtained, it was confirmed that milky mushroom is highly superior in nutritional quality. Supplementation of milky mushroom powder brought a positive trend on blood glucose, blood cholesterol, and blood pressure level of the respondents. More data is to be generated in order to validate the results further. Thus

the milky mushrooms are recommended for health and wealth and considered as “precious pearls of cookery”.

Based on the investigation, following recommendations are put forward,

Milky mushrooms are recommended as a functional food, which could bring desirable changes in the blood profile.

1) Steaming is the best method of cooking mushrooms.
2) Drying is recommended as a preservation technique for milky mushroom to obtain maximum nutrients with minimal intake.
3) Milky mushrooms are best for health and wealth.

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Appendices

APPENDIX-I

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE, VELLAYANI

DEPARTMENT OF HOME SCIENCE

Questionnaire to elicit information on the socio economic background of the selected respondents.

1. Name of the respondents :
2. Age :
3. Sex :
4. Religion :
5. Caste :
6. Address :

7. Type of family :
8. Size of family : Nuclear/Joint
9. Educational qualification :
10. Occupation :
11. Monthly income :
12. Monthly expenditure for food :
13. Monthly expenditure for health care :
14. Do you have a family history for :
    a) Diabetes
    b) Hyperlipidemic
    c) Cardiac problem
    d) Hypertension

e) Arthritis

f) Any other

15. Do you have any of the above disease :

16. If yes, how long have been affected :

17. Clinical pictures:

a) Height

b) Weight

c) BMI

d) Waist hip ratio

18. What was your blood profile last recorded for

Hyperglycemia

Hypercholestremia

Hypertension

19. Are you under any medicine : Yes/No

20. Do you consume any medicine : Yes/No

APPENDIX-I Continued

Questionnaire to elicit information on the life style pattern of the selected respondents

1. Do you have any stress and strain in your day to day life : Yes/No

2. If yes, type
   a) Occupational / family problem
   b) Health problem
   c) Financial problem
   d) Old age problem
   e) Any other

3. Do you practice any relaxation technique : Yes/No

   If yes what is it

4. Do you have the habit of doing exercise : Yes/No

   If yes, specify

   Time :

   Duration :

   Type :

APPENDIX-I Continued

Questionnaire to elicit information on the dietary intake of the selected respondents

1. Food habit : Veg /Non veg

2. No. of meals taken/ day

Two times	Three times	Four times

3. Are you following any special diet :Yes/No
   If yes, give details

4. Do you purchase convenience foods Yes/No

   How often Daily/ weekly/ monthly/ occasionally/ rarely

5. Frequency of use of various food items in the diet by the respondents

Food items	Daily	Alternative days	Twice in a week	Once in a week	Occasionally
Cereals					
Pulses					
Vegetables					
Meat					
Fish					
Fruits					
Milk& milk products					
Coffee/Tea					
Beverages					
Bakery items					

Abstract

EVALUATION OF NUTRITIONAL QUALITY AND HEALTH BENEFITS OF MILKY MUSHROOM (*Calocybe indica* P&C)

ANJU. R.P

(2011-16-103)

Abstract of the

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Department of Home Science

COLLEGE OF AGRICULTURE

THIRUVANANTHAPURAM-695 522

ABSTRACT

"Evaluation of nutritional quality and health benefits of milky mushroom" (*Calocybe indica* P&C)" was an in-depth study undertaken to determine the nutritional quality, nutrient loss during processing and also to investigate the health impact of the mushroom supplement on the blood profile of the subjects with specific disease condition.

Analysis of nutrient composition of milky mushroom indicated that, energy value of milky mushroom was 46.56 Kcal/100 g on fresh weight basis and the carbohydrate, protein and fat content were 6.70 g, 4.26 g and 0.68 g respectively on fresh weight basis. It contains all the essential amino acids, and the content of threonine was exceptionally high when compared to the reference protein. Similarly isoleucine, methionine and valine content were also found to be higher than the reference protein. Among the other amino acids present alanine was remarkably high (28.56 g) followed by cystine (18.18 g) and glycine (17.78 g). Milky mushroom was found to be superior in protein quality with respect to amino acid composition and essential amino acid content. Thiamin, riboflavin and niacin content of milky mushroom were found to be 6.20 mg, 7.50 mg and 10.0 mg/100g respectively, where as vitamin C content was 12.82 mg/100 g.

Milky mushrooms are also rich source of various minerals. Calcium content was recorded as 10.40 mg/100 g; sodium, potassium and phosphorus were found to be 11.8 mg, 415.6mg, and 622.6 mg/100g respectively. Milky mushroom contains fairly good amount of iron (10.86mg/100g). Trace elements viz. copper and zinc was estimated to be 1.9 mg and 1.47 mg/100 g respectively in milky mushroom. Moisture content of milky mushroom was 90.73 per cent and contains 3.24 g/100g fibre. Tannin and poly phenol content estimated as 1.80 mg and 3.70 mg/100g respectively.

On assessing the loss of nutrients in milky mushroom when subjected to different processing treatments revealed that all the macro nutrients decreased during processing such as boiling, steaming and frying while in dried sample nutrient concentration is more. Dried mushroom (T4) was superior to all the other processing treatments, with respect to concentration of various nutrients. Boiling and frying method recorded the maximum nutrient loss while steaming, the lowest.

Health impact of milky mushroom was ascertained through supplementation study on human volunteers with specific disease condition viz. hyperglycemia, hypercholesterolemia and hypertension. Dried mushroom powder was incorporated in the diet of the selected subjects as case study for a period of three months. Supplement was incorporated along with breakfast dishes as well as with lunch. The result of the blood profile of those subjects showed a significant decrease in the blood glucose, blood cholesterol and blood pressure level over a period of three months.

On account of the results obtained, it can be concluded that, milky mushroom is highly superior in nutritional quality and has desirable impact on the blood profile of diabetic, hypercholesterolemic and hypertensive subjects. They have appreciable taste and flavor besides provide essential nutrients for health. Hence milky mushroom could be recommended as a functional food, and considered as “precious pearls of cookery”.