ENRICHMENT OF NUTRITIONAL,MEDICINAL VALUE AND DEVELOPMENT OF FERMENTED SOYA PRODUCT BY INCORPORATING EDIBLE MUSHROOM FOR WOMEN'S HEALTH

A PROJECT REPORT

Submitted by

AANANTHI G (1110204001)

ADLIN TRINITA SHALOM P (1110204003)

GAYATHRI M (1110204012)

in partial fulfillment for the award of the degree

of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY,

COIMBATORE 641 049.

(An Autonomous Institution Affiliated to Anna University, Chennai)

APRIL 2015

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE 641 049.

(An Autonomous Institution Affiliated to Anna University, Chennai)

BONAFIDE CERTIFICATE

Certified that this project report **"Enrichment of nutritional, medicinal value and development of fermented soya product by incorporating edible mushroom for women's health"** is the bonafide work of **"AANANTHI G (1110204001), ADLIN TRINITA SHALOM P (1110204003), GAYATHRI M (1110204012)"** who carried out the project work under my supervision.

SIGNATURE

Dr. A. Manickam

HEAD OF THE DEPARTMENT

Department of Biotechnology

Kumaraguru College of Technology

P. O. Box No. 2034

Chinnavedampatti

Coimbatore – 641 049

SIGNATURE

Dr.K.Kumaresan

SUPERVISOR

Assistant Professor (SrG)

Department of Biotechnology

Kumaraguru College of Technology

P. O. Box No. 2034

Chinnavedampatti

Coimbatore – 641 049

_____ _____

Internal Examiner External Examiner

ACKNOWLEDGEMENT

We are grateful to our project guide **Dr.K.Kumaresan**, Assistant Professor (SrG), for his valuable guidance, persistent support, constant motivation, for the successful completion of this project.

We express our sincere gratitude to **Dr. A. Manickam**, Professor and Head, Department of Biotechnology for his motivation and encouragement throughout the project.

We would like to thank the **management** of Kumaraguru College of Technology and **Dr. R. S. Kumar**, Principal for providing necessary facilities for the completion of project.

We thank our review committee members **Dr. T. Sathish Kumar**, Assistant Professor (SrG) and **Mrs. S. Nithya Priya**, Assistant Professor (SrG) for all their valuable suggestions that came at the right time and greatly helped us in completing our project successfully.

Our whole hearted thanks to our Class advisor **Dr. R. Baskar**, Associate Professor and our Project Coordinator **Dr. M. Shamugaprakash**, Assistant Professor (SrG) for their undivided attention and continual encouragement during the tenure of this project work.

We wish to thank all the teaching and non-teaching staff for their unambiguous support throughout our project work. Finally, we thank our parents and friends for their immense support for the completion of project.

AANANTHI G ADLIN TRINITA SHALOM P GAYATHRI M

ABSTRACT

Soybean has proved to be a good source of protein in recent years. It is easily available and can be used to prevent malnutrition in under developed countries where malnutrition is a major problem. Soy based products like soymilk and fermented soymilk are now a days used as a substitute for cow's milk. Consumption of soy protein has proved to decrease the risk for breast cancer in women in adulthood stage.

Button mushroom has many medicinal properties and been used as a food since ancient days. Mushroom contains essential amino acids needed by our body. Water soluble polysaccharides from button mushroom have antitumour, antioxidant properties.

Our work deals with preparing extract from white button mushroom and blending it with soymilk and fermented soymilk in order to increase the medicinal value of both soymilk and fermented soymilk. Fermentation was done using Lactic Acid Bacteria. Soymilk and mushroom extract were blended in different ratios and their properties were analysed. Blend of soymilk and mushroom extract was stored at 4ºC to study the stability of the product during storage.

Key words: White button mushroom, LAB, Mushroom extract, Soya milk.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND NOMENCLATURE

Abbreviation/Symbol	Description
%	Percentage
G	Gram
mg	Milli gram
µg	Micro gram
OD	Optical density
nm	Nano meter
ml	Milli liter
W/V	Weight/Volume
V/V	Volume/Volume
µl	Micro litre
TVC	Total Viable Count
TSS	Total Soluble Solids
TS	Total Solids
EPS	Exopolysaccharides
MHC	Major histocompatability complex
APC	Antigen Presenting Cell
LAB	Lactic Acid Bacteria
DNS	Di-Nitro Salicylic Acid \

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Mushroom belongs to fungus family, a fleshy and spore bearing fruiting body having a shaft, cap, and lamellae. The parts of a mushroom are covered with reproductive cells called Basidia from which spores are produced.

According to the nature of spore forming, it may be an adjunct of Basidiomycetes(spores are produced externally from basidia) and Ascomycetes (spores are produced internally from ascus). Mushrooms can be either hypogeous or epigeous. They are naturally grown in fields, forests, manure heaps, water channels , hilly areas and woods, mostly during and just after rains.

Mushroom-producing fungi plays a major role in ecosystem. It acts as a decomposer by recycling organic matter and few of them exists as a parasite on trees. In general *Agaricus, Pleurotus,* and *Volvoriella* species of mushroom are considered edible all over the world (Rai, M *et al*., 2005).

Universally, mushroom have been used as a delicious and nutritional food. It contains a clump of nutrients, good quality of protein, vitamins, minerals and also other natural phytochemicals. Due to the presence of high amount of nutrients, it offers wide range of health benefits, nutritional values and also it has been used as a worthwhile source for medicines. Medicinal value of mushroom includes nutraceutical, antioxidative, wound healing, immunity

enhancement and tumour-retarding effects (Yaoqi Zhang *et al*.. 2004).

Several species of mushroom are cultivated for commercial purpose. The reason for it may be an enhanced medicinal and nutritive values and the presence of compounds such as carbohydrates, proteins, Amino acids, Phenol, flavonoids, alkaloids, sterols, tannins etc. Such species includes *A.bisporus, Pleurotusplatypus, P. florida , Calocybeindica,* white, crimini and Portobello (Sujatha, S *et al*., 2013)

Medicinal value, therapeutic properties and efficacy against disease increases the global interest towards mushroom from scientific community. *L.edodes* is the most leading mushroom in the global market. *L.edodes* is used for suppressing cancer, allergies due to environmental changes, cold, fungal infection, bronchial inflammation, diabetes , hepatitis. Many such health benefits are reported (Johnsy, G and Kaviyarasan, V, 2014)

Mushroom is a good source of Vitamin D. Mushroom contains ergosterol which can be easily converted to Vitamin D (ergocalciferol) by exposing it under UV light for few seconds either during processing or growing. Synthesis of Vitamin D depends on the dosage and length of exposure under UV(Haytowitz, D. B *et al*).

The antitumour and immunomodulating properties of mushroom are due to the presence of polysaccharides. These properties may be enhanced by increasing the presence of polysaccharides, particularly beta glucan. Main chain of glucan contain β (1→3) linkage and β (1→6) linkage in the branching points which are needed to make the antitumor action. Polysaccharides in the mushroom do not directly attack the cancer

cells, the effect is enhanced by inducing different immune response in the body (Wasser, S *et al*., 2002).

Chitin and Chitosan in mushroom posses strong antimicrobial activity. Chitosan showed strong antimicrobial activity against Gram-negative and Gram-positive bacteria, including *Escherichia coli, Salmonella tyohimurium, Staphylococcus aureus* and *Listeria monoytogens.* By adding chitosan, microbial cell death occurs due to the interaction of positively charged molecules and negatively charged microbial surface molecules which lead to the disruption of cell membrane and thereby resulting in the leakage of intracellular constituents. Finally microbial cell death occurs which is one of the hypothesis of Chitosan polymer (Hafdani, F. N and Sadeghinia, N, 2011).

In vivo and *in vitro* experiments have been performed to obtain a substance for anticancer, anticholestrol, antidiabetic treatments. From basidiomycetes, bioactive substance may be obtained, which has been used to produce dietary supplements. Two new dietary supplements have been produced and patented. Those supplements were found to contain cholesterol lowering, antidiabetic and immunostimulating properties (Rai, M *et al*., 2005).

1.2 *Agaricus bisporus*

Agaricus bisporus is one of the edible mushroom, belongs to the family of Agaricaceae, is a fleshy body having cap, gills and stipe with high content of polyphenols, ergothioneine, selenium and polysaccharides. It is commonly known as white button mushroom. It is commonly grown in fields and grassy areas. It is one of the commercially grown mushrooms. It has potential medicinal and nutritive value. Mushrooom contains many biological active

compounds which posses anti fungal, anti microbial, anti bacterial and anti viral properties. In general, *Agaricus* is considered as an edible mushroom all over the world. Due to the presence of proteins, Vitamin C, Vitamin D, Vitamin B6, Thiamine, Riboflavin, Niacin, Pantothenic acid, Folate, Iron and low amount of sodium, *Agaricus* may be used as a food, medicine and drug. Many *in vitro* and *in vivo* studies have been carried to prove the anti-cancer (especially breast and prostate cancer), immunomodulatory function, anti-oxidant activity, anti- microbial, anti-aromatase activity, anti-diabetic property and its capacity to lower lipoproteins.

Table 1.1 Chemical composition of *Agaricus* on cap and stipe

	CAP	STIPE
Moisture (%)	90.76	90.01
Carbohydrate (%)	20.59	31.41
Protein(%)	33.65	19.01
Fat (%)	2.48	2.00
Fibre (%)	33.11	38.08
Ash (%)	10.17	9.5
Chitin (%)	6.68	7.25

In cap and stipe, linoleic acid is unsaturated fatty acid and palmittic acid is saturated fatty acid . Calcium, iron and linoleic content in high in Stipe than cap (Naisri, F *et al*., 2013).

1.2.1 HEALTH BENEFITS

Agaricus inhibit prostate tumour growth by inducing apoptosis and suppressing proliferative acti vity. It inhibits the tumour growth by inducing apoptosis and signalling pathways such as protein kinase B, extra regulated kinase, nuclear factor kappa, protein-1 activation and

cell cycle modulation . This activity can be enhanced by the presence of polysaccharides and especially linolenic acid in mushroom (Adams, L. S *et al*., 2008).

Methanol extract of *Agaricus* posses antimicrobial activity against pathogenic bacteria by the presence of chitin and Chitosan. Microbial cell death occurs due the leakage of intracellular constituents by the interaction of positively charged molecules and negatively charged microbial surface and made the disruption of cell membrane. Phenolic compounds present in methanol extract contain few acids such as rutin, gallic acid, catechin that posses antioxidant activity. In addition, ascorbic acid and phenolic compounds attribute to antioxidant activity (Abah, S.E and Abah, G, 2010).

Agaricus posses anti-aromatase activity which will suppress the hormone responsive cancer. Estrogen plays a major role in breast cancer. Estrogen is believed to bind with estrogen receptor and induce the peptide growth factor expression which causes the proliferation of cells. Abnormal expression of aromatase is the responsible for the production of estrogen. Heat stable extract of *Agaricus* contain biological active compound, conjugated linolenic acid that inhibit the aromatase activity by binding at the active site region which will suppress the action of estrogen. *In vivo* experiments were carried out to confirm the inhibitory effects of aromatase by using mushroom and suppress the hormone responsive cancer cell proliferation (Chen, S *et al*., 2006).

Presence of compounds such as fibers, antioxidants, folates, vitamin C, vitamin D,vitamin B and polyphenols in *Agaricus* provides beneficial effects against cardiovascular diseases. Experiments were carried out in rats to confirm the reducing level of total cholesterol in

plasma, triglyceride concentration and low density lipoprotein (Jeong, S. C *et al*., 2010).

White button mushroom helps in enhancing the maturation of dendritic cell and Antigen Presenting Cell(APC). Dendritic cells and Antigen Presenting Cells play a major role in immune response. Consumption of white button mushroom increases the innate and adaptive response by elevating the maturation markers CD40,CD 80, CD 86, Major histocompatability complex(MHC), interleukin 12, Natural killer cells, Tumour necrosis factor. (Ren, Z *et al*., 2008).

1.3 SOYA BEAN

Soya bean is a legume, belongs to the family of leguminous, subfamily papiliondase and the genus glycine max which does not have cholesterol and low in saturated fatty acid and also has the capacity to fix nitrogen from the atmosphere. It has been one of the main plant foods in the world. It contain protein and all eight essential amino acids. Soya bean is also a good source of fibres, iron, calcium, zinc, vitamins, thiamine, riboflavin, niacin, folacin, isoflavones and phytoestrogen (Venter, C S. 1999).

Soya bean is widely used to alleviate malnutrition due to inexpensive and its nutritional source such as protein, vitamin and fibres etc. Presence of protein in soya bean has the ability to decrease the cholesterol level and also reduce the risk of osteoporosis. On daily consumption of soya bean, makes significant increase in concentration of hematocrit, haemoglobin, total plasma protein and plasma albumin.

Table 1.2 Composition of Soya bean (Alada, A. R. A *et al*., 2004)

Carbohydrate	32%
Fat	20%
Minerals	5%
Vitamins	5%
Fiber	3%

Soya bean is cultivated for its nutritious seeds, also used to enhance the soil fertility and its more superior to many other pulses such as grain, lentil, mash, mong, pigeon pea , cow pea. It has been considered as a universal food. Due to its nutritional value and health benefits, It's is used in the food industry and used for the manufacturing products like flour, oil, margarine, biscuits, candy, milk, meat, cheese, lecithin etc and also some products like soap, varnish, paints, lubricants, plastics are manufactured by using its bye products(Naz, R. 2012).

Due to the health benefits of soybean and also most important compound of protein, many fermented and unfermented soy products has been produced like soy milk, soy meal, soy sauce, soy cheese. For example soy meal is one of the fermented soy products . It contain 48% protein, 3% fiber and remaining dry matter. For Vegetarian, it is used as meat maker instead of chicken taste. It is made from defatted soil flour that has been cooked under pressure and then dried.

1.4 SOYA MILK

Globally, the basic concern of soya milk production is based on the deficiency of micronutrient like deficiency of vitamin A, vitamin C, iron, iodine and zinc and another main cause is malnutrition problem , malnutrition is also a lack of protein-energy and micronutrient . According to the surveillance, Approximately 125-130 million of

school children and pregnant lady belongs to the category of vitamin A deficiency in low income country, zinc is another deficiency factor in developing countries. According to World Health Organisation report , In worldwide 5 billion people are be an adjunct of iron deficient (Madukwe, E and Eme, P. E, 2012).

From the above concern , soya milk is produced from soya bean . Soya bean is one of the fermented soy products . It's just aqueous extract of soya bean resembling cow's milk. Milk is the easy way to consume by all people from child to old age people. Soya milk contain 3.20g of protein and also it's a good source for consuming more amount of iron, magnesium and potassium (Naz, R. 2012).

Soya milk has low cholesterol and saturated fatty acids with good biological value and It's a replacement of cow's milk for those who are all having allergic to the cow's milk due to its smell, especially people for lactose intolerance and also people who avoids animal products (Iancu, C *et al*., 2010).

1.5 HEALTH BENEFITS

Soya bean is the good source for consuming vitamin D especially for pregnant lady and also control obesity , blood sugars. Consumption of soy protein has the ability to decrease the concentration of cholesterol, low density lipoprotein and triglycerides . This is due to the binding of phytoestrogen(Isoflavones are the phytoestrogen producing hormone like effects , present in soy products) with estrogen receptor and produce the similar effects which is mentioned above. Soya bean prevent cancer development , due to the presence of genistein in soya bean by block the creation of blood vessel which would provide growth for tumour . Phytoestrogen in soy acts as a synthetic estrogen in women

which would prevent bone loss , calcium imbalance and also helpful to maintain healthy heart (Kristein S *et al*., 2003)**.**

1.6 FERMENTATION

Fermentation is a metabolic(break down) process, influenced by microorganism that converts sugars to acids, gases and alcohol. For example Anaerobic conversion of sugar to carbondioxide and alcohol by yeast.

Lactic acid bacteria(LAB) is majorly used for fermentation due to availability of curd and other products for isolation. LAB is widely spread in nature. In this group are included representatives of the genus *lactobacillus, lactococcus, pedococcus,* and *leuconostoc.* LAB is , a Gram-positive, non spore forming, rod shaped , fastidious organism and Generally Recognized as Safe (GRAS). LAB has been used for thousands of years for fermentation because this has the ability to change taste, texture and flavour and also the presence of antimicrobial molecules like lactic acid, acetic acid, Carbondioxide, hydrogen peroxide and bacteriocin are extensively known to avert food borne pathogen, spoilage and thus enhance the safety and shelf life of products (Patil, M. M, *et al*., 2010).

Fermentation in soya products is the recently developed process. Fermented soya milk increase the absorption , digestion, flatulence reduction, destroy undesirable pathogens (cause health problems) , reduce beany flavor, provide new textures and improve product flavor. Fermentation convert minerals like zinc, calcium, potassium, iron and copper into more soluble form and also increase the vitamin level (Iancu, C *et al*., 2010).

In this study we have planned to incorporate mushroom extract into soya milk. It is an dietary supplement for lactose intolerance

people and has been used as a substitute for cow's milk for the purpose of reducing malnutrition problem. Addition of mushroom extract into soya milk enhance the nutritional value and it adds properties like cholesterol lowering, antidiabetic, immunostimulating properties with the already existing properties of soya milk like bone mineral density, preventing menopausal symptom, breast and prostate cancer.

1.7 OBJECTIVES

The major objectives of this work are:

- To compare the efficiency of different techniques for mushroom extract.
- To produce fermented and unfermented soya milk.
- To incorporate extract from white button mushroom into soya milk and formulation of value added product.

CHAPTER-2

REVIEW OF LITERATURE

2.1 Health benefits of soymilk

Ryan-Borchers *et al*.,(2014) determined the effect of soy isoflavones on the immune function of postmenopausal women. Women in the age group 58 to 65 years were chosen for the trial. They were divided into 3 groups. Group one was given cow's milk supplemented with placebo, group two was given soymilk supplemented with placebo. Group three was given cow's milk supplemented with isoflavones. The experiment was conducted for a period of sixteen weeks. Subjects were analysed for their cytokine production, oxidative damage and lymphocyte cells population. At the end of sixteen weeks it was found that soymilk and isoflavones has modulated B cell population in subjects of group two and three. They also had reduced DNA damage.

Xaio *et al*.,(2014) did a review on the effects of soy and isoflavones on human's health. Soy proteins lowered mean cholesterol levels when administered to hypercholesterolemic men. Intake of soy protein in women increased serum level of a bone formation marker called bone specific alkaline phosphatase. Case studies have revealed that consumption of soy in adolescence can lower the risk for breast cancer in adulthood. Soy protein was found to decrease the marker for cancer development and progression in prostrate cells.

Zhang *et al*.,(2014) performed clinical trial in chinese women to find the association between coronary heart disease and soy intake. Women in the age group of 40 to 65 years were chosen for the experiment. Soy protein was given to the subjects on a daily basis for

2.5 years. Results showed an inverse association between soy intake and risk for coronary heart disease in women.

Fournier *et al*.,(2007) did clinical trial to determine the effects of soymilk and isoflavones supplement on cognitive performance in healthy postmenopausal women. Women in the age group 48 to 65 were chosen for the trial. They were divided into three experimental groups. Group one was given cow's milk and a placebo supplement. Group two was given soymilk and placebo supplement. Group three was given cow's milk supplemented with isoflavones. Every group was given their respective dosages for sixteen weeks. Their cognitive abilities were assessed through various tests before and after the trial. It was found that soy isoflavones did not improve or affect the cognitive abilities of women when they were given to them for a period of sixteen weeks.

Somekawa *et al*.,(2001) did clinical trials to determine the effects of soy intake on bone mineral density in postmenopausal women. 478 postmenopausal Japnese women were chosen for the trial. They were divided into two groups according to the years since menopause and each group was subdivided into four groups according to the amount of soy intake. At the end of the trial it was found that soy intake can increase bone mass in postmenopausal women.

2.2 Nutritive value of mushroom

Wani *et al*.,(2010) performed a review on the nutritional and medicinal importance of mushrooms. In the ancient days mushroom were used as food mainly for their tastiness. Mushrooms contain 90% water and 10% dry matter. Protein and carbohydrate constitute major part of the dry matter. Mushrooms contain essential amino acids needed by our body. Mushrooms contain water soluble polysaccharide and they have antitumour property. Unsaturated fatty acids constitute the major

amount of fat present in mushroom. They are a good source of vitamins that are needed by our body. Fruiting body of mushroom is rich in minerals. The amount of minerals present depends upon the species, size and the type of substratum used for cultivation. Mushrooms have been used to treat diseases like tuberculosis in ancient days. Lentinan is the antitumour polysaccharide present in mushroom. Mushrooms are rich in antioxidants which can protect our body from free radicals.

2.3 Value added products from soymilk

Sakhale *et al*.,(2012) fortified soymilk with mango pulp in order to increase the nutritional value of and palatability of the product. JSS-335 variety of soybean was used by them for the preparation of soymilk. Soybeans were autoclaved after soaking to suppress the beany flavour. Pulp was extracted from the mango fruit of *Kesar* variety and the partially clarified juice was used for the preparation of value added soymilk. Soymilk and mango pulp were blended in four different proportions. Cane sugar was added as a flavouring agent.TSS, moisture content, fat, protein, sugars, pH, acidity and the ash content of the four different combinations of soymilk and mango pulp and plain soymilk and mango pulp were analysed. Ascorbic acid content of soymilk increased as a result of fortification. Ascorbic acid content was higher where as protein content was lower in sample which had soymilk and mango pulp in equal proportions when compared to other combinations. Blend with equal proportion got the highest score in overall acceptability during sensory evaluation.

Naz *et al*.,(2012) produced fruit flavoured soymilk in order to mask the beany flavour of the soyamilk.Swat-84 variety of soybean was used for the preparation of fruit flavoured soymilk. Soymilk was produced as per Escuta and Benzon *et al*., 5% cane sugar is added to it

and then the soymilk was split into four different samples. One sample was maintained as a control and the other three samples were flavoured with fruits like mango, guava and banana respectively. All the samples were pasteurized and then used for analysis. pH, total soluble solids, titratable acidity, crude fat and protein were analysed for all the samples as per the standard method of A.O.A.C. The above mentioned parameters were analysed at an one week interval for 28 days for all the samples. pH of all the samples decreased on whereas total soluble solids increased in all the samples during storage. There was no significant reduction in the protein content of the samples. Guava flavoured soymilk recorded the highest reduction in fat content during storage. Organoleptic evaluation of the samples were carried out at one week interval for four weeks. Score for colour and flavour decreased during storage due to coagulation. Fruits should be added in optimal amount so as to increase the shelf life of flavoured soymilk.

Madukwe and Eme (2012) fortified soymilk with carrot powder to increase the vitamin and mineral content of soybean. Carrot is an excellent source of β-carotene which is the precursor for vitamin A synthesis. Fresh carrots were bought from the market and sundried for three days and ground with a blender to produce powder. Soymilk is prepared as per Enwere *et al*., Soymilk was separated into three samples. One sample was maintained as control and carrot powder was added to the remaining two samples. Parameters like moisture, fat, iron, zinc, ash, protein and crude fibre content was determined for all the samples according to the standard methods of A.O.A.C. Beta carotene and vitamin C content of soymilk was determined by Pearson's method. Plain soymilk had the highest moisture content when compared to other two samples. Beta carotene, iron, zinc and vitamin C content of the

soymilk increased as a result of fortification with carrot powder. Fat, protein, crude fibre, ash and carbohydrate content of the soymilk also increased as a result of fortification Sensory evaluation was carried out for all the three samples. Plain soymilk gained overall acceptability over fortified soymilk even though it's more nutritive value is lower than the fortified soymilk.

Menash-Brown *et al*.,(2014) produced chocolate flavoured soy-peanut beverage using three component constrained extreme lattice mixture design. Jenguna variety of soybean and peanut of chinese variety were used for the preparation of the beverage. Soybeans and peanut were soaked in water containing sodium bicarbonate for different durations and washed with cold water to remove the residual sodium bicarbonate. Soybean, peanut and cocoa powder were blended in ten different proportions. Parameters like protein, fat, ash, moisture, pH, titratable acidity, total solids and viscosity were analysed for all the ten different combinations. Total solids content and viscosity increased as the amount of cocoa powder increased in the sample. This is because the amount of total soluble solids in vegetable milk is lower when compared to cocoa powder. pH ranged from slightly acidic to basic. pH increased as the amount of soybean and peanut increased. This is due to the protein content of soybean and peanut. Two combinations were chosen from the optimum region and proximate analysis were done. Parameters like protein, fat, moisture, ash , carbohydrate content, pH, titratable acidity, viscosity and TSS were analysed for the above mentioned samples. Protein content was lower than soy but higher than peanut. pH is almost neutral for the two optimum products. The microbial quality of the product met the required standards. Hence it is possible to produce chocolate flavoured soy-peanut beverage.

Bisla *et al*.,(2012)produced ice creams using milk from soybean and watermelon seeds to enhance the nutritional value of ice cream. Milk from these seeds were produced as per Chakrabarthi *et al*., and Gangopadhyay *et al* ., respectively. The prepared milk was then analysed for their protein, fat, moisture, iron and vitamin C content using the standard methods of A.O.A.C Different ice creams were produced using plain soymilk, soymilk blended with watermelon seeds milk, soymilk blended with guava pulp, soymilk and watermelon seeds milk blended with guava pulp and standard milk. Proximate analysis was done for all the three different types of milk: soymilk, watermelon seeds milk and cow's milk. Protein content of soymilk was higher than cow's milk and that of cow's milk was higher than watermelon seeds milk. Fat content of watermelon seeds milk was higher when compared to soymilk and cow's milk. This is due to the presence of fatty acids in the seeds of watermelon. Ash content of cow's milk was lower in comparison with soymilk and watermelon seeds milk. Sensory evaluation was done for all the six different combinations of ice creams and the ice cream prepared from cow's milk recorded the highest score among the six different varieties. Among the blends, ice cream prepared from soymilk blended with watermelon seeds milk and guava pulp got the highest score. Proximate analysis was done for the two most accepted ice creams and their nutritional values were compared. Among the two, ice cream prepared from blend had higher amount of protein, fat, vitamin C, ash, iron content than the standard ice cream prepared using cow's milk.

Adejuyitan *et al* .,(2014) produced wara by blending soymilk and coconut milk in four different proportions. Alum was used for curdling the milk. Soymilk and coconut milk were produced and

blended in different proportions. Proximate analysis and sensory evaluation were done for all the four samples. Proximate analysis showed that wara prepared using soymilk had the highest amount of protein, fat, ash, and fibre content and lowest amount of moisture when compared to other blends. The wara made from equal proportion of soymilk and coconut milk got the best score for overall acceptability during sensory evaluation.

Ikya *et al*.,(2013) studied the effect of cooking temperature on the nutritional value of soymilk. Soymilk was produced as per Fellows *et al*., Before cooking the slurry was divided into four parts and cooked at different temperatures. Proximate analysis and sensory evaluation was done for all the four samples. As the temperature was increased moisture and fat content in the sample decreased whereas protein, fibre, carbohydrate, total solids and ash content increased since the samples became concentrated due to increase in temperature. Total viable count (TVC) was done for all the samples. It was found that TVC decreased with increase in temperature. In sensory evaluation soymilk cooked at 100ºC got the highest overall acceptability scores when compared to other samples. Even though cooking at 110ºC yielded soymilk with high nutritional value soymilk cooked at 100ºC was well accepted by consumers.

Kolapo *et al* .,(2008) fortified soymilk with corn milk and evaluated it's properties. Soybean of *Glycine max* variety was used for the production of value added product. Soymilk was produced as per Lee *et al*., Soy-corn milk was produced by using three parts of soybean and one part of corn. Both soymilk and soy-corn milk were analysed to determine the amount of total solids, protein, moisture, fat, ash and fibre. Soy-corn milk had the highest amount of protein, fat, carbohydrate

and total solids when compared to plain soymilk. Total viable count in the samples were then analysed by pour plate technique. Viable count was done for both bacteria and fungus in the samples. Colony count was determined for both bacteria and fungus and they are purified by streaking. The purified cultures were then identified as their colony morphology. Total viable counts for bacteria and fungus were higher in soymilk than soy-corn milk. Sensory evaluation was done for both the samples. Soy-corn milk got higher score for overall acceptability than plain soymilk. Thus the nutritive value of soymilk was increased by fortification with corn and it was accepted by customers when compared to soymilk.

Mandal *et al*.,(2013) fortified soymilk with extracts prepared from *Moringaolifera* leaves, spinach leaves and *Colocasia* fruit part. Methanol was used as a solvent for the preparation of the extract. The extracts are blended to produce mixed plant extract. The mixed plant extract was then blended with soymilk. Nutritional and nutraceutical composition like protein, carbohydrate, total phenolic compounds and vitamin C were analysed for all the samples. Antioxidant activity was checked for all the samples. Soymilk had high amounts of phenols than plant extracts. Blend of soymilk and plant extract had higher amounts of protein, vitamin C, carbohydrates and antioxidant activity.

2.4 Mushroom extract

Mo *et al* .,(2013) optimized the extraction of polysaccharide from *Tricholoma giganteum*. Hot water extraction was used for the extraction of polysaccharide and it was precipitated using 95% ethanol. Temperature, time, solvent-solid ratio and volume of ethanol were the variables involved in the optimization studies. Maximum yield was

obtained at a temperature of 100ºC for 3 hours with a solid-solvent ratio of 1:20(W/V) and five times the volume of ethanol.

Arora *et al* .,(2003) studied the drying kinetics of mushroom at different temperatures. The mushroom samples were blanched in boiling water for one minute. After this the mushrooms were treated with a solution containing 0.1% citric acid and 0.25% potassium meta bisulphite and then the samples were dried in a tray drier. Drying kinetics were studied using Page's model.

Tseng *et al* ., (2007)determined the antioxidant properties of cold water extracts of *Ganoderma tsugae*. The mycelia was grown in a fermenter. Then it was separated by centrifugation and then freeze dried to obtain powder. The powder was then used for extraction. Cold water extraction was carried out at 25ºC for 24 hours at 100rpm in a shaker. The residue was extracted twice by the same procedure and the fractions were pooled. The extract was freeze dried and the powder was used for analysis. The antioxidant activity of the extract was determined by conjugated diene method as per Lingnert *et al* ., Hydroxyl radical scavenging ability, chelating ability on ferrous ion were also determined for the extract. Results showed that cold water extract possessed good antioxidant activity and radical scavenging activity. These results suggest that *Ganodermatsugae* can protect the human body against oxidative damage from free radicals.

Jhonsy.G and Kaviarasa.V (2014) prepared extracts of *L. sajorcaju*. Two different solvents were used for the extraction and their efficiency were compared. The extracts were then checked for the presence of protein, carbohydrate, anthraquinones, phenolics, flavonoids, saponins, alkaloids, glycosides, sterols, triterpenes and tannins. Phenols and flavonoids were present in higher concentration in

both the extracts. Tannins and glycosides were absent in both the extracts. Antioxidant activity was checked using DDPH radical scavenging activity. Methanolic extract possessed higher radical scavenging activity and higher chelating ability for ferrous ion than water extract. Reducing power of the extracts increased with increase in concentration. Antimicrobial activity of the extracts were analysed using agar well diffusion method. It was found that the extract possessed broad spectrum antimicrobial activity.

2.5 Fermented soymilk and its properties

Iancu *et al*.,(2010) produced a symbiotic product based on soymilk. Soymilk used for the study was purchased from Dr.Otekercompany, Romania. Inulin was used as a prebiotic component and was added in different concentrations. Four different strains of lactic acid bacteria was used as an inoculum. Soymilk was inoculated with lactic acid bacteria and incubated at 37ºc for 24hours. Acidity was measured for every five hours for all the samples. After 24hours, the samples were stored at two different temperatures in order to check the stability of the fermented product. Total viable count was determined by using plating method. Rheological properties of the samples were determined using a rheometer. Sensory evaluation was done for all the samples by a panel of ten judges. Obtained sensory profile was almost closer to the desired sensory profile.

Chengcheng *et al*.,(2014) determined the physiochemical, microbiological and rheological properties of fermented soymilk. Soymilk was produced as per Wang *et al*., with some modifications. Two strains of lactic acid bacteria was used as an inoculum. Sterilized soymilk was inoculated with culture and was maintained at 37ºc for

12hours. Total viable cell count was determined before inoculating the culture and after fermentation by surface plating method. pH, titratable acidity, water holding capacity, viscosity and viscosity shear rate was determined for the fermented product. Soymilk fermented with two different strains of lactic acid bacteria was stored at different temperature and properties like pH, titratable acidity and apparent viscosity was determined during storage. Properties were checked at two days interval for three weeks. Results showed that *Lactobacillusplantarum* showed good growth in soymilk and also maintained high viability and increased the flavour of soymilk.

CHAPTER 3

MATERIALS AND METHODS

3.1 PREPARATION OF MUSHROOM SAMPLE

3.1.1 Materials

Agaricus bisporous

Distilled water.

0.1% Citric acid.

0.25% Potassium metabisulphite.

Water bath.

3.1.2 procedure

Fresh button mushrooms(*Agaricus bisporous)* were washed with water to remove dirt. The stipes were removed and the fruiting body was cut into pieces using a knife. The sliced mushroom pieces were then blanched in boiling water for one minute. The blanched mushroom pieces were then treated with solution containing 0.1% citric acid and 0.25% potassium meta bisulphite for 15 minutes. After this mushroom pieces were crushed into a paste using mortor and pestle. Crushed mushrooms were used for the preparation of mushroom extract.(Arora *et al*., 2003)

3.2 HOT WATER EXTRACTION OF MUSHROOM PASTE

3.2.1 Materials

4g of mushroom paste.

distilled water

Water bath set at 90 ˚C .

Whattman Filter paper.

3.2.2 Procedure

4grams of crushed mushroom paste was suspended in 200ml of distilled water. Extraction was carried out for 2 hours at 100 ˚C . After extraction the solution is filtered through whattman No.1 filter paper and the filtrate was used for further analysis.(Mo *et al.*,2013)

3.3 COLD WATER EXTRACTION

3.3.1 Materials

5g of mushroom paste.

Cold water.

Shaker.

Centrifuge.

Whatman filter paper.

3.3.2 Procedure

Mushroom paste was suspended in pre cooled autoclaved distilled water in the ratio 1:10(w/v) and placed in shaker at 100 rpm maintained at 25 ˚C for 24 hours. It is then centrifuged at 5000g for 15 minutes. The supernatant is filtered through whattman No.1 filter paper and the filtrate was used for further analysis.(Tseng *et al*.,2007)

3.4 SOXHLET EXTRACTION

3.4.1 Materials

20g of mushroom paste.

Distilled water.

soxhlet extractor.

Whatman Filter paper.

3.4.2 Procedure

20 grams of mushroom paste was used for the extraction . 2000 ml of distilled water as the solvent. Extraction was carried out for 4 hours at 90 ˚C .(Johnsy *et al*.,2014)

3.5 PREPARATION OF SOYMILK

Soybeans were procured from local market and they were washed in tap water. The soybeans were then soaked in solution containing baking soda for 8hours. The water was drained off. Then the soybeans were blanched for 25 minutes in steam. The blanched soybeans were grounded in a blender with the addition of small quantity of warm water to form a paste. The paste was diluted with water to 10 to 12 total solids. After dilution it was filtered through a muslin cloth. The filtrate is the soymilk and is heated for 10 minutes at 88ºC(Naz *et al.*,2012)

3.6 QUALITATIVE ANALYSIS TECHNIQUES

3.6.1 Iodine test

Materials

Iodine.

Ethanol.

Iodine reagent

Add 1gram of iodine to 100 ml ethanol solution and stir well.

Procedure

A few drops of iodine solution was added to about 1 ml of the sample solution.

3.6.2 Molisch's Test

Materials

α naphthol

Ehanol

conc.sulphuric acid.

Molisch's reagent

Dissolve 10 g of α naphthol in 100 ml of ethanol.

Procedure

- To 2 ml of sample 2 drops of molisch's reagent was added.

- conc. sulphuric acid was added along the sides of the test tubes.

3.7 QUANTITATIVE ESTIMATION PROCEDURES

3.7.1 Materials

Di-Nitro salicylic acid reagent(DNS)

Dissolve by stirring 1g Di-Nitro salicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 ml 1% NaOH. Store at 4°C. Since the reagent deteriorates due to sodium sulphite, if long storage is required, sodium sulphite may be added at the time of use.

40% Rochelle salt solution(Potassium Sodium Tartarate)

Phenol 5%

Redistilled (reagent grade) phenol (50g) dissolved in water and diluted to 1 L.

Sulphuric acid 96%(reagent grade).

Standard Glucose: stock: 100mg in 100 ml of water.

Working standard: 10 ml of stock diluted to 100 ml with distilled water.

3.7.2 Estimation of Reducing sugars by Di-Nitro Salicylic Acid method

For sugar estimation an alternative to Nelson-somogyi method is the Di-nitro Salicylic acid method- A simple sensitive and adoptable during handling of a large number of samples at a time.

Procedure

- 0.5 to 3 ml of the samples in the test tubes was pipette out and equalize the volume to 3ml with water in all the tubes
- 3 ml of DNS reagent was added to all the tubes.
- Contents in the tubes were heated in a boiling water bath for 5 mins.
- When the contents of the tubes were still warm ,1 ml of 40% Rochelle salt solution was added.

- The tubes were cooled and the intensity of the dark red colour was read at 510 nm.
- Series of standards using glucose (0 to 500µg) was ran and a standard graph was plotted.

Calculation

Amount of reducing sugars present in the mushroom sample was estimated by comparing it with the standard graph.

3.7.3 Estimation of Total carbohydrates by phenol Sulphuric acid method

In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This forms a green coloured product with phenol and has an absorption maximum at 490nm.

Procedure

- 0.2 to 1 ml of the working standards were pipette out into a series of test tubes.
- Sample solution of 0.1 to 0.2 ml was taken in two separate test tubes. The volume in each tube was made upto 1 ml with distilled water.
- 1 ml of phenol solution and 5 ml of 96% sulphuric acid was added to each tube and shook well.
- After 10 minutes the tubes were placed in a water bath at 30°C for 20 minutes.
- The colour was read at 490 nm and the amount of total carbohydrates present was calculated using the standard graph.

CALCULATION

Absorbance corresponds to 0.1 ml of the test= x mg of glucose.

100 ml of the sample solution contain=(x/0.1) x 100mg of glucose.

=y % of total carbohydrate present.

3.7.4 Estimation of protein by Folin- lowry method

Materials

2% sodium carbonate in 0.1 N sodium hydroxide.(Reagent A)

0.5% copper sulphate ($CuSO4.5H_2O$) in 1 % potassium sodium tartarate.(Reageent B)

Alkaline copper solution

Mix 50 ml of A and 1 ml of prior to use.(Reagent C)

Folin ciocalteauRreagent.(Reagent D)

Reflux gently for 10 hours a mixture consisting of 100 g sodium tungstate , 25 g sodium molybdate , 700 ml water , 50 ml of 85 % phosphoric acid and 100 ml of concentrated hydrochloric acid in a 1.5 L flask. Add 150 g lithium sulphate , 50 ml water and a few drops of bromine water. Boil the mixture for 15 minutes without condenser to remove the excess bromine. Cool , dilute to 1L and filter. The reagent should have no greenish tint.

Protein solution(Stock standard)

Weigh accurately 50 mg of bovine serum albumin and dissolve in distilled water and make up to 5o ml in a standard flask.

Working standard: Dilute 10 ml of stock solution to 50 ml with distilled water in a standard flask. 1 ml of the solution contains 200µg protein.

Principle

The blue colour developed by the reduction of the phospho molybdic-phosphotungstic components in the FCR by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Procedure

- Pipette out 0.2 to 1ml of the working standard into a series of test tubes.
- Pipette out 0.1 ml of the sample in series of tubes.
- Make up the volume to 1ml in all the tubes. A tube with 1 ml water serves as blank.
- Add 5 ml of the reagent C to each tube including the blank. Mix well and allow to stand for 10 minutes.
- Then add 0.5 ml of reagent D, mix well and incubate at room temperature in the dark for 30 minutes. Blue colour is developed. Take the readings at 660 nm.
- Draw a standard graph and calculate the amount of protein in the sample.

3.8 Estimation of ascorbic acid

Materials

0.005 mol /L iodine solution.

0.5% starch indicator solution.

Distilled water.

Preparation of iodine solution

Dissolve by stirring 2g potassium iodide,1.3 g iodine in distilled water. Shake well and transfer the solution to a volumetric flask .Make up the flask using distilled water.

Preparation of starch indicator solution

Add 0.25 g of soluble starch into a 50 ml distilled water containing conical flask. Solution heated with stirring at 79°C for 5 min and cool to room temperature.

Procedure

- 20 ml sample is transferred to a 250 ml conical flask.Add 150 ml distilled water and 1 ml of starch indicator.
- Sample is titrated with 0.005 mol/L iodine solution.
- Dark blue - black colour developed due to starch- iodine complex.

Calculation

$V_1 \times N_1 = V_2 \times N_2$

V_1=0.92

N_1=0.005

V_2=Volume of iodine solution.

N_2=Concentration of ascorbic acid.

3.9 Titratable acidity

Materials

0.1 N NaOH.

1% Phenophthalein.

Preparation of 0.1 N NaOH

Mix 0.8 g NaOH in 100 ml distilled water.

Procedure

- 10 ml sample is transferred to a 250 ml conical flask. Add 150 ml distilled water and few drops of Phenophthalein indicator.
- Sample is titrated with 0.1 N NaOH until reaching the pale pink colour.
- The total acidity was expressed at citric acid.

Calculation

% LA=(ml x 0.009) / (weight of sample).

3.10 Total solids(TS) Known amount of sample was taken in a pre-weighed watch glass and kept in hot air oven at 60°C for 6 hours. The weight of dried sample was determined.

Calculation

Total solids =(100 x W_2) / W_1.

W_1=Weight of 1ml sample.

W_2=Weight of dried product.

3.11 Total soluble solids(TSS)

Total Soluble Solids is measured using hand refractometer.

3.12 Determination of pH

pH meter was calibrated using a standard buffer then the pH of the samples were determined by immersing the glass bulb into the samples.

3.13 Isolation of Lactic Acid Bacteria

Lactic Acid Bacteria was isolated from curd sample by serial dilution and spread plate method. The diluted samples were plated on MRS agar and incubated at 37°C for 3 days. The bacterial colony was transferred to MRS broth and grown at 37°C for 24 hours.(Patil *et al*.,2010)

3.14 Preparation of fermented soymilk

48hours grown Lactobacillus culture was used as an inoculum. 4%(V/V) of the culture was inoculated into sterilised soymilk and was placed in shaker at 37ºc for 24 hours. After 24hours the soymilk was taken from the shaker and used for further analysis.(Li *et al*.,2013)

3.15 Viable cell count

Sample was plated on MRS agar and incubated at 37°C for two days and colonies were counted. (Li *et al*.,2013)

CFU= (no of colonies x dilution factor) / (Volume of culture plate).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preparation of mushroom sample

Fresh button mushroom was procured from the market and blanched in boiling water and then treated with solution containing citric acid and potassium meta bisulphite. It was then grounded in a mortar and pestle to form paste.

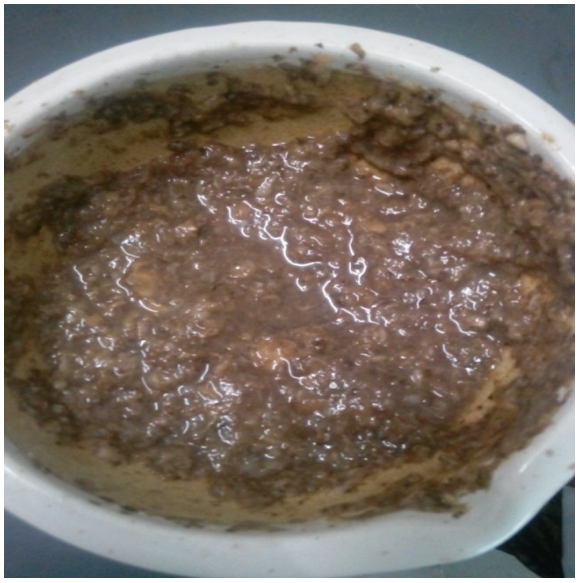

Figure 4.1 Mushroom paste

4.2 Preparation of mushroom extract

Mushroom extract was prepared by three different extraction techniques –Hot water extraction, cold water extraction, extraction using soxhlet apparatus. All the three extracts were analysed for their protein, total sugar, total soluble solids, Vitamin C content and titratable acidity. Emphasis was given to polysaccharides in the extract since the water soluble polysaccharides from mushroom has medicinal properties.

4.3 Qualitative tests

4.3.1 Molisch'stest

Ring was formed at the junction of two layers indicting the presence of carbohydrates.(Fig.2)

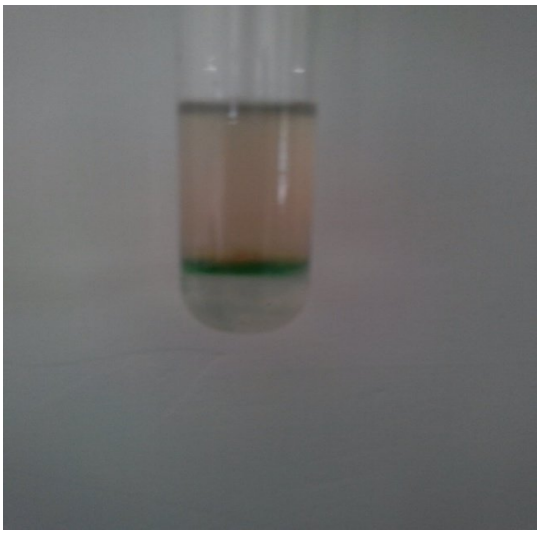

Figure 4.2 Molisch's test

4.3.2 Iodine Test

The solution did not turn into dark blue upon the addition of iodine(Fig.3).This was because the polysaccharide present in mushroom is not in spiral shaped to react with the iodine solution.

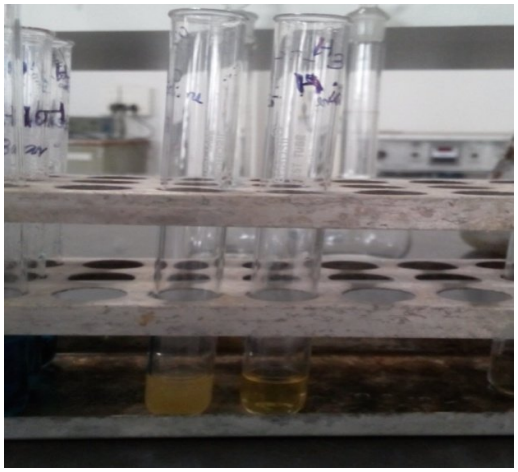

Figure 4.3 Iodine test

4.3.3 Biuret test

The sample turned violet colour upon the addition of biuret solution indicating the presence of protein.(Fig 4.4)

Figure 4.4 Biuret Test

4.4 Quantitative estimation

4.4.1 Folin-Lowry method

Folin-Lowry method was used to determine the amount of protein in all the three extracts.(Fig.4.9)The amount of protein is determined using a standard graph(Fig.4.5). Extract prepared using soxhlet apparatus had the highest amount of protein.

4.4.2 Phenol-Sulphuric acid method

This method was used to determine the amount of total sugar in the extracts.(Fig.4.8) The amount of unknown sugar in the sample is determined using a standard graph(Fig.4.6) Extract prepared using soxhlet apparatus had the highest amount of total sugar.

4.4.3 DNSA method

Reducing sugar in the sample was determined by DNSA method..The amount of reducing sugar is determined using a standard graph.(Fig.4.7)

4.4.4 Estimation of Ascorbic acid by titration method

Ascorbic content in the three different extracts were estimated and compared.(Fig.6)Extract prepared using soxhlet apparatus had the highest amount of vitamin C.

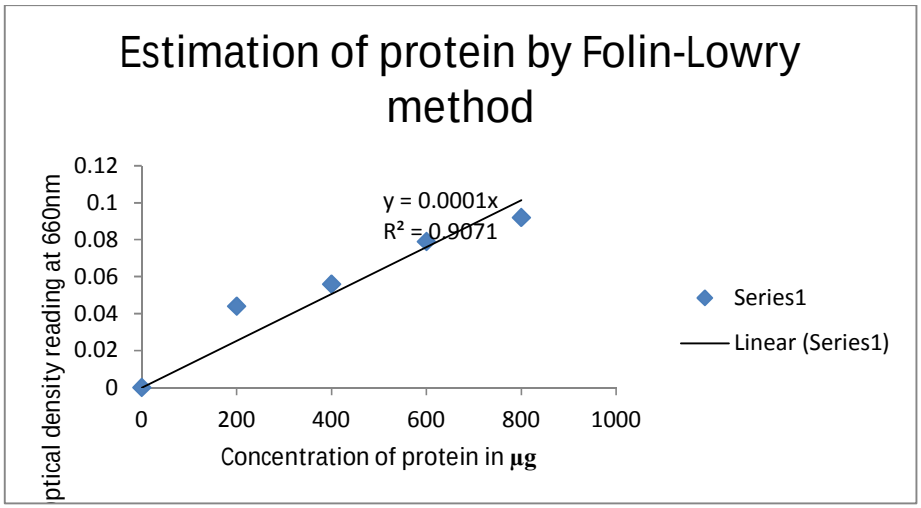

Fig 4.5 Standard curve for determination of protein in sample

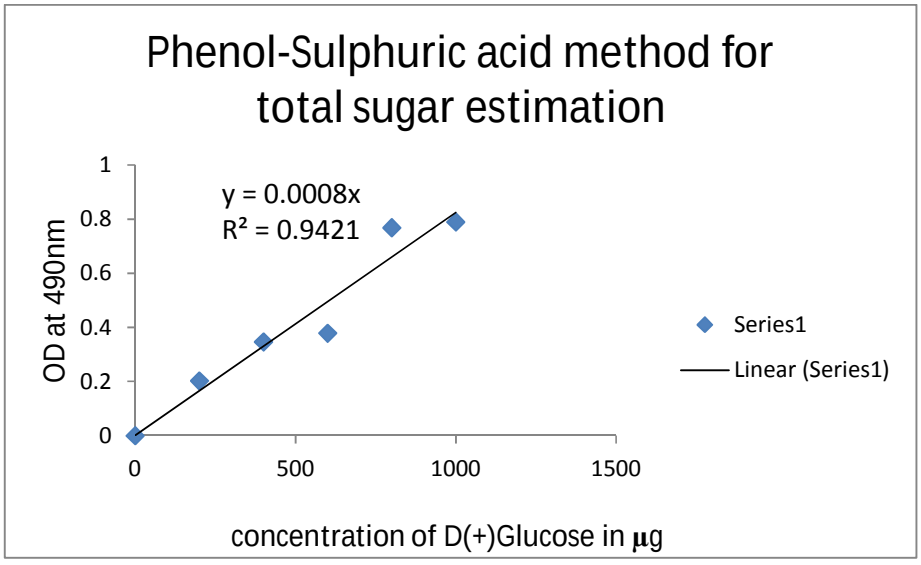

Fig.4.6 Standard curve for determination of total sugar in sample

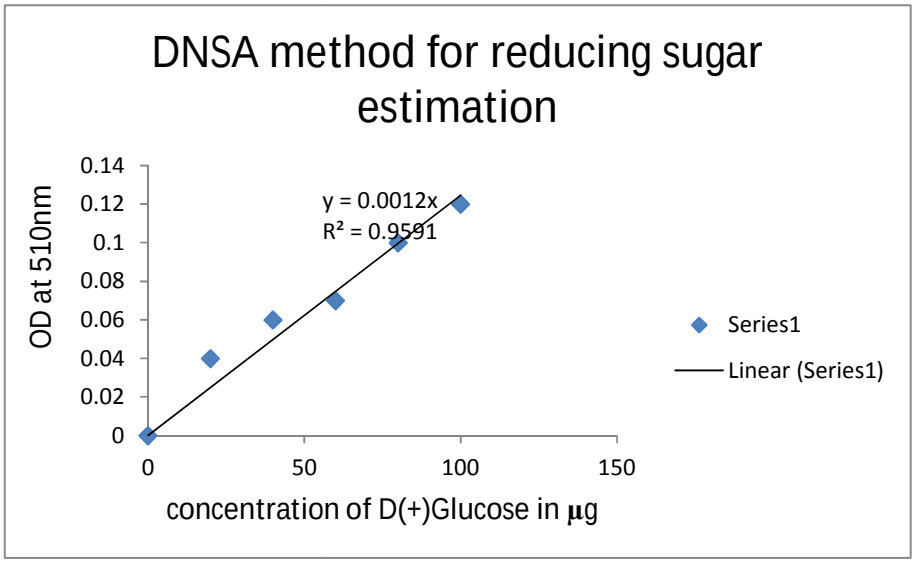

Fig.4.7 Standard curve for determination of reducing sugar in the sample.

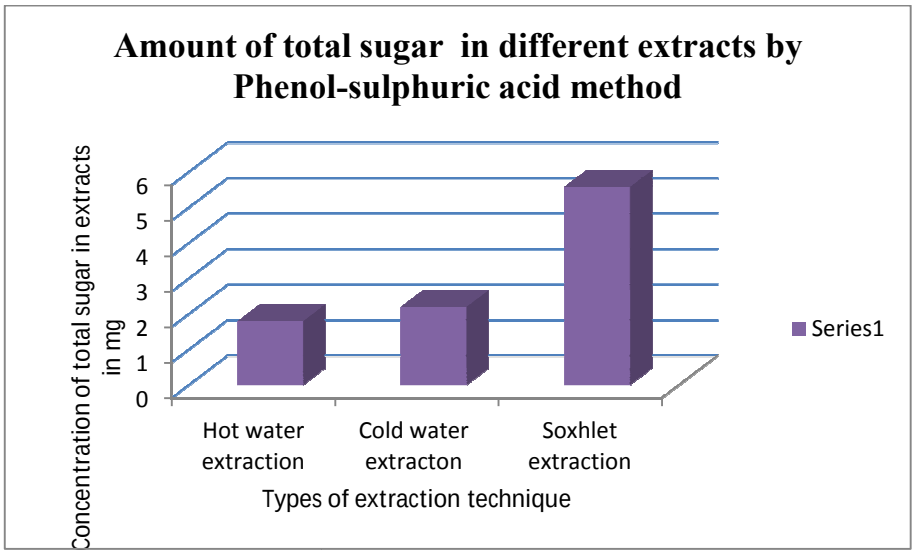

Fig4.8 Graph showing the amount of protein in different extracts

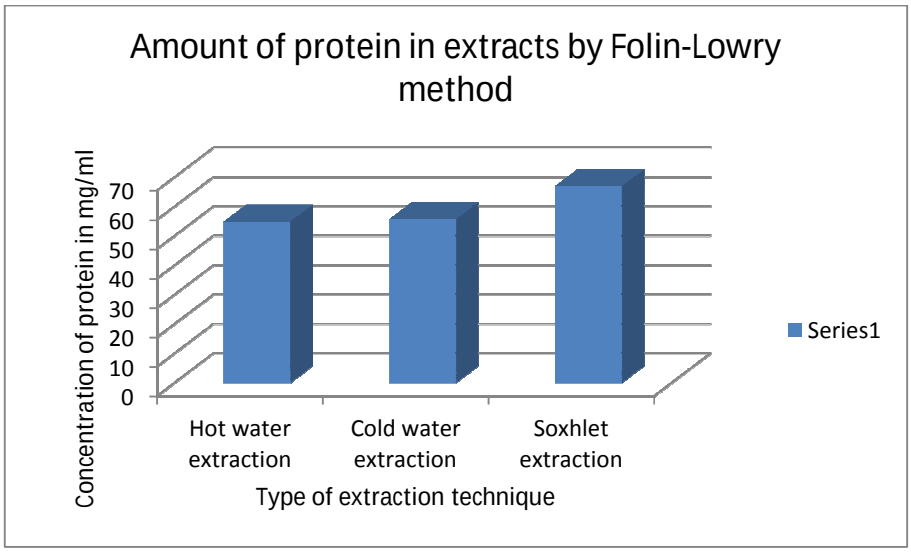

Fig.4.9 Graph showing the amount of total sugar in different extracts

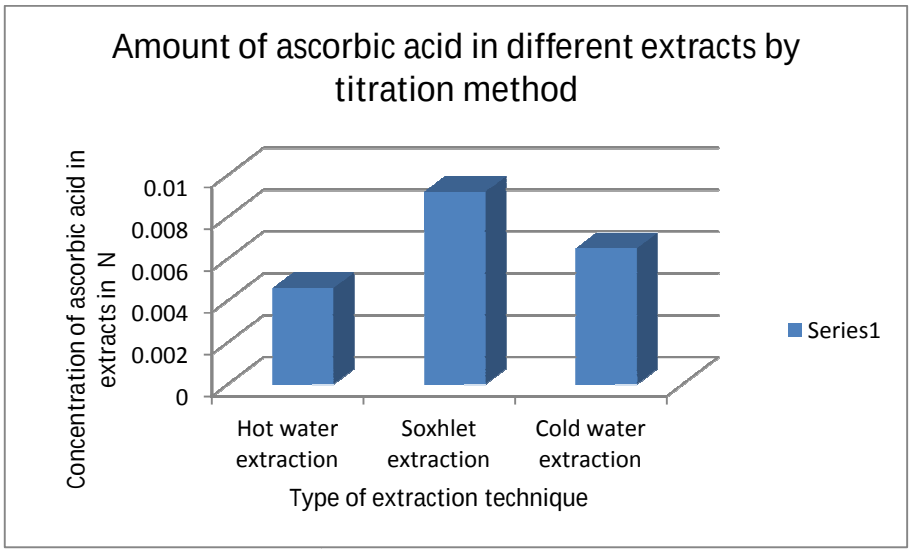

Fig.4.10 Graph showing the amount of ascorbic acid in different extracts.

4.4.5 Titratable acidity and TSS:

Soxhlet had the highest amount of TSS as shown in the table below.

Table 4.1 Amount of TSS and acidity values for the extracts

	Hot water extraction	Cold water extraction	Soxhlet extraction
Titratable acidity	0.036	0.027	0.018
TSS	0.1	0.1	0.2

4.5 Inference

Based on the results obtained, Soxhlet was found to be the better extraction technique Since it yielded the highest amount of protein, total sugar, TSS and Vitamin C. Hence extract from soxhlet was chosen for blending with soymilk and fermented soymilk.

4.6 Fortification of soymilk with mushroom extract

Soymilk and mushroom were blended in four different ratios as shown below in table 2. Plain soymilk is used as a control.

Table 4.2 Ratios for blending soymilk with mushroom

	Soymilk in ml	Mushroom extract in ml
T_1	80	20
T_2	70	30
T_3	60	40
T_4	50	50

4.7 Proximate analysis

Proximate analysis was done for all the blended samples. Protein, total sugar, reducing sugar, pH, titratable acidity, total soluble solids, total solids and ascorbic acid content were determined. The samples were refrigeratared for one week and the properties were checked again to determine the stability of the blends during storage.

Table 4.3: Proximate analysis of blends for zeroth week

	Protein (mg/ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Tritratable acidity (%)	pH	TSS	Total solids	Vitamin C (N)
T_1	385	280.95	3.505	0.279	4.48	2.9	8.21	0.002
T_2	307	225.4	2.505	0.265	4.35	2.7	7.63	0.00219
T_3	199	132	1.292	0.234	5.5	25	5.4	0.00236
T_4	116	105.4	0.462	0.194	6.3	2.2	3.95	0.0026
Soymilk	723	135	4.79	0.108	7.2	3.2	10.14	0.0017

4.8 Effect of addition of mushroom extract to soymilk

Blend containing highest amount of soymilk had the highest amount of protein content and lowest amount of soymilk had the lowest amount of protein. This is because soymilk is rich in protein hence the amount of protein has increased with increase in volume of soymilk.

Even though water soluble polysaccharides from mushroom has medicinal properties its concentration is lower when compared to the sugars present in soymilk. Hence the amount of total sugar and reducing sugar decreased with increase in volume of mushroom extract.

Soymilk had the highest amount of TSS. Hence addition of mushroom extract to soymilk decreased the amount of total soluble solids in the blends. Total soluble solids decreased as the volume of mushroom extract increased.

Mushroom extract had more amount of vitamin c than soymilk. Hence blend with more volume of mushroom extract had the highest amount of vitamin c.

Proximate analysis was done for all the samples at one week interval for two weeks and the results are shown in table 4.4 and 4.5 below

Table 4.4: Proximate analyses after one week interval

	Protein (mg/ml)	Total sugar (mg/ml)	Vitamin C (N)	pH	Titratable acidity(%)	Total soluble solids	Total solids
T_1	332	285	0.00124	4.76	0.01386	3	11.134
T_2	226	283	0.00185	4.71	0.0132	3	8.9005
T_3	175	281.4	0.00225	5.90	0.006	3	7.789
T_4	115	250.2	0.0025	7	0.00198	3	2.005

Table 4.5 Proximate analysis after 2 weeks

	Protein (mg/ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	pH	Titratable acidity	Total solids	TSS	Vitamin C (N)
T_1	181	544.6	5.44	4.8	0.01368	6.58	4	0.00071
T_2	151.5	507.4	5.22	5.21	0.0028	6.09	4	0.00131
T_3	141	326.1	3.23	9.04	0.0043	5.92	3.8	0.0020
T_4	111.45	304.2	3.12	8.55	0.0002	5.84	3.6	0.0023

4.9 Effect of storage on the blends

Protein content in the blends has decreased during storage. This was in agreeable with previous work reported by Naz *et al.*,(2012) who found a milder decrease in protein during storage.

Reducing sugar and total sugar in the blends has increased during storage. This is indicated by the increase in total soluble solids during storage. This was in agreeable with previous work reported by Naz *et al.*,2012.

There was a milder decrease in Vitamin C content in the blends during storage.

pH has increased gradually during storage. This was in contrast to previous work reported by Naz *et al.*, who fortified soymilk with fruit pulp. pH may have increased due to the addition of mushroom extract since fruits have more amount of acids in comparison with mushroom.

4.10 Fermented soymilk

Soymilk was fermented with lactic acid bacteria in order to decrease the antinutritional factors. After fermentation viable cell count was determined by spread plate method and was found to be 10^{11} cfu

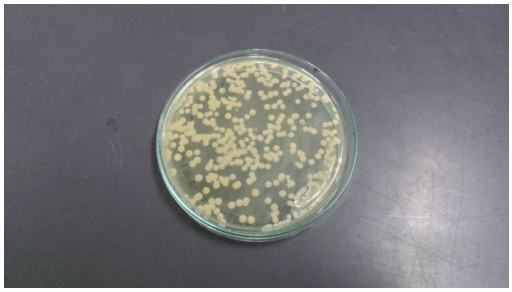

Fig no 4.11 Viable cell count

Fermented soymilk was then blended with mushroom extract in two different ratios as shown below in table.

Table 4.6 Mixing ratio for fermented soymilk and mushroom extract.

	Volume of Fermented soymilk in ml	Volume of Mushroom extract in ml
T_5	35	15
T_6	25	25

4.11 Proximate analyses for blended samples with soymilk

Proximate analyses was done for fermented soymilk and the blends.Protein, total sugar, reducing sugar, pH, titratable acidity, total soluble solids, total solids and ascorbic acid content were determined.

Table 4.7 Proximate analyses for blended samples with soymilk:

	Protein (mg/ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	pH	Titratable acidity (%)	TSS	Total solids	Vitamin C (N)
T_5	258.7	287.55	9.54	4.86	0.0096	3.1	7.921	0.00664
T_6	97	142.7	5.82	4.96	0.00468	2.3	5.35	0.0092
Fermented Soymilk	438	549.8	12.9	3.30	0.00765	4.8	9.595	0.00543

Fermented soymilk had highest amount of total soluble solids when compared to unfermented soymilk. Vitamin C content has increased as a result of fermentation. pH of the soymilk has dropped as a result of lactic acid production during fermentation. The amount of TSS

decreased as volume of mushroom extract increased. This is because mushroom extract had very low amount of total soluble solids as shown in table 4.1.

Fortification of fermented soymilk with mushroom extract increased the vitamin c content of soymilk. pH of blended samples increased due to the addition of mushroom extract.

CHAPTER 5
CONCLUSION

In this study three different extraction techniques were compared for the efficient production of mushroom extract . Soxhlet Extraction gave the best yield. Hence Mushroom extract prepared from soxhlet was blended with soymilk in different ratios. Blended samples were stored at 4°C for two weeks and Proximate analysis was done. It was found that protein and vitamin C content has decreased during storage. Soymilk was fermented with Lactic Acid Bacteria and then proximate analysis was done. Stability of the above mentioned product during storage have to be analysed. Sensory evaluation have to be done for both the products for consumer acceptance.

REFERENCES

Tseng, Y. H., & Mau, J. L. (2007), ' Antioxidant properties of cold water extracts from Ganodermatsugae', Fungal Sci, Vol. 22, No.1,2, pp. 15-25.

Mensah-Brown, H., Afoakwa, E. O., &Wadie, W. B. (2014), 'Optimization of the Production of a Chocolate-flavoured, Soy-peanut beverage with acceptable Chemical and Physicochemical Properties using a Three-component Constrained Extreme Lattice Mixture Design', Food Science and Quality Management, Vol. 23, pp. 46-54.

Bisla, G., Archana, P. V., & Sharma, S. (2012), ' Development of ice creams from Soybean milk & Watermelon seeds milk and Evaluation of their acceptability and Nourishing potential', Res. J. Dairy. Sci, Vol. 3,No. 1,pp. 371-376.

Ryan-Borchers, T. A., Park, J. S., Chew, B. P., McGuire, M. K., Fournier, L. R., &Beerman, K. A. (2006), 'Soy isoflavones modulate immune function in healthy postmenopausal women', The American journal of clinical nutrition,Vol. 83, No. 5, pp. 1118-1125.

Adams, L. S., Chen, S., Phung, S., Wu, X., & Ki, L. (2008), ' White button mushroom (Agaricus bisporus) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice', Nutritionandcancer, Vol. 60, No. 6, pp. 744-756.

Hafdani, F. N., &Sadeghinia, N. (2011), 'A review on application of chitosan as a natural antimicrobial', World Academy of SciEngg Technology, Vol. 50, No. 2,pp. 252-256.

Johnsy, G., &Kaviyarasan, V. (2014), 'Preliminary phytochemical screening,Antimicrobial and Anyioxidant activity of methanol and

waterextracts ofLentinussajor-Caju',World Journal of Pharmacy and Pharmaceutical sciences,Vol. 3, No. 3, pp. 1459-1472
Sujatha, S., Tamilselvi, S., Subha, K., &Panneerselvam, A. (2013), 'Studies on biosynthesis of silver nanoparticles using Mushroom and its antibacterial activities', InternationalJournalof CurrentMicrobiology and Applied Sciences, Vol. 2, No.12, pp. 605-614.

Abah, S. E., &Abah, G. (2010), 'Antimicrobial and antioxidant potentials of Agaricus bisporus',Advances in Biological Research,Vol. 4, No. 5, pp. 277-282.

Ren, Z., Guo, Z., Meydani, S. N., & Wu, D. (2008), ' White button mushroom enhances maturation of bone marrow-derived dendritic cells and their antigen presenting function in mice', The Journal of nutrition, Vol. 138,No. 3, pp. 544-550.

Nasiri, F., Ghiassi, T. B., Bassiri, A. R., Hoseini, S. E., &Aminafshar, M. (2013), 'Comparative study on the main chemical composition of button mushroom's (Agaricus bisporus) cap and stipe', Journal of Food Biosciences and Technology, Vol. 3, pp. 41-48.

Kristen S., Montgomery., PhD., RN.(2003), 'Soy protein',Journal of Perinatal Education, Vol.12, No. 3, pp. 42–45.
Alada, A. R. A., Akande, O. O., &Ajayt, F. F. (2004), 'Effect of soya bean diet preparations on some haematological and biochemical indices in the rat',African Journal of Biomedical Research, Vol. 7, No. 2, pp. 71-74.

Patil, M. M., Pal, A., Anand, T., &Ramana, K. V. (2010), 'Isolation and characterization of lactic acid bacteria from curd and cucumber', Indian journal of biotechnology, Vol. 9, No. 2, pp. 166-172.

Adejuyitan, J. A., Olanipekun, B. F., &Moyinwin, O. A. (2014), 'Production and evaluation of cheese-like product from the blend of soy milk and coconut milk',Archives of Applied Science Research, Vol. 6, No. 4, pp. 263-266.

Wani, B. A., Bodha, R. H., &Wani, A. H. (2010), 'Nutritional and medicinal importance of mushrooms', J Med Plants Res, Vol. 4, No.24, pp. 2598-2604.

Madukwe, E., &Eme, P. E. (2012), 'Chemical evaluation and sensory attributes of soymilk fortified with carrot powder', African Journal of Food Science, Vol. 6, No. 20, pp. 483-486.

Naz, R. (2012), 'Development and evaluation of fruit flavored soymilk',Pakistan Journal of Food Sciences, Vol. 22, No. 3, pp.154-160.

Fournier, L. R., Ryan-Borchers, T. A., Robison, L. M., Wiediger, M., Park, J., Chew, B. P., .&Beerman, K. A. (2007), 'The effects of soy milk and isoflavone supplements on cognitive performance in healthy, postmenopausal wome',Journal of Nutrition Health and Aging, Vol. 11, No. 2, pp.155.

Xiao, C. W. (2008), 'Health effects of soy protein and isoflavones in humans',The Journal of nutrition, Vol. 138, No.6, pp. 1244-1249.

Mo, M., Hu, S., Xu, X., Ma, Z., Ni, Y., Wei, Y., &Nie, J. (2013), 'Optimization of Extraction Technology of Polysaccharide of Tricholoma giganteum',Pharmacology& Pharmacy, Vol. 4, No. 05, pp. 1.

Zhang, X., Shu, X. O., Gao, Y. T., Yang, G., Li, Q., Li, H., ... &Zheng, W. (2003), 'Soy food consumption is associated with lower risk of coronary heart disease in Chinese women',The Journal of nutrition,Vol. 133,No. 9, pp. 2874-2878.

Arora, S., Shivhare, U. S., Ahmed, J., &Raghavan, G. S. V. (2003), 'Drying kinetics of Agaricus bisporus and Pleurotus floridamushrooms',Transactions-American Society of Agricultural Engineers,Vol. 46,No. 3, pp. 721-724.

Iancu, C., Barbu, V., Nicolau, A., &Iordachescu, G. (2010), 'Attempts to obtain a new symbiotic product based on soy milk',Innovative Romanian Food Biotechnology, Vol. 7,pp. 21-29 .

AL, K., &Oladimeji, G. R. (2008), 'Production and quality evaluation of soy-corn milk', Journal of Applied Biosciences,Vol. 1, No. 2, pp.40-45.

Somekawa, Y., Chiguchi, M., Ishibashi, T., &Aso, T. (2001), 'Soy intake related to menopausal symptoms, serum lipids, and bone mineral density in postmenopausal Japanese women',Obstetrics& Gynecology, Vol. 97, No. 1, pp. 109-115.

Sakhale, B. K., Pawar, V. N., &Ranveer, R. C. (2012), 'Studies on development of soymilk based mango RTS beverage', EJEAFChe, Vol. 11, No. 5, pp. 523-528.

J, K. I., Gernah, I., Ojobo, E., & Oni, K. (2013), 'Effect of Cooking Temperature on Some Quality Characteristics of Soy Milk',Advance Journal of Food Science and Technology, Vol. 5, No. 5, pp. 543-546.

Jeong, S. C., Koyyalamudi, S. R., & Pang, G. (2012), ' Dietary intake of Agaricus bisporus white button mushroom accelerates salivary immunoglobulin A secretion in healthy volunteers',Nutrition, Vol. 28,No. 5, pp. 527-531.

Wasser, S. (2002), ' Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides',Applied microbiology and biotechnology, Vol. 60, No. 3 ,pp. 258-274.

Jose Alves, M., CFR Ferreira, I., Dias, J., Teixeira, V., Martins, A., &Pintado, M. (2013), 'A review on antifungal activity of mushroom (basidiomycetes) extracts and isolated compounds.',Current topics in medicinal chemistry, Vol. 13, No.21,pp. 2648-2659.

Jeong, S. C., Jeong, Y. T., Yang, B. K., Islam, R., Koyyalamudi, S. R., Pang, G., ... & Song, C. H. (2010), 'White button mushroom (Agaricus bisporus) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats', Nutrition Research,Vol. 30, No. 1, pp. 49-56.

Yaoqi Zhang, Wei Geng, YueqinShen, Yanling Wang and Yu-Cheng Dai. (2014), 'Edible mushroom cultivation for food security and rural developmentin china: Bio-innovation, Technological

dissemination and marketing' ,Sustainability, Vol. 6, pp. 2961-2973.

Li,C., Li,W., Chen,X. ,Feng,M., Rui,X., Jiang,M.,& Dong,M. (2014), 'Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains', LWT-Food Science and Technology, Vol. 57 ,No. 2, pp. 477-485.

Rai, M., Tidke, G., & Wasser, S.P. (2005), 'Therapeutic potential Mushrooms', Natural Product Radiance, Vol. 4, No.4 , pp. 246-257.

Iancu, C., Barbu, V., Nicolau, A., & Iordachescu, G. (2010), 'Attempts to obtain a new symbiotic product based on soy milk', Innovative Romanian Food Technology, Vol. 7, pp. 21-29.

Haytowitz, D. B. 'Vitamin D in Mushroom', Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Belsville MD. USDA-ARS .

Chen, S., Oh, S. R., Phung, S., Hur, G., Ye, J. J., Kwok, S. L., & Williams, D. (2006), ' Anti-aromatase activity of phytochemicals in white buttom mushrooms (*Agaricus bisporus*)', Cancer Research, Vol. 66,No. 24, pp. 12026-12034**.**

Venter, C S. (1999), 'Health benefits of soy bean and soy produts: a review', Journal of Family Ecology and Consumer Sciences/ Tydskrif vir Gesinsekologie en Verbruikerswetenskappe, Vol. 27,No. 2.

Mandal, S.K., kumar , V., Banerjee, M., Mishra, B., & Suneetha, V.(2013) 'Evaluating the nutritive properties of mixed plant derived products with and without soymilk for pharmacological usage' , Asian journal of pharmaceutical and Clinical Research, Vol. 6, No. 4, pp.74-77