COMPARATIVE ANALYSIS OF VITAMIN D2 RETENTION IN OYSTER MUSHROOM (*Pleurotus ostreatus*) USING HOME-BASED DRYING METHODS AND DEVELOPMENT OF INCORPORATED RECIPES

ST. TERESA'S COLLEGE
(AUTONOMOUS)
ESTD 1925

DISSERTATION SUBMITTED

In partial fulfillment of requirement for the award of the degree of

MASTER'S PROGRAMME IN
CLINICAL NUTRITION AND DIETETICS

By

REBECCA MANUEL

(Register No: SM23MCN012)

DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS

ST. TERESA'S COLLEGE (AUTONOMOUS)
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APRIL 2025

CERTIFIED AS BONAFIDE RESEARCH WORK

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DECLARATION

I hereby declare that the project entitled "**COMPARATIVE ANALYSIS OF VITAMIN D2 RETENTION IN OYSTER MUSHROOM (*Pleurotus ostreatus*) USING HOME-BASED DRYING METHODS AND DEVELOPMENT OF INCORPORATED RECIPES"** submitted in partial fulfilment of the requirement for the award of the degree of Master's Programme in Clinical Nutrition and Dietetics is a record of original research work done by me under the supervision and guidance of **Dr. Samja Sabu**, Assistant Professor, Department of Clinical Nutrition and Dietetics, Women's Study Centre, St. Teresa's College (Autonomous), Ernakulam and has not been submitted in part or full of any other degree/diploma/fellowship or the similar titles to any candidate of any other university.

Place: Rebecca Manuel

Date:

CERTIFICATE

I here certify that the dissertation entitled **"COMPARATIVE ANALYSIS OF VITAMIN D2 RETENTION IN OYSTER MUSHROOM (*Pleurotus ostreatus*) USING HOME-BASED DRYING METHODS AND DEVELOPMENT OF INCORPORATED RECIPES"** submitted in partial fulfilment of the requirement for the award of the degree of Master's Programme in Clinical Nutrition and Dietetics is a record of original work done by Ms. **Rebecca Manuel** during the period of the study under my guidance and supervision.

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ABSTRACT

Oyster mushrooms (*Pleurotus ostreatus*) are popular edible mushrooms known for their numerous health benefits including antioxidant, immunomodulatory, anticancer and antidiabetic properties. These mushrooms are rich in nutrients such as B-vitamins, vitamin D, protein, potassium, iron and zinc. They are also excellent sources of fiber and bioactive compounds. Owing to these benefits, oyster mushrooms can be dried and converted into powder for the value-addition in food products. Vitamin D deficiency is a global health concern and one of the most common micronutrient deficiencies in India. It can result from inadequate dietary intake, limited exposure to sunlight and certain health conditions that hinder the absorption of vitamin D. This deficiency can lead to diseases such as osteoporosis and rickets. Studies also suggest a link between vitamin D deficiency and chronic illnesses like cardiovascular diseases and diabetes. Fortification of staple foods with vitamin D is one strategy to combat this deficiency. The present study was conducted to compare oven-drying and sun-drying to determine which drying method better retained the nutrients in oyster mushrooms. The dried oyster mushroom samples and fresh oyster mushroom were analyzed for their nutrient content, specifically, protein, calcium, potassium, vitamin D2 and vitamin B2. It was revealed that sun-dried oyster mushroom powder retained vitamin D2 better than oven-dried mushroom powder but oven-drying resulted in higher retention of other nutrients such as protein, calcium, potassium and vitamin B2. The developed oyster mushroom powder was incorporated into products like cupcake, hummus, appam and bread pakoda. Sensory evaluations of these products were conducted to assess the acceptability of these products.

Keywords: *Pleurotus ostreatus*; vitamin D2; sun-drying; oven-dying; oyster mushroom powder incorporation

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Nature alone is antique, and the oldest art a mushroom.

- Thomas Carlyle

Scottish Essayist and Historian

A mushroom is a fungus which can be seen with the naked eye. They are sporophytes. They belong to the family Agaricaceae and have been part of the human cuisine for millions of years. There is evidence of mushroom consumption and cultivation dating back thousands of years in various regions, including China, Europe and the Americas. They are rich in protein, vitamin D, B vitamins, especially riboflavin, niacin and folic acid, potassium and fiber. Mushrooms have immense health benefits. They promote immune function, boost health, lower the risk of cancer, inhibit tumour growth, keep off viruses, balance the blood sugar, bacteria and fungi and reduce inflammation.

Owing to its distinct flavour, mushrooms are regarded as gourmet foods worldwide. It is a functional food of high nutritional value and is quite popular because of its organoleptic advantages, medicinal characteristics and high economic importance (Ibrahim *et al.,* 2022; Valverde *et al.,* 2015; Ergönül *et al.,* 2013). They are low in calories (250-350 calories/kg) since fresh mushrooms have a low-fat content (2-6%), a protein content of 19–35% and a carbohydrate content of 50-65% (Ibrahim *et al.,* 2022; Rathore *et al.,* 2017). According to Muswati *et al*. (2021), Yao *et al.* (2019) and Zied *et al.* (2017), non-starchy carbohydrates are thought to be abundant in mushrooms. Mushrooms also contain all the essential amino acids such as lysine, tryptophan, aspartic acid, glutamic acid and arginine making them an excellent alternative to animal products (Ibrahim *et al.,* 2022; Zhang *et al.,* 2021; Murugesan, 2017). The amount of vitamin D in mushrooms is significantly higher than the vitamin D content in fortified food products (Ibrahim *et al.,* 2022).

There are 2000 edible varieties of mushroom worldwide. There are a few varieties that are known to be toxic (Kadam *et al.,* 2023; Igile *et al.,* 2020). China is said to be the greatest mushroom producer in Asia, contributing approximately 35% to the global mushroom market. Asian countries contribute up to 76% of mushroom production, followed by Europe (17.2%) and the United States (5.9%). Mushroom production necessitates suitable conditions for their optimal growth and yield. Different types of mushrooms require different environmental conditions to be grown. In the mushroom industry, a number of species are grown for

commercial use. For example, oyster mushrooms are easily grown in lowlands and sold in markets, button and shiitake mushrooms are cultivated in the highlands and cold environment (Mahari *et al.,* 2020; Haimid *et al.,* 2013). The medicinal mushroom, Ling Zhi has a significant market value. Growing on maple, oak or tree roots, this hard bracket fungus has a strong potential to prevent cancer (Mahari *et al.,* 2020; Lin and Hsu, 2016; Pegler, 2002).

It has been reported by Elengoe (2024), Royse *et al.* (2017) and Sanchez (2010), that oyster mushrooms are one of the most cultivated mushrooms species in the world and the second largest commercially produced mushroom worldwide. The Pleurotus genus consists of about 40 species (Golak-Siwulska *et al.,* 2018; Kues and Liu, 2000). According to Waktola and Temesgan, 2020, oyster mushrooms are good sources for the development of food additives and antibacterial agents. They consist of 2.50% fat, 17-44% sugar, 25-30% protein, 7-38% myocellulose and approximately 8-12% mineral (potassium, calcium, phosphorus and sodium) (Muswati *et al.,* 2021; Stanly and Odu, 2012). They are also very rich sources of dietary fibre (Muswati *et al.,* 2021; Cheung, 2013; Deng G, 2009; Lemieszek and Rzeski, 2012). According to Golak-Siwulska (2018), they also exhibit anti-diabetic, anti-neoplastic, immunostimulatory, anti-atherosclerotic, anti-inflammatory, anti-oxidative and antibacterial properties.

Turning fresh mushrooms into powder help preserve mushrooms which would otherwise spoil quickly. Mushroom powder is important due to its various health and culinary benefits. It is rich in various nutrients such as vitamin D, protein and vitamin B. It is used in various dishes so as to add an umami flavour and to improve the nutrient profile of these dishes. Nowadays, the usage of mushroom powder in homes has increased due to the previously mentioned benefits. The present study was undertaken to determine the better drying method out of oven-drying and sun-drying that allows better retention of the nutrients in mushrooms while drying them at home. This can help people to use the method to make mushroom powder at home rather than getting it at the store.

Calin-Sánchez *et al*. (2020), Rahman (2020) and Szychowski *et al.* (2018), reported that drying is a traditional and unique physical food preservation method that is utilised in the food industry for both direct product preparation and additional processing. It has long been a beneficial and widespread conservation technique. In the past, drying was a straightforward and natural process which was driven by sun energy. This has now become more complex as it uses a lot of equipment and the drying parameters are carefully examined and optimized at

every stage of the process. It involves the removal of water from both solid and liquid products, usually with the help of heat, in order to produce solid-dried products.

There are 2 forms of moisture found in fresh foods namely, bound moisture, which is defined as the liquid that is retained in the microstructure of the solid part and unbound moisture, which is represented by the excess of the bound water. A liquid solution trapped within a solid matrix represents the bound moisture. As a result, many processes coexist when fresh food products are dried thermally. Initially, the fresh product receives energy from the hot drying agent. This is followed by the evaporation of the unbounded moisture and eventually water particles bounded within the cellular structure gradually migrate and diffuse to the product's surface, where the water eventually evaporates (Calin-Sanchez *et al.,* 2020; Pydi Setty and Ramana Murthy, 2003). Eliminating moisture from the fresh product prolongs its shelf life by inhibiting bacterial development and multiplication. Additionally, the drying process has an impact on the microbial development, sensory qualities and enzymatic activity. (Calin-Sanchez *et al.,* 2020; Ozbek and Dadali, 2007).

The methods that are most commonly used in the food industry include spray drying, convective drying, vacuum microwave drying, sun-drying, conventional dryers, fluidized bed dryers, spray dryers, freeze, vacuum or microwave drying, microwave-vacuum, microwave-freezing, puffing, superheated steam, instant controlled pressure drop, infrared radiation, radio frequency, electrohydrodynamic, ultrasonic and supercritical CO2 drying. In addition to this, combinations of the above mentioned methods are also considered as novel technologies used for the production of good quality dried food products. The microflora in vegetables is associated with Gram-negative bacteria. There are various drawbacks to eating vegetables untreated owing to this microbial load such as food poisoning or foodborne infections. These products may also have a limited shelf-life and may be easily degraded. As a result, many drying techniques are employed to maintain food quality. Drying also helps halt enzymatic and non-enzymatic browning reactions in foods (Alp and Bulantekin, 2021).

Mushrooms, due to the fact that they are highly perishable, are not commonly cultivated and utilized. This necessitates the turning of mushrooms into value added goods so as to cater to the protein and micronutrient requirement of the people and to tackle the issues of short shelf-life and post-harvest losses of these mushrooms (Markam, 2014).

The importance of vitamin D for the human body lies in its effect on both the formation of the bone system in addition to numerous extra - skeletal effects of cholecalciferol. A lack of

vitamin D has been linked to a higher risk of diabetes mellitus, hypertension, heart failure, peripheral artery disease, acute myocardial infarction, different types of cancer, autoimmune and inflammatory diseases, decreased immune defenses and increased mortality (Saloxiddinovna, 2024). Vitamin D is a class of fat–soluble vitamins, which has been traditionally recognized for its role in maintaining the homeostasis of calcium and phosphorus. There are 2 forms of vitamin D, namely, vitamin D2 and vitamin D3. Vitamin D3, also known as cholecalciferol, is synthesized de novo in the skin when exposed to UV-B radiation. It is also available from animal source foods (Balachander *et al.,* 2021; Ross *et al.,* 2011). Vitamin D2 or ergocalciferol, is derived from plants, particularly, mushrooms and yeast (Balachander *et al.,* 2021; Bikle, 2014). Studies have shown that 90% of vitamin D in the body is vitamin D3 and 10% is vitamin D2 (Ibrahim *et al.,* 2022; Taofiq *et al.,* 2017; Philips *et al.,* 2012).

According to Amrein *et al*. (2020), the testing of vitamin D and the use of vitamin D supplements have increased markedly in recent years. Most studies suggest that serum plasma 25(OH)D levels less than 75 nmol/l is associated with Vitamin D deficiency. A cutoff of less than 25 or less than 30 nmol/l is associated with an increased risk of osteomalacia and nutritional rickets. Van Schoor and Lips (2011), reported that the status of vitamin D in Europe is determined by latitude, season and skin pigmentation and is higher in Northern Europe than in Southern Europe. Lower levels of serum 25(OH)D were reported in Eastern Europe when compared to Western Europe. In the case of the Middle East, boys and girls aged 14 to 18 had higher vitamin D levels. Studies from Africa generally report adequate or high mean serum 25(OH)D levels, although population-based data are not available. However lower levels were observed in Tunisia, with non-veiled women having lower levels when compared to that of veiled women. Low serum levels were observed in Mongolia and in Chinese girls of 12-15 years especially in winter. Low levels were also observed in hip fracture patients and in India, especially in women. In a study conducted in Indian children and adolescents, it was found that the mean vitamin D concentration of 25(OH)D3 was below 50 nmol/l. The results also showed that in Maharashtra, Vitamin D deficiency was most prevalent among children and adolescents. Tamil Nadu and Chhattisgarh had the most vitamin D sufficient children and adolescents (Khadilkar *et al.,* 2022).

The recommended dietary allowances for vitamin D vary with age. The RDA for children aged 1–9 years ranges from 390–630 μg/day. For men and women above 18 years the RDA is 1000 μg/day and 840 μg/day respectively. In the case of pregnant and lactating women

it is 900 µg/day and 950 µg/day respectively (Indian Council of Medical Research-National Institute of Nutrition [ICMR-NIN], 2024).

Vitamin D deficiency is a global health concern. Oyster mushrooms being rich in vitamin D2 can be added to food products that are consumed, offering a plant-based alternative to help tackle vitamin D deficiency. They can also be used as a meat substitute especially for vegans due to their umami flavour and high protein content. Drying is a preservation method that has long been used for the preservation of food products.

The present study was conducted so as to determine the best method of drying mushrooms at home. Oyster mushrooms can be easily cultivated and require minimal resources. They are considered as environmentally sustainable food products. The mushroom powder which is developed was then used to enrich food products that are commonly consumed such as appam, hummus, bread pakoda and cupcakes. The enrichment of these food products with mushroom powder can improve the vitamin D2, protein and other nutrient concentrations of these foods.

The study entitled ‘‘Comparative Analysis of Vitamin D2 Retention in Oyster Mushroom (*Pleurotus ostreatus*) Using Home-Based Drying Methods and Development of Incorporated Recipes’’ was undertaken with the following objectives,

Objectives

- To analyse the effect of sun drying and oven drying on the vitamin D2 content of oyster mushrooms.
- To analyse the nutritional composition of dried mushroom powder.
- To develop and evaluate the acceptability of common food products prepared by the incorporation of mushroom powder.

2. REVIEW OF LITERATURE

The literature pertaining to the study **"Comparative Analysis of Vitamin D2 Retention in Oyster Mushroom (*Pleurotus ostreatus*) Using Home-Based Drying Methods and Development of Incorporated Recipes"** is presented under the following heads

2.1 Oyster Mushrooms

2.1.1 Nutritional Values

2.1.2 Therapeutic Properties

2.1.2.1 Antioxidants

2.1.2.2 Anti-Carcinogenic and Anti-Tumour

2.1.2.3 Anti-Microbial

2.1.2.4 Anti-Diabetic

2.1.2.5 Immuno-modulators

2.1.2.6 Anti-Hypercholesterolemic and Hypolipidemic Activity

2.1.3 Anti-Nutritional Factors

2.1.4 Bioactive Compounds

2.1.4.1 Phenols and Flavonoids

2.1.4.2 Terpenes and Terpenoids

2.1.4.3 Polysaccharides

2.2 Vitamin D

2.3 Different Drying Methods

2.3.1 Hot-Air Drying

2.3.2 Infrared-Drying

2.3.3 Microwave Drying

2.3.4 Combination Drying

2.3.5 Solar and Sun-drying

2.3.6 Tray Drying

2.3.7 Oven Drying

2.3.8 Freeze Drying

2.3.9 Vacuum Drying

2.3.10 Spray Drying

2.1 Oyster Mushrooms

Oyster mushrooms (*Pleurotus* spp.) are one of the most cultivated species of mushroom. They are sometimes referred to as "Dhingri" in India. They belong to the class Basidiomycetes and family Agaricaceae (Nongthombam *et al.,* 2021; Randive, 2012). *P. ostreatus* and *P. pulmonarius* are among the most important *Pleurotus* species cultivated in large scale (Correa *et al.,* 2016; Bazanella *et al.,* 2013). Their nutrition, taste and physiological function make them popular for having all the properties that would be usually expected from a food product. Thus, they are appreciated for their excellent nutritional profile and sensory characteristics (Correa *et al.,* 2016). Studies have reported that the largest producer of oyster mushrooms in the world is China (Muswati *et al.,* 2021; Kortei *et al.,* 2018). According to Golak-Siwulska *et al.* (2018) and Fernandes *et al.* (2015), oyster mushrooms are the world's third most cultivated species of mushrooms. Few other studies reported that oyster mushrooms are the second most cultivated species of mushrooms (Lesa *et al.,* 2022; Hoa and Wang, 2015).

Many valuable scents and flavour attributes of their fruiting body and mycelial biomass are determined by the terpenes, lactones, amino acids and carbohydrates that make up their composition (Correa *et al.,* 2016; Smiderle *et al.,* 2008). *P. ostreatus* is commonly used for preparing food products like soups. They are also used in stir fry recipes with soy sauce or eaten stuffed. Another species, namely, *P. eryngii* is used for the preparation of vegetarian dishes, being served sauteed, grilled, braised, stewed or boiled (Correa *et al.,* 2016; Reis *et al.,* 2012). The moisture content of fresh fruiting bodies of *Pleurotus* spp. ranges from 85-90%. The moisture percentage depends on the mushroom species and on the parameters related to harvest, growth, culinary and storage conditions (Correa *et al.,* 2016; Khan and Tania, 2012; Reis *et al.,* 2012).

2.1.1 Nutritional Values

These mushrooms are well known for having high levels of vitamin C and vitamin B complex as well as 1.6 – 2.5% protein and mineral salt, all of which are vital for human health (Nongthombam *et al.,* 2021; Randive, 2012). Khatun *et al.* (2015), reported that *P. florida* has a higher protein content and better antioxidative action than *P. pulmonarius* and *P. citrinopileatus*.

The catalase, phenolics and peroxidase contents in these mushrooms contribute to their function as an antioxidant (Khatun *et al.*, 2015). The protein content of mushrooms depends on their genetic makeup and the physical and chemical properties of the growing medium (Majesty *et.al.,* 2019; Adejumo *et al.,* 2015; Sanmee *et al.,* 2003). The amino acid composition also varies. Majesty *et.al.* 2019, pointed out that the amino acid content of the mushrooms was as follows: leucine (5.10 g/100g), lysine (2.90 g/100g), isoleucine (2.01 g/100g), phenylalanine (3.20 g/100g), valine (3.18 g/100g), proline (1.89 g/100g), arginine (2.96 g/100g), tyrosine (7.03 g/100g), histidine (1.01 g/100g), tryptophan (0.13 g/100g) cysteine (2.04 g/100g), alanine (4.90 g/100g), methionine (0.72 g/100g), glutamic acid (8.12 g/100g), glycine (4.20 g/100g), threonine (4.27 g/100g), serine (0.18 g/100g), and aspartic acid (6.10 g/100g).

2.1.2 Medicinal Values

Waktola and Temesgen (2020) reported that mushrooms are used in healthcare for treating common diseases like skin diseases and pandemic diseases such as AIDS. Owing to its high nutritional content and potential medical applications, oyster mushrooms are the most known functional food for human health. They also added that these mushrooms have over 100 medicinal uses and functions such as hepatoprotective, antifungal, antiviral, antiparasitic, antibacterial, detoxifying, immunomodulating, cardiovascular protection, antioxidant, antiallergic, anticancer, antidiabetic, antitumor and anti-inflammatory effects.

In a study conducted by Lesa *et al.* (2022), it was found that between 6% and 10% of edible oyster mushrooms are thought to have therapeutic uses and have been utilised as significant natural ingredients in pharmaceutical products.

2.1.2.1 Antioxidants

Numerous researchers have explored the natural antioxidant properties of various edible and medicinal mushrooms, which exhibit significant free radical scavenging activity (Lesa *et al.,* 2022; Gupta *et al.,* 2018). In a study it was found that an extract of *Pleurotus*

ostreatus enhanced catalase gene expression and reduced free-radical-induced protein oxidation in aged rats, thereby mitigating age-related disorders. The ethanolic extract of these mushrooms have been shown to exhibit antioxidant activity both in vitro and in vivo. This activity includes scavenging superoxide and hydroxyl radicals, inhibiting lipid peroxidation, chelating ferrous ions, reducing ferric ions and quenching 2,3-diazabicyclo. Additionally, reports have shown that the superior antioxidant properties can be attributed to the carbohydrate component particularly, β-glucan (Zhang *et al.,* 2012). It can also be used as for developing antioxidants in food industries as food additives. According to Ahmed *et al.* (2016) and Weigand-Heller *et al.* (2012), the antioxidant property of mushrooms can also be attributed to the presence of ergothioneine in them. Another study reported that the myoconstituent triterpenoid in *Ganoderma lucidum* might be responsible for the antioxidant activity (Pandimeena *et al.,* 2015; Paterson, 2006).

2.1.2.2 Anti-Carcinogenic and Anti-Tumour

Devi *et al.* (2024), pointed that the anti-cancer activity of oyster mushrooms is due to the bioactive molecules such as polysaccharides, *β*-glucans, lectin, peptides, lovastatin, phenolics and flavonoids present in them.

Deepalakshmi and Mirunalini (2013), reported that the anti-tumour properties of *P. ostreatus* polysaccharides were first reported by Yoshioka *et al.* (1972). These mushrooms contain tumour-suppressing compounds that have undergone clinical trials, with all forms of edible mushrooms showing potential for preventing prostate and breast cancers. As a nutraceutical and medicinal treatment, *P. ostreatus* has been utilized in carcinogenic patients. Its dietary mycelia-derived proteoglycans have been shown to enhance natural killer (NK) cell cytotoxicity in mice, stimulate macrophages to produce nitric oxide, and interact strongly with glucose or mannose-specific lectin concanavalin A (Con A), indicating the presence of terminal sugars with glucose and mannose. These proteoglycans, along with a water-soluble heteroglucan folded into a triple-helical structure, were suggested to exhibit immune cell activation and significant anti-tumour effects in tumour-bearing mice models. Thus, the anti-tumour effects of *P. ostreatus* are closely associated with its heteroglucan production (Deepalakshmi and Mirunalini, 2013).

Bioactive compounds in *P. ostreatus*—such as glucans, ergosterol, amino acids (arginine and glutamine), and proteoglycans—are linked to its anti-tumour activities. Their potential mechanisms of action include inhibiting tumour-induced neovascularization in

sarcoma 180 cells in vitro, activating and promoting T cell clonal expansion, enhancing NK and T helper (THC) cell activity, stimulating interleukin synthesis, boosting immune function, prolonging cell survival, and increasing tolerance to chemotherapy. Various extracts of *P. ostreatus* have demonstrated anticancer effects in different cancer cell lines (CCLs) and experimental animal models through multiple mechanisms. Among these, water-soluble extracts have shown the most significant cytotoxicity and apoptotic induction in human androgen-independent PC-3 prostate cancer cells. In a study conducted on the mycelia of oyster mushroom it was concluded that the mycelial hot water extracts could be used for developing nutraceuticals and for designing innovative myco-therapeutics and phytocosmetics applications (Lesa *et al.*, 2022; Galappaththi *et al*., 2021).

2.1.2.3 Anti-Microbial

Mushrooms need antibacterial and antifungal compounds for survival. They have an anti-bacterial spectrum against Gram-negative and Gram positive-bacteria. Ethyl acetate extract was investigated for their antimicrobial properties (Lesa *et al.,* 2022). According to Onuegbu *et al.* (2017), n-hexane extracts possess broad-spectrum antimicrobial activities. A laccase purified from *P. ostreatus* mushroom is capable of inhibiting the Hepatitis C virus entry into the peripheral blood cells and into the hepatoma Hep G2 cells and inhibited its replication. A novel ubiquitin-like protein found in *P. ostreatus* mushrooms was found to have an inhibitory activity towards the HIV-1 reverse transcriptase (Waktola and Temesgen, 2020; Wang and Ng, 2000).

2.1.2.4 Anti-Diabetic

Oyster mushrooms are ideal for the prevention of hyperglycemia which can be attributed to their low-fat content, high dietary fiber and protein (Lesa *et al.,* 2022). Another study investigated the effects of King Oyster mushrooms on glycemic metabolism in alloxan-induced hyperglycemic mice. The results showed that the extracts of these mushrooms increase the glycogen and insulin concentrations and recover injured β-cells and reduce free radical damage (Li, 2014).

2.1.2.5 Immuno-Modulators

A study which investigated the antitumor and immunomodulatory activity of *Pleurotus eryngeii* extract confirmed that the extract not only inhibits growth of breast cancer cells and mice tumours but also can increase some immune activities (Xue *et al.,* 2015). Another study

which demonstrated the in vivo immunostimulatory capacities of five edible mushrooms, including *Lentinula edodes, Agaricus bisporus, Pleurotus ostreatus , Pleurotus columbinus* and *Pleurotus sajor-caju* using Wistar Albino rats confirmed the immunostimulatory effect of the mushrooms by histopathological examination of the rats' spleen and thymus tissues which showed marked proliferation of lymphocytes which was particularly obvious in *Pleurotus ostreatus, Lentinula edodes* and *Agaricus bisporus* at high doses (Elhusseiny *et al.,* 2022).

2.1.2.6 Anti-Hypercholesterolemic and Hypolipidemic Activity

According to Abidin *et al*. (2017), Pleurotus spp., both as food or extracts from fruiting bodies or mycelium, can prevent and treat hypercholesterolemia, hypertension and oxidative stress. *P. ostreatus* was found to be the most effective for the prevention and treatment of atherosclerosis as they contain a large amount of ergothioneine, chrysin and lovastatin. *Pleurotus ostreatus* extracts lowered plasma cholesterol and triglyceride levels after a 28-day administration in hypercholesterolemia induced adult rats (Iqbal *et al.,* 2024).

2.1.3 Anti-Nutritional Factors

Hydrogen cyanide is a component which is very toxic at low concentration in animals. It can precipitate the dysfunction of the central nervous system, cardiac arrest and respiratory failure (Oly-Alawuba and Obiakor-Okeke, 2014; D'Mello 2000).

Phytates form complexes with calcium, zinc, iron and magnesium and render them unavailable for absorption and utilization. Its concentration in mushrooms was found to be ten times lower than the safe limit (Oly-Alawuba and Obiakor-Okeke, 2014; Afiukwa *et al.,* 2013).

Oxalates also form complexes with minerals such as calcium, iron and magnesium rendering them unavailable for absorption.

Trypsin inhibitors, present in oyster mushrooms reduce the proteolytic activity of trypsin enzyme, leading to the formation of irreversible trypsin and trypsin inhibitor complexes (Oly-Alawuba and Obiakor-Okeke, 2014; Ogbe and Obeka, 2013).

2.1.4 Bioactive Compounds

Lesa *et al. (*2022), reported that the bioactive compounds present in oyster mushrooms provide a wide range of health benefits. Indigestible fermentable polysaccharides, such as β-glucans are converted into short-chain fatty acids (SCFAs), which further help modulate the gut microbiota, leading to improved plasma glucose levels, adipose tissue function and overall

energy homeostasis. As a result, mushrooms exhibit anti-diabetic and anti-obesity properties. Additionally, mushrooms contain low and high molecular weight proteins and peptides, terpenes, quinolones, steroids and polysaccharides like β-glucans and mannans, that contribute to anti-fungal, anti-microbial, anti-cancer and anti-tumor activities. Nutraceuticals derived from mushrooms also enhance the efficiency of protein and muscle development. β-glucans also activate macrophages and natural killer cells, which help in boosting the immune system.

2.1.4.1 Phenols and Flavonoids

Phenol, flavonoid and tannins are secondary metabolites. These are collectively known as phenolic compounds and can exhibit antioxidant properties (Devi *et al.,* 2024; Rahimah *et al.,* 2019).

2.1.4.2 Terpenes and Terpenoids

These are compounds that are found naturally in plants and are major constituents of essential oil from plants. They possess antioxidant, anti-tumor, antiviral, anti-cytotoxicity, insecticidal, anti-inflammatory and nematicidal activity. Studies have shown that sterols, another class of terpenoids are present *in P. ostreatus* strains (Devi *et al.,* 2024; Prastiyanto *et al.,* 2020).

2.1.4.3 Polysaccharides

Oyster mushrooms are a source of polysaccharides including α-glucans, β-glucans, heteroglycans, and proteoglycans. They possess antioxidant, anti-tumor and anti-inflammatory effects. Polysaccharide-K (krestin) and polysaccharide peptide found in *Coriolus versicolor*, exhibit immunomodulatory effects (Devi *et al.,* 2024; G. Venturella *et al.,* 2021).

Polysaccharides extracted from grey oyster mushrooms contain β-glucans that enhance neutrophil-killing activity against *Candida albicans.* Extracts from *Pleurotus ostreatus* demonstrated tumor growth inhibition by increasing tumor necrosis factor-α and nitric oxide levels which highlights their potential as immune-stimulating agents. Sulphated polysaccharides enhanced plasma clot inhibition through intrinsic and extrinsic pathways (Devi *et al.,* 2024).

2.2 Vitamin D

Vitamin D is known for its role in maintaining calcium homeostasis and bone health, as it increases the production of calcium transport proteins in the small intestine, which

improves dietary calcium absorption and lowers the incidence of rickets and osteomalacia in children and adults respectively (Cardwell *et al.,* 2018).

Vitamin D plays a role in regulating the differentiation and activation of CD4 lymphocytes. It also plays a role in improving the number and function of regulatory T cells, in vitro inhibition of the differentiation of monocytes in dendritic cells, reduction in the production of cytokines, interferon-g, IL-2 and TNF-α by Th1 cells, stimulation of the function of Th2 helper cells, inhibition of the production of IL-17 by Th1 cells and in vitro and in vivo stimulation of NK cells (Marques *et al.,* 2010).

Studies have linked poor vitamin D status with increased risk of osteoporotic and stress fractures, increased risk of developed cardiovascular diseases and some cancers, and the pathogenesis of immune mediated inflammatory diseases (Durrant *et al.,* 2022).

2.3 Different Drying Methods

Drying is the oldest food preservation technique. Initially, water was extracted from fruits, cereals, meats and herbs using sun, smoky fire and wind. Food dehydration is the process of drawing water out of the food product by passing hot air through it. This inhibits the growth of bacteria and enzymes in them. Dried foods are delicious, wholesome, portable, easy to prepare and convenient to store and use. Less energy and space are also required and it has very little effect on the food's nutritional content (Ahmed *et.al.,* 2013). According to Lee *et al.* (2016), the drying method selected can influence the quality index of dried agricultural products such as colour values, rehydration rate and hardness.

2.3.1 Hot-Air Drying

This is one the most frequently used method for vegetables and fruits, commercially. In this process, convective air flow passes over the surface of the product not through the products. Due to this, the thermal conductivity of an agricultural product determines the heat transfer from the surrounding environment to its internal section. Hot-air drying may lead to loss in quality and requires a long drying time with low energy efficiency. The high-altitude temperature and velocity play an important role in the reduction in drying time and the deterioration of quality index such as colour values (Lee *et al.,* 2016).

2.3.2 Infrared Drying

It is widely used in drying and pasteurization, and for the evaluation of the quality and safety of agricultural products. Infrared (IR) radiation has the wavelength, 0.78-1000 μm and is accounted as an efficient alternative to drying methods like sun drying, hot air drying and vacuum drying. Additionally, this procedure involves the amplification of the drying rate of the agricultural products and is used to dehydrate thin slices of fruits and vegetables as it facilitates the exposure of large surface areas to IR radiation. The moisture and composition of agricultural products determine their efficiency of IR energy absorption (Lee *et al.,* 2016). In a study, it was concluded that this method of drying can increase the drying efficiency and energy efficiency of oyster mushrooms (Nurmawati *et al.,* 2023).

2.3.3 Microwave Drying

Microwave (MW) heating technology is used for thermal processing operations in the food industry due to its ability to reduce processing time. The MW power generator, the magnetron, has led to the use of microwave ovens. In this process internal heat is generated within the food materials through the rotation if dipolar water molecules and ion conduction caused by changes in the electromagnetic field (Lee *et al.,* 2016). Rapid removal of surface moisture takes place using this technique (Motegaonkar *et al.,* 2024). According to Bashir *et al.* (2020), microwave drying is associated with lower mineral retention in oyster mushrooms.

2.3.4 Combination Drying

According to Lee *et al.* (2016), both microwave and infrared drying are effective at reducing drying time when operated at high power levels. However, they can also lead to quality deterioration in the final products due to elevated temperatures in the dryer. To alleviate such damage, combination methods such as microwave with vacuum, IR with hot air and microwave with hot air have been developed.

2.3.5 Solar and Sun-Drying

This is a commonly used method for drying fruits and vegetables as it is a natural and affordable drying method. It is one of the oldest drying techniques. However, this technique has certain disadvantages such as contamination, infections and microbial spoilage. It also takes a long time for fruits and vegetables to dry using this method (Motegaonkar *et al.,* 2024).

2.3.6 Tray Drying

The procedure's simple and economical design makes it one of the most widely used techniques of drying. The products are spread over different trays at an appropriate thickness so as to achieve uniform drying of the product. The trays are heated by the passage of hot air passing over them, through convection from the heated trays or via radiation from heated surfaces. It is a batch process of drying (Motegaonkar *et al.,* 2024).

2.3.7 Oven Drying

Oven drying was considered as a very popular drying method in the last decade. This could be attributed to it being a low-cost method and energy efficient. It was found that nutrients like beta-carotene was found to be higher in oven dryers and fluidized bed dryers. It was also found that oven-dried strawberries had more rehydration ability and better structural characteristics (Hasan *et al.,* 2019).

2.3.8 Freeze Drying

This method prevents the shrinkage of food products. This method uses the principle of sublimating frozen products, which leads to the inhibition of biochemical and microbiological processes at a low temperature and the production of a high-quality product (Motegaonkar *et al.,* 2024).

2.3.9 Vacuum Drying

This technology is important in the pharmaceutical, chemical, biotechnology and food industries particularly for drying heat-sensitive materials. Heat is generated and water vapor is removed, in this process. Reduced pressure facilitates faster evaporation of water, with heat being provided either through radiation or contact with a heated metal surface (Motegaonkar *et al.,* 2024).

2.3.10 Spray Drying

Liquid food materials are converted into powder form in this technique. This is process is undertaken under controlled temperature conditions and is a single step process. The resultant products will be more stable and would have low moisture content and water activity. Few studies have been conducted to develop mushroom powder using this method (Motegaonkar *et al.,* 2024).

3. METHODOLOGY

The present study entitled **"Comparative Analysis of Vitamin D2 Retention in Oyster Mushroom (*Pleurotus ostreatus*) Using Home-Based Drying Methods and Development of Incorporated Recipes"** was conducted and the methodology adopted is discussed under the following headings

3.1 Selection and Collection of Raw Materials

3.1.1 Collection of Fresh Oyster Mushroom

3.1.2 Collection of Raw Materials

3.2 Development of Oyster Mushroom Powder

3.2.1 Development of Oven-Dried Oyster Mushroom powder

3.2.2 Development of Sun-Dried Oyster Mushroom powder

3.3 Nutritional Analysis

3.4 Development of Oyster Mushroom Powder-Incorporated Products

3.4.1 Cupcakes

3.4.2 Hummus

3.4.3 Appam

3.4.4 Bread Pakoda

3.5 Organoleptic Evaluation

3.5.1 Selection of Panel Members

3.5.2 Preparation of Scorecard

3.5.3 Sensory Evaluation of Oyster Mushroom Powder-Incorporated Products

3.6 Statistical Analysis

3.1 Selection and Collection of Raw Materials

Oyster mushrooms are rich in antioxidants, flavonoids, phenolic compounds, and polysaccharides which contribute to their antioxidant activity. these mushrooms also have antibacterial, antiviral, anti-inflammatory and other effects.

3.1.1 Collection of Fresh Oyster Mushrooms

Fresh oyster mushrooms were obtained from a store in Ernakulam, Kerala. These were dried and converted into powder. This dried mushroom powder was incorporated with selected recipes to check their acceptability.

Plate 1. Fresh oyster mushrooms

3.1.2 Collection of Raw Materials

The remaining ingredients for product development were eggs, atta, oil, sugar, milk, baking powder, baking soda, wheat bread, besan, onion, chilly, coriander leaves, coriander powder, chilly powder, oil, salt, yeast, rice flour, coconut, sugar, water, chickpea, sesame seeds, lemon juice, garlic and olive oil. All the ingredients were purchased from supermarkets.

3.2 Development of Oyster Mushroom Powder

Oyster mushroom powder is rich in minerals, antioxidants, B vitamins and vitamin D. Incorporating mushroom powder into food products can enhance the latter’s nutritional value and provide an earthy flavour to the food product.

3.2.1 Development of Oven-Dried Oyster Mushroom Powder

The oven-dried oyster mushroom powder was prepared as follows:-

- Wash the oyster mushrooms. Slice them into small pieces around 2-3mm thick.
- Place them on a baking tray and keep inside the oven at 60°C for 5 hours.
- Once the mushroom slices are crispy enough to be snapped, they are ready.
- Then transfer the dried slices to a mixer grinder and grind to a powder.

Plate 2. Preparation of oven-dried oyster mushroom powder

3.2.2 Development of Sun-Dried Oyster Mushroom Powder

The sun-dried oyster mushroom powder was prepared as follows:-

- Wash the oyster mushrooms and slice them into small pieces around 2-3mm thick.
- Place these on a winnower and place it under the sun for 2 days.
- Once the mushroom slices are crispy enough to be snapped, they are ready.
- Then transfer the dried slices to a mixer grinder and grind to a powder.

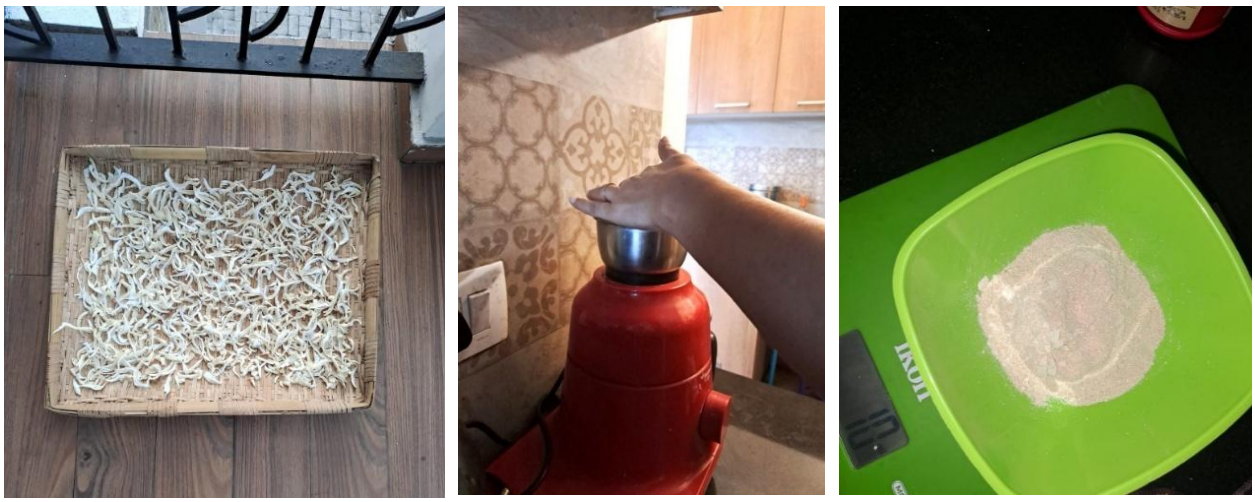

Plate 3. Preparation of sun-dried oyster mushroom powder

3.3 Nutritional Analysis

Nutritional analysis is a method of identifying the nutrient content of a food product. The samples were analysed to identify the amount of protein, calcium, potassium, vitamin D2 and vitamin B2 present in them.

Table 1. Methods adopted for analysis

Parameters	Unit	Method adopted
Protein	%	AOAC 21st EDITION 991.20
Calcium	mg/100 g	IS 4285 : 2003
Potassium	%	IS 9497 : 1980 RA 1998
Vitamin D2	mg/kg	IPL.CH.INS.STP.23
Vitamin B2	mg/kg	IPL.CH.INS.STP.34

The shelf-life study of fresh mushrooms, oven-dried oyster mushroom powder and sun-dried oyster mushroom powder was conducted for 10 days.

Plate 4. Nutritional analysis of dried oyster mushroom powders and fresh oyster mushroom

3.4 Development of Oyster Mushroom Powder-Incorporated products

The recipes selected for the incorporation of oyster mushroom powder were cupcakes, hummus, appam and bread pakoda.

3.4.1 Cupcakes

These are small individual cakes made with flour, baking powder, baking soda, butter/oil, eggs, milk and sugar. They are fluffy and come in an array of flavours. Cupcakes can be made healthier by preparing them with healthier alternatives to the ingredients that are traditionally used, like replacing refined flour, sugar and butter with wheat flour, brown sugar or honey and oil, respectively. Incorporating mushroom powder into cupcakes adds vitamin D to the cupcakes, making them more nutritious.

T0

T1

T2

T3

Plate 5. Oyster mushroom powder-incorporated cupcakes

3.4.2 Hummus

Hummus is a thick dip or spread, native to the Middle East. It is made from chickpeas, sesame seeds, garlic, olive oil and lemon juice and is rich in fiber, protein, polyunsaturated fatty acids, vitamin A, vitamin C, vitamin E, magnesium, iron, potassium and folate. It is associated with weight control, reduced blood glucose levels, improved total cholesterol and low-density lipid cholesterol levels and improved gastrointestinal tract health. Hummus is generally served together with crackers, fresh vegetables or pita bread. Adding mushroom powder to hummus improves its nutritional value by adding vitamin D and B vitamins, making it even healthier. It can also add a savoury umami flavour to the hummus, making it more delicious.

T0

T1

T2

T3

Plate 6. Oyster mushroom powder-incorporated hummus

3.4.3 Appam

Appam are lacy pancakes made from a batter of ground, fermented rice, coconut and yeast. They have thin, crispy edges with a soft, fluffy center and are typically eaten for breakfast in South India, especially in Kerala. They are low in fats since they are prepared by steaming. Appam, being a fermented food, promotes the growth of healthy gut bacteria and aids in digestion. Fortifying appam with mushroom powder increases their vitamin D and protein content. Additionally, it adds beneficial β-glucans in the appam.

T0

T1

T2

T3

Plate 7. Oyster mushroom powder-incorporated appam

3.4.4 Bread Pakoda

Bread pakoda is a popular Indian snack that is widely enjoyed across the country as a street food. It is prepared by coating bread slices in a batter prepared from gram flour, onions, coriander leaves and a mixture of spices. Incorporating mushroom powder into the batter can improve the nutritional value of the snack by adding B vitamins, vitamin D and some extra protein.

T0

T1

T2

T3

Plate 8. Oyster mushroom powder-incorporated bread pakoda

3.5 Organoleptic Evaluation

3.5.1 Selection of Panel Members

Serial dilution test was first conducted in order to select a panel of judges for the organoleptic evaluation. The solutions were prepared by setting up 7 cups each of which contained 10g, 20g, 30g, 40g, 50g, 60g and 70g of dissolved sugar in 200ml of water. An extra cup was also kept which contained normal water. This was used as a palate cleanser. The participants were asked to taste the different solutions and fill up a form in which they had to rank the solutions according to the sweetness. Among the participants, 10 who obtained the highest scores for the serial dilution test were selected as panel members for sensory evaluation of the developed products.

Plate 9. Serial dilution test

3.5.2 Preparation of Scorecard

A scorecard was prepared for the sensory evaluation of the developed products. A 9-point hedonic scale was prepared which evaluated the acceptance for different attributes such as appearance, texture, taste, flavour, colour, odour, from ‘like extremely’ to ‘dislike extremely’.

3.5.3 Sensory Evaluation of Oyster Mushroom Powder-Incorporated Products

Organoleptic evaluation involves assessing different attributes of a product such as appearance, colour, flavour, texture, taste and overall acceptability. Each judge was asked to fill out a scorecard with different characteristics of the products such as appearance, colour, flavours, texture, taste and overall acceptability. They were asked to score each attribute out of 9.

Plate 10. Sensory evaluation

3.6 Statistical Analysis

The statistical analysis was done using IBM SPSS version 20. The numeric variable, overall acceptability of different variations was represented in mean and SD.

To test the statistical significance of sensory evaluation between each variation with control of each product, independent sample t-test was applied. A p-value of less than 0.05 was considered to be statistically significant.

4. RESULTS AND DISCUSSION

Salient features of the study entitled **'Comparative Analysis of Vitamin D2 Retention in Oyster Mushroom (*Pleurotus ostreatus*) Using Home-Based Drying Methods and Development of Incorporated Recipes'** were discussed under the following headings.

4.1 Nutrient Composition of the Dried Oyster Mushroom Powders and Fresh Oyster Mushrooms

4.1.1 Nutrient Analysis of Oven-Dried Oyster Mushroom Powder

4.1.2 Nutrient Analysis of Sun-Dried Oyster Mushroom Powder

4.1.3 Nutrient Analysis of Fresh Oyster Mushrooms

4.2 Shelf-life Quality of the Dried Oyster Mushroom Powders and Fresh Oyster Mushrooms

4.3 Nutrient Calculation of the Oyster Mushroom Powder-Incorporated Products

4.3.1 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Cupcakes

4.3.2 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Hummus

4.3.3 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Appam

4.3.4 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Bread Pakoda

4.4 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Products

4.4.1 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Cupcakes

4.4.2 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Hummus

4.4.3 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Appam

4.4.4 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Bread Pakoda

4.1 Nutrient Composition of the Dried Oyster Mushroom Powders and Fresh Oyster Mushrooms

The nutritional composition of sun-dried and oven-dried oyster mushroom powders and fresh oyster mushrooms was analyzed. The different nutrients such as protein, calcium, potassium, vitamin D2, vitamin B2 and vitamin B6 were estimated.

4.1.1 Nutrient Analysis of Oven-Dried Oyster Mushroom Powder

The result of the nutritional analysis of oven-dried oyster mushroom powder is given in table 2.

Table 2. Nutrient analysis of oven-dried oyster mushroom powder

Sl. No.	Nutrients	Result
1.	Protein	28.6 g
2.	Calcium	58.6 mg
3.	Potassium	3050 mg
4.	Vitamin D2 (ergocalciferol)	6.3 µg
5.	Vitamin B2 (riboflavin)	1.074 mg

As depicted in table 2, the oven-dried oyster mushroom powder contained 28.6 g protein, 58.6 mg calcium, 3050 mg potassium, 6.3 µg vitamin D2 (ergocalciferol) and 1.074 mg vitamin B2 (riboflavin) in 100 g. In a study by Salamatullah *et al.* (2020), it was reported that there was an increase in the total phenolic and flavonoid content of oven dried red and white onions compared to undried onions.

4.1.2 Nutrient Analysis of Sun-Dried Oyster Mushroom Powder

The result of the nutritional analysis of sun-dried oyster mushroom powder is given in table 3.

Table 3. Nutrient analysis of sun-dried oyster mushroom powder

Sl. No.	Nutrients	Result
1.	Protein	22.8 g
2.	Calcium	56.5 mg
3.	Potassium	2120 mg
4.	Vitamin D2 (ergocalciferol)	299 µg
5.	Vitamin B2 (riboflavin)	0.223 mg

As presented in table 3, the sun-dried oyster mushroom powder contained 22.8 g protein, 56.5 mg calcium, 2120 mg potassium, 299 µg vitamin D2 (ergocalciferol) and 0.223 mg vitamin B2 (riboflavin) in 100 g. Several studies have pointed out that UV-B radiation exposure enhances the vitamin D content of oyster mushrooms (Moutia *et al.,* 2024).

4.1.3 Nutrient Analysis of Fresh Oyster Mushrooms

The result of the nutritional analysis of fresh oyster mushrooms is given in table 4.

Table 4. Nutrient analysis of fresh oyster mushrooms

Sl. No.	Nutrients	Result
1.	Protein	3.59 g
2.	Calcium	32.2 mg
3.	Potassium	590 mg
4.	Vitamin D2 (ergocalciferol)	1.3 µg
5.	Vitamin B2 (riboflavin)	0.224 mg

As depicted in table 4, fresh oyster mushrooms contained 3.59 g protein, 32.2 mg calcium, 590 g potassium, 1.3 µg vitamin D2 (ergocalciferol) and 0.224 mg vitamin B2 (riboflavin) in 100 g.

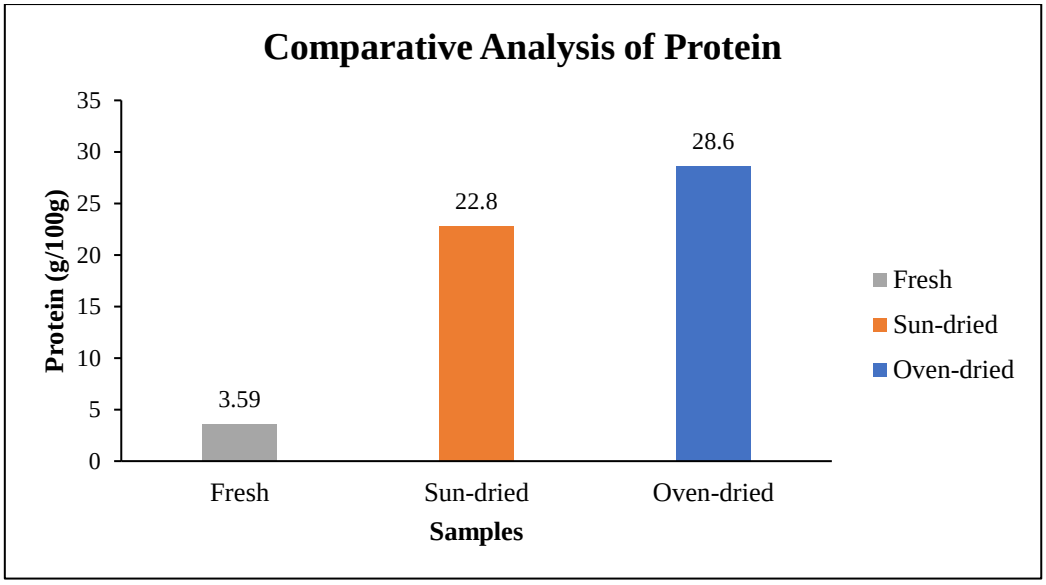

Figure 1. Comparative analysis of protein

From figure 1, it is evident that oven-dried oyster mushroom powder had the highest amount of protein (28.6 g), while fresh oyster mushroom had the lowest amount (3.59 g). These results were similar to the results obtained by Chukwu and Shaba *et al.* (2009), in their study on the effect of different drying methods on the nutritional content of catfish, which said that oven-drying improved the protein quality in catfish.

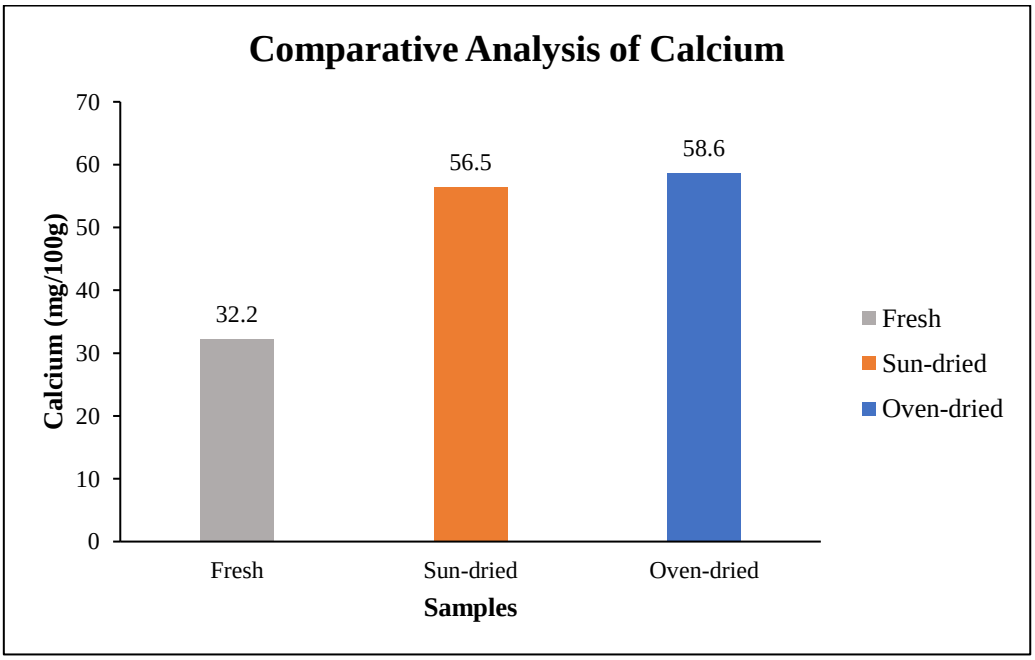

Figure 2. Comparative analysis of calcium

As depicted in figure 2, the oven-dried oyster mushroom powder contained the highest amount (58.6 mg) of calcium and fresh mushrooms had the lowest amount (32.2 mg) of

calcium. In a study aimed at investigating the effect of different methods of drying on the micronutrient content of green leafy vegetables, it was concluded that calcium was lower in sun-dried green leafy vegetables (Khatoniar *et al.,* 2019).

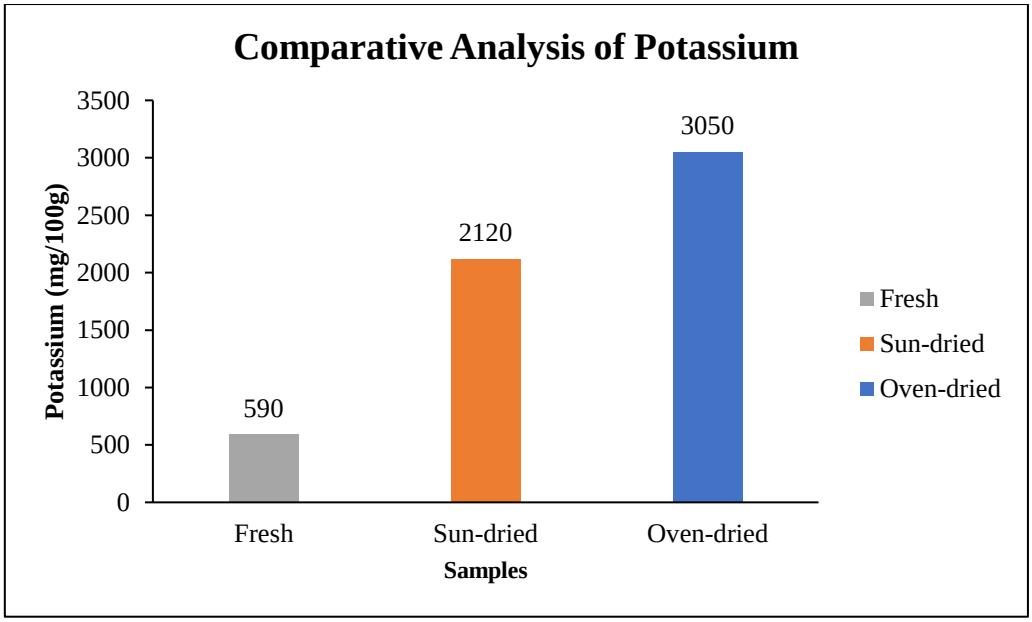

Figure 3. Comparative analysis of potassium

As depicted in figure 3, potassium was highest in oven-dried mushroom powder (3050 mg) and lowest in sun-dried oyster mushroom powder (2120 mg). Yarkwan and Uvir (2015) reported that oven-dried plantain sample had a higher potassium content compared to fresh sample.

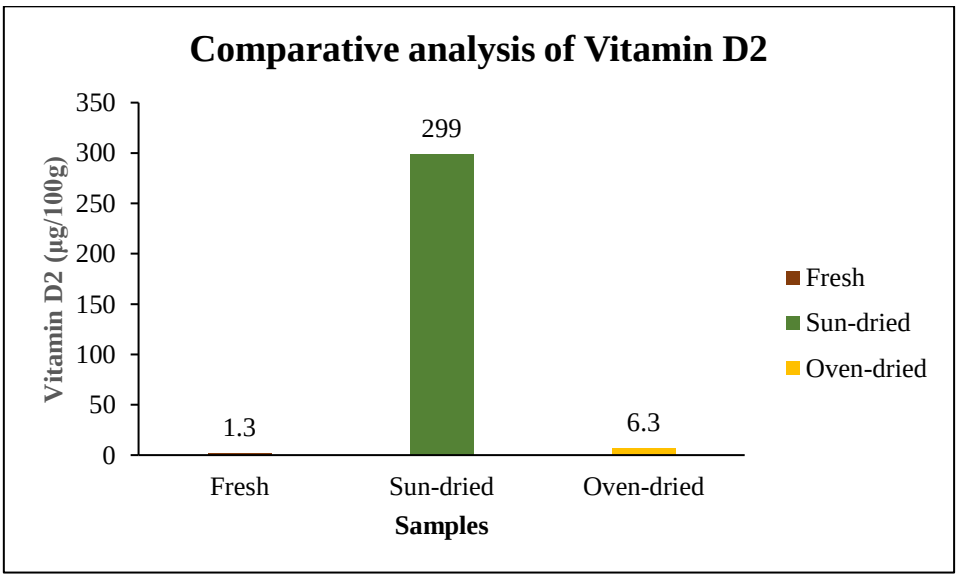

Figure 4. Comparative analysis of vitamin D2

From figure 4 it is evident that vitamin D2 was highest in sun-dried oyster mushroom powder (299 µg) and lowest in the fresh mushroom sample (1.3 µg). These findings are in line with the results obtained by Ibrahim *et al.* 2022, who mentioned that fresh oyster mushroom exposed to sunlight had a higher content of vitamin D2 compared to the fresh mushroom samples that were not exposed to sunlight.

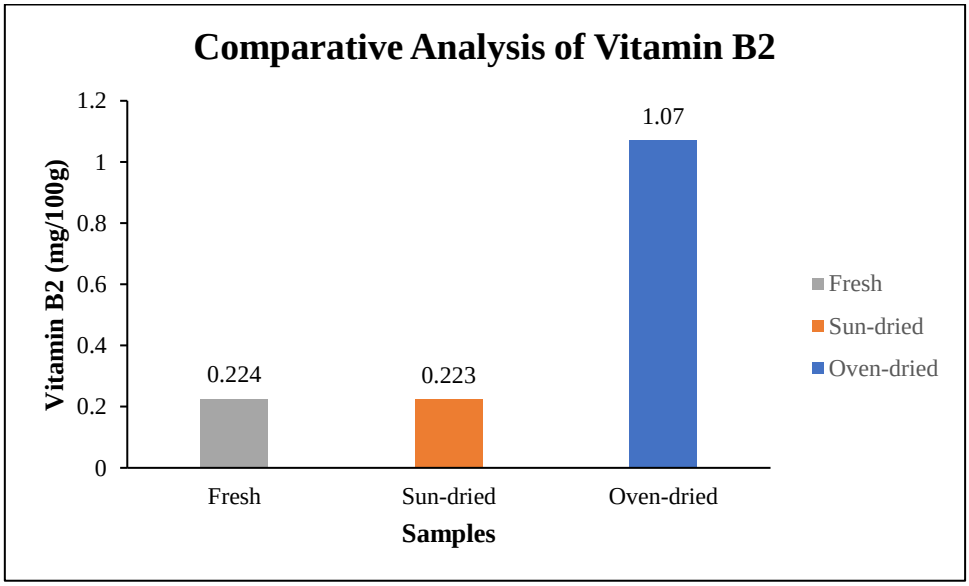

Figure 5. Comparative analysis of vitamin B2

As depicted in figure 5, vitamin B2 was highest in oven-dried oyster mushroom powder (1.07 mg) and lowest in sun-dried mushroom powder (0.223 mg). In a study conducted to investigate the effect of drying on water-soluble vitamin content of bulgur, it was found that oven-drying retained more vitamin B2 than sun-drying (Kadakal *et al.,* 2007).

In a study aimed at studying the effects of sun-drying and oven-drying on the nutrients, anti-nutrients and bioavailability of some minerals in certain capsicum varieties it was found that the sun-drying method had retained the minerals better when compared to the oven-drying method (Shokunbi *et al.,* 2021). The higher protein content in dried oyster mushroom powders, when compared to fresh mushroom, may be due to the fact that approximately 700 g of fresh oyster mushroom is required to get 100 g of dried oyster mushroom powder.

4.2 Shelf-life quality of the Dried Oyster Mushroom Powders and Fresh Oyster Mushrooms

The shelf-life of a food product food is defined as the maximum duration for which it remains both safe to eat and meets quality standards based on multiple evaluation criteria.

These criteria include nutritional value, consumer sensory perception and hygiene standards all while ensuring food safety. Accuracy in predicting and determining a product's shelf-life under specific condition helps in selecting appropriate storage and transportation methods (Li *et al.,* 2024). Total plate count test was done to assess the microbiological quality of the mushroom powders and fresh oyster mushrooms. The results are presented in table 5.

Table 5. Shelf-life quality assessment of the dried oyster mushroom powders and fresh oyster mushrooms

Sl. No.	Products	Total plate count (cfu/g)	Colour	Odour	pH
1.	Oven-dried oyster mushroom powder	860	Beige	Slightly fishy	6.48
2.	Sun-dried oyster mushroom powder	970	Beige	Slightly fishy	6.31
3.	Fresh oyster mushrooms	3100	White to off white	Agreeable	5.99

The shelf-life quality assessment was conducted for 10 days. From table 5 it is evident that, fresh oyster mushrooms have the highest microbial load (3100 cfu/g), indicating higher microbial activity, which may be attributed to their moisture content. Oven-dried oyster mushroom powder was found to have the lowest microbial load (860 cfu/g). These results are similar to the results obtained by another study which aimed at comparing the microbial load in mushrooms exposed to different drying methods. The study pointed out that oven-dried tomato slices had lowest viable fungal count compared to fresh slices (Omorodion and Obiobu, 2025).

4.3 Nutrient Calculation of the Oyster Mushroom Powder-Incorporated products

4.3.1 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Cupcakes

The selected nutrients for the oyster mushroom powder-incorporated cupcakes were calculated and depicted in Table 6.

Table 6. Nutrient calculation of oyster mushroom powder-incorporated cupcakes

Variations	Nutrients				
	Protein (g)	Calcium (mg)	Potassium (mg)	Vitamin D2 (µg)	Vitamin B2 (mg)
T0	15.49	77.45	535.60	9.95	0.38
T1	23.78	118.04	1457.97	7.68	0.44
T2	24.67	151.71	1530.29	8.62	0.46
T3	17.21	133.53	788.86	8.48	0.39

Table 6 indicates that the protein content was highest in T2 (24.67 g) and lowest in T0 (15.49 g). The calcium content was found to be highest in T2 (151.71 mg), while it was the lowest in T0 (77.45 mg). Potassium was highest in T2 (1530.29 mg) and was the lowest in T0 (535.60 mg). The vitamin D2 content was found to be highest in T0 (9.95 µg) and lowest in T1 (7.68 µg). This may be due to the high vitamin D2 content in wheat flour which was used in the highest quantities in T0. Vitamin B2 was the highest in T2 (0.46 mg) and the lowest in T0 (0.38 mg).

4.3.2 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Hummus

The selected nutrients for the oyster mushroom powder-incorporated hummus were calculated and depicted in Table 7.

Table 7. Nutrient calculation of oyster mushroom powder-incorporated hummus

Variations	Nutrients				
	Protein (g)	Calcium (mg)	Potassium (mg)	Vitamin D2 (µg)	Vitamin B2 (mg)
T0	14.32	367.07	587.95	15.08	0.12
T1	16.76	444.60	859.45	20.00	0.1181
T2	16.56	449.27	800.2	19.95	0.1189
T3	14.73	357.72	706.45	15.19	0.1198

Table 7 suggests that the protein content was highest in T1 (16.76 g) and lowest in T0 (14.32 g). The calcium content was highest in T2 (449.27 mg) and lowest in T3 (357.72 mg). Potassium was highest in T1 (859.45 mg) and lowest in T0 (587.95 mg). Vitamin D2 was found

to be highest in T1 (20.00 µg) and lowest in T0 (15.08 µg). Vitamin B2 was the highest in T0 (0.121 mg) and lowest in T1 (0.1181 mg).

4.3.3 Nutrient calculation of Oyster Mushroom Powder-Incorporated Appam

The selected nutrients for the oyster mushroom powder-incorporated appam were calculated and depicted in Table 8.

Table 8. Nutrient calculation of oyster mushroom powder-incorporated appam

Variations	Nutrients				
	Protein (g)	Calcium (mg)	Potassium (mg)	Vitamin D2 (µg)	Vitamin B2 (mg)
T0	7.06	15.02	147.6	0	0.048
T1	11.54	26.75	741.2	0.83	0.11
T2	10.42	23.81	592.8	0.62	0.09
T3	9.30	20.88	444.4	0.41	0.07

Table 8 suggests that the protein content was highest in T1 (11.54 g) and lowest in T0 (7.06 g). The calcium content was highest in T1 (26.75 mg) and the lowest in T0 (15.02 mg). The potassium content was highest in T1 (741.2 mg) and the lowest in T0 (147.6 mg). Vitamin D2 was highest in T1 (0.83 µg) and the lowest in T3 (0.41 µg). Vitamin B2 was highest in T1 (0.11 mg) and lowest in T0 (0.048 mg).

4.3.4 Nutrient Calculation of Oyster Mushroom Powder-Incorporated Bread Pakoda

The selected nutrients for the oyster mushroom powder-incorporated bread pakoda were calculated and depicted in Table 9.

Table 9. Nutrient calculation of oyster mushroom powder-incorporated bread pakoda

Variations	Nutrients				
	Protein (g)	Calcium (mg)	Potassium (mg)	Vitamin D2 (µg)	Vitamin B2 (mg)
T0	18.37	92.24	508.5	0.36	0.06
T1	18.45	94.54	763.3	0.96	0.082
T2	18.43	93.96	699.6	0.81	0.076
T3	18.41	93.39	635.9	0.66	0.071

Table 8 suggests that the protein content was highest in T1 (18.45 g) and the lowest in T0 (18.37 g). The calcium content was highest in T1 (94.54 mg) and the lowest in T0 (92.24 mg). The potassium content was highest in T1 (763.3 mg) and lowest in T0 (508.5 mg). The vitamin D2 content was highest in T1 (0.96 µg) and lowest in T0 (0.36 µg). The vitamin B2 content was highest in T1 (0.082 mg) and lowest in T0 (0.06 mg).

4.4 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Products

Organoleptic evaluation involves the examination of properties – texture, flavour, taste, appearance, smell, etc. – of a product or food through the different senses of the panelists and is considered to be very useful in the development of new foods (Ruiz-Capillas and Herrero, 2021).

The process of sensory evaluation involves scoring different aspects of the developed products such as appearance, colour, taste, flavour, texture and overall acceptability using a 9-point hedonic rating scale. The scoring was done by a panel of 10 trained judges.

4.4.1 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Cupcakes

The results for the oyster mushroom powder-incorporated cupcakes were presented in table 10.

Table 10. Sensory evaluation report of oyster mushroom powder-incorporated cupcakes

	T0 (control)	T1 (40%)	T2 (30%)	T3 (20%)	p-value
Appearance	8.57±0.75	6.79±1.12	7.29±1.06	7.79±1.25	T0 Vs T1 -<0.001* T0 Vs T2 -0.014* T0 Vs T3 -0.338
Colour	7.43±1.01	7.29±0.91	7.14±1.35	7.71±1.20	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Taste	7.43±1.39	6.64±1.39	7.14±1.09	8.07±1.43	T0 Vs T1 -0.760 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Texture	7.00±1.41	7.00±1.30	6.50±1.34	7.57±1.55	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Flavour	7.57±1.22	6.93±1.14	7.14±1.09	7.86±1.46	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Overall acceptability	7.29±1.06	6.50±1.01	6.79±1.18	8.07±1.38	T0 Vs T1 -0.49 T0 Vs T2 -1.00 T0 Vs T3 -0.494

Appearance

The appearance of a food product is defined by its visual attributes such as colour, shape, size, surface texture and overall presentation. The appearance and colour of a product are often the primary factors used by consumers to assess the quality if different food products (Carneiro *et al.,* 2022).

Considering the appearance of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 (40% incorporation) and T2 (30% incorporation) obtained significantly lowest scores (6.79±1.12, p=<0.001 and 7.29±1.06, p=0.014) as compared with the control. Thus, they were not accepted by the judges. T3 was not observed for any significant variation for its score in appearance as compared with the control.

Colour

The colour of a food product is a visual attribute linked to the spectral distribution of light as it interacts with matter (Silva *et al.,* 2022). It plays a crucial role in influencing consumer acceptance, as it, along with the appearance of a food product, is often the first characteristics judged (Carneiro *et al.,* 2022; Kutlu *et al.,* 2022). It can either stimulate a person's appetite or dissuade them from consuming the product (Kutlu *et al.,* 2022).

Considering the colour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.71±1.20). T1, T2 and T3 were not observed for any significant variation for their score in colour as compared with the control.

Taste

The taste of a food product is a sensation that is perceived through the taste buds that are present on the tongue. It is a very important characteristic of food. Considering the taste of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (8.07±1.43). T1, T2 and T3 were not observed for any significant variation for their score in taste as compared with the control.

Texture

The procedure of assessment of texture relies on a person's ability to interpret and describe their sensory experiences. Visual, tactile and auditory cues greatly influence the perception of food texture. The visual aspect is determined by prior experiences with similar foods, while the tactile component is determined by both mouthfeel and hand sensations. The auditory factor is determined by sounds produced by the food product, with lower-pitched sounds associated with crunchiness and higher-pinched sounds linked to crispiness (Rustagi, 2020).

Considering the texture of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.57±1.55). T1, T2 and T3 were not observed for any significant variation for their score in texture as compared with the control.

Flavour

The flavour of a food product is the sensory perception of food arising from the activation of chemical senses of taste and smell. It refers to the complete set of sensations experienced when consuming food or beverages. It includes a substance's aroma, taste and any physical characteristics that can be detected (Saqqa, 2022).

Considering the flavour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.86±1.46). T1, T2 and T3 were not observed for any significant variation for their score in flavour as compared with the control.

Overall acceptability

Considering the overall acceptability of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (8.07±1.38). T1, T2 and T3 were not observed for any significant variation for their score in overall acceptability as compared with the control.

The overall results of sensory evaluation of the oyster mushroom powder-incorporated cupcakes with control were presented in Figure 6.

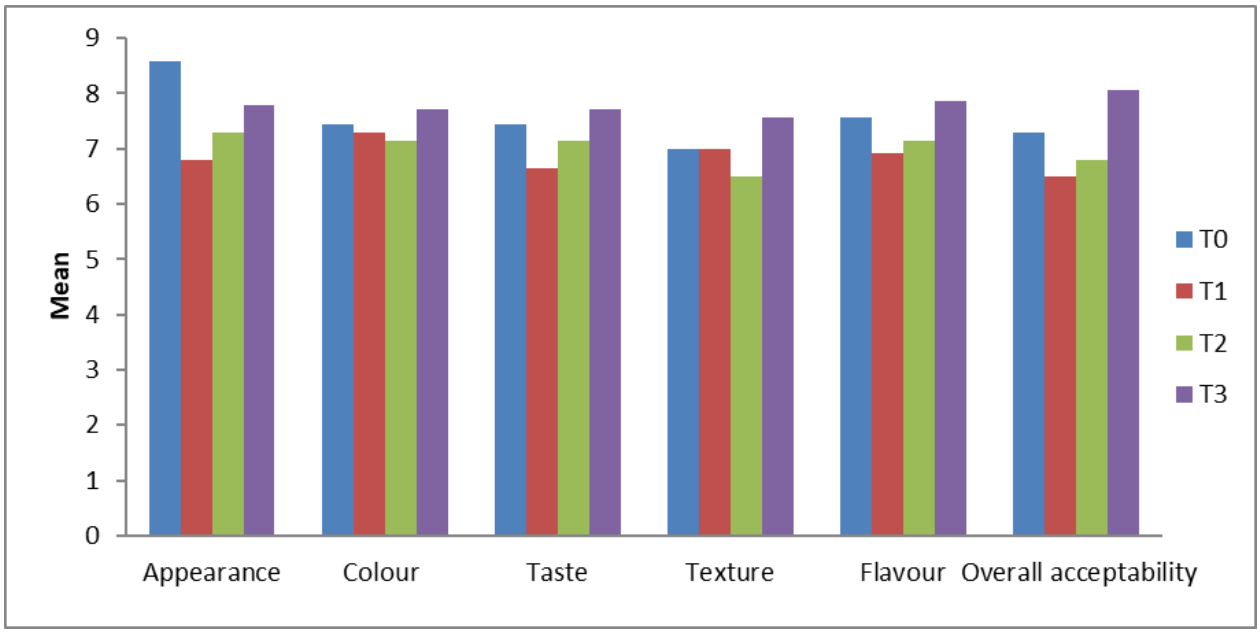

Figure 6. Sensory evaluation of oyster mushroom powder-incorporated cupcakes

From the overall analysis of quality attributes for the cupcakes the only attribute which had significantly lower score as compared with the control was the appearance. The appearance of T1 and T2 were observed for significantly lower scores of 6.79±1.12 and 7.29±1.06 respectively. All the other attributes such as colour, taste, texture, flavour and overall acceptability did not exhibit any significantly different score for the incorporated variations of T1, T2 and T3. It indicated overall consumer acceptability of the oyster mushroom powder-incorporated cupcake.

4.4.2 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Hummus

The results for the mushroom powder incorporated hummus were presented in table 11.

Table 11. Sensory evaluation report of oyster mushroom powder-incorporated hummus

	T0 (control)	T1 (40%)	T2 (30%)	T3 (20%)	p-value
Appearance	8.14±1.02	7.21±1.25	6.71±1.59	7.93±0.82	T0 Vs T1- 0.282 T0 Vs T2 -0.017* T0 Vs T3 -1.00
Colour	8.00±1.30	7.07±1.07	6.86±1.40	7.86±0.86	T0 Vs T1 -0.253 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Taste	7.64±1.78	6.21±1.31	6.14±1.40	7.71±0.82	T0 Vs T1 -0.049* T0 Vs T2 -0.034* T0 Vs T3 -1.00
Texture	7.71±1.68	7.36±1.15	6.93±1.49	7.86±0.77	T0 Vs T1 -1.00 T0 Vs T2 -0.729 T0 Vs T3 -1.00
Flavour	7.79±1.47	6.50±1.34	6.07±1.43	7.71±0.72	T0 Vs T1 -0.064 T0 Vs T2 -0.005* T0 Vs T3 -0.093
Overall acceptability	7.71±1.59	7.29±1.43	6.21±1.36	7.79±0.69	T0 Vs T1 -1.00 T0 Vs T2 -0.024* T0 Vs T3 -1.00

Appearance

The appearance of a food product, which refers to the visual characteristics of that particular product, is a key factor that influences the culinary experience of an individual (Suzuki *et al.,* 2021). Considering the appearance of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T2 obtained a significantly low score (6.71±1.59, p=0.017) as compared with the control. Thus, it was not accepted by the judges. T1 and T3 were not observed for any significant variation for their score in appearance as compared with the control.

Colour

Visual cues such as colour, shape, etc. affect food selection and acceptance. It also greatly determines flavour perception, particularly flavour recognition or flavour intensity (Hoppu *et al.,* 2018). Considering the colour of the developed products, the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder, did not have any significantly lower or higher score as compared with that of the control. This indicated that the colour of the three variations was accepted for consumer evaluation.

Taste

Taste remains the primary factor influencing consumers' food and beverage purchasing decisions. It refers to sensations such as sweet, salty, bitter, sour and umami with the potential inclusion of fat and starchy/carbohydrate perceptions. Individuals' food choices are greatly determined by their taste perceptions (Nolden and Feeney, 2020).

Considering the taste of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.71±0.82). T1 and T2 obtained significantly low scores (6.21±1.31, p=0.049 and 6.14±1.40, p=0.034) as compared with the control. T3 was not observed for any significant variation for its score in taste as compared with the control.

Texture

Beyond taste and appearance, texture and mouthfeel play a significant role in the acceptance of food by an individual. Although taste and appearance are the primary aspects

that draw attention during food production and influence purchasing decisions, texture is essential in shaping food preferences and can determine food choices (Pereira *et al.,* 2021).

Considering the texture of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.86±0.77). T1, T2 and T3 were not observed for any significant variation for their score in texture as compared with the control.

Flavour

It is the most important sensory quality in food and combines the perception of aroma, taste, texture and other sensations. Considering the flavour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T2 obtained a significantly low score (6.07±1.43, p=0.005) as compared to the control. Thus, it was not accepted by the judges. T1 and T3 were not observed for any significant variation for their score in flavour as compared with the control.

Overall acceptability

Considering the overall acceptability of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% mushroom powder respectively, T3 had the highest score (7.79±0.69) and T2 obtained a significantly low score as compared with the control (6.21±1.36, p=0.024). T1 and T3 were not observed for any significant variation for their score in overall acceptability as compared with the control.

The overall results of sensory evaluation of the oyster mushroom powder-incorporated hummus with control were presented in Figure 7.

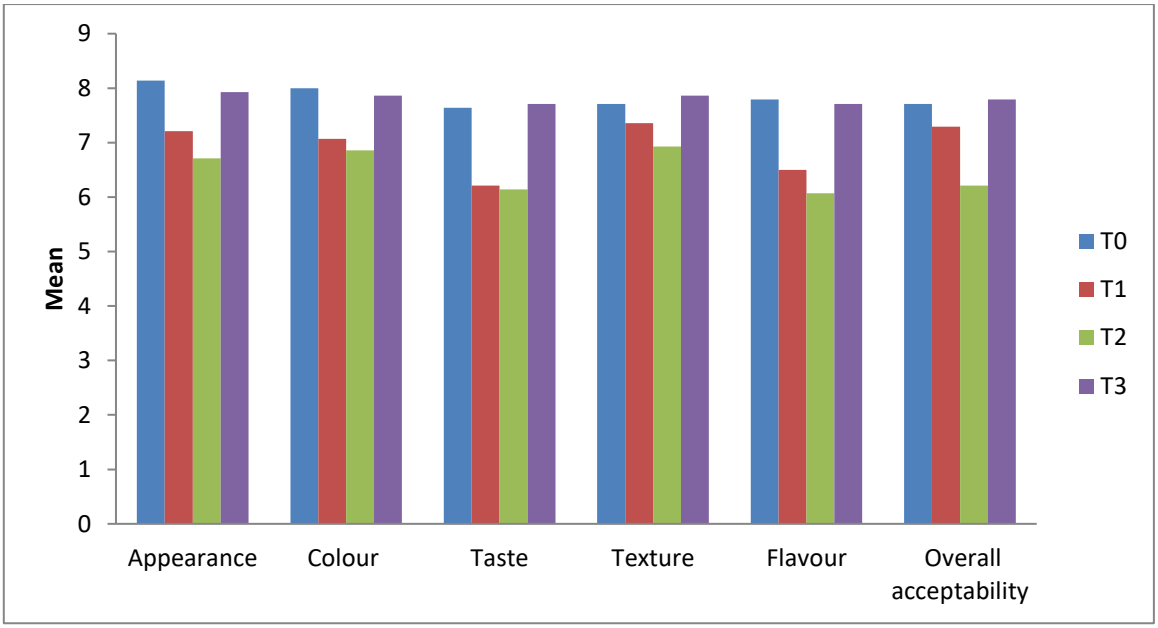

Figure 7. Sensory evaluation of oyster mushroom powder-incorporated hummus

From the overall analysis of quality attributes for hummus, appearance, taste, flavour and overall acceptability were the attributes which had significantly lower score as compared with the control. The appearance of T2, taste of T1 and T2, flavour of T2 and the overall acceptability of T2 was observed for significantly lower scores of 6.71±1.59, 6.21±1.31, 6.21±1.31 and 6.14±1.40, 6.07±1.43 and 6.21±1.36 respectively. The other attributes such as colour and texture did not exhibit any significantly different scores for the incorporated variations of T1, T2 and T3. It indicates overall consumer acceptability of the oyster mushroom powder-incorporated hummus.

4.4.3 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Appam

The results for the oyster mushroom powder incorporated appam were presented in table 12.

Table 12. Sensory evaluation report of oyster mushroom powder-incorporated appam

	T0	T1 (40%)	T2 (30%)	T3 (20%)	p-value
Appearance	7.50±1.09	3.64±2.09	5.57±1.50	6.14±1.87	T0 Vs T1 -<0.001* T0 Vs T2 -0.023* T0 Vs T3 -0.228
Colour	7.64±2.30	4.07±2.43	5.71±2.01	6.07±1.43	T0 Vs T1 -<0.001* T0 Vs T2 -0.252 T0 Vs T3 -0.085
Taste	7.50±1.45	3.00±2.41	4.86±1.99	5.71±2.05	T0 Vs T1 -<0.001* T0 Vs T2 -0.006* T0 Vs T3 -0.135
Texture	7.07±2.16	3.50±1.99	5.71±2.09	5.93±1.73	T0 Vs T1 -<0.001* T0 Vs T2 -0.471 T0 Vs T3 -0.821
Flavour	7.00±2.25	2.71±2.05	4.93±2.33	5.64±2.62	T0 Vs T1 -<0.001* T0 Vs T2 -1.33 T0 Vs T3 -0.771
Overall acceptability	7.64±1.00	3.57±2.17	5.36±2.06	6.29±1.68	T0 Vs T1 -<0.001* T0 Vs T2 -0.008* T0 Vs T3 -0.301

Appearance

The appearance of a food product is the first sensory attribute that consumers perceive, often influencing their perception of its quality. Considering the appearance of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 and T2 obtained significantly low scores (3.64±2.09, p=<0.001 and 5.57±1.50, p=0.023) as compared with the control. Thus, they were not accepted by the judges. T3 was not observed for any significant variation for its score in appearance as compared with the control.

Colour

The colour of a food product can help consumers perceive the flavour, ripeness, nutritional value, texture, taste and safety of that particular food. Considering the colour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 obtained a significantly low score (4.07±2.43, p=<0.001) as compared to the control. Thus, it was not accepted by the judges. T2 and T3 were not observed for any significant variation for their score in colour as compared with the control.

Taste

This attribute of a food product determines the acceptance of foods. Genetic and environmental factors, metabolic diseases, cancer and metabolic syndrome can lead to individual differences in sensory perceptions (Tepper and Barbarossa, 2020). Considering the taste of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T0 had the highest score (7.50±1.45). T1 and T2 obtained significantly low scores (3.00±2.41, p=<0.001 and 4.86±1.99, p=0.006). T3 was not observed for any significant variation for its score in taste as compared with the control.

Texture

The texture of foods is an important attribute that triggers a psychological response. This influences its quality and acceptability. The perception of texture is determined by the physical characteristics of the food, including its structure, composition and the rate at which it deforms in the mouth. The sensory mechanisms that contribute to texture perception involve discriminative touch, sense of jaw position and movement, sensation of pain and the perception of temperature (Rustagi S., 2020).

Considering the texture of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T0 had the highest score (7.07±2.16). T1 obtained a significantly low score (3.50±1.99, p=<0.001) as compared to the control. T2 and T3 were not observed for any significant variation for their score in texture as compared with the control.

Flavour

The flavour of foods plays a crucial role in determining food palatability and significantly impacts dietary choices, nutrition and overall health (Tepper and Barbarossa, 2020). Considering the flavour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T0 had the highest score (7.00±2.25). T1 obtained a significantly low score (2.71±2.05, p=<0.001) as compared to the control. T2 and T3 were not observed for any significant variation for their score in flavour as compared with the control.

Overall acceptability

Considering the overall acceptability of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% mushroom powder respectively, T1 (40% incorporation) and T2 (30% incorporation) obtained significantly low scores (3.57±2.17, p=<0.001 and 5.36±2.06, p=0.008) as compared to the control. Thus, they were not accepted by the judges. T3 was not observed for any significant variation for its score in overall acceptability.

The overall results of sensory evaluation of the oyster mushroom powder-incorporated appam with control were presented in Figure 8.

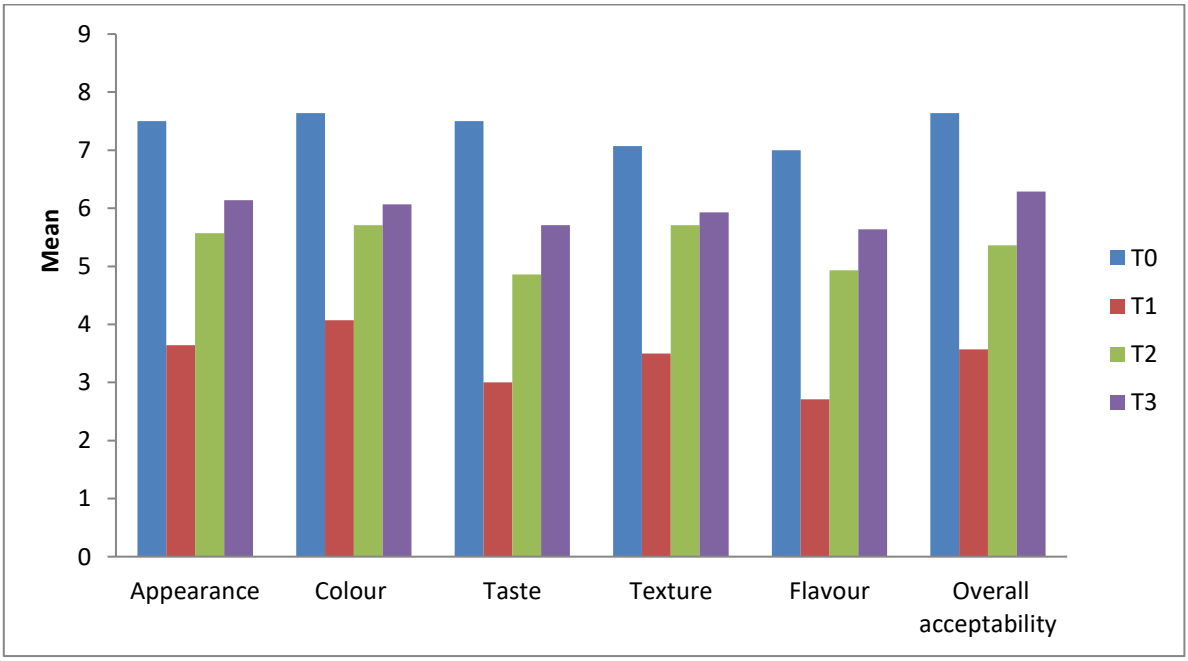

Figure 8. Sensory evaluation of oyster mushroom powder-incorporated appam

From the overall analysis of quality attributes for appam, T1 and T2 showed significantly low scores for all attributes as compared with the control. This indicated that T2 was the most accepted variation.

4.4.4 Organoleptic Evaluation of Oyster Mushroom Powder-Incorporated Bread Pakoda

The results for the oyster mushroom powder incorporated bread pakoda were presented in table 13.

Table 13. Sensory evaluation report of oyster mushroom powder-incorporated bread pakoda

	T0 (control)	T1 (40%)	T2 (30%)	T3 (20%)	p-value
Appearance	8.29±0.99	6.57±1.55	7.00±1.24	8.00±0.67	T0 Vs T1 -0.002* T0 Vs T2 -0.03* T0 Vs T3 -1.00
Colour	8.50±0.94	6.64±1.39	6.93±1.14	7.93±0.61	T0 Vs T1 -<0.001* T0 Vs T2 -0.002* T0 Vs T3 -0.962
Taste	7.14±1.61	7.14±1.40	7.79±1.36	7.57±1.69	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Texture	7.57±1.01	7.79±0.89	7.57±0.85	7.50±1.28	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Flavour	7.07±1.43	7.07±1.26	7.57±1.08	7.64±1.59	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00
Overall acceptability	7.07±1.54	7.29±1.43	7.79±1.31	7.64±1.59	T0 Vs T1 -1.00 T0 Vs T2 -1.00 T0 Vs T3 -1.00

Appearance

Appearance is a crucial attribute evaluated by consumers and is closely associated with the quality of a food product. Considering the appearance of the three variations, T1, T2 and

T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 and T2 obtained significantly low scores (6.57±1.55, p=0.002 and 7.00±1.24, p=0.03) as compared with the control. Thus, they were not accepted by the judges. T3 was not observed for any significant variation for its score in appearance as compared with the control.

Colour

The colour of the food product indicates their taste and flavour. Considering the colour of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 and T2 obtained significantly low scores (6.64±1.39, p=<0.001 and 6.93±1.14, p=0.002) as compared with the control. Thus, they were not accepted by the judges. T3 was not observed for any significant variation for its score in colour as compared with the control.

Taste

Taste is a crucial attribute of the overall experience with food. From the moment a bite is taken, the sensory system becomes actively engaged allowing for the perception and distinction of flavours, textures and aromas that make each dish unique (Helen V., 2023). Considering the taste of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T2 had the highest score (7.79±1.36). T1, T2 and T3 was not observed for any significant variation for their score in taste as compared with the control.

Texture

Texture is a property of food that greatly influences that product's consumer acceptance. It also determines the selection of foods and the eating behaviour. Considering the texture of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T1 had the highest score (7.79±0.89). T1, T2 and T3 was not observed for any significant variation for their score in texture as compared with the control.

Flavour

The flavour of a food product involves a combination of different senses like smell, touch and taste. Considering the flavour of the three variations, T1, T2 and T3 which were

prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T3 had the highest score (7.64±1.59). T1, T2 and T3 was not observed for any significant variation for their score in flavour as compared with the control.

Overall acceptability

Considering the overall acceptability of the three variations, T1, T2 and T3 which were prepared with the incorporation of 40%, 30% and 20% oyster mushroom powder respectively, T2 had the highest score (7.79±1.31). T1, T2 and T3 was not observed for any significant variation for their score in overall acceptability as compared with the control.

The overall results of sensory evaluation of the oyster mushroom powder-incorporated bread pakoda with control were presented in Figure 9.

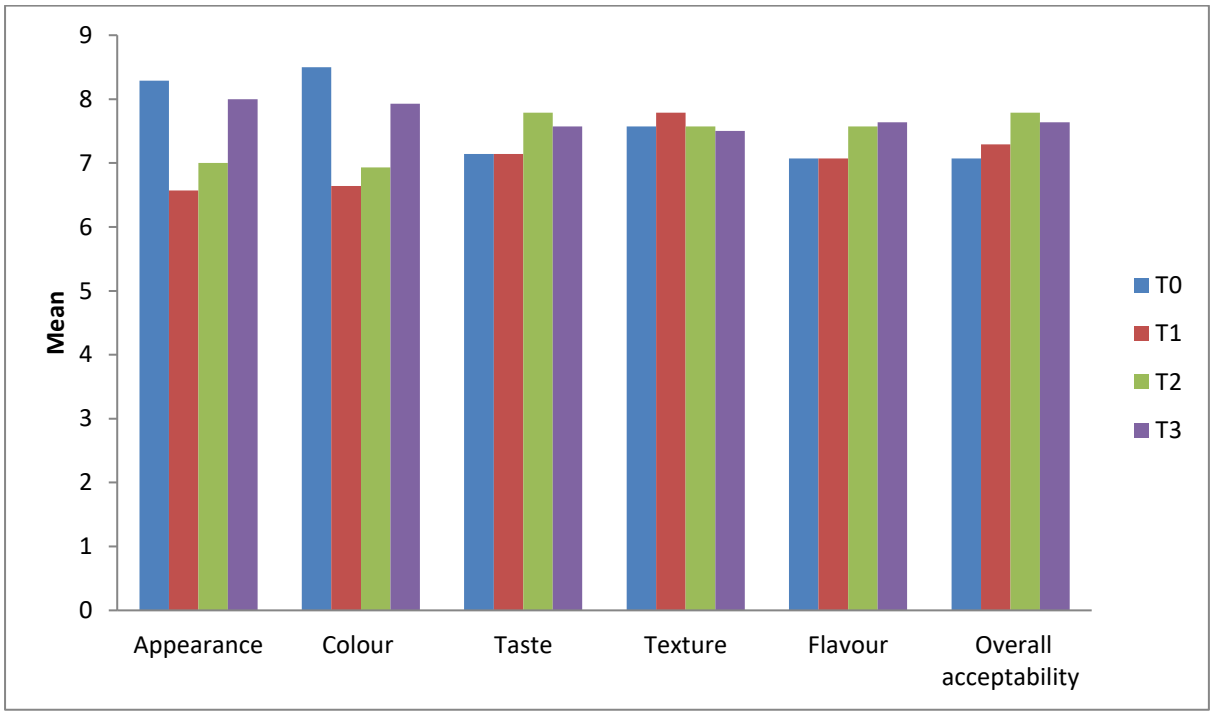

Figure 9. Sensory evaluation of oyster mushroom powder-incorporated bread pakoda

From the overall analysis of quality attributes for the bread pakoda, the attributes which had significantly lower score as compared with the control was the appearance and colour. The appearance of T1 and T2 and colour of T1 were observed for significantly lower scores of 6.57±1.55, 7.00±1.24 and 6.64±1.39 respectively. All the other attributes such as taste, texture, flavour and overall acceptability did not exhibit any significantly different score for the incorporated variations of T1, T2 and T3. It indicated overall consumer acceptability of the oyster mushroom powder-incorporated bread pakoda.

5. SUMMARY AND CONCLUSION

Oyster mushrooms are considered to be very good sources of vitamins especially, vitamin D2, certain B-vitamins and minerals like potassium and calcium and possess various health benefits like promoting heart health, improving immunity and reducing the risk of cancer among others. They are also good sources of antioxidants. Owing to more and more people switching to plant-based diets, mushrooms have attracted significant attention in recent research. Studies on using mushrooms as an alternative to meat have also been conducted. They are highly valued, not only due to their array of nutritional benefits, but also due to the savoury, umami flavour that makes any bland food taste better. Converting mushrooms into dried powders can greatly improve the shelf-life of fresh mushroom.

Vitamin D deficiency is a major public health problem even in a tropical country like India. This may be due to limited intake of vitamin D from the diet, insufficient exposure to sunlight or certain disease conditions. It is associated with a higher prevalence of certain diseases. This has been a driving factor for an increasing number of products being fortified with vitamin D in the recent years. Mushrooms, being rich in vitamin D can be used as a valuable option especially for vegetarians or for those with limited exposure to sunlight.

The aim of this study was to find out the which method, out of sun-drying and oven-drying, would better retain the nutrient content of oyster mushrooms, particularly vitamin D2. The dried mushrooms were then powdered and incorporated in food products like cupcakes, hummus, appam and bread pakoda, in three different variations (20%, 30% and 40%). Organoleptic evaluation of the prepared products was then carried out to judge their consumer acceptability.

Based on the result obtained from the nutritional analysis, it is evident that vitamin D2 content was highest in sun-dried mushroom powder. This is attributed to the conversion of pro-vitamin D2 to vitamin D2 in the presence of sunlight. But other nutrients like potassium, calcium, protein and vitamin B2 were found to be highest in oven-dried mushroom powder followed by sun-dried mushroom powder and fresh mushroom. The high nutrient content retention in oven-dried mushroom powder may be due to the fact that a huge amount (approximately 700 g) of fresh mushroom would be needed to make at least 100 g of dried oyster mushroom powder. Even though there would be minute losses in the nutrients, there would still be a significant amount of nutrients still remaining in the mushrooms after oven-drying.

The shelf-life quality assessment was done using total plate count method and it was found that oven-dried oyster mushroom powder was found to have the lowest microbial load, while fresh mushroom was found to have the highest microbial load.

The nutrient calculation of mushroom powder incorporated cupcakes showed that the all other nutrients except vitamin D2 was found to be higher in T2 (30% incorporation). Vitamin D2 was highest in T0 which may be due to the high vitamin D2 content in wheat flour. In the case of the mushroom powder-incorporated hummus protein, potassium and vitamin D2 was found to be the highest in T1 (40% incorporation). Calcium and vitamin B2 were highest in T2 (30% incorporation) and T0 (control) respectively. The nutrient calculation of mushroom powder incorporated appam suggested that T1 (40% incorporation) had the highest content of all the nutrients. The nutrient calculation of bread pakoda also showed that all the nutrients were highest in T1 (40% incorporation).

The results of the organoleptic evaluation of the mushroom powder incorporated cupcakes suggested that, the only attribute which had significantly lower score as compared with the control was the appearance. The appearance of T1 (40% incorporation) and T2 (30% incorporation) were observed for significantly lower scores. In the case of mushroom powder incorporated hummus, appearance, taste, flavour and overall acceptability were the attributes which had significantly lower score as compared with the control. The appearance of T2 (30% incorporation), taste of T1 (40% incorporation) and T2 (30% incorporation), flavour of T2 (30% incorporation) and the overall acceptability of T2 (30% incorporation) was observed for significantly lower scores. This indicated the consumer acceptability of the hummus. In the case of appam, T1 (40% incorporation) and T2 (30% incorporation) showed significantly lower scores for all the attributes, indicating that these variations were not accepted by the judges. The results of the overall analysis of quality attributes of bread pakoda, the attributes which had significantly lower score as compared with the control was the appearance and colour. The appearance of T1 (40% incorporation) and T2 (30% incorporation) and colour of T1 (40% incorporation) were observed for significantly lower scores. It indicated overall consumer acceptability of the mushroom powder-incorporated bread pakoda. From the above results it is evident that the most preferred variation of incorporation of mushroom powder in the products was 20%.

Future research could focus on how the different colours that different varieties of oyster mushroom have would affect the vitamin D2 synthesis in them.

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APPENDIX 1

NUTRIENT ANALYSIS OF THE FRESH OYSTER MUSHROOM AND DRIED OYSTER MUSHROOM POWDER

ESTIMATION OF PROTEIN

1. **Protocol:** AOAC 21st Edition 991.20
2. **Procedure:**
    - Weigh accurately 0.7 to 2.2g of the sample into the digestion flask and add 0.7g mercury oxide or copper sulphate and 15g potassium sulphate or anhydrous sodium sulphate, and 25 ml sulphuric acid.
    - Each gram of fat consumes 10 ml and each gram of carbohydrate 4 ml sulphuric acid during digestion.
    - Place the flask in an inclined positing on a heater and heat gently until foaming ceases. Then boil vigorously until the solution becomes clear and then continue boiling it for 1 to 2 hours.
    - Distillation- Cool the digested sample and add about 200 ml distilled water into it. Add a few zinc granules/glass beads to prevent bumping, incline flask, and add 50% NaOH solution. Immediately connect flask to distillation bulb or trap on condenser, and add with tip of the condenser immersed in a measured quantity standard acid in the receiver, rotate flask to mix the contents thoroughly then heat immediately until all ammonia has distilled over (at least 150 ml distilled).
    - Lower the receiver before stopping distillation and wash tip of condenser with distilled water.
    - Back-titrate excess acid with standard 0.1 N Sodium hydroxide, using methyl red as indicator. Correct for blank determination in reagents.
3. **Calculation:**

$$\text{Protein} = \frac{(\text{TV for blank} - \text{TV for sample}) \times 1.4007 \times \text{normality of NaOH} \times 6.25}{\text{Weight of sample}}$$

where TV = Titration volume

6.25 = Nitrogen to protein conversion factor

ESTIMATION OF CALCIUM

1. **Protocol:** IS 4285 : 2003
2. **Scope:** This standard prescribes the method for the volumetric determination of calcium by the potassium permanganate method.
3. **Reagents:**
    - Ammonium Oxalate Solutions – (a) saturated, and (b) 1 percent (w/v)
    - Methyl Red Indicator Solution -Dissolve 0.15 g of methyl red in water and dilute to 500 ml
    - Dilute Ammonium Hydroxide - approximately 5 N
    - Dilute Sulphuric Acid - 1:4 (v/v)
    - Standard Potassium Permanganate Solution - 0.02 N
    - Concentrated Hydrochloric Acid - conforming to IS : 265-1962
    - Dilute Hydrochloric Acids - (a) 1:4 (v/v), and (b) 1:100 (v/v)
4. **Procedure:**
    - Take a clear weakly acidic solution as prepared containing not more than 50 mg of calcium.
    - Dilute to 200 ml and add 2 to 3 drops of methyl red indicator.
    - Add 5 ml of concentrated hydrochloric acid.
    - Heat to boiling and add with constant stirring an excess of hot ammonium oxalate solution.
    - Heat to 70 to 80°C and add with constant stirring dilute ammonium hydroxide (5 N) dropwise until the colour changes from red to yellow.
    - Allow the solution to stand at 70 to 80°C for one hour and filter through filter paper.
    - Wash the precipitate with cold ammonium oxalate solution (1 percent).
    - Dissolve the precipitate in 50 ml of hot 1:4 hydrochloric acid.
    - Wash the paper with hot 1:100 hydrochloric acid and dilute to 200 ml.
    - Carry out precipitation of calcium oxalate as described above.
    - Allow the solution to stand for 4 hours and then filter through a filter paper.
    - Wash the precipitate with cold ammonium oxalate solution (1 percent).
    - Dissolve the precipitate in 50 ml of hot 1:4 hydrochloric acid.
    - Wash the paper with hot 1:100 hydrochloric acid and dilute to 200 ml.

- Carry out precipitation of calcium oxalate as described above.
- Allow the solution to stand for 4 hours and then filter through a filter paper.
- Wash the precipitate with cold ammonium oxalate solution (1 percent) until it is free from chlorides and then with minimum quantity of hot water until free from oxalates.
- Pierce the apex of the filter paper with a stirring rod and wash down the bulk of the precipitate into a conical flask.
- Dissolve the precipitate by pouring warm dilute sulphuric acid.
- Finally, wash the filter paper thoroughly with hot water.
- Add about 30 ml of dilute sulphuric acid 10 the washings, dilute to about 200 ml, heat to 60°C and titrate while hot with standard potassium permanganate solution to an end point of pink colour persisting for 30 seconds.

DETERMINATION OF POTASSIUM (FLAME PHOTOMETRIC)

1. **Protocol:** IS 9497 : 1980 RA 1998
2. **Scope:**
   This standard prescribes method for the estimation of potassium by flame photometer.
3. **Apparatus:** Flame Photometer
4. **Reagents:**
   - Concentrated Hydrochloric Acid -See IS : 265-19767
   - Concentrated Nitric Acid - See IS : 264-1976%.
   - Hydrofluoric Acid
   - Potassium Chloride
   - Sodium Chloride
5. **Procedure:** The material is powdered and homogenized so as to pass through 150 micron IS Sieve. A known mass of the air-dried sample is suitably decom- posed in acid mixture, filtered and diluted to a definite volume. This test solution or its suitable dilutions are then aspirated in the flame photometer. From the galvanometer reading the concentration of sodium or potassium can be obtained by referring to a calibration curve. Ground water sample or its suitable dilution can be directly aspirated and alkali metals determined.

APPENDIX 2

PREPARATION OF RECIPE

CUPCAKE

INGREDIENTS	CONTROL	T1	T2	T3
Wheat Flour	70 g	42 g	49 g	56 g
Mushroom powder	-	28 g	21 g	14 g
Egg	1 no.	1 no.	1 no.	1 no.
Brown sugar	70 g	70 g	70 g	70 g
Baking powder	½ tsp	½ tsp	½ tsp	½ tsp
Baking soda	A pinch	A pinch	A pinch	A pinch
Salt	A pinch	A pinch	A pinch	A pinch
Oil	50 ml	50 ml	50 ml	50 ml
Milk	45 ml	45 ml	45 ml	45 ml
Vanilla essence	1 tsp	1 tsp	1 tsp	1 tsp

Procedure

- Preheat the oven to 180°C.
- Arrange paper cups on a baking tray.
- Take flour, baking powder, baking soda, and salt in a sifter and sieve it into a bowl.
- Take milk, oil and vanilla essence in a bowl and mix well.
- Take egg in a bowl and whisk well.
- Add sugar, little at a time and keep whisking till light and fluffy.
- Add dry ingredients and wet ingredients and mix well.
- Now pour the batter into the cupcake liners and bake for 20-25 minutes, or until a skewer inserted into the center of the cake comes out clean.
- Once cooled, they are ready to be served.

HUMMUS

INGREDIENTS	CONTROL	T1	T2	T3
Chickpeas	50 g	30 g	35 g	40 g
Mushroom powder	-	20 g	15 g	10 g
Sesame seeds	22.5 g	22.5 g	30 g	30 g
Olive oil	15 ml	15 ml	15 ml	15 ml
Lemon juice	15 ml	15 ml	15 ml	15 ml
Garlic	1 no.	1 no.	1 no.	1 no.
Salt	A pinch	A pinch	A pinch	A pinch
Water	2-3 tbsp	2-3 tbsp	2-3 tbsp	2-3 tbsp

Procedure:

- Cook the chickpeas.
- Lightly toast the sesame seeds in a pan over low heat until golden. Let them cool.
- Grind the toasted sesame seeds into a paste.
- In a blender combine chickpeas, sesame paste, olive oil, lemon juice, garlic and salt. Lightly sauté mushroom powder and add that into the blender. Blend until smooth.
- Adjust the consistency by adding water.
- Add more lemon juice, salt or olive oil if needed.
- Transfer to a bowl. Drizzle with olive oil and optionally sprinkle paprika or extra sesame seeds.
- Transfer to a bowl and serve with a drizzle of olive oil and optionally sprinkle paprika or extra sesame seeds.

APPAM

INGREDIENTS	CONTROL	T1	T2	T3
Rice flour	70 g	42 g	49 g	56 g
Mushroom powder	-	28 g	21 g	14 g
Coconut	60 g	60 g	60 g	60 g
Warm water	As required	As required	As required	As required
Yeast	½ tsp	½ tsp	½ tsp	½ tsp
Sugar	37.5 g	40 g	37.5 g	37.5 g

Procedure:

- Grind together rice flour and coconut adding water to make a smooth batter.
- Mix sugar and yeast in warm water and keep for 5 minutes to raise.
- After 5 minutes add this to the previously ground mixture.
- Add some more sugar to the mixture.
- Keep it for 10-12 hours.
- Use this batter to prepare appam.

BREAD PAKODA

INGREDIENTS	CONTROL	T1	T2	T3
Wheat bread	3 nos.	3 nos.	3 nos.	3 nos.
Mushroom powder	-	20 g	15 g	30 g
Oil	For frying	For frying	For frying	For frying
Chickpea flour	50 g	30 g	35 g	40 g
Ajwain	¼ tsp	¼ tsp	¼ tsp	¼ tsp
Green chilli	1 tsp	1 tsp	1 tsp	1 tsp
Onion	50 g	50 g	50 g	50 g
Coriander leaves	2 tbsp chopped	2 tbsp chopped	2 tbsp chopped	2 tbsp chopped
Coriander powder	½ tsp	½ tsp	½ tsp	½ tsp
Turmeric powder	¼ tsp	¼ tsp	¼ tsp	¼ tsp
Chili powder	¼ tsp	¼ tsp	¼ tsp	¼ tsp
Salt	¼ tsp	¼ tsp	¼ tsp	¼ tsp

Procedure:

- Mix all the ingredients together with water to make a thick batter without lumps.
- Cut the 3 slices of bread into triangles and dip them in the prepared batter. Deep fry them in oil till golden brown.

APPENDIX 3

SCORE CARD FOR SERIAL DILUTION TEST

SL. NO:	SAMPLE	GRADE (1,2,3,4,5,6,7)
1.	A	
2.	B	
3.	C	
4.	D	
5.	E	
6.	F	
7.	G	

*Mark above given solution based on the intensity of sweetness

NAME:

SIGNATURE:

APPENDIX 4

SCORE CARD FOR SENSORY EVALUATION

TREATMENTS	PARTICULATES					
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
T_1						
T_2						
T_3						
T_0						

***Kindly indicate your rating between 1-9 (1 stands for poor and 9 stands for excellent)**

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

NAME:

SIGNATURE: