

FORMULATION OF PROTEIN AND FIBRE ENRICHED INSTANT SOYA MUSHROOM SOUP MIX

PUJA DAS
(20PFD024)

Thesis submitted to



**Avinashilingam Institute for Home Science and
Higher Education for Women,
Coimbatore – 641 043**

**In partial Fulfillment of the Requirements for the
Degree of Master of Science in
FOOD SERVICE MANAGEMENT AND DIETETICS**

May 2022

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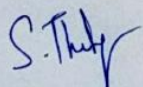
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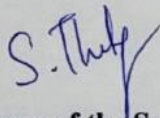
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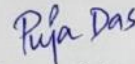
Signature of the Head of the Department

CERTIFICATE

This is to certify that the thesis entitled, "**Formulation of Protein and Fibre Enriched Instant Soya Mushroom Soup Mix**" submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in Partial fulfilment of the requirements for the award of the degree of Master of Science in Food Service Management and Dietetics, is a record of original research work done by **Ms. Puja Das** with Register Number 20PFD024 during the period of this study under the Supervision and Guidance of **Dr. S. Thilagamani**, Assistant Professor (SG), Department of Food Service Management and Dietetics, Avinashilingam Institute For Home Science and Higher Education For Women, Coimbatore- 641 043, Tamil Nadu, India.



Signature of the Supervisor



Signature of the Candidate

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CONTENTS

Chapter No.	Title	Page No.
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF ANNEXURES	
I	INTRODUCTION	1-7
II	REVIEW OF LITERATURE	8-25
	A. Importance of Protein and Fiber Rich Foods	8-13
	B. Nutritional Benefits of Oyster Mushrooms and Soya Beans	13-21
	C. Need for Ready to Eat and Ready to Serve Foods in Indian Market	21-25
III	METHODOLOGY	26-37
	A. Selection of ingredients	26-27
	B. Standardization process of instant ready to use soup mix	28-34
	C. Food Costing and Packaging of the instant ready to use soup mix	34-35
	D. Acceptability of the formulated ready to use soup mix	36
IV	RESULTS AND DISCUSSION	38-57
	A. Selection of ingredients	38-39
	B. Standardization process of instant ready to use soup mix	39-50
	C. Food Costing and Packaging of the instant ready to use soup mix	51-55
	D. Acceptability of the formulated ready to use soup mix	56
V	SUMMARY AND CONCLUSION	57-60
	BIBLIOGRAPHY	61-67
	ANNEXURES	68-81

LIST OF TABLES

Table No.	Title	Page No.
I	Different variations of the soup mix per serving	32
II	Formulation of trial 1 (AAA)	39
III	Formulation of trial 2 (BBB)	40
IV	Formulation of trial 3 (CCC)	41
V	Formulation of trial 4 (DDD)	42
VI	Total sensory evaluation score for trials by ten semi trained panel members	46
VII	Mean sensory acceptability score of the trials for instant soy mushroom soup mix	46
VIII	Total sensory evaluation score by ten semi trained panel members	48
IX	Mean sensory acceptability score of the instant soy mushroom soup mix for the developed variations	48
X	Nutritional composition of soy mushroom soup mix	50
XI	Shelf life study of instant soy mushroom soup mix	51
XII	Calculation of food cost of the developed product	52
XIII	Mean sensory acceptability score of the instant soy mushroom soup mix among homemakers	56

LIST OF FIGURES

Figure No.	Title	Page No.
1	Oyster mushrooms in fresh and dried form	27
2	Soya flour	27
3	Selection of raw ingredients	27
4	Sun drying of oyster mushrooms	29
5	Formulation of trials	31
6	Different variations of the formulated soy mushroom soup mix	32
7	Preparation of sachets	33
8	Sensory evaluation of four developed variations	33
9	Food label design of the soup mix	35
10	Sensory evaluation among homemakers	36
11	Selection of ingredients for soy mushroom soup mix	38
12	Trial 1 (AAA)	40
13	Trial 2 (BBB)	41
14	Trial 3 (CCC)	42
15	Trial 4 (DDD)	43
16	Preparation process of instant soya mushroom soup mix	44
17	Sensory acceptability score of trials for soup mix	47
18	Sensory acceptability score of soy mushroom soup mix	49
19	Packaging and labeling of the instant soya mushroom soup mix	55

LIST OF ANNEXURES

Annexure No.	Title	Page No.
I	Human Ethical Committee Certificate	68
II	Plagiarism Report	69
III	Nutrient analysis test report	70
IV	Procedure for nutrient analysis	71-79
V	Recipe of the instant soy mushroom soup mix	80
VI	Sensory score card	81

I. INTRODUCTION

Proteins are necessary for human nutrition and health, and they are commonly used in the food business to create a variety of nutritious diets. Amino acids serve as building blocks for body tissue and produce physiological enzymes that help the body function properly by regulating chemical and biological interactions. Dietary proteins are the primary supply of nitrogen, and amino acids are the building blocks of biological tissue (Qin et al., 2022).

Dietary fibre is made up of plant components or carbohydrates that resist digestion and absorbed by the small intestine and ferment entirely or partially in the large intestine. Dietary fibre is an important part of any food item or food group. It has a variety of functional qualities and is classed as both soluble and insoluble fibre (S et al., 2020).

Fibre-rich diets, such as those high in cereals, fruits, and vegetables, have been linked to a lower incidence of a number of diseases due to benefits such as increased faecal bulk, decreased intestinal transit time, cholesterol, and glycemic levels, and the trapping of potentially harmful substances in the human body (mutagenic and carcinogenic agents) (Dhingra et al., 2012).

Edible mushrooms have long been valued not only as a delicacy, but also as a source of nutrition in human diets. Mushrooms are high in protein, lipids, amino acids, glycogen, vitamins, and minerals, according to studies. The mineral salt content of mushrooms, is superior to meat and fish, and roughly double the quality of the most common veggies (Abulude et al., 2013).

Mushrooms have a protein composition ranging from 20 percent to 40 percent on a dry matter basis and are high in critical amino acids like lysine and leucine, which are scarce in cereal grains. It is a unique plant meal since it has very little dietary sugar, making it good for diabetics. Mushrooms are especially high in vitamin B12 (cobalamine), which is rarely found in plant diets, making them a good vegetarian option. Because of its balanced content of protein, fat, carbohydrate, minerals, vitamins, amino acids, and active substances, it's a good choice for dietary supplements. As a result, mushrooms can be a healthy addition to cereals and are

found in a variety of meats, vegetables, health drinks, soups, cakes, and bakery items (Srivastava et al., 2019).

In India, mushroom cultivation began in the early 1960s and has evolved through time as composting techniques have improved. In India, total mushroom production is currently around 0.13 million tonnes (Sharma et al., 2017). Fresh mushrooms contain 85-95 percent moisture, 3.0 percent protein, 4.0 percent carbohydrates, 0.3-0.4 percent fat, and 1.0 percent minerals and vitamins (Chandra and Samsher, 2005). Mushrooms are particularly perishable in nature due to their high moisture content. After harvest, they continue to breathe, and several changes such as browning, liquefaction, moisture loss, and texture occur, resulting in lower market value and unacceptability (Amuthan et al., 1999).

Pleurotus mushrooms are delicate and sensitive, and they begin to deteriorate within one day of being harvested. Mushrooms have a short shelf life under normal refrigeration conditions, limiting their distribution and marketing, making mushroom shelf life extension a never-ending quest. They lack a cuticle, which protects them from physical and microbial harm as well as water loss. After harvesting, the mushrooms undergo a number of physiological and morphological changes that render them unfit for ingestion. The mushrooms' enzyme activity could cause taste degradation. The most common postharvest changes in mushrooms are browning, veil opening, weight loss, and microbiological deterioration, all of which result in significant financial losses.

As a result, establishing proper postharvest technology, such as drying, is crucial to extending the product's life. Drying is a traditional food preservation method based on the premise that the water activity of the product must be decreased to particular levels in order to maintain microbiological and physicochemical stability. Drying is a simple procedure that has been used for many years to improve the shelf life of food. Dried mushrooms are suitable for long-term preservation and transportation since they can be dried in a variety of ways.

The market's availability of dried and safe mushrooms may improve public knowledge of the commercial and nutritional importance of this item, as well as boost its production and use in the country (Tolera and Abera, 2017).

By removing toxins and eliminating or suppressing infections, processing and preparing foods can make them safer to eat. Pathogens can be slowed or stopped by using techniques including refrigeration, freezing, fermenting, drying, and adding salt or sugar (2019, Mihretie).

Mushrooms may play an important role in overcoming protein deficit in underdeveloped countries when animal-derived high-quality proteins are either inaccessible or unsuitable due to religious beliefs. Mushrooms can be a fantastic way to enhance cereals in your diet. Because of their high nutritional and digestible characteristics, mushrooms are becoming increasingly popular in today's healthy diet. Polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, lipids, and their derivatives have all been detected in this mushroom (Tolera and Abera, 2017). Mushrooms' nutritional content, antioxidant activity, and health-beneficial characteristics, as well as their flavour and texture properties, have piqued industry interest in using them as viable substitutes for a variety of ingredients and additives in processed foods (Rangel-Vargas et al., 2021).

Soybean (*Glycine max*), a legume species, is a solid source of high-quality protein (43.2 percent) with low saturated fat, 31.3 percent carbs, and appropriate minerals and vitamins. Soybean contains roughly 18 percent oil, the majority of which is linoleic acid, which accounts for about half of total fat, whereas most oilseeds include 40-50 percent oil. Soybean is superior to other plant proteins in terms of amino acid profile since it includes the majority of essential amino acids, with the exception of methionine, which is rich in cereals. Antihypercholesterolemic, hypertension, diabetic control, antioxidant defence system alleviation, immunomodulatory activities, and chemopreventive properties have all been connected to bioactive compounds in soybean. Isoflavone is one of the important components of soybean that helps to human health. (Naresh, 2019). Soybeans are a great addition to supplementary foods like biscuits, bread, pasta, and other cereal items because of their healthy characteristics (Hegstad, 2008).

Ready to consume Dehydrated soup mixtures are versatile food items made up of a variety of ingredients such as corn starch, spices, salt, flavours, and flavour enhancers (Hafeel et al., 2013). A 'soup' is a dish made by simmering meat or

vegetables in stock or hot/ boiling water until the flavour has been extracted and a broth is formed. Soup is frequently used to aid in the recovery of sick people, especially if they can only digest liquids. A soup can also be served as an appetiser at the start of a meal to help stimulate the stomach's hunger and the flow of digestive juices (Singh and Chaudhary, 2015).

The ready-to-eat food market in India is quickly expanding, with approximately 80% of demand in metropolitan areas. India is Asia's second largest customer and one of the top five markets for ready-to-eat food variety (Meena et al., 2019). In 2017, India's ready-to-eat and ready-to-cook food industry was worth \$261 million, and it's predicted to grow to \$647 million by 2023, with a CAGR of over 16 percent. (CARG). Because of the abundance of fast foods on the market and their response to fresh and simple to prepare products, modern people's lifestyles and behaviours have shifted toward goods that are ready-to-eat and ready-to-cook.

As a result of their convenience, customers have been drawn to Ready to Eat and Ready to Cook snacks in the previous five years, which has been the fastest growing food category. Consumer interest was stimulated by these food products' attractiveness, reasonable pricing, flavour, look, texture, and other features. Various technologies have been extensively used in the creation of Ready to Eat and Ready to Cook snacks due to their simple operation methods, which do not require further cooking or preparation and instead focus on beautiful packaging. Fast food, baked food, dried or preserved food, extruded food, sweet, salty, fried, canned food and so on are all examples of Ready to Eat items. Traditional cereal consumption is dominated by bakery food, extruded food, rapid snacks, fast food, breakfast cereals, biscuits and bars, and other foods. Because these food compositions are shelf stable, lightweight, nutritionally fortified, and easy to shop for and store, they have a high level of customer approval (Temgire et al., 2021).

People's lives have become more stressful as a result of urbanisation. They don't have enough time to cook and have grown accustomed to fast food and other convenience foods. The bulk of these items are junk foods because of their high sugar, fat, and salt content, as well as their low nutritional value in terms of protein, fibre, vitamin, and mineral content. Malnutrition and related illnesses result from

consuming these nutrient-deficient meals. Furthermore, a cereal-based diet may exacerbate this illness. This problem could be solved by providing nutritious foods that are simple to prepare. Dried soup powder is one of the easy-to-cook foods accessible in our country, and it plays a significant role in meeting current and future social consumer demands.

Other variables that contributed to the rise of convenience food in many countries include nuclear families, active female engagement, inventive manufacturers, persuasion advertising, individualism, ownership of kitchen technology, time usage, hesitancy, and deteriorating culinary abilities (Raj et al., 2021).

One of the drawbacks of using mushrooms as a blend is that they are particularly perishable due to their high water content and significant postharvest respiration. When mushrooms are utilised as a substitute ingredient, a drying method can be performed to lengthen their shelf life. Drying is a classic way for preparing edible fungi, and both natural and technology-assisted drying processes are routinely utilised. However, the drying techniques used to extend the shelf life of mushrooms may alter not only their nutritional value, but also their flavour and bioactive properties.

The drying and grinding procedures make the mushrooms particularly ideal for use as a flour ingredient in pasta, bread, and snack goods. Several works have used mushroom powders instead of wheat flour (Rangel-Vargas et al., 2021).

Mushrooms are traded in processed forms around the world, such as frozen, canned, pickled, dried, and so on, which extends their shelf life and market value. Mushrooms can be used to make soup powder, pickles, chips, paste, ketchup, noodles, spaghetti, biscuits, nuggets, mushroom-based flavour enhancers, and as an addition in beverages and beauty products. Personal Care and Beverage Products To extract flavour and bioactive compounds, water or alcohol can be utilised, which can then be used to manufacture mushroom beers, wines, spirits, and prophylactic beverages. Not only must losses be reduced, but revenue must also be increased through value-added services and mushroom consumption must be increased. Other mushroom-based food products have been developed to increase mushroom consumption among children

and the elderly, such as bread, cake, roasted mushrooms in oil, mushroom pate, and so on (Jahan and Singh, 2019).

Many Indians today, especially those who live in big cities, require a fast-paced and practical lifestyle in practically every aspect of their lives, including food preparation, processing, and presentation. It fosters a society that values instant food products like ready-to-cook and ready-to-eat meals. Functional soup is one of the things that could be developed into an instant food. Dried soups play an important function in meeting the social needs of consumers (Upadhyay and colleagues, 2017).

Dry soup mixes are in high demand on the global market. In general, the physiochemical and rheological qualities of instant soup at the moment of preparation play a big role in commercial production. Rheological properties are those that deal with the deformation and flow of matter. As a result, rheological characteristics are critical for understanding food structure behaviour during processing (Islam and colleagues, 2018).

Soup is a traditional food that can be served as an appetiser or as a warm meal when you're unwell. Because making soup is a time-consuming operation, commercially manufactured instant soup has largely supplanted homemade soup in the modern world. Soup is made by combining elements like vegetables, green leaves, juice, water, or another liquid with other ingredients. The extraction of flavours from solid components in liquids in a pot, resulting in a broth, distinguishes hot soups even more. Soups and broths are frequently thickened with rice, lentils, wheat, and grains.

As the population and industries grew, more people chose to work alone after finishing their schooling. They have less time available to prepare food. As a result, most of them eat foods that take less time to prepare without thinking about the health benefits. Instant soups play an important role in balancing the nutrients that individuals require to stay healthy, and they can also be utilised as a breakfast option (Ansari and colleagues, 2021).

Dehydrated foods, particularly dry soup mixes, may provide protection against enzymatic and oxidative spoilage as well as long-term flavour stability at room temperature. They also don't require any preservatives or refrigeration to keep them

fresh. It is high in nutrients. Furthermore, they can be reconstituted in a short period of time (Sudarshan and colleagues, 2017).

Instant soup can be used as a breakfast substitute since it provides the body with sufficient energy and nutrients, is easy to prepare, and takes only a few minutes to serve.

Instant mixes have gained popularity in recent years as a result of their convenience, hygienic nature, long shelf life, and ease of transportation. As a result, it saves money on shipping and is available in a variety of containers. It also takes very little time to make food (Niththiya and colleagues, 2014).

Dried soup powders have the benefit of being resistant to enzymatic and oxidative deterioration, as well as having long-term flavour stability at room temperature (up to 12 months). Working families, hotels, hospitals, restaurants, and institutions, as well as military rations, can quickly alter them. Additionally, they are lightweight for shipping and are accessible all year. The bulk of locally accessible soups, on the other hand, do not exceed nutritional guidelines. To increase nutritional quality, introduce plant-based protein, mineral, and vitamin sources that are suitable to a wide range of people. Soybean and oyster mushroom are wonderful sources to consider because of their great nutritious quality (Farzana et al., 2017).

Thus keeping all these perspectives the present study was conducted for standardization and evaluation of acceptability of the soup mix using oyster mushrooms and soya bean flour as the key ingredients and shelf life of the soup mix was studied with the following objectives:

- To formulate protein and fibre enriched instant soy mushroom soup mix
- To analyze nutritional composition, sensory evaluation & shelf life of the soup mix
- To study the acceptability of the soup mix

II. REVIEW OF LITERATURE

The review of literature pertaining to the study titled “**Formulation of protein and fiber enriched instant soy mushroom soup mix**” constitutes of three major headings:

- A. Importance of Protein and Fiber Rich Foods
- B. Nutritional Benefits of Oyster Mushrooms and Soya Beans
- C. Need for Ready to Eat and Ready to Serve Foods in Indian Market

A. Importance of Protein and Fibre Rich Foods

Proteins are nitrogen-containing substances which are made up of amino acids. They act as the major structural component of the muscle and other tissues within the body. Moreover, they serve to produce hormones, enzymes and hemoglobin. Proteins can also be used as an energy source, but they are not the primary choice as an energy source. For proteins to be used by the body, they must be metabolized in their simplest form, amino acids. Twenty amino acids were found to be necessary for human growth and metabolism. Twelve of these amino acids (eleven in children) are termed as nonessential which means that they can be synthesized by our body and do not need to be consumed in the diet. The remaining amino acids cannot be synthesized in the body and are described as essential meaning that they should be consumed in our diet. Lack of any of these amino acids compromises the ability of tissue to grow, be repaired or be maintained (Hoffman and Falvo, 2004).

One of the major public health concern nowadays is obesity and it is associated with the development of metabolic diseases such as cardiovascular disease, nonalcoholic fatty liver disease, and Type II Diabetes Mellitus in both children and adults. One of the main factors controlling and preventing obesity and related chronic diseases is diet, for example, protein rich diets. Diets higher in protein that is, 30 percent of energy intake have been shown to improve body composition, improve glycemic response, increase satiety, and increase postprandial energy metabolism, which are all mediating factors of weight loss (Baum et al., 2020).

Many studies have identified proteins as a key nutrient for older adults. Low protein intake is related to reduced physical capacity in older adults. However, there is

general agreement that moderately increasing daily protein intake beyond $0.8 \text{ g} \times \text{kg}(-1) \times \text{d}(-1)$ may enhance muscle protein anabolism and provide a means of reducing the progressive loss of muscle mass with age. The beneficial effects of resistance exercise in aging populations are unequivocal (Jones et al., 2008).

Accumulated research has revealed that the gut microbiota mediate the crosstalk between protein metabolism and host immune response. Gut microbes are involved in the digestion, absorption, metabolism and transformation process of dietary protein in the gastrointestinal tract. Amino acids can be metabolized into numerous microbial metabolites, and these metabolites participate in various physiological functions related to host health and diseases. The components of dietary protein impact the gut microbiota composition and microbial metabolites. The source, concentration, and amino acid balance of dietary protein are primary factors which contribute to the composition, structure and function of gut microbes (Zhao et al., 2019).

Over the past decade, the potential muscle-related benefits achieved by consuming higher-protein diets (that is, greater than Recommended Dietary Allowance but within the Acceptable Macronutrient Distribution Range) have become increasingly clear. Increased protein intake contributes to greater strength and muscle mass gains when coupled with resistance exercise, allows for greater muscle mass preservation when consumed during periods of negative energy balance, limits age-related muscle loss, and, to a lesser extent, provides a greater muscle protein synthetic response when evenly distributed across meals (Carbone and Pasiakos, 2019).

Many epidemiological studies have found a significant positive relationship between protein intake and bone mass or density. Similarly, isotopic studies have also demonstrated greater calcium retention and absorption by individuals consuming high-protein diets, particularly when the calcium content of the diet was limiting. High-protein intake may positively impact bone health by several mechanisms, including calcium absorption, stimulation of the secretion of IGF-1 (Insulin-like growth factor 1), and enhancement of lean body mass (Kerstetter et al., 2011).

Dietary fibre is that part of plant material in the diet which is resistant to enzymatic digestion which includes cellulose, noncellulosic polysaccharides such as

hemicellulose, pectic substances, gums, mucilages and a non-carbohydrate component lignin. The diets rich in fibre such as cereals, nuts, fruits and vegetables have a positive effect on health since their consumption has been related to decreased incidence of several diseases. Dietary fibre can be used in various functional foods like bakery, drinks, beverages and meat products. Influence of different processing treatments (like extrusion-cooking, canning, grinding, boiling, frying) alters the physico- chemical properties of dietary fibre and improves their functionality. Dietary fibre can be determined by different methods, mainly by: enzymic gravimetric and enzymic-chemical methods. The two basic categories of dietary fibre are soluble and insoluble fibres. Cellulose, hemicellulose and lignin- are not soluble in water whereas pectins, gums and mucilages- become gummy in water.

The importance of food fibres has led to the development of a large and potential market for fibre-rich products and ingredients and in recent years, there is a trend to find new sources of dietary fibre that can be used in the food industry. To enhance fibre content of foods supplementation has been used. Supplementation has been mainly focused on cookies, crackers and other cereal-based products, enhancement of fibre content in snack foods, beverages, spices, imitation cheeses, sauces, frozen foods, canned meats, meat analogues and other foods also has been investigated (Dhingra et al., 2012).

The recommended allowance of dietary fibre is 35 to 40 g per day for an adult and should be taken in small amounts but at regular intervals (Singh and Singh, 2015). Several non-starch food provide up to 20 to 35 g of fibre per 100 g dry weight and other those containing starch provide about 10 g per 100 g of dry weight and the content of fibre of fruits and vegetables is 1.5 to 2.5 g per 100 g of dry weight (Dhingra et al., 2012).

Lambo et al. (2015) reported cereals to be one of the main sources of dietary fibre, contributing to about 50 percent of the fibre intake in western countries, 30 to 40 percent dietary fibre may come from vegetables, about 16 percent from fruits and the remaining 3 percent from other minor sources.

The diets with a high content of fibre, such as those rich in cereals, fruits and vegetables have a positive effect on health since their consumption has been related to

a decreased incidence of several types of diseases as due to its beneficial effects like increasing the volume of fecal bulk, decreasing the time of intestinal transit, cholesterol and glycaemic levels, trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents), stimulating the proliferation of the intestinal flora etc. (Dhingra et al., 2012).

Yang et al., (2012) Dietary fiber intake can increase stool frequency in patients with constipation. Soluble fiber absorbs water to become a gelatinous, viscous substance and is fermented by bacteria in the digestive tract. Insoluble fiber has a bulking action. Dietary fiber is the product of healthful compounds and has demonstrated some beneficial effect. Increase of dietary fiber intake has been recommended to treat constipation in children and adults.

Reynolds et al., (2020) stated that higher-fibre diets are an important component of diabetes management, resulting in improvements in measures of glycaemic control, blood lipids, body weight, and inflammation, as well as a reduction in premature mortality. These benefits were not confined to any fibre type or to any type of diabetes and were apparent across the range of intakes, although greater improvements in glycaemic control were observed for those moving from low to moderate or high intakes. Increasing daily fibre intake by 15 g or to 35 g might be a reasonable target that would be expected to reduce risk of premature mortality in adults with diabetes.

Dietary fibre supplementation may aid to weight loss through reduced frequency of eating and food consumption. Based on current published evidence, dietary fibre appears to associate only with small improvements in body weight, and evidence for changes in body composition (including fat mass) is less clear. A small reduction in abdominal adiposity (as reflected by changes in waist circumference) likely also associates with dietary fibre intake and is probably reflective of changes in overall body weight. Although some evidence suggests that reduction of food intake (both frequency and size of meals) associates with increased fibre intake, future studies should explore the mechanisms that mediate effects of dietary fibre on body weight and composition (for example, through effects on appetite regulation).

A high intake of soluble dietary fibre appears to have additional metabolic benefits, including improved glycaemic index of carbohydrate-rich foods and lipid profiles. Other effects of dietary fibre that may also have an impact on overall metabolic health include the release of various gut hormones, adipokines, bile acids and the metabolic signatures of amino acids. Based on the current scientific evidence from prospectively designed and controlled studies, dietary fibre does appear to associate with improvements in insulin sensitivity and overall metabolic status (Barber et al., 2020).

The gut microflora consists of around 100 trillion microbes that co-evolved with our hominid ancestors over millions of years. A healthy and diverse gut microflora underlies normal physiology, including normal immune development, metabolic and appetitive pathways and even regulation of normal mental and emotional functioning. Gut dysbiosis underlies much of 21st century chronic ill health through effects on chronic inflammatory pathways and immune dysfunction, the latter resulting in atopy, food intolerances and autoimmune conditions. Fortunately, our gut microflora is modifiable through lifestyle factors, primarily our diet (Oduro-Donkor et al., 2020). We can all, therefore, improve our future health prospects through improving our gut flora. One excellent way to achieve this is through optimizing our dietary fibre intake. Much of our evidence for the role of dietary fibre on the gut microbiota and the implications for health stems from rodent-based studies. These include the effects of dietary fibre intake on colonic health (Desai et al., 2016).

It has been postulated that low intake of dietary fibre is a risk factor for both local and systemic chronic inflammation. The current dogma suggests that limited dietary fibre intake stymies the establishment and maintenance of a healthy, viable and diverse colonic microbiota that, in turn, limits the local production of Short Chain Fatty Acids, including butyrate. Signalling pathways that implicate nuclear factor kappa-B (NF- κ B) and inhibition of deacetylase influence inflammatory processes both locally (including gut-wall leakiness and colonic inflammation in patients with inflammatory bowel disease) and systemically, and both are likely influenced by levels of butyrate within the colon. Furthermore, butyrate may improve oxidative stress within the colon through effects on gene expression implicated in glutathione and uric acid metabolism (Barber et al., 2020).

Threapleton et al., (2013) stated that with inclusion of 22 prospective cohort studies reporting on associations between dietary fibre intake and coronary heart disease or Cardiovascular Disease, with a minimum follow-up period of 3 years. There was an inverse association between total dietary fibre intake (including insoluble fibre and fibre from cereal and vegetable sources) and risk of Cardiovascular Disease (risk ratio 0.91 per 7 gram per day (95 percent CI 0.88 to 0.94)), and coronary heart disease (risk ratio 0.91 per 7 g per day (CI 0.87 to 0.94)). Greater dietary fibre intake is associated with a lower risk of both cardiovascular disease and coronary heart disease.

B. Nutritional Benefits of Oyster Mushrooms and Soya Beans

Mushrooms are considered to be a complete and safest food, suitable for all age groups. This nutrient dense versatile food can be taken as a substitute of meat, fish, fruits, and vegetables. They are excellent source of protein, vitamins, dietary fibers, minerals and is low in fat. On dry matter basis, protein content in mushrooms ranges from 20 percent to 40 percent and contain an abundance of essential amino acids like lysine and leucine which are limited in cereal grains. It is a unique plant food in that they are very low in dietary sugar making them ideal for diabetic patients. Mushroom is also an excellent source of vitamin B12 (Cobalamine), which is generally not present in plant foods and ideal choice for the vegetarians. The balanced status of protein, fat, carbohydrate, minerals, vitamins, amino acids, and active ingredients makes it an ideal choice for food supplementation (Farzana et al., 2017).

Carbohydrates are one of the most important components of mushrooms constituting between 50 percent and 60 percent of the dry matter. These mushrooms contain both high and low molecular weight carbohydrates. The high molecular weight carbohydrates are polysaccharides such as chitin and glucan. The low molecular weight carbohydrates are monosaccharides, disaccharides, and sugar alcohols (polyols), such as arabitol, glucose, mannitol, and trehalose. Moreover, the carbohydrate contents of *Pleurotus ostreatus* are significantly affected by the method used to dry them: oven-dried *Pleurotus ostreatus* recorded a higher carbohydrate value (43.64 percent) than open sun-dried mushrooms (39.99 percent).

There is higher protein content in mushrooms than in many vegetables. Also, mushrooms contain some of the essential amino acids that are often present in animal proteins. Because of these reasons, mushrooms can be consumed as an alternative to animal meat. Protein is a significant component of mushroom's dry weight. The protein content of mushrooms depends on the species of the mushroom, the composition of the substrate, the harvesting time, and the size of pileus. The protein content of *Pleurotus ostreatus* is also significantly affected by the method of drying, as oven-dried *Pleurotus ostreatus* recorded a less protein content (24.99 percent) than open sun-dried mushrooms (27.14 percent). The protein content of mushrooms is also better than many common legumes such as peanuts, soybeans, and protein-yielding vegetables. *Pleurotus* mushrooms consist of essential and non-essential amino acids. Mushrooms are especially rich in essential amino acids such as lysine and leucine which are lacking in most cereal foods (Galappaththi et al., 2021).

Das et al., (2021) stated that edible mushroom is considered as a novel source of dietary fiber due to the presence of non-starch polysaccharides.

Trace elements are essential for human health, which having important physiological effect on different organs and cellular mechanisms. Mushrooms fruit bodies are rich in vitamins, mainly vitamin-B1, vitamin- B2, vitamin-C and vitamin-D2. The vitamin of group B are abundant particularly thiamine, riboflavin, pyridoxine, pantoic acid, nicotinic acid, nicotinamide, folic acid and cobalamin as well as other vitamins such as ergosterol, biotin, phytoquinone and tocopherols. With respect to thiamine content, mushrooms are a bridge between yeast and other food products of vegetal origin. *Pleurotus ostreatus* contains more folacin, vitamin B1, vitamin B3 but less vitamin B12 than other mushroom species (Deepalakshmi and Mirulalini 2014).

Galappaththi et al., (2021) stated that mineral levels of wild edible mushrooms are affected by the interval between the fructification and the age of mycelium. Also mineral elements are unevenly distributed within a fruit body. Trace elements such as arsenic (As), barium (Ba), cobalt (Co), copper (Cu), rubidium (Rb), silver (Ag), thallium (Tl) and vanadium (V) were observed in edible mushrooms.

Humans have used mushrooms for medical purposes since the Neolithic and Paleolithic eras (Samorini 2001). The medicinal properties of mushrooms have

valuable healthcare applications, from being used for simple conditions like skin diseases, to complex diseases like AIDS. Mushrooms also have anti-tumor, anti-cancer, anti-bacterial, anti-fungus, anti-viral, anti-inflammatory, genoprotective, anti-oxidant, immuno-modulatory, anti-diabetic, anti-allergic, anti-mitogenic, anti-hypertensive and anti-hyper cholesterolemic properties that make them valuable for treating a wide range of conditions.

The bioactive compounds such as ergosterol, glucans, amino acids (arginine and glutamine), and proteoglucans present in *Pleurotus* have been correlated with antitumor activities. A possible mechanism of action of these substances is through the inhibition of neovascularization induced by the tumor growth in Sarcoma 180 cells in vitro, the activation and clonal expansion of T cells, increase in NK and T Helper cells and stimulation of the synthesis of interleukins, stimulation of immune function, prolonging cell survival, and promoting greater tolerance to chemotherapy (Fortes et al., 2006).

Galappaththi et al., (2021) discussed that different types of extracts from *Pleurotus* mushrooms have demonstrated anticancer abilities in different cancer cell lines and experimental animals through a number of different mechanisms of action. Protein fractions extracted from *Pleurotus ostreatus* show antitumor activity against different tumors of mice. Water-soluble extracts of *Pleurotus ostreatus* showed the most significant cytotoxicity and induced apoptosis in human androgen-independent prostate cancer PC-3 cells. Hot water extracts of *Pleurotus ostreatus* show inhibition in proliferation of MCF-7 human breast cancer cells. Aqueous polysaccharide extracts of *Pleurotus ostreatus* induce pro-apoptotic and anti-proliferative effects on HT-29 colon cancer cells. The methanol extracts of *Pleurotus ostreatus* show suppression of the proliferation of breast cancer and colon cancer cells. Protein extracts of *Pleurotus ostreatus* exhibited therapeutic efficacy against human colorectal leukemia cell lines (THP-1 cells) by inducing apoptosis in SW 450 cells.

Iwalokun et al., (2007) stated that the effectiveness of mushroom antibacterial activity varies according to the solvent used to extract the compounds of mushrooms. Petroleum ether extracts (PE) and acetone extracts of *Pleurotus ostreatus* are effective antimicrobial agents for *Bacillus subtilis* and *Escherichia coli*.

Elhusseiny et al., (2021) discussed that the antiviral effect of mushrooms is mostly due to interfering with viral uptake, replication, enzyme activity and functioning peptides as well as potentiating the host immune system. The β -glucan in the polysaccharide fraction of *P. ostreatus* may be responsible for its antiherpetic effect through pre- and post-treatment effects. The anti-HIV effect of the aqueous extracts of *P. ostreatus* may be caused by inhibition of the reverse transcriptase enzyme by ubiquitin-like protein and lentin, respectively.

Inflammation is a protective response to tissue injury and is usually abates after healing. As a result of these kinds of defensive reactions, other tissue injuries may occur. In such cases, people use anti-inflammatory drugs, however many currently available anti-inflammatory drugs cause side effects. Because of this, scientists are investigating the anti-inflammatory activity of mushrooms. A polysaccharide known as pleuron isolated from the fruit bodies of *Pleurotus* has demonstrated anti-inflammatory activity, Lovastatin contained in *Pleurotus* species has exhibited anti-inflammatory activity, and *Pleurotus ostreatus* demonstrated protective effects on inflammatory pathologies in diabetic rats (Jayasuriya et al. 2020).

Extracts of *Pleurotus* mushrooms exhibit DNA-protecting activities. For example, *Pleurotus cornucopiae* extracts significantly reduce H₂O₂-induced DNA damage in lung cells of the Chinese hamster, and *Pleurotus ostreatus* suppresses DNA damage in artificially mutated *Drosophila*. Cadmium (Cd) is a highly toxic heavy metal that is harmful to both human and animal health. Cadmium that is bound to proteins in kidneys causes nephropathy and renal proximal tubular dysfunction due to heavy metal accumulation. However, *Pleurotus ostreatus* extract reduces the cadmium level in renal tissues and restores DNA fragmentation in rats.

Antioxidants are chemical compounds that protect the cell from damage by powerful oxidants known as free radicals. Free radicals have an ability to cause damage to compounds such as proteins, lipids, carbohydrates, and DNA, and are also involved in cancers and mutations. Antioxidant activities of compounds are positively correlated with their total polyphenol content. Methanol extracts *Pleurotus ostreatus* showed antioxidant activities that were higher than other commercially cultivated mushroom species. Internal polysaccharides and exopolysaccharides isolated from

Pleurotus ostreatus also showed high antioxidant properties (Galappaththi et al., 2021).

Ravi et al., (2012) reported that the consumption of *Pleurotus ostreatus* produced a significant hypoglycemic effect in diabetic mice and it is capable of improving hyperlipidemia and the impaired kidney functions in alloxan-induced diabetic mice. Thus, indicating that the ethanolic extract of *P. ostreatus* could be added in the list of medicinal preparations beneficial in diabetes mellitus.

Alam et al., (2009) reported that feeding of hypercholesterolemic rats a 5 percent powder of oyster mushrooms reduced the plasma total cholesterol level by 37 percent and reduced the triglyceride level by 45 percent. Low Density Lipoprotein/High Density Lipoprotein ratio decreased by 45 percent for *Pleurotus ostreatus* fed rats. Mushroom feeding also reduced body weight in hypercholesterolemic rats. However, it had no adverse effect on plasma bilirubin, creatinin and urea nitrogen level. Mushroom feeding also increased the total lipid and cholesterol excretion in the feces.

The macronutrient composition of the soybean differs markedly from other legumes as it is much higher in fat, moderately higher in protein and much lower in carbohydrate (Messina, 2016).

Soy protein is one of the least expensive sources of dietary protein and is a high-quality protein with a protein digestibility-corrected amino acid score (PDCAAS) of 1.00, which is close to some of the proteins from animal sources, such as meat and dairy. Soy proteins contain well-balanced essential amino acids except for sulfur-containing ones like methionine. They also have desirable textures with endless possibilities to formulate various sophisticated soy-based food products. Due to their high protein content and versatility in developing food products, soy proteins are the primary supply of plant-based proteins and are widely consumed by diverse populations worldwide (Qin et al., 2020).

The low carbohydrate content of the soybean makes it beneficial for people with diabetes. Also, much of the carbohydrate present in soybean is comprised of oligosaccharides (predominately stachyose), which, because they are poorly digested

by intestinal enzymes, travel to the colon where they are able to stimulate the growth of bacteria such as bifidobacteria, that are considered to be beneficial to the host. For this reason the soybean oligosaccharides are classified as prebiotics. Although oligosaccharides can lead to flatulence, research suggests that concerns about the flatulence-producing effects of beans may be exaggerated. Furthermore, this concern is not applicable to many soy products because as a result of processing the oligosaccharide content is greatly reduced or eliminated (Messina, 2016).

The fat content of soybeans is comprised of approximately 10 to 15 percent saturated, 19 to 41 percent monounsaturated and 46 to 62 percent polyunsaturated fatty acids (PUFA) (per 100 g soybeans that equates to approximately 2.9, 4.4 and 11.3 g saturated, monounsaturated and PUFA, respectively) (Slavin et al., 2009).

Soybean is a good source of vitamins and minerals, especially for example, potassium. The potassium content is notable because intake of this mineral is often suboptimal. It is important to recognize however that the nutrient content of individual soy products, depending upon the manufacturing method employed can differ markedly from one another and from the bean itself.

Soybeans are relatively high in iron which is not surprising because although it is often not recognized, the iron content of plant-based diets is similar to or higher than the iron content of omnivorous diets. The absorption of calcium and iron from soy is an important consideration because soyfoods often replace foods that are good sources of these minerals. Despite the presence of both phytate and oxalate, two compounds that inhibit mineral absorption, calcium absorption from soybeans is quite good (Messina, 2016).

Isoflavones are found in many different plant foods but among commonly consumed foods the soybean contains rich amounts of isoflavones. Soy isoflavones contain 12 different isoforms that are divided into four chemical forms: aglycone (daidzein, genistein and glycitein), glucoside (daidzin, genistin and glycitin), acetylglucoside (acetyldaidzin, acetylgenistin and acetylglycitin) and malonylglucoside (malonyldaidzin, malonylgenistin and malonylglycitin). Soy isoflavones were demonstrated to possess numerous biological functions, such as

antioxidant, inhibitory on cancer cell proliferation, anti-inflammatory and preventive of coronary heart disease and osteoporosis (Huang et al., 2010).

Each gram of soy protein in soybeans and traditional soy foods is associated with approximately 3.5 mg of isoflavones. Consequently, one serving of a traditional soy food, such as 100 g of tofu or 250 mL soymilk, typically provides about 25 mg isoflavones. In more refined products, such as Isolated Soy Protein, as much as 80 to 90 percent of the isoflavone content can be lost as a result of processing (Messina, 2016).

The first human study on the cholesterol-lowering effect of soy protein was reported in 1967 and demonstrated that replacement of mixed proteins by mainly isolated soy protein products at an intake of 100 g per day reduced mean cholesterol levels by greater than 2.59 mmol per L in hypercholesterolemic men (Xiao, 2008).

Soy foods are associated with improved markers of bone health and improved outcomes, especially among Asian women. Although the optimal amounts and types of soy foods needed to support bone health are not yet clear, dietary pattern evidence suggests that regular consumption of soy foods is likely to be useful for optimal bone health as an integral part of a dietary pattern that is built largely from whole plant foods (Lanou, 2011).

Ho et al., (2003) demonstrated that, among women after the initial few years postmenopausal, soy protein/isoflavones intake had a modest but significant association with hip Bone Mineral Density as well as total body Bone Mineral Content.

The menopausal transition is often associated with complaints. The most prominent are hot flashes, sleeping problems, fatigue, and stiffness of muscles and joints. Although soy products contain several bioactive phytochemicals, most cancer research has focused on isoflavones and genistein in particular. The substantially lower breast cancer prevalence in populations with a high soy consumption (one-third reduction in both pre- and postmenopausal breast cancer risk), as well as the increased risk observed upon migration and westernization of Asian people, initiated soy-breast cancer research 20-30 years ago. Both significant and null results have been obtained

regarding the (protective) impact of soy isoflavones on hormone-related breast cancer risk factors (Bolca et al., 2012).

Kurzer, (2008) stated that consumption of 30 mg per day of soy isoflavones (or at least 15 mg genistein) reduces hot flashes by up to 50 percent.

Prostate cancer is the second most commonly diagnosed cancer in men worldwide, and the fourth most commonly diagnosed cancer overall. Intervention studies involving prostate cancer patients generally show that isoflavone exposure slows the rise in prostate specific antigen (PSA) levels. In contrast, in long-term trials isoflavone exposure did not affect the biochemical recurrence of prostate cancer after radical prostatectomy or the progression from high-grade prostatic intraepithelial neoplasia to cancer. However, the design weaknesses of each study limit the implications of the findings. In the former, patients were exposed to only 24 mg per day genistein, a relatively low dose for men who had already undergone radical prostatectomy for the treatment of prostate cancer. In the other study, in addition to the treated group consuming isoflavone-rich soy protein, participants were administered supplements of vitamin E and selenium. There is evidence that both of these micronutrients stimulate the development of prostate cancer, although the risk associated with vitamin E is much less clear (Messina, 2016).

Chronic kidney disease (CKD) is a serious universal problem that is the main risk for several diseases including cardiovascular disease. Some evidence has revealed that altering the quantity and the types of dietary protein exert the main effect on renal failure. Soybeans supply high-quality plant protein and exclusive isoflavones (genistein and daidzein). Soy protein contains a unique amino acid profile that is different from animal and soy peptides including 4 to 20 amino acids which may have been very worthy effects on high BP and hyperlipidemia; therefore, soy peptides may affect renal function. In the animal, it seems to be well established the ability of soy protein to reduce proteinuria and consequently, to lower the progression of renal disease. Some investigators have shown in rats that were received a soy protein diet, a considerable recovery of creatinine clearance and a significant decrease in proteinuria compared with rats that were fed with casein, although the effect of soy protein is well studied in rats, this statement is not true for humans (Kopaei et al., 2017).

The impact of soy and more specifically isoflavones, on skin health including wrinkles, is another area for which encouraging preliminary data exist. A study conducted by Izumi et al., (2007) involved only 26 premenopausal Japanese women, over a 3-month period, use of supplements that provided 40 mg per day isoflavones led to a statistically significant decrease in fine wrinkles, whereas no change occurred in the placebo group.

C. Need for Ready to Eat and Ready to Serve Foods in the Indian Market

Ready-to-eat (RTE) foods are a group of food products that are pre-cleaned, precooked, mostly packaged and ready for consumption without prior preparation or cooking. According to the 2009 US Food code (FDA, 2009), RTE foods should be in an edible form without an additional preparation step to achieve food safety (Huang and Hwang, 2012).

Ready to eat food are packages foods, a variety of these food products are available in markets at any part of the city. Ready to eat food are processed food which can be consumed with a slight warming or cooling upon the consumers preference. Both vegetarian and non vegetarian food varieties are available being ready to eat with simple cooking processes (Meena et al., 2019).

Consumer interest in ready-to-eat (RTE) snack and ready-to-serve (RTS) food is growing due to their convenience, value, attractive appearance, taste and texture (Pawar et al., 2014).

The ready to eat food industry in India is growing rapidly as there is existing about 80 percent demand in the urban areas. India is the second largest consumer in Asia and ranks one among the five main market of ready to eat food varieties. (Meena et al., 2019)

The cooking style and eating habits in India varies drastically from southern part of India to northern part of India. Due to lifestyle pressure nowadays people prefer easy short way of cooking food rather spending too much time on spending in cooking. Non-availability of raw materials to prepare masala and tedious process involved in doing so, has influenced people to choose such products. There is no specific category and market potential for these products. The product portfolio varies

from noodles, pasta, chips, dairy products, fish & meat products, ready cooking dosa, parota items, masala dishes, pre cooked dishes etc.

There are peoples, who are migrating to cities for job and education and these people have find the Ready-to-eat products are comfortable to eat rather than depending on restaurants. Most of the dual income (both husband and wife are office goers) families want to spend much less time on cooking because of less availability of time. During weekends they want to spend time with their kids and outing, whereas in weekdays the office duration is large and these factos forced them to go for buying such products. Other factors influences this products is availability of different flavours and dishes. Consumers who are looking for different dishes and flavours now depend on these products. This products brings variety to their eating's and palatable too. There is no conclusion which one precedes-whether the availability or taste or time constraint, all these factors complement each other in driving these products (Vijayabhaskar and Sundaram, 2012).

Over the last 15 years Indian cooking and way of life have experienced huge changes as India becomes the centre of many multinationals and there's a quick change in individual's ways of life. Nowadays, due to lifestyle pressure, people prefer an easy, fast way of cooking food rather than spending too much time cooking. The emergence of Ready-To-Eat (RTE) food in the global industry, and now in the Indian markets, has seen a shift in consumption patterns over the years. Consumers are becoming more aware of food products and so intentions and perceptions of Ready-to-Eat food products are changing in the Food Retail Industry. Ready-to-Eat meal demand has outpaced a huge amount of India's food retail market. Ready to eat foods can be refrigerated or shelf-stable. It requires minimal heating or no heating at all. Ready-to-Eat food has made people's life easier. Initially, these foods were consumed by defensemen, disaster victims, trekkers, hikers, and hunters who needed food in a short time and on the road. But now, Ready-to-Eat food has become popular among most busy people in modern cities (Goinda et al., 2021).

As consumers work longer hours, spend more time commuting, and aspire to use their time effectively, they demand products and services to ease and support their busy lifestyles. Ready to eat foods economic importance is increasing with respect to

demand, sales and export values. Food retailers have expanded their product assortments of ready to eat food options to react to this increasing demand. Increased female workforce participation, time pressures brought by jobs single and households and lack of cooking skills motivate consumers to purchase ready to eat foods. Ready to eat foods can be defined as complete meals that require little efforts and quickly replace with home-made food.

Employment status, household size, income level, perceived time pressure, and female workforce participation influence ready to eat food consumption. The Theory of Planned Behavior explained consumers' ready to eat food behaviors. According to this, although attitudes and subjective norms influence the intention to consume ready to eat products, perceived behavioral control had no significant effect. Several psychological factors also affect food choice, including convenience orientation, food-related lifestyles, and food choice orientation (Gurce and Friedman, 2020).

Price also takes an important place in ready to eat food consumption behavior. Swoboda & Morschett (2001) found that convenience food consumers were less price-sensitive than non-convenience-oriented ones. As a result, consumers of ready to eat foods were willing to pay extra for the meals. Affective attitudes such as satisfaction, pleasure, and excitement appear to influence the intention to consume ready to eat foods, including investigations of intention to consume ready to eat foods. In determining consumers' behaviors towards ready to eat foods, taste and price is a critical element on ready to eat food choices.

Consumers who are concerned with their health usually try to avoid consuming ready to eat foods. Ready to eat food has traditionally been associated with unhealthy ingredients and perceived as being of lower quality and less healthy than home-made foods (Gurce and Friedman, 2020).

Mandelkar et al., (2018) have found that convenience is the most affecting intrinsic factor for the purchase behavior of ready to eat foods, followed by extrinsic factors such as availability and product advertisement. The remaining factors, including price, product quality, health consciousness, and the taste did not affect the purchase intention of consumers in Bangkok for ready to eat foods. However,

Buckley et al., and De Boer et al., found that health consciousness negatively affects consumers' ready to eat food purchase decisions.

There is a positive outlook towards the Ready-To-Eat food industry and is one of the fastest growing areas in the food market not only in developed countries but also in developing countries. This is because people don't have time to cook in their busy and hectic schedule. Most of the people prefer Ready-To-Eat food products as it is very convenient and easy to use. It also saves a lot time. Majority of the people consume Ready-To-Food occasionally and not frequently as many of them are health conscious. The price and quality of RTE food products are satisfactory at some level which indicates that as quality increases in value, the price will also increase in value and vice versa. The packaging variable and freshness variable of Ready-To-Eat food products are very important factors that are considered while purchasing Ready-To-Eat food products. If the packaging is up to the mark, the food products will be fresh.

Nowadays most of the families are earning dual-income and prefer spending their little free time with their children rather than cooking and that's why Ready-To-Eat food products have become so popular. The findings show that working employees and students preferred Ready-to-eat food products. Apart from convenience, time-saving, and a relative decline in habitual food and eating habits, television has played a huge role in creating awareness about Ready-To-Eat food products (Goinda et al., 2021).

An increase in working class people, projection in the economical background of the families and changing lifestyles of the upcoming generation of the nation are the major causes of these developments in the food patterns. These factors continuously and steadily affect the food pattern of urban peoples. These foods varieties are also been considered the boom for the travelers and transient migrating peoples. These food have advantageous as the saves time, convenient to consume at any time, and relieves the stress of long time procedural cooking (Meena et al., 2019).

Today's health-conscious consumers demand products that are convenient and promote health. There is a demand for high quality, convenient, shelf-stable and ready-to-use processed food products. Instant soups fall in the category of key dried

foods. Soup powders are convenient food items and comprised of ingredients such as corn starch, spices, salt, flavors, and flavor enhancers. Instant soup mixes gained popularity among all dehydrated products as they are very practical in preparation and available in various packages. Soups are consumed for their nutritional benefits and by patients whose intake of solid is reduced due to various physiological conditions. Soup formulations can be made nutritionally balanced by incorporating whole cereals, vegetables, and pulses. Instant soup mixes can be used as an alternative food for breakfast as it can fulfill the adequacy of energy and nutrient required by the body. Being a liquid-food, a good soup powder should be cooked within minimum time, retaining nutrients and palatability almost similar to freshly cooked products. Dried soup mixes have an advantage of protection from oxidative and enzymatic spoilage and flavor stability for longer periods up to one year at room temperature. They do not need refrigeration; exert lightweight for shipping and available all around the year. Functional ingredients can be incorporated into soup powder to provide health benefits (Joshi et al., 2020).

Formulation food products are increasing day by day to meet the requirements of the consumers. The formulation on value added products are now the main target of the consumers because it can supply the nutritional value as well as delicious taste to consumers. Instant soup is a major element of instant food which is highly preferred food of modern society for simple, easy and instant preparation characteristics. Generally, people of developing country are suffering from malnutrition. Instant soup may be a potential source to meet the demand of nutritional requirements (Islam et al., 2018).

3. METHODOLOGY

The methodology pertaining to the study titled “**Formulation of protein and fibre enriched instant soy mushroom soup mix**” four phases are furnished under the following headings:

Phase 1 : Selection of Ingredients

Phase 2 : Standardization process of instant ready to use soup mix

Phase 3 : Food Costing and Packaging of the instant ready to use soup mix

Phase 4 : Acceptability of instant ready to use soup mix among homemakers

Ethical approval for the study was obtained from the Institutional Human Ethics Committee (IHEC), Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore with approval number AUW/IHEC/FSMD-21-22/XPD-22 (Appendix I).

Phase 1 : Selection of Ingredients

Fresh oyster mushrooms were selected based on their local availability, seasonal availability and nutrient. The balanced status of protein, fat, carbohydrate, minerals, vitamins, amino acids, and active ingredients makes it an ideal choice for food supplementation (Srivastava al., 2019).

Soybean, a species of legume, is an excellent source of good quality protein (43.2 percent) with minimal saturated fat, 31.3 percent carbohydrates and adequate amounts of minerals and vitamins. The presence of bioactive compounds in soybean has been associated with antihypercholesterolemic, antihypertensive, regulation of diabetes, alleviation of antioxidant defense mechanism, immunomodulatory activities, and chemopreventive effects. Isoflavone is one of the key components of soybean that contributes to the human health (Farzana and Mohajan, 2015).

Hence fresh oyster mushrooms and soya bean flour were selected as key ingredients for the development of protein and fibre enriched instant soy mushroom soup mix. The key ingredients were selected based on their nutritional properties.



Fresh Oyster Mushroom (2000 gm)



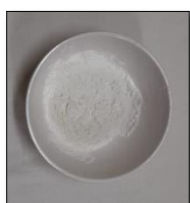
Dried Oyster Mushrooms (104 gm)

Fig 1: Oyster mushrooms in fresh and dried form



Fig 2: Soya Flour

The other ingredients selected are corn flour, milk powder, onion powder, garlic powder, ginger powder, coriander powder, cumin powder, salt, sugar, black pepper and oregano.



Corn flour



Milk powder



Onion powder



Ginger powder



(Garlic powder



Cumin powder



Coriander powder



Black pepper



Oregano



(Salt

Fig 3: Selection of Raw Ingredients

Phase 2 : Standardization Process of Instant Ready to use Soup Mix

I. Cleaning, slicing, drying and grinding the ingredients

Cleaning

The selected fresh oyster mushrooms were washed thoroughly for the removal of dust particles. They were then submerged in a bowl containing water and then washed, it was repeated for three times to ensure complete removal of any dirt present.

Slicing

The fruiting body of the mushrooms were separated from the bunch and was sliced separately in small pieces.

Drying

Drying is a classical method of food preservation, based on the principle that the water activity of the product must be lowered until defined levels that guarantee the microbiological and physicochemical stability. Mushrooms can be dried by different methods and dried mushrooms are convenient for long-term storage and transportation (Tolera and Abera 2017).

Giri and Prasad (2013) stated that freeze-drying produces a high-quality product, but being an expensive process, its application for mushroom drying is limited.

Walde et al., (2006) reported that fluidized bed drying seems to be a promising method for drying mushrooms, when comparing the lower drying time and good-quality products to the faster microwave drying.

Kurozawa et al., (2012) reported that the air drying of fresh and osmotically dehydrated mushroom in Brazil showed the influence of temperature and air velocity on drying kinetics and color of dried samples.

Aishah and Wan Rosli (2013) reported that low heat air blow drying method is recommended in reducing water activity and increasing proximate contents.

Maray et al., (2017) stated that sun drying was the best method for drying of the mushroom and the results showed that sun drying is the best in the chemical composition, physico-chemical properties, sensory properties, mineral elements content and finally in the amino acid contents.

All the mushrooms was then placed on the plates separately and then covered using a clean cotton cloth to avoid any dust and insects intruding. The plates were kept outside where there is direct contact with the sunlight. They were sundried for about three days till there is no moisture surrounded by them. After sun drying for three days the weight of the mushrooms was reduced from 2 kilograms to 104 grams. These were then transferred to a clean air tight container respectively.



Fig 4: Sun drying of Oyster Mushrooms

Granulating the mushrooms

The dried mushroom was then powdered into granules by using a mixer. The powdered granules were collected for further process.

II. Standardization process of instant ready to soup premix

Standardization of the recipes is a recipe that was tried, adapted and retried several times for use to produce the same good results and yield every time when the exact procedures are used with the same type of equipment and the same quantity and quality of ingredients.

Standardization of recipes is necessary for many reasons. Product was consistent in quality each time they are prepared using the standard recipes. By using

standardized recipe the planned number of serving was produced. Nutritional values per serving was consistent in standardized recipes. For the food cost control standardized recipes provide consistent information because of the same ingredients and quantities of ingredients used each time the recipe is made. It is useful for purchasing as the quantity and quality of food required for production is easily calculated from standardized recipe. Exact number of labor requirement can be predicted and even maximum use of their time can be utilized for the particular day.

As a part of standardization process, four trials were done to quantify the ingredients, pre-preparation process, cooking time, method of cooking and yield.

Standardization process trial 1 (AAA)

In the first trial soup mix was prepared using oyster mushroom powder, soy flour, corn flour, milk powder, coriander, salt, sugar, black pepper, oregano. The result was that the soup was sweet in taste and was not thick in consistency.

Standardization process trial 2 (BBB)

In the second trial soup mix was prepared using the same ingredients as used in first trial but the quantity of the corn flour was increased for the consistency and the quantity of the milk powder was reduced to reduce the sweetness of the soup. The result was that the consistency of the soup was good but the taste of soup was same as first trial.

Standardization process trial 3 (CCC)

In the third trial few more ingredients were added like onion powder, ginger powder and garlic powder which will help to enhance the flavour of the soup. Sugar was excluded in this trial to remove the sweetness of the soup. The result was that after addition of the spices the taste of the soup was better.

Standardization process trial 4 (DDD)

In the fourth trial more spices were added like coriander and cumin powder to enhance the taste. The result was that the sensory evaluation for this trial scored the highest.

After four time's trial, the finalized soup mix was prepared for three times for standardization to ensure the yield as same amount while repeating the process.



Trail I



Trail II



Trail III



Trail IV

Fig 5: Formulation of Trials

The sensory attributes which were appearance, colour, consistency, flavour, taste, overall acceptability was evaluated for the developed products. It was done to choose a product to continue the further process to develop into different variations. The sensory evaluation was done against each trial. The sensory evaluation for the four trials was done to choose the most liked one.

Formulations of soup mix containing four varying proportions was prepared by dry mixing of oyster mushroom powder, soy flour, corn flour, milk powder, salt, black pepper, onion powder, ginger powder, garlic powder, coriander powder, cumin powder and oregano.

Mushroom soup can be prepared by heating it with two cups of water for 2.5 minutes with stirring.

The different variations of the soup mix per serving is given in table I.

TABLE I
DIFFERENT VARIATIONS OF THE SOUP MIX PER SERVING

Ingredients	Variation I (g)	Variation II (g)	Variation III (g)	Variation IV (g)
Mushroom powder	6	4	8	2
Soy flour	4	6	2	8
Corn flour	4	4	4	4
Milk powder	8	8	8	8
Onion powder	1	1	1	1
Ginger powder	1	1	1	1
Garlic powder	1	1	1	1
Cumin powder	1	1	1	1
Coriander powder	1	1	1	1
Salt	3	3	3	3
Black pepper	1	1	1	1
Oregano	0.5	0.5	0.5	0.5
Butter	3	3	3	3

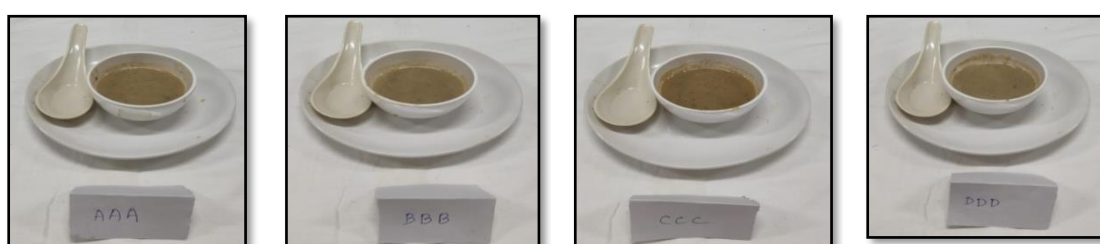


Fig 6: Different Variations of the Formulated Soy Mushroom Soup Mix

The ingredients were packed in two sachets. Sachet one contains oyster mushroom powder, soy flour, onion powder, ginger powder, garlic powder, coriander powder, cumin powder, salt, black pepper and oregano and sachet two contains corn flour and milk powder.



Sachet I



Sachet II

Fig 7: Preparation of sachets

Sensory evaluation of the soup

The sensory evaluation of the standard five scale hedonic scale was used to evaluate the soup mix with 25 semi trained panel members. The sensory attributes which are appearance, colour, consistency, flavour, taste, overall acceptability was evaluated. The output was recorded according to the response of the panel members. For each level of likeness it was given a score from the scale of 5 to 1 in which, there is a gradual decrease of the likeness. The hedonic scale values are, 5- likes very much, 4 -liked moderately, 3- Neither liked nor disliked, 2- dislike moderately and 1-dislike very much. The panel member was provided with water before and after testing the product to evaluate the sensory characteristics of the developed products in the laboratory. This is to ensure that the taste of the one product does not affect the taste of the other product. The score card for sensory evaluation is given in appendix VI.

The sensory evaluation was done using five-point hedonic scale; for all the formulations developed.



Fig 8: Sensory Evaluation of Four Developed Variations

By analyzing the sensory evaluation, the variation which has scored high and had overall acceptability has been standardized.

Nutrient analysis

The nutritive value was given per 100 grams of soup mix. Moisture, crude fat, ash, fibre, protein, calcium, iron, thiamine, riboflavin and cobalamin in different soup mix samples was determined and the shelf life of the soup mix was determined. The nutrient content was analyzed according to the nutrients which are prominent in the oyster mushroom and soy bean flour. The procedure adopted for nutrient analysis is given in appendix IV.

Phase 3: Food Costing and Packaging of the Instant Ready to use Soup Mix

The Shelf-Life of the Developed Product:

The developed and dried product was analysed for shelf life. 30g of the developed product which was fully developed was kept in an air-tight packaging. The packets were first washed thoroughly and then they were dried without leaving any moisture content. The packets were wiped with clean white cotton cloth to prevent any dust and moisture as these may invade as a cause for the spoilage of the product. Then the product was added in the dried form and it was made sure that the packets were closed properly. The container was then kept in a dry place in room temperature. The product was kept for thirty days and was checked for the shelf life of the product. The shelf-life of the product was analyzed for 30 days. Each day the packet was opened and checked for the spoilage. Any changes in the colour, flavour, and smell were noted.

Standardised recipes portion food cost were calculated with the help of an MRP (Material Requirement Program) system and the unit prices that were used on food cost analysis were taken from purchasing list of the major and minor ingredients from the local store market. Food Cost was calculated by taking into consideration the gross weights of the ingredients and the prices were indexed to the Indian Rupees.

Packaging maintains the quality of food products for storage, transportation, and end use. Packaging prevents deterioration in the quality of foods and beverages

due to environmental influences and contributes to efficient distribution, sales, and consumption. The primary functions of food packaging are to achieve preservation and the safe delivery of food products until consumption. Food packaging extends shelf life and maintains the quality and safety of the food products. Based on the overall acceptability score the soup premix was selected for packaging. The soup mix was packed using food grade polythene packets. Each pack contains two servings. The soup mix was packed inside the polyethylene cover and then they were sealed.

The soup mix was packed into two sachets and then food labelling was done. The labelling contained of all the needed information for the food product developed. The labelling contains of the name of the product, nutritional facts, ingredients, cooking instructions, manufacture date, expiry date, maximum retail price of the product and batch number. The food design label is as follows:

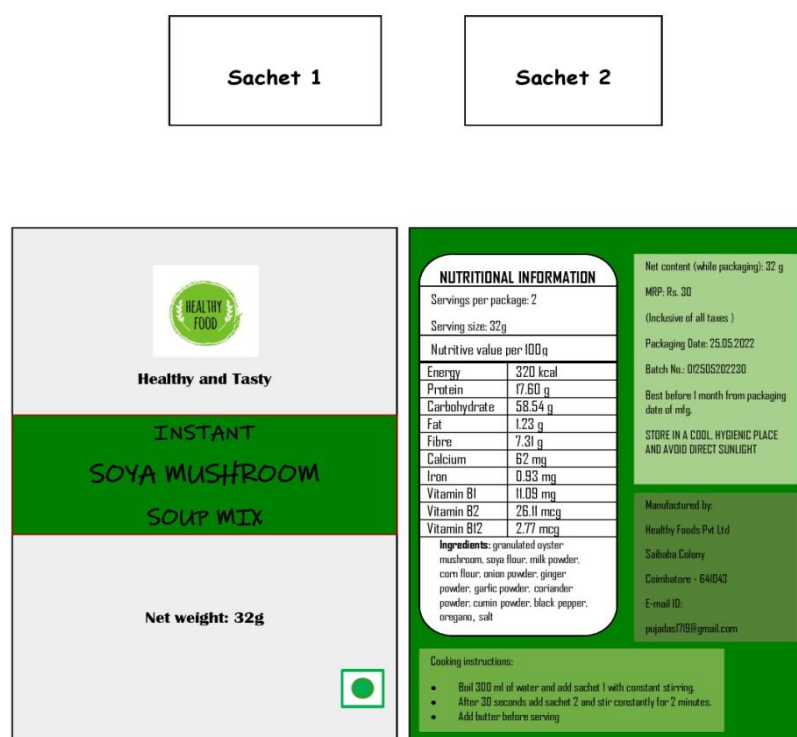


Fig 9: Food design label of the soup mix

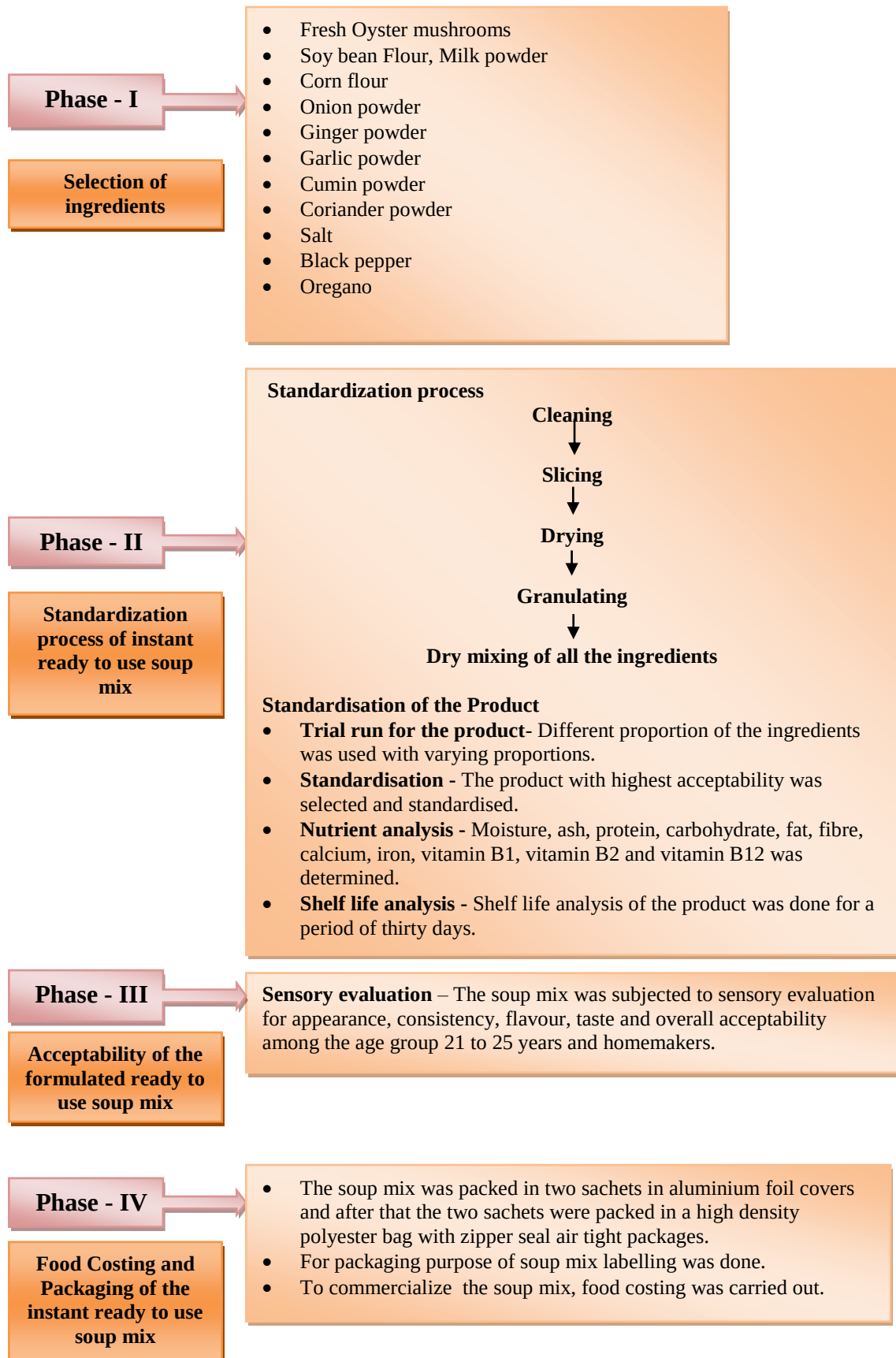
Phase 4: Acceptability of Instant Ready to Use Soup Mix among Homemakers

The overall acceptability of the instant soy mushroom soup mix was done among homemakers in Coimbatore. Five point- Hedonic scale was used to give scores (1- dislike very much, 2- dislike slightly, 3- neither like nor dislike, 4- like slightly, 5- like very much). They were asked to evaluate appearance, colour, consistency, flavor and taste for the developed soup mix. For sensory evaluation ten members were selected. Recipes were displayed neatly with cutlery and water. Water is provided to rinse mouth before tasting food and after tasting food. Score card was given to evaluate the recipes.



Fig 10: Sensory Evaluation among Home Makers

Flowchart of the study on Formulation of Protein and Fibre Enriched Instant Soya Mushroom Soup Mix



IV. RESULT AND DISCUSSION

The results obtained in the present research study entitled “**Formulation of Protein and Fibre Enriched Instant Soy Mushroom Soup Mix**” are discussed under the following headings:

- A. Selection of Ingredients
- B. Standardization process of instant ready to use soup mix
- C. Food Costing and Packaging of the instant ready to use soup mix
- D. Acceptability of the instant ready to use soup mix among homemakers

A. SELECTION OF INGREDIENTS

The instant soy mushroom soup mix was formulated to develop protein and fibre enriched instant soy mushroom soup mix. Soup is considered as an appetizer and can be fortified with essential nutrients to make it beneficial to health.

In the formulation of Soy Mushroom soup mix two main ingredients were selected. Fresh oyster mushroom and soya bean flour were the two main ingredients which had been selected based on their nutritional composition for the formulation of soy mushroom soup mix.



Fig 11: Selection of Ingredients for Soy Mushroom Soup Mix

The minor ingredients include milk powder, corn flour, onion powder, ginger powder, garlic powder, coriander powder, cumin powder, black pepper, oregano, salt and butter. Corn flour was added to thicken the soup which will help to maintain the consistency of the soup. Milk powder was added to balance the taste of the soup as

oyster mushrooms are slightly bitter in taste. Onion powder was added because it makes the soup richer and more savoury. Garlic powder and ginger powder was added to the soup because it act as a flavouring agent and it helps to enhance the flavor of the soup. Cumin powder was added as it gives a earthy, nutty and spicy taste to the soup. Coriander powder was added because it has a mid aroma which instigates the appetite. Black pepper was added as it adds flavor to the soup. Salt was added to enhance the flavor to the soup and butter was added as a little fat helps in enhancing the flavor of the soup.

B. STANDARDIZATION PROCESS OF INSTANT READY TO USE SOUP MIX

As a part of standardization process, four trials were done to quantify the ingredients, pre-preparation process, cooking time, method of cooking and yield.

Standardization process trial 1 (AAA)

Formulation of first trial (AAA) is presented in table II.

TABLE II
FORMULATION OF TRIAL 1 (AAA)

Ingredients	Quantity (g)
Oyster mushroom powder	10
Soy flour	5
Corn flour	10
Milk powder	12.5
Salt	4
Sugar	1.5
Black pepper	1
Oregano	1

Preparation

In the first trial soup mix was prepared using oyster mushroom powder, soy flour, corn flour, milk powder, salt, sugar, black pepper, oregano. The ingredients were dry mixed and added in 300 ml water and was boiled for 2 minutes with constant stirring.

Observation

It was observed that the soup was sweet in taste as sugar was added and was not thick in consistency. The appearance of the soup mix was dull and not very appealing. The colour of the prepared soup was very dull.



Fig 12: Trial 1 (AAA)

Standardization process trial 2 (BBB)

Formulation of second trial (BBB) is given in table III.

TABLE III
FORMULATION OF TRIAL 2 (BBB)

Ingredients	Quantity(g)
Oyster mushroom powder	10
Soy flour	5
Corn flour	15
Milk powder	12.5
Salt	4
Sugar	1.5
Black pepper	1
Oregano	1

Preparation

In the second trial soup mix was prepared using the same ingredients as used in first trial but the quantity of the corn flour was increased for the consistency and the quantity of the milk powder was reduced to reduce the sweetness of the soup. The ingredients were dry mixed and added in 300 ml water and was boiled for 2 minutes with constant stirring.

Observation

It was observed that the consistency of the soup was thick as the quantity of the corn flour was increased but there was no difference in taste and flavour of the soup as compared to the first trial. The appearance and colour of the soup was also same as the first trial.



Fig 13: Trial 2 (BBB)

Standardization process trial 3 (CCC)

Formulation of third trial (CCC) is presented in table IV.

TABLE IV
FORMULATION OF TRIAL 3 (CCC)

Ingredients	Quantity (g)
Oyster mushroom powder	6
Soy flour	4
Corn flour	4
Milk powder	8
Onion powder	1
Ginger powder	1
Garlic powder	1
Salt	3
Black pepper	1.5
Oregano	0.5

Preparation

In the third trial few more ingredients were added like onion powder, ginger powder and garlic powder which will help to enhance the flavour of the soup. Sugar was excluded in this trial to remove the sweetness of the soup. The ingredients were dry mixed and added in 300 ml water and was boiled for 2 minutes with constant stirring.

Observation

It was observed that after addition of the spices the taste of the soup was better compared to trial 1 and trial 2 but the taste of mushroom was dominating the flavor of the spices. The consistency of the soup was thick and the surface and colour of the soup was bright and appealing.



Fig 14: Trial 3 (CCC)

Standardization process trial 4 (DDD)

Formulation of fourth trial (DDD) is given in table V.

TABLE V
FORMULATION OF TRIAL 4 (DDD)

Ingredients	Quantity (g)
Oyster mushroom powder	6
Soy flour	4
Corn flour	4
Milk powder	8
Onion powder	1
Ginger powder	1
Garlic powder	1
Cumin powder	1
Coriander powder	1
Oregano	0.5
Black pepper	1.5
Salt	3
Butter	3

Preparation

In the fourth trial more spices were added to enhance the taste. The product was developed using oyster mushroom, soy flour, milk powder, corn flour, onion powder, ginger powder, garlic powder, coriander powder, cumin powder, black pepper, oregano, salt and butter. The dry ingredients were mixed and added in 300 ml water and was boiled for 2 minutes with constant stirring and at the end butter was added.

Observation

It was observed that the taste of the soup was good and scored the highest acceptability score. The flavor of the spices enhanced the flavor of the soup which made the soup highly acceptable. The consistency of the soup was thick and the surface and colour of the soup was bright and shiny due to the addition of butter at the end and it was also appealing.



Fig 15: Trial 4 (DDD)

Among all the four trials, the fourth trial scored the highest score and was finalized. After four time's trial, the finalized soup mix was prepared for three times for standardization to ensure the yield as same amount while repeating the process.

The fourth trial was further developed into four variations. The variations were prepared by varying the proportion of oyster mushroom powder and soy flour.

Variation (AAA) was prepared using 6g oyster mushroom powder, 4g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g garlic powder, 1g ginger powder, 1g coriander powder, 1g cumin powder, 3g salt, 1.5g black pepper and 0.5g oregano.

Variation (BBB) was prepared using 4g oyster mushroom powder, 6g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g garlic powder, 1g ginger powder, 1g coriander powder, 1g cumin powder, 3g salt, 1.5g black pepper and 0.5g oregano.

Variation (CCC) was prepared using 8g oyster mushroom powder, 2g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g garlic powder, 1g ginger

powder, 1g coriander powder, 1g cumin powder, 3g salt, 1.5g black pepper and 0.5g oregano.

Variation (DDD) was prepared using 2g oyster mushroom powder, 8g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g garlic powder, 1g ginger powder, 1g coriander powder, 1g cumin powder, 3g salt, 1.5g black pepper and 0.5g oregano.

Among all the variations, variation (AAA) scored the highest sensory score and was finalized for further analysis.

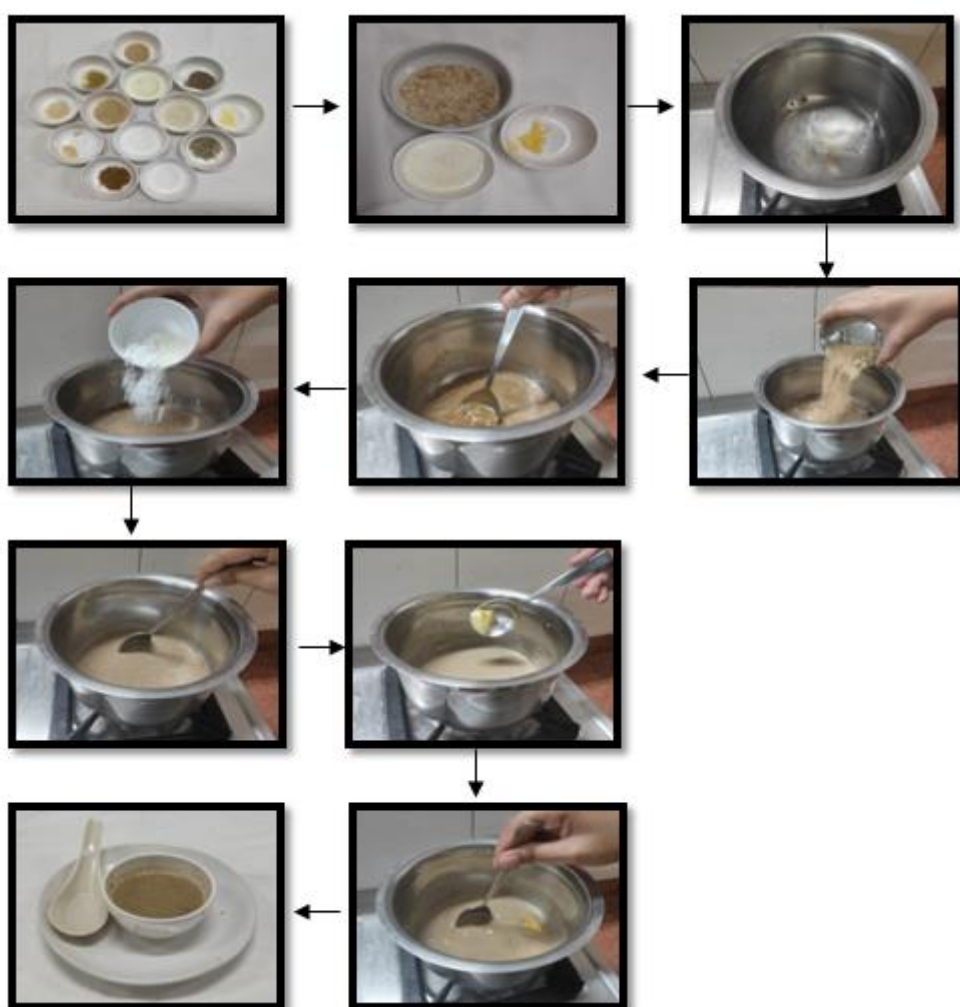


Fig 16: Preparation process of instant soy mushroom soup mix

All the ingredients were weighed separately. The ingredients were dry mixed into two separate sachets. Dry mixing of the ingredients was done by adding oyster

mushroom powder, soy flour and all the spices including onion powder, garlic powder, ginger powder, coriander powder, cumin powder, salt, black pepper and oregano in one bowl and another bowl contains corn flour and milk powder. The soup was prepared by adding 2 cups of water in a pan and then the first sachet was added to the water with stirring. After 15 seconds the second sachet was added and stirred continuously for 2 minutes and after that butter was added at the end.

Acceptability of the soup mix

Sensory evaluation had been done for four developed products. The sensory evaluation was done using five-point hedonic scale rating to find out the best variation of the developed product. The products were evaluated and the mean scores for the sensory attributes like appearance, texture, flavour, taste and overall acceptability of all the variations were done by the semi-trained panel members. From the sensory result the product that scored higher had been finalized and undergo for preparation of products using different variations of oyster mushroom powder and soy flour.

The total sensory evaluation score for trials by ten semi trained panel members is given in table V.

TABLE V
TOTAL SENSORY EVALUATION SCORE FOR TRIALS BY TEN SEMI
TRAINED PANEL MEMBERS

Code	Appearance	Colour	Consistency	Flavor	Taste	Overall Acceptability
AAA	43	42	36	37	39	197
BBB	44	43	43	38	39	207
CCC	42	44	45	42	43	216
DDD	47	48	49	46	48	238

The sample (DDD) scored 238 as higher score followed by sample (CCC) scored as 216, sample (BBB) scored as 207 and sample (AAA) scored as 197. These scores were collected from ten semi trained panel members of Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore.

The scores obtained had evidently showed that the fourth trial (DDD) has a higher acceptability than the other three trials. Hence the fourth trial (DDD) has been

finalized for the standardization of soy mushroom soup mix. This Sensory had been carried out to select the highly accepted sample to standardize the instant soy mushroom soup mix.

The mean acceptability score of the trials for instant soy mushroom soup mix is presented in table VI.

TABLE VI
MEAN SENSORY ACCEPTABILITY SCORE OF THE TRIALS FOR
INSTANT SOY MUSHROOM SOUP MIX

Variations	Appearance	Colour	Consistency	Flavor	Taste	Overall Acceptability
AAA	4.3 ± 0.48	4.2 ± 0.42	3.6 ± 0.52	3.7 ± 0.48	3.9 ± 0.57	19.7 ± 1.25
BBB	4.4 ± 0.52	4.3 ± 0.48	4.3 ± 0.48	3.8 ± 0.42	3.9 ± 0.57	20.7 ± 1.16
CCC	4.2 ± 0.42	4.4 ± 0.52	4.5 ± 0.53	4.2 ± 0.42	4.3 ± 0.48	1.16 ± 21.6
DDD	4.7 ± 0.48	4.8 ± 0.42	4.9 ± 0.32	4.6 ± 0.52	4.8 ± 0.42	23.8 ± 1.23

From the above table it shows that, the sample (DDD) scored higher in all the criteria as Appearance (4.7 ± 0.48); Colour (4.8 ± 0.42); Consistency (4.9 ± 0.32); Flavour (4.6 ± 0.52); Taste (4.8 ± 0.42) and overall acceptability (23.8 ± 1.23). The sample (CCC) scored lower than the sample (DDD) in all criteria as Appearance (4.2 ± 0.42); Colour (4.4 ± 0.52); Consistency (4.5 ± 0.53); Flavour (4.2 ± 0.42); Taste (4.3 ± 0.48) and Overall Acceptability (21.6 ± 1.26). The sample (BBB) scored lower than both (CCC) and (DDD) sample in all the criteria as Appearance (4.4 ± 0.52); Colour (4.3 ± 0.48); Consistency (4.3 ± 0.48); Flavour (3.8 ± 0.42); Taste (3.9 ± 0.57) and Overall Acceptability (20.7 ± 1.16). The sample (AAA) scored lowest than (BBB), (CCC) and (DDD) sample in all the criteria as Appearance (4.3 ± 0.48); Colour (4.2 ± 0.42); Consistency (3.6 ± 0.52); Flavour (3.7 ± 0.48); Taste (3.9 ± 0.57) and Overall Acceptability (19.7 ± 1.25).

This sensory evaluation score analysis shows that sample (DDD) had scored highest than (AAA), (BBB) and (CCC) samples.

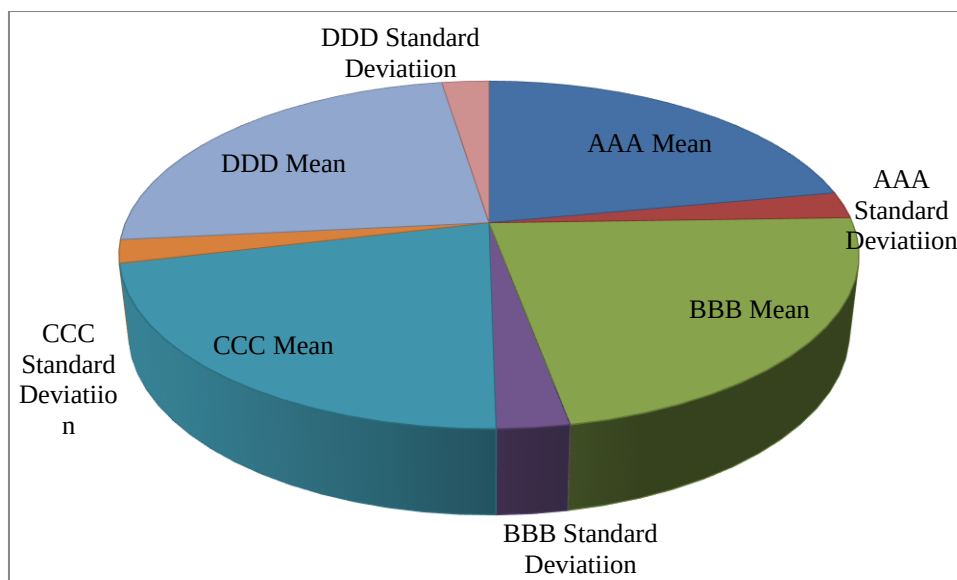


Fig 17: Sensory Acceptability score of Trials for Soup Mix

Sensory evaluation had been done for the four developed variations. From the sensory result the product that scored the highest had been finalized for further analysis.

The total sensory evaluation score for by ten semi trained panel members is given in table VII.

TABLE VII
TOTAL SENSORY EVALUATION SCORE BY TEN SEMI TRAINED
PANEL MEMBERS

Code	Appearance	Colour	Consistency	Flavor	Taste	Overall Acceptability
AAA	38.5	38.5	40	38.5	38	193.5
BBB	35.5	37.5	38.5	35	34.5	181
CCC	35	34.5	36	34	34	173.5
DDD	34	36	36	30	32	168

The sample (AAA) scored 193.5 as higher score followed by sample (BBB) scored as 181, sample (CCC) scored as 173.5 and sample (DDD) scored as 168. These scores were collected from ten semi trained panel members of Avinashilingam Institute of Home Science and Higher Education for Women, Coimbatore.

The mean acceptability score of the instant soy mushroom soup mix is presented in table VIII.

TABLE VIII
MEAN SENSORY ACCEPTABILITY SCORE OF THE INSTANT SOY
MUSHROOM SOUP MIX FOR DEVELOPED VARIATIONS

Variations	Appearance	Colour	Consistency	Flavor	Taste	Overall Acceptability
AAA	4.81 ± 0.37	4.81 ± 0.37	5 ± 0	4.81 ± 0.37	4.75 ± 0.46	24.18 ± 1.52
BBB	4.43 ± 0.49	4.68 ± 0.45	4.81 ± 0.37	4.37 ± 0.51	4.31 ± 0.45	22.62 ± 1.4
CCC	4.37 ± 0.44	4.31 ± 0.45	4.5 ± 0.53	4.25 ± 0.37	4.25 ± 0.37	21.68 ± 1.75
DDD	4.25 ± 0.46	4.5 ± 0.53	4.5 ± 0.53	3.75 ± 0.46	4 ± 0.75	21 ± 1.69

From the above table it shows that, the sample (AAA) scored higher in all the criteria as Appearance (4.8 ± 0.37); Colour (4.8 ± 0.37); Consistency (5 ± 0); Flavour (4.81 ± 0.37); Taste (4.75 ± 0.46) and overall acceptability (24.18 ± 1.52). The sample (BBB) scored lower than the sample (AAA) in all criteria as Appearance (4.43 ± 0.49); Colour (4.68 ± 0.45); Consistency (4.81 ± 0.37); Flavour (4.37 ± 0.51); Taste (4.31 ± 0.45) and Overall Acceptability (22.62 ± 1.4). The sample (CCC) scored lower than both (BBB) and (AAA) sample in all the criteria as Appearance (4.37 ± 0.44); Colour (4.31 ± 0.45); Consistency (4.5 ± 0.53); Flavour (4.25 ± 0.37); Taste (4.25 ± 0.37) and Overall Acceptability (21.68 ± 1.75). The sample (DDD) scored lowest than (AAA), (BBB) and (AAA) sample in all the criteria as Appearance (4.25 ± 0.46); Colour (4.5 ± 0.53); Consistency (4.5 ± 0.53); Flavour (3.75 ± 0.46); Taste (4 ± 0.75) and Overall Acceptability (21 ± 1.69).

This sensory evaluation score analysis shows that sample (AAA) had scored highest than (BBB), (CCC) and (DDD) samples.

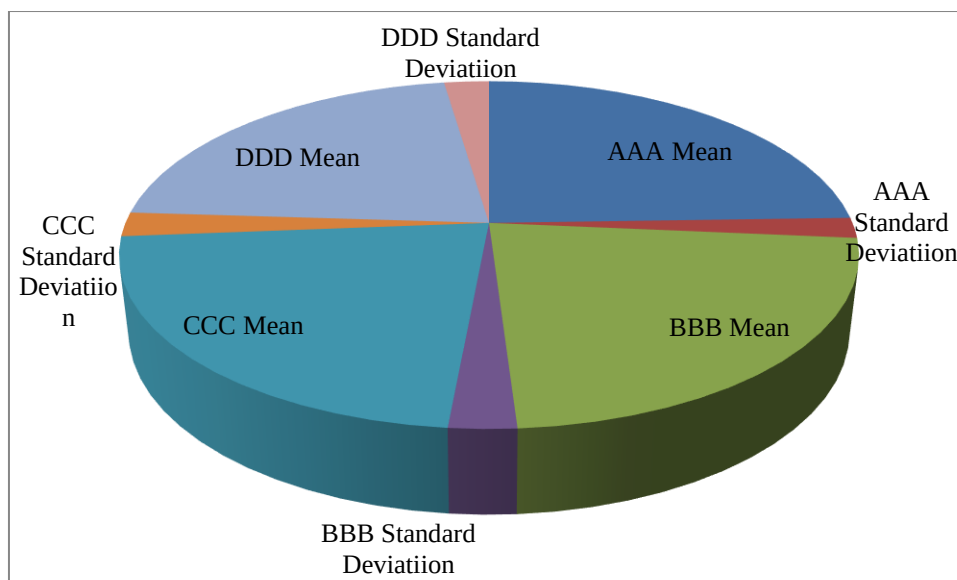


Fig 18: Sensory acceptability score of soy mushroom soup mix

Nutrient Analysis of Soy Mushroom Soup Mix

The nutrient analysis was done for the variation that scored the highest acceptability score that is, variation (AAA). The major nutrients such as the Energy, carbohydrate, protein, fat, fiber was analyzed. The minor nutrient such as the calcium, iron, thiamine, riboflavin and cobalamine (vitamin B12) were analyzed. The nutrient content was analyzed according to the nutrients which are prominent in the selected ingredients.

The amount of the nutrients present in the variation (AAA) is given in the table IX.

TABLE IX
NUTRITIONAL COMPOSITION OF SOY MUSHROOM SOUP MIX

Nutrients	Composition
Moisture	6.15%
Ash	16.48%
Energy	320 Kcal
Carbohydrate	58.54g
Protein	17.60g
Fat	1.23g
Fibre	7.31g
Calcium	62.8 mg
Iron	0.93 mg
Thiamine	11.09 mg
Riboflavin	26.11 mcg
Vitamin B12	6.77 mcg

From the above table it is observed that the instant soy mushroom soup mix has a moisture content of about 6.15%. According to Srivastava et al., (2019), moisture is an important factor in maintaining food quality because increased moisture facilitates the growth of microbes and ultimately destroy quality. Moisture content is an important factor for microorganism's death. Microorganisms cannot grow when moisture content is below 8 percent. The ash content of the soup mix is 16.48%. The values of the energy, carbohydrate, protein, fat, fibre were found to be 320 kcal, 58.54g, 17.60g, 1.23g, 7.31g respectively. The protein and fibre content of the instant soy mushroom soup mix was quite high. The calcium content of the soy mushroom soup mix was also high that is, 62.8 mg per 100 g. the iron content was 0.93 mg per 100g. The thiamine, riboflavin and vitamin content were found to be 11.09 mg, 26.11 mcg and 6.77 mcg respectively.

C. FOOD COSTING AND PACKAGING OF THE INSTANT READY TO USE SOUP MIX

The developed instant soy mushroom soup mix was analyzed for shelf-life. 32g of the developed instant soy mushroom soup mix was kept in an air-tight packaging. The packets were first washed thoroughly and then they were dried without leaving any moisture content. The packets were wiped with clean white cotton cloth to prevent any dust and moisture as these may be invaded as a cause for the spoilage of the product. Then the instant soy mushroom soup mix was added in the dried form and it was made sure that the packets were closed properly. The packets were then kept in a dry place in room temperature (32°C). The shelf life of instant soy mushroom soup mix was analyzed at room temperature. Direct method has been used to analyze the shelf life of the product.

The Developed soup mix was kept in room temperature for thirty days and was checked for the shelf life of the product. The shelf life of the product kept in dry place at room temperature was analyzed for thirty days. Each packet was opened and checked for the spoilage. Any changes in the colour, flavour and smell were noted. The growth of the mould or microbes was also noted to analyze the shelf-life of the product.

The shelf life study of the instant soy mushroom soup mix is presented in table X.

TABLE X
SHELF LIFE STUDY OF INSTANT SOY MUSHROOM SOUP MIX

Dates of Observation of Microbial Growth	Appearance	Colour	Texture	Flavor	Taste	Overall acceptability
1 to 5	X	X	X	X	X	X
6 to 10	X	X	X	X	X	X
11 to 15	X	X	X	X	X	X
16 to 20	X	X	X	X	X	X
21 to 25	X	X	X	X	X	X
26 to 30	X	X	X	X	X	X

X- The product was acceptable with the absence of Microbial Growth.

Y- The product was not acceptable with the presence of Microbial Growth.

The products were assessed and evaluated. The shelf-life of the products for the period of thirty days shows that the product was stable and had no changes in their sensory attributes. There were no changes in between the period of analysis. In the period of thirty days, there was no microbial contamination and no molds or fungus formation found. The product did not have any change in their attributes and holds the shelf-life.

Food cost analysis of the developed product

Food costing is important to know as it has a direct effect on the profit. It is the cost of the ingredients used and does not include other costs. Food costing is an essential tool in determining whether food cost targets are being met.

Recipe costing helps us to know how much food cost is incurred to prepare a soup mix. This gives us a clear view of how much one can earn per soup mix. It plays a significant role in deciding the selling price of the product.

The food cost of the product is calculated by considering all the ingredients used for the purchased amount which was then converted to one serving. The product was packed for two serving and so the maximum retail price of the product was calculated for two serving.

Cost of ingredients used for preparing soup mix is given below in table XI.

TABLE XI
CALCULATION OF FOOD COST OF THE DEVELOPED PRODUCT

Ingredients	Quantity (g)	Cost (Rs.)
Oyster mushroom powder	6	23
Soy flour	4	0.24
Corn flour	4	0.32
Milk powder	8	3.36
Onion powder	1	1.98
Garlic powder	1	0.92
Ginger powder	1	2.47
Cumin powder	1	0.6
Coriander powder	1	0.34
Black pepper	1.5	1.8
Oregano	0.5	1.28
Salt	3	0.04
Total	32	36

Calculation:

Total yield - 300g

Weight per portion - 32 g

Total servings - 2

Cost per portion = Total cost / Number of Serving X Overhead cost (1.66)

Cost per portion = Rs. 36/2 X 1.66

= Rs. 18 X 1.66

= Rs. 30

Cost per portion = Rs 30

The total cost of soy mushroom instant soup mix for two serving is Rs. 30.

The selling price of soy mushroom instant soup mix per serving is Rs 30. In each packet, the product was packed for 2 serving. So, the maximum retail price for the product was calculated for two serving.

A very high quantum of polymeric materials, besides Cellulosics and Aluminium foil are used for packaging. Paper board and metal containers are also used for certain applications. Although a variety of packaging materials are available, the ultimate choice of the wrapper depends upon the required shelf-life. The most common choice of packaging medium is plastic which is generally flexible as it provides the required protection and preservation, grease resistance, physical strength, machinability and printability. Plastics being lighter in weight are, therefore, the most preferred material for packaging of powders.

There are many changing trends in the packaging of food products. Plastic films and their laminates are increasingly replacing waxed papers due to better properties and Aluminium foil laminates due to price and better flex crack property.

A package intended for a food product has to perform several functions during distribution, storage and sales. Essentially, the package has to preserve the quality attributes of the product and afford protection against chemical and microbiological deteriorative reactions. For soup mix, the major functional packaging requirements include protection from, dust, dirt, other contaminating agents, moisture / water vapours pickup or loss resulting in sugar and fat bloom, stickiness, hardening,

desiccation, rancidity due to interaction with moisture and oxygen, Colour and aroma loss and tainting, physical damages like dusting and formation of lump.

Labeling requirements for packaged food products as laid down in the part VII of the Prevention of Food Adulteration (PFA) Rules, 1955, and the Standards of Weights and Measures (Packaged Commodities) Rules of 1977, require that the labels contain the following information: Name, trade name or description; Name of Ingredients used in the product in descending order of their composition by weight or volume; Name and complete address of manufacturer / packer, importer, country of origin of the imported food (If the food article is manufactured outside India, but packed in India); Nutritional Information; Information relating to Food Additives, Colours and Flavours; Instructions for use; Vegetarian or Non-Vegetarian Symbol; Net weight, number or volume of contents, Distinctive batch, lot or code number; Month and year of manufacture and packaging; Month and year by which the product is best consumed; Maximum retail price.

All declarations may be printed in English or Hindi on a label securely affixed to the package, or made on an additional wrapper containing the imported package, or printed on the package itself, or may be made on a card or tape affixed firmly to the package and bearing the required information prior to customers clearance.

According to the FSS Packaging and Labelling Regulation 2011, “Prepackaged” or “Pre packed food” including multi-piece packages, should carry mandatory information on the label. Hence, mandatory has been included in the designed labelling for the developed instant soy mushroom soup mix.

The packaging and labeling of the instant soya mushroom soup mix is as follows:



Fig 19: Packaging and labelling of the instant soya mushroom soup mix

D. ACCEPTABILITY OF THE INSTANT READY TO USE SOUP MIX AMONG HOMEMAKERS

The overall acceptability of the instant soy mushroom soup mix was done among homemakers. Five point- Hedonic scale was used to give scores (1- dislike very much, 2- dislike slightly, 3- neither like nor dislike, 4- like slightly, 5- like very much). They were asked to evaluate appearance, colour, consistency, flavor and taste for the developed soup mix. For sensory evaluation ten members were selected. Score card was given to evaluate the recipes.

The mean acceptability score of the for instant soy mushroom soup mix among homemakers is presented in table XII.

TABLE XII
MEAN SENSORY ACCEPTABILITY SCORE OF THE INSTANT SOY
MUSHROOM SOUP MIX AMONG HOMEMAKERS

Variations	Appearance	Colour	Consistency	Flavor	Taste	Overall Acceptability
AAA	4.7 ± 0.48	4.5 ± 0.71	4.6 ± 0.69	4.7 ± 0.71	4.7 ± 0.48	23 ± 2.4

From the above table it shows that, the sample (AAA) scored lower in all the criteria as Appearance (4.7 ± 0.48); Colour (4.5 ± 0.71); Consistency (4.6 ± 0.69); Flavour (4.7 ± 0.71); Taste (4.7 ± 0.48) and overall acceptability (23 ± 2.4) as compared to acceptability scores among the semi trained members of age group 20 to 25 as Appearance (4.8 ± 0.37); Colour (4.8 ± 0.37); Consistency (5 ± 0); Flavour (4.81 ± 0.37); Taste (4.75 ± 0.46) and overall acceptability (24.18 ± 1.52) .

V. SUMMARY AND CONCLUSION

Proteins are essential to human nutrition and health and are widely used in the food industry to formulate various healthy diets for nutritional demand. Dietary fiber is defined as the edible part of the plant or carbohydrates that are resistant to digest and absorb in the human small intestine with complete or partial fermentation in the large intestine. The diets with a high content of fibre, such as those rich in cereals, fruits and vegetables have a positive effect on health since their consumption has been related to a decreased incidence of several types of diseases as due to its beneficial effects like increasing the volume of fecal bulk, decreasing the time of intestinal transit, cholesterol and glycaemic levels, trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents), stimulating the proliferation of the intestinal flora etc.

Soups are considered as an appetizer and can be fortified with essential nutrients to make it health promoting. Various types of instant soup mixes are available in the market which contain different ingredients, mostly corn starch, spices, salt, flavours and flavor enhancers. Instant soups play an important role in balancing the nutrients required for the people to stay healthy and can become an alternative food for breakfast. The study titled **“Formulation of protein and fibre enriched instant soya mushroom soup mix”** was carried out with objectives to formulate protein and fibre enriched instant soy mushroom soup mix, to analyze nutritional composition, sensory evaluation and shelf life of the soup mix and to study the acceptability of the soup mix among homemakers.

The methodology comprises of selection of various ingredients based on their nutritional properties to formulate the instant soy mushroom soup mix, Standardization process of instant ready to use soup mix comprises of processing of the soup mix which include cleaning, slicing, drying and powdering the ingredients and trial run of the product was carried out. Variations were done by adding varying proportions of oyster mushroom powder and soy flour from the selected trail. The product with highest acceptability was selected for nutrient analysis. The nutrients that were analyzed are energy, protein, carbohydrate, fat, fibre, calcium, iron, thiamine, riboflavin and cobalamine. Food Costing and Packaging comprises of

calculation of food cost of the highly accepted soup mix followed by labeling and packaging of the soup mix and Acceptability of the formulated ready to use soup mix was done among ten homemakers and the age group 21 to 25 years at Coimbatore.

The salient findings of the study are summarized below:

- The major ingredients that were selected for the development of the soup mix were fresh oyster mushrooms and soybean flour and the minor ingredients included corn flour, milk powder, onion powder, ginger powder, garlic powder, coriander powder, cumin powder, black pepper, oregano and salt.
- The selected ingredients were processed through different systems right from selection of the ingredients, cleaning, slicing, powdering and standardization of the product as soup mix.
- Trial 1 was prepared using 10g oyster mushroom powder, 5g soy flour, 10g corn flour, 12.5g milk powder, 4g salt, 1.5g sugar, 1g black pepper, 1g oregano.
- Trial 2 was prepared using 10g oyster mushroom powder, 5g soy flour, 15g corn flour, 12.5g milk powder, 4g salt, 1.5g sugar, 1g black pepper, 1g oregano.
- Trial 3 was prepared using 6g oyster mushroom powder, 4g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g ginger powder, 1g garlic powder, 3g salt, 1.5g black pepper, 0.5g oregano.
- Trial 4 was prepared using 6g oyster mushroom powder, 4g soy flour, 4g corn flour, 8g milk powder, 1g onion powder, 1g ginger powder, 1g garlic powder, 1g coriander powder, 1g cumin powder, 3g salt, 1.5g black pepper, 0.5g oregano, 3g butter.
- Trial run of the soup mix was carried out and the sensory score of the trials was evaluated. Among all the four trials, trial IV scored the highest acceptability score and standardization was done for the accepted trial.
- Trial 1 scored the least mean score for appearance 4.3, colour 4.2, consistency 3.6, flavor 3.7, taste 3.9 with the overall acceptability of 19.7. Trial IV scored the highest mean score for appearance 4.7, colour 4.8, consistency 4.9, flavor 4.6, taste 4.8 with the acceptability score of 23.8. Thus trial IV was selected for standardization.

- Four variations were standardized and were coded as variation (AAA), variation (BBB), variation (CCC) and variation (DDD). Each developed variation was evaluated for sensory attributes using five point hedonic scale by ten members.
- Among all the four variations, variation (DDD) scored the least mean score for appearance 4.25, colour 4.5, consistency 4.5, flavor 3.75, taste 4 with the overall score of 21 and variation (AAA) scored the highest mean score for appearance 4.81, colour 4.81, consistency 5, flavor 4.81, taste 4.75 with overall acceptability of 24.18. Thus variation (AAA) was selected for further analysis.
- Variation (AAA) was evaluated for sensory attributes among homemakers as it scored the highest acceptability score using five point hedonic scale to check the acceptability among the homemakers. The mean score for appearance, colour, consistency, flavor taste and overall acceptability of 4.7, 4.5, 4.6, 4.7, 4.7 and 23 respectively.
- Nutrient analysis was done for variation (AAA) which scored the highest acceptability score. The nutrients that were analyzed include energy, protein, carbohydrate, fat, fibre, calcium, iron, thiamine, riboflavin and cobalamine.
- The values of the energy, carbohydrate, protein, fat, fibre were found to be 320 kcal, 58.54g, 17.60g, 1.23g, 7.31g respectively. The protein and fibre content of the instant soy mushroom soup mix was quite high. The calcium content of the soy mushroom soup mix was also high that is, 62.8 mg per 100 g. The iron content was 0.93 mg per 100g. The thiamine, riboflavin and vitamin content were found to be 11.09 mg, 26.11 mcg and 6.77 mcg respectively.
- The soup mix was packed in two sachets. Sachet 1 contained granulated oyster mushrooms, soybean flour, onion powder, ginger powder, garlic powder, coriander powder, cumin powder, black pepper, oregano and salt and sachet 2 contains corn flour and milk powder.
- The food cost of the soup mix was analyzed. The total cost of soy mushroom instant soup mix for two serving is Rs 30. The cost per portion is Rs. 30. In each packet, the product was packed for 2 serving.

- The shelf life of the soup mix was assessed and evaluated. The shelf-life of the product for the period of thirty days showed that the product was stable and had no changes in their sensory attributes. There were no changes in between the period of analysis.

Conclusion:

Formulation of such convenience foods packed with nutrients will serve as a healthy choice for the consumers saving time as as an appetizer in the menu course of all age groups especially women whose requirements for protein, calcium iron, thiamin, riboflavin and vitamin B12 (cobalamine) are higher.

The formulated instant soy mushroom soup mix will help in balancing the nutrients required for the people to stay healthy and can become an alternative food option. It will also be free from enzymatic and oxidative spoilage and flavour stability at room temperature over a long period of time as it was packaged as dry soup mix. Also, they do not need any preservatives or refrigerator to preserve them. It has high nutritive value and health benefits

Scope for Future Research:

- Development of various Ready-to-Eat and Ready-to-Serve products such as cookies, bread, pasta, bars, cake, muffins, jam, chips etc. with incorporation of oyster mushroom powder and soya flour.
- Supplementation study of developed instant soya mushroom soup mix can be carried out among malnourished children.
- Creating awareness among people about the locally available and natural foods such as oyster mushrooms in the processed forms.

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APPENDIX I
HUMAN ETHICAL COMMITTEE CERTIFICATE

INSTITUTIONAL HUMAN ETHICS COMMITTEE



Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3
of UGC Act 1956) Re-accredited with 'A++' Grade by
NAAC. Recognised by UGC Under Section 12 B
Coimbatore-641 043, Tamil Nadu, India

24th March 2022

Chairman

Dr. Sudha Ramalingam
Director-Research & Innovation,
Professor-Community Medicine,
PSG Institute of Medical Sciences
& Research, Coimbatore

Member Secretary

Dr. S. Uma Mageshwari
Professor and Head,
Department of Food Service
Management & Dietetics

Members

Mr. K. Arunmoli (Legal Expert)
Dr. Subhashini K. Sripathi
Dr. A. Saraswathy (Medical Officer)
Ms. D. Kavitha
Dr. A. R. Sudamani Ramasamy
Dr. G. Victoria Naomi
Dr. Judith Justin
Dr. Anitha Subash

To
Ms. Puja Das
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Puja Das,

Ref: Your proposal No. IHEC/21-22/FSMD-22 entitled
“Formulation of Protein and Fibre Enriched Instant Soya Mushroom
Soup Mix” resubmitted for approval to IHEC on 18.03.2021.

The Institutional Human Ethics Committee of our University
hereby grants approval to your research proposal No. IHEC/21-22/
FSMD-22 entitled “Formulation of Protein and Fibre Enriched
Instant Soya Mushroom Soup Mix” resubmitted by you. The
Approval number for the same is AUW/IHEC/FSMD-21-22/XPD-
22.

We wish you all the best in your research endeavours.

Regards,


Dr. S. Uma Mageshwari
Member Secretary



APPENDIX II

PLAGIARISM REPORT

INTRODUCTION

ORIGINALITY REPORT

9%

SIMILARITY INDEX

5%

INTERNET SOURCES

5%

PUBLICATIONS

5%

STUDENT PAPERS

PRIMARY SOURCES

1	Kumela D. Tolera, Solomon Abera. " Nutritional quality of Oyster Mushroom () as affected by osmotic pretreatments and drying methods ", Food Science & Nutrition, 2017 Publication	1%
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APPENDIX III

NUTRIENT ANALYSIS TEST REPORTS



ALPHA
LABS & TECHNOLOGIES
FOOD / WATER / SOIL TESTING

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TEST REPORT

REPORT NO:	ALT/17249/2022	REPORT DATE:	09.05.2022
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Customer Details :	Ms. HARINI.A.S AVINASHILINGAM UNIVERSITY, COIMBATORE.		
Sample Description:	MUSHROOM SOUP MIX		
Sample Code no:	ALT/APR/22/17249	Sample Received Date	29.04.2022
Sample Analysis Date	29.04.2022	Sample Completed Date	09.05.2022

RESULTS OF THE ANALYSIS

S.NO	PARAMETERS	UNIT	METHOD	RESULTS
1.	Moisture	%	AOAC 20 th edition 925.10	6.15
2.	Ash	%	AOAC 20 th edition 923.03	16.48
3.	Fat	%	AOAC 20 th edition 2016 920.85	1.23
4.	Protein	%	IS 7219	17.60
5.	Carbohydrate	%	AOAC 20 th edition 986.25	58.54
6.	Energy	Kcal	ALT/SOP/03/01:2016	320.55
7.	Fibre	%	AOAC 20 th edition,920.86	7.31
8.	Calcium	mg/100g	AOAC 21 st EDITION - 944.03	62.8
9.	Iron	mg/100g	AOAC 21 st EDITION 944.02:	0.93
10.	Vitamin B1	mg/kg	AOAC 21 st Edition 974.29	11.09
11.	Vitamin B2	mcg/100g	AOAC 21 st Edition 974.29	26.11
12.	Vitamin B12	mcg/100g	AOAC 21 st Edition 974.29	6.77

*** End of Report ***



S.S.G.
Authorized Signatory
S.S.Gayathri
Technical Manager

The test results relate only to the items tested. The test report will not be reproduced in full or part without the written consent of Alpha Labs & Technologies. Lab is not responsible for Legitimacy of xerox or photo copied test reports. Unless informed by the Customer, the test items will not be retained for more than 14 days from the date of issued the test report.

APPENDIX IV

NUTRIENT ANALYSIS

1. Determination of energy value of food

Scope:

The SOP provides the method for determination of Energy value I the products.

Method:

The energy of the food product is analysed by calculation method

Calculation:

Food energy values in both kilocalories (kcal) and kilojoules use the same factors.

Energy (kcal/100g) = (Carbohydrate X 4) + (Protein X 4) + (Fat X 9)

2. Determination of Total Carbohydrate by Anthrone method

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into simpler sugars. The carbohydrates content can be measured by hydrolysing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Materials:

- 25 N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 mL of ice -cold 95%

- H₂SO₄ . Prepare fresh before use. Standard glucose : Stock - Dissolve 100 mg in 100 mL water. Working standard - 10ml of stock diluted to 100 mL with distilled water. Store in refrigerator after adding a few drops of toluene

Procedure:

- Weigh 100 mg of the sample into a boiling water
- Hydroxyl by keeping it in a boiling water bath for three hours with 5mL of 2.5
- N HCl and cool to room temperature. Neutralise it with solid sodium carbonate until the effervescence ceases.
- Make up the volume to 100 mL and centrifuge.
- Collect the supernatant and take 0.5 and 1 mL aliquots for analysis.
- Prepare the standards by taking 0., 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard '0' serves as blank.
- Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water.
- Then add 4 mL of anthrone reagent
- Heat for eight minutes in a boiling water bath.
- Cool rapidly and read the green to dark green colour at 630 nm.
- Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph calculate the amount of carbohydrate present in the sample tube.

Calculation:

Amount of carbohydrate present in 100 mg of the sample

$$= \text{mg of glucose} \quad \times \quad 100 \quad \underline{\hspace{2cm}}$$

Volume of test sample

3. Protein Estimation by Lowry's method

Protein can be estimated by the methods as given by Lowry and also by estimating the total nitrogen content. No method is 100% sensitive. Hydrolysing the protein and estimating the amino acids alone will give the exact quantification. The method developed by Lowry et al (2010), is sensitive enough to give a moderately

constant value and hence largely followed. Protein content of enzyme extracts is usually determined by this method.

Principle:

The blue colour developed by the reduction of the phosphomolybdic - phosphotungstic components in the Folin-ciocalteu reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cuprictartrate are measured in the Lowry's method.

Materials:

- 2% sodium Carbonate in 0.1 N Sodium Hydroxide (Reagent)
- 0.5% Copper Sulphate, ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartrate (Reagent B)
- Alkaline copper solution: Mix 50 mL of A and 1 mL of B prior to use (Reagent C)
- Folin-ciocalteu reagent (reagent D); Reflux gently for 10 hours a mixture consisting of 100 g sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), 25 g sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)
- 700 mL water, 50 mL of 85% phosphoric acid, and 100 mL of concentrated hydrochloric acid in a 1.5 L flask. Add 150 g lithium sulphate, 50 mL water and a few drops of bromine water. Boil the mixture for 15 min without condenser to remove excess bromine cool, dilute to 1 L and filter. The reagent should have no greenish tint. (determine the acid concentration of the reagent by titration with 1 N NaOH to a phenolphthalein end point)
- Protein solution (Stock standard) Weigh accurately 50 mg of bovine serum albumin (Fraction V) and dissolve in distilled water and make up to 50 mL in a standard flask.

Working standard

Dilute 10 mL of the stock solution to 50 mL with distilled water in a standard flask. One mL of this solution contains 200 μg protein.

Procedure

- Extraction of protein from sample Extraction is usually carried out with buffers used for the enzyme assay.
- Weigh 500mg of the sample and grind well with a pestle and mortar in 5 - 10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of protein

- Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes.
- Pipette out 0.1ml and 0.2 ml of the sample extract in two other test tubes.
- Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank.
- Add 5 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10min.
- Then add 0.5ml of reagent D, mix well and incubated at room temperature in the dark for 30 min. Blue colour is developed.
- Take the reading at 660nm.
- Draw a standard graph and calculate the amount of protein in the sample.

Calculation

Express the amount of protein mg/g or 100g sample.

4. Estimation of Crude Fibre

Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It presents only 60-80% of the cellulose and 406% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and live stock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

Principle:

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre control.

Materials

Sulphuric acid solution (0.255 ± 0.005 N): 1.25g concentrated sulphuric acid diluted to 100mL (Concentration must be checked by titration)

Sodium hydroxide solution (0.313 ± 0.005 N): 1.25g sodium hydroxide in 100mL distilled water (Concentration must be checked by titration with standard acid)

Procedure

Extract 2g of ground material with ether or petroleum ether to remove fat (Initial boiling temperature 35-38 °C and final temperature 52°C) If fat content is below 1% extraction may be omitted.

After extraction with ether boil 2g of dried material with 200mL of sulphuric acid for 30min with bumping chips.

Filter through muslin and wash with boiling water until washings are no longer acidic

Boil with 200mL of sodium hydroxide solution for 30 min.

Filter through muslin cloth again and wash with 250 mL of boiling 1.25% H₂SO₄ there 50mL of portions of water and 25mL alcohol.

Remove the residue and transfer to ashing dish (Prew weighed dish W1) Dry the residue for 2 h at $130 \pm 2^\circ$ C. Cool the dish in a desiccator and weigh (W2).

Ignite for 30 min at $600 \pm 15^\circ$ C.

Cool in a desiccators and reweigh (W3).

Calculation

% crude fibre in ground sample

= Loss in weight on ignition (W2-W1) - (W3-W1) / weight of sample x 100

5. Estimation of Calcium

Titrimetric method

Principle

Calcium is precipitated as oxalate and is titrated with standard potassium permanganate.

Reagents

- 4% ammonium oxalate solution
- Dilute ammonia solution: 2 ml of liquor ammonia with 98 ml water.
- 1N H₂SO₄
- 0.01 N Potassium permanganate solution
- 0.01 N oxalic acid: Sodium oxalate is dried in an oven at 100-105°C for 12 hours. Exactly 0.67 g is dissolved in redistilled water. Five ml of concentrated H₂SO₄ is added and solution made up to 1 L after it has cooled down.

Standardisation of potassium permanganate solution: 25 ml of 0.01 N oxalic acid is transferred to an Erlenmeyer flask. One ml of concentrated H₂SO₄ is added, warmed to about 70°C and titrated against KMnO₄ solution, till the pale pink colour remains. The normality of KMnO₄ solution = $(25 \times 0.01) / \text{Titre value (ml)}$

Procedure

91 Two ml of sample is taken into a 15 ml centrifuge tube. Add 2 ml of distilled water and 1 ml of ammonium oxalate solution and mix thoroughly and leave overnight. Again the contents are mixed and centrifuged for 5 min at 1500 rpm. The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube is wiped with a piece of filter paper. The precipitate is stirred and the sides of the tubes are washed with 3 ml of dilute ammonia. It is centrifuged again and drained as before. The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate is dissolved in 2 ml of 1N H₂SO₄. The tube is heated by placing it in a boiling water bath for 1 min and titrated against 0.01 N KMnO₄ solution to a definite pink colour persisting for at least 1 min.

Calculation

1 ml of 0.01 N KMnO_4 is equivalent to 0.2004 mg of calcium.

$$\text{mg of calcium/ 100 ml serum} = [(X-b) \times 0.2004 \times 100] / 2$$

where, X = Volume in ml of 0.01 N KMnO_4 required to titrate the sample, b = Volume in ml of 0.01 N KMnO_4 required to titrate 2 ml of H_2SO_4 (blank). If the normality of KMnO_4 is 'a', the value obtained in the above formula should be multiplied by the factor, a/ 0.01.

6. Estimation of Iron using Atomic Absorption Spectrophotometry

Principle: Test portions are dried and then ashed at 450°C under a gradual increase ($\leq 50^\circ\text{C/h}$) in temperature 6m HCL (1+1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1m HNO_3 and the analytes are determined by flame and graphite procedures.

Apparatus

- a. Atomic absorption spectrophotometer - with an air - acetylene burner or nitrous oxide - acetylene burner for flame and a graphite furnace for electro thermal determinations, with appropriate back ground (non atomic) correction
- b. Hollow cathode or electrode less discharge lamps for all elements determined
- c. Furnace - programmable, or muffle furnace with thermostat maintaining $450 \pm 25^\circ\text{C}$. If muffle furnace is used, a separate pre-ashing device is required
- d. Hot plate - with heating control, to heat up to about 300°C
- e. Lamp - IR 250W, fixed to a retort stand in a way that allows adjustment of the distance to the plate
- f. Ceramic plate - eg. Desiccators plate on a low stand, with a diameter that suits the hot plate
- g. Glass cover - eg., crystallizing dish, 185mm diameter, 100mm height, to fit on (f) for equivalent
- h. Wash - bottle - "Scrubber", containing H_2SO_4 for purification of air
- i. Quarts or platinum crucibles - 50- 75ml
- j. Polydiamine bottles - with leak-proof closures, 100ml, carefully clean and rinse all glassware and plastic ware with HNO_3 or HCL to avoid metal

contamination. Cleaning procedure for glass and plastic ware - Acid solution: 500ml concentrated HNO₃ C (c) +4500ml deionized water c(a) wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4-5 times with deionised water.

Reagent

Reagents should be at least analytical reagent grade (p.a) preferably ultrapure (Suprapur) or equivalent.

Water - Redistilled or deionized, resistivity $\geq 18\text{M } \Omega\cdot\text{cm}$

Nitric acid - 65% (w/w)

Nitric acid - 0.1M dilute 7 ml HNO₃ (c) with water (a) to 1h.

Procedures:

Pre-treatment - Aomogenize if necessary using non containing equipment check for leaching metals if the apparatus consists of metal parts.

Drying - In a crucible, weight 10-20g test portion to nearest 0.01g. Dry in a drying oven, on a water - bath, or a hot plate at 100C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace

Ashing

Ashing in a programmable furnace - place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum tae of 50°C /h to 450°C. Let dish stand for at least 8h or overnight. Continue according to (c)

Ashing in a muffle furnace with thermostat following drying and pre-ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in β (d) - (h) place crucible with the test portion covered with the glass tube sweep over the product. Put IR lamp down at the cover. Pre-ash product sample by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should than be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200-250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8h or overnight. Take crucible out of furnace and let cool.

Wet ash with 3ml water and separate on water or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50 -100°C/h) to 450°C. Proceed with ashing 450°C for 1-2h or longer. Repeat procedure until product is completely ashed, ie.ash should be white /gray or slightly covered. Number of repetitions varies depending on type of product. Add 5ml 6M HCL, c(b), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water - bath or hot plate. Dissolve residue in 10.0 - 30.0ml, to the nearest 0.1ml, of 0.1M HNO₃ (d) Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1-2h then stirring solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blank with each analytical batch.

Atomic absorption spectrophotometry - Pb and Cd in foods generally require graphite furnace AAS for determination of iron

Wavelength, gas mixture/ temperature program and other instrumental parameters that are most appropriate for each metal are found in the manual provided with the instrument.

Background correction must always be used in flameless AAS and for flame application at low concentrations.

When results are outside for the linear range, the test solutions should be diluted with 0.1M HNO₃, c(d).

Flame technique - prepare calibration curves from a minimum of 3 standards.

Graphite furnace (flameless) technique - The method of addition should always be used. Measurements must be made in the linear range when method of addition is used. Measurements are preferably made with peak area rather than peak height.

APPENDIX V

RECIPE

The method for preparation of instant soy mushroom soup mix is given below:

- Boil 300 ml of water and add sachet 1 with constant stirring.
- After 30 seconds add sachet 2 and stir constantly for 2 minutes.
- Add butter before serving

APPENDIX VI

Questionnaire on Sensory Evaluation Score Card for Evaluation of Developed Product (Cooked)

Name : Contact Number :
Date : Address :
Email ID :

The sensory evaluation is done using five-point hedonic scale, for all the varieties developed.

Variation	Appearance	Colour	Consistency	Flavor	Taste	Overall acceptability
AAA						
BBB						
CCC						
DDD						

Appearance : 5 - Smooth layer; 4 - Good; 3 - Average; 2 - Irregular layer; 1 - Very irregular layer.

Colour : 5 - Bright and colourful; 4 - Good; 3 - Average; 2 - Dull; 1 - Very dull

Consistency : 5 - Thicker; 4 - Good; 3 - Average; 2 - Slightly thicker; 1 - Very thinner

Flavor : 5 - Excellent flavor; 4 - Good Flavour; 3 - Moderate flavor; 2 - Unpleasant flavor; 1 - Very unpleasant flavor

Taste : 5 - Good; 4 - Moderately good; 3 - Slightly good; 2 - Less good; 1 - very bad

Five point hedonic scale:

- 5 -liked very much
- 4 -liked moderately
- 3 -neither liked nor disliked
- 2 -dislike moderately
- 1 -dislike very much

Signature of Participant