Preparation of Ready-to-eat Oyster Mushroom (*Pleurotus pulmonarius)* Soup in the Retort Pouch Packaging

KHAVINISHA A/P SILVAKUMAR

F18B0298

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions

Khavinisha

Student

Name: Khavinisha a/p Silvakumar

Date: 24.2.2022

I certify that the report of this final year project entitled "**Preparation of Ready-to-eat Oyster Mushroom (*Pleurotus pulmonarius)* Soup in the Retort Pouch Packaging**" by **Khavinisha a/p Silvakumar**, matric number **F18B0298** have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Malaysia.

Approved by:

Supervisor

Name: Dr Noor Hafizoh Binti Saidan

Date:

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ABSTRAK

Apabila dunia semakin membangun, gaya hidup manusia juga turut berubah. Makanan yang sedia untuk dimakan ialah istilah yang menggambarkan gaya hidup pantas dunia dan teknologi yang semakin berkembang yang dituntut oleh orang ramai. *Pleurotus pulmonarius* yang biasa dikenali sebagai cendawan tiram, adalah pek dengan nilai pemakanan. *P. pulmonarius* sangat mudah rosak, cendawan segar diketahui mempunyai daya simpan yang rendah. Oleh itu, dengan menghasilkan produk nilai tambah jangka hayat cendawan dapat dilanjutkan dan mengurangkanpembaziran. Dalam kajian ini, sup cendawan *Pleurotus pulmonarius* dinilai dengan 3 formulasi perbezaan (500g, 300g, dan 100g) cendawan. Sup cendawan sedia dikomersialkan digunakan sebagai kawalan. Sup cendawan *Pleurotus pulmonarius* telah dimasak dan dibungkus dalam pembungkusan kantung retort dan menjalani proses retort selama 15 minit pada suhu 121 °C. Nilai pH bagi kesemua 3 formulasi adalah melebihi 4.6 selepas proses retort yang menunjukkan sup adalah makanan rendah asid. Nilai kelikatan telah menurun selepas retort daripada julat 2.0- 1.9 Pa.c kepada 1.1- 0.5 Pa.c. Analisis warna menunjukkan 3 formulasi mempunyai nilai L* yang hampir sama iaitu melebihi ±72.00 dan nilai a* dan b* bagi ketiga-tiga formulasi telah meningkat selepas retort. Protein yang mengandungi kesemua 3 formulasi adalah lebih tinggi daripada sampel kawalan. Selain itu, kandungan lemak ketiga-tiga formulasi adalah lebih tinggi daripada sampel kawalan. Berdasarkan analisis mikrob, terdapat kehadiran mikroorganisma pada hari 1 dan ia meningkat pada hari ke-7 secara drastik. Berdasarkan penilaian deria, kesemua 3 formulasi sup cendawan *P. pulmonarius* mempunyai skor yang tinggi dari segi rasa, warna, aroma, tekstur dan penerimaan keseluruhan berbanding sampel kawalan. Formulasi 2 sup cendawan mempunyai kebolehterimaan yang lebih baik. Berdasarkan hasil yang diperoleh, sup cendawan *Pleurotus pulmonarius* telah diterima oleh pengguna. Kesimpulannya, objektif kajian ini iaitu untuk membangunkan sup *Pleurotus pulmonarius* telah dicapai dan sifat deria telah ditentukan.

Kata kunci: *Pleurotus pulmonarius*, penilaian deria, sifat fizikokimia, pembungkusan kantung retort, sedia untuk dimakan, sup

ABSTRACT

As the world is developing, the lifestyle of people is also changing. Food that is ready to eat is a term that describes the world's fast-paced lifestyle and evolving technology which people are demanding. *Pleurotus pulmonarius* which commonly known as the oyster mushroom, is pack with nutritional value. *P. pulmonarius* are highly perishable, the fresh mushroom is known to have low shelf live. Therefore, by producing value-added products the shelf life of the mushroom can be extend and reduce waste. In this study, *Pleurotus pulmonarius* mushroom soup were evaluated with 3 differences formulations (500g, 300g, and 100g) of mushroom. A commercialized ready to mushroom soup were used as control. The *Pleurotus pulmonarius* mushroom soup was cooked and packed in retort pouch packaging and underwent retort process for 15 minutes at 121 °C. The pH values of all 3 formulations were above 4.6 after retort process which indicates the soup is a low acid food. The value for viscosity has decreased after the retort from the range of 2.0- 1.9 Pa.c to 1.1- 0.5 Pa.c. The color analysis show that 3 formulations have almost the same L* value which is more than ±72.00 and a* and b* value of all three formulations have increased after the retort. The protein contain of the all 3 formulation are higher than the control sample. Moreover, fat content of the three formulations are higher than the control sample. Based on microbial analysis, there were presences of microorganism in day 1 and it increased in day 7 drastically. Based on the sensory evaluation, all 3 formulation of *P. pulmonarius* mushroom soup have high score in term of taste, color, aroma, texture and overall acceptance compare to control sample. Formulation 2 mushroom soup had better acceptability. According to the result obtain the *Pleurotus pulmonarius* mushroom soup were accepted by the consumers. In a conclusion, objective of this study which was to develop *Pleurotus pulmonarius* soup have been accomplished and the sensory attribute haven been determined.

Keyword: *Pleurotus pulmonarius*, mushroom, oyster mushroom, sensory evaluation, physicochemical properties, retort pouch packaging, ready-to-eat, soup

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LIST OF ABBREVIATION AND SYMBOLS

ANOVA	Analysis of Variance
CFU	Colony Forming Units
SPSS	Statistical Package for the Social Science
±	Plus-minus
TFTC	Too Few To Count
a*	Redness/greenness
L*	Lightness
b*	Blueness/yellowness
N	Sample size
mL	Millilitre
L	Litter
%	Percentage
°C	Celcius
TPC	Total Plate Count

CHAPTER 1

INTRODUCTION

1.1 Research Background

In the past few years, ready-to-eat food products are highly in demand due to the fast-developing world. These days, mankind has a very busy lifestyle. Therefore, they are looking for instant food that can be grabbed in one go. For example, soup is the most filling and easily available food in the market currently. Meanwhile, during the fasting month, the Muslim people are already tired from work and often look for a ready available cooked food product. Students are not allowed to cook in their hostel room, so they always look for alternatives. Soup, in general, is a portion of comfort food that can be consumed by all ages and can be easily cooked. Soups are a diverse liquid made from vegetables, fish, meat, water, juice, or stock and certain thickening agents. However, the soups available in the market are in premix, dry, or canned form. These soups have to be added cream, milk, or water to cook it so that it could be consumed. Urbanization leads to rapid and practical activities, like the production and preparing of food, nearly in all ways.

According to Vlachos & Georgantzis (2016), consumers now request healthier versions of convenient food. However, ready-to-eat meals can be seen on the shelf of a convenience store, and the shelf life of ready-to-eat meals is prolonged because they undergo several processes where the microbes are killed. Fundamentally, there are different type of packaging material and technology that were used to pack ready-to-eat food, such as modified atmosphere packaging (MAP) and vacuum packaging where the food product can be protected from microbes and other food contamination because it is a moisture barrier and heat resistant that allows the food to have longer shelf life without using any food preservative and additives. For heat-processed food, a flexible retort pouch is a great alternative to metal containers (Manju et al., 2014). Retort packaging is made up of laminated three layers that are treated like can. Hence, it will be able to be shelf-stable and work as a boil in the bag. The packaging is crafted of an outer layer polyester film, mid-layer of aluminum foil, and an inner layer of a modified polypropylene. In addition, retort pouch-packed food products need significantly less heat than cans to achieve commercial sterility because retort pouch packaging has thinner material than cans. Shelf stability, lower weight, storage capacity, the convenience of opening and preparation, and reduced heat exposure are advantages of retort packaging, which makes it a better quality (Majumdar et al., 2017).

Since old occasions, mushrooms have been developed for their dietary benefit and flavor, particularly in the earlier civilization. Mushrooms are nutrient-dense, environmentally sustainable crops with various medicinal properties. Edible mushroom cultivation is crucial in today's society, especially in population growth and environmental pressures (Chakravarty, 2011). Malaysia, have different types of mushroom available for mankind to consume such as *Auricularia spp*., *Agaricus spp.*, and *Pleurotus spp.* These mushrooms are cultivated while *Termitomyces spp*, *Ganoderma spp*, and *Polyporus spp* are being collected in the wild. These fungi species were often eaten for their medicinal or dietary benefits, such as high fiber, calcium, and vitamin content, low fat, cholesterol, and sodium content (Samsudin et al., 2018).

Pleurotus pulmonarius is a type of oyster mushroom which is similar to *Pleurotus ostreatus* but has some differences. They have longer steam compare to *Pleurotus ostreatus,* and also *P. pulmonarius'* caps are paler and narrower than those of *P. ostreatus* (Bonito Lab, 2018). Furthermore, *P. pulmonarius* is an oyster mushroom species that has grown to be the world's second most popularly cultivated mushroom (Chiu et al. 1998). *P. pulmonarius*, like *P. ostreatus*, requires warmer weather and can emerge in late summer. *P. pulmonarius* is found all over the world in subtropical and temperate forests. Meanwhile, because of the high amount of proteins, vitamins, and minerals found in *Pleurotus* mushrooms, they are considered a healthy source of nutrition. It not only has a high nutritious benefit, but it also has therapeutic properties due to its low fat and cholesterol content (Agarwal et al., 2017).

The development of *P. pulmonarius* soup would be an innovative product that has a prolonged shelf life. Hence, the research aimed to study the shelf-life of the developed product after retort packaging and evaluate the consumer acceptance towards ready-to-eat oyster mushroom soup developed from *Pleurotus*.

1.2 Problem Statement

Oyster mushroom (*Pleurotus pulmonarius)* is widely available in the market. However, the mushroom has a short shelf life because mushrooms are high in moisture which attracts the microorganism to grow and deteriorate the mushroom very easily. Moreover, the fresh oyster mushroom cannot be kept for too long in the chiller either because it will start to change color and spoil within few days. Fruits and vegetables deteriorate, wasting resources such as energy, land, and water that were utilized in their production which increase the price of the vegetables because it reduces the export potential (Chow, 2020). Therefore, developing a value added product using oyster mushroom could extend the mushroom's shelf life and reduce the wastage of the mushroom. The development of oyster mushroom soup in retort pouch packaging would be an excellent value added product. This is because the mushroom soup will undergo thermal process where all the spoilage microorganism were killed to extend the shelf life of the mushroom soup. Low acid foods such as vegetable soup are prone to microbial deterioration are retorted, which involves heating them in hermetically sealed containers to increase their shelf life. The purpose of retort processing is to use heat to achieve commercial sterilizing. Generally, mushroom soup is made with button mushroom, and it is available in premix and canned form in the market. On the other hand, premixed and canned foods are not fresh, and there were added with preservatives in powdered and canned soup to extend the shelf life of the product, which is not so healthy for humans to consume frequently.

1.3 Hypothesis

H_O: Different amount of mushroom content do have significant differences in sensory and physicochemical properties of mushroom soup prepared by a mixture of ingredients and *Pleurotus pulmonarius* mushroom.

H_1: Different amount of mushroom content have significant differences in sensory and physicochemical properties of mushroom soup prepared by a mixture of ingredients and *Pleurotus pulmonarius* mushroom.

1.4 Significance of Study

In this modern world ready-to-eat meals are widely available in grocery stores. Still, according to Times of India, 2019 regular consumption of these meals is not healthy because various alternative meals are not healthy before it reaches the consumers. There are different types of food additives, artificial food color and preservatives were added into the food product to make it look more presentable. Extending the shelf life by frequently consuming this food product can cause health issues.

The finding of this study will produce liquid mushroom soups made from *Pleurotus pulmonarius* mushrooms. Moreover, the soup will be packed in retort pouch packaging; helps prolong the soup's shelf life. Thus, innovating oyster mushrooms into a value-added product can extend the shelf life of the mushroom, because similar to other vegetables, mushrooms have high water content, making them to have a shorter shelf life. Moreover, there was no preservative added in the *Pleurotus pulmonarius* mushrooms soup making the additives-free mushroom soup which makes the soup as a saver option for people who are looking for healthy eating lifestyle. Besides that, *Pleurotus pulmonarius* mushrooms are known to have high protein content which could make the mushroom soup as a healthy ready-to-eat mushroom soup compare to the commercialized mushroom soup tht used button mushroom and Shiitake mushroom.

1.5 Objectives

The objectives of this study are:

1. To develop different formulations of mushroom soup from *Pleurotus pulmonarius* mushroom.
2. To evaluate the physicochemical properties of 3 different formulations of ready-to-eat *Pleurotus pulmonarius* mushroom soup.
3. To determine sensory attributes of the soup made from *Pleurotus pulmonarius* mushroom.

1.6 Scope of study

From this study, a ready to eat oyster mushroom soup will be produced using *Pelutorus pulmoneras* mushroom and some other basic soup ingredients. There will be 3 formulations created for this study. The end product is packed in retort pouch packaging, which is similar to the canning process. The end product is packed in retort packaging to extend the shelf life of the product. Moreover, retort pouch packaging is a flexible packaging which would be easily carried around, and it only will take less time to cook it or reheat it. The end product is used to determine the physicochemical properties (pH, color, viscosity, protein and fat) and microbial and sensory evaluation.

CHAPTER 2

LITERATURE REVIEW

2.1 *Pleurotus pulmonarius*

Pleurotus pulmonarius is also known as oyster mushroom in general. Since mushrooms are fungi, they lack chlorophyll and cannot produce food using solar energy (Okwulehie et al., 2014). Mushrooms have been described as an excellent food supply for alleviating malnutrition in developing countries due to their flavor, texture, nutritious value, and high productivity (Ma et al., 2014; Sufer et al., 2016; Islam et al., 2017). Edible mushrooms are also known to have medicinal benefits and are free of side effects. It can be used as a meat replacement making soups and sauces as meat substitutes and flavorings. They're also high in vitamins and minerals (Oie, 2003; Okwulehie et al., 2007).

Figure 2.0: *Pleurotus pulmonarius* mushroom

2.2 Characteristic of Oyster Mushroom

Oyster mushrooms have a trumpet-like shape above a short, off-centered stem or even an oddly shaped ear. Oyster mushrooms come in a variety of colors from all over the world. When it was in the early stage, it was white to light grey, and when they're dry, they're light brown to golden. These mushrooms can grow to be more than a half-foot long and prosper in overlapping clusters, usually on rotting hardwoods. Besides that, *Pleurotus* mushrooms come in some 40 different varieties (Chirinang and Intarapichet, 2009).

2.3 Nutritional Value

Oyster mushrooms are high in nutrients and therefore beneficial to people's health. Moreover, carbohydrates, calcium, fiber, and vitamins A, C, B, D, and K are all found in oyster mushrooms. However, it also contains low in fat and 13 potassium, sodium, copper, phosphorus, calcium, and iron, making them ideal for low-calorie diets (Wani et al., 2010). As a result, mushrooms have become popular as a source of nutritionally beneficial foods and drug production.

2.4 Food Products developed from *Pleurotus pulmonarius*

The oyster mushroom is consumed either cooked or preserve. Mushrooms are preserved by drying them to prolong their shelf life. There are available soup premixes in the market that are generally made with button mushroom only. There are also oyster mushrooms that were capsuled and used for medicinal purposes, and sometimes oyster mushroom was produced as pickles. However, the Asian peoples prefer to cook the oyster mushroom with vegetable stir fry and fry them (Priyadarsini and Mishra, 2020).

2.5 Soup

Soup, a common ingredient in both food and herbal medicine, has nutritional benefits and stomach-filling nutrients. Soup generally will be in the form of liquid and creamy, and full of flavor. Soup usually will be served warm or hot. Moreover, soups are made by boiling vegetables and meat. The main ingredient of the soup determines the flavor of the soup. They are served before the meal as an appetizer. In addition, soups are divided into two categories: thick and thin soup, divided into subcategories (The Culinary Cook, 2019). However, consumers prefer soup as a healthy and ready-to-eat meal in this modern world because it is easy to prepare and does not consume time.

2.6 *Pleurotus pulmonarius* Soup Ingredients

2.6.1 Onion and Garlic

Garlic (*Allium sativum*) and onion (*Allium cepa*) are among the oldest cultivated plants and are used for both food and medicine (Lanzotti, 2006). Onion and garlic are popular spices in many cuisines since they add a pungent, spicy taste to the cooked food. These vegetables, though, do more than just improve the taste of the food. Eating onion and garlic has been attributed to various health effects, including reduced cholesterol, blood pressure, and the risk of cancer (Shalpik, 2020).

2.6.2 Olive Oil

Olive oil is used during the preparation of *Pleurotus pulmonarius* soup. Olive oil plays a major role in cooking and adding nutritional benefit to the oyster mushroom soup. Olive oil is made from the fruits of olive tree. Olives are a typical Mediterranean crop. Whole olives are pressed to produce olive oil. Extra-virgin olive oil's health benefits are well-documented. The flavorful oil, a part of the Mediterranean diet, is a favorite of experienced chefs and home cooks alike. Extra virgin olive oil is the primary source of fat in the Mediterranean diet, with a different fatty acid structure and higher antioxidant level than other edible oils. These molecules, called polyphenols, are mainly responsible for the health benefits. When used in moderate cooking, olive oil reserves much of its nutritional value (Rathod, 2020).

2.6.3 Chicken Stock Powder

Chicken stock powders were used in *Pleurotus pulmonarius* soup to enhance the soup's flavor because it has salty, pale yellow crumbly powder with a concentrated chicken broth flavor. Asian brands of chicken stock powder, unlike cubed chicken broth stock, often contain monosodium glutamate (MSG) and dehydrated meat stock. Moreover, the chicken stock powder is a taste enhancer often used in basic Chinese dishes like salads, soups, and stir-fries. In daily cooking, the chicken powder is handier and offers a simple way to improve the flavor of stir-fried dishes (Zhu, 2014). By using chicken stock powder, the taste of the oyster mushroom soup can be enhanced.

2.6.4 Evaporated Milk

Evaporated milk is the marketing term for sterilized unsweetened condensed milk, which is made from fresh cow's milk that has had a good portion of the water removed. It can be used in place of fresh milk in recipes when combined with an equal amount of water (Deysher et al., 1994). The secret ingredient is evaporated milk, which thickens the soup and adds a deep, milky taste. Adding some evaporated milk in *P. pulmonarius* soup will give a fine and creamy texture that might attract people.

2.6.5 Salt

Salt is very basic ingredients that were used in any type of cooking. Salt comprises of two components: sodium and chloride, with sodium responsible for up to 40% of the molecule. Humans must attain their sodium and chloride needs from their diet; however, having too much salt will lead to health problems. Salt's value in food production derives from its preserving capabilities and its flavor, texture, and color contributions. After a layer of seawater becomes contained and the water evaporates due to the sun's heat, salt forms naturally in seawater and rock salt deposits (Durack, 2008). Adding enough salt to *P. pulmonarius* soup will develop the taste of the oyster mushroom soup.

2.7 Retort Process

Retort processing is a popular thermal preservation technology for extending the shelf life of foods, especially in the manufacturing of ready-to-eat meals (Gokhale and Lele,.2012). Retort pouch processing technique has long been acknowledged as a reasonable alternative to metal cans for manufacturing shelf-stable, thermally processed foods (Sabapathy et al., 2001; Abhishek et al., 2014). The advantage of retort technology is that it combines the processing of food and packaging, resulting in a more commercially stable filled product. The heating of low acid foods that is susceptible to microbial decomposition in a hermetically sealed container to extend shelf life. Ready-to-eat oyster mushroom *(P. pulmonarius*) soup that undergoes thermal process will have an extended shelf live.

2.7.1 Retort packaging

Retort pouches (figure 2.2) have become a popular alternative to traditional canning methods for preserving food and keeping it fresh for many years due to their strength, flexibility, and light weight. These pouches use less than 5% of the packaging material of typical rigid tin cans while also helping to improve food quality, texture, flavor, and aroma (Nalini et al,.2014). The reportable pouch is a flexible laminated pouch that can resist high temperatures during processing and combines the benefits of metal cans with plastic containers. Flexible reportable pouches are a one-of-a-kind packaging solution for sterile, shelf-stable items (Sabapathy and Bawa, 2003; Abhishek et al., 2014). Customers and food manufacturers generally benefit from the advantages of retort pouches over canned and frozen food products. The benefit are pouch profile, packaging cost, storage and preparation efficiency, transportation savings, enhanced flavor, and energy savings (Kumar et al., 2007; Abhishek et al., 2014). Hence, by using flexible retort pouch packaging for ready-to-eat oyster mushroom *(P pulmonarius*) soup it could benefit the consumer and at the same time preserve the nutritional value of the soup.

Figure 2.2**:** Retort pouch packaging

2.8 Nutritional Compositions of *Pleurotus pulmonarius* Soup

Convenient food or ready-to-eat meals are currently in trend in this fast developing world. This is because, mankind are have a busy life style, males and females are working so hard that they have no time to cook at home so they always looks for a ready-to-eat meals. Currently, they are so many types of convenient food available in the market but not all of the ready-to-eat food products are healthy. Moreover, oyster mushrooms have a protein composition ranging from 20% to 40% (Chang and Buswell, 1996; Changand Mshigeni, 2001; Kurtzman, 2005) and are high in important amino acids like lysine and leucine. Therefore, ready-to-eat *P.pulmonarius* mushroom soup contains high protein and healthy mushroom soup.

2.9 Physicochemical Analysis

2.9.1 Determinations of pH analysis

The number of free hydrogen ions present in a portion of food determines its pH content. Microorganisms, such as bacteria, molds, and yeasts are sensitive to the pH of food. However, microbial growth is inhibited by very low or extremely high pH (McGlynn, 2016). Hence, the pH analysis is important to ensure the shelf life of the ready-to-eat oyster mushroom soup.

2.9.2 Determination of Color

The chemical, microbial, physical, and biochemical changes during development, maturation, postharvest handling, and food processing determine the food color. Color is an essential quality attribute that affects customer choice and preferences in the food and bioprocessing industries. According to Pankaj et al. (2021), color measurement of food products has been used as an indirect measure of other consistency attributes such as flavor and pigment content. Hence, color is an important aspect in determining consumer acceptance.

2.9.3 Protein Content

Together with carbohydrates and lipids, proteins are the energy-giving components in the diet and play an important role in human growth and maintenance (Mæhre et al., 2018). Measuring the protein content of food is more significant than ever before because of its critical role in diet and health (Ickes, 2015). According to Mæhre et al. (2018) when protein content is measured based on amino acid residue analysis, it is known as direct protein determination. Thus, it is import determine the protein content in oyster mushroom (*P. pulmonarius)* soup.

2.9.4 Fat Content

Fat is a subject that consumers, commercial food producers, and government officials are all interested in, but for very different reasons. Consumers are worried about reducing total fat, saturated fat, and cholesterol consumption to improve human healthy life (Chao et al, 1991; Eller & King, 1996). Carbohydrates, protein, and fats are the three foods that provide calories however a gram of fat contains 9 calories. The amount of calories intake is important; taking more calories that required will change the calorie into fat to keep it in the body. Hence, it is important to know about calories before consuming ready-to-eat meals.

2.10 Microbial Analysis

Microbial analysis has a wide range of scope of study. Microbiological testing has been used to discover and prevent harmful bacteria that can deteriorate food as well as assure foodborne illness safety. These microorganisms can thrive in harsh environments where no other living organism could. They can grow and live in severe temperatures such as boiling points and extreme freezing circumstances such as -20°C and -30 °C (Brock et al., 1968) Therefore, it is important to conduct microbial analysis in ready-to-eat oyster mushroom soup even though it went through thermal processing. Total plate count was selected to determine the variable count in ready-to-eat oyster mushroom soup. The total plate count is a method of counting aerobic, mesophillic organisms that develop in aerobic circumstances at 20-45°C. This includes any aerobic bacteria, yeast, moulds, or fungi found on the agar (Liu et al., 2017).

2.11 Sensory evaluation

Sensory evaluation is the study of measuring, analyzing, and interpreting people's reactions to food products as experienced via their senses. It's a way of figuring out whether product differences are noticeable, what causes them, and whether one product is preferred over another. The science's usefulness comes in its ability to make conclusions based on a small number of customers that can be confidently projected to larger groups (Stone, 2018). Therefore, it would be helpful in determining the consumer preferences towards the food product.

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Chemical and Reagent

The chemical that were used in this research was distilled water, ethanol (70%), peptone, nutrient agar, potato dextrose agar (PDA), Sulphuric acid, boric acid, Kjeldhal catalyst tables Copper, petroleum ether

3.1.2 Equipment

There are several apparatuses that are used in this experiment, such as stove, pot, chopping board, plate, spatula, knife, bowl, weighing balance, retort pouch packaging, beaker, analytical balance, glass rod, pH meter, measuring cylinder, incubator, petri dish, glass cell spreader, Bunsen burner, parafilm, media bottles, aluminum foil, sterile pipette tips, conical flask, hot plate, retort stand, chroma-meter, pH meter, beaker, test tube with lids, soxhlet extractor, condenser, thimble, filter funnel, desiccator, hot plate, auto clave machine 35 litter-digital counter pressure.

3.1.3 Raw material

The raw material involved when conducting this experiment were oyster mushroom (*Pleurotus pulmonarius*), onions, garlic, salt, water, evaporated milk, chicken stock, flour, black pepper, butter, and olive oil.

3.2 Method

3.2.1 Experimental Design

The experiment starts with cleaning and sorting the *Pleurotus pulmonarius* mushroom to the cooking process. The 3 formulation of oyster mushroom soup has developed. There are a few experiments that were conducted after the formulations begin. To analyze the nutritional value of the product, protein content and lipid content was analyzed and *P. pulmonarius* soup was measured in triplicate using AOAC guideline (Association of Official Analytical Chimistry, 1999). The physicochemical properties, color, viscosity and pH content of the formulated sample were performed after all. Moreover, a microbial analysis was tested after the retort process to ensure that there were no microorganisms in the soup after thermal process. A commercialized ready-to-eat mushroom soup was used as control to compare with the formulation. Sensory evaluation was conducted to know the customer's preferences in oyster mushroom soup.

3.2.2 Development of *Pleurotus pulmonarius* Mushroom Soup

Pleurotus pulmonarius mushroom has been collected from the located market at Jeli, Kelantan. The mushroom was cleaned under the tap water. The cooking process starts by adding the entire ingredient required, such as 40 g of garlic, 200g onion, 2 mL of olive oil, 110 g butter, 1 L water, 30.5 g of chicken stock powder, a pinch of salt and pepper for taste and 178 mL of evaporated milk and finally the 500 g, 300g and 100g mushrooms has added and let it completely cook.

Table 3.1: Three different formulation of *Pleurotus pulmonarius* mushroom soup

Ingredient	**Formulation 1**	**Formulation 2**	**Formulation 3**
*Pleurotus pulmonarius*Mushroom	500g	300g	100g
Onions	300g	300g	300g
Garlic	40g	40g	40g
Salt	5g	5g	5g
Water	1L	1L	1L
Evaporated milk	780ml	780ml	780ml
Chicken stock	30.5g	30.5g	30.5g
Black pepper	6g	6g	6g
Butter	110g	110g	110g
Olive oil	2ml	2ml	2ml
TOTAL	1774.5g	1574.5g	1374.5g

3.2.3 Retort process

The cooked mushroom soup has carefully filled into flexible laminated retort pouch packaging and sealed using heat sealing machine. Each pouch contains 100 g of mushroom soup. Once it was sealed, the pouches were taken for the thermal process. The retort machine that was used for retort process is The Counter Pressure Retort and the pouches were heated for 15 minutes at 121 °C and the pressure is 109 (Nalini et al.,2018). When is retort process is done it was immediate cooled down before removing the pouches from the retort machine.

3.3 Physicochemical Analysis

3.3.1 Determination of pH

The determination of the pH analysis of mushroom soup has conducted to maintain the quality and stability of the new product (Heng et al., 2015). A pH meter was used to determine the pH of the soup. The pH meter was calibrated using fresh pH 7.00 buffer and it was let to stabilize the pH reading. Once the meter indicates that it was stable the buffer solution was rinse using distilled water and dry the pH meter. The *Pleurotus pulmonarius* mushroom liquid soup sample was placed on flat sensor where the pH reading was obtained.

3.3.2 Determination of color

The appearance of fresh and packaged foods, goods, and their advertising is essential for sensory consistency. The Konica Minolta Chroma Meter is used to determine the color strength of the ready-to-eat oyster mushroom soup. The liquid samples were placed in a clear glass plate to enable the chroma meter to detect the color strength. Since it is simpler, faster, and correlates well with other physicochemical properties, color measurement of food products has been used as an indirect indicator of different consistency attributes such as flavor and pigment content (Pathare, Opara, & Al-Said, 2013).

3.3.3 Viscosity

The mechanical resistance of shear is characterised as food viscosity. Foods texture is described as a set of physical features that can be perceived by touch and are formed from the composition of the food. Viscosity is measured using viscometer. The *Pleurotus pulmonarius* mushroom soup sample was filled in the container that are provided and was let the thin sensor plates were completely cover in the sample will cause little sample texture disruption, allowing for the detection of consistent viscosity measurements.

3.3.4 Determination of Protein content

To analyze the protein content, the Kjeldahl method is used. Firstly, about 1 gram of sample has weighed and placed into a digestion tube. Next, together with 12 mL of Sulphuric acid (H_2SO_4), one gram of catalyst tablets added into the digestion tube. Then, to set the ratio of catalyst and H_2SO_4 catalyst tablet added for the best result. Due to a quicker digestion process, a catalyst tablet will be included.

Then, after the preparation of the sample in a digestion tube, the sample has been inserted into the digester system. For 2 to 3 hours, the sample has been allowed to rest. To avoid a bubbling occur, the sample has been digested at a low temperature. Moreover, the digestion has been carried on until a complete digest was achieved. The sample has been heated again until all organic compounds had fully broken down and the solvent had become translucent. After the samples are separated from the digester it was allowed to cool for 15 to 20 minutes.

Regardless, in a conical flask, 50 mL of 4% boric acid (H_3BO_3) was added and placed as an indicator as a receiver on the distillation instrument. The digested sample has mixed with 80 mL distilled water and 40% sodium hydroxide (NaOH) (50 mL) to begin the distillation process. As a result, the distillation process continues until all of the ammonia in the sample has been released.

Move on to the next, the receiver conical flask were introduced as the final step in the titration procedure. Until the titration, a standardized 0.1 N hydrochloric acid (HCl) was prepared. The distillate was titrated until a pink color appeared, and the amount of HCl was recorded for later calculations (Nielsen, 2010). The protein content (%)was calculated using the following formula:

% Protein = % nitrogen x factor

$$\% \text{ Nitrogen} = \frac{(\text{mL standard acid - mL blank}) \times \text{N of acid} \times 14}{\text{Weight of sample}}$$

3.3.5 Determination of Fat Content

The sample was dried and transformed into powder form before the analysis began (Geeth et al., 2020). In an extraction thimble with petroleum ether, 2g of powdered mushroom sample were placed. The weight of the pre-dried round blotted sample was then measured. The samples were then covered in a sheet of de-fatted cotton. The samples were then inserted into the extraction machine section before the magnets were added. Extraction started in the Soxhlet extractor at a rate of 5 or 6 drops per second condensation for around 4 hours after heating the solvent inside the circular bottled flask.

The more the time the sample spends in contact with the solvent, allowing all of the fat in the sample to dissolve. To allow the solvent to enter the sample completely, the sample must be finely processed (Min & Ellefson,2010). The extracted fat, and the flask, were placed into the oven for 30 minutes at 105 °C, and the weight was recorded as the final step. Then it was cooled and weighed again in the desiccator. The weight had to be maintained in order to prevent further weight loss. The fat content was determined based on the weight of the contents in the receiver flask. The calculation of percentage crude fat was calculated using the following formula:

$$\% \text{ Crude fat} = (W2 - W1) \times \frac{100}{\text{Sample (g)}}$$

W1 = Weight of empty flask

W2 = Weight of extracted fat and flask

S = Sample

3.4 Microbial analyses

During storage, bacteria, yeast, and fungus contaminate foods. The microbes thrive in settings that are conducive to them, rendering the food unsafe for human consumption. For adequate quality control of retort pouches packed mushroom soup, identification of these organisms is required. 10 ml of ready-to-eat oyster mushroom soup sample were weighed into 90 ml pepton water. Later the media bottle was shaken 25 times to homogenized the sample. The dilutions were taken up to 10^{-6} in 6 different 10 ml test tube were prepared. The sample was then obtained from dilution; 0.1ml of the dilution was transfers to petri dish with agar by using a glass spreader the dilution were spread in the petri dish. The microbial growth test was repeated twice in a sterile Petri dish for each dilution. After 48 hours at 37 °C, the bacterial count was obtained, (Maturin & Peeler, 2001). Nutrient agar media is a typical general-purpose medium for the culture of a wide variety of bacteria. It's a basic medium made up of animal tissue peptic digest, beef extract, yeast extract, sodium chloride, and agar (Jadhav et al., 2018).

$$\text{CFU/ml} = \frac{colonies\ formed}{Dilution \times volume\ plated\ (ml)}$$

3.5 sensory evaluations

In the sensory evaluation, thirty untrained panelists of male and female panelist have been used to estimate the acceptability of the *Pleurotus pulmonarius* mushrooms soup before the retort process. The assessors are asked to score how much they preferred the taste, color, flavor, texture, and overall acceptability of the *Pleurotus pulmonarius* mushrooms soup on a hedonic scale ranging from 1 (extreme dislike) to 7 (like extremely). The samples were served in transparent plastic cups with three-digit random numbers written on them. Between sample evaluations, the panelists were advised to properly rinse their mouths with drinkable water and taste the soup samples one by one.

3.6 Statistical Analysis

To get the precise consumer acceptance outcomes, the collected data was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) after sensory evaluation of ready-to-eat oyster mushroom (*Pleurotus pulmonarius*) soup and Paired Samples t Test were performed to compare differences of means of pH, color and viscosity analysis. All of the tests were done in triplicate.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Development of ready to eat oyster mushroom soup

Development of ready to eat oyster mushroom soup was created with three formulations and it was compared with commercialized ready- to- eat mushroom soup (Lioco food Industries Sdn Bhd). The difference between the three formulations is the amount of mushroom added in each formulation. This is to get to know the sensory attributes of each formulation. The mushroom soup was cooked and packed in retort pouch packaging and underwent retort process for 15 minutes at 121 °C by using Digital Counter Pressure Retort Machine. The time taken to reach the desirable temperature is 20 minutes after it was hold for 15 minutes at 121 °C at the desirable temperature. The pouches were quickly cooled after the thermal processing using circulating water. This rapid cooling avoids overcooking as well as thermopile survival. The retort pouches were kept at room temperature for further study. Thermal processing is mostly used to inactivate microorganisms in foods in order to assure microbiological safety while minimizing product quality deterioration. Thermal processing is required to achieve commercial sterility of the product. The heat-resistant organism *Clostridium botulinum* will be killed during retort processing (Shihab et al., 2013). Hence, the product can be kept at room temperature.

4.2 Physicochemical Analysis

4.2.1 pH Analysis

The pH scale is used to determine how acidic or basic a component is when dissolved in water. The pH scale ranges from 0 to 14. A value of 0 indicates that the material is extremely acidic; a value of 7 indicates that the substance is neither acidic nor basic, but neutral. However, 14 are more alkaline (Karastogianni et al., 2016). A low-acid food has a pH of greater than 4.6, whereas a high-acid food has a pH of less than 4.6. Because of one specific bacterium, *Clostridium botulinum*, which creates a dormant form known as a spore, hence pH reading is essential. These spores are incredibly hard to eliminate and can live for years, waiting to develop.

Table 4.1: pH Analysis of Ready-to-eat *Pleurotus pulmonarius* mushroom soup before and after retort process.

Sample	pH	
	Before	After
Formulation 1	5.50±0.17[a]	5.20±0.26[a]
Formulation 2	4.83±0.15[c]	4.63±0.15[d]
Formulation 3	5.30±0.26[b]	4.93±0.15[c]
Control	-	5.93±0.57[b]

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different (p<0.05).

In conducting this study, a control was chosen in order to compare oyster mushroom *P. pulmonarius* soup formulations with mushroom soup that are commercialized and shelf-stable which was stated in the packaging of the control. The table above clearly shows that all 3 formulation of ready-to-eat oyster mushroom soup has above 4.6 pH value before the retort process, which indicates that the soup formulations are low acid food. However, formulation 1 has the highest value of pH which is 5.50±0.17 before the thermal process. This might be due to amount of mushroom used in formulation 1. Generally, vegetables such as mushrooms are alkaline which have pH value above 7. Hence, this proves why formulation 1 has high pH value compared to other formulation. However, formulation 2 before the retort process has the lowest pH value. This might be due to the amount of mushroom and onions used in the formulation. In formulation 2 the same amount of mushroom and onion were used. In formulation 3 the amount of onion is more compared to the amount of mushroom. Therefore, this might be the reason for the pH value for formulation 2 has low pH compare to formulation 3.

Moreover, the table above clearly shows that the value of pH has decreased after the retort processing. This might be due to the retort processing. Once the pouches were sealed it went through a thermal process where it was heated at 121 °C for 15 minutes so that spoilage bacteria's can be killed and the ready -to-eat oyster mushroom soup would have longer shelf life. This statement was supported by (Reineke et al., 2011). Temperature causes a drop in pH. However, this does not imply that soup becomes more acidic as temperatures rise. If there are more hydrogen ions than hydroxide ions in a solution, it is acidic (Reineke et al., 2011).

The final pH value in the ready-to-eat oyster mushroom soup might be also be the reason for the packaging to bloated. This is because all the three formulations have pH value of more than 4.6, which indicates it is a low acid and is a desirable pH value for the spoilage microorganism to grow. Besides, when compared to control, control has a higher pH value which is 5.93±0.57. However, the control mushroom soup is still in good condition due to the amount of preservatives used in control. According, to its packaging, salt, sugar, sodium, and oregano are used to make control soup. All ingredients play as natural preservatives to prevent foodborne bacteria and yeasts to grow. Hence, this might be the reason the shelf life of control is long and stable. However, for ready-to-eat oyster mushroom soup, no such ingredients were added except for salt. There was a statistically significant difference ($p \leq 0.05$) between the formulations of ready-to-eat oyster mushroom soup and control before and after the retort process.

4.2.2 Determination of color

Food's visual qualities add to its overall sensory attributes and attraction to consumers. Therefore, CIE Lab color space or coordinates L*, a*, and b* were used to measure the color intensity of the soup samples. In the L*a*b* color space, L* indicates brightness, a* designates red and green coordinates (-), and b* indicates yellow and blue (-) coordinates.

Table 4.2: Color Analysis of ready-to-eat *Pleurotus pulmonarius* mushroom soup before retort process

Sample	Colour (Before)		
	L*	a*	b*
Formulation 1	74.43±0.310[b]	0.610±0.286[b]	15.84±0.812[b]
Formulation 2	75.85±0.517[a]	0.670±0.185[b]	15.77±0.176[c]
Formulation 3	73.29±0.549[c]	1.686±0.035[a]	15.93±0.150[a]
Control	-	-	-

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different (p<0.05).

Table 4.3: Color Analysis of ready-to-eat *Pleurotus pulmonarius* mushroom soup after retort process

Sample	Colour (After)		
	L*	a*	b*
F1	72.05±1.635[c]	1.350±0.847[a]	20.50±0.527[c]
F2	72.15±1.560[a]	1.803±0.526[a]	21.51±0.507[a]
F3	72.14±0.701[b]	1.966±0.760[a]	21.14±0.254[b]
C	50.48±1.187[d]	2.310±0.278[a]	12.29±0.363[d]

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different (p<0.05).

Food's visual qualities add to its overall sensory attributes and attraction to consumers. Therefore, CIE Lab color space or coordinates L*, a*, and b* were used to measure the color intensity of the soup samples. In the L*a*b* color space, L* indicates brightness, a* designates red and green coordinates (-), and b* indicates yellow and blue (-) coordinates.

Based on table 4.2 and 4.3, the result that the L* value of before and after the retort process has decreased. The L* indicates the brightness of the soup where the higher the value the lighter the soup color will be. In this case, the L* value have decrease shows that after the retort process the color of the mushroom soup turns to be slightly darker. This might be due to the thermal process of the mushroom soup. According to Maskan,. (2006), during thermal processing, the food material may be subjected to temperatures that have a negative impact on quality, causing color degradation in food product. Hence, this proves that the retort process could affect the ready-to-eat mushroom soup's color. Besides, after the retort process all three formulations have almost the same L* value which is more than ±72.00. This might because of all the formulation undergo thermal process at the same time and temperature.

Moreover, a* and b* value of all three formulations have increased after the retort process. This might be due to the non-enzymatic browning of the onion and mushroom. Mushroom and onion might undergo the Maillard reaction. The reaction between reducing sugars and proteins under the influence of heat is a very complicated process. The reaction begins with a reducing sugar reacting with an amine to form glycosylamine that can change the color of the food product (Feiner, 2006). This reaction increase the a* and b* value. The more the positive a* value the higher the redness and the higher the positive b* value the highest the yellowness. Based on the result obtained, all three formulations have a higher b* value, which shows that the ready –to eat oyster mushroom soup has more yellowness. However, formulation 1 have the lowest L* value (72.05±1.635), a* (1.350±0.847), and b* (20.50±0.527). Control has the lowest L*,a*,b* when compared to control. This is due to the type of mushrooms and herbs used by control. L* value indicates control sample has darker color soup.

4.2.3 Viscosity

The viscosity is important because it influences the texture of the food product. The intrinsic barrier to the flow of a fluid is called viscosity. A viscous fluid pours slowly and seems thicker than a viscous fluid. The viscosity and quality vary when a material's characteristics, such as molecular weight and density, change (Johnson et al., 1975). Measurement range of the viscosity is 0.3 – 10,000mPa.s.

Table 4.4: Viscosity of ready-to-eat *Pleurotus pulmonarius* mushroom soup before and after retort process

Sample	Viscosity (Pa.s)	
	Before	After
Formulation 1	2.016 ± 0.110^{a}	1.150 ± 0.866^{b}
Formulation 2	2.423 ± 0.020^{a}	2.226 ± 0.230^{a}
Formulation 3	1.953 ± 0.345^{a}	0.593 ± 0.516^{b}
Control	1.523 ± 0.680^{b}	1.533 ± 0.577^{a}

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different ($p<0.05$).

The table above shows that the value for viscosity has decreased after the retort process. This might be due to the ready-to-eat oyster mushroom soup's thermal process. Moreover, the influence of temperature on viscosity is important, with viscosity decreasing as temperature rises. The internal friction of the liquid reduces as the temperature rises because the molecules in the liquid move around more and spend less time in contact with one other (Sahi, 2014). The measurement range of formulation 2 is higher than the other formulations. This might be due to the amount of mushroom and onion used in the formulation because the mushroom and onion were chopped finely. According to Duan. et al., (2021), particle size and particle interaction significantly impact the soup viscosity. The viscosity values were greater because fine particles can be better diffused in the soup. The interaction between particles became stronger as particle sizes decreased. Formulation 3 has the lowest viscosity value because the amount of mushroom was less compare to formulation 1 and 2. Hence, based on the table above, viscosity was significantly different ($p \leq 0.05$) for all the formulations and control.

4.2.4 Protein content

Based on the Table 4.4, Protein content was determined for all the ready-to-eat oyster mushroom (*Pleurotus pulmonarius)* soup samples which are formulations 1,2, and 3 including the control sample. The technique comprises three basic steps: digestion, distillation, and titration, indirectly estimates total protein content from nitrogen measurement. The Kjeldahl method was selected in this research because the analytical principle is acknowledged as the official method for determining food protein by the AOAC International. Upon measuring nitrogen, the crude protein concentration is determined using a conversion factor (Mæhre etal., 2018) .

Table 4.5: Protein content of ready-to-eat *Pleurotus pulmonarius* mushroom soup

Formulation	Protein Content ± standard deviation (%)
Formulation 1	1.493±0.530[a]
Formulation 2	1.040±0.235[a]
Formulation 3	0.993±0.270[a]
Control	0.653±0.808[a]

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different ($p<0.05$).

Based on Table 4.5, formulation 1 exhibits the highest protein content 1.49% than formulation 2, 3, and control. Both formulations 2 and 3 have higher protein content compared to control. This might be due to the amount of oyster mushroom (*Pleurotus pulmonarius*) and evaporated milk used in each formulation. However, formulation 1 contains 500g of mushroom, formulation 2 contains 300g of mushroom and formulation 3 contains 100g of mushrooms and each formulation contains the same amount of evaporated milk. The results shows decreased in protein content (1.49 % to 0.99 %) because the amount of mushroom was reduced for each formulation. The result shows that oyster mushroom do contain high protein content. This statement was supported by Khatun et al. (2015) state that *P. pulmonarius* is one of the most extensively farmed mushrooms globally, with low cholesterol, protein-rich diet, and nutritious. Besides that, the control shows the lowest amount of protein containing 0.6% might be due to the amount and type of mushroom used in the control sample. Besides, based on Table 4.5 the protein content of all three formulations of oyster mushroom soup and control do not have significant differences ($p \leq 0.05$).

4.2.5 Fat content

From Table 4.6, fat content of all the ready-to-eat oyster mushroom soup samples which are formulation 1, formulation 2, formulation 3 and control were tested in order to evaluate their fat content. The determination of fat content was done using the Soxhlet method. The Soxhlet method is a basic way to extract lipids from foods.

Table 4.6: Fat content of Ready-to-eat Oyster mushroom soup (*Pleurotus pulmonarius)*

Formulation	Fat Content ± standard deviation (%)
Formulation 1	19.66±6.429^{a}
Formulation 2	19.00±1.732^{d}
Formulation 3	19.00±6.928^{c}
Control	4.333±0.577^{b}

Note: Values are expressed as mean ± standard deviation (n=3). Mean values with superscript of different letter are significantly different (p<0.05).

Moreover, based on Table 4.6 the fat content in formulation 1 is slightly higher than other formulations. This might be because of the amount of mushroom and onion that were added in the soup. Formulation 2 and 3 has the same amount of fat content because the amount of butter and evaporated milk used in all 3 formulations were the same. Butter and evaporated milk plays and important role for the fat content of ready-to-eat oyster mushroom soup. Butter is a dairy product manufactured solely from pasteurized cream separated from cow’s milk and it is known to have high fat content (Sergiu Pădure, 2021). Moreover, the use of evaporated milk also could lead to the high fat content. This is because evaporated milk is produced by evaporating, condensing, homogenizing, and sterilizing whole milk. The fat content of evaporated milk fat is around 7.5–9.0 % (Fuquay et al, 2011). This indicates why the fat content in all 3 formulations were higher than control.

Based on control packaging, there was no butter and evaporated milk used in mushroom soup instead, vegetable oil and milk powder was used in the making of mushroom soup. Form here, shows that different types of milk sources and oil sources would lead to differences in fat contain a value. Therefore, table 4.6, it shows there were significant differences in fat content between the 3 formulations and control.

4.3 Microbial analysis

Microbial analysis were carried out in this study by using total plate count method to determine the total viable count inside the retorted processed *Pleurotus pulmonarius* mushroom soup formulation and control. The total plate count (TPC) is a technique for determining the total number of microorganisms (mould, yeast, and bacteria) in a sample and it was widely used in food industries (Ariffinet et al., 2019). Nutrient agar can determine a wide range of bacteria, including potato dextrose agar the most commonly used media for fungal and bacteria cultivation, was used in this experiment. Besides, the spread plate method was used in this experiment because it provides a high colony count and provides accurate and high colony count compared to the pour plate method. TFTC refers as Too Few to Count were the colony present in the agar was less than 30. Plates with more than 300 colonies are difficult to count, whereas plates with less than 30 colonies produce statistically inaccurate counts.

The microbiology test was conducted on day 1 and day 7 after the retort process of the mushroom soup. Based on the table below there were colonies present in the plate on day 1 however it was too few to count in all three formulation. Generally, there should not be any colonies present in the plate because all three formulations underwent retort process where the samples are hold for 15 minutes at 121 °C temperature. The time and temperature used is default. However, according to USDA, (2015) *C. botulinum's* heat-resistant spores can only be killed if they are exposed to the right temperature and pressure for enough period of time. To destroy spores, temperatures between 115°C and 121°C are required. The result obtain in this study is contrarily. However, the control sample shows that there were no colonies present in the agar plate. Therefore, control sample are complied with the statement.

Table 4.7: Colony count of Ready-to-eat Oyster mushroom soup (*Pleurotus pulmonarius)*

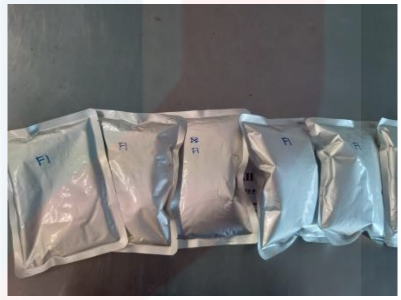
Sample	Colony Count (CFU/mL		
	DAY	NA	PDA
Formulation 1	1	TFTC	TFTC
Formulation 2	1	TFTC	TFTC
Formulation 3	1	TFTC	TFTC
Control	1	NIL	NIL
Formulation 1	7	2.5×10^{6}	1.13×10^{5}
Formulation 2	7	5.4×10^{5}	3.4×10^{4}
Formulation 3	7	2.15×10^{4}	6.3×10^{4}
Control	7	NIL	NIL

Note: Values are expressed as mean ± standard deviation. NIL: No Value. TFTC: Too Few To Count

Based on the Table 4.7, on the day 7, clearly shows that the number of colonies has increase drastically in all 3 formulations. This might be due to the protein denature that caused by the retort process. The Maillard reaction (non-enzymatic browning) happens between free amine and carbonyl groups within the food system and is one of the most common reactions that occur when protein foods are heated. Moreover, the Maillard reaction reduces the nutritional quality of proteins by reducing the availability of certain amino acids, notably lysine, or by causing the production of toxins or mutagens. The Maillard reaction causes nutritional changes such as reducing in protein digestibility and the production of growth inhibitory and/or toxic compounds (Dutson et al., 1984). The colonies form in potato dextrose agar, also show that there were spoilage fungi present. This might be due to the processing and handling of the mushroom soup during cooking and packaging. Moreover, filamentous fungi also were recognized for their resistance to temperature changes. Some ascomycetes' sexual spores can withstand intense heat, surviving even the conditions employed to make high-acid, shelf-stable foods that are "commercially sterile" (Snyder et al.,2019). Considering *P. pulmonarius* mushroom soup as low acid food product there are greater changes for fungi to survive and grow.

Besides that, storage temperature might be the reason for bacteria to grow rapidly in the ready-to-eat *P. pulmonarius* mushroom soup. After the retort process, all the samples were stored at room temperature. According to USDA, pathogenic bacteria are microorganisms that are capable of causing foodborne illness and room temperature is ideal for these microbes to grow. Therefore, bacteria grow inside the retort pouch, which results in gas production as the gas cannot escape from the sealed pouch; it builds up within, increasing the pressure and bloating of the pouches (Table 4.8). For the Table 4.7, it shows that the higher the amount of mushroom the faster the packaging bloated due to the amount of protein content that denature due to high temperature. However, control samples show that there is no colonies form on the plate on day 1 and 7. This might be due to the ingredients used in the control samples. Salt, sugar, oregano, and sodium act as preservatives which could limit the bacteria growth in the food product.

Table 4.8 shows the observation of packaging bolted

Days	Observation
4	 Formulation 1
6	 Formulation 2
9	 Formulation 3

4.4 Sensory Evaluation

Table 4.9: Sensory score of Ready-to-eat Oyster mushroom soup (*Pleurotus pulmonarius)*

Samples	Sensory Attributes				
	Aroma	Colour	Texture (Creaminess)	Taste	Overall Acceptability
F1	6.13±0.89	6.30±0.74	5.80±1.09	5.76±1.04	6.03±0.76
F2	5.56±1.35	6.10±1.21	5.96±1.88	5.83±1.26	6.10±1.15
F3	5.86±1.38	6.13±1.27	6.10±1.39	5.80±1.563	6.03±1.40
C	4.13±2.08	4.17±1.79	4.00±2.01	2.60±1.73	3.36±1.99

Note: Values are expressed as mean ± standard deviation. Mean values with superscript of different letter are significantly different ($p<0.05$).

Table 4.9 shows the results obtain from sensory evaluation of ready-to-eat oyster mushroom soup. There were 3 formulation of ready-to-eat oyster mushroom soup compare with commercialized control sample. The differences between the 3 formulation are the amount of mushroom used in each formulation (F1: 500g, F2: 300g and F3: 100g). The overall acceptability sensory score was obtained by Formulation 2.

Aroma is an important attribute of food product where sense of smell can trigger the brain before consuming the oyster mushroom soup. The mean score for aroma for ready-to-eat *P.pulmonarius* soup 6.13±0.89 to 4.13±2.08. For the highest range score of aroma is formulation 1 6.13±0.89 which indicates that it is moderately liked by the panelist compare to the other formulations including control. The lowest score were obtained by control 4.13±2.08. This might be due to ingredients used in *Pleurotus pulmonarius* soup and the amount of mushroom used. F1 contains 500 g of mushroom. When the amount of mushroom is higher mushroom aroma would also be more. This shows that the panelist likes the aroma of the mushroom. Cooked mushrooms have mild and bittersweet aroma when it was combined with chicken stock and black pepper it gives a pleasant aroma. As for formulation 2 and 3 only have slights deferens score. Hence, there were significant different between control and formulation 1,2 and 3 in term of aroma.

Color is an important aspect of food products. Based on the result obtained, F1 have the highest mean score 6.30±0.74. This shows that the panelist likes the slightly yellowish color of soup. This is because F1 contain a high amount of mushroom where it alters the soup's color. During cooking of mushroom soup, the onion and mushroom sauté to get light brown color resulted from caramelization; hence the color of mushroom soup became slight darker. When compared to F2 and F3, F3 has obtained the second-highest result. This might be due to the reduction of the amount of mushrooms and increased amount of onion. Once again, due to the caramelization, the color could be mildly darker. However, control has the lowest score. The control soup is darker in color and doesn't look pleasing to the panelist compare to oyster mushroom soup. Therefore, there were significant different between formulation 1, 2, 3 and control in term of color.

According to texture result, the mean score for texture for ready-to-eat *P. pulmonarius* soup 6.10±1.39 to 4.00±2.01. The highest range score was obtained by formulation 3, which is 6.10±1.39, which shows that the panelist liked the light and smooth, creamy texture soup. This might be due to the amount of mushroom added in other formulations. The more the mushroom contents, the thicker and creamy the soup will be. Besides, formulation 3 has the least mushroom. Hence, this makes the soup to be thin and smooth texture. When compared to control, control have the least score 4.00±2.01. This shows that the panelist prefers the chunky and thin texture of the soup.

Taste is important attributes that play an important role in urging people to consume a food product. According to the result, the mean score for aroma for ready-to-eat *Pleurotus pulmonarius* soup 5.83±1.26 to 2.60±1.73. The higher score was obtained by formulation 2 (5.83±1.26). This shows that the panelist prefers the soup to have a stable taste because formulation 2 has the same amount of mushroom and onions, making the soup have perfect balance taste of creamy mushroom and sweetness due to the onion. The least mean score were obtained by the control sample. This is because the control sample contains various ingredients including herbs that make the soup have a strong taste.

Overall acceptances, the panelist prefers F2, while F1 and F3 obtain the same overall acceptability. The result shows that the consumers prefer a balance taste of mushroom and an onion, which makes the soup have a mild and creamy taste and texture. Hence, tere were significant differences between control and formulation 1,2 and 3 in terms of overall acceptances.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 conclusions

In conclusion, the objectives of this study which were to develop *Pleurotus pulmonarius* soup have been accomplished, and the sensory attribute haven been determined. The sensory results showed that the panelist preferred *P. pulmonarius* soups compared to the control mushroom soup made from Shitake, button and Portobello mushroom. However, the shelf life of the retort pouch-packed *P. pulmonarius* soup has low shelf life in room temperature. This is primarily due to the microbial and chemical change that occurs during the retort process. On the other hand, the physicochemical properties of the *P.pulmonarius* mushroom soup prove that the mushroom contains a high amount of protein.

Meanwhile, this soup cannot be considered healthy, because it contains high-fat content. This is due to the ingredient used in making the soup. The main contributor to the high-fat content in oyster mushrooms is butter and evaporated milk. *P. pulmonarius* mushroom soup has a lighter and yellow color based on color analysis. According to the microbial analysis, it clearly shows the presence of bacteria that cause the retort pouch packaging bloated.

5.2 Recommendation

From this study, it can be concluded that further investigation must be performed on the thermal process of the oyster mushroom soup to obtain a shelf-stable, long shelf life less chemical changes that could trigger the microbial growth in the packaging after the thermal process. By using the right temperature, time, and right ingredient, this product could produce a shelf-stable product. This is because the sensory score shows that the panelist accepts this product. Moreover, it is also recommended to study the stability of the product under controlled conditions to ensure the shelf life of the *P. pulmonarius* mushroom soup.

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APPENDIX A

Figure A 1**:** *Pleurotus pulmonarius* soup

Figure A 2: Retort processed *Pleurotus pulmonarius* soup

APPENDIX B

Mean pH

Sample		Before	After
F1	Mean	5.5000	5.2000
	N	3	3
	Std. Deviation	.17321	.26458
F2	Mean	4.8333	4.6333
	N	3	3
	Std. Deviation	.15275	.15275
F3	Mean	5.3000	4.9333
	N	3	3
	Std. Deviation	.26458	.15275
C	Mean	.0000	5.9333
	N	3	3
	Std. Deviation	.00000	.05774
Total	Mean	3.9083	5.1750
	N	12	12
	Std. Deviation	2.37504	.52419

Mean: Viscosity

Sample		Before	After
F1	Mean	2.0167	1.1500
	N	3	3
	Std. Deviation	.11015	.08660
F2	Mean	2.4233	2.2267
	N	3	3
	Std. Deviation	.02082	.23007
F3	Mean	1.9533	.5933
	N	3	3
	Std. Deviation	.34530	.51627
C	Mean	1.5233	1.5333
	N	3	3
	Std. Deviation	.06807	.05774
Total	Mean	1.9792	1.3758
	N	12	12
	Std. Deviation	.36857	.66720

Mean Stand deviation Color

Report

Samples		L	a	b
F1_before	Mean	74.4367	.6100	15.8400
	N	3	3	3
	Std. Deviation	.31005	.28618	.81265
F2_Before	Mean	75.8567	.6700	15.7733
	N	3	3	3
	Std. Deviation	.51791	.18520	.17673
F3_Before	Mean	73.2900	1.6867	15.9367
	N	3	3	3
	Std. Deviation	.54991	.03512	.15044
C_Before	Mean	.0000	.0000	.0000
	N	3	3	3
	Std. Deviation	.00000	.00000	.00000
F1_After	Mean	72.0500	1.3500	20.5000
	N	3	3	3
	Std. Deviation	1.63502	.84788	.52735
F2_After	Mean	72.1567	1.8033	21.5100
	N	3	3	3
	Std. Deviation	1.56033	.52691	.50715
F3_After	Mean	72.1467	1.9667	21.1433
	N	3	3	3
	Std. Deviation	.70117	.76002	.25482
C_After	Mean	50.4867	2.3100	12.2967
	N	3	3	3
	Std. Deviation	1.18779	.27875	.36350
Total	Mean	61.3029	1.2996	15.3750
	N	24	24	24
	Std. Deviation	24.91475	.85560	6.70528

Fat Mean Stand deviation

Report

Fat_Contant

SAMPLE	Mean	N	Std. Deviation
F1	19.6667	3	6.42910
F2	19.0000	3	1.73205
F3	19.0000	3	6.92820
C	4.3333	3	.57735
Total	15.5000	12	7.89131

ANOVA

Fat_Contant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	499.667	3	166.556	7.189	.012
Within Groups	185.333	8	23.167		
Total	685.000	11			

Protein Mean Stand deviation

Report

Protein_Contant

SAMPLE	Mean	N	Std. Deviation
F1	1.4933	3	.53003
F2	.9933	3	.27006
F3	1.0400	3	.23580
C	.6533	3	.08083
Total	1.0450	12	.41597

ANOVA

Protein_Contant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.071	3	.357	3.434	.072
Within Groups	.832	8	.104		
Total	1.903	11			

Sensory evaluation

Report

Sample		Color	Aroma	Texture	Taste	Overall_acceptance
F1	Mean	6.3000	6.1333	5.8000	5.7667	6.0333
	N	30	30	30	30	30
	Std. Deviation	.74971	.89955	1.09545	1.04000	.76489
F2	Mean	6.1000	5.5667	5.9667	5.8333	6.1000
	N	30	30	30	30	30
	Std. Deviation	1.21343	1.35655	1.18855	1.26173	1.15520
F3	Mean	6.1333	5.8667	6.1000	5.8000	6.0333
	N	30	30	30	30	30
	Std. Deviation	1.27937	1.38298	1.39827	1.56249	1.40156
C	Mean	4.1667	4.1333	4.0000	2.6000	3.2667
	N	30	30	30	30	30
	Std. Deviation	1.76329	2.08001	2.01717	1.73404	1.99885
Total	Mean	5.6750	5.4250	5.4667	5.0000	5.3583
	N	120	120	120	120	120
	Std. Deviation	1.55657	1.66356	1.68500	1.97888	1.84161

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Color	Between Groups	91.692	3	30.564	18.031	.000
	Within Groups	196.633	116	1.695		
	Total	288.325	119			
Aroma	Between Groups	71.558	3	23.853	10.734	.000
	Within Groups	257.767	116	2.222		
	Total	329.325	119			
Texture	Between Groups	87.400	3	29.133	13.493	.000
	Within Groups	250.467	116	2.159		
	Total	337.867	119			
Taste	Between Groups	230.467	3	76.822	37.835	.000
	Within Groups	235.533	116	2.030		
	Total	466.000	119			
Overall_acceptance	Between Groups	175.092	3	58.364	29.629	.000
	Within Groups	228.500	116	1.970		
	Total	403.592	119			

Thesis draft

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