Development of Mushroom (*Pleurotus soju-cajo*) Patty Integrated with Different Binders (dried tofu, TVP and Soy pulp)

MUHAMMAD AMIR AZMI BIN MOHD SALLEH

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A Thesis Submitted in Fulfilment of the Requirement for The Degree of Bachelor of Applied Science (Food Security) with Honours

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DECLARATION

I declare that this thesis entitled "Development of Mushroom (Pleurotus soju-cajo) Patty Integrated with Different Binders (dried tofu, TVP and Soy pulp)" is the results of my own research except as cited in the references.

Signature : ___________________________

Student's Name : Muhammad Amir Azmi Bin Mohd Salleh

Date : ___________________________

Verified by:

Signature : ___________________________

Supervisor's Name : TS. Dr. Maryana Binti Mohamad Nor

Stamp : ___________________________

Date : 16hb January 2022

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ABSTRACT

Mushroom has been globally used in the market as an ingredient, most applicable in the food industry to be incorporated in meals, side dishes due to its meat like taste and also act as a binder to other meat based processed food. A wide variety of food products can be produced from this ingredient such as mushroom soup, snacks and even for seasoning fast foods. This project was proposed to make mushroom patty with different binders by using *Pleurotus soju-caju* as the main ingredient. The samples were shaped into patty form and formulated by mixing the mushroom with three different binders which are Textured Vegetable Protein (TVP), Soy Pulp and Dried Tofu at two different percentages of binders (15% and 20%). The value of cooking yield was first carried out by calculating the difference of the weight before and after cooking. Next, the effects of using different types of binders with different percentages of binders in patty samples were obtained by performing physicochemical analysis and proximate analysis. Findings showed that the incorporation of mushroom as the main ingredient in the patties had significantly decreased the hardness and gumminess but these values increased with the addition a higher percentage of the binders. However, the recorded results showed no significant changes in terms of chewiness and cohesiveness. Next, the use of higher percentages of binder had also increased the lightness (L*), yellowness (a*), and redness (b*). Additionally, the proximate analysis conducted had found that both the protein and fat values had increased with the use of higher percentages of binder. Further studies may be done to gain input on the sensory evaluation of the mushroom patty with different binders to further expand the potential of commercializing this product.

Keywords: binders, mushroom, patty, proximate, yield

Pembangunan Cendawan (Pleurotus soju-cajo) Patty Bersepadu dengan Binders yang Berbeza (tauhu kering, TVP dan pulpa soya)

ABSTRAK

Cendawan telah digunakan secara global di pasaran sebagai bahan, yang paling terpakai dalam industri makanan untuk dimasukkan ke dalam makanan, hidangan sampingan kerana dagingnya seperti rasa dan juga bertindak sebagai pengikat kepada makanan diproses berasaskan daging yang lain. Pelbagai produk makanan boleh dihasilkan daripada bahan ini seperti sup cendawan, makanan ringan dan juga untuk makanan segera yang berpengalaman. Projek ini dicadangkan untuk membuat patty cendawan dengan pengikat yang berbeza dengan menggunakan Pleurotus soju-caju sebagai bahan utama. Sampel telah dibentuk menjadi bentuk patty dan dirumuskan dengan mencampurkan cendawan dengan tiga pengikat yang berbeza iaitu Protein Sayur Bertekstur (TVP), Pulp soya dan Tauhu Kering pada dua peratusan pengikat yang berbeza (15% dan 20%). Nilai hasil memasak pertama kali dilakukan dengan mengira perbezaan berat sebelum dan selepas memasak. Seterusnya, kesan menggunakan pelbagai jenis pengikat dengan peratusan yang berbeza pengikat dalam sampel patty diperolehi dengan melakukan analisis fizikokimia dan analisis yang dekat. Penemuan menunjukkan bahawa penggabungan cendawan sebagai bahan utama dalam patties telah mengurangkan kekerasan dan gumminess dengan ketara tetapi nilai-nilai ini meningkat dengan penambahan peratusan pengikat yang lebih tinggi. . Walau bagaimanapun, keputusan yang direkodkan menunjukkan tiada perubahan ketara dari segi kunyah dan perpaduan. Seterusnya, penggunaan peratusan pengikat yang lebih tinggi juga telah meningkatkan kecerahan (L *), kekuningan (a*), dan kemerahan (b*). Di samping itu, analisis proksi yang dijalankan telah mendapati bahawa kedua-dua protein dan nilai lemak telah meningkat dengan penggunaan peratusan binder yang lebih tinggi. Kajian lanjut boleh dilakukan untuk mendapatkan input mengenai penilaian deria patty cendawan dengan pengikat yang berbeza untuk mengembangkan lagi potensi mengkomersialkan produk ini.

Kata kunci: pengikat, cendawan, patty, dekat, hasil

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CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

For a long time, mushroom extracts have been widely used in different agricultural products as food or food additives. This mushroom is planted on the top of the substrate on a decaying organic material and is edible. The dry matter of mushrooms contains over 25% protein, under 3% crude fat and almost 50% total carbohydrate (Kotwaliwale et al., 2007). Mushrooms were nutritious since the amount of salt, fat and cholesterol is low in calories. Consequently, they constitute an important dietary element for a population with atherosclerosis. It also provides substantial levels of dietary fibre, β-glucan, vitamin B and D groups and additional nutrients. Blood cholesterol levels and the glycaemic reactance level were often correlated with the ability to minimise soluble and insoluble dietary fibre (SDF) (Finimundy et al., 2018) The capacity of a β-glucans for showing important immo-modulative properties, improved antioxidant activity and free radicals were also associated with scavenging (Synytsya et al., 2009). Dietary fibres in mushrooms were associated with the acceleration of the passage of bowel waste, raising the length, frequency, and ease of faecal removal. This mushroom was also claimed to defend the body from irritable bowel and colon syndrome. In addition, carbohydrates and plant product fibres have been used to lower formulation costs and enhance texture while reducing the cost of formulation. (Zhang et al., 2021)

1.2 PROBLEM STATEMENT

In Malaysia nowadays, we have normalize consuming more foods that can result in unhealthy behavior towards our body. Fast food restaurants are also rampant around the nations. There were more animal based food product comparison to plant based. People usually are not interested in plant-based foods, but veggie base is much healthier. In Malaysia, plenty of wastage of unused mushrooms got thrown away. Previously a study identified development of veggie burger that lack of binder that bind the burger forming well-shaped patties. The usability of mushroom as functional food of have not been explored by people nowadays and mostly they consume the mushroom as a salad or just as a side dish with the main dish. Alternatively, mushrooms can be developed as the main ingredients for making ready-to-eat food. This type of food is easy to be prepared and reduce the preparation time for consumers. Pleurotus fungi provide high protein and vitamin content and are also low in fat.(Bonatti et al., 2004). This new formulation could help those who need to losing weight and to support a healthier lifestyle. The protein content and fibre content in the grey oyster mushroom that can be exploited and make an innovation that can give high impact to the consumer and the vegetarian. In the process of making of the mushroom patty, suitable binders need to be used to form the ingredients in a patty form. Previous study showed that lack of a binder leads to veggie burger not forming solid forms as it needs binding agents to keep it together. Previous research found that the soft texture mainly reflects the dilution effects of meat ingredients on meat protein systems (Tsai',1998)

1.3 HYPOTHESIS

H_0: There is no significant difference in terms of the physicochemical properties and the proximate analysis of the mushroom patty when combined with different type of binders (soy pulp, textured vegetable protein and dried tofu) and the different percentages of binders (15% and 20%).

H_1: There is significant difference in terms of the physicochemical properties and the proximate analysis of the mushroom patty when combined with different type of binders (soy pulp, textured vegetable protein and dried tofu) and the different percentages of binders (15% and 20%).

1.4 RESEARCH OBJECTIVE

1. To produce different formulations of mushroom patties using different types of binders (soy pulp, textured vegetable protein and dried tofu) and different percentages of binders (15% and 20%).
2. To compare physicochemical properties of mushroom patties such as Cooking Yield, Texture Profile Analysis (TPA) and Colour.
3. To investigate the nutritional facts of mushroom patties.

1.5 SCOPE/LIMITATION OF THE STUDY

One of the limitations of this research would be on conducting a proper sensory evaluation among the students and staff here in University Malaysia Kelantan (Jeli Campus), Kelantan, Malaysia. Due to the pandemic, it was difficult to gather the data of the sensory evaluation, hence this study was not able to conclude the preference or the perception on Mushroom Patty in terms of its colour, taste, and overall liking.

1.6 SIGNIFICANT OF STUDY

The outcome of this research gives insight to the innovative development of food using oyster mushroom as the main ingredient and they can directly increase the variability of vegetarian food products in the market. This creation of more vegetarian foods may offer many benefits to the food industry and its uses to the vegan groups. For example, having to use mushroom patties as an option may improve the texture, taste, and colour of the product. The result from the study suggest that the introduction of new formulations is highly potential for entering food market. In addition, the development of downstream products by using the mushroom can also reduce wastage especially when the commodity is in excess and cannot be marketed fresh due to some factors. The innovation can solve the disadvantage of mushroom that highly perishable food product. The shelf-life of the mushroom is very short and needs to be sold immediately after harvest before it wilts. Thus, food innovation such as the burger patty using the mushroom can expand its usability and reduce loss to the mushroom.

CHAPTER 2

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LITERATURE REVIEW

2.1 Mushrooms

2.1.1 Type of Mushrooms

2.1.1.1Chanterelles

These mushrooms are easy to recognize, and just by the appearance of them. The chanterelles are fruity and peppery and are their striking golden colour (but often in various shades). They are sensitive in taste and texture and fit well with the eggs. Chanterelles can withstand more than 10 days in the refrigerator than other mushrooms. They are rich in moisture and should be prepared in dry sauté when cooked as fast as possible, so they emit their own water. These vitamins have a strong balance, including thiamine, riboflavin and a high concentration of trace minerals and ascorbic acid and vitamin D2, and are well protein content. Their calories and their fat are low, and their dietary fibre is important. It is widely appreciated for its beautiful fruity, apricot like fragrance, which is particularly coveted for European cooking. In addition to the taste and the scent, it has a good feel. For the north-east of Portugal, the chanterelle is of considerable economic concern because its harvest is not just a valuable commercial source but also a means of livelihood for its people. (Valentão et al., 2005)

2.1.1.2 Cremini

Creminis were a slightly more mature white button fungus, also sold as baby portobellos. They have a similar appearance, maybe much larger, but their colours are a visible distinction which creminis are a light brown colour. It is a medium mushroom in taste and a perfect replacement for white buttons on a finer taste of your favourite dishes. Cremini mushroom had slightly higher concentrations than Shiitake; although it was spread throughout the fruiting body as in Cremini, it was localised to Shiitake in the hymenophore area. (Seyfferth et al., 2016)

2.1.1.3 Morels

Maybe it does not look good, but this mushroom is very tasty and savoury. It may look like a dead wine, but it is alive with taste and worth it to overcome the fear of the hideous. They taste a little chewy with butter and perfect sauté. Morels need to be cooked slowly to release the volatiles that provide the flavour of these mushrooms. Where this cannot be done, the mushroom should be blanched for 1 to 2 minutes before use. Cooking often eliminates the possibility of stomach discomfort after raw stomachs have been consumed. (Stott et al., 2004)

2.1.1.4 Portobello

This fungus is the middle stage with a fully grown cap in the white button mushroom. The portobello has a slight taste but a meaty feel. It works best in some dishes as a substitute for meat and particularly flavours when grilled.

2.1.1.5 Enoki

There are long stalks and small cape mushrooms. They look like bean sprouts at first sight, but they have got even more flavour. In Asian cuisine, they are popular and available fresh and canned. They have spicy soups, and they go great in salads. so, they are crisp. They come from Japan and are delicious.

2.1.1.6 Shiitake

Shiitake mushrooms mostly grow in Japan, China and Korea and are so prevalent in Asian cuisine for one reason. They are delicious and meaty, adding an umami taste to food. Shiitakes may be used to prepare meat and improve soup and sauces. The mushroom has also been developed into powder form to add mushroom taste and flavour to cooking instantaneously.

2.1.1.7 Button

The button mushrooms are the most popular in supermarkets is button mushroom known as white mushrooms. They are picked when they are young, and they have a very subtle, earthy taste.

2.1.1.8 Porcini

In Italian cuisine, porcini is also used as a meaty mushroom such as portobello. It is mildly nutty and smooth and has a sauerkraut fragrance. Slightly brown, porcini can vary from 1 to 10 centimetres in size. The mushroom is sold either fresh, dried or canned. The dried mushroom needs to rehydrate in water before cooking.

2.1.1.9 Hen of The Woods

These small, flowery mushrooms are also considered to be maitake - which means "dancing mushroom" in Japanese - filled with flavour. It is rich, terrestrial, and dreamy. It grows at the base of the trees and is used in Japanese and western cuisine, particularly in oaks. When fried, they keep their form well, so they are perfect for soup and frying.

2.1.1.10 Oyster

Oysters may tend to be one of the most threatening mushrooms since they do not look like the typical button mushroom. They are easy to make and to give a sweet and delicate taste. Oysters may also emit anise-like flavour depending on the time of year they are dried up. This fungus, known as the water bivalve mollusc, can easily be grown and is one of the more economical types of mushrooms.

2.1.2 *Pleurotus soju-caju*

Pleurotus sajor-caju, an edible mushroom. The real Pleurotus sajor-caju is a separate species of mushroom, which was returned to the genus Lentinus by Pegler in 1975. Fungi of the Pleurotus genus present a high content of proteins and vitamins and low content of fat. (Bonatti et al., 2004). Pleurotus sajor-caju has been receiving great interest in research because of its biologically active compounds, such as polysaccharides (e.g., pleurane, a β-glucan), proteoglycans, phenolic acids, terpenes, proteins, and sterols, becoming a source of nutraceuticals   Pleurotus can be easily dried and preserved and can be grown on a variety of agricultural wastes, which are easily available in the villages. It offers one of the best prospects for producing food rich in protein and vitamins from wastes (Bisaria & Madan, 1983). The active ingredients in Pleurotus sajor-caju have hypertensive effects on the renin-angiotensin-system. The overall amount of plasma cholesterol and triglyceride is very successful in decreasing (Alam et al., 2008).

2.1.3 Plantation of *Pleurotus soju- cajo*

Mushroom is Malaysia's modern and small company. This industry is therefore rising to be a food supply and a new wealth source for farmers. The study discuss the potential of this industry, particularly the challenges and problems that could delay its growth. From the growing point of view, the market is more than the supply of the farmers to the oyster mushroom. The region of Klang Valley, there was no competition to sell the oyster mushroom. Increased demand led to a higher price. Marketers decide the price of oyster mushrooms. The mushroom industry is expected to grow due to increased demand from the local and global consumers (Haimid et al., 2013)

2.1.4. Significant of *Pleurotus soju- cajo*

Protein and carbohydrate composition have the only major variations. The mushroom cultivated has the lowest protein content (26,6% dry) and the protein generated with cotton waste is comparatively higher. On the other hand, the raw fat and energy content of *Pleurotus soju-cajo* are lower and the fibre level is higher than that of all the four mushrooms, and the overall carbohydrate content can be compared with that of Lentinus edodes. *Pleurotus soju-cajo's* content is very similar, and the content of thiamine and ascorbic acid is almost zero and niacin content comparatively high.

2.2 Patty

Meat burger patty is one of the most common meat items, consumed in Asian countries as fast foods. The most popular meat raw material widely used for burger patty processing is beef. The increasing prices of beef as basic meat materials required to produce meat products encourage food manufacturing companies to assess the possibility of using such cheap and high-quality meat sources as cow meat, in Asian and African countries where cow meat is available and considered more efficient in food production than other domestic animals (Abdel-Naeem & Mohamed, 2016)

2.2.1 Nutritional value in patty

The features of the fungi burger patty appeared to most participants. The findings suggest, as diffusion of knowledge focuses on environmental benefits, that fungal products raised using agricultural waste can become accepted products. In addition, the chewiness and bitterness of the substance must be reduced if it is approved commercially. The use of certain non-digestible plant carbohydrates will substitute fat for food while preserving some or all the desired technical and sensory qualities in the original full-fat product. The desired goals of fat substitution are high-fat foods that are widely eaten in the diet, including meat products (Piers et al., 2003) However, little detail on the satiating capacity of foods in which fat is substituted for nondigestible carbohydrates is available in literature. Inulin is an example of a non-digestible food component of carbohydrate and is currently used in consumer purposes as a fat replacer. A plant-derived polymer of fructose which is not digested in the human top gastrointestinal tract (Niness, 1999), inulin is known to be in line with the new 'Dietary Fibre Complex' definition (Cherbut, 2002). Chicory roots are widely used to use commercial inulin to produce a white, smell less, very sweet powder (Ho et al., 1997).

2.3 Binders in food

2.3.1 Textured Vegetable Protein (TVP)

Protein products are categorised as edible-protein foods, including soy grit, soy protein isolates and concentrate of soy protein, with or without appropriate nutritional or technology added ingredients. Protein products and protein materials are manufactured from edible protein ingredients. Texturized vegetable protein is the wide range of items with a wide range of textures and different manufacturing techniques.

2.3.2 Soy Pulp

Soy pulp is the most common vegetable protein used in meat products due to its high biological factors and strong functional properties, which enhance the capacity to bond to seawater and thus enhance the texture and acceptability of the final product. However, soy food or other food that contains soy was found to be prohibited for persons with a genetic disease called favism. Whereas the overall soy protein improved the paper strength by 46% compared to paper without an additional binder, wheat gluten increased its strength to as much as 60%. Although gluten is successful in improving the tear strength of the paper, the authors concluded that soy protein is more suitable as a bulk binder because it is less costly than gluten. Whereas these proteins have not increased the tensile strength enough to replace the existing chemical binders, the volume of synthetic binder required by this plant-driven protein binder can be decreased.

2.3.3 Dried Tofu

Lean frankfurters with tofu powder contained less moisture and colour, greater texture, and acceptability overall with no taste variations. Except for fracture ability, there were no variations between standard and control frankfurters regarding texture parameters by Texture Profile Analysis (TPA), but the texture of the lean frankfurters has improved. Nothing about the use of tofu as a food product is visible.

Ability to use dried tofu powder in cooked meat products as an ingredient. The effect of tofu powder in frankfurter and sausage patties on physical, chemical and palatability properties were tested at different levels of fat.

2.4 Tapioca Flour

Among the plant-based starches/binders, tapioca, a starch produced from cassava root, is one of the most regularly used items in ground meat. Tapioca flours have been proven in studies to change the physical characteristics, sensory texture, and taste aspects of meat products. When tapioca starch was added to frankfurters, the starch reduced cook loss while increasing emulsion stability and overall taste intensity. Tapioca starch boosted the succulence and softness of low-fat beef patties, according to Desmond et al.(Chatterjee et al., 2019)

2.5 Shelf life

To save and protect meat because of the shorter shelf life there are limits to food intake, the production of sausages is evolving. Beef, ham, and poultry are all often used in the manufacture of sausage as a meat product and both spices and seasonings. Flavour, bacterial growth slow and patty yield raise non-meat ingredients, including water, salt, succulent, non-fat dried milk, spices, and spicy products (Mousavi et al., 2019). Beef burger patties are a highly peregrinate foodstuff, with 3 days maximum shelf life at 4°C, as consistency parameters and microbial growth are quickly decreasing.

CHAPTER 3

METHODOLOGY

3.1 Mushroom Preparation

Grey Oyster Mushroom (*Pleurotus sajor caju)* was provided by a student company in UMK Jeli. The mushroom was steamed process in the digital steamer for 6 to 9 minutes with a temperature of 90°C for the inactivation of the enzyme polyphenoloxidase. Then, the mushrooms were kept in room temperature to cool down. Next, the mushroom stems were cut off because the stems will not be able to give the patty a good texture as it will only cause a chewier texture. The mushrooms were then chopped into smaller sizes before it can be processed further in food Process Panasonic MK-F800. Manual mode was used to make sure the mushrooms not too fine so that the mushroom is able to form a better texture in the patty

3.2 Binders Preparation

3.2.1 Soy Pulp

For the soy pulp preparation, the soybeans were boiled for 30 minutes until the beans were puffed. The beans were then filtered with the coriander and were left to cold in room temperature. Next, the boiled beans were then inserted into the blender and were processed to cut into small and fine size.

3.2.2 Texture Vegetable Protein (TVP)

Textured Vegetable Protein (TVP) was chosen to be one of the binders as it was used to extend ground meat as a means of reducing the cost without reducing nutritional value (Samard et al., 2021). This ingredient was purchased from Qingsu Groceries, it was a commercialized product that can be easily bought at the store.

3.2.3 Dried Tofu (Fucuk)

Dried tofu (fucuk) is a ready to eat product. This product was bought from the local grocery shop here in Jeli, Kelantan. The dried tofu was processed in a dry blender and blended until it turned into powder form. The process was done as it was able to produce a better texture of the patty.

3.1 Formulation that used to form mushroom patties with different binders and different percentages of binders.

Table 1 shows the formulation that use different binders and different percentages of binders.

Recipe %	F0	F1	F2	F3	F4	F5	F6	F7
Minced Meat	70.18	-	-	-	-	-		
Mushroom	-	70.18	66.5	66.5	66.5	61.5	61.5	61.5
Textured Vegetable Protein (TVP)	-	-	15	-	-	-	-	-
Dried Tofu	-	-	-	15	-	-	-	-
Soy Pulp	-	-	-	-	15	-	-	-
Textured Vegetable Protein (TVP)	-	-	-	-	-	20	-	-
Dried Tofu	-	-	-	-	-	-	20	-
Soy Pulp	-	-	-	-	-	-	-	20
Tomato Paste	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500
Tapioca Powder	-	-	10.00	10.00	10.00	10.00	10.00	10.00
Canola Oil	1.875	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Minced Cloves	1.250	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Salts	0.750	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Chilli	0.750	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Cumin	0.500	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Paprika	0.250	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Onion Powder	0.250	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Garlic Powder	0.250	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Black Pepper	0.125	15.00	15.00	15.00	15.00	15.00	15.00	15.00

3.3 Development of Product Formation

The steamed mushroom was transferred into the food processor for as long as 6 seconds in a single rotation (6 one-second pulse) which had resulted in a particular uniform size which was 105mm. Some of the particles were much bigger than 105mm, hence those were then minced again in order to eliminate the bigger sized particles, hence by then end - particles of the same size were then obtained. All the ingredients in Table 1 were then mixed by using the food processor and then neatly formed into a patty shape with each weighing 80g. Lastly, all the patties were then stored in the freezer (-18°C) before proceeding with the next analysis.

3.3.1 Determination of Physical and Chemical Properties of mushroom patty

All the analysis was carried out in the laboratories of University Malaysia Kelantan, Jeli Campus, Kelantan, Malaysia. Several analysis for physicochemical determination were carried out to determine the quality of acceptance of mushroom patty which were:

1. Cooking yield
2. Colour
3. TPA Texture Analysis

3.3.2 Determination of cooking yield measurement

Percent cooking yield was determined by calculating weight differences for samples before and after cooking (Murphy et al. 1975):

$$\text{Cooking yield (\%)} = \frac{\text{cooked weight}}{\text{raw weight}} \text{ x } 100$$

The calculation of cooking yield percentage using this specific formulation is important to identify the sample which has the similarity to the control.

3.3.3 Determination of colour

The colour measurements of the uncooked patty and cooked patty were measured using Thermo Scientific Evolution 600 UV–Vis Spectrophotometer. Colour properties of L* had indicated lightness; a* indicated redness, and b* indicated yellowness. The measurement of the colour properties were carried out of each batch sample against a white plate.

3.3.4 Determination of Texture profile analysis (TPA)

Texture profiles analysis such as chewiness, hardness, springiness, and cohesiveness were measured by using the TA-HDi (Stable Micro Systems, Ltd., UK). Before the TPA measurement, cooked patty was tempered to 20 ºC and was cut into slices with a size of 2 cm x 2 cm. Platen compressions were then used, cuts and compressed twice. The patty thickness was varied and measured concerning its original thickness to provide an analogous condition induced by the cooking process and comparable calculation in the sensory examination.

3.4 Proximate analysis

3.4.1 Determination of protein content

This protein determination test was carried out using the standard method. The method involved 3 stages. First stage was digestion of 1g sample, 1 mL of sulphuric acid (H_2SO_4) and the Kjehdahl tablet added into a tube. The tube was heated at 400 ºC for 3 hours and cooled for 15 minutes at room temperature. Second stage was distillation. After digestion, 80 mL of distilled water and 50 mL of 40% NaOH was added into the tube. The 25 mL of 4% boric acid solution and 2-3 drops methyl red and bromocresol green was then added into a conical flask. The tube was related to the one end of the condenser and conical flask and connected towards the end. The volumetric flask was heated continuously until the conical flask filled. The third stage was titration. The conical flask was disconnected and taken for titration. It was titrated against 0.1N HCl solutions. The end point indicated by pink colour and was calculated using the following formula:

% Protein = % nitrogen x factor (6.25)

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

3.4.2 Determination of fat

The fat content was analyzed following the standard soxhlet method (Virot, 2007). 2 g of powdered mushroom sample was placed into the extraction thimble along with petroleum ether. The weight of the pre-dried round blotted sample was recorded. Then, a layer of defatted cotton was placed on the sample. After that, the sample was inserted into the extraction unit until attached to the magnets. The solvent was heated within the round bottled flask and extraction began in the soxhlet extractor at a rate of 5 or 6 drops per second, condensation for roughly 4 hours. This higher contact time between the sample and the solvent had given time for all the fat found in the sample to be dissolved. The sample needed was finely processed as possible in order for the solvent to penetrate the sample thoroughly (Min & Ellefson, 2010).

At the final stage, the extracted fat and flask were replaced in the oven for 30 minutes at 105°C, and the weight was recorded. Then, the extracted fat was cooled and weighed. The data was recorded and used to calculate crude fat using the formula below:

fat = (W2 - W1) x 100 ÷ Sample(g)

W1 = Weight of empty flask

W2 = Weight of extracted fat and flask

S = Sam

3.5 Statistical Analysis

All the recorded data obtained from the analysis were calculated to find the mean and standard deviation. The readings were then properly tabulated. IBM SPSS version 21.0 programme was used for the purpose of data analysis where one-way variance analysis (ANOVA) was performed to find the variation of the means. The $p < 0.05$ was set to significant standard.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Analysis of Physicochemical Properties

The physicochemical properties involved in this study were Colour, Texture Profile Analysis (TPA), cooking yield and proximate analysis for the determination of protein and fat content.

4.1.1 Colour

Based on the data, in comparing the lightness of the patty samples, there were no significant changes (p<0.05) between the colour of the beef patty (control) and the samples of patty which uses different type of binders except for the sample of patty with 20% Soy Pulp. The colour of sample which incorporated with 20% of soy pulp was brighter.

As for the a* which notes the positive values for the colour red, there were significant changes between the beef patty (control) and the patty samples that used the binder of TVP and Soy Pulp. Based on the data, it was seen that as the percentage of the binder increased, the redness of the patty sample that uses the binder of TVP or Soy Pup will decrease. The reason was because the colour of the binder itself are brighter and covers up the mushrooms. On the other hand, the use of the Dried Tofu as a binder had not shown any significant changes when compared to the beef patty (control). The data also showed that the use of higher percentage of Dried Tofu cause of a much more similar intensity of the redness just like the beef patty. The Dried Tofu was bright yellowish in colour and came in a powder form. In this case, the Dried Tofu may be able to not cause much difference in the colour of the patty due to its powder form. During the experiment, it was found that since the Dried Tofu was in powder form, it was much easier for the binder to be mixed together with the ingredients. Overall, it had formed a more consistent and blended mixture with no obvious or visible chunks of the binder as what had happened with the use of TVP or Soy Pulp.

In comparing the b* which notes the positive values for the colour yellow, there were significant changes (p<0.05) between the beef patty (control) and the patty samples only when the patty samples were added with 20% of binder. Based on the data, it was seen at first that there were no significant changes that occur when using 15% of the binder however there were significant changes when 20% of binder was used. In this case, it can be said that the different of the percentage of binders would cause an effect to the intensity of the colour yellow since all patty samples are affected according to the percentage despite using different types of binder.

4.1.1 Table of Colour Analysis for mushroom patties formulations.

Formulation	Types of Binder	Percentage of Binder Added (%)	Colour Attribute		
			L*	a*	b*
F0	Control	-	35.77±2.91	14.79±1.24	17.18±1.23
F1	No Binder	-			
F2	TVP	15.00	32.19±2.40	10.84±0.78	11.51±2.11
F3	Dried Tofu	15.00	36.59±5.03	12.67±0.69	14.94±4.40
F4	Soy Pulp	15.00	34.36±3.38	9.89±1.29	12.68±2.54
F5	TVP	20.00	33.82±0.81	9.47±0.92	13.49±1.98
F6	Dried Tofu	20.00	38.78±0.84	12.75±0.73	17.01±1.65
F7	Soy Pulp	20.00	32.99±1.05	11.98±0.65	13.25±0.45

4.1.2 Cooking Yield.

There was a significant difference (P > 0.05) in cooking yield between the beef patty (control) and the samples of patty which used different type of binders. The techniques to cook the patty had affected the weight after cooking. The mushroom patties were fried in cooking oil. Based on the table, dried tofu had the lowest percentage of cooking yield, this was because dried tofu itself does not contain any water holding properties in it. The cooking yield of the mushroom patty with the binder of 20% textured vegetable protein (TVP) has the highest value to be compared with beef patty (control). It was also found that the mushroom patty with 20% binder of dried tofu had the similar value of cooking yield with the beef patty (control). For the beef patty (control), fat was quickly lost after cooking, which was likely owing to a low-density beef protein matrix and high fat instability. This was consistent with prior study which investigated the influence of grind size and levels on the physicochemical and sensory properties of low-fat ground buffalo meat patties (Suman and Sharma, 2003).

4.1.2. Table of Cooking yield of mushroom patties formulations.

Formulation	Types of Binder	Percentage of Binder Added (%)	Weight (g)		Cooking Yield (%)
			Before Cooking	After Cooking	
F0	Control	-	82.16±0.88	61.88±0.49	75.32
F1	No Binder	-			
F2	TVP	15.00	81.99±0.76	79.40±0.77	96.84
F3	Dried Tofu	15.00	85.92±0.58	67.99±0.79	79.13
F4	Soy Pulp	15.00	83.35±0.45	68.31±0.66	81.96
F5	TVP	20.00	83.49±1.21	80.15±0.57	95.99
F6	Dried Tofu	20.00	85.83±0.75	68.41±1.06	79.70
F7	Soy Pulp	20.00	82.76±1.08	69.16±0.56	83.57

4.1.3 Texture profile Analysis.

Table 4.1.3 shows the results of the texture profile study for patty samples made with various types of binders. The qualities of hardness, cohesiveness, chewiness, and gumminess are all considered in texture profile analysis. The results showed that, with the exception of hardness and gumminess, the textural characteristics were not substantially ($p<0.05$) altered by the application of various binders.

In comparing the hardness of the patty samples, there were significant changes ($p<0.05$) between the hardness of the beef patty (control) and the samples of patty which uses different type of binders. Patty mushrooms were less hard in texture than the beef patty (control). The value of hardness of samples mushroom patties had increased when the percentage of binders increased. The highest value of hardness recorded was 4784.33±6.80 which is for the mushroom patty with 20% of textured vegetable protein (TVP). A study by Tsai et al., (1998) had mentioned that the dilution effect of non meat ingredients in meat protein systems primarily accounted for soft texture. Besides that, another study done by Riaz (2011) had also stated that products manufactured from this raw material can be used in the same way as those prepared from defatted soy flour or soy concentrate, but they will have deeper hues and softer textures due to significant heat treatment and reduced protein content. According to Kotwaliwale et al., (2007), the reduction of hardness due to the incorporation of the mushroom as one of the ingredient may be due to the higher moisture content.

Next, in comparing the gumminess of the patty samples, it was found that there were significant changes ($p<0.05$) between the gumminess of the beef patty (control) and the samples of patty which uses different type of binders. The results showed that the patty mushrooms were less gummy when compared with the beef patty (control). The value of gumminess of samples mushroom patties increased when the percentage of binders increased. The highest value of gumminess recorded was the mushroom patty with 20% Dried Tofu which is 5167.33±235.26 while the lowest value recorded was the mushroom patty with 15% Soy Pulp which is 1075.33±69.81. These results were supported by previous studies as it was showed that the incorporation of Dried Soy Tofu Powder had significantly increased the gumminess of frankfurters and cooked pork sausage patties when compared with their controls (Ho et al., 1997).

Moving on, in comparing the value of cohesiveness, there were no significant changes ($p<0.05$) between the cohesiveness of the beef patty (control) and the samples of patty which uses different type of binders except for the sample which uses 20% Soy Pulp and 20% Dried Tofu. The results found were different with previous research, where a study by (Rosli et al., 2011) has indicated that the amount of oyster mushroom used lowered the consistency of chicken patties correspondingly. In order to better understand the influence of various binders on the patties, (Bakhsh et al., 2021) conducted research that discovered that substituting 10 to 40% TVP resulted in a considerable drop in cohesion. Another study by Gujral et al., (2002) has also stated that the use of TVP in patties has shown the greatest effect of cohesiveness on both raw and baked patties. However, different from the findings presented in Table 4.1.2, it was seen that the use of TVP as a binder had shown no significant changes for the values of cohesiveness.

Lastly, in comparing the chewiness of the patty samples, there were no significant changes ($p<0.05$) between the chewiness of the beef patty (control) and the samples of patty which uses different types of binders. Previous studies had shown that the chewiness of meatless burger patties integrated with TVP were significantly lower when compared with the control patty (Samard et al., 2021). Another study had also supported that statement, where a study on soy based meat analogs had shown that the overall texture which includes the hardness, cohesiveness, chewiness and cohesiveness would all decrease when the moisture content increases (Lin et al., 2000). Based on previous research, it was expected that there were to be significant differences ($p<0.05$) in the value of the chewiness but this was in contrast with the results given in Table 4.1.2 as there were no significant differences between the control and the samples. A study by Kamani et al., (Kamani et al., 2019) had stated that patties sourced from beef or pork have much higher values in terms of hardness, chewiness, and gumminess due to the presence of myofibril proteins, which causes a tougher network formation internally and thus increasing compression resistance, which overall supports the statement that there should be a significant difference between the beef patty (control) and the sample patties.

4.1.3 Table of Texture Profile Analysis mushrooms patties formulations.

Formulation	Types of Binder	Percentage of Binder Added (%)	Hardness (g)	Cohesiveness	Gumminess (g)	Chewiness (mJ)
F0	Control	-	7942.16±398.66	0.7±0.010	6048.00±391.80	282.67±21.80
F1	No Binder	-				
F2	TVP	15.00	3345.00±120.90	0.85±0.04	2762.00±3.05	127.90±5.00
F3	Dried Tofu	15.00	3841.00±93.32	0.68±0.02	2919.00±48.05	1109.00±484.92
F4	Soy Pulp	15.00	1672.50±81.00	0.58±0.01	1124.00±69.81	49.50±1.61
F5	TVP	20.00	4781.00±6.80	0.68±0.07	3531.00±116.00	117.70±2.51
F6	Dried Tofu	20.00	4308.50±100.50	1.20±0.21	5122.00±235.25	321.40±1.25
F7	Soy Pulp	20.00	3161.00±42.93	1.11±0.07	3407.00±16.65	172.00±1.36

4.2 Proximate Analysis

The patty samples were sent to be tested by KBioCorp, Kedah BioResources Corporation Sdn Bhd. The proximate analysis was focused on obtaining the results of fat and protein. The results showed that the use of different types of binder with different percentages did affect the protein and fat content. The Beef Patty which acts as the control contained only 8g of protein. However, the alternative use of mushroom patty using the binder of Dried Tofu produced a patty with much higher content of protein. By incorporating 15% of Dried Tofu, the amount of protein had increased up to 10.8g. However, by the addition of 20% of Dried Tofu, the number was then further increased to as much as 13.4g. This shows that the amount of binder used played a very significant role in obtaining a patty sample that was able to contain as much protein that would be beneficial to our dietary intake. In the use of Dried Tofu for the purpose of making patties, it can be said that as the percentage of binder increased, the protein content had also increased. However, a different trend was observed for the patty samples that used the binder of Soy Pulp. It was seen that for this case, the amount of protein drops as the percentage of the binder increased.

As for the fat content, the table of analysis shows that the patty samples using the binder of TVP had obtained the lowest amount of fat content. By using 15% of TVP, the fat content obtained was 1.8g and by using 20% of TVP, the number further dropped to only 0.8g. This concludes that the higher the percentage of TVP as binder was used, the lower the fat content will be. The second binder with the lowest amount of fat content was Soy Pulp which was 2.9g for 15% and 1.3g for 20%, with the highest being Dried Tofu with the content of 5.8g for 15% and 7.8g for 20%. However, these readings were all much lower as compared to the beef patty (control) as it contained as much as 10g of fat, which was even higher than its protein content.

In choosing the best formulation that has potential to be an alternative to the Beef Patty, it can be said that the patty samples using the binder Dried Tofu may be a good choice as it contains the highest amount of protein. The consumption of patty as a part of a human's dietary plan functions to act as a source of protein which is why, among other analysis – the protein content should be prioritized. However, it is also important to note that the fat content of using the Dried Tofu is also highest as compared to the other binders. The analysis above also shows that increasing the percentage of Dried Tofu in the samples will increase both the protein and fat content.

4.2 Table of Proximate Analysis of mushrooms patty.

Formulation	Types of Binder	Percentage of Binder Added (%)	Parameters Protein (g)	Fat (g)
F0 Control		-	8.0	10.0
F1	No Binder	-		
F2	TVP	15.00	4.7	1.8
F3	Dried Tofu	15.00	10.8	5.8
F4	Soy Pulp	15.00	9.8	2.9
F5	TVP	20.00	11.8	0.8
F6	Dried Tofu	20.00	13.4	7.8
F7	Soy Pulp	20.00	6.6	1.3

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

In conclusion, grey oyster mushrooms were successfully made patty with different binders which are dried tofu, textured vegetable protein and soy pulp. It was found that the value of hardness, cohesiveness, gumminess and cooking yield changed when the percentage of binder increased.

First, the hardness of the mushroom patty with the 20% binder of textured vegetable protein (TVP) found that the value is the highest among the other binders. Yet, this mushroom patty also contains the highest amount of protein. The protein content should be prioritized for the human's dietary plan.

Secondly, the cohesiveness of the mushroom patty with the binder 20% of dried tofu is the highest value comparable to the beef patty (control). This may help the patty maintain to be in a patty form. The gumminess of the mushroom patty has increased when the percentage of binder has increased.

Lastly, the cooking yield of the mushroom patty with the binder of 20% textured vegetable protein (TVP) has the highest value to be compared with beef patty (control). It was also found that the mushroom patty with 20% binder of dried tofu has the similar value of cooking yield with the beef patty (control).

So, mushroom patty with the binder of dried tofu and textured vegetable protein (TVP) chosen because these formulations meet the criteria as similar to the control patty. Also mushroom patties with these two binders can give more protein, less fat and are safe to consume for all people.

RECOMMENDATION

Further research could be conducted in improving the functionality and stability of the products. Next, sensory analysis should be carried out and conducted to other people for consumer satisfaction purposes because it may help in analysing the mushroom patty product besides testing the mushroom patty using a machine. Moreover, the use of mould in mushroom patty production should have the same shape to prevent bias in result and be easy to conduct.

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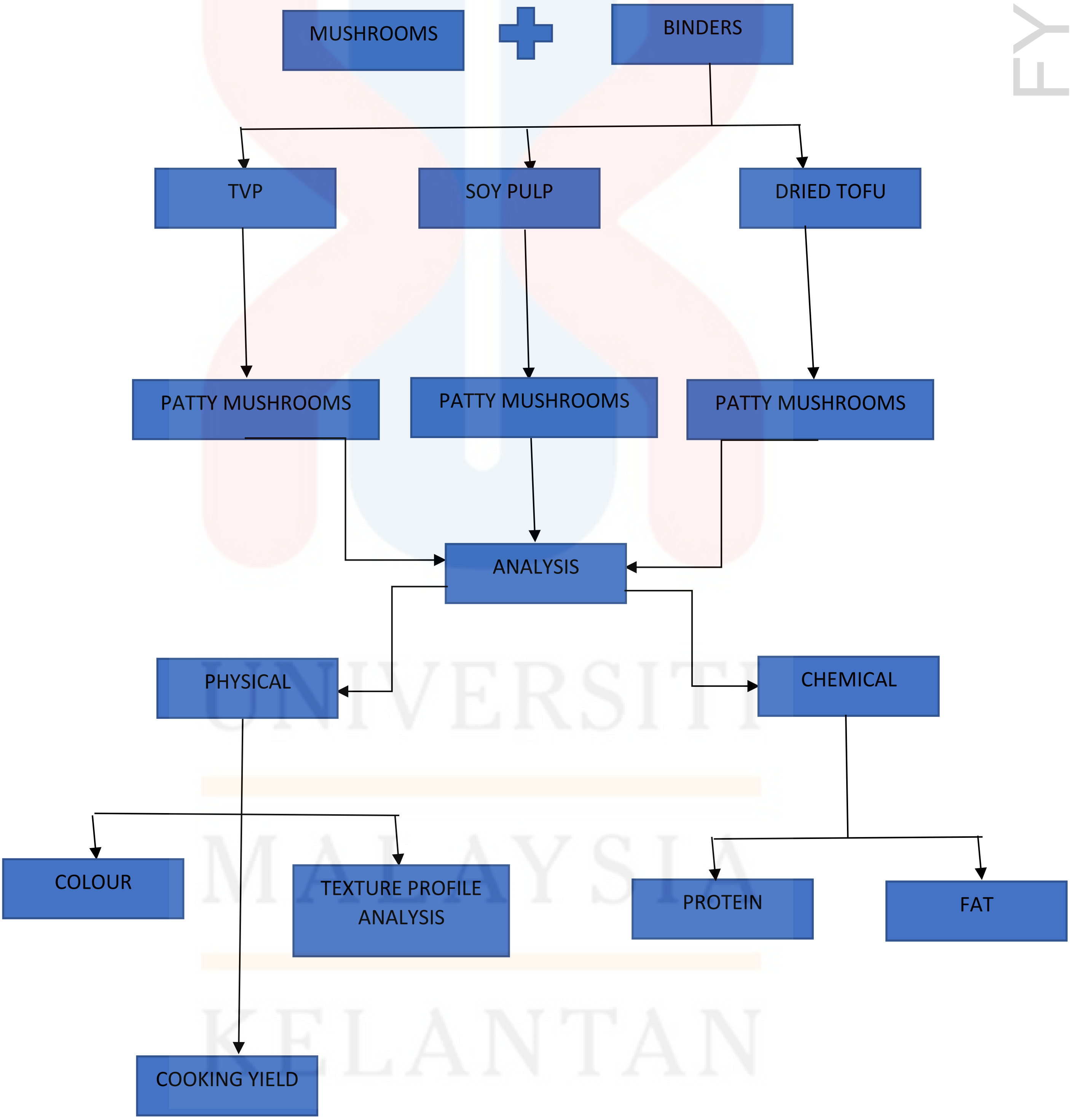
APPENDIX

FLOW CHART OF EXPERIMENT



Type of Samples	Formulation	Types of Binders	Percentages of the binders (%)	Pictures
Mushroom patties	F1	Textured Vegetable Protein	15.00	
	F2	Dried Tofu (Fucuk)	15.00	
	F3	Soy Pulp	15.00	
	F4	Textured Vegetable Protein	20.00	
	F5	Dried Tofu (Fucuk)	20.00	
	F6	Soy Pulp	20.00	

A) ANOVA of The Hardness (g) of The Mushroom Patties

ANOVA

Hardness (g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	77322801.333	6	12887133.556	449.335	.000
Within Groups	401526.833	14	28680.488		
Total	77724328.167	20			

B) ANOVA of The Gumminess (g) of The Mushroom Patties

ANOVA

Gumminess (g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48215311.810	6	8035885.302	244.801	.000
Within Groups	459566.000	14	32826.143		
Total	48674877.810	20			

C) ANOVA of the Cohesiveness of The Mushroom Patties

ANOVA

Cohesiveness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.704	6	.117	14.625	.000
Within Groups	.112	14	.008		
Total	.816	20			

D) ANOVA of The Chewiness (ml) of The Mushroom Patties

ANOVA

Chewiness (mJ)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	506243.737	6	84373.956	2.506	.073
Within Groups	471336.519	14	33666.894		
Total	977580.256	20			

E) ANOVA of The Weight (g) Before Cooking of The Mushroom Patties.

ANOVA

Weight (g) Before Cooking

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1253.799	6	208.966	285.317	.000
Within Groups	10.254	14	.732		
Total	1264.052	20			

F) ANOVA of The Weight (g) After Cooking of The Mushroom Patties.

ANOVA

Weight (g) After Cooking

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2174.614	6	362.436	686.592	.000
Within Groups	7.390	14	.528		
Total	2182.005	20			

G) ANOVA of The Colour (L*) Lightness of The Mushroom Patties.

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	235.683	6	39.280	5.145	.005
Within Groups	106.895	14	7.635		
Total	342.578	20			

H) ANOVA of The Colour (a*) yellowness of The Mushroom Patties.

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68.775	6	11.462	13.054	.000
Within Groups	12.293	14	.878		
Total	81.068	20			

I) ANOVA of The Colour (b*) redness of The Mushroom Patties.

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	899.440	6	149.907	28.452	.000
Within Groups	73.762	14	5.269		
Total	973.202	20			