THE EFFECT OF DIFFERENT COOKING TECHNIQUES OF OYSTER MUSHROOM ON PHYSICOCHEMICAL AND SENSORY PROPERTIES AND ITS APPLICATION IN BEEF PATTY

By

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ABSTRAK

Warisan makanan Malaysia sering didorong oleh pengaruh antarabangsa yang menyebabkan Malaysia bergantung kepada import daging merah. Penggunaan bahan pengganti bukan berasaskan daging seperti cendawan bagi formulasi burger yang secara praktikalnya berpotensi kerana mengandungi pelbagai nilai pemakanan. Kesan teknik memasak yang berbeza pada sifat fizikal dan deria cendawan tiram dan aplikasinya dalam patti daging lembu telah disiasat. Keputusan menunjukkan burger daging lembu digantikan dengan cendawan tiram mentah (kawalan), mencatatkan hasil masakan yang paling tinggi (85;40%). Mengenai ciri-ciri fizikal yang lain, burger daging lembu yang telah digantikan dengan cendawan tiram yang di celur (BOM), mencatatkan pengekalan kelembapan yang tertinggi (33;32%). Analisis warna burger daging lembu yang digantikan dengan 25% daripada cendawan tiram yang di kukus (SOM) adalah lebih cerah kerana ia mempunyai nilai L * (cerah) yang lebih tinggi berbanding dengan burger daging lembu mentah yang diganti dengan BOM tetapi kecerahan kurang berbanding burger kawalan. Burger daging lembu yang digantikan dengan BOM merekodkan nilai terendah a* (kemerahan) berbanding dengan burger daging lembu yang digantikan dengan SOM dan kawalan masing-masing. Sementara itu, burger daging lembu yang digantikan dengan SOM merekodkan tertinggi nilai b * (kekuningan) berbanding dengan burger daging lembu yang digantikan dengan BOM dan kawalan masing-masing. Penilaian deria produk mendedahkan perbezaan signifikan ($p < 0.05$) bagi warna, juiciness, rasa, penerimaan keseluruhan sifat-sifat. Sebaliknya, produk tidak signifikan ($p > 0.05$) terhadap aroma dan ciri-ciri kelembutan. Rata-rata bagi warna burger daging lembu yang digantikan dengan SOM tidak berbeza ($p > 0.05$) daripada kawalan. Secara ringkasnya, teknik memasak yang berbeza digunakan untuk cendawan tiram

sebelum digantikan dalam burger daging lembu itu tidak menjejaskan beberapa sifat-sifat fizikal dan sensori.

ABSTRACT

The inheritance of Malaysian food is frequently driven by international influences which cause Malaysia to rely on imports of red meat. The utilization of non-meat-based substitute such as mushroom to the patty formulation is potentially practical since it contain various nutritive values. The effect of different cooking techniques on physical and sensory properties of oyster mushroom and its application in beef patty were investigated. Result shows beef patty replaced with raw oyster mushrooms (control), recorded the highest cooking yield (85.40%). On the other physical traits, beef patty which was replaced with blanched oyster mushroom (BOM), recorded the highest moisture retention (33.32%). The color analysis of beef patty substitute with 25% of steamed oyster mushrooms (SOM) was brighter as it had higher L* (lightness) value compared with raw beef patty substitute with BOM but less brightness compared to the control patty. Beef patty substituted with BOM recorded the lowest a* value (redness) compared to beef patty substituted with SOM and control, respectively. Meanwhile, beef patty substituted with SOM recorded the highest b* value (yellowness) compared to beef patty substituted with BOM and control, respectively. Sensory evaluation of the products revealed significant ($p<0.05$) differences for color, juiciness, flavor, overall acceptance attributes. Conversely, the products had no significant ($p>0.05$) effect on aroma and tenderness attributes. The score for color of beef patty substituted with SOM was not significantly different ($p>0.05$) from the control. In summary, different cooking techniques applied to the oyster mushroom before substituted in the beef patty did not jeopardize several physical properties and sensory properties attributes.

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LIST ABBREVIATION AND SYMBOLS

%	: percentage
°C	: degree celcius
g	: gram
ml	: mililiter
ppm	: part per million
ANOVA	: Analysis of varience
PSC	: *Pleurotus sajor-caju*
ROM	: raw oyster mushroom
BOM	: blanched oyster mushroom
SOM	: steamed oyster mushroom
BPROM	: beef patty substitutes with raw oyster mushroom
BPBOM	: beef patty substitutes with blanched oyster mushroom
BPSOM	: beef patty substitutes with steamed oyster mushroom

CHAPTER 1
INTRODUCTION

1.1 Background of Study and Problem Statements

Malaysian diet often pervades unity within the multicultural civilization. The inheritance of Malaysian food is frequently driven by international influences which cause Malaysia to rely on imports of food and food ingredients for food processing and local production. For example the demand for red meat remains to increase. Malaysia mostly imports red meat from India, Australia and New Zealand due to products from these countries are fulfilling Halal and food safety aspects. According to Australian Trade Commission (ATC), International Trade Centre stated that, Australian red meat contributed 24.34 per cent in value of total meat imported into Malaysia in 2011. ATC continue that the prospect for more high quality Australian red meat has increased to gain access to Malaysia market as expanding consumer appeal for Halal meat. Furthermore, Australia's Halal integrity program support this due to it is benchmarked against Malaysia's Halal standard. Before distributing its meat products into Malaysia, the Islamic Development Foundation of Malaysia (JAKIM) will approved after the slaughterhouses have been scrutinized (Australian Trade Commission, 2014). The meat demand is also related to the meat-based patty production. Hence, to reduce dependency of imported meat, non-meat ingredients can be used as alternative sources in the burger patty.

The high level of meat and saturated fat intake in high income countries surpasses nutritional needs and contributes to high rates of chronic diseases such as cardiovascular disease, diabetes mellitus and some type of cancers. Prosperous citizens in middle- and low-income countries are adopting similar high-meat diets and experiencing increased rates of these same chronic diseases (Walker, 2005).

Asgar *et al.* (2010) mentioned that, plant-based ingredient can be expended directly as a meat substitutes or can lengthen meat products while providing an economical, functional, and high-protein food ingredient. Meat substitutes from plant based ingredient are effective because of their healthy image (cholesterol free), meat-like texture, and low cost (Asgar *et al.*, 2010). Therefore, the production of meat-based substitute to the patty product is potentially practical by using more economical and healthier ingredients including mushroom.

The origin of hamburger or burger is unknown but during the 19th century, Hamburg, Germany became famous for their beef which produce Hamburg beef that was generally minced or chopped and combined with garlic, onions, salt, pepper, and molded into patties then grilled or fried (Avey, 2013). Nevertheless, in the mid 1800's, two pieces of bread launched the Hamburg steak into nationwide popularity which difference between Hamburg steaks and hamburgers. The hamburger continual growing in popularity throughout the subsequent decades, but unfortunately then suffering with the food shortages and meat rationing during World War II. American soldiers brought hamburgers overseas with them during the war. In the 1940s, the McDonald brothers opened their Burger Bar Drive-In in San Bernardino, California as the hamburger made its authorized introduction in the suburbs. As the results, McDonald's had sold over 100 million hamburgers by that late 1950s (Avey, 2013). Currently hamburgers can be found in virtually every part of the world. Over time the idea has developed, and meat patties are decorated with an infinite selection of creative and scrumptious garnishes. Besides, nowadays there are researches that develop the healthier version of burger for health concern without require the consumers to avoid burger in their diet.

Edible and therapeutic mushrooms are amazingly common foods in most nations as the ideal health foods. Their delicate taste and flavor are well valued and are consumed both in the fresh and processed forms (Zahid *et al.*, 2009). Mushrooms are perish in a short shelf life period (1–3 days at room temperature), associated to the occurrence of post-harvest fluctuations. These situations are because of the high activity of enzymes such as protease or polyphenol oxidase and the high moisture content of the carpoforus which liable for a browning reaction throughout storage and for protein and sugar decline (Manzi *et al.*, 2004). Furthermore, edible mushrooms may contribute massively to the supply of both macro- and micro-nutrients in consumer diet; hence they are possible to contribute tremendously to food value of consumer habitual diet. Mushroom consumers are usually found to have better intake levels of most vitamins and minerals and in certain cases to eat less alcohol, fat and sodium. Beneficial value of mushrooms is accredited to their high content of essential amino acids, vitamins, minerals and low lipid content (Zahid *et al.*, 2009). Mushrooms comprise significant amounts of dietary fibre, even though they are not high in protein or fat, predominantly vital for the regulation of physiological functions in the human organism. Whereas, functional compounds in mushrooms also have recently been highlighted and studied. These constituents are capable to modify the immune system, reduce cholesterolemia, and prevent tumoral growth. Particularly, chitin (N-acetyl-d-glucosamine polymer), a nitrogen-containing polysaccharide of the fungal cell walls, and chitosan, its deacetylated derivative, are liable for declining the physiological cholesterol (Manzi P. *et al.*, 2004). Moreover, comestible mushrooms have used for long times as traditionally seasoning ingredients in soups and sauces, because of their extraordinary and delicate flavors. The usual flavor of mushrooms comprises of nonvolatile components and volatile compounds. The taste of palatable mushrooms is mainly due to the existence of numerous small water soluble elements, as well as soluble sugars, free amino acids and 50-nucleotides (Li *et al.*, 2013).

1.2 Research Objective

1.2.1 General Objective

- To determine the effect of different cooking techniques of oyster mushroom on physicochemical and sensory properties and its application in beef patty.

1.2.2 Specific objectives

- To determine the nutrient content of oyster mushroom prepared with different cooking techniques.
- To evaluate the physical properties of oyster mushroom prepared with different cooking techniques.
- To elucidate the sensory properties of beef patty incorporated with treated oyster mushroom.

1.3 Research Questions

- What are the effect of different cooking techniques of oyster mushroom on physicochemical properties and its application in beef patty?
- What are the sensory properties of beef patty incorporated with treated oyster mushroom?
- Which type of nutrients of oyster mushroom prepared with different cooking techniques retained the most?

1.4 Research Hypothesis

- There is significant effect of different cooking techniques of oyster mushroom on physical properties and its application in beef patty.

1.5 Significance of Study

First and foremost, the purpose of the study is to determine the effect of different cooking techniques towards the oyster mushroom which will be added into beef patty as partial substitutes of beef. Processed food especially beef patty is assumed can be significantly affected by processing, raw material and ingredient factors either from nutritional value or overall acceptability by consumer. Beef patty which was substituted with 25% of Pleurotus sajor-caju (PSC) give the highest cooking yield (76.62%) and moisture retention (59.80%) respectively, compared to beef patty containing 50% of PSC and control (Wan rosli and Solihah, 2012). Hence, the level PSC at 25% will be used for product formulation in beef patties as recommended by the researchers (Wan Rosli and Solihah, 2012).

In this research, nutrient content and sensorial qualities of oyster mushroom after being undergoing pre-treatments (blanching and steaming), and also after being form into beef patty will be determined. Furthermore, for the economic perspective, by using the oyster mushroom as one of key ingredient in the beef patty formulation, the local beef patties producer may reduce the dependency of imported meat from other countries. Scientist and industry progressively recognized that oyster mushroom and their components can deliver health benefits beyond their nutritional value. Whereas, the different cooking techniques may contribute to the different palatability effect for beef patty when oyster mushroom is being added in it. Blanching in hot water and in steam, in order to diminish or disregard the bitterness of the vegetables and acid components that are mutual in leaves, and in order to make it more palatable (Oboh, 2005).

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom

Mushrooms are saprophytic fungi, a class of original plants that are lacking of chlorophyll. Fresh mushroom is soft textured and greatly perishable and must be either consumed or processed immediately after harvest (Srivastava *et al.*,2009). In natural world mushrooms grow wild in every nation on all types of soils, pastures, forests, cultivated fields or water lands from snowy mountains to sandy deserts. They grow in all seasons, primarily during the rainy climate, as long as organic matter or its decomposition products are present (Asghar *et al.*, 2007).

Mushroom cultivators in Malaysia commonly use a sawdust-based medium that prepared from local waste biomass like sawdust and/or rice bran to grow the mushroom. This cultivation method is popular and simple process in tropical areas. However, sometime serious problem has occurred involving either very slow growth rate of inoculate mycelium in the prepared medium or the complete failure of the inoculated mycelium to grow. The main cause of this problem appears to be the utilization of sawdust which is used as the major component of the medium preparatium (Yokota and Ree, 2009).

Mushrooms have been presented as part of the typical human diet for thousands of years and the volumes of a huge number of species consumed have grown prominently in contemporary times (Jayakumar *et al.*, 2011). Mushrooms also have been a food supplement in many cultures and they are cultivated and consumed for their palatability and gracefulness. Mushrooms are assumed to give significant nutritional function in the human diet as they are high in non-starchy carbohydrates, dietary fibre, minerals, vitamin-B, amino acids and fat are relatively low in fat value (Dunkwal & Singh, 2007; Konuk, 2006) and they fall among the top sources of vegetables and animal protein. The essential amino acids, water-soluble vitamins and all the essential minerals are also present. The protein value of mushrooms is double as much as asparagus and potatoes, four times as much as tomatoes and carrots, and six times as much as oranges. Currently, mushrooms are being considered as source of alternative food to offer sufficient nutrient content to world's growing population (Konuk, 2006). Though mushrooms have important role as biopharmaceuticals for human diseases, they are largely used as delicious and nutritious food, hence mushrooms can be used as source of alternative food in addition to fortification or supplementation of diet for enhanced nutrition (Mukhopadhyay & Guha, 2015).

In addition, according to Mukhopadhyay & Guha (2015), the efforts from both public and private organizations are required to increase the mushroom production and export which will guarantee positive effect on country's economy as well as quality alternative food or supplement.

Mushrooms are very deprived in lipid and very high in carbohydrate, protein, ash, fibre, and minerals. Former study reported that mushrooms are very decent nutrition source for mankind who search for innovative and alternative food and nutrition source all the time. They possibly will be very convenient for vegetarians and comprise several essential amino acids which are found in merely animal proteins (Verma *et al.*, 1987). It could be very useful in removing dependency on animal protein once they are enhanced with mushroom to overwhelm the amino acid deficiency. Several other benefits of oyster mushroom include containing low sodium and cholesterol levels, being of low calorific value, and being a prospective source of prebiotics (Aida *et al.*, 2009).

Furthermore, a review by Khatun *et al.* (2015), mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals due to their antioxidant, antitumor and antimicrobial properties. Substances that may be considered a food or part of a food and that provide medical or health benefits like the prevention and treatment of diseases are referred to as nutraceuticals.

2.2 Oyster Mushroom

National Horticulture Board (NHB) shows that oyster mushroom (*Pleurotus sp.*) belong to Class Basidiomycetes and Family Agaricaceae. The oyster mushrooms generally have three distinctive parts which include a fleshy shell or spatula shaped cap (pileus), a short or long lateral or central stalk called stipe and long ridges and furrows beneath the pileus called gills or lamellae. The gills stretch from the edge of the cap down to the stalk and endure the spores. The spores are smooth, cylindrical and germinate very easily on any kind of mycological media within 48-96 hrs. The mycelium of *Pleurotus* is pure white in colour (NHB, 2011).

Oyster mushrooms (*Pleurotus spp.*) are currently cultured and consumed globally (Vullioud *et al.*, 2011). Oyster mushrooms are proliferates that have a good taste compared to most other food fungus. Oyster mushrooms are cultivated on diverse agricultural wastes because of its compatibility and produce high yield in varied climate (Asghar *et al.*, 2007). Unfortunately, they are easily perishable due to a high respiration and transpiration rates leading to fast post-harvest deterioration (Vullioud *et al.*, 2011). The most renowned species of *Pleurotus* are P. *ostreatus*, P. *florida,* P. *eryngii*, P. *cystidiosis,* P. *flabellatus,* P. *cornucopie,* and P. *sajor-caju*. P. *sajor-caju* is acknowledged as a tremendous mushroom because it can be cultivated within a wide range of temperatures on different natural resources and agricultural wastes (Asghar *et al*., 2007).

Oyster mushrooms (Pleurotus spp.) are ranked second in terms of mushroom world production. In a study, reported that the nutritional parameters of P. *sajor-caju* SCP grown in alternative fermentation medium deproteinized whey. It may be concluded that essential amino acid profile in the P. *sajor-caju* SCP meets the requirements recommended by FAO except sulphur containing amino acids. Additionally, utilization of lactose of whey by this fermentation procedure provides an excellent way to reduce the BOD of whey and environmental pollution. Relatively low nucleic acid content, presence of essential minerals and PUFA (linoleic acid) and high amount of folic acid in the SCP of P. *sajor-caju* makes it an attractive candidate for alternative food source or fortification of diets low in nutritive value (Mukhopadhyay & Guha, 2015).

2.3 The Pharma-Nutritional Roles of Edible Mushroom

Mushrooms and their extracts are commonly well-tolerated with some, or if any, side-effects although there have been relatively few direct intervention trials of mushroom consumption in humans.

There is a briskly rising volume of *in vitro* and *in vivo* animal trials relating a sort of potential health benefits including immunomodulatory, anti-tumor, anti-microbial effects and hypocholesterolemic effects, although there are restricted direct human intervention trials. Part of review by Roupas *et al.* from Hetland *et al.* (2011), mentioned that, there have been stated mushrooms may prevent tumor growth by stimulating the immune system through activation of macrophages, by balance of T helper cell populations and subsequent effects on natural killer (NK), cells and correspondingly through cytokinesome, due to the more valuable compounds in mushrooms which are 1,6-branched 1,3-b-glucans (Roupas *et al.*, 2012).

In addition, immunomodulatory effects (increased NK cell activity, effects on IgG, IgM, neutrophil and leukocyte counts) in humans from oral consumption of dietary polysaccharides (glucans) from several varieties of mushrooms has been reported (Ramberg *et al.* 2010). Inhibition of aromatase activity by mushroom extracts (Grube *et al.*, 2001; Chen *et al.*, 2006) and consequent declining of estrogen, which is a possible adjuvant therapy for breast cancer patients with estrogen receptor positive tumors were also reported before. Besides, bioactive proteins from mushrooms (such as lectins, fungal immunomodulatory proteins (FIP), ribosome inactivating proteins (RIP), ribonucleases and other proteins have also been reported to possess similar anti-tumor, anti-viral and immunomodulatory activities due to the effects and underlying mechanisms of mushroom polysaccharides in health outcomes have been more extensively evaluated by Xu *et al.* (2011).

Moreover, Borchers (2008) has associated polysaccharides with varying sugars (beta- and alpha-glucans). Although structure–function relationships have been described between anti-tumor activities and structural characteristics of b-D-glucans, further explanation of the mechanisms by which these polysaccharides utilize their immunomodulatory effects are not entirely clear, these mushroom polysaccharides commonly do not utilize cytotoxic effects on tumor cells, however have been shown to boost host-mediated immunomodulatory responses stated by Cheung *et al.* (2011).

2.4 Meat Alternative

Vegetarian groups provide evidence of lower morbidity and mortality; hence, vegetarian diets tend to be viewed as healthy by consumers. Typically, vegetarian groups most likely to consume diets those are higher in fibre, fruits and vegetables. Lower disease risk, predominantly cardiovascular disease (CVD) and cancer are linked with diets that characterized by high intakes of plant foods, including fruits and vegetables. The persistent consumer concern in vegetarianism and more generally in the selection of occasional meat-free meals as portion of a various diet, are the key driving forces behind consumer request for high quality and convenient meat alternative products. Industry has responded to this request with technological progresses that have supported the use of an increasing variety of main ingredients in the production of meat alternative products. Meat substitute ingredients are healthful with some offering specific health benefits. Such products consequently have the prospective to contribute to overall public health, as well as increasing consumer choice. Furthermore, the prior survey also highlights consumer awareness that meat-free alternatives compromise positive health benefits (Sadler, 2004).

A study that supports promoting the main issue for meat-free alternative product is to reduce fat or lipid content, by evaluating the effect of oyster mushrooms on blood lipid profile in humans. Animal studies have suggested that continuous treatment with oyster mushrooms improved the lipid profile in hamsters and rats. Hence, reliable with the animal studies, the results of this study support the assumption that a diet with oyster mushrooms has a positive influence on the lipid profile in humans. The data suggest that the blood lipid profile of humans was positively affected by consumption of oyster mushrooms. However the results should be validated in forthcoming intervention studies (Schneider *et al.,* 2011).

In addition, the physical manipulation of the meat by means of massaging or mincing and the incorporation of non-meat ingredients into the formulation are among the solutions used to minimize problems related to fat reduction and salt reduction (Saricoban *et al.*, 2009).

2.5 Cooking Techniques

Blanch is defined as to dip a food briefly into boiling water; water must reach 100°C, a temperature at which water bubbles rapidly (Brown, 2011). Blanching technique could be described as the process of heating vegetables to a temperature high enough to destroy enzymes present in the tissue (Oboh, 2005). The shelf-life of fresh oyster mushrooms is affected by browning reactions and dehydration within a few days, even if refrigerated. Polyphenol oxidase (PPO) is an important enzyme involved in browning. Hence, oyster mushrooms can be treated to be preserved for long times. Blanching is also a mutual step in most processes, as it diminishes microbial load, inhibits enzymatic browning, persuades contraction in size and air leaking, and produces a better product for further industrial operations. Conversely, some disadvantages of blanching are weight loss (water, dry matter, nutrients), color and texture changes (Vullioud *et al.*, 2011).

Steaming is generally a technique which any food heated by direct contact with the steam generated by boiling water. A regular process for steaming is to place food in a rack or steamer basket above boiling water and to cover the pot or pan with a lid in order to trap the steam. This technique helps to retain texture, color, taste and nutrients, hence, cooked vegetables are at their best when steamed (Brown, 2011).

CHAPTER 3

METHODOLOGY

3.1 Research Design

This work is based on an experimental study. Oyster mushroom was pre-treated by different cooking techniques (blanching and steaming). Untreated raw oyster mushroom was used as control. Experimentation conducted was designed to determine the effect of different cooking techniques on oyster mushroom and its application in beef patty. The nutritional composition of oyster mushroom prepared with different pre-treatment and its application in beef patty were also determined.

3.2 Materials and Methods

3.2.1 Sample preparation

Sample of oyster mushroom (*Pleurotus sajor-caju*) were obtained from local supermarket around Kota Bharu town, Kelantan state of Malaysia.

Pre-treatments. Sample preparation was begin by cleaning process of oyster mushroom. The samples were weighed first. Then, the sample were rinsed with clean water and tossed to drain the excessive water. Sliced mushrooms were subjected to two pretreatments prior to formulation of beef patties. Sample sizes of 300 g of mushroom (Dunkwal & Singh, 2007) were prepared for each cooking techniques by using 500 ml boiled water (Oboh, 2005).

Control. Samples used without any treatment (untreated samples).

Blanching. Samples were immersed in boiling water at 100°C for two minutes, cooled instantly in cold water and drained (Dunkwal & Singh, 2007).

Steaming. Samples were steamed for five minutes according to Andress & Harrison (2006).

3.2.2 Patties formulation

The patty formulations were prepared by addition with oyster mushroom (after being prepared by different cooking techniques) at 25% of beef substitution according to Wan Rosli and Solihah (2012) with slight modification.

Beef patty formulation:

Three beef patty formulations are compared. Each of them contains 25% of pre-treatments oyster mushrooms. The percentages of other ingredients are unchanged compared to the control sample, whereas the percentage of beef (hind quarter) decreases with the increase of oyster mushrooms content. The oyster mushrooms are incorporated into the beef patties using the formulations described in Table 1. The finished beef patties was stored in a freezer at -18 °C until further analysis. Oyster mushrooms were prepared in the Nutrition Laboratory of the School of Health Sciences, Universiti Sains Malaysia Health Campus. Beef were purchased from local wet market (*Wet market* of *Wakaf Che Yeh, Kota Bharu district, Kelantan, Malaysia*). Other dry materials were purchased from local suppliers.

Table 1: Beef patty formulated with pre-treatments Oyster Mushrooms (Wan Rosli & Solihah, 2012).

	Pleurotus sajor-caju level (%)		
Ingredients (%)	Control (0)	25	50
Beef (hind quarter)	54.0	40.5	27.0
Fat	9.0	9.0	9.0
Water	26.0	26.0	26.0
Potato starch	6.0	6.0	6.0
Ground PSC	0.0	13.5	27.0
Isolated soy protein	3.0	3.0	3.0
Salt	1.0	1.0	1.0
Spices and seasoning	1.0	1.0	1.0
Total	100	100	100

Processing:

The beef (hind quarter) were manually cut using a cleaver and mince through a 4 mm-diameter grinder plate. The minced beef fleshes were stored at –18 °C until processing time. The emulsion prepared (called pre-emulsion) is kept in a chiller (2-5oC) until ready for use. Salt was added to the frozen minced beef and mixing is carried out using a heavy mixer for 3 min. Then spices, potato starch, bread crumb were mixed and oyster mushrooms are added and mix for another 2 min. The pre-emulsion is then added and mixing continued for another 2 min. The finished beef batters are then weighed into 80g portions, and then manually molded to produce a uniform patty. The raw beef patties are then frozen in a freezer at –18°C until further analyses.

Cooking procedure:

Beef patties were thawed at 4 °C for 12 h. Beef patties were then cooked on a pan-fried for 7-8 min until an internal temperature of 72 ± 1°C was achieved.

3.2.3 Nutritional composition

Nutritional composition analysis; moisture and fat was used to compare values between before and after the oyster mushroom is added in the beef patty to determine the moisture retention and fat retention. Proximate composition was conducted using (AOAC, 1996) for moisture and crude fat content using the semi-continuous extraction [Soxhlet] method (AOAC, 1996).

Determination of Moisture:

In determination of moisture content, air-oven method or drying method (AOAC, 1996) were followed. In this method, a homogenous ground sample was dried in an oven (usually at 105 – 110oC) until constant weight was achieved. The difference between the initial weight and constant final weight after drying is considered as the moisture lost. Therefore, this different is recorded as moisture content of the sample. The method is frequently used and should give fairly accurate results when considered on a comparative basis. However, it should be noted that in the process of heating until constant weight, not only water evaporates from the sample, but also volatile substances such as volatile oils. On the other hand, with some foodstuffs only a portion of water present is lost at this drying temperature. Hence the results obtained in this method may not be a true measure of the water content of the sample.

Procedure :

The samples were finely homogenized in a blender. Aluminium dishes with cover were let dried for 3 hours in an oven at 105°C. Then, were cooled in a dessicator and were weighed soon after it has attained room temperature as W1. The homogenized samples were weighed 5.000+ 0.0001g into aluminium dish (W2). Dish with sample, uncovered and were placed in a 105 °C oven overnight. The lid was replaced while dish is still in oven; dish was removed from oven. Then, was cool in dessicator and weighed soon after attaining room temperature (W3). The steps were repeated until constant weight is obtained. The moisture percentage was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{(W2-W3)}{(W2-W1)} \times 100$$

Determination of Crude Fat:

The fat content was measured in triplicates by gravimetric technique with chloroform/ methanol/water according to the method described by Kinsella et al. (1977). The yields of the extracted samples were calculated based on the difference in the mass before and after lipid extraction.

Procedure :

The universal bottles were weighed. The samples were weighed at 30.000 + 0.0001 g. The solution; methanol and chloroform were added 60 ml. The samples were grounded and homogenized. After 2 minutes, the solvent; chloroform was added 30 ml. The samples were homogenized again. After 30 seconds, distilled water was added 30 ml. The homogenate were stirred with a glass rod and were filtered through Whatman No. 1 filter paper on a Buchner funnel with slight suction. The filtrate was transferred into separatory funnel. The lipid extract were collected into universal bottle. The collected lipid was evaporated to dryness. The universal bottles with dry lipid extract were weighed. The fat percentage was calculated using the formula:

$$\text{Fat} = \frac{(W2-W1)}{\text{Weight sample (g)}} \times 100$$

3.2.4 Sensory Evaluation

Sensory evaluation for beef patty added with oyster mushroom from each cooking techniques was conducted to evaluate and differentiate the taste of beef patties. All beef patty samples were evaluated by each untrained consumers according to the hedonic scaling method outlined by Piggott (1989). Sensory evaluations were carried out by 60 untrained consumers consisting of students and staff of the School of Health Sciences, Universiti Sains Malaysia Health Campus. The cooked patty samples were equally divided into 6 portions. Each portion of product sample was placed in sensory cups with lids coded with 3 digit random numbers. Permutation sample presentation is applied to the patties before presented to the panellists. The attributes analyzed were colour, juiciness, elasticity, flavour and overall acceptance on a 7 point scale (1 = dislike extremely and 7 = like extremely). Significance is established at $P \leq 0.05$ (Wan Rosli and Solihah, 2012).

3.2.5 Physical traits

Colour analysis

The colour of the oyster mushrooms after undergoes pre-treatment, raw beef patties substitutes with oyster mushroom, and cooked beef patties substitutes with oyster mushroom were quantified by using a Colorimeter (Minolta Spectrophotometer). The colour meter was set to CIE Standard Illuminant C. Then its colour are measured by using the ground material colour measurement apparatus of the instrument. The colour of the liquid is also measured for comparison purpose. L*, a* and b* values was measured to describe three dimensional colour space and interpreted as follows: L* is the brightness/lightness or whiteness ranging from no reflection for black (L = 0) to perfect diffuse reflection for white (L = 100). The instrument was calibrated with a yellow CR-A47Y standard tile: L* = 85.46, a* = −0.13, and b* = 54.58. The value a* is the redness ranging from negative values for green to positive values for red. The value b* is the yellowness ranging from negative values for blue and positive values for yellow. The data were presented as means of nine independent measurements for each treatment (Soysal et al.,2009).

Cooking characteristics:

Cooking yield

Cooking yield of beef patties were determined by measuring the weight of six patties for each treatment/batch and calculations of weight differences for patties before and after cooking, are as follows (El-Magoli *et al.,* 1996):

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

Moisture retention (percent)

The moisture retention values represent the amount of moisture retained in the cooked product per 100 g of raw sample, These values are calculated according to the following equations (El-Magoli *et al.,* 1996):

$$\text{Moisture retention (\%)} = \frac{\text{(percent yield x \% moisture in cooked patties)}}{100}$$

Diameter reduction (%)

Change in beef patties' diameter was determined using the following equation:

$$\text{Diameter reduction (\%)} = \frac{\text{raw beef patties diameter – cooked chicken patties diameter x 100}}{\text{raw beef patties diameter}}$$

3.2.6 Statistical Analysis

Data obtained were analyzed according to ANOVA procedure by using program SPSS 22.0 (USA). Results were expressed as mean ± standard deviation. All measurements were carried out in triplicate (n = 3). Significant level established at P ≤0.05.

CHAPTER 4

RESULTS

4.1 Nutritional Composition Analysis

Nutritional composition analysis is only focuses on the analysis of moisture and fat. The nutritional composition analysis of oyster mushroom, raw beef patties substitutes with oyster mushroom and cooked beef patties substitutes with oyster mushroom is shown in Table 4.1.1, 4.1.2, and 4.1.3.

Table 4.1.1. Moisture and fat analyses of oyster mushroom.

	ROM (control) (%)	BOM (%)	SOM (%)
Moisture composition	90.70 ± 0.14^{b}	90.89 ± 0.03^{b}	88.45 ± 0.06^{a}
Fat composition	0.16 ± 0.02^{a}	0.13 ± 0.04^{a}	0.15 ± 0.07^{a}

$^{a\text{-}b}$ Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
ROM = raw oyster mushroom, BOM = blanched oyster mushroom, and SOM = steamed oyster mushroom

The table 4.1.1 shows the moisture and fat composition analysis of oyster mushroom (raw and pre-treated). The moisture compositions of oyster mushroom were ranged from 88.45% to 90.89%. The moisture composition of ROM showed the highest content of moisture at 90.89%. Conversely, it was not significantly different with the SOM. The highest content of fat was recorded by ROM, (0.16%) and the lowest content of fat was recorded by BOM, (0.13%). In addition, there was not significance different among all of the raw and pre-treated oyster mushroom in fat content.

Table 4.1.2. Moisture and fat analyses of raw beef patty substitutes with 25% oyster mushroom.

	BPROM (control) (%)	BPBOM (%)	BPSOM (%)
Moisture composition	67.47 ± 0.44^{b}	65.95 ± 0.09^{a}	66.22 ± 0.32^{a}
Fat composition	4.50 ± 2.15^{a}	5.30 ± 1.60^{a}	4.29 ± 2.03^{a}

$^{a-b}$ Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
BPROM = beef patty raw oyster mushroom, BPBOM = beef patty blanched oyster mushroom, and BPSOM = beef patty steamed oyster mushroom

Table 4.1.2 shows the moisture and fat composition analysis of raw beef patty substitutes with 25% oyster mushroom (raw and pre-treated). There was no significant different of moisture composition for beef patties substitutes with pre-treated oyster mushroom. The mean percentage of moisture content of BPSOM is, 66.22% while BPROM (control) had moisture content of 67.47%. The lowest mean percentage of moisture content value was recorded by BPBOM, (65.95%). In other nutrient, the fat compositions of raw beef patty substitutes with 25% oyster mushroom are ranged between 4.29% and 5.30%. The lowest fat content of raw beef patty was 4.29% belong to BPSOM, whereas the highest fat content of raw beef patty was 5.30% (BPROM). However, there was no significance different between all of raw patties formulated with oyster mushroom which treated with different method.

Table 4.1.3. Moisture and fat analyses of cooked beef patty substitutes with 25% oyster mushroom.

	BPROM (control) (%)	BPBOM (%)	BPSOM (%)
Moisture composition	38.32 ± 0.69^{a}	39.22 ± 0.73^{a}	39.04 ± 0.30^{a}
Fat composition	6.02 ± 1.40^{a}	5.05 ± 2.88^{a}	4.02 ± 2.56^{a}

^{a} Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
BPROM = beef patty raw oyster mushroom, BPBOM = beef patty blanched oyster mushroom, and BPSOM = beef patty steamed oyster mushroom

Table 4.1.3 shows the moisture and fat composition analysis of cooked beef patty substitutes with 25% oyster mushroom (raw and pre-treated). There was no significant different in moisture content among cooked patty substitutes with oyster mushroom which treated with different techniques. The mean percentage of moisture content of BPBOM was the highest, 39.22%. The lowest mean percentage of moisture content value was BPROM, 38.32%. In addition, the range between fat composition analyses of raw beef patty substitutes with 25% oyster mushroom were between 4.02% and 6.02%. There was no significance different between all of cooked beef patty. The highest fat content of raw beef patty was 6.02% (BPROM), whereas the lowest fat content of raw beef patty was 4.02% (BPSOM).

4.2 Physical Traits

Physical traits that were determined which including cooking yield, diameter reduction, and moisture reduction in percentage value. Table 4.2.1 shown physical traits of beef patty substituted with pre-treated oyster mushroom.

Table 4.2.1 Physical traits of beef patty substitutes with 25% pre-treated oyster mushroom.

Physical traits (%)	BPROM (control) (%)	BPBOM (%)	BPSOM (%)
Cooking Yield	85.40 ± 1.00^{a}	84.94 ± 1.03^{a}	83.77 ± 0.40^{a}
Diameter Reduction	10.43 ± 3.48^{a}	9.88 ± 0.49^{a}	11.32 ± 0.45^{a}
Moisture Retention	32.73 ± 0.85^{a}	33.32 ± 0.76^{a}	32.71 ± 0.25^{a}

[a] Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
BPROM = beef patty raw oyster mushroom, BPBOM = beef patty blanched oyster mushroom, and BPSOM = beef patty steamed oyster mushroom

Table 4.2.1 shows the physical traits of beef patty substitutes with 25% pre-treated oyster mushroom. Among all beef patties, the beef patty which was replaced with 25% of raw oyster mushroom recorded the highest cooking yield (85.40%) compared to beef patty which was replaced with 25% of steamed oysters mushroom which had the lowest cooking yield (83.77%). The cooking yield attribute show there was no significance different between all beef patties.

Diameter reduction attribute of beef patty substitutes with 25% pre-treated oyster mushroom was ranged from 9.88% to 11.32%. The lowest mean percentage of diameter reduction was recorded by BPBOM (9.88%). Meanwhile, the highest mean percentage of diameter reduction was indicated from BPSOM (11.32%). However, there was no significance different of diameter reduction of all beef patties.

Furthermore, there was also no significance different of moisture retention between all beef patties that substitutes with raw and pre-treated oyster mushroom. As the beef patty substitutes with blanched oyster mushroom had highest value which was 33.32%, and the beef patty substitutes with steamed oyster mushroom had lowest value which was 32.71%.

4.3 Color Analysis

Color analysis is using five replicate measurements were taken for each sample, following the guidelines for color measurements from American Meat Science Association (Hunt and Kropf, 1987). Table 4.3.1, 4.3.2, and 4.3.3 is shown the color analysis of oyster mushroom, raw and cooked beef patties substitutes with oyster mushroom respectively.

Table 4.3.1 Color analysis of oyster mushroom

Color analysis	ROM (control) (%)	BOM (%)	SOM (%)
L*	55.30 ± 0.01^{a}	59.75 ± 0.00^{c}	58.52 ± 0.00^{b}
a*	2.21 ± 0.01^{c}	1.24 ± 0.01^{a}	1.26 ± 0.00^{b}
b*	15.17 ± 0.00^{c}	11.41 ± 0.01^{a}	14.20 ± 0.01^{b}
C	15.33 ± 0.00^{a}	11.47 ± 0.01^{b}	14.26 ± 0.01^{c}
H	81.73 ± 0.01^{a}	83.79 ± 0.05^{b}	84.93 ± 0.01^{c}

$^{a\text{-}c}$ Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
ROM = raw oyster mushroom, BOM = blanched oyster mushroom, and SOM = steamed oyster mushroom

Table 4.3.1 shows the color analysis of oyster mushroom (raw and pre-treated). The color properties of oyster mushroom (Lightness L*, redness a*, yellowness b*) treated with different treatment was shown. Blanched oyster mushroom were significantly lighter (higher L*) compared to raw oyster mushroom and steamed-oyster mushroom. Oyster mushroom that had lower L* value were ranged from 55.30 – 58.52. There was significance different among all oyster mushroom samples (treated with either steaming or blancing techniques).

Pre-treated oyster mushroom shows nearly same value that lower in color a* (redness), compared to the raw oyster mushroom. There was significance different between each of oyster mushroom. The raw oyster mushroom had the highest a* value at 2.21 compared to pre-treated oyster mushroom which had value in the range of 1.24 to 1.26.

The raw oyster mushroom was significantly higher in color b* (yellowness) compared to the pre-treated oyster mushroom. The raw oyster mushroom had the highest b* value at 15.17 compared to pre-treated oyster mushroom which had value in the range of 11.41 to 14.20. There was also significance different between each of oyster mushroom.

Table 4.3.2 Color analysis of raw beef patty substitutes with 25% oyster mushroom.

Color analysis	BPROM (control) (%)	BPBOM (%)	BPSOM (%)
L*	49.70 ± 0.22^a	50.06 ± 1.24^a	52.00 ± 1.15^a
a*	7.20 ± 0.69^a	8.06 ± 0.63^a	7.56 ± 0.12^a
b*	19.48 ± 0.22^a	20.03 ± 0.90^a	20.09 ± 0.72^a
C	20.77 ± 0.42^a	21.60 ± 0.71^a	21.46 ± 0.69^a
H	69.72 ± 1.63^a	68.06 ± 2.21^a	69.37 ± 0.67^a

[a] Mean values within the same row bearing different superscripts after differ significantly ($P < 0.05$)
BPROM = beef patty raw oyster mushroom, BPBOM = beef patty blanched oyster mushroom, and BPSOM = beef patty steamed oyster mushroom

Table 4.3.2 shows the color analysis of raw beef patty substitutes with 25% oyster mushroom (raw and pre-treated). Raw beef patty substitutes with steamed oyster mushroom were significantly lighter (higher L*) compared to raw BPROM and raw BPBOM. Raw beef patty had lower L* values are ranging from 49.70 – 50.06. There was no significance