PHYSICOCHEMICAL PROPERTIES OF SOY- AND PEA-BASED IMITATION SAUSAGE PATTIES

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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IMITATION SAUSAGE PATTIES

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a candidate for the degree of Master Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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PHYSICOCHEMICAL PROPERTIES OF SOY- AND PEA-BASED IMITATION SAUSAGE PATTIES

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ABSTRACT

The objective of this thesis is to better understand how changing levels of soy or pea protein isolates (SPI)(PPI) (3%, 6% and 9%) and king oyster mushrooms (KOM) (0%, 3.5% and 7%) affect the physicochemical properties of imitation sausage patties using textured soy or pea proteins as the main base ingredient. After all materials were blended evenly and formed into circular patties, the raw patties were then fried on the pan with corn oil for 5 minutes on each side. Samples from each blend were done in triplicate and conducted the measurements on the same day. Cooking and textural properties, Hunter color, pH value, water activity and water holding capacity were considered to evaluate the contributions of the main ingredients.

Altering the SPI or PPI level did not decrease the cooking yield; however, KOM did lower the yield. Lightness and yellowness of the meatless sausage were attributed to the addition of SPI or PPI but these properties were not affected by KOM. Combining KOM and a high level of soy or peat protein created redness and showed the greatest value. Texture profile parameters, excluding adhesiveness, showed higher value when both 3.5% and 7% KOM were combined with 9% SPI; when 3% PPI mixture without KOM. Water activity, pH value and cooking shrinkage did not differ significantly on dependent interactions.

SPI or PPI did not affect shrinkage percentage. Water holding capacity decreased as the amount of KOM and SPI or PPI increased.

CHAPTER 1

INTRODUCTION

1.1 Background

In 1999, the United States Food Drug Administration (FDA) recognized the benefits of using soy in food products allowing manufacturers to attach a label saying: “Consuming 25 grams of soy protein a day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease (NSRL 2014). With this statement from FDA, manufacturers increased attempts to develop soy-based protein or to include soy protein products as ingredients of products (Hollingsworth 2002). Following the FDA approval of soy foods, as a health benefit, the Soyfoods Association of North America (SANA) reported that the sales and consumption of soybean and its products had increased over a 15 year period to 2011 (SANA 2011). A survey (Soyatech 2012) covering 2010 to 2011, reported an increase of 1.1% in total sales of soy products reaching $5.17 billion in sales, while the sale of meat alternatives increased 2.0% in 2011 after a 4.2% increase in 2010. Moreover, SANA also reported that 42% of Americans consumed soyfood or soy beverages once a month or more in 2013, compared to 30% in 2006 (SANA 2013). According to Northern Pulse Growers Association, 2012 US Pulse Quality Survey, production of yellow pea were increased two-fold compared to 2011 (USA Dry Pea Lentil Council 2012). On the other hand, during the 2011 to 2012, on study of Economic Research Service (ERS), in line with higher production, the per capita use of all mushrooms rose 4.4% to 3.99 pounds (USDA 2012).

The National Soybean Research Laboratory (NSRL) at the University of Illinois refers to soybean plants as Edamame stating that they are among the few plant foods that provide a complete protein; hence, that they have all the essential amino acids human beings require. With no cholesterol and very little saturated fat, Edamame are low in sodium with RDA percentages that are generally high in vitamin C, K, manganese and folate. They are also a good source of dietary fiber, iron, calcium, thiamin, magnesium, phosphorus and copper (NSRL 2014).

Peas, usually yellow peas, also known as split peas, are legumes and often referred to as "pulses", are rich in protein (20-30%) and an excellent source of dietary fiber, low molecular weight carbohydrates, essential amino acids, polyunsaturated fatty acids, and a range of micronutrients (US Pulse Quality Survey 2012). They are also low in fat. Yellow peas have a milder flavor than green peas and are an excellent source of Vitamin B and several minerals essential for human health.

As for king oyster mushrooms' properties, the characteristics of insoluble dietary fiber gives the chewability and firmness of mouth-feel (Kasabian and Kasabian 2005) due to the fiber arrangement (Ogawa and others 2012) and mechanical strength (Ogawa and others 2012). There are also meaty, umami flavor (Mau and others 1998; Zhang and others 2013) after cooked due to their MSG-like amino acids such as aspartic acid, glutamic acid ,5'-guanosine monophosphate (5'-GMP), 5'-inosine monophosphate (5'-IMP), and 5'-xanthosine monophosphate (5'-XMP) (Sommer 2008a) . Considerable evidence had confirmed and reported the health benefits of consuming mushrooms. Like many other mushrooms, king oyster mushrooms also might help to reduce the risk of cancer, heart disease, blood cholesterol problems, control hyperglycemia and boost our immune system (Rop and others 2009; Alam and others 2011; Kang and others 2012; Zeng and others 2012; Chen and others 2013; Liang and others 2013; Yang and others 2013; Lin and others 2014).

1.2 Motivations and objectives

1.2.1 Motivations

When this research began its work on developing plant-protein-based imitation sausage patties, it was determined that the current sausage analogs lacked the consistency of mouth-feel products. Both the soy bean and yellow spilt pea are high in protein and low in fat with no lactose, gluten or GM ingredients. These are the main advantages of their use as a material in meatless patties, and they can both deter risks of heart-related disease when the recommended daily intake (RDI) is followed by consumers. Mushrooms are considered to be one ingredient that enhances mouth-feel (Kasabian and Kasabian 2005) of plant-protein-based imitation sausage patties based on its nutritional benefits and meat-like chewing properties. King oyster mushrooms provide high protein and fiber content, low fat, they also provide umami flavor (Mau and others 1998; Kim and others 2011; Zhang and others 2013). Umami is a Japanese word, which describes a meaty or savory taste. Dr. Kikunae Ikeda discovered umami in 1908 calling it the fifth taste joining the four traditional flavors of sour, sweet, salty and bitter.

Combining edible mushrooms with plant derived protein of the products may begin a new the trend in the United States.

1.2.2 Objectives

This literature survey found most of the vegetable proteins, either soy or pea, were used as fillers or extenders to enhance the texture, stability of emulsion, replace the fat and to lower the cost. So far, there were abundant reviews show the development of the best acceptable ratio of meat to replacers or texture-improving agents in reduced/ low fat meat products but very little research has been undertaken on the use of textured soy proteins (TSP) and textured pea proteins (TPP) as main ingredient in processing imitation sausage patties. More research could be conducted on their physical properties and more non-company related tests should be made by researchers free from any conflict of interest.

The objectives of this research were to gain an understanding of the physicochemical properties of adding rehydrated king oyster mushroom and soy or pea protein isolate as the variances in soy- or pea-based imitation sausage patties. For this purpose, tests were conducted adding dried king oyster mushroom and protein isolate to the soy mixture and then measuring cooking yield, cooking loss, color, water activity (Aw), pH value, texture profile analysis (TPA), water holding capacity (WHC) of textured soy protein (TSP) and textured pea protein (TPP), of the final product and cooking shrinkage.

CHAPTER 2

LITERATURE REVIEW

2.1 Driving Forces Toward Meat Analog

Meat has enjoyed its popularity as a foodstuff for a long time; however, a number of adverse impacts on human health, environmental quality and animal welfare have growing concerns to consumers (New Harvest 2013). Moreover, as the global populations continue to increase, food supply would gaining more attention around the world. Plant proteins offer hope for countries where food is in short supply if cost can be controlled and production technology transferred to those who are willing to promote meatless products as a way to feed and provide jobs for the impoverished masses.

Health concerns over the connection of meat over-consumption to cardiovascular diseases (Sacks and others 2006), blood cholesterol levels, obesity and other diseases (FDA 1993) have prompted consumers to be more aware of the risks linked to high-fat and high-caloric diet. These heart diseases are now responsible for a third of global mortality (WHO 2001). Over-consumption of meat may be responsible for a quarter of all ischemic heart disease, or 1.8 million deaths, annually (Key and others 1999; WHO 2001). According to Barnard and others (1995), the annual medical costs related to over-consumption of meat are believed to be between 30 and 60 billion dollars. In light of these implications, disadvantages of eating excessive meat and meat products, the World Health Organization (WHO) has drawn up the following nutritional recommendations: fat should provide between 15 and 30% of the calories in the diet; saturated fat should provide not more than 10% of these calories, and cholesterol intake should be limited to 300 mg/day (WHO 2003). The American Heart Association's (AHA) Dietary Guidelines for Americans also emphasize the importance of consuming a variety of fiber sources to obtain the different types of fibers found in foods rather than supplements (Krauss and others 2000).

So far, meatless products (also known as meat analogs) not only satisfy nutrition concerns but also boost our sense of well-being as we see its development work towards the reduction of animal slaughter and carbon dioxide emission. According to the Livestock, Environment and Development Initiative, livestock production accounts for 18 percent of global greenhouse gas emissions, including 9 percent of carbon dioxide and 37 percent of methane gas emissions worldwide (Brooks 2006; FAO 2006). Moreover, the Nutrition and Forestry reported (1997) that 1.4 billion tons of farm animal wastes are produced in the United States annually (United States Senate Committee on Agriculture 1997). de Haan and others (1997) also reported that together with animal feed production, meat production is responsible for the emissions of nitrogen and phosphorus, pesticide contamination of water, heavy metal contamination of soil, and acid rain from ammonia emissions.

Pimentel and Pimentel (1996) mentioned that given the inputs required to house, transport, and slaughter animals; transport and process feed grains; and transport and process meat, intensive meat production is only 25% as energy efficient as soybean production. Plant protein ingredients usually cost less than the muscle proteins due their accessibility and energy efficiency.

Foodborne pathogens and diseases are usually found in meats and cause the illness, such as *Salmonella*, *Campylobacter*, pathogenic *E. coli*, avian influenza, and Bovine spongiform encephalopathy (BSE). Mead and others (1999) concluded that foodborne diseases -the most common causes of which are contaminated meats - are responsible for over 76 million episodes of illness, 325,000 hospitalizations, and 5,000 deaths each year. Animal diseases, like Mad Cow Disease is a persistent threat despite efforts to prevent such outbreaks from happening. Animal diseases and fear of genetically modified animals have become driving forces behind an increased demand for vegetable proteins to replace animal proteins in the world's food supply.

FAO (2002) reported 75% of existing fishing stocks are either fully- or over-exploited. National Agricultural Statistics Service also reported (2003) that 9 billion farm animals are killed each year in the United States to produce meat and 60 billion are killed worldwide, not including unaccounted marine life (USDA 2003).

Islam population has been increasing rapidly around the world. Halal food regulations allow the consumption of some meat, if it is properly slaughtered, but it does not allow consumption of pork, which opens up a potential market for meatless products including imitation sausage patties.

In summary, the major forces driving consumers to buy meatless products and the reasons why soy or pea protein ingredients have received such great acceptance are due to the following concerns: 1) global demands for foods, 2) nutrition-related diseases, 3) environmental pollutions, 4) efficient and cheaper protein sources, 5) foodborne pathogens, 6) animal welfare and 7) religious diet restrictions. That awareness created a demand for low-fat and low-caloric food without changing taste and eating habits significantly. To balance the health concerns of those consumers who want to cut or reduce their meat intake, high protein, high fiber and low fat foods are believed to become a potential market in United States and even around the world. Furthermore, Oberbeil (1999) mentioned that plant proteins are easily converted to amino acids and are up to 60 percent more bioactive than the protein in pork, beef or ground meat due to the acids in vegetable products stimulate the secretion of stomach acid to break down proteins to amino acids (Gallagher and Schugt 2012).

2.2 Meat Analog Ingredients

Typical meat analog ingredients and fillers or extenders in food industries are summarized in the following table (Table 2.2.1).

Table 2.2.1 Typical meat analog ingredients.

Ingredients	References
Textured vegetable proteins	Deliza and others 2002; Gujral and others 2002; Kassama and others 2003; Rentfrow and others 2004; Kayayama and Wilson 2008; Qammar and others 2010
Non-textured vegetable proteins	Yang and others 1995; Cofrades and others 2000; Taylor and Walsh 2002; Parmer and others 2004; Lee and Chin 2009; Rehrah and others 2009; Walsh and Carpenter 2009
Surimi	Cavestany and others 1994; Fátima Henriques Lourenço and others 2012
Plant starches/ flours	Reitmeier and Prussa 1991; Mohammadi and Oghabi 2012
Fibers	Cengiz and others 2005; Campagnol and others 2012
Water	Claus and Hunt 1991; Egbert and Borders 2006
Carbohydrates or hydrocolloids	Mittal and Barbut 1993; Chin and others 2000; Pietrasik and Duda 2000; Lin and Huang 2003; Kumar and Sharma 2004; Cierach and others 2009; Jiménez-Colmenero and others 2012 ; Jiménez-Colmenero and others 2013
Fats/oil	Gujral and others 2002
Binding agents,	Carballo and others 1996; Gujral and others 2002; Modi and others 2003; Ahmed and others 2007
Flavoring agents and coloring agents.	Deliza and others 2002

2.2.1 Textured Vegetable Proteins

Textured vegetable proteins were made from defatted soy or pea flour, which is extracted from soybean or split pea or other plant proteins. Little to no fat, high protein and dietary fiber are the health benefits of meatless products. Textured vegetable proteins are an economical and high protein quality (Hamdy 1974; Egbert and Borders 2006) source which has been applied in school lunch programs and in prison lunch programs to balance the demand of nutrition.

The United States Department of Agriculture (USDA) has defined textured vegetable protein products for use in school lunch program as “ food products made from edible protein sources and characterized by having a structural integrity and identifiable structure which allows each unit to withstand hydration and cooking, as well as other procedures used in preparing the food for consumption" (USDA 1971).

2.2.1.1 TVP Manufacturing and Use Instruction

Textured vegetable proteins, derived from soybean or pea, are usually made by cleaning, heating, cracking the seed, dehulling and removing the oil with hexane. This is the process which produce the white flakes needed to go on to the next step of processing. The processing steps, needed to obtain textured soy protein and textured pea protein also include extraction by alkali and acid, extrusion and drying. Extruded products can be made into any shape depending on the type of die used. These shapes include irregular granules, flakes, cubes chunks, nuggets, grains, fibers and strips. Colored and nutrition fortified textured soy or pea proteins are also widely used in food industry. Finished textured vegetable proteins present no allergic components such as peanuts, tree nut, milk and gluten. Throughout the various steps of processing including rehydration (Guy 2001), cooking and other processes, a meatless product could retain its structural integrity, texture and mouth-feel properties (Egbert and Borders 2006).

2.2.1.2 Limitations of TVP

. Up to 30% of soy materials were incorporated into the U.S. school lunch program by the United States Department of Agriculture (USDA) (Riaz 2001; USDA 1971; Edmondson and Graham 1975). USDA notice 219 permitted the use of maximum of 30% of dehydrated TVP in meat dishes served to approximately 25 million children (Edmondson and Graham 1975). In food industries, the incorporation of textured soy proteins or textured pea proteins into meat products usually as an extender (Wilding 1974; Ali and others1982; Orcutt and others 2006) and thus reduce costs (Singh and others 2008). When textured vegetable proteins are used as a binder, they improve cooking yield, and when used as an emulsifier they enhance stability of the products. The overall acceptance of low-fat products has ranged from 10% to 30% (Deliza and others 2002; Gujral and others 2002; Rentfrow and others 2004; Kilic and others 2010).

2.2.1.3 Applications of TVP

Soy products have better yields, easier handling, better yields, and lower transportation, and preservation costs (Singh and others 2008) as compared to meat (Kadane 1979). A textured vegetable protein diet (Riaz 2004) is economically feasible and the high protein ingredients provide a variety of choices.

Combined theTVP into meat patties (Deliza and others 2002; Kassama and others 2003) or other meat products (Gujral and others 2002; Rentfrow and others 2004; Kilic and others 2010) were the most common approaches in food industries. Kayayama and Wilson (2008) use textured soy protein (TSP) and vegetable-based flavors to produce consumer acceptable “chicken” or “shrimp” flavored TSP using heat application processes. Qammar and others (2010) use chicken flavored TSP substitution for chicken meat in pizza toppings.

2.2.2 Non-textured Vegetable Proteins

Vegetable proteins usually refer to soybeans, but can also apply to cotton seeds, peas, wheat or oats, all of which are utilized in processing textured products (Strahm 2006) or are processed directly to form various products. Plant protein isolates (Egbert and Payne 2009) can be used as a textured modifier via protein-polysaccharide interaction and form complexes where electrostatic complexation of oppositely charged protein and polysaccharide occurs (Akseowan 2002; Shahiri Tabarestani and Mazaheri Tehrani 2012).

Beverages, meat analogs, energy bars, snacks, cereals and other low-fat products are common applications of non-textured plant proteins in United States (SANA 2013).

2.2.2.1.1 Soybean

Soybean and its products have been consumed for many years in Eastern countries (Barrett 2006) like Japan, Taiwan, Korea, China and Southern Asian countries as traditional foods; these foods include soybean milk (Fukushima 1994), tofu (Fukushima 1981), and fermented produce (Fukushima 1981), like tempeh, miso and soy sauce. In contrast to the eating history of Eastern countries, Western countries have utilized soybeans within their diet dramatically, especially after the approval of food-labeling health claim for soy proteins in the prevention of coronary heart disease by the US FDA in 1999.

As more nutritional characteristics about soybean understood, the soybean contains almost little or no in cholesterol, fat or lactose, which gives consumers a healthy diet and serves as alternatives to animal proteins. According to the US Soybean Export Council (USSEC), Quality Report for 2013 (Naeve and others 2013): "US soybean meal had lower protein content than Brazilian soybean meal, but better quality of protein – higher concentrations of essential amino acids (Park and Hurburgh 2002; Thakur and Hurburgh 2007; Bootwalla 2009). Although soybeans from the US are generally lower in crude protein, both US soybeans and soybean meal contained higher concentrations of essential amino acids (Thakur and Hurburgh 2007), thus making their protein fraction of higher quality".

2.2.2.1.2 Health Benefits of Soybean

Unlike other legume proteins, soy proteins' health benefits reach far beyond just providing amino acids (NSRL 2014). The US Food and Drug Administration (FDA) has claimed in 1999 that the consumption of 25g of soy protein a day, as part of a diet low in saturated fat and cholesterol can reduce the risk of heart disease (FDA 2013). Friedman and Brandon (2001) and Zhan and Ho (2005) have also mentioned the health benefits and the importance of consuming plant proteins, focusing on their effect on soy-induced lowering of serum total cholesterol, LDL cholesterol, against obesity, diabetes and others. Another benefit includes the reduced recurrence and death rate of breast cancer (Shu and others 2009; Guha and others 2009; He and Chen 2013).

Soy protein have good functional characteristics in food products providing the good digestibility (Kiers and others 2000; Hong and others 2004; Wang and others 2009; Teng and others 2012), after either by chemical or enzymatic processing of raw soy. Soy proteins have a protein quality (DuPont Nutrition & Health 2011) comparable to eggs. According to this study, soy protein has a protein digestibility-corrected amino acid score (PDCAAS) of 1.00, meaning it is a high-quality protein that meets the needs of both children and adults. Eggs, dairy and meat proteins also have a PDCAAS score of 1.0. However, soy protein is the only widely available high-quality plant-based protein that achieves this score (DuPont Nutrition & Health 2011).

2.2.2.1.3 Anti-nutrient and Sensory Issues of Soybean

The anti-nutritional factors, mainly trypsin and chymotrypsin (Jiao and others 1992) and lectins were reduced after processing although not entirely (Foley and others 2013) which in turn of aids digestibility (Kiers and others 2000; Hong and others 2004; Wang and others 2009). Potential disadvantages of soybean products such as beany flavor, lacks of the consistency in mouth-feel, and high purine content (Havlik and others 2010) became the issues to be addressed. Off-flavor could be controlled by blanching or lipoxygenase-2 to reduce its beany flavor (Matoba and others 1985) or by lactic acid fermentation (Schindler and others 2012) or by genetic modification (Fukushima 2004). Grinding soybeans at high temperature is effective to control lipoxygenase activities and results in a reduction of off-flavor generation and maximizes protein extraction (Wilkens and others 1967).

2.2.2.1.4 Applications of Soy Protein Derivatives

The literature search begins in 1970 with a review of various soy protein products, their preparation and use by the meat industry is reported by Rakosky, who defined soy protein isolates (Rakosky 1970) as edible isolated soy protein (ISP) produced by extracting a white flake with water or mild alkali (Meyer 1966). Soy protein isolates (Rakosky 1970; Bookwalter 1978; Welsh 1979; Campbell 1981; Ledward and Lawrie 1984; Ahn and others 1999; Liu 2000; Egbert and Borders 2006; Walsh and Carpenter 2009; Ahmad and others 2010) and soy protein concentrates' (Rakosky 1970; Bookwalter 1978; Welsh 1979; Campbell 1981; Liu 2000; Tömösközi and others 2001; Egbert and Borders 2006) properties were widely discussed and investigated in reduced- fat meat and meat analog products.

Soy protein being an economical source of food proteins has been used extensively in meat products as a binder for improving yield, as a gelling agent to enhance emulsion stability and as a meat replacement to reduce costs (Das and others 2008). Ahn and others (1999) also showed soy proteins, especially soy protein isolates (SPI) additives incorporated into comminuted meat products for their water and fat binding ability, enhancement of emulsion stability, and increased yield. Addition of soy protein isolates can change the texture (Akesowan 2002), juiciness and color of buffalo meat sausage emulsion, which was confirmed by Ahmad and others (2010). Exemptions of the incorporation or application of soy derivatives had also been shown through many studies (Quass 1979; Kotula and Berry 1986; Chin and others 2000; Pietrasik and others 2000; Cengiz and others 2005; Das and other 2008; Akesowan 2010; Nantapatavee and others 2011).

2.2.2.2.1 Pea

According to Northern Pulse Growers Association, 2012 US Pulse Quality Survey, yellow pea acreage and production was 344,596 acres and 294,802 metric tons, respectively, in 2012 compared to total acreage of 190,650 acres and production 142,276 metric tons in 2011 (USA Dry Pea Lentil Council 2012).

Peas (*Pisum sativum*) are good sources of protein and are increasingly recommended as part of the diet in order to promote general well-being and reduce the risks of diseases. Additionally they are low in sodium while offering an excellent source of soluble and insoluble fiber, complex carbohydrates, B vitamins and minerals, such as calcium, iron and potassium (Nutralys® 2008). Pea proteins, with its light, fluffy texture and slightly sweet taste, are highly digestible (Rubio and others 2013), has a low-potential for allergic response (Nowak-Wegrzyn and others 2003; Natural Food Benefits), low level of anti-nutritional factors and phytosteroids (Nutralys® 2008) and also low cost (Plummer 2012). They are also low glycemic index (GI) food and have been shown to improve glucose and lipid levels in people with diabetes (USA Dry Pea Lentil Council 2012).

2.2.2.2.2 Health Benefits of Split Pea

Yellow split peas are highly digestible (Rubio and others 2013) and contain low phytosteroids or isoflavones; which reduce possible estrogenic effects (Nutralys® 2008). Later, Agboola and others (2010) reported some moieties of polyphenols obtained from the extraction responsible to the antioxidant and antihypertensive actives. More currently, some studies also demonstrated that pea protein derivatives are capable of stabilizing blood sugar levels (Li and others 2011), improving the arterial function and serum cholesterol levels (Zahradka and others 2013) to maintain heart health in animal and human.

2.2.2.2.3 Applications of Pea Protein Derivatives

Pea protein isolates (Tömösközi and others 2001; Fukushima 2004; Watson 2009; Aluko and others 2009; Osen and others 2012) and pea protein concentrates' (Watson 2009) properties were widely researched and applied to manufacture reduced- fat meat and meat analog products. Tabarestani and Tehrani (2012) utilized split pea flour as part of fat replacer system in low-fat burger. The application of pea protein derivatives in meat analog still limited compared to soy protein derivatives.

2.2.3 Starch

Starches (Skrede 1989; Brewer 2012), in either their native form or in a modified form, often serve as filler, fat-replacers and texture-enhancing agents in formulation. Tabarestani and others (2012) showed that soy flour in combination with starch leads to an increase in cooking yield and addition of split-pea flour in mixed formula decreases shrinkage while improving texture properties. Shewry and Tatham (2000) also claimed that starches and flours have been studied extensively as potential fat replacers because of their ability to bind and retain moisture.

2.2.4 Tofu Powder, Oatmeal and Fibers

Tofu powder, oatmeal (Kerr and others 2004; Yang and others 2007; Yang and others 2010) and fibers (Cengiz and others 2005; Campagnol and others 2012) have been added in low-fat meat products. Yang and others' results (2006) showed that dried tofu powder reduce the fat content which increases protein contents but weaken the internal texture of meat products.

Oats, a great source of soluble fiber, have been applied to several food products based on their excellent functional and physiology effect. The main purpose of adding hydrated oatmeal was to improve water holding capacity, cooking loss, texture, moisture adsorption capacity, and sensory evaluation of the final products. Kerr and others (2005) showed that up to 15% hydrated oat in low-fat sausage can be made to improved flavor and texture as compared to control without oat. Yang and others (2007) showed that the decrease in hardness of oatmeal-added sausage products may be due to the higher water-retention properties of oatmeal in response to heat treatment. They also demonstrated that an acceptable low-fat pork sausage can be made where pork is replaced with up to 25% hydrated oatmeal (Yang and others 2007) as textured-modifying agents in low fat pork sausages. Later, Yang and others (2010) showed the addition of 10% hydrated oatmeal as a fat substitute was more effective for decreasing hardness and increasing cooking yield of beef meat than pork and chicken. Altering textural properties by decreasing the hardness of a product was also shown by tests that focused on the addition of oatmeal (Pszczola 1991; Dawkins and others 2001).

Fibers have proven capable of holding the water content while cooking because of their ability to retain moisture and prevent meat from drying when cooked. Campagnol and others (2012) reported that fermented sausages with healthier characteristics can be produced without quality loss by reducing fat from 15 to 10% and by adding 1% soy fiber. Cengiz and Gokoglu (2005) reported the frankfurter-type sausages did not have significant difference between sausages treated with citrus fiber and those with soy protein concentrate.

2.2.5 Carrageenan

Carrageenan, are linear sulfated high molecular weight polysaccharides, extracted from certain type of red algae, could enhance water-holding capacity, cooking loss, juiciness and slicing properties and also acts as a gelling and thickening agent of various food products (Imeson 2000). It is classified as GRAS by the United States Food and Drug Administration (FDA 1973) .In the food industry, many other hydrocolloids are also applied, such as guar gum, cellulose gum, gum Arabic, xanthan gum and konjac (Chin and others 2000; Lin and Huang 2003) to enhance the cooking properties of products or as fat replacements. Hydrocolloids also improve the hardness, cohesiveness, chewiness, gumminess, sliceability, water holding capacity, binding ability, emulsification stability, and juiciness of meat products. They also lower the weight loss, cooking loss and shrinkage of low-fat products (Egbert and others 1991; Barbut and Mittal 1992; Bater and others 1992; Brewer and others 1992; Trius and Sebranek 1996; Xiong and others 1999; Hsu and Chung 2001; Candogan and Kolsarici 2003; Kumar and Sharma 2004; Ayadi and others 2009; Cierach and others 2009; Modi and others 2009). Concluded from those studies, around 0.5% carrageenan were considered as optimum level due to the best cooking properties, overall acceptable and textural changes; however, study from Hsu and Chung (2001) claimed less than 2% addition had significantly affected the cooking and textural properties to low-fat emulsified meatballs.

2.2.6 Binding Agents

Widely used binders in meat-based or meatless products include legume flour (Modi and others 2003), sodium caseinate, whey protein concentrate, liquid whole egg (Gujral and others 2002), egg white powder (Ahmed and others 2007) and egg white (Carballo and others 1996). Processing technology also enhances the binding ability of ingredients; e.g. high pressure restructuring meat is more effective than binders (Hong and others 2006).

2.3 Other Classified Meat Analogs

2.3.1 Mycoproteins

In the United Kingdom (UK), mycoprotein, a high-quality imitation protein is the main ingredient in Quorn food products, and its popularity lies in the fact that it is free from cholesterol, low in fat and saturates and contains no trans-fats. It also contains all nine essential amino acids making it a first class protein (Marlow Foods Ltd. 2008). “Quorn” was developed by Marlow Foods in Stokesley, North Yorkshire, UK. It has a texture which resembles the texture of meat and is recognized by the Food and Drug Administration as Generally Recognized as Safe (GRAS) (FDA 2001). Danny and others (2009) showed that mycoprotein is a high protein ingredient with high fiber and low-fat food materials fermented from the filamentous fungus *Fusarium venenatum*.

Asgar and others (2010) reported that 120 to 140 grams mycoprotein can reduce cholesterol by about 10%. Amounts required to acquire the promising health benefits (Asgar and others 2010; Marlow food 2008) have not been recommended. However, Turnball and Ward (1995) issued a test meal of 20 g mycoprotein per day along with a control meal to 19 healthy adults to investigate the glycemic response. The meals were given in random order with a 7-day washout period between the two meals (Marlow Foods 2009). Turnball and Ward (1995) observed that the serum glucose response was lower throughout the entire 120 min post-prandial period following the mycoprotein meal compared to the control. The insulin response was also lower. Furthermore, the only nutritional difference between the test meals was that the mycoprotein meal contained 11.2 g more dietary fiber; hence, the viscous polysaccharides were given credit for reducing postprandial glycemia and insulinemia. Turnbill and others also attributed lower cholesterol to mycoprotein-based Quorn (Turnbill and others 1992). Despite Marlow Food's extensive coverage of their products' benefits, origin and process, many people still not familiar with mycoprotein as an alternative protein source to which can serve as a substitute for meat when combined with other ingredients (Rodger 2001). Disadvantages, such as a long culture time, and high costs for media component (Danny and others 2008) have led some consumers to find other vegetarian products although Quorn is popular in Europe, Canada and the U.S.

2.3.2 Seitan

Seitan, also called gluten or wheat meats, is derived from the protein portion of wheat. When simmered in a traditional broth of soy sauce or tamari, ginger, garlic, and kombu (seaweed), it is the so called seitan (Nussinow 1996). Though it considered as one of the good vegetarian foods source, it is an allergic food for some wheat allergy consumers (FARE 2013).

2.4 Mushrooms

2.4.1 Edible Mushrooms and King Oyster Mushrooms

Edible mushrooms include many species, such as shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus ostreatus*), black polar mushroom (*Agrocybe aegrita Singer*), beech mushroom (*Lyophyllum ulmarium*), button mushroom (*Agricus bisporus*), king oyster mushroom (*Pleurotus eryngii*), enokitake (*Flammulina velutipes*), black mushroom (*Auricularia auricular judae*).

King oyster mushroom (*Pleruotus eryngii*), also known as king trumpet mushroom, French horn mushroom, king brown mushroom, boletus of the steppes and trumpet royale, is an edible mushroom native to the Mediterranean regions of Europe, Asia, the Middle East and North Africa. It has a trumpet-like tan cap and a thick, meaty white stem and as the largest species, a king oyster mushroom can have up to an 8-inch stem.

2.4.2 Consumption and Sale Trend of Mushrooms

Mushrooms has been consumed in Asian countries and Mediterranean regions of Europe for many decades for their nutritional values, nutraceutical values and for medical purposes (Chang and Miles 2004; Dikeman and others 2005). Production of mushrooms gradually increases over time, with China being the largest producer (Aida and others 2009) and consumer (USITC 2010) of mushrooms with a market share of 47 % (Harsh and Joshi 2008). According to Economic Research Service (ERS), from 1960s to 2000s, the per capita use of mushrooms has waned due to consumers' economic, social and demographic characteristics (Lucler and others 2003). From 2011 to 2012, the ERS reported in line with higher production, that the per capita use of all mushrooms rose 4.4% to 3.99 pounds (USDA 2012).

2.4.3 Nutrition of Mushrooms

Mushrooms are rich in protein with little or no fat. They are rich in essential amino acids (EAA) and fibers (Reis and others 2012). Well known for their excellent nutritional content, mushrooms provide vitamins, B1, B2, B12, C, D and E and selenium, potassium (Duyff 2006; Mushroom Council, CA) , which classifies them as nutraceuticals. The health properties of mushroom are especially helpful to diabetics (Deshmukh 2013), but they also provide benefits for all consumers with low calories, high protein, and little or no fat (Ranogajec and others 2010). They are also cholesterol-free, and they have been reported to lower the risk of gout (Lyu 2003; Choi 2004; Choi 2010; Chuang 2011). They are also very low in sodium (Tong and others 2008; CSIRO 2012).

2.4.4 Health Benefits of Mushrooms

For the medical properties, according to the International Society for Mushroom Science (ISMS) and Agriculture Marketing Resource Center (AgMRC), benefits of mushrooms include antitumor, anti-cancer attributes. The ISMS and AgMRC also report that they boost the immune system and help in digestion. They also reduce hypertension and other therapeutic properties, which are well-known benefits of consuming mushrooms (Harsh and Joshi 2008). These potential benefits are attributed to the content of β-glucans, ergothioneine and lovastatin in the king trumpet mushroom (Dikeman and others 2005). The health benefits of mushrooms can be documented by several studies (Mallavadhani and others 2006; Rop and others 2009; Alam and others 2011; Zeng and others 2012; Kang and others 2012; Chen and others 2013; Liang and others 2013; Yang and others 2013) and had reported the components and the validity of health benefits mentioned above.

2.4.5 Applications on Meat Products

Wan Rosli and others (2011) utilized oyster mushrooms in chicken patties for its proximate composition and sensory evaluation. In the work of Cha and others (2012), white jelly mushroom (*Tremella fuciformis*) served as meat substitute to reduce greasy and oily issues in pork patty formulation.

2.5 Physicochemical Properties Measurement

2.5.1 Textural Properties

Texture is considered one of the vital properties by which the food industry and customers render both quality and acceptability of food products. The term "texture" includes a variety of kinesthetic sensory characteristics: those perceived prior to mastication (particle size, oiliness), those perceived during mastication (tenderness, juiciness) and those perceived after mastication (fibrous, residue, mouth coating) (Brewer 2012). Food texture could be attributed to and associated with the entire food processing chain, so called "From Farm to Table", which includes all the steps leading to the harvest procedures and ending with the processing operation. Food internal properties, such as water, fat and protein are content, also associated with the texture (Drewnowski 1987; Shieh and others 2004) of foods.

Instrumental tests have been used in research and the food industry to assess food texture of the products, and different probes are used to sense the sample depending on the form, shape, and its own properties. Texture profile analysis (Bourne 1978; Chen and Opara 2013) is the most common method to qualify the properties, which hardness, cohesiveness, adhesiveness, springiness, fracturability, chewiness, gumminess, and resilience, of meat analogs or low- or reduced-fat meat products.

CHAPTER 3
MATERIALS AND METHODS

3.1 Ingredients

Textured soy protein (unflavored, caramel colored and minced 180, Code: 165-218, ADM, Decatur, IL, USA) and textured pea protein (unflavored, uncolored, irregular granules, University of Missouri, Columbia, MO, USA) were used to provide the water binding ability, the texture or mouth-feel, the protein source and the insoluble fiber of the product (Egbert and Borders 2006). Other ingredients such as corn oil, salt, fresh egg white, mushroom soy sauce, sesame oil, sugar, and onion powder were purchased from local grocery stores (HyVee). Garlic powder (Dehydrated garlic powder, Silva, Momence, IL, USA) was added to enhance the flavor of imitation patties. Potato starch (Bob's Red Mill Natural Foods, Milwaukie, OR, USA) served as fillers of the imitation sausage patties. Kappa -carrageenan (Grindsted ® Meatline 1725, Danisco, DuPont Nutrition and Health, St. Louis, MO, USA) was added to bind water, reduce cooking loss of imitation patties and also served as an emulsifier (Egbert and Borders 2006). Dried king oyster mushroom (Ivory Portabella, Woodland Foods, Waukegan, IL, USA) were used to provide chewability and fiber source to imitation patties. Soy protein isolates (Solae, St. Louis, MO, USA) and pea protein isolates (Roquette, New Bern, NC, USA) were employed as a part of binders, emulsifier and nutritional enhancer (Egbert and Borders 2006).

3.2 Experimental Design

A full experimental design containing two independent variables, each had three different levels, was used. The two independent variables were soy or pea protein isolates (3, 6 and 9%) and dried king oyster mushrooms (0, 3.5 and 7%). The level of textured soy or pea proteins were fixed at 15% based on preliminary tests. Other ingredients were corn oil, fresh egg white, carrageenan, potato starch, salt, sesame oil, soy sauce, sugar, onion powder and garlic powder, were kept constant at 4, 7, 0.5, 0.5, 0.3, 0.3, 0.3, 0.2 and 0.2%, respectively (Appendix 9). Table 3.1 shows the total amount of water needed to be added. Total amount of water minus the hot water used to rehydrate textured vegetable protein are the amount of water added into mixture. One hundred percent minus all the ingredients mentioned above is the amount of potato starch. With one exception, the 7% king oyster mushrooms mixed with 9% soy protein isolates, which the amount of egg white is 2.7% instead of 7%. The levels for each independent variable and textured soy or pea proteins were determined based on the results from the preliminary tests.

3.3 Processing of soy- and pea-based Imitation Sausage Patties

The percentages of various ingredients were based on the percentage of the final weight of imitation sausage patties, approximately 45 gram of each. Dried king oyster mushrooms were 0, 3.5 and 7%; soy and pea protein isolates were 3, 6 and 9%. Dried king oyster mushrooms were washed without soaking in excess water and then cut into small pieces (approximately 0.5 cm long x 0.3 cm wide). The percentage of textured soy protein (TSP) (Nutrition fact: moisture 9% max, protein 53%, fat 3%, total dietary fiber 18%, carbohydrates 32% and 270 calories per 100g) and textured pea protein (TPP) in the formulation were fixed at 15%. Due to differences in size, shape and moisture content they had different requirements of hot water for rehydration (Guy 2001). From the results of preliminary tests, the ratios of hot water to textured soy protein (TSP) and textured pea protein (TPP) were 2.5:1 and 1.5:1, respectively. Other ingredients were corn oil, fresh egg white, carrageenan, salt, seasoning (sesame oil, mushroom flavor soy sauce and sugar), garlic powder and onion powder were kept identical to 4, 7, 0.5, 0.5, 0.9 (0.3 of each), 0.2 and 0.2%, respectively (Appendix 9). The total amount of water, includes hot water used to rehydrate TVP, added in the imitation patties was shown in Table 3.1. The potato starch was used, in addition to other ingredients listed above, to make up 100% of imitation patties. At 7% king oyster mushroom and at 9% soy protein isolate, only 2.7% fresh egg white were added due to minimum solubility requirement of the ingredients to maintain the quality of formed raw patties. Each treatment was mixed manually in a stainless steel bowl until the mixture was uniform. The mixtures were then formed into patties with an acrylic circular mold (63 mm in diameter and 12mm in height). The molded imitation patties were cooked in a pan with a medium heat for 5 minutes on each side. Samples from each treatment were prepared in triplicate and tested on the same day.

Table 3.1.Total amount of water added in Imitation patties formulation.

Dried king oyster mushroom(%) [1]	SPI or PPI(%) [1,2]	TSP [1,2]	TPP [1,2]
		Water(%)[1]	
0	3	51.0	43.0
	6	54.0	46.0
	9	57.0	49.0
3.5	3	52.5	44.5
	6	55.5	47.5
	9	58.5	50.5
7	3	54.0	46.0
	6	57.0	49.0
	9	60.0	52.0

[1] The percentage of soy- or pea-based imitation sausage patties.
[2] SPI, soy protein isolates; PPI, pea protein isolates; TSP, textured soy protein; TPP, textured pea protein.

3.4 Methods

3.4.1 Cooking Yield and Cooking Loss

The weight of each imitation patty sample was measured before and after cooking to determine cooking yield and cooking loss. Cooking yield and cooking loss were calculated as follows.

Cooking yield (%) = (weight after cooking / weight before cooking) x100%   Eq.1

Cooking loss= [(weight before cooking- weight before cooking)/weight before cooking] x100%   Eq. 2

3.4.2 Color Measurement

The surface color of cooked patties was measured by a Konica Minolta's CR-410 Chroma Meter (Sensing Inc., Japan) after standardized with a white tile. Two measurements, turned 90 degree after the first measurement, were taken for each sample and three samples were used from each treatment. The averages of L, *a* and b values were reported. L are lightness with values ranging from 0 (black) to 100 (white), while *a* and b means redness and yellowness, respectively.

3.4.3. Texture Profile Analysis (TPA)

For texture profile analysis (TPA), a TA-HDi texture analyzer (Texture Technologies, Crop, New York) was used to measure the hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience. Three imitation sausage patties from each treatment were used. They were cut into two cubic samples (2.5x2.5x1.2 cm). A 50.98 mm cylindrical probe was used with the following testing conditions: pre-test speed, test speed and post-test speed was 5.00 mm/s, 1.00mm/s and 1.00mm/s, respectively and each sample was compressed to 50% of initial height (1.2cm); using a 5 kg load cell. The following parameters were determined: hardness(N/cm^2), the peak force at the first compression and the force needed to deform the sample; springiness(cm), the ability of a sample to recover its original form after a deforming force is removed; cohesiveness, the extent to which a sample could be deformed before rupturing; adhesiveness(N s), the force needed to pull the compressing plunger away from the sample; gumminess(N/cm^2), the force needed to conform a sample for swallowing; chewiness(N/cm), the force needed to chew the sample before swallowing; and resilience, how well a product regains its original position after measurement.

3.4.4 Water Activity

Water activity was determined by a water activity meter (Decagon's AquaLab CX-2, Pullman, WA, USA), which uses the dew point method to determine water activity. The sample cup was filled to slightly less than its half capacity with the sample. Test results were acquired from the CX-2 meter screen after several minutes. Every sample's water activity value and temperature (^{o}C) were recorded in triplicate for each treatment.

3.4.5 pH Value

The pH value of imitation sausage patties was determined by mixing 10 g of sample with 90 mL of distilled water for one minute using a hand blender (Hamilton Beach Model 59762, Southern Pines, NC, USA). The pH of the suspension was recorded by a glass electrode with a digital pH meter (Meridian, MR-10, Denver Instruments). Three samples were used for each treatment and the average was reported.

3.4.6 Cooking Shrinkage

Shrinkage test was conducted by determining the difference in diameter between raw (mold diameter) and cooked imitation patties using a six-inch digital caliper (Pittsburgh, China). Three samples were taken for each treatment and each sample was measured twice, rotating 90^{0} after the first measurement and the average was reported.

3.4.7 Water holding/hydration capacity (WHC) of textured soy protein (TSP) and textured pea protein (TPP)

Water holding capacity (WHC) was determined by adding 30 g TSP or TPP into a 400-mL beaker with 150 mL of $4^{o}C$ water. Samples were refrigerated for 1 h. The beaker was emptied onto a mesh screen and titled 25° angle and allowed to drain for 3 min. A paper towel was used to remove excess water before the samples were weighed (Troy and Lawrence 2001). WHC was calculated by:

WHC (%) = [(hydrated sample weight-dry sample weight)/ dry sample weight] x 100%. Eq. 3

3.4.8 Water holding/hydration capacity (WHC) of product

The WHC modified AACC (56-30.01) (2000) procedure and the Dagbjartsson and Solberg WHC method (1972) were applied as follows. Five grams of samples were mixed with 10 mL of water, and then placed in a 50 mL centrifuge tube, weighed, vortexed for 1 min, and centrifuged for 10 min (2000g, 15^{o}C; Centrifuge, Model J2-21M/E, BECKMAN, USA). After removing the supernatant, the residue weight was determined. WHC of samples was calculated as follows:

WHC%= [(weight of sample after centrifuging- weight of sample before centrifuging)/ weight of the sample before centrifuging] x100% Eq. 4

The retained weight was expressed as the amount of water absorbed per gram of sample on dry weight basis. Three samples were taken from each treatment.

3.5 Statistical Analysis:

All experimental data were expressed as a mean of at least three measurements. Analysis of Variance (ANOVA) and Least Squares Means were used to determine the significance of the independent variable (SAS, version 9.3, Gary, NC, USA). Significance of results were determined at the $P<0.05$ level for all data analyses. All data was used to generate the 3D plot using the Sigma Plot (Systat software Inc. version 12.0, San Jose, CA, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary Tests

4.1.1 Initial Formulation

The very first formulation of the soy-based imitation sausage patties was developed and designed from the patents (Coöperatie Koninklijke Cebeco Groep U.A. 2000; Hargarten and others 2004). The first patent used dried champignons, pea protein, wheat fiber, chicken egg protein, oil, salt carrageenan seasoning and textured pea protein to make sausage. The second patent used water, vegetable protein products, vital wheat gluten, soy/canola salad oil, modified starch B990, dextrose, methylcellulose, spice and color to make emulsion based meat analogue.

Based on these two formulas, textured soy protein, vital wheat gluten, king oyster mushroom, corn oil, fresh egg white, salt, carrageenan, seasoning, garlic powder, onion powder and water were used as the initial soy-based imitation sausage patties formulation.

4.1.2 Formula Modification

Considering the food allergy problems in United States, even though vital wheat glutens helped providing chewability and texture for the imitation sausage patties, they were replaced by soy protein isolates in soy-based imitation sausage patties. In addition, textured pea protein and pea protein isolates were used in pea-based imitation sausage patties. By doing so, all the ingredients in formulation were gluten free in the production of plant-protein-based imitation sausage patties.

Textured soybean or pea proteins usually undergo processes like extrusion and drying, which had been developed to retain a fibrous and spongy structure thereby boosting the juiciness and succulence of the end-product by certain degrees. However, excessive use of textured vegetable proteins, where they take up 20% to 30% of total weight reduced the texture properties and acceptability. The negative effects include off-flavor (Rentfrow and others 2004; Katayama and Wilson 2008) and hard texture (Heywood and others 2002; Deliza and others 2002), though it could improve cooking properties (Kilic and others 2010). Therefore, this research set the textured soy and pea proteins at 15% of total weight.

King oyster mushrooms were added to enhance the fibers content, digestion and improve mouth-feel. However, up to 8% of king oyster mushroom weakens the binding ability and integrity of mixture; hence, the maximum king oyster mushroom was 7%.

4.1.3 Final Formulation

The goal was to determine the influences of additional soy or pea protein isolates and king oyster mushrooms on the properties of imitation sausage patties. Therefore, the two independent variables were soy or pea protein isolates (3, 6 and 9%) and dried king oyster mushrooms (0, 3.5 and 7%). The level of textured soy or pea proteins were fixed at 15% based on preliminary tests. From the results of preliminary tests, the ratios of hot water to textured soy protein (TSP) and textured pea protein (TPP) were was 2.5:1 and 1.5:1, respectively. Other ingredients were corn oil, fresh egg white, carrageenan, salt, sesame oil, soy sauce, sugar, onion powder and garlic powder, were kept constant at 4, 7, 0.5, 0.5, 0.3, 0.3, 0.3, 0.2 and 0.2%, respectively. The amount of cold water (Table 3.1) added in imitation patties formulation came from a series trials to acquire dough integrity and good solubility among ingredients. The potato starch was used, in addition to other ingredients listed above, to make up 100% of imitation patties. With one exception, the 7% king oyster mushrooms mixed with 9% soy protein isolates, where the amount of egg white is 2.7% instead of 7%.

4.2 Effect on Product Properties

4.2.1 Effect on Cooking Yield or Cooking Loss of the Products

Animal meat loses water during heat treatment and thus has a lower cooking yield or high cooking loss in the end-products. This is because water exists within animal tissues and cannot be held under thermal treatment. However, this is not the case with meatless produce and protein-protein-based products due to different patterns structured to keep water within vegetable additives. Cooking loss and cooking yield show the degrees of water evaporated or released during heat treatment and are widely used as the indicators to determine what degree of water can be retained within structure of imitation sausage patties.

Statistical results showed a significant difference ($p<0.05$) between soy-based and pea-based imitation sausage patties. Different shape and minced degree lead to different capacities for binding water, or for interacting with other ingredients; hence, different percentages of water are forced out when heating at different temperatures. Kilic and others (2010) presented the ability of textured vegetable protein to decrease cooking loss and evaporative loss in meat products. Differences between soy-based and pea-based imitation sausage patties also related to the results of water holding capacity (WHC) of textured vegetable proteins. WHC of textured soy proteins and textured pea proteins were 2.72 and 0.68, respectively.

Table 4.2.1.1: Average values of cooking yield percentage for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

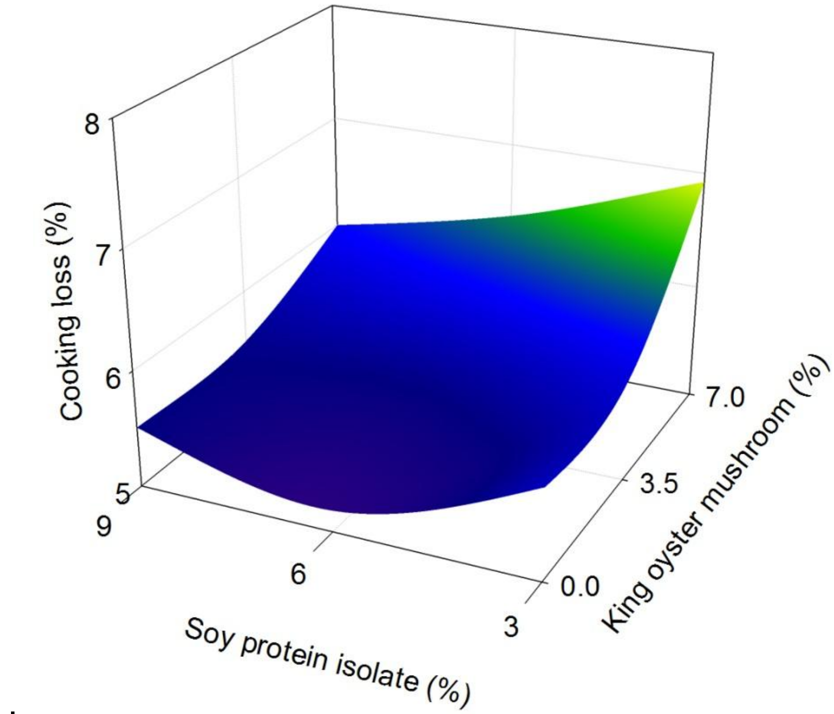
Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	94.22^{A}_{a}	94.15^{A}_{a}	93.07^{A}_{b}
6	94.82^{A}_{a}	94.45^{A}_{a}	93.64^{AB}_{b}
9	94.48^{A}_{a}	94.45^{A}_{a}	94.00^{B}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

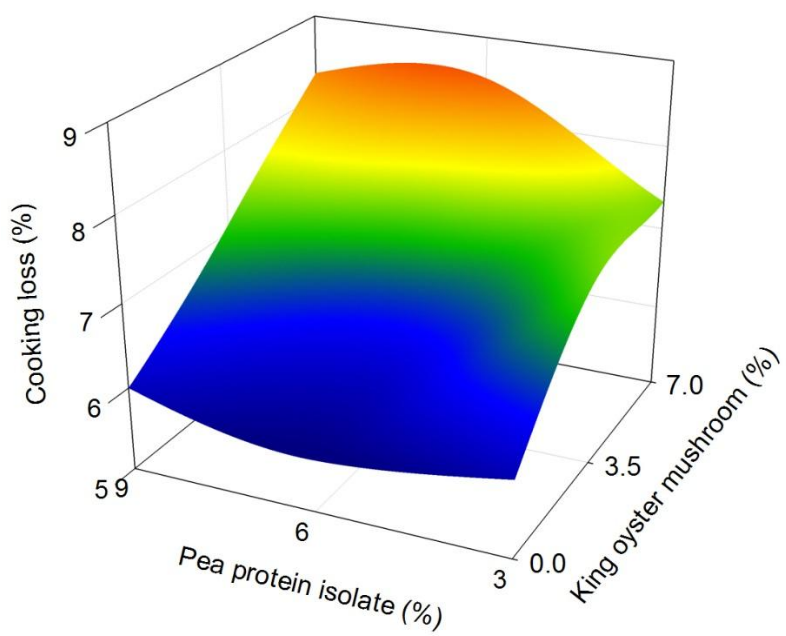
(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	94.08^{A}_{a}	92.75^{A}_{a}	92.67^{A}_{b}
6	94.38^{A}_{a}	93.56^{B}_{b}	91.48^{B}_{c}
9	94.00^{A}_{a}	92.90^{AB}_{b}	91.70^{B}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.



(a)



(b)

Fig 4.2.1.1: Average values of cooking loss percentage for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.1.1 Soy-based Imitation Products

For the soy-based imitation sausage patties shown in Table 4.2.1.1(a) and Fig 4.2.1.1(a), 0 and 3.5% king oyster mushroom groups were not significantly different ($p>0.05$) with increased level of soy protein isolates; however, the level of statistical significance were significantly increased ($p<0.05$) between 3% and 9% soy protein isolates as 7% king oyster mushroom incorporated. The higher significance is thought to be due to a higher percentage of soy protein isolates added into the formulation, leading to a compact and firm structure and a higher cooking yield compared with others within the 7% king oyster mushroom group.

The 3 and 6% soy protein isolate groups showed a significant decrease ($p<0.05$) in cooking yield as the level of king oyster mushrooms increased to 7%, but not when 9% soy protein isolates were combined with 7% king oyster mushrooms. Improvement in statistical significance had a direct connection to the combination containing the highest amount of king oyster mushrooms and soy protein isolates. This combination slightly reduced the amount of water forced out during heat treatment.

4.2.1.2 Pea-based Imitation Products

For the pea-based imitation sausage patties (Table 4.2.1.1 (b); Fig 4.2.1.1 (b)), it did not make significant differences ($p>0.05$) in cooking yield without king oyster mushrooms. The level of statistical significance were significantly increased ($p<0.05$) only between 3% and 6% pea protein isolates as 3.5% king oyster mushroom incorporated. The 7% king oyster mushroom group showed a significant decrease ($p<0.05$) in cooking yield as the 6% and with 9% pea protein isolates were mixed individually. It is possible that the pea protein isolates lessen the strength of structure.

All three pea protein isolate groups showed decreased significance ($p<0.05$) in cooking yield with boosted level of king oyster mushrooms, with the exception wherein 3% pea protein isolate combined with 7% king oyster mushrooms.

4.2.1.3 Further Discussion on Cooking Properties

Under the highest amount of king oyster mushrooms and soy protein isolates seemed to slightly increase the yield; and pea protein isolates seem slightly decrease the yield. All these findings contribute to the body of knowledge on textured soy or pea proteins as discussed by Gujral and others (2002) and Kasssm and Emara (2010). The research also adds to the body of knowledge on soy or pea protein isolates (Kotula and Berry 1986; Akesowan 2010; Kilic and others 2010; Silva and others 2011; Shahiri Tabarestani and Mazaheri Tehrani 2012), potato starch (Carballo and others 1995; Pietrasik 1999; Shewry and Tatham 2000) and carrageenan (Brewer and others 1992; Bater and others 1992; Xiong and others 1999; Hsu and Chung 2001; Candogan and Kolsarici 2003; Kumar and Sharma 2004; Cierach and others 2009; Garcia and others 2013), all of whom were interested in how to better retain or bind the water within the vegetable structures when looking for high cooking yield or low cooking loss, high rigidity and low degree of expressible water. Cha and others (2012) found that addition of grinded white jelly mushrooms had significant higher cooking yield and oil binding capacity than control, which had different result to this study. The possible reason would be the size of mushrooms added to formulation causes the differences on cooking yield.

4.2.2 Effect on Color of the Products

Color plays as an indicator to judge the acceptance and also influences food products marketing strategy based on first impressions of consumers. Food manufacturers want to know if the added king oyster mushrooms and different levels of soy and pea protein isolate could cause any changes to its color or appearance, which is why measuring the surface color by a colorimeter or other techniques are widely applied by food manufacturers. Color depends on prior mixing with other ingredients, in addition to the textured soy proteins and textured pea proteins, which are brown and light yellow, respectively. Other ingredients that affect the color index are soy protein isolates, pea protein isolates and potato starches, which are ivory-white with slightly yellow, pale-yellow and white, respectively. Frying and the addition of spices are two possible factors that produce different colors of end-products after heat treatment.

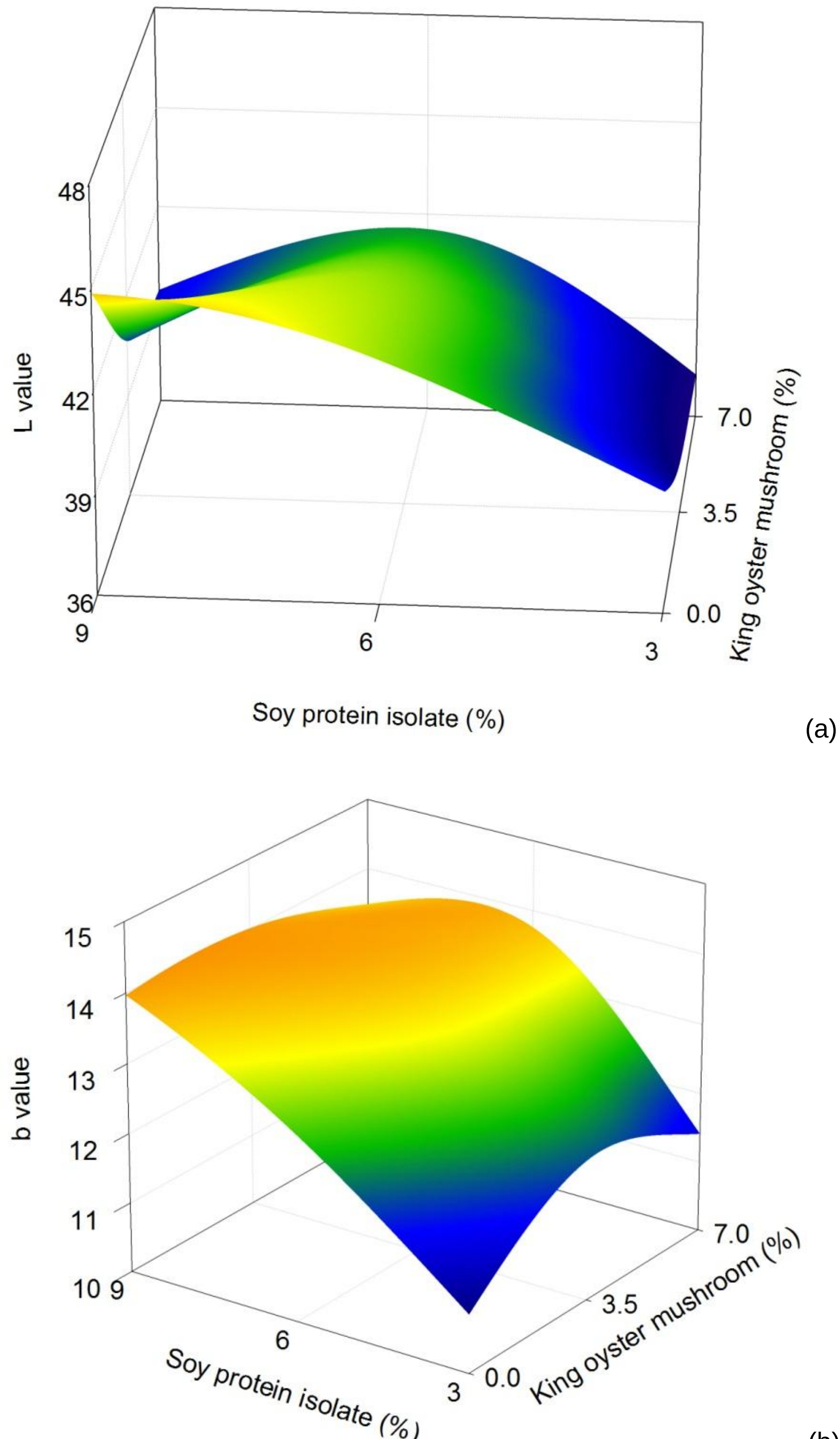


Fig. 4.2.2.1: Average values of color index, L (a) and b (b) value, for each combination of king oyster mushrooms and soy protein isolates.
L value indicates lightness: 0 yields black and 100 indicate diffuse white and b value indicates yellowness when positive and blueness when negative.

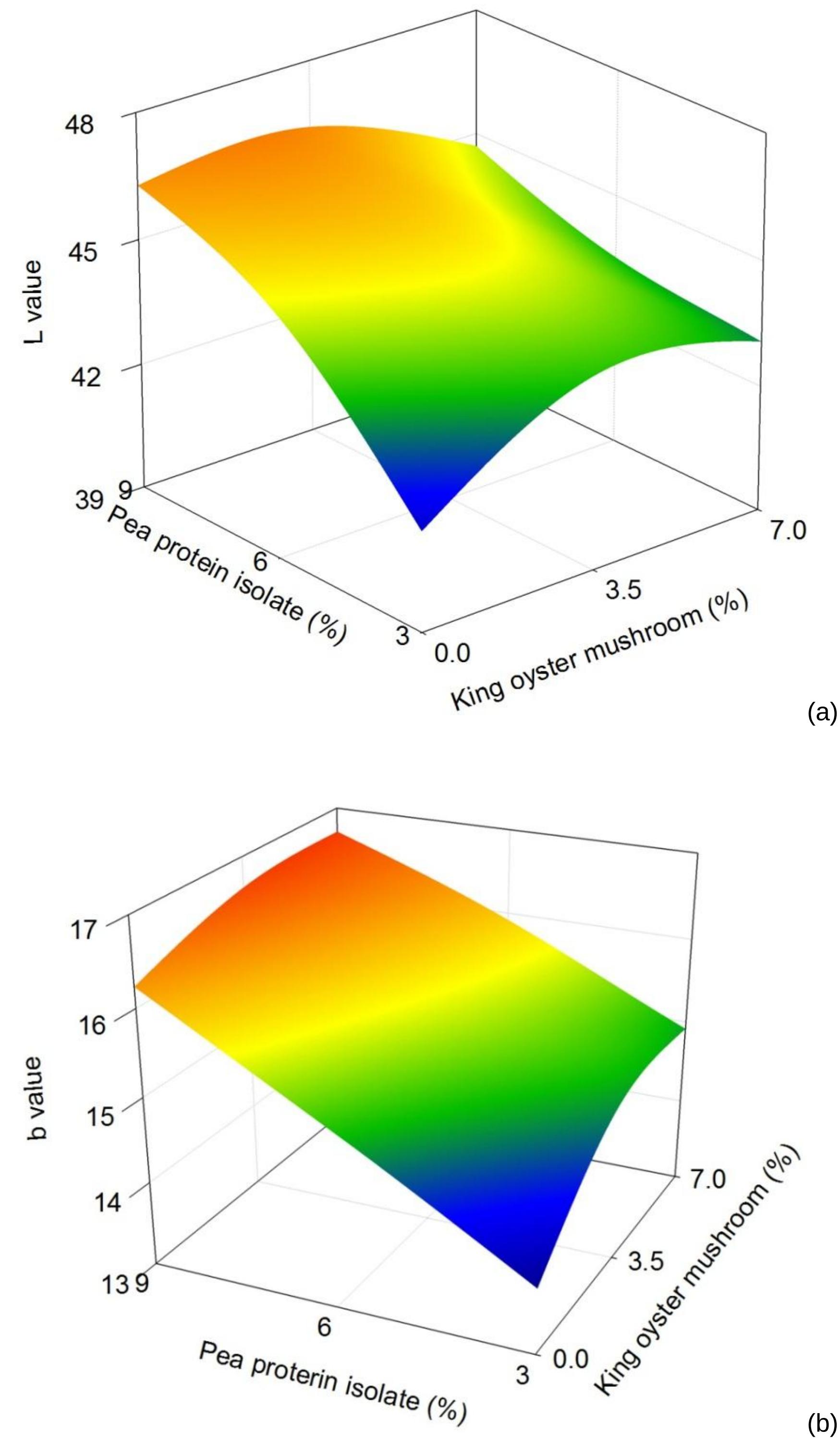


Fig. 4.2.2.2: Average values of color index, L (a) and b (b) value, for each combination of king oyster mushrooms and pea protein isolate.
L value means lightness: 0 yields black and 100 indicates diffuse white and b value means yellowness when positive and blueness when negative.

.4.2.2.1 The L and b Value of Soy-based Imitation Products

The L and b values were not significantly different ($p>0.05$) for both soy-based and pea-based imitation sausage patties. However, 0% king oyster mushroom group had significantly ($p<0.05$) higher lightness, and 3% soy protein isolate group had significantly lower lightness in soy-based imitation sausage patties (Fig. 4.2.2.1 (a); Appendix 1(a)) independently.

The three soy protein isolate groups had significantly different yellow values when compared to each other ($p<0.05$) and the three king oyster mushroom groups did not show significantly different ($p>0.05$) yellow value in soy-based imitation sausage patties (Fig. 4.2.2.1 (b); Appendix 1(b)) independently.

4.2.2.2 The L and b Value of Pea-based Imitation Products

For pea-based imitation sausage patties (Fig. 4.2.2.2 (a) and (b)), three pea protein isolate groups were significantly different ($p<0.05$) in both yellowness and lightness when compared to each group independently; and the three king oyster mushroom groups did not show a significantly different ($p>0.05$) color independently.

4.2.2.3 Further Discussion on the L and b Value

Conclusions can be drawn that king oyster mushrooms slightly increased the lightness but not significantly ($p>0.05$) after cooking; nevertheless, soy or pea protein isolates significantly ($p<0.05$) increased the lightness and yellowness in most of cases. These results were similar to other works (Ahmad and others 2010; Shahiri Tabarestani and Mazaheri Tehrani 2012) but the finding Pietrasik and Duda (2000) and Akesowan (2010).

Table 4.2.2.1: Average values of *a* value for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	3.81^{A}_{a}	5.56^{A}_{b}	5.11^{A}_{c}
6	4.30^{B}_{a}	4.60^{B}_{b}	5.56^{B}_{c}
9	4.19^{B}_{a}	5.99^{C}_{b}	5.85^{C}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.
a value indicates redness when positive and greenness when negative.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	7.50^{A}_{a}	7.17^{A}_{b}	7.43^{A}_{ab}
6	7.11^{B}_{a}	7.19^{A}_{a}	8.06^{B}_{b}
9	7.66^{A}_{a}	7.55^{B}_{a}	8.16^{B}_{b}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.
a value indicates redness when positive and greenness when negative.

4.2.2.4 The *a* Value of Soy- and Pea-based Imitation Products

Excluding two exceptions, 0% king oyster mushroom mixed with 9% soy protein isolates and 9% soy protein isolates incorporating 7% king oyster mushrooms, redness was significantly ($p<0.05$) different but with no trend in each formulation of the soy-based meat sausage patties (Table 4.2.2.2.1 (a); Appendix 2(a)) .

For pea-based imitation sausage patties (Table 4.2.2.2.1 (b); Appendix 2 (b)), the amount of pea protein isolates did not seem to correlate with the redness without king oyster mushroom; however, at the3.5 and 7% king oyster mushroom groups significantly ($p<0.05$) increased the redness with increased amount of pea protein isolates.

A possible explanation of the redness would be the Maillard reaction during cooking on pan. The Maillard reaction is not a single reaction, but a complex series of reactions between amino acids and reducing sugars, usually at increased temperatures (FOOD-INFO 2013). Increased amounts of king oyster mushrooms (Reis and others 2012) and of soy or pea protein isolates (Shahiri Tabarestani and Mazaheri Tehrani 2012) render a color change. Other explanations for the color would be the effect of spices or the after effect of thermal treatment involving textured soy or pea proteins.

4.2.3 Effect on Texture Profile of the Products

4.2.3.1 Hardness of Plant Protein-based Imitation Products

4.2.3.1.1 Hardness of Soy-based Imitation Products

Textural properties defined the character of the products and also referenced the mouth-feel to some degree. Table 4.2.3.1.1 (a) shows the hardness of the soy-based imitation sausage patties. At 0%, the king oyster mushroom group's hardness decreased not significantly ($p>0.05$), but increased significantly ($p<0.05$) at 3.5% and 7% king oyster mushroom group, especially when 9% soy protein isolates are incorporated, causing the meatless sausage patties to attain their highest hardness.

As the level of king oyster mushrooms increased, hardness declined significantly ($p<0.05$) both at 3% and 6% soy protein isolate group. However, the hardness increased significantly ($p <0.05$) at 9% soy protein isolate group, which is 1606.974, 1916.676 and 2005.966 N/cm^2, respectively.

For soy-based imitation sausage patties (Table 4.2.3.1.1 (a)), soy protein isolates play a certain role in strength hardness. On the other hand, king oyster mushroom lowered the hardness as combined with 3 and 6% SPI individually. The highest level of soy protein isolates and the fair to highest amount of king oyster mushrooms combined exhibited the highest hardness of soy-based Imitation sausage patties.

4.2.3.1.2 Hardness of Pea-based Imitation Products

As for the pea-based imitation sausage patties (Table 4.2.3.1.1 (b)), hardness waned ($p<0.05$) significantly with increased pea protein isolates besides the formulation mixed with 9% pea protein isolates only. In the case of the pea protein isolate mixtures of 3, 6 and 9%, hardness also decreased ($p<0.05$) significantly and the highest value were recorded when the 3% pea protein isolates' mixture was not made up of king oyster mushrooms. For pea-based imitation sausage patties, both pea protein isolates and king oyster mushrooms weaken the hardness of pea-based imitation sausage patties.

4.2.3.1.3 Further Discussion on Hardness

Recent research has favored soy or pea protein isolates and starches for texture improvement according to the literature (Das and others 2008; Ahmad and others 2010; Matos and others 2014). Shahiri Tabarestani and Mazaheri Tehrani (2012) reported that higher hardness was attributed to firm texture of hydrated flours and starch in low-fat burger, which corresponds to the result of 3% soy or pea protein isolate without king oyster mushroom due to higher amount of potato starch mixed in formulation. Chung and others (2010) showed that fish paste added with king oyster mushroom paste had significant decrease on hardness, which corresponds to some part of result of this study. Matos and others (2014) also reported the muffins containing pea protein isolates were softer. Studies have also shown how carrageenan can enhance the texture of meat and meatless products (Barbut and Mittal 1992; Trius and Sebranek 1996; Xiong and others 1999; Hsu and Chung 2001; Ayadi and others 2009; Cierach and others 2009).

Table 4.2.3.1.1: Average values of hardness (N/cm^2) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	1764^{A}_{a}	1297^{A}_{b}	966^{A}_{b}
6	1637^{A}_{a}	1358^{A}_{a}	1214^{A}_{b}
9	1607^{A}_{a}	1917^{B}_{b}	2006^{B}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

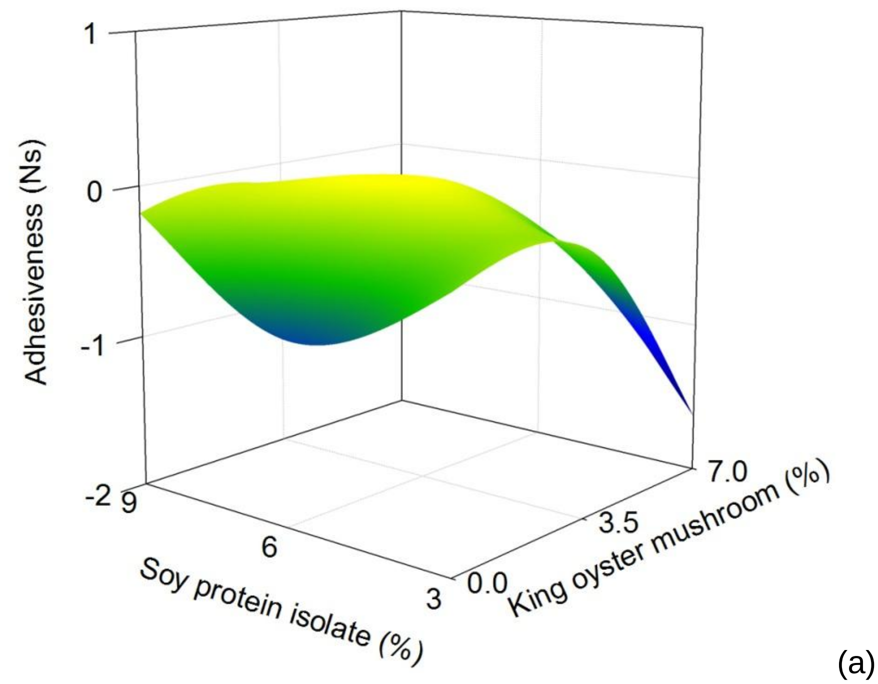
(b)

Pea protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	2811^{A}_{a}	2710^{A}_{a}	2110^{A}_{b}
6	1972^{B}_{a}	1838^{B}_{a}	1489^{B}_{b}
9	1950^{B}_{a}	1341^{C}_{b}	810^{C}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

4.2.3.2 Adhesiveness of Imitation Products

Figure 4.2.3.2.1 and Appendix 3 show the adhesiveness of soy-based (a) and pea-based (b) imitation sausage patties. Not significantly different ($p>0.05$) results were shown in either soy-based or pea-based imitation sausage patties. My own research has proven that imitation sausage patties are apt to pull away or break down under external forces with no respect to the amount of king oyster mushrooms and soy or pea protein isolates. This is a problem that has to be overcome in future research.



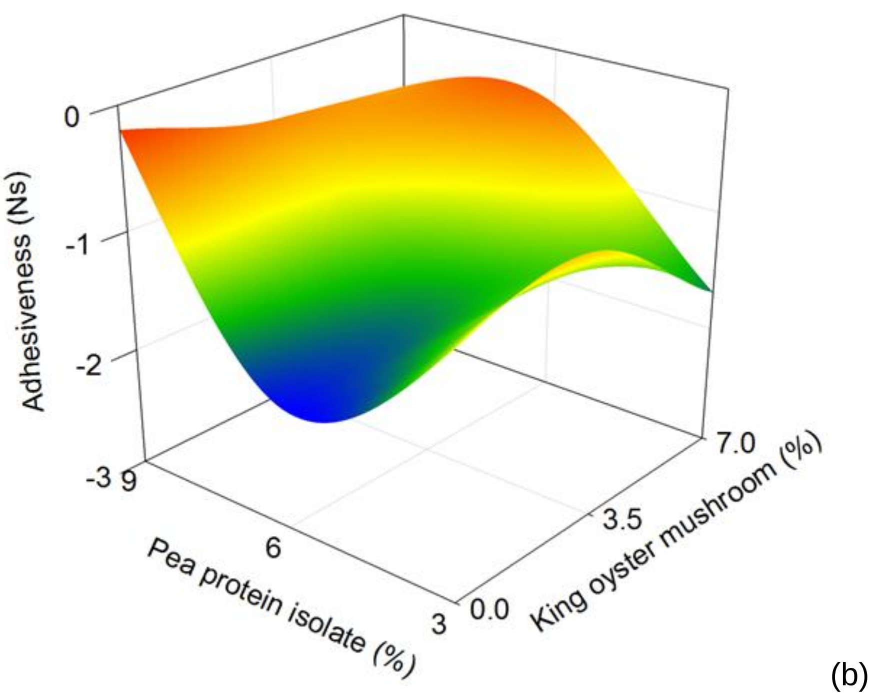


Fig. 4.2.3.2.1: Average values of adhesiveness (Ns) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.3.3 Springiness of Plant Protein-based Imitation Products

4.2.3.3.1 Springiness of Soy-based Imitation Products

According to Table 4.2.3.3.1 (a), soy-based imitation sausage patties were significantly different ($p<0.05$) in springiness only between 3% and 6% soy protein isolates mixed without king oyster mushrooms individually. The springiness significantly increased ($p<0.05$) with a king oyster mushroom content of 3.5% and 7% especially significant at the 7% king oyster mushroom rate, with an increased amount of soy protein isolates.

As the king oyster mushroom amount increased, the 3% soy protein isolate group showed a significant decrease in springiness ($p<0.05$); the 6% soy protein isolate group's springiness was significantly decrease ($p<0.05$) only between 0 and 3.5% king oyster mushroom were incorporated. The 9% soy protein isolate group had significant improvement ($p<0.05$) in springiness between control (0% king oyster mushrooms) and other mixtures (3.5% and 7% king oyster mushrooms showed good results). When a mixture of 9% soy protein isolates added 7% addition king oyster mushrooms, springiness of soy-based imitation sausage patties were at their best. Past research (Trius and Sebranek 1996; Das and others 2008; Cierach and others 2009; Ahmad and others 2010; Chung and others 2010; Shahiri Tabarestani and Mazaheri Tehrani 2012) had confirmed the texture improving ability of soy protein isolates, king oyster mushrooms, starch and carrageenan.

4.2.3.3.2 Springiness of Pea-based Imitation Products

For pea-based imitation sausage patties (Table 4.2.3.3.1 (b)), a significantly decreased ($p<0.05$) springiness was shown in all levels of variances. Proper explanation would be that both pea protein isolates and king oyster mushrooms did no helps on springiness of pea-based imitation sausage patties. The studies (Trius and Sebranek 1996; Cierach and others 2009; Chung and others 2010; Shahiri Tabarestani and Mazaheri Tehrani 2012) verified the texture altering and improving ability of pea protein isolates, king oyster mushrooms, starch and carrageenan. However, springiness was not affected by the pea protein isolates, which contradict with the study from Matos and others (2014).

Table 4.2.3.3.1: Average values of springiness (cm) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	0.628^{A}_{a}	0.503^{A}_{b}	0.405^{A}_{c}
6	0.541^{B}_{a}	0.485^{A}_{b}	0.531^{B}_{ab}
9	0.592^{AB}_{a}	0.752^{B}_{b}	0.735^{C}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	0.770^{A}_{a}	0.737^{A}_{a}	0.676^{A}_{b}
6	0.657^{B}_{a}	0.587^{B}_{b}	0.531^{B}_{c}
9	0.547^{C}_{a}	0.460^{C}_{b}	0.349^{C}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

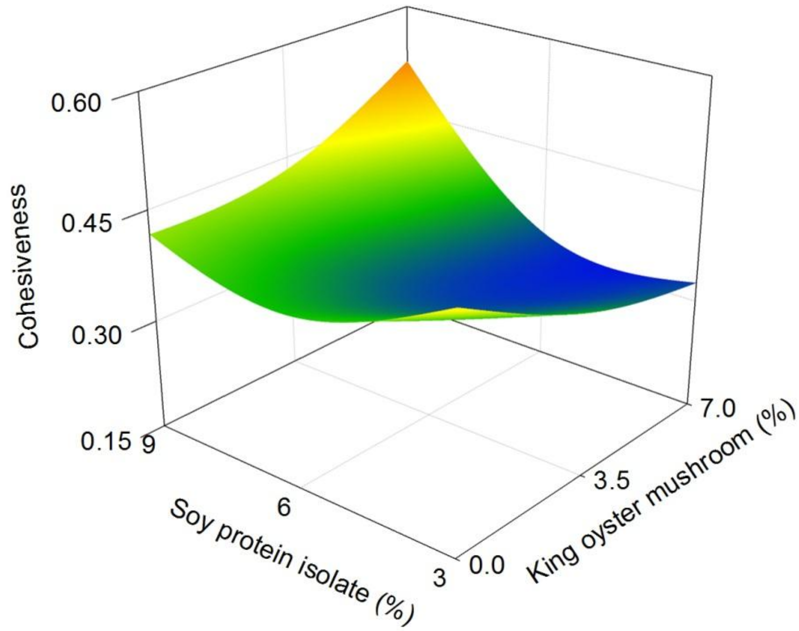
4.2.3.4 Cohesiveness of Plant Protein-based Imitation products

4.2.3.4.1 Cohesiveness of Soy-based Imitation Products

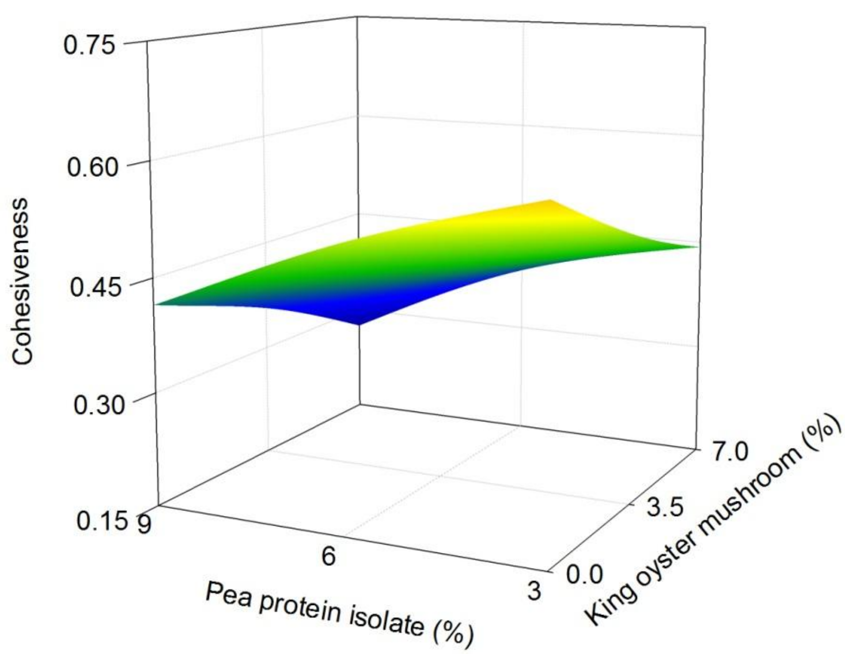
Cohesiveness indicates the ability of samples to deform before finalization. Statistical results pointed out the cohesiveness of three king oyster mushroom groups increasing significantly ($p<0.05$) when soy protein isolates were added up to 9%, though the lowest value was reported with 6% soy protein isolates combined without king oyster mushrooms.

Increased king oyster mushroom amounts showed a decreased ($p<0.05$) trend as 3% and 6% soy protein isolate mixed individually, but not at 9%. A mix of 9% soy protein isolates and 7% king oyster mushrooms gave higher ($p<0.05$) values in cohesiveness of soy-based meat free sausage (Fig. 4.2.3.4.1 (a); Appendix 4 (a)).

Previous research (Xiong and others 1999; Chin and others 2000; Gujral and others 2002; Kassama and others 2003; Ayadi andf others 2009; Cierach and others 2009; Shahiri Tabarestani and Mazaheri Tehrani 2012) has verified texture ameliorating ability of soy protein isolates, textured vegetable proteins and carrageenan.



(a)



(b)

Fig. 4.2.3.4.1: Average values of cohesiveness for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.3.4.2 Cohesiveness of Pea-based Imitation Products

Pea-based imitation sausage patties, Fig. 4.2.3.4.1 (b) and Appendix 4 (b), presented a significantly decreased ($p<0.05$) value of cohesiveness despite increased amounts of king oyster mushrooms. Slightly different from results for soy-based imitation sausage patties, both the pea protein isolates and king oyster mushrooms weakened the cohesiveness of pea-based imitation sausage patties, though carrageenan was present. Tabarestani and Tehrani (2012) shed light on utilizing pea protein as an ingredient or main material for manufacturing their end-products or serving an enhancement agents to low fat burger.

4.2.3.5 Gumminess of Plant-protein-based Imitation Products

Gumminess characterizes the force necessary to destroy a sample for swallowing. Ability of binders to maintain the integrity and completeness of imitation sausage patties after frying define gumminess. Components of tertiary structure, textured vegetable proteins, vegetable-based protein isolates, hydrocolloids, potato starches and fresh egg whites, could influence the textural, gel-forming ability when denatured. Table 4.2.3.5.1 (a) and (b) show the gumminess of soy-based and pea-based imitation sausage patties, respectively.

4.2.3.5.1 Gumminess of Soy-based Imitation Products

Increased amounts of soy protein isolate presented an upside curve trend of gumminess both at 0% and 3.5% king oyster mushroom content. However, at 7% king oyster mushroom content, gumminess showed an ascending straight line as amounts of soy protein isolates were added. Although no significant difference was noticeable ($p>0.05$) between 3% and 6% soy protein isolates combination.

On the other hand, gumminess decreased significantly ($p<0.05$) in 3% and 6% soy protein isolates group when the 7% king oyster mushroom content was realized, but this did not occur in the 9% soy protein isolates group. This exception shows an increased trend of gumminess at increased king oyster mushroom levels, though not significantly different ($p>0.05$) between the 3.5 and 7% king oyster mushrooms levels. A combination of 9% soy protein isolates and 7% king oyster mushrooms showed the highest ($p<0.05$) gumminess of all.

Potato starches, soy protein isolates and textured soy proteins can form a fine strength structure when aided with binders and gums (Xiong and others 1999; Chin and others 2000; Hsu and Chung 2001; Kumar and Sharma 2004; Das and others 2008; Ayadi and others 2009; Cierach and others 2009; Ahmad and others 2010; Shahiri Tabarestani and Mazaheri Tehrani 2012), but the strengthen ratios begin changing as different ratio of the soy protein isolates, king oyster mushrooms and potato starch are subjected to experiments.

Table 4.2.3.5.1: Average values of gumminess (N/cm^2) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	821.196^{A}_{a}	479.737^{A}_{b}	322.488^{A}_{b}
6	622.787^{A}_{a}	448.634^{A}_{ab}	401.820^{A}_{b}
9	673.445^{A}_{a}	828.329^{B}_{a}	1089.351^{B}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	1655.370^{A}_{a}	1367.259^{A}_{b}	938.586^{A}_{c}
6	1021.879^{B}_{a}	807.323^{B}_{b}	574.920^{B}_{c}
9	813.958^{C}_{a}	478.644^{C}_{b}	224.196^{C}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

4.2.3.5.2 Gumminess of Pea-based Imitation Products

For pea-protein imitation sausage patties, Table 4.2.3.5.1 (b), gumminess significantly decreased ($p<0.05$) with increase of both king oyster mushroom levels and pea protein isolates. This supports the results from Tabarestani and Tehrani (2012) and are similar to others studies (Trius and Sebranek 1996; Kumar and Sharma 2004; Cierach and others 2009). In general, pea protein isolates lowered the gumminess more obviously than soy protein isolates did since different interaction degree of ingredients. King oyster mushrooms also lessened the gumminess clearly in pea-based imitation sausage patties, more so than in soy-based products.

4.2.3.6 Chewiness of Plant Protein-based Imitation Products

Chewiness (N/cm) is the work needed to chew the sample for swallowing. The intent of this study was to improve the lack of the consistency in mouth-feel and to provide the chewability of the soy-based (Table 4.2.3.6.1 (a)) and pea-based (Table4.2.3.6.1 (b)) imitation sausage patties by adding king oyster mushrooms.

4.2.3.6.1 Chewiness of Soy-based Imitation Products

Increased soy protein isolate amounts did not have significantly different ($p>0.05$) without king oyster mushrooms. However, chewiness significantly increased ($p<0.05$) when 3.5% and 7% king oyster mushroom mixed with 9% soy protein isolate individually.

Chewiness decreased ($p<0.05$) when 3% soy protein isolate was incorporated; but did not differ significantly ($p>0.05$) when 6% soy protein isolate was added; nevertheless, a significantly increased ($p<0.05$) occurred when 9% soy protein isolate combined, with raised levels of king oyster mushrooms.

This result indicated that the chewiness had a significant correlation with the combination of high level soy protein isolates (Das and others 2008; Akesowan 2010; Tabarestani and Tehrani 2012), carrageenan (Cierach and others 2009) and high amount king oyster mushrooms (Ogawa and others 2012).

Table 4.2.3.6.1: Average values of chewiness (N/cm) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	521.855^{A}_{a}	245.782^{A}_{b}	131.794^{A}_{b}
6	340.088^{A}_{a}	219.762^{A}_{a}	215.254^{A}_{a}
9	402.357^{A}_{a}	625.002^{B}_{b}	833.164^{B}_{c}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	1279.149^{A}_{a}	1014.613^{A}_{b}	639.966^{A}_{c}
6	677.162^{B}_{a}	486.189^{B}_{b}	306.346^{B}_{b}
9	455.921^{C}_{a}	223.144^{C}_{b}	83.902^{C}_{b}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

4.2.3.6.2 Chewiness of Pea-based Imitation Products

As pea-based imitation sausage patties (Table 4.2.3.6.1 (b)), showed a significantly lower ($p<0.05$) trend with increased pea protein isolate levels. Significant differences ($p<0.05$) were reported between control, no king oyster mushrooms were mixed, and the addition of king oyster mushrooms within each pea protein isolate group.

Showing the interactions of pea protein isolates (Tabarestani and Tehrani 2012; Matos and others 2014), the addition of king oyster mushrooms, and other ingredients include potato starch and carrageenan (Carballo and others 1995; Cierach and others 2009) did little helps on the chewiness of pea-based imitation sausage patties.

4.2.3.7 Resilience of Plant Protein-based Imitation Products

4.2.3.7.1 Resilience of Soy-based Imitation Products

The formulation without king oyster mushrooms showed an upside curve trend, meaning high amounts of potato starches and low levels soy protein isolates formed a relative higher resilience ($p<0.05$) as levels of soy protein isolates increased. Though 3.5% and 7% king oyster mushroom groups also presented an upside curve trend as the control groups, the highest ($p<0.05$) resilience appears in 9% soy protein isolates.

Resilience decreased ($p<0.05$) significantly with increased amount of king oyster mushrooms in the 3% and 6% soy protein isolate group; however, an increased trend was reported in the 9% soy protein isolate group, which increased significant ($p<0.05$) when results were combined with 7% king oyster mushrooms results. With a combination of 9% soy protein isolates and 7% king oyster mushrooms, we were able to render the highest resilience, indicating that both ingredients boosted the resilience of soy-based imitation sausage patties (Fig. 4.2.3.7.1 (a); Appendix 5 (a)) well.

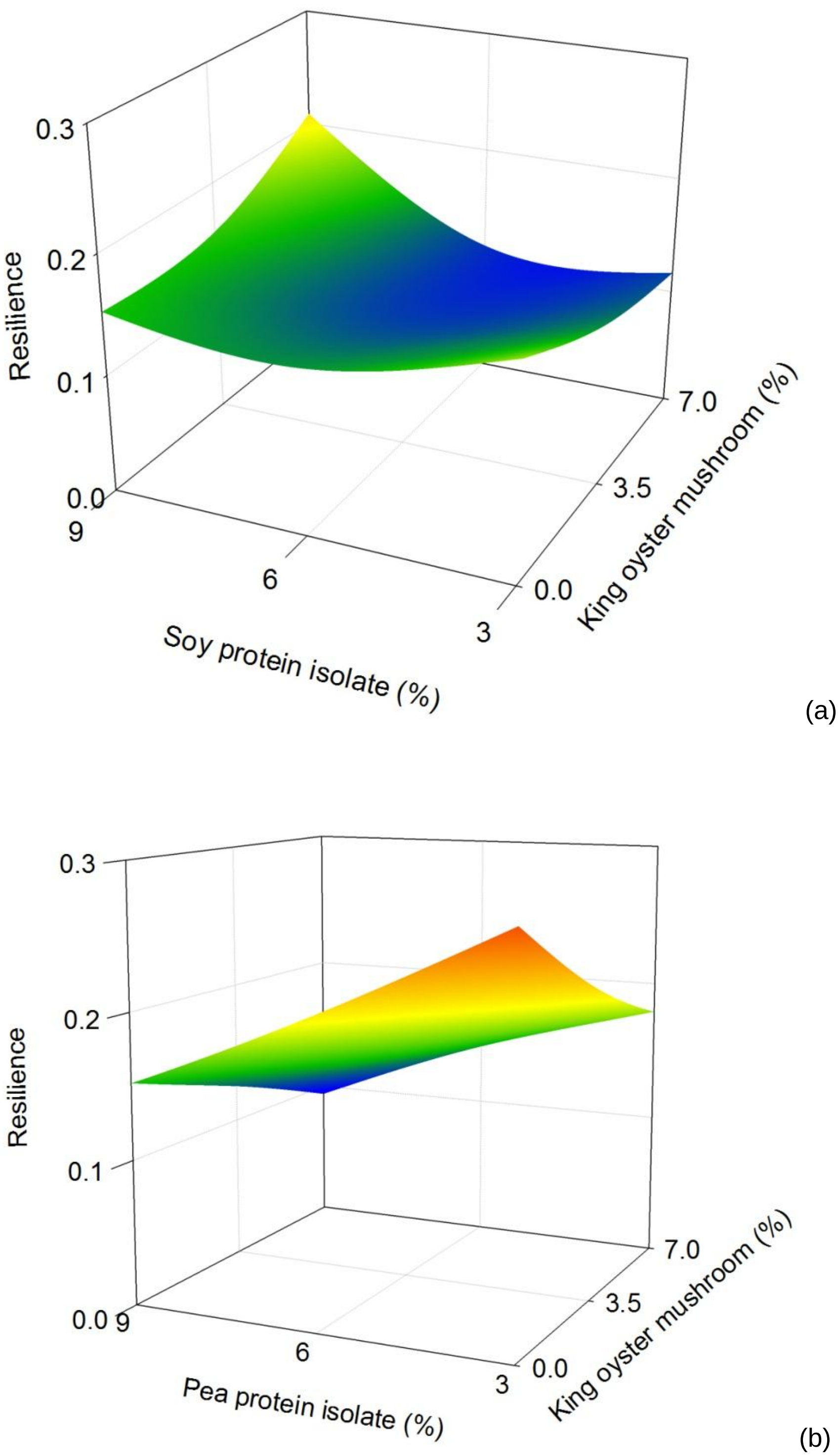


Fig. 4.2.3.7.1: Average values of resilience for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.3.7.2 Resilience of Pea-based Imitation Products

Fig. 4.2.3.7.1 (b) and Appendix 5 (b) show the obvious results of pea-based imitation sausage patties where resilience decreased significantly ($p<0.05$) with increased pea protein isolates in three groups of king oyster mushrooms. Changing the amounts of king oyster mushroom lowered the resilience significantly ($p<0.05$) in three groups of pea protein isolates as well. It goes without saying that the pea protein isolates and king oyster mushrooms did little to help on in adding resilience to the imitation sausage patties.

4.2.4 Effect on Water Activity of Plant-protein-based Imitation Products

Intrinsic property, as it relates to water activity refers to the ratio of water vapor pressure in equilibrium with a food to the saturation vapor pressure of water at the same temperature. Water activity describes the degree to which water is bound in the food, the energy status in food, and its availability to act as a solvent and participate in chemical or biochemical reactions and growth of microorganisms (Labuza 1977). Hence, it was widely used as an indicator of stability and food safety with respect to microbial growth, rate of deterioration, and chemical or physical properties. Basically, water has one water activity, for fresh meat, fruit, meat and their produce which have 0.96 to 0.99 water activity; bread has 0.96 water activity; flour has 0.72 water activity, and intermediate moisture food has 0.65 to 0.85 water activity. High water activity is usually related to high incidence of non-enzymatic browning, lipid oxidation, vitamin degradation, enzymatic reactions, and protein denaturation. Texture also depends on water activity.

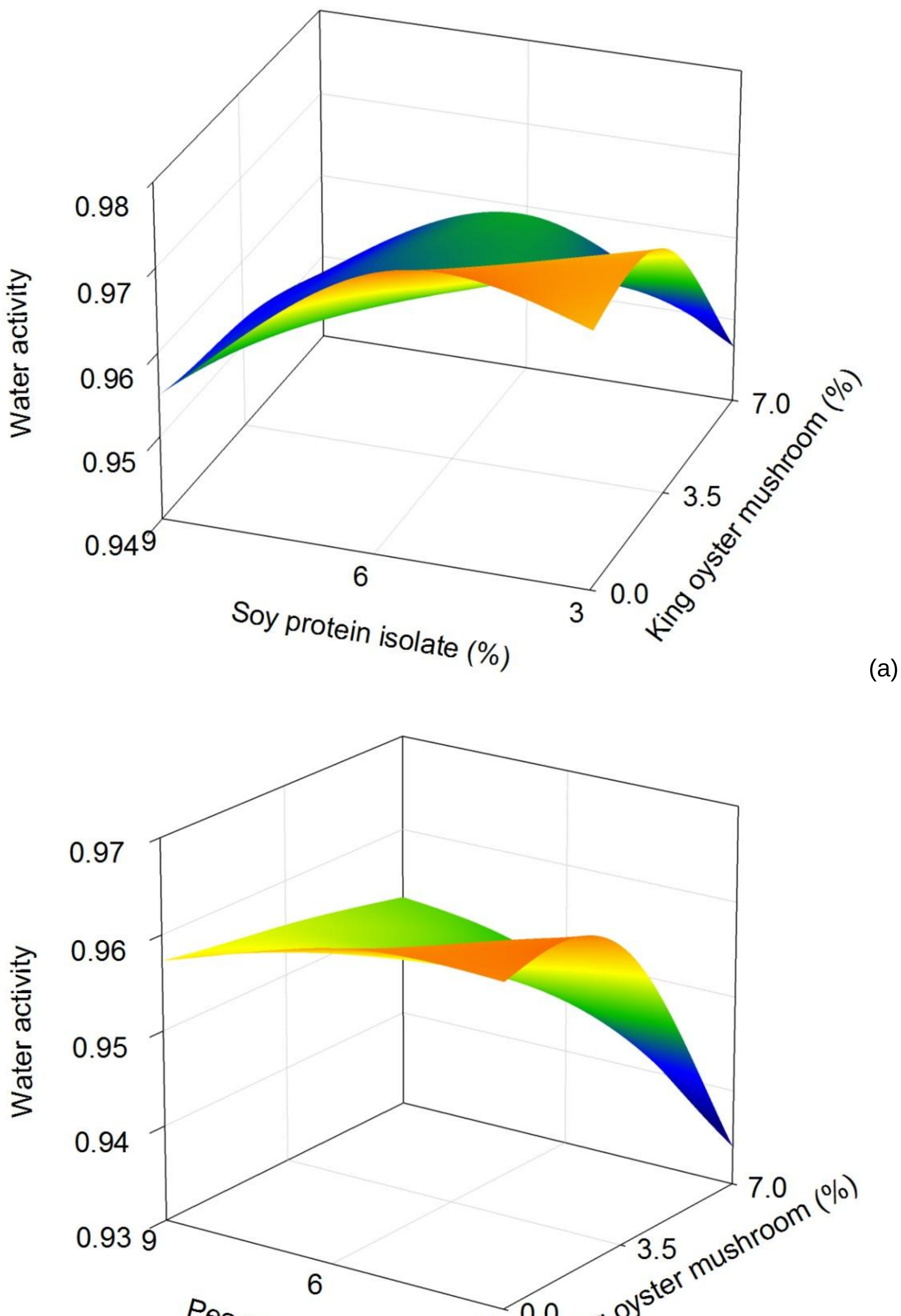


Fig. 4.2.4.1 Average values of water activity for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.4.1 Water Activity of Soy- and Pea-based Imitation Products

According to the results from water activity measurement, neither soy-based (Fig. 4.2.4.1 (a); Appendix 6 (a)) nor pea-based (Fig. 4.2.4.1 (b); Appendix 6 (b)) imitation sausage patties were differed significantly ($p>0.05$) dependently.

The 9%soy protein isolate group presented a significantly decreased ($p<0.05$) in water activity, not shown in figures, but showed no significant differences ($p>0.05$) within the three pea protein isolate groups. All three of the king oyster mushroom groups did not pose significant differences ($p>0.05$) in both soy-based and pea-based imitation sausage patties. Soy protein isolates probably played a slightly influential role in the pH value of soy-based imitation sausage patties.

Generally, all data located around 0.930 to 0.975 water activity, indicate a relatively low stability and lack of safety under improper storage and consumer abuse. Therefore, water activity serves as a reference to the labeling instructions on the package and storage temperature requirement in retail stores or in the home.

4.2.5 Effect on pH value of Plant Protein-based Imitation Products

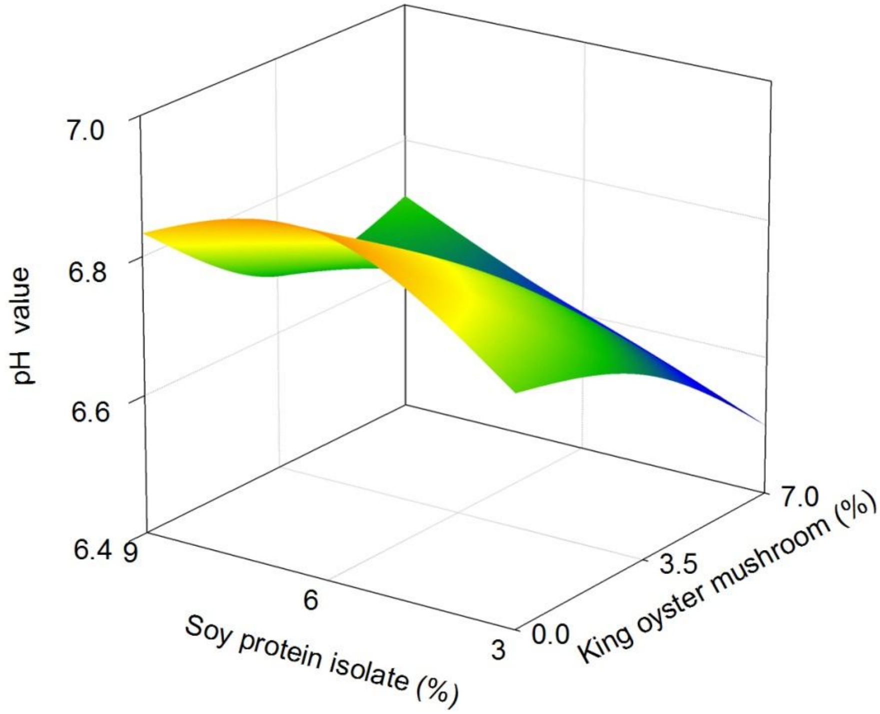
4.2.5.1The pH value of Soy- and Pea-based Imitation Products

The pH value of soy-based (Fig. 4.2.5.1 (a); Appendix 7 (a)) and of pea-based (Fig. 4.2.5.1(b); Appendix 7 (b)) imitation sausage patties did not present a significant difference ($p>0.05$) in dependent interaction. Though, soy-based imitation sausage patties had a lower average pH and had similar trends, as different levels of king oyster mushrooms and soy or pea protein isolates mixed, to pea-based imitation sausage patties.

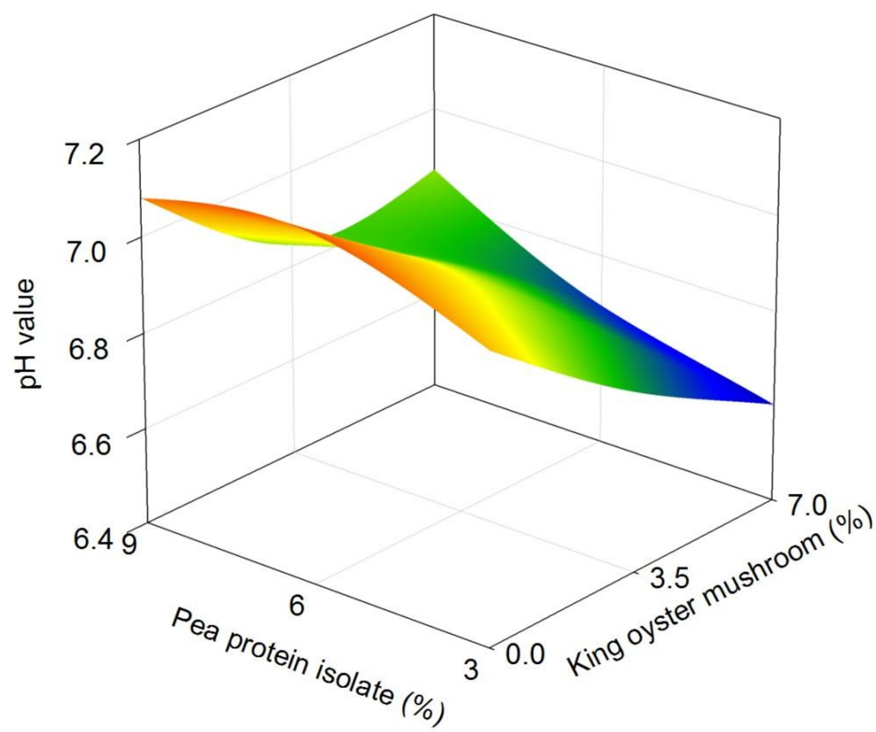
On independent point of view, not shown in the figures relates to the fact that-three groups of soy protein isolates or of pea protein isolates did not affect the pH significantly ($p>0.05$), while of the king oyster mushrooms did influence the pH significantly ($p<0.05$).

Addition of soy protein isolates did not increase the pH significantly compared to control samples (Porcella and others 2001); on the other hand, adding king oyster mushrooms did run down the pH value significantly ($p<0.05$). Though Ahmad and others (2010) showed pH slightly affected by SPI in buffalo meat emulsion sausage.

Concerning the food safety point, both soy-based and pea-based imitation sausage patties are subjected to microorganisms, such as mold, yeast and bacteria, easily due to the pH value deterioration under proper temperature and consumer abuse.



(a)



(b)

Fig. 4.2.5.1: Average values of pH value for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

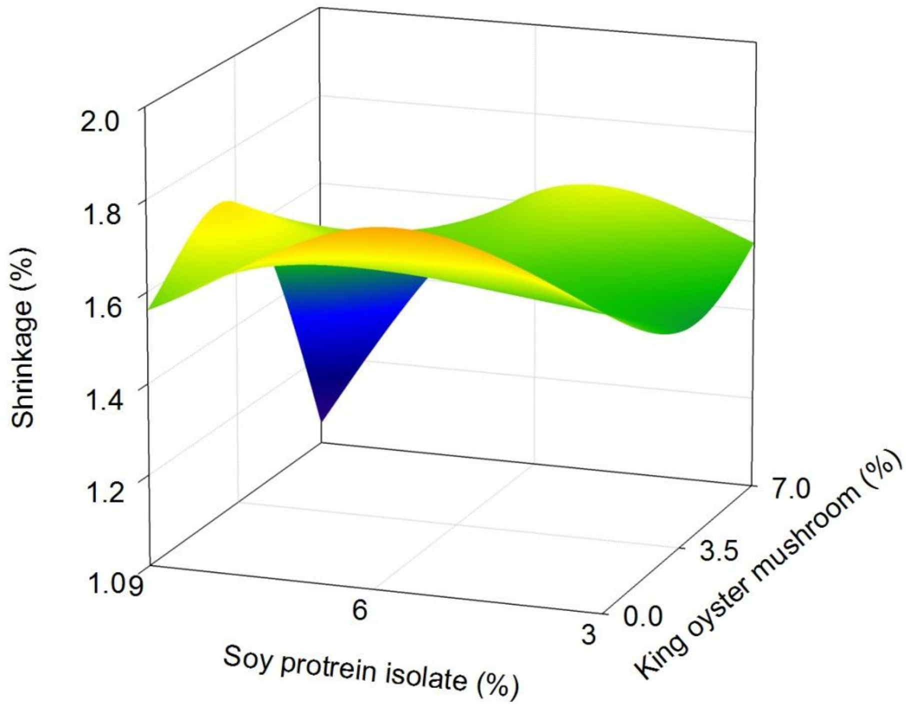
4.2.6 Effect on Cooking Shrinkage of Plant Protein-based Imitation Products

Cooking treatment usually induces liquid and soluble materials loss in meat products, known as the cooking loss. Heat-induced protein denaturation occurs when heating products, causing less water to be entrapped within the structure. Serdaroglu and Degirmencioglu (2004) reported that fat level affected the shrinkage of hamburger and recommended decreasing fat levels. Figure 4.2.6.1 and Appendix 8 show the shrinkage percentage of soy-based (Fig. 4.2.6.1 (a); Appendix 8 (a)) and pea-based (Fig.4.2.6.1 (b); Appendix 8 (b)) imitation sausage patties.

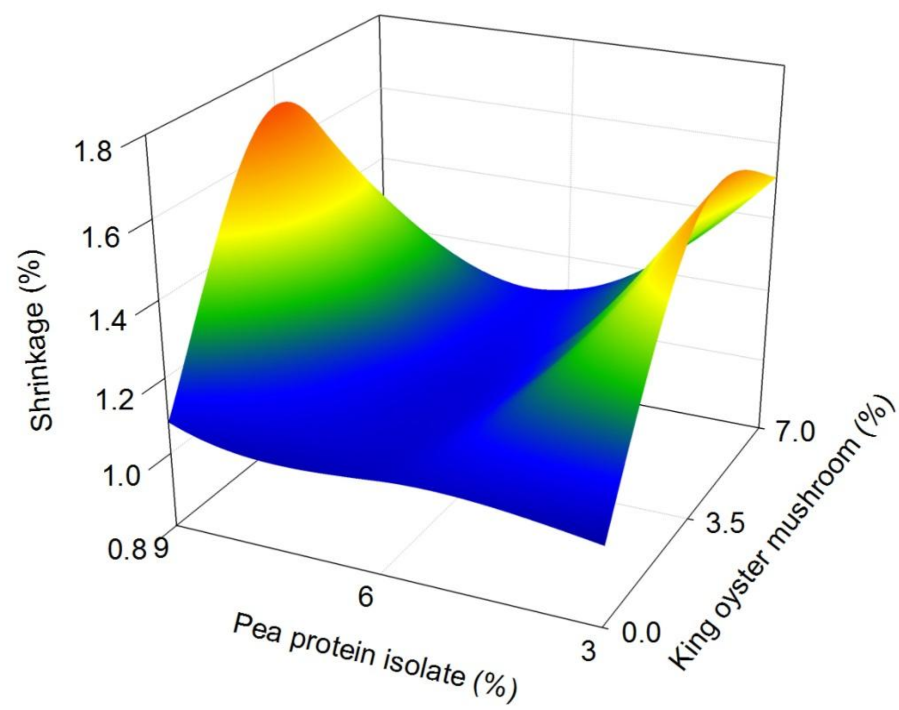
4.2.6.1 Cooking Shrinkage of Soy-based Imitation Products

No significant differences ($p>0.05$) were recorded for either both soy-based or pea-based imitation sausage patties, indicating that the diameter did not reduce or defect dramatically due to the addition of soy or pea protein isolates and king oyster mushrooms.

Many researchers (Bater and others 1992; Kotula and Berry 1986; Carballo and others 1995; Shewry and Tatham 2000; Gujral and others 2002; Candogan and Kolsarici 2003; Kumar and Sharma 2004; Akesowan 2010; Tabarestani and Tehrani 2012) have reported anti-shrinkage ingredients, including textured soy or pea proteins, soy or pea protein isolates, carrageenans, potato starches and egg whites, which when applied in this research were able to prevent shrinkage.



(a)



(b)

Fig. 4.2.6.1: Average values of cooking shrinkage percentage for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

4.2.7 Effect on Water Holding Capacity (WHC) of Plant-protein-based Imitation Products

Protein is well-known for its ability to hold oil and water and form a stable emulsion due to the lipophilic and hydrophilic groups in the same polymer chain. Physical and chemical properties, such as size, shape, amino acid composition and sequence, net charges, hydrophobicity to hydrophilicity ratio, structures, molecular flexibility and the ability to interact with other components, govern protein functionality. Hydrodynamic and surface-related properties render the functionality of protein. For imitation sausage patties, protein hydration, solubility, emulsification and gelation are the most important characteristics needed to qualify the stability and acceptance of end products. However, owing to the product's required heat procedure of product, these characteristics can undergo functionality changes throughout the heating processes. The three-dimensional structure of proteins can be altered by denaturation, leading to changes of water holding capacity for plant proteins like soy, pea and others (Egbert and Payne 2009; Brewer 2012). Textured vegetable protein also contributes greatly toward water holding capacity because of their fibrous structure and capacity to absorb liquid.

From the preliminary test, water holding capacity of textured soy protein and textured pea protein was 2.72 and 0.68, respectively. Higher value is linked with greater water holding capacity of the sample. Soy-based (Table 4.2.7.1 (a)) and pea-based (Table 4.2.7.1 (b)) shows the water holding capacity (WHC) of imitation sausage patties.

Table 4.2.7.1: Average values of water holding capacity for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	0.75^{A}_{a}	0.49^{A}_{b}	0.37^{A}_{c}
6	0.64^{B}_{a}	0.56^{B}_{b}	0.27^{B}_{c}
9	0.49^{C}_{a}	0.34^{C}_{b}	0.31^{AB}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%) [1]		
	0	3.50	7
3	0.58^{A}_{a}	0.48^{A}_{b}	0.41^{A}_{b}
6	0.52^{A}_{a}	0.42^{A}_{b}	0.36^{A}_{b}
9	0.51^{A}_{a}	0.35^{B}_{b}	0.21^{B}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample means with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

4.2.7.1 Water Holding Capacity (WHC) of Soy-based Imitation Products

From Table 4.2.7.1 (a), water holding capacity of 0% and 3.5% king oyster mushroom group significantly decreased ($p<0.05$) as increased amounts of soy protein isolates were added, indicating that the three dimensional structure weaken when the amount of potato starch decreased. For the 7% king oyster mushroom group, water holding capacity show a significant decrease ($p<0.05$) only between 3% and 6% soy protein isolates mixed.

With increased amounts of king oyster mushrooms, all three groups of soy protein isolate show significantly decreased ($p<0.05$) water holding capacity.

Good water holding capacity needs the efforts of high level potato starch (Carballo and others 1995; Shewry and Tatham 2000) to strengthen the tertiary structure; and soy protein isolates play a minor role in boosting water holding capacity of soy-based imitation sausage patties. Pietrasik and Duda (2000) reported lower fat content is usually accompanied by a significant reduction in WHC Das and others (2008) reported nuggets with soy paste had significantly higher expressible water content, indicating a lower WHC than that of the control groups and nuggets with soy granules had higher water content due to the protein and water interaction. A slightly different scenario was set up by other researchers (Kotula and Berry 1986; Ahmad and others 2010; Tabarestani and Tehrani 2012), who applied soy proteins in low- or reduced-fat meat products to improve WHC; meanwhile carrageenan utilized to enhance water holding capacity successfully of fat reduction meat products (Egbert and others 1991; Barbut and Mittal 1992; Bater and others 1992; Trius and Sebranek 1996; Candogan and Kolsarici 2003; Kumar and Sharma 2004; Verbeken and others 2005; Ayadi and others 2009).

4.2.7.2 Water Holding Capacity (WHC) of Pea-based Imitation Products

Table 4.2.7.1 (b) presents the water holding capacity of pea-based imitation sausage. In contrast to the 0% king oyster mushrooms group of soy-based imitation sausage patties, water holding capacity did not differ significantly ($p>0.05$) as pea protein isolates were increased. This means the ratio of potato starches and pea protein isolates did not pose great influence on the ability of vegetable structures to retain water. For 3.5% and 7% king oyster mushrooms groups, water holding capacity lowered significantly ($p<0.05$) as 9% pea protein isolates were incorporated. This research also reported a significant decrease ($p<0.05$) between 0% king oyster mushroom and additional king oyster mushroom combination in all three pea protein isolate groups.

Water holding capacity due to high level potato starches (Carballo and others 1995; Pietrasik 1999; Shewry and Tatham 2000) strengthened the structure of soy-based imitation sausage patties; however, the WHC was not noticeable when altering the ratio of potato starches and pea protein isolates. Other researchers have reported that pea proteins improve the WHC of low-fat products (Tabarestani and Tehrani 2012; Osen and others 2014). Other research has confirmed that pea proteins improve the water holding capacity of low-fat products. Research has also shown how carrageenan enhances the ability of food mixtures to retain water (Egbert and others 1991; Barbut and Mittal 1992; Bater and others 1992; Trius and Sebranek 1996; Candogan and Kolsarici 2003; Kumar and Sharma 2004; Verbeken and others 2005; Ayadi and others 2009). Though carrageenan used in this research did not show as excellent WHC as in low-fat meat products.

4.2.7.3 Further Discussion on Water Holding Capacity (WHC)

From a broad point of view, structures were strengthened from rehydrated textured soy or pea proteins, potato starch and carrageenan but they were slightly weakened as soy or pea protein isolates and king oyster mushrooms added.

Although previous studies utilized soy or pea protein to enhance the ability of materials to hold water in reduced- or low-fat meat products, additional soy or pea protein isolates in this study did not improve the water holding capacity after cooking. King oyster mushrooms could absorb the water and provide chewy mouth-feel of imitation sausage patties; nevertheless, it somehow creates “holes” for water to be forced out during heat treatment. All the data were above zero. Although not high, the data showed that imitation sausage patties still had a fair ability to retain water against heat-induced mass loss.

CHAPTER 5

CONCLUSIONS

5.1 Conclusions

Physicochemical properties can change when adding different levels of plant protein isolates and king oyster mushrooms to imitation sausage patties; therefore, cooking properties, color measurement, texture profile analysis, pH value, water activity, shrinkage percentage and water holding capacity were considered to evaluate the contributions of the variances. Cooking properties, cooking yield or cooking loss did not differ significantly ($p>0.05$) in most of formulations as plant protein isolates were increased; however, soy-based imitation sausage patties presented significantly higher ($p<0.05$) average cooking yield than pea-based one. King oyster mushrooms decreased the cooking properties of imitation sausage patties as a result. Color index, L, *a* and b value, of imitation sausage patties were influenced by the ingredients. Lightness and yellowness, L and b, increased as soy protein isolates or pea protein isolates were added independently ;and did not influenced the meatless sausage dramatically when king oyster mushrooms were incorporated independently. Redness, *a* value, changed greatly as increased king oyster mushroom amounts and highest levels of soy protein isolates or pea protein isolates were added together. Highest hardness occurred in the highest percentage of soy protein isolates and king oyster mushrooms formulation and in the lowest percentage of pea protein isolates and king oyster mushrooms formulation. Specifically, soy protein isolates interaction with king oyster mushrooms somehow increased ($p<0.05$) the hardness, while pea protein isolates and king oyster mushrooms alone decreased ($p<0.05$) the hardness. Adhesiveness had no significant ($p>0.05$) influence on imitation sausage patties. Greatest springiness appeared when highest soy protein isolates amounts and fair-to-highest king oyster mushrooms levels interacted together, and when lowest levels of pea protein isolate and lowest amounts of king oyster mushrooms interacted together. Cohesiveness reached highest value as highest soy protein isolates amounts and fair to highest king oyster mushrooms levels interacted together, and as lowest levels of pea protein isolates and lowest amounts of king oyster mushrooms interacted together. Gumminess, chewiness and resilience showed highest value as the highest levels of soy protein isolates and medium to highest amounts of king oyster mushroom combinations worked together; and as lowest levels of pea protein isolates and lowest levels of king oyster mushrooms interacted together. Water activity did not differ significantly ($p>0.05$) dependently. Nevertheless, from an independent point of view, the highest amounts of soy protein isolates had lower ($p<0.05$) than average water activity; pea protein isolates and king oyster mushrooms had no relationship with the values. The pH values did not differ significantly ($p>0.05$) dependently. Cooking shrinkage did not differ significantly in all soy- and pea-based formulations. Increased levels of king oyster mushrooms led to a declined trend of water holding capacity. Increased soy protein isolates or pea protein isolates rendered lower water holding capacity of imitation sausage patties due to less potato starches to build a compacted structure.

5.2 Further Research

Future development could conduct a microbiological, organoleptic and nutritional analysis to further understand the storage stability and market acceptance of imitation sausage patties. Further research should use other legume or plant sources as ingredients to enhance the textural properties and water holding capacity, which are promising alternative approaches to open up a wider range of gluten-free, soy-free and GM-free vegetarian products to meet the various consumer demands. Other further study could use different cooking treatments such as steam cooking, deep frying, baking or grilling to optimize best mouth-feel and physicochemical properties of imitation sausage patties.

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Appendices:

1. Average values of L value for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	39.61^{A}_{a}	37.55^{A}_{a}	37.31^{A}_{a}
6	43.20^{A}_{a}	42.92^{A}_{a}	41.55^{A}_{a}
9	44.87^{A}_{a}	40.68^{A}_{a}	39.44^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	41.40^{A}_{a}	43.64^{A}_{a}	43.07^{A}_{a}
6	44.79^{A}_{a}	45.41^{A}_{a}	43.51^{A}_{a}
9	46.28^{A}_{a}	46.42^{A}_{a}	44.70^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

2. Average values of b value for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	10.84^{A}_{a}	12.02^{A}_{a}	11.41^{A}_{a}
6	12.68^{A}_{a}	13.60^{A}_{a}	13.76^{A}_{a}
9	13.97^{A}_{a}	14.01^{A}_{a}	13.48^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	13.77^{A}_{a}	14.85^{A}_{a}	14.92^{A}_{a}
6	15.04^{A}_{a}	15.62^{A}_{a}	15.92^{A}_{a}
9	16.24^{A}_{a}	16.65^{A}_{a}	16.72^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

3. Average values of adhesiveness for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	-0.394^{A}_{a}	-0.814^{A}_{a}	-1.959^{A}_{a}
6	-0.976^{A}_{a}	0.000^{A}_{a}	-0.991^{A}_{a}
9	-0.179^{A}_{a}	-0.515^{A}_{a}	-1.093^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	-0.987^{A}_{a}	-0.811^{A}_{a}	-2.020^{A}_{a}
6	-2.409^{A}_{a}	-1.562^{A}_{a}	-0.543^{A}_{a}
9	-0.229^{A}_{a}	-0.784^{A}_{a}	-1.266^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

4. Average values of cohesiveness for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	0.465^{A}_{a}	0.371^{A}_{b}	0.326^{A}_{c}
6	0.379^{B}_{a}	0.328^{B}_{b}	0.330^{A}_{b}
9	0.418^{C}_{a}	0.433^{C}_{a}	0.523^{B}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	0.590^{A}_{a}	0.505^{A}_{b}	0.443^{A}_{c}
6	0.517^{B}_{a}	0.435^{B}_{b}	0.386^{B}_{c}
9	0.415^{C}_{a}	0.357^{C}_{b}	0.275^{C}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

5. Average values of resilience for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	0.181^{A}_{a}	0.137^{A}_{b}	0.117^{A}_{c}
6	0.140^{B}_{a}	0.116^{B}_{b}	0.113^{A}_{b}
9	0.153^{B}_{a}	0.158^{C}_{a}	0.209^{B}_{b}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	0.270^{A}_{a}	0.216^{A}_{b}	0.179^{A}_{c}
6	0.207^{B}_{a}	0.165^{B}_{b}	0.143^{B}_{c}
9	0.153^{C}_{a}	0.125^{C}_{b}	0.094^{C}_{c}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

6. Average values of water activity for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	0.971^{A}_{a}	0.969^{A}_{a}	0.947^{A}_{a}
6	0.973^{A}_{a}	0.960^{A}_{a}	0.959^{A}_{a}
9	0.955^{A}_{a}	0.953^{A}_{a}	0.948^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	0.963^{A}_{a}	0.961^{A}_{a}	0.934^{A}_{a}
6	0.963^{A}_{a}	0.955^{A}_{a}	0.948^{A}_{a}
9	0.957^{A}_{a}	0.955^{A}_{a}	0.953^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

7. Average values of pH value for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	6.73^{A}_{a}	6.67^{A}_{a}	6.50^{A}_{a}
6	6.89^{A}_{a}	6.76^{A}_{a}	6.60^{A}_{a}
9	6.83^{A}_{a}	6.68^{A}_{a}	6.72^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	7.01^{A}_{a}	6.78^{A}_{a}	6.60^{A}_{a}
6	7.12^{A}_{a}	6.91^{A}_{a}	6.69^{A}_{a}
9	7.08^{A}_{a}	6.85^{A}_{a}	6.87^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

8. Average values of cooking shrinkage (%) for each combination of king oyster mushrooms and soy protein isolates (a); and pea protein isolates (b).

(a)

Soy protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7
3	1.65^{A}_{a}	1.48^{A}_{a}	1.55^{A}_{a}
6	1.78^{A}_{a}	1.55^{A}_{a}	1.62^{A}_{a}
9	1.56^{A}_{a}	1.68^{A}_{a}	1.05^{A}_{a}

[1] The percentage of soy-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

(b)

Pea protein isolate (%)[1]	King oyster mushroom (%)[1]		
	0	3.50	7.00
3	1.01^{A}_{a}	1.62^{A}_{a}	1.51^{A}_{a}
6	1.05^{A}_{a}	1.09^{A}_{a}	1.10^{A}_{a}
9	1.08^{A}_{a}	1.71^{A}_{a}	1.08^{A}_{a}

[1] The percentage of pea-based imitation sausage patties.
Sample mean with at least one superscript letter in common among columns or subscript letter in common among rows (within the same box) are not significantly different at 5% level by Fischer's protected LSD test.

9. The formulations of soy-based (a) and pea-based (b) imitation sausage patties.

(a)

Soy-based products	0,3	0,6	0,9	3.5,3	3.5,6	3.5,9	7,3	7,6	7,9
TSP	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
Hot water	16.88	16.88	16.88	16.88	16.88	16.88	16.88	16.88	16.88
KOM	0.00	0.00	0.00	1.58	1.58	1.58	3.15	3.15	3.15
Corn oil	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Fresh egg white	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	1.22
SPI	1.35	2.70	4.05	1.35	2.70	4.05	1.35	2.70	4.05
Carraggenan	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Potato starch	7.97	5.27	2.57	5.72	3.02	0.32	3.47	0.77	0.00
Salt	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Seasioning	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Onion powder	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Garlic powder	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Water	6.08	7.43	8.78	6.75	8.10	9.45	7.43	8.78	10.13

(b)

Pea-based products	0,3	0,6	0,9	3.5,3	3.5,6	3.5,9	7,3	7,6	7,9
TPP	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
Hot water	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13	10.13
KOM	0.00	0.00	0.00	1.58	1.58	1.58	3.15	3.15	3.15
Corn oil	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Fresh egg white	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15
PPI	1.35	2.70	4.05	1.35	2.70	4.05	1.35	2.70	4.05
Carraggenan	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Potato starch	11.57	8.87	6.17	9.32	6.62	3.92	7.07	4.37	1.67
Salt	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Seasioning	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Onion powder	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Garlic powder	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Water	9.23	10.58	11.93	9.90	11.25	12.60	10.58	11.93	13.28

All the components are in gram of soy- or pea-based imitation sausage patties (total weight: 45g of each sample).
The first number in the first rows means the percentage of king oyster mushrooms and the second number in the first rows means the percentage of soy or pea protein isolates.
TSP, textured soy proteins; TPP, textured pea proteins; SPI, soy protein isolates; PPI, pea protein isolates; KOM, king oyster mushrooms.
Hot water was used to rehydrate TSP or TPP.
Seasoning includes sugar, soy sauce and sesame oil.
The first number in the first rows means the percentage of king oyster mushrooms and the second number in the first rows means the percentage of soy or pea protein isolates.

10. Proximate analysis of soy- (a) and pea-based (b) imitation sausage patties.

(a)

Soy-based products	0,3	0,6	0,9	3.5,3	3.5,6	3.5,9	7,3	7,6	7,9
Protein(g)	4.89	6.23	7.39	5.12	6.28	7.44	5.17	6.33	7.30
Carbohydrate (g)	22.68	20.42	18.17	20.92	18.67	16.42	19.13	16.92	16.26
Dietary fiber (g)	1.32	1.32	1.32	1.37	1.37	1.37	1.42	1.42	1.42
Fat (g)	4.58	4.63	4.81	4.72	4.81	4.80	4.72	4.76	4.72
Ash(g)	0.84	0.89	0.94	0.82	0.87	0.93	0.81	0.86	0.92

(b)

Pea-based products	0,3	0,6	0,9	3.5,3	3.5,6	3.5,9	7,3	7,6	7,9
Protein (g)	4.92	6.29	7.48	6.69	7.71	8.92	6.64	7.79	8.98
Carbohydrate (g)	17.22	16.98	16.74	17.12	16.88	16.64	17.02	16.78	16.54
Dietary fiber (g)	1.32	1.32	1.32	1.37	1.37	1.37	1.41	1.41	1.41
Fat (%)	4.57	4.61	4.65	4.58	4.62	4.66	4.60	4.61	4.65
Ash(g)	0.84	0.87	0.91	0.82	0.86	0.90	0.81	0.85	0.89

Data was calculated based on the following proximate data table and the weight of soy- and pea-based imitation sausage patty is 45 gram of each.
The first number in the first rows means the percentage of king oyster mushrooms and the second number in the first rows means the percentage of soy or pea protein isolates.
Data was calculated by Genesis R&D Product Development and Labeling Software (Elizabeth Stewart Hands and Associates Inc., version 9.7.0, Salem, OR, USA).

Proximate data.

	TSP	TPP	KOM	Corn oil	Fresh egg white	SPI	PPI	Carrageenan
Protein (g)	50	50	3	0	9.8	86	88	0
Carbohydrate (g)	32	32	8	0	1.05	0	2	90
Dietary fiber (g)	18	18	3	0	0	0	0	30
Fat (g)	3	3	1	100	0	4	3	0
Ash (g)	6	6	0	0	0.6	5	4	0

	Salt	Sugar	Soy sauce	Sesame oil	Onion powder	Garlic powder	Potato starch
Protein (g)	0	0	6.67	0	9.5	15.3	0
Carbohydrate (g)	0	100	33.33	0	82	74.1	83.3
Dietary fiber (g)	0	0	0	0	17.3	17.9	0
Fat (g)	0	0	0	93.3	0.4	4	0
Ash (g)	99.8	0.5	17.78	0	3.98	3.91	0.5

TSP, textured soy proteins; TPP, textured pea proteins; SPI, soy protein isolates; PPI, pea protein isolates; KOM, king oyster mushrooms.
The data was based on 100 gram of each ingredient.