# Effects of Food Ingredients and Food Additives to the Texture of Plant-based Ingredients Meat Balls

By

Ms. Pavita Olakit

6219604

A thesis summited to Theophane Venard School of Biotechnology, Assumption University in part of fulfillment of the Requirements for the Degree of Master of Science in Food Biotechnology

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Pavita Olakit

# Abstract

Developing plant-based protein products has become a challenge for the food industry. Many have tried but the acceptance in mimicking meat texture is still an issue. To modify the texture of protein, transglutaminase (TG, EC 2.3.2.13) has been widely introduced to food production industry and plays important role in improving texture of protein by improving water-binding property of protein. The effect of employing transglutaminase at different concentration and using a variety of plant-based ingredients to the texture of plant-based protein meatballs were investigated. The usage of transglutaminase at different concentrations (0.1%, 0.2%, and 0.3%) were applied to soy protein meatballs to select an appropriate concentration of enzyme. Then plant-based protein meatballs treated with transglutaminase were studied for the textural characteristics and color analysis when incorporated with various plant-based ingredients including soy protein, mung bean protein, pea protein, konjac, unripen jackfruit, and cauliflower. Selected plant-based ingredient including soy protein isolate, mung bean protein, konjac, and cauliflower had been using to formulate plant-based ingredient meat balls using design of experiment. Statistical analysis from texture analysis (TPA) of 0.2% and 0.3% transglutaminase showed no significant differences but affected significantly when decreasing concentration to 0.1% ($p \leq 0.05$). Treating at 0.2% was selected due to its high potential and cost effectiveness. The plant-based protein meatballs were evaluated for texture on hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience by using texture analyzer. Hardness, cohesiveness, chewiness, and resilience showed significantly difference between different plant-based sources ($p \leq 0.05$) but no significant difference found in adhesiveness. After formulated plant-based ingredient using mixture design, the formulation which contained 10% soy protein isolate, 7.5% cauliflower, and 5% mung bean protein showed great amount of adhesiveness, springiness, cohesiveness, chewiness, and resilience. Also, this formulation obtained the highest liking score of appearance, color, texture, overall taste, and overall liking in sensory test. Not only that, this formulation received the highest preference at 65.4%. Therefore, the plant-based ingredients to be mixed in the plant-based products should be selected according to the food application.

**Keywords:** Plant-based protein, Transglutaminase (TG, EC 2.3.2.13)

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# Introduction

Protein is an important nutrient for the human body which contribute to the muscle building and repairing of damaged tissues. Human bodies should gain an appropriate amount of protein in order to function the bodies efficiently. In general, protein can be obtained from natural sources of protein such as meat, eggs, and some type of plants. However, people tend to change their lifestyles in these recent years. Meat consumption shows a decrease due to many reasons including diet, following doctor's order, social concerning on animal's welfare, religious beliefs, the believes that consuming animal's meat cause the diseases, or social media forces which push the customers to consume clean meal rather than meat. In addition, Thailand is now an aging society which consist of population having problem with the digestive system or facing the hardness of chewing. This group of people tends to consume less meat.

Nowadays, many food suppliers put their interest in this group of people, so the suppliers start to produce food products that do not contain meat but give the protein contents comparable to the meat, known as Faux meat or alternative meat. Recently, several food companies acquire brands to get prime products in the alternative protein space and launch a variety of new products from plant-based burgers to nuggets. More than 16 billion dollars have been invested in U.S. plant-based meat companies in the past 10 years and 13 billion dollars from this amount was spent in 2017 and 2018 (Byington, 2020). Alternative meat trends are predicted to rise up to 240 billion in a further 20 years (Bloomberg, 2019). The main ingredients or main sources of protein for alternative meat will be plant proteins such as legume group including soybean, pea, bean or mung bean. Grains are one of the important sources such as wheat, barley, or rye, which contained special plant protein called gluten. Gluten's structure is quite similar to animals' muscle structure. Thus, it had been used in various types of food products in foreign countries. Even though alternative meat seems to be popular in Western countries, it has just started to gain popularity in Asian countries. It is rarely found in the market or can be found only in the vegetarian festival period. Furthermore, people who avoid meat consumption or have allergy to legume consumption might be susceptible to deficiency in essential amino acids.

In Thailand, alternative meat or plant-based protein foods offer a few varieties of products. Thai consumers are only familiar with tofu and textured vegetable protein produced from soybeans. Therefore, developing food products into Asian styles food such as fish ball without using meat as the ingredients. Moreover, this product also can add the value to the agricultural product of Thailand. But to produce acceptable taste and texture in products from plant-based proteins is still a challenge.

To modify the texture of protein, transglutaminase enzyme (transglutaminase, TG, EC 2.3.2.13) has been widely introduced to food production industry and plays important role in improving texture of protein by improving water-binding property of protein. Transglutaminase enzyme is mostly used to improve protein-containing food's texture especially in food products including dairy products, fishery products, meat products, bread, bakery products, noodles, pasta, and tofu. The products that treated by transglutaminase enzyme will have better elastic texture because the enzyme access through the structure and improve bonding of amino acid including glutamine and lysine in protein (Dube, 2007). Soy protein can be treated with a plant-based protein containing both lysine and glutamine. This enzyme can be used as the key to improve the texture of plant-based product.

With all above reasons, developing plant-based protein balls which have acceptable overall characteristic and sensory could be an alternative choice to respond the plant-based protein market that rapidly grows in recent years.

# Aim

To develop plant-based ingredients meat balls which have overall characteristics similar to fish ball and can be acceptable for consumers

# Objectives

1. To study the effect of different plant-based ingredients on characteristics of plant-based ingredients meat balls
2. To study the suitable process and formulation for producing plant-based ingredients meat balls
3. To develop plant-based ingredients meat balls prototype
4. To study physical characteristics of product
5. To study the sensory analysis of the product

# Literature Review

## 1. Meat Analogue or Alternative Meat

Meat analogue or alternative meat is the food product which has appearance, texture, and taste similar to meat but not containing meat as part of ingredients. Most of the meat analogue is made from soybean protein and grain protein or gluten but sometimes eggs protein, fish protein, or dairy can be added to make protein contents comparable to meat protein. Meat analogue is not only developed for vegetarians but also for a group of people who want to reduce the amount of meat consumption. Furthermore, acceptance in taste and texture of meat analogue still be the biggest challenge for food producers (Egbert and Borders, 2006). Thus, textured vegetable protein is normally used to produce a meat analogue to improve the texture both elastic, tenderness, and chewiness. Also, the meat analogue's flavor can improve by adding seasoning and flavor to have similar flavors as meat.

## 2. Plant-Based Ingredient Sources

### 2.1 Soy Protein

Soy protein has been used widely to make meat analogue since soy protein can normally be found in the market and also considered a popular choice in Thailand. Moreover, soy protein gives the texture of products quite similar to animal meat with 25-50% of protein content (Siddique, 2000). Soy protein contains more than 50% of protein but the protein content tends to decrease after the drying process. Soy protein can be classified into 2 groups including soy protein concentrate which contains protein up to 65% and soy protein isolate which contained up to 90% of protein (Kolar *et al.*, 2010). Nowadays, soy flour has been developed and contain protein of up to 50%. Different types of soy protein are suitable for different types of products depending on the decided properties.

Furthermore, heat-induced protein gel is one of the important structures of soy protein which plays an important role in the texture of the product. Glycinin and conglycinin in soy protein have a complex quaternary structure which can easily form gel in association-dissociation form. Conglycinin in soy protein has an unusual structure and can form gel polymer more than glycinin gel which mostly has double spirals structure. Not only that, soy protein can form gel better with salts in aggregated structure but the swollen characteristic of protein after spray drying will not be the same as normal protein gel (Hermansson, 1986).

## 2.2 Jackfruit Protein

Jackfruit is one of the interesting plant protein sources. Almost every part of the fruits can be used to substitute the animal protein. The jackfruits are rich in carbohydrates as well as protein and fiber. Especially, unripe fruit pulps have a smooth texture and tasteless which allow absorption of flavor. Also, cooked unripe jackfruit has similar texture to that of chicken which enable local people to use in local recipes. The use of unripe jackfruit in foods can increase the carbohydrates contents giving the energy with lower in calories comparing to beef. It also increases moisture content, thereby increase the juiciness of food products, and also fiber content (Abdullah, 2017).

Not only that, jackfruit seeds also can be used as a plant-based protein source. According to Kumar *et al.* (1988), jackfruit's seed flour contained 78% of carbohydrate and 11.2% of protein which is comparable to wheat flour. Also, the combination of jackfruit's seed flour and wheat flour in bread making showed good paste forming ability after passing heat treatment (Vanna *et al.,* 2002).

## 2.3 Mung Beans Protein

Mung beans are an agricultural product that normally found in Thailand. Mung beans contained 20-25% of protein including essential protein for human bodies except for methionine, cystine, and tryptophan. Mung beans are usually used for making glass noodles. The gelling property depends on the concentration of protein. The concentration of protein less than 8% cannot form heat-induced gel while 8-9% of protein can raise viscosity up but still cannot form gel. The least concentration for gel formation is 10% when heating at 80°C for 10 minutes before cooling down to 0°C. Moreover, the gel strength will be increased due to the higher amount of protein concentration (Coffmann & Garciaj, 2007).

## 2.4 Pea Protein

Pea is one type of plant which is usually used as an alternative protein. Pea contains 18-30% of protein content. The previous research on pea protein isolated resulting in 80.7% protein content and at least 19.6% of pea protein recovered after passing through 93°C heat at pH 7.1 with 2% salt which was the suitable condition for gel-forming (Shand *et al.*, 2007). These results showed the effect of concentration of protein, heat, and others including salt concentration on gel formation of pea protein.

## 2.5 Cauliflower

Cauliflower is now becoming one of the most interesting plant-based ingredient sources. Since cauliflower considered as low-carb vegetables, contains protein, and high in dietary fiber (U. S. Food and Drug Administration, 2019), so in recent food products selected cauliflower as one of ingredients to produce plant-based products. Not only that, cauliflower contains glucosinolate which is the sulfur derivative found in cruciferous vegetables. Glucosinolate can break down into active compounds including indoles, nitriles, thiocyanate, and isothiocyanates that act as antioxidant and anticancer (Johnson, 2002).

Moreover, cauliflower is great substitute for gluten-containing ingredients to including cereal, pasta, gnocchi, pizza, pretzel, etc. Also, cauliflower can be used as fat substitute in chicken nuggets and show no significantly difference from 100% chicken nuggets in terms of overall acceptability on sensory evaluation. (Ayman *et al.*, 2020)

## 2.6 Wheat Protein

Wheat is one type of grain which is widely used for a long period of time especially wheat flour which contains special protein, gluten. Gluten has a fibrous structure which extremely suitable for the bakery industry. It is also used to mimic alternative because it provides the texture that is similar to muscle protein. Gluten also contains high protein up to 75% and cost-effective (Day *et al.*, 2006). These reasons supported the widely use of wheat protein in food industry but the limitation of using wheat protein can be found. Many people cannot consume gluten due to allergy problem, as a result, some of food suppliers recently try to avoid using wheat protein.

Unfortunately, there is no other protein which has a structure similar to gluten and has only soy protein that has a structure quite resemble gluten.

**2.7 Mucuna Beans or Velvet Beans (เมล็ดหมามุ่ย)**

Mucuna beans or velvet beans (*Mucuna Pruriens*) belong to the legume group which is quite well known in Thailand. Mucuna beans have several medical benefits for example in the research of Kasetsart University in 2011. The authors found out that mucuna beans contained hormones and neurotransmitters including Catecholamines which can improve reproductive systems. But raw beans can cause an increase in the body's temperature, dermatitis, vomiting, and diarrhea.

Not only that, extracting mucuna beans protein at pH 4 can obtain protein up to 96% with have gel-forming property. Besides, pH affected the gel-forming property of mucuna beans protein. At pH 4, gel formation is stronger in its association form than at pH 2 and pH 10 (Adebowale, 2003).

**2.8 Mycoproteins**

Mycoprotein is the protein developed by Quorn company and obtained from the fungi, *Fusarium venenatum*. Mycoproteins have a fibrous structure similar to the animal's muscles because of mycelia structure in this fungus. Alternative protein from mycoproteins are mixed with binding agents and seasoning before cooking. After passing heat treatment, mycoproteins are going to change the structure then form gel and attached with hyphae of fungi resulting in texture and taste which similar to animal's meat. Nowadays, Quorn company has produced various types of products such as patties, nuggets, jerky, and minced alternative meat (Quorn company, 2020).

**3. Transglutaminase Enzyme**

Transglutaminase enzyme (EC 2.3.2.13) or protein-glutamine γ-glutamyltransferase is the enzyme in the transferase group which normally can be found in natural sources in animal tissues, plants, and microorganisms. This enzyme enhances the formation of isopeptide bonds between proteins. Transglutaminase enzyme improves the formation of isopeptide bonds between glutamine in the group of γcarboxamides side and ε-amine groups in the first order of proteins including lysine (Buettner *et al.,* 2012). The cross-linking can be formed by the bonding of polypeptide chains due to transferring of acyl onto a lysine residue. This cross-linking is the intramolecular cross-links of ε-(γ-Glu)Lys (Kashiwagi *et al.* 2002). In food industry, this enzyme has also been used to improve polymer structure which results in better texture, gel formation, and increase nutrition value to food products (Kieliszek & Misiewicz, 2013).

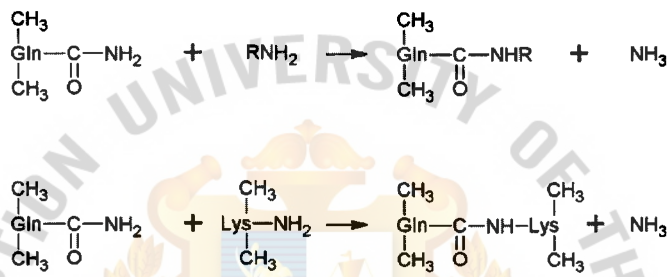


Figure 1: acyl transfer reaction (upper), cross-linking reaction between Gln and Lys residues of proteins or peptides (lower)

According to Soares *et al.* (2004), using transglutaminase enzyme with soy protein isolated can reduced protein solubility with help improve gel formation of protein. Furthermore, transglutaminase help improving gel formation by glycinin and β-conglycinin in soy protein isolate. Glycinin in soy protein is responsible for in fracturability, chewiness, and gumminess of gel while the β-conglycinin is accountable in the attachment of structure and gel recovery. Transglutaminase enzyme also improves gel formation by helping in covalent bonding between the hydrophobic side and H-bond and disulfide bonds which improve gel strength of glycinin. While β-conglycinin will be maintained by the hydrophobic side and H-bonding (Tang *et al.,* 2006). Not only that, using transglutaminase enzyme and maltodextrin with soy protein help in better gel formation due to glycosylation and also improve water holding capacity (Zhao & Luo, 2019).

## 4. Texture Profile Analysis

Texture profile analysis (TPA) is the texture analysis of the products using the probe which has a larger diameter than the product with compression force. The compression force will be pushed into the sample 2 times to mimic the human tooth when chewing. The texture profile analysis is normally used to determine the texture of food products such as meat, ham, sausage, butter, vegetables, fruits, tofu, and jelly in order to determine hardness, cohesiveness, gumminess, resilience, springiness and chewiness (Tathma *et al.*, 2019). Also, texture profile analysis results can be used corporately with sensory evaluation.

The results of texture profile analysis will come out as a graph between force in Newton (N) and time. The TPA graph can describe food texture with different parameter as shown in Figure 2.

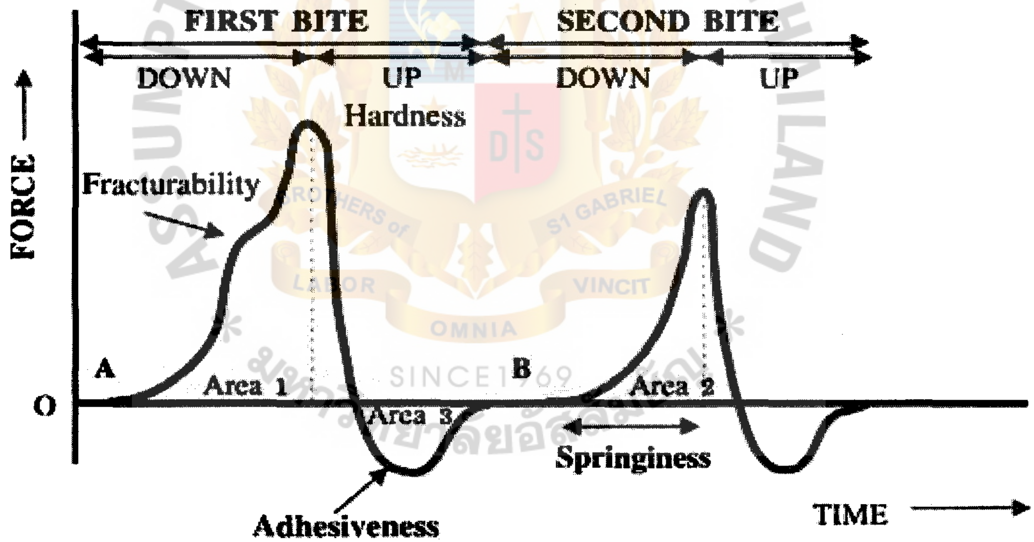


Figure 2: Texture Profile Analysis Graph

Fracturability comes out from the first force or first peak of the graph which can break the sample in the first bite or first compression. Sometimes fracturability can be called brittleness and the unit of this force is Newton (N).

Hardness is the first highest peak of the graph which resulting while compressing the sample that can compare to the first biting of human teeth. The unit of this force is Newton (N).

Cohesiveness is the attaching force in the product which can be calculated from the area under graph between first bite and second bite on the positive side. (Cohesiveness = Area 2/Area 1)

Gumminess is the force that can break down the semi-solid product which lows in hardness but high in cohesiveness. The gumminess can be calculated by multiply hardness value and cohesiveness value. (Gumminess = hardness x cohesiveness)

Springiness is the value that responds to the recovery ability of the product after deformation in the first time. Sometimes springiness can refer to the elasticity of the product. The springiness value can be described in many ways but popularly described in the ration of time or distance that product deform. The springiness value can be obtained by the highest compression of the product in the second turn.

The chewiness is the energy that use to chew the product and can be calculated by multiply hardness with cohesiveness and springiness.

Adhesiveness is work that a texture analyzer uses to pull back the probe which can compare to work those human teeth needed to remove from the food product. Adhesiveness can refer to the stickiness of the product and can be obtained from the area under graph between first bite and second bite on the negative side. The unit of adhesiveness value is N.s (Sirisomboon, 2006).

**5. Mixture Design**

Mixture design is an experimental design which is considered as one kind of factorial design. This experimental design is useful for formulation problems in order to determine the effect of ingredients as the result of factorial design cannot be used in the case of the ratio of ingredients. The mixture design is suitable for 3 or more ingredients that have an effect on each other. It is performed by changing the ingredient ratios when the sum of these ingredients ($x_1+x_2+x_3$) must be equal to 1.0 or 100%. Each ingredient will be scoped from 0 to 1. These ratios of ingredients can be plotted on the tri-linear coordinate system in figure 3 and the peak of each angle showed 100% of each ingredient.

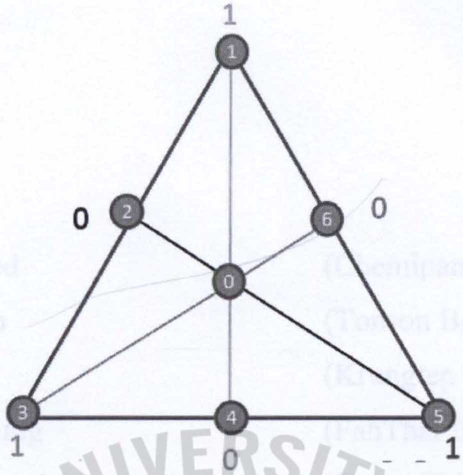


Figure 3: Tri-linear Coordinator Graph

However, the limitation of this design is the ingredients must be dry materials. If not, the ingredients must be studied in dry basis with mixture design prior to the real experiment (Tipvarakarnkoon, 2019).

# Materials and Methods

**Materials**

**1. Sample**

| | |
|---|---|
| - Soy protein isolated | (Chemipan Brand) |
| - Mung bean protein | (Tonson Brand) |
| - Konjac | (Krungtep Chemi Brand) |
| - Vegetarian Seasoning | (FahThai Brand) |
| - Salt | (Prung Thip Brand) |
| - Sugar | (Lin Brand) |
| - Tapioca flour | (Fish Brand) |
| - Soy bean oil | (Emerald Brand) |
| - Unripen jackfruit | |
| - Cauliflower | |
| - Transglutaminase Enzyme | |
| - Water | |

**2. Equipment**

The equipment was provided by Faculty of Biotechnology, Assumption University.

**3. Instrument**

- Kjedalh Machine
- Incinerator and Desiccator
- Texture Analyzer (TA-XT plus, Charpa Techcenter Co., Ltd)
- Colorimeter
- Refrigerator
- Food Processing Machine

## 4. Chemical Reagents

The chemical agents will be provided by Faculty of Biotechnology, Assumption University.

## 5. Sensory Evaluation

The sensory evaluation was done using plastic cups, plastic spoons, drinking water, questionnaire and pens. The sensory analysis was performed at sensory room (E52 at E building of Assumption University, Huamak Campus).

# Method

## 1. Optimization of Transglutaminase

### 1.1 Sample Preparation

The 22.5% of soy protein isolated was mixed with 1% salt and 1.6% sugar then slightly added 5.5% of soy bean oil while kneading with mixing machine. After that, warm water was slowly added in half amount then 5.2% of tapioca flour was added. After kneaded into paste, transglutaminase enzyme was added in different concentration including 0%, 0.1%, 0.2%, and 0.3%. Ingredients kneaded together until dough was formed then shaped the mixture into 7-gram balls before incubating at room temperature for 20 minutes. The plant-based meat balls were boiled in boiling water for 10 minutes and rapidly cooled down using cold water then drained for 15 minutes before using in further analysis.

### 1.2 Texture Profile Analysis

The samples were analyzed using texture analyzer (TA-XT plus, Charpa Techcenter Co.,Ltd). The P/100 probe was used to measure hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness of sample in TPA test. The analysis was done in quintuplicate and further analyzed using statistic method in SAS program.

## 2. Effect of Plant-based Ingredients on Plant-based Ingredient Meat Balls

### 2.1 Sample Preparation

The 12.5% of soy protein isolate and 10% of plant-based protein ingredients including mung beans protein, pea protein, konjac flour, unripen jackfruit, and cauliflower were mixed with 1.6% salt and 1% sugar differently each batch. Then slightly added 5.5% of soy bean oil while kneading with mixing machine. After that, warm water was slowly added in half amount then adding dissolved 5.2% of tapioca flour following by the rest amount of water. After kneading into paste, 0.2% transglutaminase enzyme was added. All ingredients were kneaded until dough was formed then shaped the mixture into 7-gram balls before incubating at room temperature for 20 minutes. The plant-based ingredients meatballs were boiled in boiling water for 10 minutes and rapidly cooled down using cold water then drained for 15 minutes before using in further analysis.

### 2.2 Texture Profile Analysis and Color Analysis

The samples were analyzed using texture analyzer (TA-XT plus, Charpa Techcenter Co.,Ltd). The P/100 probe was used to measure hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness of sample in TPA test. The analysis was done in quintuplicate and further analyzed using statistic method in SAS program.

After calibration, the colorimeter was used to measure the L*, a*, and b* for at least 5 points of sample. The measurement was done in quintuplicate.

## 3. Formulation of Plant-based Ingredients Using Design of Experiment

### 3.1 Formulation of Plant-based Ingredient Meat Balls Design of Experiment

The mixture design was used as the experimental design in order to formulate the plant-based protein balls. After selected suitable plant-based ingredients and preliminary study to find the limitation and properties of each plant-based ingredients meat balls, some of plant-based ingredient sources were eliminate. Limitation of cauliflower, mung beans, and konjac flour were used to formulate using mixture design.

Table 1: The plant-based ingredient meat balls formulation from mixture design

| Plan | Water | SPI | Oil | Tapioca | Seasoning | C | M | K |
|---|---|---|---|---|---|---|---|---|
| A | 64 | 10 | 5.5 | 5.2 | 2.6 | 7.5 | 5 | 0 |
| B | 64 | 10 | 5.5 | 5.2 | 2.6 | 5 | 7.5 | 0 |
| C | 64 | 10 | 5.5 | 5.2 | 2.6 | 5 | 5 | 2.5 |
| D | 64 | 10 | 5.5 | 5.2 | 2.6 | 5.84 | 5.84 | 0.81 |

Note: C is referred to Cauliflower, M is referred to Mung beans, and K is referred to Konjac flour

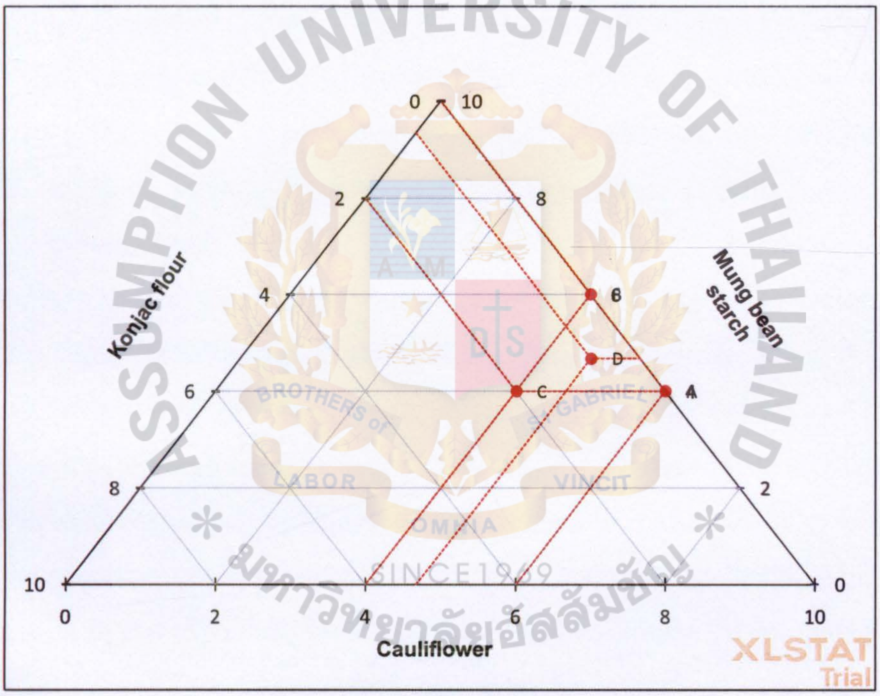


Figure 4: Tri-linear Coordinator Graph of Plant-based Protein Meat Balls

### 3.2 Texture Profile Analysis and Color Analysis

The samples were analyzed using texture analyzer (TA-XT plus, Charpa Techcenter Co.,Ltd). The P/100 probe was used to measure hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness of sample in TPA test. The analysis was done in quintuplicate and further analyzed using statistic method in SAS program.

After calibration, the colorimeter was used to measure the L*, a*, and b* for at least 5 points of sample. The measurement was done in quintuplicate.

### 3.3 Sensory Evaluation

One ball in each formulation was tested by each panelist. The 9-points hedonic score and most preference test was evaluated by 30 panelists. The color, texture, overall taste, and overall liking were used as attributes.

# Result and Discussion

**1. Effect of Difference Concentration of Transglutaminase on Texture of Plant-based Ingredient Meatballs**

In general, the transglutaminase was required at 0.1-0.3% in order to improve polymer structure which results in better texture, gel formation, and increase nutrition value to food products (Kieliszek & Misiewicz, 2013). The result showed in Table 1 pointed out that the concentration of transglutaminase used affected the texture of plant-based meatballs significantly in hardness, adhesiveness, springiness, chewiness, and resilience ($p \leq 0.05$) but did not show significantly difference on cohesiveness ($p \geq 0.05$). The hardness, springiness, chewiness, and resilience of plant-based ingredient meatballs increased with the increasing of concentration of transglutaminase. The improvement of texture after transglutaminase addition was due to the transglutaminase help improving gel formation by glycinin and β-conglycinin in soy protein isolate. Glycinin in soy protein is responsible for in fracturability, chewiness, and gumminess of gel while the β-conglycinin is accountable in the attachment of structure and gel recovery. Transglutaminase enzyme also improves gel formation by helping in covalent bonding between the hydrophobic side and H-bond and disulfide bonds which improve gel strength of glycinin. While β-conglycinin will be maintained by the hydrophobic side and H-bonding (Tang *et al.,* 2006). However, the adhesiveness of the plant-based ingredient meatballs decreased with the increasing of concentration of transglutaminase which can referred to more stickiness of the product. Although the adhesiveness of plant-based ingredient meatballs more sticky comparing to commercial fish balls, at 0.2% and 0.3% of transglutaminase showed the efficiency in providing the desirable texture in other parameters

Table 2: Texture Profile Analysis of Different Concentration of transglutaminase Used in Plant-based Ingredient Meatballs

| Concentration | Hardness | Adhesiveness | Springiness | Cohesiveness | Chewiness | Resilience |
|---|---|---|---|---|---|---|
| Commercial Fish balls | 4168.865±1219.82$^{a}$ | -0.254±0.28$^{a}$ | 0.857±0.15$^{a}$ | 0.825±0.03$^{a}$ | 3019.715±1115.35$^{a}$ | 0.530±0.05$^{a}$ |
| 0% | 1290.519±168.75$^{c}$ | -1.395±1.96$^{a}$ | 0.255±0.04$^{c}$ | 0.791±0.05$^{a}$ | 1560.203±424.61$^{b}$ | 0.281±0.03$^{c}$ |
| 0.1% | 2676.954±652.46$^{b}$ | -1.452±3.25$^{a}$ | 0.486±0.09$^{b}$ | 0.821±0.05$^{a}$ | 2134.037±680.31$^{ab}$ | 0.354±0.03$^{b}$ |
| 0.2% | 4076.924±1009.41$^{a}$ | -23.671±29.09$^{b}$ | 0.746±0.17$^{a}$ | 0.834±0.05$^{a}$ | 2536.037±1224.36$^{a}$ | 0.517±0.06$^{a}$ |
| 0.3% | 4236.958±353.33$^{a}$ | -26.700±12.67$^{b}$ | 0.776±0.16$^{a}$ | 0.851±0.02$^{a}$ | 2735.037±163.04$^{a}$ | 0.537±0.09$^{a}$ |

Remark: The same letter of superscript in each row referred to no significant difference

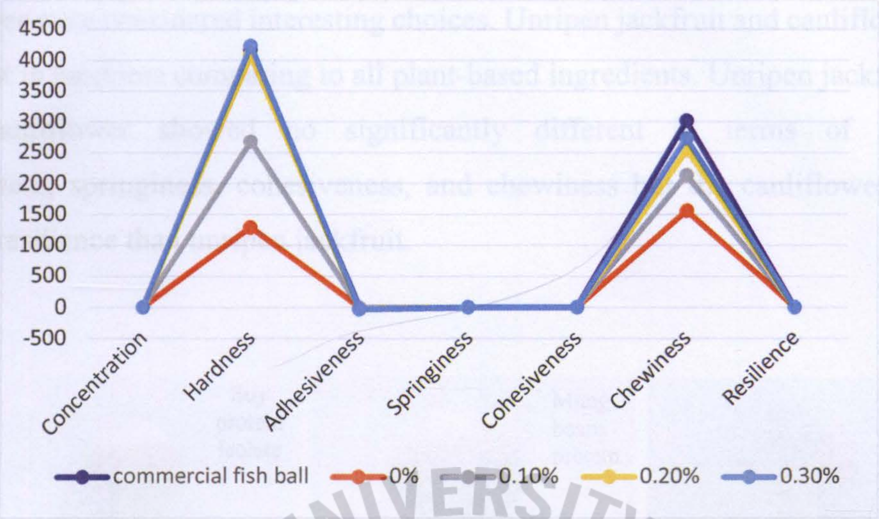


Figure 5: Texture Profile Analysis Graph of Different Concentration of transglutaminase Used in Plant-based Ingredient Meatballs

**2. Effect of Difference Plant-based Ingredients on Texture and Color of Plant-based Ingredients Meatballs**

Table 3 shows that different plant-based ingredients used affected the texture of plant-based ingredients meatballs differently in each attribute. Using only soy protein as the plant-based ingredient with 0.2% transglutaminase showed the high potential to improve the texture most similar to commercial fish balls because soy protein isolate contained high protein with all essential amino acid which most suitable for used cooperated with transglutaminase (Kolar *et al.,* 2010). But soy protein had the unique flavor which was strong and unacceptable in some group of people. Using mung bean protein showed good potential texture improvement. Even mung beans protein has no glycinin but high in lysine (Yi-Shen *et al.,* 2018). So, transglutaminase can use glycinin in soy protein only to bind with the rest lysine which might drop down the potential of binding. But the mung bean protein has good potential to form gel (Coffmann & Garciaj, 2007) which resulting in better adhesiveness than using only soy protein isolate. Furthermore, because of the amino acid composition and gel formation in pea protein and konjac (Leterme *et al.*, 1990 and Anderson et al., 1986) the texture profile showed similar potential to mung beans protein in terms of hardness, adhesiveness, springiness, and chewiness. Although plant-based protein showed quite high potential to make plant-based protein meatballs, excessive hardness in texture made the texture incomparable to the real fish balls. Therefore, plant-based ingredients including unripen jackfruit and cauliflower were considered interesting choices. Unripen jackfruit and cauliflower gave the lowest in hardness comparing to all plant-based ingredients. Unripen jackfruit and

cauliflower showed no significantly different in terms of hardness, adhesiveness, springiness, cohesiveness, and chewiness but the cauliflower showed better in resilience than unripen jackfruit.

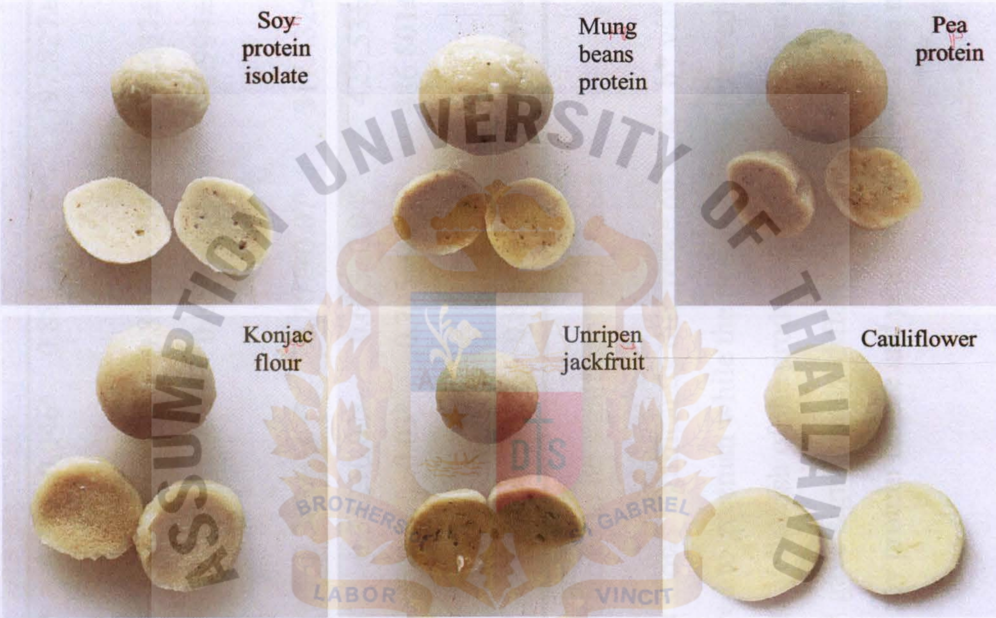


Figure 6: Plant-based Ingredient Meatballs Using Different Type of Plant-based Ingredients

Table 3: Texture Profile Analysis of Different Type of Plant-based Ingredients Used in Plant-based Ingredient Meatballs

| Plant-based ingredients | Hardness | Adhesiveness | Springiness | Cohesiveness | Chewiness | Resilience |
|---|---|---|---|---|---|---|
| Commercial Fish balls | 2288.615±558.80[a] | -1.304±0.74[a] | 0.387±0.046[a] | 0.889±0.022[ab] | 797.827±272.47[a] | 0.588±0.029[ab] |
| Soy protein | 4076.954±1312.47[b] | -58.561±10.90[a] | 0.746±0.17[a] | 0.811±0.054[ab] | 2539.037±1224.36[a] | 0.517±0.06[bc] |
| Mungbeans protein | 2200.715±343.55[c] | -16.169±20.85[a] | 0.375±0.044[b] | 0.854±0.01[a] | 712.994±182.26[b] | 0.545±0.02[ab] |
| Pea protein | 1179.053±12.31[e] | -1.186±0.91[a] | 0.383±0.03[b] | 0.707±0.07[b] | 317.432±37.98[b] | 0.404±0.06[c] |
| Konjac | 1911.201±226.86[cd] | -40.903±38.84[a] | 0.342±0.02[b] | 0.655±0.04[c] | 425.53±43.18[b] | 0.320±0.03[d] |
| Unripen jackfruit | 1540.092±177.44[de] | -0.906±0.55[a] | 0.396±0.03[b] | 0.795±0.02[ab] | 486.801±83.44[b] | 0.473±0.02[bc] |
| Cauliflower | 1133.733±152.99[e] | -1.046±0.71[a] | 0.404±0.03[b] | 0.838±0.03[a] | 385.056±67.27[b] | 0.565±0.03[a] |

Remark: The same letter of superscript in each row referred to no significant difference

The color of plant-based ingredient meatballs shows significantly difference on L, a*, b* values ($p \leq 0.05$). L value referred to the lightness of the product, the minus value of a* referred to greenish while the positive value referred to redness, and the minus value of b* referred to blueish while the positive value referred to yellowish of the product. Table 4 shows that commercial fish balls and cauliflower resulted the highest lightness of the product. The a* gave significantly difference in all type of plant-based ingredients used but the soy protein isolate showed the most greenish. Moreover, soy protein isolate and pea protein showed the most yellowish of the product.

Table 4: Color Analysis of Plant-based Ingredient Meatballs Using Different Type of Plant-based Ingredients

| Plant-based Ingredients | L | a* | b* |
|---|---|---|---|
| Commercial Fish balls | 92.03±0.23$^{a}$ | -2.89±0.24$^{c}$ | 6.03±3.42$^{f}$ |
| Soy protein isolate | 89.93±2.34$^{b}$ | -7.28±0.58$^{f}$ | 25.93±1.34$^{a}$ |
| Mung beans protein | 87.87±1.23$^{d}$ | -0.5±2.42$^{b}$ | 19.08±1.43$^{c}$ |
| Pea protein | 84.00±0.23$^{f}$ | -0.23±0.94$^{a}$ | 25.46±0.38$^{a}$ |
| Konjac | 89.04±1.40$^{c}$ | -3.51±1.42$^{d}$ | 19.69±0.92$^{b}$ |
| Unripen Jackfruit | 85.84±0.42$^{e}$ | -3.95±0.93$^{e}$ | 16.89±1.34$^{d}$ |
| Cauliflower | 91.94±0.24$^{a}$ | -3.61±0.74$^{d}$ | 11.78±0.21$^{e}$ |

Remark: The same letter of superscript in each row referred to no significant difference

According to the results above, different types of plant-based ingredients used resulted in different texture and color. However, due to the protein content and essential amino acid in soy protein isolate so soy protein was selected to use as the base ingredient for further development. Also, the properties of mung beans protein and konjac were interesting to select and use combine with the lighter color and softer texture given from cauliflower.

**3. Formulation of Plant-based Ingredient Meat Balls from Design of Experiment**

The mixture design had been used as the experimental design to formulate plant-based ingredients meat balls formulation as showed in Table 1.

**3.1 Texture Profile Analysis and Color Analysis of Plant-based Ingredients Meat Balls Formulated by Using Mixture Design**

As the result shows in Table 5, difference plant-based ingredient formulations show significantly difference in hardness, adhesiveness, springiness, cohesiveness, chewiness, and resilience ($p \leq 0.05$). The formulation C and D contained konjac which had relatively good in gel forming resulting in higher in hardness of the products. Besides, the formulation A contained highest amount of cauliflower followed by mung bean and soy protein isolate resulted in highest of adhesiveness, springiness, cohesiveness, chewiness, and resilience.

Table 5: Texture Profile Analysis of Plant-based Ingredient Meat Balls Formulated by Using Mixture Design

| Formulation | Hardness | Adhesiveness | Springiness | Cohesiveness | Chewiness | Resilience |
|---|---|---|---|---|---|---|
| A | $2050.871\pm154.22^{b}$ | $-10.439\pm3.75^{a}$ | $0.534\pm0.01^{a}$ | $0.689\pm0.03^{a}$ | $752.070\pm43.32^{a}$ | $0.373\pm0.03^{a}$ |
| B | $2462.995\pm624.27^{b}$ | $-58.747\pm49.78^{ab}$ | $0.469\pm0.2^{b}$ | $0.568\pm0.06^{b}$ | $643.865\pm109.83^{b}$ | $0.252\pm0.05^{b}$ |
| C | $2983.244\pm433.63^{a}$ | $-185.279\pm97.95^{c}$ | $0.478\pm0.03^{b}$ | $0.424\pm0.02^{c}$ | $610.908\pm139.54^{b}$ | $0.159\pm0.01^{c}$ |
| D | $2892.406\pm207.94^{a}$ | $-111.666\pm17.49^{bc}$ | $0.569\pm0.05^{a}$ | $0.446\pm0.02^{c}$ | $732.074\pm65.88^{ab}$ | $0.184\pm0.01^{c}$ |

Remark: The same letter of superscript in each row referred to no significant difference

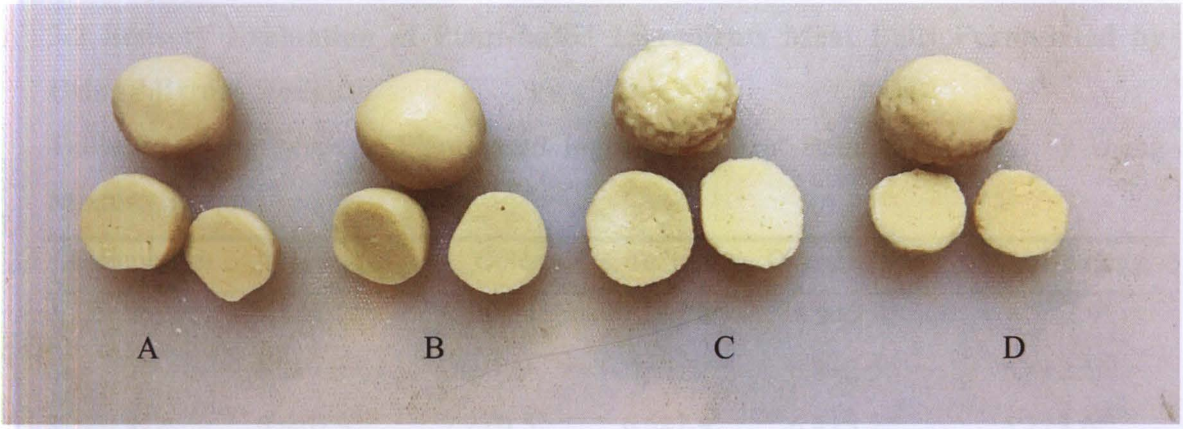


Figure 7: Plant-based Ingredients Meat Balls Formulated by Using Mixture Design

Table 6: Color Analysis of Plant-based Ingredients Meat Balls Formulated by Using Mixture Design

| Formulation | L | a* | b* |
|---|---|---|---|
| A | 92.93±1.02[c] | -5.04±0.92[b] | 14.1±0.86[b] |
| B | 92.72±0.91[c] | -5.86±0.28[b] | 16.58±0.93[a] |
| C | 95.24±1.22[a] | -3.77±1.12[a] | 10.36±1.01[c] |
| D | 94.83±0.29[b] | -3.31±0.39[a] | 9.04±0.92[d] |

Remark: The same letter of superscript in each row referred to no significant difference

The color of plant-based Ingredient meat balls showed significantly difference in L, a*, and b* values ($p \leq 0.05$). Formulation C and D contained konjac flour which gave the products lighter in color also slightly greenish. However, formulation B which contained highest amount of mung bean protein resulting the most yellowish of the product.

### 3.2 Sensory Evaluation of Plant-based Ingredients Meat Balls Formulated by Using Mixture Design

Table 7: Liking Score of Plant-based Ingredients Meat Balls Formulated by Using Mixture Design

| Formulation | Appearance | Color | Texture | Overall Taste | Overall Liking |
|---|---|---|---|---|---|
| A | 7.8±1.2$^{a}$ | 8.1±0.4$^{a}$ | 7.3±1.1$^{a}$ | 6.9±2.4$^{a}$ | 6.5±2.9$^{a}$ |
| B | 7.3±0.8$^{ab}$ | 8±0.5$^{b}$ | 7±1.5$^{b}$ | 6.8±2.5$^{ab}$ | 6.3±2.5$^{ab}$ |
| C | 6.8±0.9$^{b}$ | 7.2±0.5$^{c}$ | 6.5±2.4$^{b}$ c | 6.4±3.2$^{ab}$ | 5.7±3.6$^{ab}$ |
| D | 6.1±1.4$^{b}$ | 7.8±0.9$^{c}$ | 6.2±3.1$^{c}$ | 6.1±2.1$^{b}$ | 5.5±2.4$^{b}$ |

The sensory test was done using 9-point hedonic score and most preference test in order to observe the liking score and the formulation that most of panelists preferred. As the result shows in Table 7, formulation A shows the highest liking score in all attributes. Formulation A received liking score of color more than 8 which can consider as consumer very like the color of the product. Also, moderately like appearance and texture and slightly like the taste and overall product. Moreover, the preference test shows that formulation A was the most preferable formulation at 65.4% followed by formulation B at 34.6%.

According to the results both physical analysis and sensory taste, formulation A received the highest score in texture and sensory, so formulation A can be used for further development in order to improve the taste and texture.

### 3.3 Estimated Nutrition Composition of Plant-based Ingredients Meat Balls

The estimated nutrition composition of Plant-based Ingredients Meat Balls Formulation A obtained from the calculation using the data from Food Data Central of U.S. Department of Agriculture (U.S. Department of Agriculture, 2019). The calculated result showed that the highest nutrition composition of the plant-based ingredients meat balls is protein at 9.924 g, followed by carbohydrate, fat, and fiber. Since the main ingredient of plant-based ingredients meat balls is soy protein which contained 90.5% of protein and mung bean flour which contained 10% protein.

Table 8: Estimated Nutrition Composition of Plant-based Ingredients Meat Balls

| Estimated Nutrition Composition<br>For 100 grams of Plant-based Ingredients Meat Balls | |
|---|---|
| Calories | 121.781 kcal |
| Total Fat | 5.505 g |
| Carbohydrates | 8.135 g |
| Protein | 9.924 g |
| Fiber | 0.15 g |

**3.4 Estimated Cost Production of Plant-based Ingredients Meat Balls Per 100 g**

The estimated cost production is 4.698 Baht per 100 grams of Plant-based Ingredients Meat Balls. The most expensive ingredient is transglutaminase enzyme but the usage was at only 0.2% so the soy protein isolate which used at 10% showed highest cost.

Table 9: Estimated Cost Production of Plant-based Ingredients Meat Balls Per 100 g

| **Ingredients** | **Cost per 1 gram** | **Total (Baht)** |
|---|---|---|
| Water | 0.002 | 0.152 |
| Soy Protein Isolate | 0.18 | 1.8 |
| Soy Bean Oil | 0.04 | 0.22 |
| Tapioca Flour | 0.08 | 0.416 |
| Sugar | 0.035 | 0.056 |
| Salt | 0.014 | 0.014 |
| Cauliflower | 0.12 | 0.9 |
| Mung Bean Flour | 0.14 | 0.7 |
| Transglutaminase | 2.2 | 0.44 |
| **Total Cost** | | 4.698 |

# Conclusion

In conclusion, 0.2% of transglutaminase gave the highest potential to improve the texture of plant-based ingredients meatballs and also cost effectiveness to use. However, the different plant-based ingredients showed different effect on texture which resulting in distinctive characteristics of the plant-based ingredients. Therefore, the selection of plant-based ingredients should depend on the food application. In this study, soy protein was selected to use as the base ingredient. The mung bean protein, konjac, and cauliflower also selected to formulate plant-based ingredient meat balls using mixture design. Formulation A which contained 10% soy protein, 7.5% cauliflower, and 5% mung bean protein as the plant-based ingredient shows highest in adhesiveness, springiness, cohesiveness, chewiness, and resilience of the texture. Also, formulation A obtained the highest score in sensory test in appearance, color, texture, overall taste, and overall liking. Moreover, formulation A was the most preferable formulation from panelists at 65.4%.

# Suggestion

Even the characteristic of plant-based ingredients meatballs can be determined the texture using machine. But sensory analysis should be further studied or parallel-studied in order to study the acceptance of the product in any steps. Also, every plant-based ingredient gave difference in characteristics, so to use different plant-based ingredients cooperate with others might result in various texture of the plant-based ingredients products. To select the plant-based ingredients should depend on the product design and the food application for further development.

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# Appendix

**1. Raw Data of Texture Profile Analysis**

Data were kept as electronic file and stored in the attached thumb drive. To retrieve this data, please contact atittayatnd@au.edu.

**2. Raw Data of Color Analysis**

Data were kept as electronic file and stored in the attached thumb drive. To retrieve this data, please contact atittayatnd@au.edu.

**3. Sensory Evaluation**

**3.1 Raw Data of Sensory Evaluation**

Data were kept as electronic file and stored in the attached thumb drive. To retrieve this data, please contact atittayatnd@au.edu.

### 3.2 Sensory Evaluation Questionnaire

**Sensory Analysis of Plant Based Protein Balls**

**Instruction**

1. Please rinse the mouth with water before starting. You may rinse again at anything during the test you need to.
2. Please taste the samples in the order presented, from **left to right**.
3. Please rate the samples from the most preferred to lest preferred using the following numbers (9-point hedonic)

9 = Like extremely
8 = Like very much
7 = Like moderately
6 = Like slightly
5 = Neither like nor Dislike
4 = Dislike slightly
3 = Dislike moderately
2 = Dislike very much
1 = Dislike extremely

| Attributes | Sample Code | | | |
|---|---|---|---|---|
| | | | | |
| Appearance<br>ลักษณะ | | | | |
| Overall Taste<br>รสชาติโดยรวม | | | | |
| Overall Texture<br>เนื้อสัมผัสโดยรวม | | | | |
| Overall Liking<br>ความชอบโดยรวม | | | | |

Which sample you preferred the most? ______

Comment: ______________________________