SHELF LIFE OF THE FREEZE-DRIED SAI-KROK-ISAN WITH TOM-YUM INGREDIENT

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MR. SORAWIT ANANTAKUL

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A special project submitted to School of Biotechnology. Assumption University in part fulfillment of the requirements of Degree of Bachelor of Science in Biotechnology.

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Sincerely yours,

Sorawit Anatakul

ABSTRACT

Sai-Krok-Isan is one types of Thai fermented sausage which made from minced pork, lard, cooked rice or glutinous rice and incubated at room temperature for 2 days. The aim of this project studied the effect of adding natural antioxidant (vitamin E or oregano) and two storage methods (vacuum packed or oxygen absorber) to extent shelf-life of the Freezed-dried Sai-Krok-Isan. The product formula and freeze dry processing were obtained by Srinin (2014) and Taepaiboon (2014), respectively. The samples were designed as 4x2 RCBD experiment including four treatments (control, 0.6% vitamin E, 1.2% vitamin E and 0.5% oregano and two storage methods (vacuum pack in nylon pack and aluminium foil adding oxygen absorber). All samples were analyzed the color measurement (Hunter L*, a*, b*), Texture Profile Analysis (TPA), water activity, moisture content, total acidity, pH, oxidative rancidity acid value (AV), aerobic plate count (PCA) and mold and yeast plate count (PDA) for every 0, 30, 60 and 90 days of shelf-life when kept in room temperature. Unfortunately, studying plan was incomplete because of pandemic Corona-19 virus. Samples was able to measure at day-0, some in day-30 and day-100. There were significant different in all analysis results ($p > 0.5$). Results was found that adding of 0.5% oregano as antioxidant might reduce the oxidative rancidity acid value of sample, adding of 0.6% of vitamin E in vacuum pack can reduced water activity. The color parameter of control sample was shown color change and the vacuum pack method might reduce oxidative rancidity acid value of sample.

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INTRODUCTION

Sai-Krok-Isan (SKI) or Thai fermented sausage is one of the local foods that most of Thai people prefer to consume and well known in street food. The purpose of this special project, SKI-TY (the SKI mixed with Tom-Yum ingredients) and continue to freeze dry process and called the freeze-dried SKI-TY. Freeze-dried process is one of preservative food product to gain longer shelf-life, lighter weight and keep in room temperature.

Rancidity is one of the problems that usually occur in high fat food. It always affects to shorter shelf-life, off-flavor or off-color etc. The Freeze-dried SKI-TY in plastic bags have been detected rancid, off-flavors problems after storage over 45 days and affected to shelf-life and quality of product.

For the previous study, the Freeze-dried SKI-TY was controlled the rancidity of product only 2 months. Because SKI-TY is contained lard as one of main ingredient and caused of rancidity of the product. The storage conditions also prevent the rancidity of high fat product by keeping away from sunlight, low oxygen and water content. Therefore, this study was aimed to prevent the rancidity that will occur in Freeze-dried SKI-TY during storage by controlling the packing condition such as vacuum packing to reduce oxygen gas inside packaging; using the opaque packaging to prevent sunlight; add oxygen absorber to absorb oxygen gas inside package to prevent oxidation reaction during storage. Although there are many methods to prevent the rancidity of product, this research will find others method that will help to prevent the rancidity of product and increase shelf-life of product and prevent the rancidity that might occur on this product as long as possible.

OBJECTIVES

1. To study shelf-life of Freeze-dried SKI-TY (Sai-Krok-Isan with Tom-yum) by adding antioxidants (Liquid Vitamin E) or spices (dried oregano) and varying storage condition (vacuum packing and packing with oxygen)
2. Review "Effect of the packaging method on dry fermented sausage"
3. Review "Effect of spices for preventing oxidation on dry fermented sausage"
4. Review "Shelf-life of freeze-dried fermented sausage"
5. Review "Function of tocopherols and tocotrienol in lipid oxidation"
6. Review "Effect of water activity on freeze-dried meat"

LITERATURE REVIEW

Traditional Fermented Foods in Thailand

1. Traditional fermented Thai foods

Traditional fermented Thai food (fish, meat and vegetable products), were produced with different processes and consumed in many parts of Thailand. The Lactic acid bacteria are responsible for souring and ripening of the product. The homofermentative strains of *L. plunkrum*, *Pediococcus pentosuceus and Lucfobucihs penfosus* are dominant in foods which contain low salt. On the other hand, *P. hulophilus* strains are present in foods which contain high salt. The strains of *Lucfobucillus suke,* other *ucidiluctici, Lucfobucillus spp*. and *P. uritiueequi* are frequently found. Heterofermentative strains of *L. confusus, L. breuis, L. fermenhm, L. vuccinoskrcus,* other *Leuconostoc spp*., and *Luctobucillus spp*. are distributed as a minor bacteria and strains of *Skzphyfococcus, Enkrococcus* and *HuZobucterium* (Tanasupawat, 1995). In dairy life of Thai people, they consume many types of fermented food such as fish sauce, shrimp paste, Pla-Ra (salt fermented fish) as ingredient in food and Thai people also consume fermented meat product such as Nham, Pla-som, Sai-Krok-Isan and etc (Yongsmith, 2016).

2. Sai-Krok-Isan

Sai-Krok-Isan is Thai traditional fermented sausage made from mince pork, lard, pork rind, garlic, pepper, salt, cook rice, chili (optional) and starter culture (optional). This product can be consumed in raw and cooked products. *Lactobacilli, Pediococci* and micrococci are common bacteria which found in Sai-Krok-Isan (Thieavattanamontri *et al.,* 1998). The development of this meat fermented product has been focused on the application of defined starter culture to increase the process control and product consistency (Campbell-Platt, 1995). Moreover, it difficult to obtain the product consistency because the pathogenic in the raw fermented sausage still affect to food safety problems (Noonpakdee, 2003).

Ingredients:

1. **Pork and lard** are one of meat that Thai people usually consume in their daily life, which use as main ingredient of fermented sausage. Pork meats was given the texture, sensory, flavor, and nutritional quality of Sai-Krok-Isan. Lard is a high fat contain from pork that help to develop good flavor of fermented sausage and work as a binding of Sai-Krok-Isan (Visessanguan, 2005).
2. **Pork rind** fat is one of important in the processed meat product to improve texture and mouth feels of the product. From Briggs and Schweiger indicated that pork fat was add in fermented sausage has about of 40 % saturated fatty acid. Thai fermented sausage also one of the products that added pork skin into the making process. The pork skin also consumed as snack by fried the skin this product is very popular in northern of Thailand (S. S. Abiola, 2001).
3. **Rice (*Oryza sativa L*)** is one the most important cereals crops in the world and it also main food that Thai people always consume in every meal. It is source of carbohydrate which use in process of making fermented sausage. The rice was uses to give texture of fermented sausage and use as a source of lactic acid bacteria for fermented process of sausage. (S.M.A. Basra, 2005)
4. **Glutinous rice** (*Oryza sativa*) is a staple food of Asian people. Especially, in Thailand, glutinous rice was consumed in many parts of Thailand. The glutinous rice has sticky characteristics which use as a binding agent in the Sai-Krok-Isan (Keeratipibul, 2008).
5. **Garlic** is one of the edible plants which has generated a lot of interest throughout human history as a medicinal plant. A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown sensitive to crushed garlic preparations. Chemical analyses of garlic cloves have revealed an unusual concentration of sulfur-containing compounds (1–3%). Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl sulfides. The anti1bacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria species such as *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus,* and *Clostridium*. Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic. Garlic extracts are also effective

to *Helicobacter pylori*, which cause of gastric ulcers. Garlic extracts can also prevent the formation of *Staphylococcus* enterotoxins. On the other hand, it seems that garlic is not effective against toxin formation of *Clostridium botulinum*. Cavallito and Bailey were the first to demonstrate that the antibacterial action of garlic is mainly due to allicin. The sensitivity of various bacterial and clinical isolates to pure preparations of allicin is very significant (Ankri, 1999).

Tom-Yum ingredients:

1. **Chili** is a common ingredient in Asian and Latin American food. Capsaicin is an active component of chili that triggers a painful and burning sensation in the human gut via transient receptor potential vanilloid-1 or TRPV1 receptors which are expressed throughout the digestive tract mucosa with variable distribution in each organ. Chili is one of main food ingredients in Thai food because of their pungency and aroma properties. which help to improve flavor and aroma in food (Patcharatrakul, 2016).
2. **Lemongrass** (*Cymbopogon spp., Poaceae*) is tufted perennial C4 grasses with numerous stiff stems arising from a short, rhizomatous rootstock. Lemongrass is indigenous in tropical and semi-tropical areas of Asia, and it also cultivated in South and Central America, Africa and other tropical countries. *Cymbopogon flexuosus* (*Steud.) Wats*., also known as East Indian or Cochin lemongrass, and *Cymbopogon citratus Stapf.* Which produce up to 75 to 85% of citral in their essential oils. Citral is a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (transcitral, citral A) and neral (cis-citral, citral B). In Thai cooking, lemongrass is chopped to form marinades, soups or stir-fries. Because it has a citrus aroma and lemony flavor. It can use in dried, powdered and fresh (Lewinsohn, 1998).
3. **Galangal root** is a spice native of Southern Asia. Galangal refers to the root of several plants of the Zingiberaceae family. Lesser galangal, or *Alpinia officinarum*, are commonly used. Similar to ginger and turmeric, galangal can be eaten fresh or cooked and is a popular addition to many Chinese, Indonesian, Malaysian, and Thai dishes.
4. **Kaffir leave** are used in assortment of Thai cuisine and famous for its distinctive citrus scent, which is irreplaceable. Kaffir lime leaves are probably one of the most

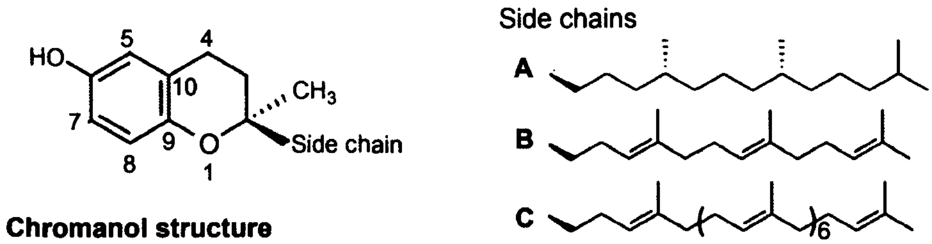
aromatic herbs and a vital addition to several Thai curry, stir-fry and soup. Kaffir lime leaves is a part of authentic Thai cuisine. The dark green leaf comes with two interconnecting parts, which impart a lemony fragrance. The leaves are added whole or bruised in watery simmered cuisine, whereas the leaves are cut into fine slivers for dry dishes.

Antioxidant compound:

Lipid oxidation is an autocatalytic reaction that compromises the functional and nutritional properties of fatty acids containing double bonds, especially the highly unsaturated ones. The rate and extent of lipid oxidation-induced changes can be significantly inhibited by the presence of antioxidants. Vitamin E is the common term of tocopherols and tocotrienols which coexist with lipids in different biological settings. They are well known as a natural antioxidant for lipids in foods and biological systems. Scientists are interested in the antioxidant mechanisms commonly believe that tocopherols and tocotrienols, especially *α-tocopherol*, exert their protective actions by interrupting the autocatalytic reaction cycles by donation of phenolic hydrogen to peroxyl radicals, and by stabilization of preformed hydroperoxides (Kamal-Eldin, 2015). During lipid oxidation process free radicals, lipid hydroperoxide and secondary oxidant product (aldehydes, ketones, alcohols, hydrocarbons, core aldehyde) were formed. The secondary oxidant products were responded to change of aroma and flavor of food by lipid oxidation. Among these compound vinyl ketones and trans, cis-alkadienals have the lowest flavor thresholds in oil, while a hydrocarbons (alkanes and alkenes) have the highest. Therefore, the hydrocarbons are not considered to the flavor significant.

Rancid is commonly uses to define of off-flavor and off-odor of food product, which cause by the lipid oxidation, but it can use to describe the off-flavors that develop by the hydrolytic liberation of short-chain fatty acids in dairy products. In addition, the meaning in term of rancid may depend on food product. Additional, since the lipid oxidation cause to the formation of a large number of secondary oxidation products in several sensory attribute was used to describe off-flavor that develop during lipid oxidation (Jacobsen, 1999).

1. **Tocopherols and Tocotrienols** are derivatives from *6-chromanol*, different in number and position of methyl substituents in phenolic ring and chains at C-2. Figure.1 shows the structures and some important properties of the molecules. The radical-scavenging properties of tocopherols and related compounds was determined by the donation of the phenolic hydrogen, or its Bond Dissociation Energy (BDE), which enhanced by methyl substituents in the two ortho positions, in addition the alkoxy substitution in the para position. Thus, α-tocopherol is a stronger hydrogen donor than either β- or γ-tocopherols, which are more potent than δ-tocopherol with no ortho methyl substituent. An antioxidant potency in order of $\alpha > \beta > \gamma > \delta$ would be expected, but this order was not consisted and it affected inter alias by the concentrations of tocopherols. The unsaturated side chains of tocotrienols are believed to allow them to have greater access to interfacial regions and contribute to its antioxidation and scavenging activities. Tocotrienols were found act synergistical with tocopherols, polyphenols, sterols, and other compounds against lipid peroxidation. Tocotrienols were claimed to have a better antioxidative activity than tocopherols, especially α-tocopherols. From the reported of other studied show that tocopherols and tocotrienols were have similar effect which protect oil against oxidation (Chow and Draper, 1970). Because of the antioxidant activity of tocopherols and tocotrienols, play in protecting the mono- and polyunsaturated fatty acids (PUFAs) from oxidation. Moreover, some of edible oil may also contain of tocotrienols. Lipid fraction of oilseed and nuts is a major natural dietary source of tocopherols and tocotrienols. The stability of tocopherols and tocotrienols may affect by the present of them and in food by fatty acid composition of food lipid fraction which different in cultivars of the same crop, by the storage and processing of the feed stocks and by cooking (Shahidi, 2016).



Tocopherol/ tocotrienol	Structure		CAS #	Log P	BDE (Kcal/mole)	Vitamin E Value
	Methyl substitution	Side chain				
TROLOX	5,7,8	COOH	53188-07-1	3.071	unknown	unknown
2,2,5,7,8-Pentamethyl-6-chromanol (PMC)	5,7,8	CH_3	950-99-2	4.201	unknown	unknown
α-tocopherol	5,7,8	A	59-02-9	9.043	75.8	100
β-tocopherol	7,8	A	16698-35-4	8.982	77.7	50
Y-tocopherol	5,8	A	54-28-4	8.982	78.2	10
δ-tocopherol	8	A	119-13-1	8.602	79.8	3
α-tocotrienol	5,7,8	B	493-35-6	9.089	unknown	30
β-tocotrienol	7,8	B	490-23-3	9.030	unknown	5
Y-tocotrienol	5,8	B	14101-61-2	9.030	unknown	unknown
δ-tocotrienol	8	B	25612-59-3	8.671	unknown	unknown
Plastochromaol-8	5,8	C	4382-43-8	10.487	unknown	unknown

Log P = logarithm of the octanol-water partition coefficient (a measure of lipophilicity calculated at www.molinspiration.com. BDE = bond dissociation energy of the phenolic hydrogen (Wright *et al.*, 2001).

Figure a. Structures and selected properties of tocopherols and tocotrienols and some synthetic analogues.

Source: (Kamal-Eldin, 2015).

2. **Oregano** (*Origanum vulgare*) is a native herb of Mediterranean region, this herb is rich in phenolic compounds with strong antioxidant and antibacterial activity. The antioxidant activity depends on amounts of compounds that can delay or prevent the oxidation of lipids and other molecules. From the research of (Zeng and Wang 2001) reported that oregano had 3-20 times higher antioxidant activity than other herbs. The studied according to ORAC method, and higher total phenolic content. In addition, the oregano has 42 times more antioxidant activity than apples, 30 times more than potatoes, 12 times more than oranges and 4 times more than blueberries. Herb as oregano have been used to extent the shelf life of food product. Dried oregano is commonly used in food industry (Jałoszyński, 2008). From the report of (Fernandes RPP, Trindade MA, Tonin FG, Pugine SMP, lima CG, Lorenzo JM, de Melo MP 2017) The main bioactive compound of oregano were rosmarinic acid,4-(3,4-Dihydroxybenzoyloxymethyl)phenyl-β-D-glucopyranoside, catechin and luteolin-7- *O*-rutinoside (Munekata, 2020).

Processing:

1. **Sample preparation:**

Thai fermented sausage was made by grinding pork and lard together, mixing with cooked rice and cooked sticky rice, pork rind and seasoning. In this process the starter culture or Nham powder will add to the sausage to help in the fermentation process. Then the sausage was shape into round shape with 2 cm. diameter. Fermentation process the fermented sausage by using air dryer to help to control the temperature which suitable to the fermented sausage around room temperature. Food drying is method that use to preservation food. Drying help to prevent the growth of bacteria, mold and fungi by removing water content from food product. There are many types of drying method for example conventional air drying, freeze dried, spray dried, sunlight and etc.

2. **Conventional air drying** is common drying process that approximately 85% use in industry drying process. The relative drying time depend on temperature, moisture content and product structure. Too high temperature can lead to degrading of valuable nutrient and the product structure will be change to hard crust of outside of product because during drying process water was removed (Kumar, 2014).

3. **The vacuum freeze-drying process** is well-known and established technology and it has been done with widely research and development. The energy consumption, product quality and thermal and physical parameters during the drying process are some areas. The vacuum freeze-drying process is used as a benchmark regarding product quality. Vacuum freeze drying has advantage of very highly valued dried products, with nutritional characteristics well preserved. This was confirmed by Marques *et al.*, who investigated the physical properties of vacuum freeze drying of tropical fruits. As expected, vacuum freeze-dried foods have high values of porosity, low values of apparent density and conserved color, flavor, and taste. For the disadvantages are the high fixed and operational costs. Vacuum operations are mainly batchwise, which represents to another additional cost, together with the demanding requirements of apparatus operated under vacuum. The important part

Analysis method:

1. **Texture profile analysis** is a double compression which use to determine texture of food product. The textural identity of any food products is rarely simple matter of understanding a singular attribute for example Hardness: the measure of how resistant solid matter to various permanent changed of shape when the force applied, Cohesiveness: the ratio of first compression to the second compression force area, Springiness: the height that food can recover the end of first bite and the beginning of second bite, Fracturability: the crisp of the outer shell of food which unique in sample food, Chewiness: defined to the energy which use to chew the solid food. The texture of any food product is multi-faceted and tied to consumers' sensory expectations. This method is not sufficient to deliver of food product with a target hardness, springiness and others textural identity value if consumers do not like the product and the product does not meet their expectations for that food type.

   Texture is one of the most important properties of the product quality. The factor that effect to texture of product are moisture content, composition, variety and sample dimensions. The chemical change is associated with the change of texture, the crystallization of cellulose, degradation of pectin and starch gelatinization in fruit and vegetable. High temperature caused the complex chemical and physical change to surface and formation of hard impermeable skin which call case harden in fruit, vegetable and meat (Valentina, 2016).

2. **Moisture content** affects the processability, shelf-life, usability and quality of a product. The accurate moisture content is play as a key role in ensuring quality in many industries including food, pharmaceuticals and chemicals products. Furthermore, the maximum permissible moisture content in certain products may be governed by legislation for example national food. Moisture content analysis is a critical component of material quality and essentially a function of quality control in most production and laboratory facilities. From biological research organizations, pharmaceutical manufacturers, food producers and packers, the moisture content can affect to physical properties and product quality of all product substances and materials at all stages of processing and final product existence.

Storage condition:

There are many techniques to prevent the rancidity that will occur during packed in the packaging for example use the packaging that made from Aluminium coat, dark opaque package and other packaging that prevent light pass through the package. Light is one of the causes of rancidity occur in food, it is a powerful influence of deterioration of fat. Which capable to producing the rancidity of fats.

1. **Vacuum packing** is way to increase shelf life of food products. This method the product is placed in air-tight pack, the air removed and sealed package. The levels of oxygen in the packaging are reduced, inhibit microbial growth and spoilage of product due to the lipid oxidation. A certain amount of oxygen will remain, however, because it is not possible to create a total vacuum.

2. **Using the oxygen absorber,** Food in a package has a headspace with some gas such as Oxygen which damages packaged foods, cause of off-flavors and effected to freshness of food product. Oxygen absorbers can remove oxygen from within a sealed package, whereas oxygen scavengers are irreversible chemical absorbers of molecular oxygen.

   The oxygen absorber is a various sachet which used to keep products safe from things like mold and extend shelf life by remove out of a sealed package. These oxygen absorbers can be found in many different types of things from pharmaceuticals to industrial metals, but probably the most common use for them is with food.

MATERIALS AND METHOD

List of ingredients

- Pork meat
- Pork lard
- Pork rind/pork skin
- Cooked rice and glutinous rice
- Garlic
- Chili
- Salt and pepper
- Galangal
- Lemongrass
- Kaffir leave

List of chemical reagents and meat binders

- Nham powder (Lobo®)
- Potassium hydrogen phthalate (KHP)
- 0.1 N. Sodium hydroxide (NaOH)
- Phenolphthalein indicator
- 0.1 N KOH
- Solvent mixture of 95% ethanol and diethyl ether

List of Equipment

- Meat grinder (SavioliLelio S.n.c Model SAV-TRC22BE)
- Freeze dryer (program version PFY900)
- Texture profile analyzer (TAXT plus Stable Micro System®, 50 kg) (Probe P/50)
- Colorimeter
- Hot air dryer
- pH meter (Hanna HI222)
- Vacuum packaging

Process flowchart SKI-TY (Sai-Krok-Isan with Tom-yum).

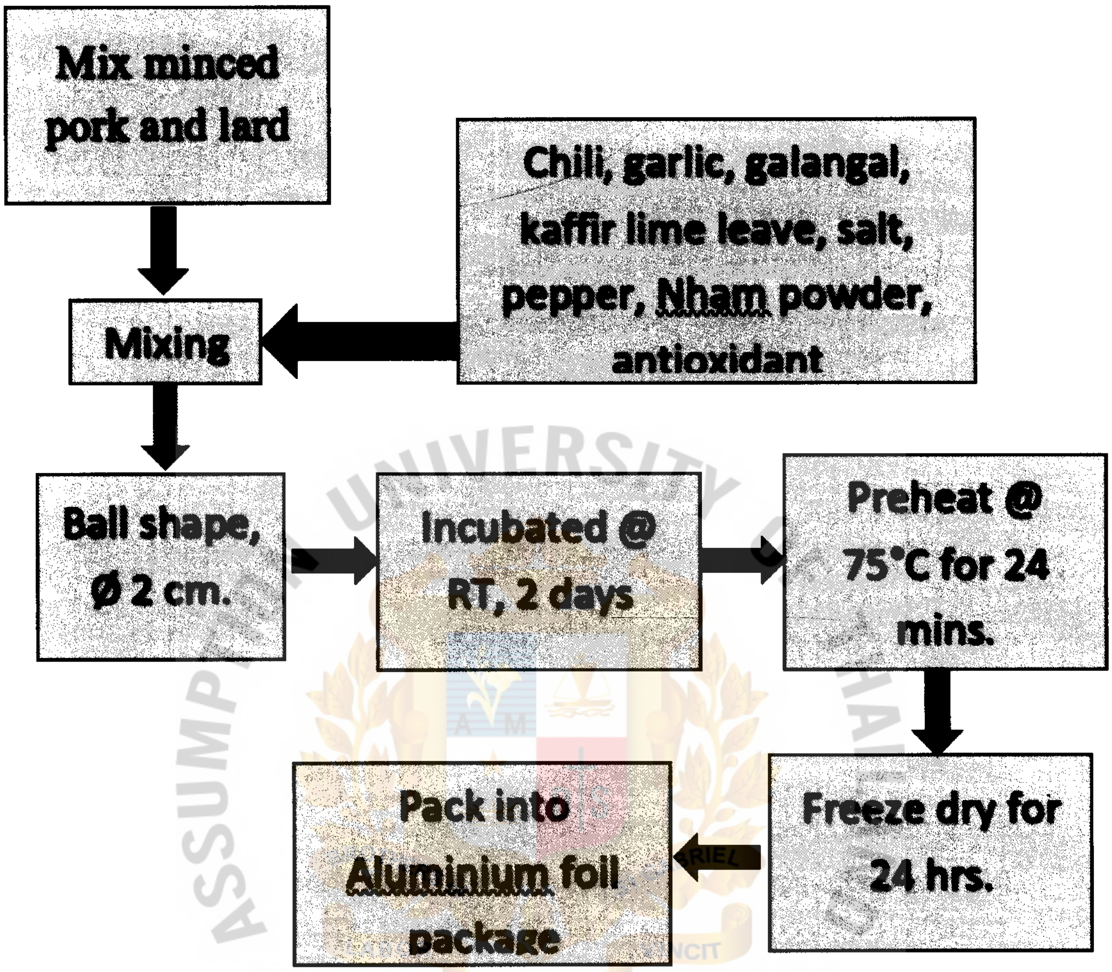


Figure b. Process flowchart of Freeze-dried Sai-Krok-Isan.

EXPERIMENTAL DESIGN AND METHOD

Process of Thai fermented sausage with Tom-yum spices

The formula of Thai fermented sausage with Tom-yum obtained from previous special project Srinin T., 2014 and Taepaiboon, Korn. 2016. Mince pork, pork lard and pork rind buy from local supermarket and keep in refrigerator at 5°C. All of Tom-yum spices lemon glass, galangal, kaffir leave, chili and garlic buy from local supermarket. All Tom-yum ingredients were washed and blended by using food blender. Rice and sticky rice washed and steamed for 15 minutes. All raw material mixed together and added salt, pepper and Nham powder (LOBO) as starter culture. Then the mixture was grinded by using grinder machine, and form in ball shape 1-1.5 cm diameter, and incubated at room temperature for 1-2 days to allow the lactic acid bacteria improve texture and flavor of sausage.

1. Varying storages conditions of SKI-TY.

The sample was separated into 4 different sample control sample, 0.6% vitamin E, 1.2% vitamin E and 0.5% oregano. Each sample was storage in two different storage condition vacuum packed and packed with adding oxygen absorber. Pre-heat the sausage by using hot air dryer machine to replace the cooking process as cooker due to ability that control specific temperature, constant air flow and time that applied on the product. The initial temperature will set at 75°C for 30 minutes. The samples were designed as 4x2 RCBD experiment including four treatments (control, 0.6% vitamin E, 1.2% vitamin E and 0.5% oregano and two storage methods (vacuum pack in nylon pack and aluminium foil adding oxygen absorber). All the samples will Freeze-dried at temperature -35°C for 24 hours. After freeze dried all sample quick pack into package with 2 storage condition: vacuum pack and add the oxygen absorber. All sample will choose for statistical analysis of variance for texture analysis, moisture content in product, oxidative rancidity AV test, range of pH and acidity of product.

Table 1. Sample Code, Antioxidant and Storage condition.

No.	Sample Code	Antioxidant	Storage condition
1	Control A	-	NylonVacuum Pack
2	Control B	-	Al. foil +Oxygen Absorber
3	0.6% Vit E_A	0.6% Vit E	NylonVacuum Pack
4	0.6% Vit E_B	0.6% Vit E	Al. foil +Oxygen Absorber
5	1.2% Vit E_A	1.2% Vit E	NylonVacuum Pack
6	1.2% Vit E_B	1.2% Vit E	Al. foil +Oxygen Absorber
7	0.5% Oreg_A	0.5% Oreg	NylonVacuum Pack
8	0.5% Oreg_B	0.5% Oreg	Al. foil +Oxygen Absorber

3.Statistical analysis

The data collect from chemical and physical test will analyze with program SASs using one-way analysis of variance evaluates with 95% confidence ($p<0.05$). RCBD 4 x 2 test compared to the significant effect of antioxidant and its concentration and packing technique. R program T-test analysis of two different storage condition vacuum packed and packed with adding oxygen absorber.

4.Determination of moisture content

AOAC international (2007) Official method of analysis to determine amount of moisture content in food product. Pre heat evaporate to 110°C, record weight of each moisture cans and samples, place it in evaporator at 110°C for 8 hours and transfer moisture cans that contain samples to desiccator cool down before weight measurement. All of sample were duplicate. The percent moisture content was calculated from ratio in weight loss and weight before heat with percentage. The percent total solid can be calculated by ratio of sample weight after over before evaporates weight and multiplied by one hundred to convert to percentage.

5.Acidity and pH value

Standardize 0.1N sodium hydroxide (NaOH) solution by titrate with 1g. of potassium hydrogen phthalate (KHP) dissolved in 40ml of distilled water. Grind sample and mix in 40ml of distilled water for 30 minutes before titrations, Add 1-2 drops of phenolphthalein as indicator. Titrate sample with standardized NaOH solution, record volume that use for titration. Do it duplicate for all samples. The predominant microorganisms of Thai fermented sausage are lactic acid bacteria, that measure by method of AOAC 15.004 report percent acidity of lactic acid by weight for every 1ml of 0.1N. NaOH equivalent to 90.08 mg of lactic acid. Recorded pH will measure by using pH meter (Hanna HI222).

6.Texture profile analysis

The texture profile analysis is applied a direct force to sample by using compress method to stimulate a jaws sensation with a disc probe at constant crosshead speed 0.5 mm per second, 5g initial force, and 70% strain compressed. Stimulate front teeth biting by using penetration method with 2mm. stainless steel cylindrical probe, speed 0.5 mm per second, 5g. initial force, and 1cm. penetrate. The samples will test and average value of hardiness, fracturability, cohesiveness, and chewiness were reported.

8.Oxidative rancidity AV test.

The determination of Acid value (AV) is the method to determined mg of potassium hydroxide which require to neutralize free fatty Acid in 1g. of fat. By using 0.1N. KOH, solvent mixture of 95% ethanol and diethyl ether and Phenolphthalein as reagent. Add the indicator solution to require amount of solvent in ratio 2ml to 125ml and with alkali to permanent pink color record as blank, weigh specific amount of sample approximate 2g. mix sample with solvent mixture into Erlenmeyer flask. Titrate the mixture with standard alkali until change to pink color and record the result.

RESULT AND DISCUSSION

Process of SKI-TY (Sai Krok Isan with Tom-yum spices)

The process of making Sai-krok-isan mixed and grinded process by meat grinder and food blender to blend the Tomyum spice for reducing process time. The meat grinder was reduced size of meat and others ingredient and the meat grinder also help to mix the ingredient before incubating the mixture and to cover by lid or food plastic wrap for keeping the moisture of mixture and prevent the surface of mixture contact with the air, which cause hard surface of mixture. After incubation of Sai-Krok-Isan, mixture was more stick and easy to form ball shape.

Discussion color

From the result of color measurement in the *table 2 and 3*, there were significantly different in color attributes in L*a*b* of all sample ($p < 0.05$). Color was changed during storage period. L* or lightness of all sample was increased from day-0 to day-100: 32.74 to 40.66 in control A, 33.10 - 39.62 in control B, 33.83 to 39.52 in 0.6% Vit E_A, 33.77 to 40.60 in 0.6% Vit E_B, 32.50 to 38.41 in 1.2% Vit E_A, 31.32 to 37.81 in 1.2% Vit E_B, 33.38 to 36.41 in 0.5% oregano A and 34.12 to 37.21 0.5% oregano B. But L* value was decrease from day-0 to day-30 of Control A 32.74 to 31.88, 1.2%Vit. E_A 32.50 to 31.62 and 1.2% Vit E_B 31.32 to 30.29 but Control B sample was increased 33.10 to 35.66. The a* value slightly decreased during storage period from day-0 to day-100, except control A, control B and 1.2& Vit E _B were increase of a* value at day-30 8.96 to 11.76, 7.92 to 9.36 and 9.87 to 12.16 in order, on the others the a* value of these samples were decrease at day-100 except the control B. The b* value of all samples also deceased during storage period from day-0 to day-100 but the b* of control A, B and 1.2% vitamin E A were increase 33.86 to 36.64, 34.18 to 34.94, 36.24 to 36.69. The increasing of L*, a* and b* value during day-30 of sample, may affect by the air which left in the package after the sample was packed into the package by the sample absorb the air inside the package increase the color value of sample. The increasing of L* value was affected to the color of sample has lighter than day-0. On the others hand the decreasing of b* value was affected to the yellow color of sample has fade out from day-0.

There were significant different ($p < 0.05$) between in all sample in day-0, day-30 and day-100 in each sample when compare in row of each sample. The change of color of sample may affect to the satisfied of the consumer, if the sample was sold as the product in the market. The study of prevent the change of color during storage would be study further in future study of this sample.

Table 2. Compare result of color measurement compare all of sample in column

Sample	Color Day 0			Color Day 30			Color Day 100		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control A	32.74 ± 3.05^{ab}	8.96 ± 1.14^{b}	33.86 ± 1.41^{b}	31.88 ± 2.91^{b}	11.76 ± 8.00^{a}	36.64 ± 1.70^{a}	40.66 ± 1.77^{a}	8.08 ± 0.84^{b}	24.36 ± 0.98^{a}
Control B	33.10 ± 2.78^{ab}	7.92 ± 2.04^{c}	34.18 ± 2.15^{b}	35.66 ± 2.72^{a}	9.36 ± 0.79^{a}	34.94 ± 1.63^{a}	39.62 ± 1.68^{ab}	9.53 ± 0.82^{a}	24.92 ± 1.04^{a}
0.6% Vit E_A	33.83 ± 2.24^{a}	7.60 ± 0.59^{c}	31.10 ± 1.41^{d}	-	-	-	39.52 ± 2.36^{ab}	6.45 ± 0.56^{d}	21.35 ± 0.97^{c}
0.6% Vit E_B	33.77 ± 1.69^{a}	7.90 ± 0.79^{c}	32.59 ± 1.36^{c}	-	-	-	40.60 ± 2.94^{a}	6.72 ± 0.87^{cd}	22.79 ± 0.85^{b}
1.2% Vit E_A	32.50 ± 1.83^{ab}	9.82 ± 1.26^{a}	36.24 ± 1.51^{a}	31.62 ± 1.98^{b}	9.51 ± 0.89^{a}	36.69 ± 1.88^{a}	38.41 ± 1.40^{bc}	8.56 ± 0.80^{b}	24.78 ± 1.15^{a}
1.2% Vit E_B	31.32 ± 2.77^{b}	9.87 ± 0.99^{a}	36.88 ± 1.51^{a}	30.29 ± 2.00^{b}	12.16 ± 4.43^{a}	36.59 ± 7.37^{a}	37.81 ± 1.61^{cd}	9.83 ± 0.90^{a}	25.02 ± 1.50^{a}
0.5% Oreg_A	33.38 ± 2.25^{a}	7.39 ± 0.54^{c}	30.78 ± 1.00^{d}	-	-	-	36.41 ± 1.80^{d}	7.22 ± 0.48^{c}	22.07 ± 0.94^{bc}
0.5% Oreg_B	34.12 ± 2.36^{a}	7.77 ± 0.72^{c}	30.86 ± 1.86^{d}	-	-	-	37.21 ± 2.05^{cd}	6.92 ± 0.56^{cd}	21.72 ± 1.42^{c}

Table 3. Compare result of color measurement of control and 1.2 % Vit_ E in row.

Sample	Color Day 0			Color Day 30			Color parameter Day 100		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control A	32.74 ± 3.05^{c}	8.96 ± 1.14^{b}	33.86 ± 1.41^{b}	31.88 ± 2.91^{c}	11.76 ± 8.00^{a}	36.64 ± 1.70^{a}	40.66 ± 1.77^{a}	8.08 ± 0.84^{b}	24.36 ± 0.98^{c}
Control B	33.10 ± 2.78^{c}	7.92 ± 2.04^{b}	34.18 ± 2.15^{b}	35.66 ± 2.72^{b}	9.36 ± 0.79^{ab}	34.94 ± 1.63^{b}	39.62 ± 1.68^{a}	9.53 ± 0.82^{ab}	24.92 ± 1.04^{c}
1.2% Vit E_A	32.50 ± 1.83^{b}	9.75 ± 1.26^{b}	36.19 ± 1.51^{b}	31.62 ± 1.98^{bc}	9.51 ± 0.89^{b}	36.69 ± 1.88^{b}	39.52 ± 2.36^{a}	6.45 ± 0.56^{c}	21.35 ± 0.97^{c}
1.2% Vit E_B	31.36 ± 2.77^{bc}	9.93 ± 0.99^{b}	36.88 ± 1.51^{b}	30.12 ± 2.00^{c}	11 ± 0.97^{a}	38.45 ± 1.41^{a}	40.60 ± 2.94^{a}	6.72 ± 0.87^{c}	22.79 ± 0.85^{c}

Discussion texture

From the result of texture analysis in *table 4 and 5.* There were no significant different ($p > 0.05$) between sample and 2 storage period of hardness attribute. There were significant different ($p < 0.05$) between sample control A and 1.2% vitamin E A at day-0 and no significant different between sample at day-100 of Fracturability attribute. There were significant different ($p < 0.05$) between sample control A and 1.2% vitamin E A at day-0 and between sample control A and 1.2% vitamin E A at day-100 of springiness attribute. There was significant different ($p < 0.05$) of 1.2% vitamin E A sample at day-0 and no significant different between all sample at day 100 of cohesiveness attribute. Lastly, there was significant different ($p < 0.05$) of 1.2% vitamin E A sample at day-0 and no significant different ($p > 0.05$) between sample at day-100 of chewiness attribute. From the result of T-test of texture attribute of control sample and 1.2% Vit E. The graph (a), (b), (c), (d) and (e) showed all of texture attribute changed during storage period hardness, cohesiveness and chewiness attribute of same are decrease. The same are more fracturability and springiness attribute at day-100.

From the result of pairing T-test in all attribute of control and 1.2% Vit E sample in *table 6 and 7.* There was significant different ($p < 0.05$) of control A at day-0 and day-100 in hardness attribute. There were significant different ($p < 0.05$) of control A and B at day-0 and day-100 in fracturability attribute. There were significant different ($p < 0.05$) of control A and B at day-0 and day-100 in springiness attribute. There was significant different ($p < 0.05$) of 1.2% Vit E_A at day-0 and day-100 in cohesiveness attribute. Lastly, there was significant different ($p < 0.05$) of control A at day-0 and day-100 in chewiness attribute.

It cannot conclude which formula of sample is the best and the change of texture effect to the quality of each sample during shelf-life of sample. In addition, the acceptable of sensory test, because of the COVID-19 situation was effect to cancelled of the sensory test of the sample.

Table 4. Compare result of texture profile analysis Hardness, Fracturability and Springiness attribute in column.

Sample	Hardness		Fracturability		Springiness	
	Day 0	Day 100	Day 0	Day 100	Day 0	Day 100
Control A	35057.27 ± 5.22^{a}	27494.93 ± 37.54^{b}	14946.18 ± 32.76^{ab}	13075.82 ± 20.99^{b}	0.27 ± 8.07^{ab}	0.29 ± 20.285^{bc}
Control B	27416.80 ± 17.75^{a}	23281.75 ± 24.37^{b}	7637.70 ± 32.45^{b}	$10230.46 \pm 22.15b^{c}$	0.22 ± 22.03^{b}	0.32 ± 12.31^{b}
0.6% Vit E_A	-	24405.14 ± 21.31^{b}	-	20510.58 ± 31.62^{a}	-	0.14 ± 19.69^{d}
0.6% Vit E_B	-	24378.64 ± 11.99^{b}	-	14768.63 ± 31.39^{b}	-	0.15 ± 13.67^{d}
1.2% Vit E_A	40634.36 ± 36.37^{a}	29556.54 ± 20.03^{b}	10603.68 ± 53.24^{ab}	14274.10 ± 30.36^{b}	0.32 ± 43.98^{ab}	0.30 ± 15.70^{bc}
1.2% Vit E_B	48564.76 ± 13.05^{a}	39960.25 ± 25.279^{a}	6715.94 ± 29.88^{a}	7116.06 ± 31.09^{c}	0.43 ± 8.35^{b}	0.45 ± 9.411^{a}
0.5% Oreg_A	-	22351.85 ± 29.45^{b}	-	20636.13 ± 23.44^{a}	-	0.23 ± 58.86^{c}
0.5% Oreg_B	-	25158.16 ± 30.54^{b}	-	12608.64 ± 16.33^{b}	-	0.14 ± 11.60^{d}

Table 5. Compare result of texture profile analysis Cohesiveness and Chewiness attribute in column.

Sample	Cohesiveness		Chewiness	
	Day 0	Day 100	Day 0	Day 100
Control A	0.21 ± 18.65^{b}	0.16 ± 38.63^{b}	2051 ± 14.90^{b}	1621.06 ± 81.20^{b}
Control B	0.24 ± 16.48^{b}	0.15 ± 29.94^{b}	1624 ± 49.01^{b}	1315.58 ± 51.82^{b}
0.6% Vit E_A	-	0.14 ± 34.60^{b}	-	563.08 ± 65.03^{b}
0.6% Vit E_B	-	0.15 ± 33.77^{b}	-	568.79 ± 45.40^{b}
1.2% Vit E_A	0.28 ± 20.98^{ab}	0.15 ± 24.52^{b}	3978.27 ± 58.56^{ab}	1457.88 ± 50.42^{b}
1.2% Vit E_B	0.34 ± 2.11^{b}	0.27 ± 21.959^{a}	7286.99 ± 22.93^{a}	5299.90 ± 50.71^{a}
0.5% Oreg_A	-	0.18 ± 64.52^{b}	-	1007.58 ± 100.95^{b}
0.5% Oreg_B	-	0.17 ± 34.81^{b}	-	742.83 ± 75.74^{b}

Table 6. Compare Result of texture profile analysis Hardness, Fracturability and Springiness attribute of control and 1.2% Vit E sample in paring T-test.

Sample	Hardness		Fracturability		Springiness	
	Day 0	Day 100	Day 0	Day 100	Day 0	Day 100
Control A	26290 ± 5.22[a]	27495 ± 37.54[b]	14946.18 ± 32.76[a]	13075.82 ± 20.99[b]	0.27 ± 8.07[b]	0.29 ± 20.285[a]
Control B	27021 ± 17.75[ns]	23282 ± 24.37[ns]	7637.70 ± 32.45[b]	10230.46 ± 22.15[a]	0.22 ± 22.03[b]	0.32 ± 12.31[a]
1.2% Vit E_A	40634.36 ± 36.37[ns]	29557 ± 20.03[ns]	10603.68 ± 53.24[ns]	14274.10 ± 30.36[ns]	0.32 ± 43.98[ns]	0.27 ± 15.70[ns]
1.2% Vit E_B	48564.76 ± 13.05[ns]	39960.25 ± 25.279[ns]	6715.94 ± 29.88[ns]	7116.06 ± 31.09[ns]	0.43 ± 8.35[ns]	0.15 ± 9.411[ns]

Table 7. Compared Result of texture profile analysis Cohesiveness and Chewiness attribute of control and 1.2% Vit E sample in paring T-test.

Sample	Cohesiveness		Chewiness	
	Day 0	Day 100	Day 0	Day 100
Control A	0.21 ± 18.65[ns]	0.16 ± 38.63[ns]	2051 ± 14.90[a]	1621.06 ± 81.20[b]
Control B	0.24 ± 16.48[ns]	0.15 ± 29.94[ns]	1624 ± 49.01[ns]	1315.58 ± 51.82[ns]
1.2% Vit E_A	0.28 ± 20.98[b]	0.30 ± 24.52[a]	3978.27 ± 58.56[ns]	1457.88 ± 50.42[ns]
1.2% Vit E_B	0.34 ± 2.11[ns]	0.45 ± 21.959[ns]	7286.99 ± 22.93[ns]	5299.90 ± 50.71[ns]

The graph show comparison of texture analysis attribute (Figure c.) Hardness, (Figure d.) Fracturability, (Figure e.) Springiness, (Figure f.) Cohesiveness and (Figure g.) Chewiness. of control and 1.2% Vit E sample in row.

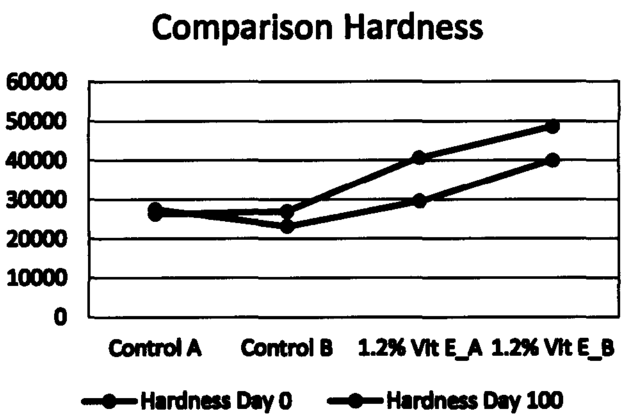


Figure c.

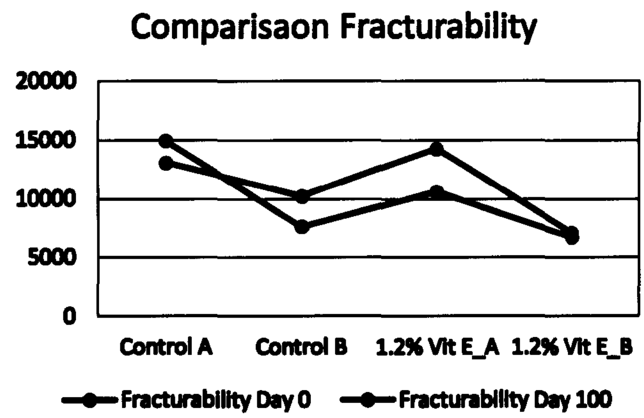


Figure d.

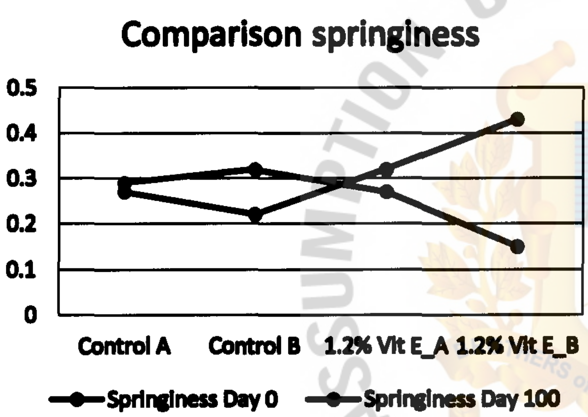


Figure e.

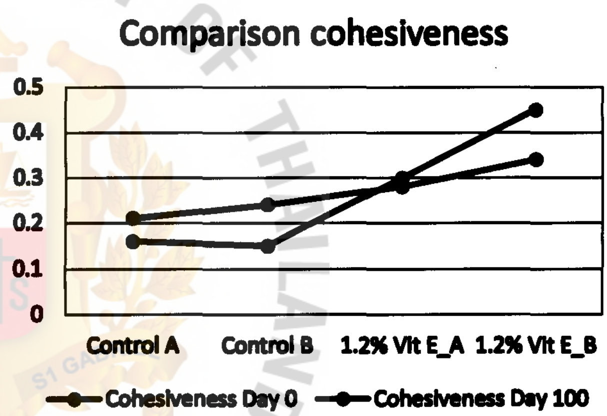


Figure f.

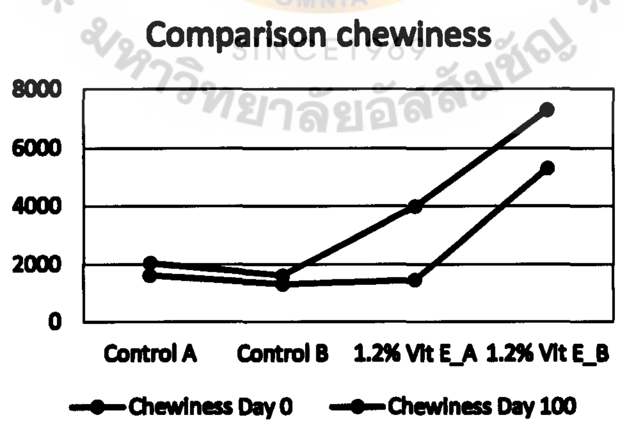


Figure g.

Discussion %moisture content and water activity.

From the result in *table 8*, show the effect of storage conditions to the moisture content and water activity of sample. The sample was packed and storage with vacuum have lower moisture content and water activity than the sample was packed with adding the oxygen absorber. The sample which add the 0.6% vitamin E were has lowest moisture content and 0.6% vitamin E and 0.2% oregano have lowest water activity. On the others hand the sample was added 1.2% vitamin E has the highest of moisture content. The added too much of vitamin E may affect to the moisture content and quality of sample. There were no significant different ($p > 0.05$) of water activity between all sample. The lower of moisture content and water activity of sample which packed in vacuum was may cause by the vacuum packed was removed all of gas and the moisture out from the package. On the others hand, the packed with adding oxygen absorber did not remove all of air out of package like vacuum and it can have the moisture left in the package and the sample would be absorb the moisture inside the package.

The same with moisture content result the sample was added 1.2% vitamin E has the highest of water activity. The effect of increased and decreased of moisture content and water activity may cause by other factors like the water transparent of the package, which factor may study in the future study of the experiment to find the effect of water transparent of each package to the sample.

Discussion total acidity, pH oxidative rancidity acid value (AV)

From the result in *table 9*, the total acid of all samples decreased during storage period. There are significant different ($p < 0.05$) between total acidity of sample at day-100. The pH value of all samples slightly increased during storage period. From the result show that the sample was adding 0.5% oregano have the lowest oxidative rancidity acid value, the oregano may have the properties to inhibit the lipid oxidation of sample which contained high fat. The graph (f), (g) and (h) show comparison changed of total acid, pH and acid value of control sample from day-0 to day-100. The total acid slightly decreased and the pH value slightly increased but for the acid value are increase approximate 2 time of the acid value of day-0 at day-100.

The result from *table 10,* showed the paring t-test of % total acidity, pH and oxidative rancidity of control A and B sample. There was significant different ($p < 0.05$) of % total acidity of control A and the control B was significant different ($p < 0.05$) in pH and oxidative rancidity.

The decreased of % total acidity and increase of pH value may affect to the inhibit of microbial growth of sample during storage in both storage condition. The acid value of all sample cannot tell that the sample which has lowest acid value is the best. The product should test with the sensory test to find which sample is chose and accepted by the sensory test.

Table 8. Compared result of % moisture content and water activity in column.

Sample	% moisture content day 0	% moisture content day 100	Water activity day 100
Control A	6.66 %	6.39 %	0.48 ± 0.00^{d}
Control B	6.5 %	7.44 %	0.51 ± 0.00^{b}
0.6% Vit E_A	-	4.44 %	0.33 ± 0.00^{g}
0.6% Vit E_B	-	4.77 %	0.35 ± 0.00^{f}
1.2% Vit E_A	-	7.09 %	0.50 ± 0.00^{c}
1.2% Vit E_B	-	8.02 %	0.56 ± 0.00^{a}
0.5% Oreg_A	-	6.17 %	0.33 ± 0.00^{g}
0.5% Oreg_B	-	6.78 %	0.36 ± 0.00^{e}

Table 9. Compared result of % total acidity, pH and oxidative rancidity acid value in column.

Sample	% total acidity		pH		Acid value (av)	
	Day 0	Day 100	Day 0	Day 100	Day 0	Day 100
Control A	0.23% ± 0.09[a]	0.06% ± 0.01[ab]	5.33 ± 0.05[a]	5.46 ± 0.08[a]	179.40 ± 51.61[a]	308.8 ± 44.05[a]
Control B	0.22% ± 0.07[a]	0.06% ± 0.01[ab]	5.12 ± 0.02[b]	5.54 ± 0.13[ab]	167.35 ± 30.56[a]	293.03 ± 13.04[a]
0.6% Vit E_A	-	0.07% ± 0.01[ab]	-	5.20 ± 0.11[c]	-	76.35 ± 20.49[d]
0.6% Vit E_B	-	0.07% ± 0.00[ab]	-	5.01 ± 0.04[d]	-	44.45 ± 20.37[d]
1.2% Vit E_A	-	0.07% ± 0.00[ab]	-	5.50 ± 0.04[ab]	-	135.87 ± 60.02[c]
1.2% Vit E_B	-	0.09% ± 0.00[a]	-	5.43 ± 0.06[b]	-	201.79 ± 48.10[b]
0.5% Oreg_A	-	0.05 ± 0.00[b]	-	5.22 ± 0.02[c]	-	57.41 ± 6.54[d]
0.5% Oreg_B	-	0.07 ± 0.02[ab]	-	5.08 ± 0.14[cd]	-	33.01 ± 12.21[d]

Table 10. Compare result of % total acidity, pH and oxidative rancidity acid value of control in paring T-test.

Sample	% total acidity		pH		Acid value (av)	
	Day 0	Day 100	Day 0	Day 100	Day 0	Day 100
Control A	0.23 % ± 0.09^{a}	0.06 % ± 0.01^{b}	5.33 ± 0.05^{ns}	5.46 ± 0.08^{ns}	179.40 ± 51.61^{ns}	308.8 ± 44.05^{ns}
Control B	0.22 % ± 0.07^{ns}	0.06 % ± 0.01^{ns}	5.12 ± 0.02^{a}	5.54 ± 0.13^{b}	167.35 ± 30.56^{b}	293.03 ± 13.04^{a}

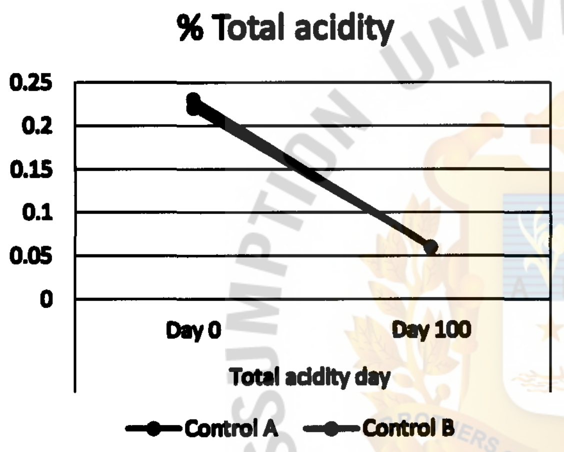


Figure h.

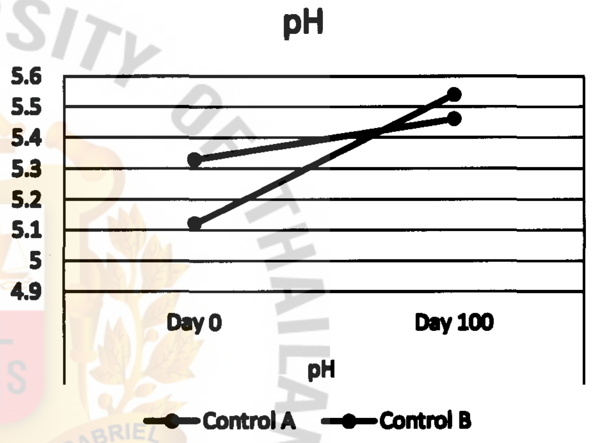


Figure i.

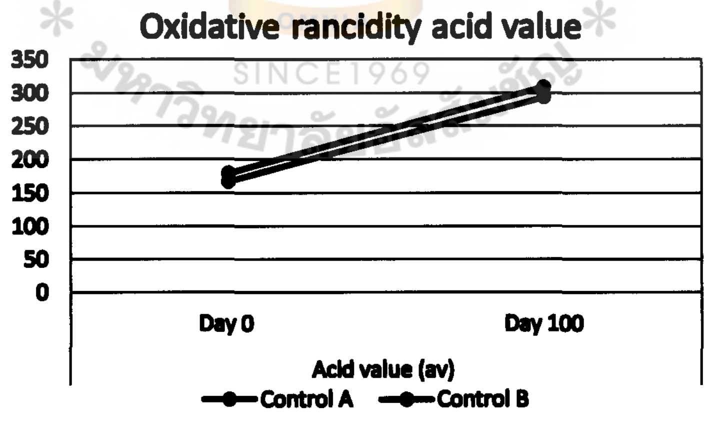


Figure j.

The graph show comparison of (Figure h.) % total acidity, (Figure i.) pH, (Figure j.) oxidative rancidity acid value of control and 1.2% Vit E sample in row.

Table 10. Compare result of % total acidity, pH and oxidative rancidity acid value of control in paring T-test.

Sample	% total acidity		pH		Acid value (av)	
	Day 0	Day 100	Day 0	Day 100	Day 0	Day 100
Control A	0.23 % ± 0.09[a]	0.06 % ± 0.01[b]	5.33 ± 0.05[ns]	5.46 ± 0.08[ns]	179.40 ± 51.61[ns]	308.8 ± 44.05[ns]
Control B	0.22 % ± 0.07[ns]	0.06 % ± 0.01[ns]	5.12 ± 0.02[a]	5.54 ± 0.13[b]	167.35 ± 30.56[b]	293.03 ± 13.04[a]

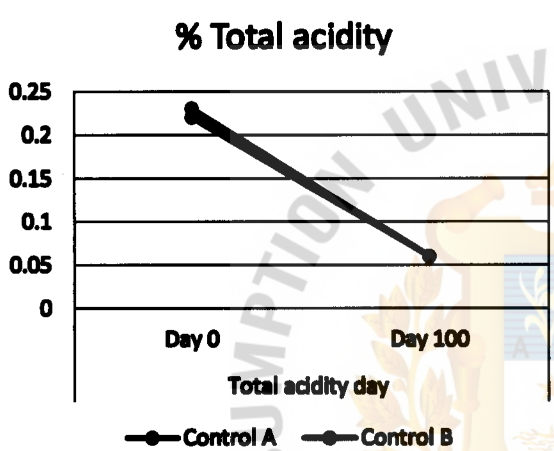


Figure h.

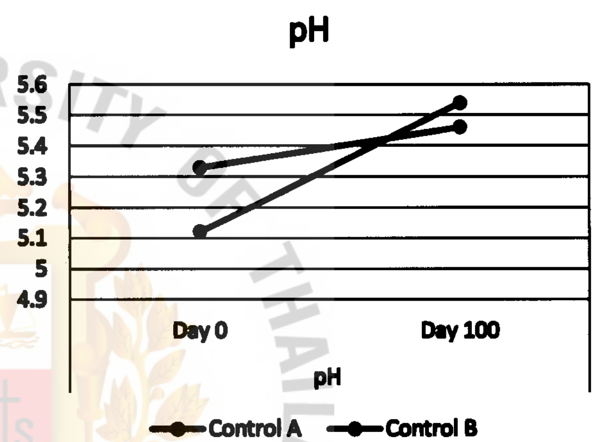


Figure i.

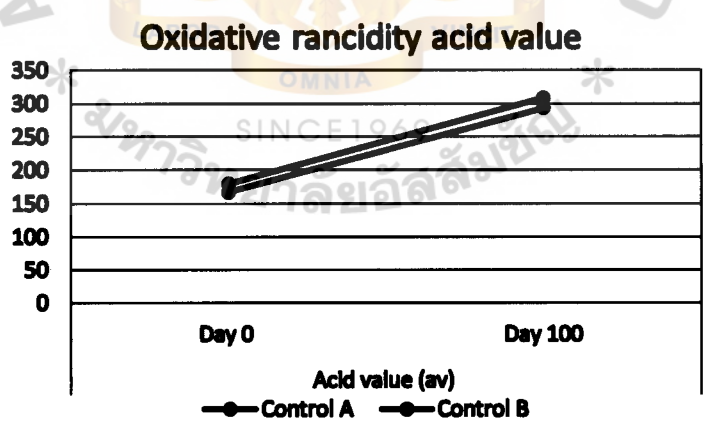


Figure j.

The graph show comparison of (Figure h.) % total acidity, (Figure i.) pH, (Figure j.) oxidative rancidity acid value of control and 1.2% Vit E sample in row.

Discussion aerobic plate count and mold and yeast plate count

From the result in *table 10,* it showed that, they have microbial growth in sample control B and 1.2% vitamin E_B and mold growth in control A, B and 1.2% vitamin E_A and B at day-0. At day-30 there are only control A and B have the mold growth with mold and yeast plate count. At day-100 all of sample have the microbial growth with aerobic plate count. For the mold and yeast plate count control B is only sample which has mold growth. The vacuum pack storage method may inhibit the growth of microorganisms than packed with adding oxygen absorber. Because there are lower of number microbial growth on the vacuum pack storage than adding with oxygen absorber packed. In addition, the adding of 0.5% oregano has the lowest antimicrobial activity to inhibit microbial growth. Because the adding of 0.5% oregano have highest number than others sample. The result was like the review result of effect of adding oregano essential oil, garlic and tomato preparations separately and in combination on the stability of vacuum-packed minced pork during storage. It showed that the adding of oregano oil can affect a little to inhibit the microbial growth. The increased of percentage adding of oregano in the formula may help to improve the inhibit microbial growth property of oregano in the sample.

Table 11. Compare result of microbial growth of aerobic plate count and mold and yeast plate count of all sample in column.

Sample	Aerobic plate count day0 CFU/ml	Mold and yeast plate count day 0 CFU/m	Aerobic plate count day 30CFU/ml	Mold and yeast plate count day 30 CFU/m	Aerobic plate count day 100 CFU/ml	Molds and yeasts plate count day 100 CFU/m
Control A	-	1.1×10^4	-	5.13×10^3	3.3×10^1	-
Control B	8×10^3	1.38×10^3	-	6×10^2	5×10^3	6.67×10^2
0.5% Vit E_A	-	-	-	-	1.6×10^3	-
0.5% Vit E_B	-	-	-	-	9×10^2	-
1.2% Vit E_A	-	2.51×10^3	-	-	5.33×10^2	-
1.2% Vit E_B	3.3×10^1	4.08×10^3	-	-	3.3×10^1	-
0.5% Oreg_A	-	-	-	-	3.71×10^3	-
0.5% Oreg_B	-	-	-	-	1.06×10^4	-

CONCLUSION

In conclusion, the adding of 0.6% vitamin E and 0.5% oregano can help to reduce the oxidative rancidity acid value but the adding of 0.5% oregano has the lowest property to inhibit the microbial growth the sample with 0.5% oregano was storage with oxygen absorber has lower acid value than vacuum storage. The sample with adding 0.6% vitamin E and storage with vacuum packed is the lowest moisture content and water activity. From the result the adding of 0.6% vitamin E and storage with vacuum is the recommend to extent the shelf life of Sai-Krok-Isan.

Due to the COVID-19, it affected to the analysis of all sample, in the beginning the sample was planned to analyze at day 0, 30, 90 and 120. The result can analyze only day 0 and day 100-110, there are only control A, B, 1.2 % vit E_A and B that have result of day 30 on color measurement, %moisture content and microbial growth.

In overall assessment, it cannot conclude which sample was the better sample of this study. Because it has not enough of analysis data to analyze the complete result of this study.

REVIEW

1.Review "Effect of the packaging method on dry fermented sausage"

- Vacuum package can affect to the lipid oxidation and color stability of dry sausage. The vacuum packed and suitable modified atmosphere method can improve shelf life in the sausage which rich in MUFA and PUFA (Rubio, 2008).

2.Review "Effect of spices for preventing oxidation on dry fermented sausage"

- The main benefit of adding oregano essential oil to help to lower lipid oxidation indicator. Normally the oregano was known it has strong antimicrobial properties but this study the essential oil had little effect to microbial growth. It may cause by the high initial population of mivcorbial in the product sample (Magdalena Michalczyk, 2015).

- The oils extracted from plants of the genus Origanum have been shown to have antimicrobial activity in vitro and in food. The oil of *Origanum vulgare* at 1/4000 dilution can reduced level of *S. aureus* from 8 $\log_{10}$ cfu ml^{-1} to 0 within 60 mins in the nutrient broth and the 1/*10000 dilution was required 24 hrs. to destruct of all viable cell in the nutrient broth (Richard A. Holley, 2005).

3.Review "Shelf-life of freeze-dried fermented sausage"

- Sensorial Characterization of Foods Before and After Freeze-drying, time, temperature and pressure were the most important of freeze dried. It can affect to the quality of the product if the condition was set not suitable for the product type. Pre-treatment is very important to prevent the browning of the product and limit the time of freeze dry. (Valentina V, 2016)

4.Review "Function of tocopherols and tocotrienol in lipid oxidation"

- The optimum antioxidant concentrations of tocopherols in oils and fats indicated that the optimal level for α-tocopherol is usually lower than other tocopherols, the less of α-tocopherol was needed for the maximum antioxidant protection (Christine M. Seppanen, 2007).

5.Review "Effect of water activity on freeze-dried meat"

- Water activity has the potential to impact the stability of freeze-dried beef during storage. The lipid oxidation was favored at a low aw range and 49 °C. In the point of protein solubility medium range of water activity caused the decreased of protein extractability. The low of a_w and storage temperature preserved protein solubility than other storage condition (Q. SUN, 2002).

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APPENDIX 1

Appendix 1.1: RCBD DUNCAN Texture profile analysis Hardness, Fracturability, Springiness, Cohesiveness and Chewiness attribute of control, 0.6% Vit E, 1.2% Vit E and 0.5% oregano day-100

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: hardness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1448767357	131706123	2.39	0.0241
Error	36	1983367338	55093537		
Corrected Total	47	3432134696			

R-Square	Coeff Var	Root MSE	hardness Mean
0.422118	27.41621	7422.502	27073.41

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	5	109591397	21918279	0.40	0.8470
sample	6	1339175961	223195993	4.05	0.0033

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	5	109591397	21918279	0.40	0.8470
sample	6	1339175961	223195993	4.05	0.0033

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: fracturability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	779391506	70853773	3.33	0.0031
Error	36	766182948	21282860		
Corrected Total	47	1545574454			

R-Square	Coeff Var	Root MSE	fracturability Mean
0.504273	32.59718	4613.335	14152.56

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	5	64951083.5	12990216.7	0.61	0.6925
sample	6	714440423.0	119073403.8	5.59	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	5	64951083.5	12990216.7	0.61	0.6925
sample	6	714440423.0	119073403.8	5.59	0.0004

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: springiness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.50195108	0.04563192	11.01	<.0001
Error	36	0.14924958	0.00414582		
Corrected Total	47	0.65120067			

R-Square	Coeff Var	Root MSE	springiness Mean
0.770809	24.97274	0.064388	0.257833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	5	0.01846242	0.00369248	0.89	0.4976
sample	6	0.48348867	0.08058144	19.44	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	5	0.01846242	0.00369248	0.89	0.4976
sample	6	0.48348867	0.08058144	19.44	<.0001

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: cohesiveness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.08571400	0.00779218	1.83	0.0854
Error	36	0.15351081	0.00426419		
Corrected Total	47	0.23922481			

R-Square	Coeff Var	Root MSE	cohesiveness Mean
0.358299	37.08953	0.065301	0.176063

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	5	0.01403544	0.00280709	0.66	0.6572
sample	6	0.07167856	0.01194643	2.80	0.0243

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	5	0.01403544	0.00280709	0.66	0.6572
sample	6	0.07167856	0.01194643	2.80	0.0243

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: chewiness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	107133852.5	9739441.1	6.68	<.0001
Error	36	52480431.5	1457789.8		
Corrected Total	47	159614284.1			

R-Square	Coeff Var	Root MSE	chewiness Mean
0.671205	76.79526	1207.390	1572.219

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	5	5302927.9	1060585.6	0.73	0.6074
sample	6	101830924.7	16971820.8	11.64	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	5	5302927.9	1060585.6	0.73	0.6074
sample	6	101830924.7	16971820.8	11.64	<.0001

Appendix 1.2: RCBD DUNCAN Texture profile analysis Hardness, Fracturability, Springiness, Cohesiveness and Chewiness attribute of control and 1.2% Vit E day-0

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: hardness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	849323409	141553902	1.28	0.3871
Error	6	665064832	110844139		
Corrected Total	12	1514388241			

R-Square	Coeff Var	Root MSE	hardness Mean
0.560836	27.61348	10528.25	38127.23

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	3	156806794.7	52268931.6	0.47	0.7132
sample	3	692516614.7	230838871.6	2.08	0.2040

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	3	124940324.9	41646775.0	0.38	0.7741
sample	3	692516614.7	230838871.6	2.08	0.2040

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: fracturability

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	168385433.6	28064238.9	1.41	0.3438
Error	6	119500311.6	19916718.6		
Corrected Total	12	287885745.2			

R-Square	Coeff Var	Root MSE	fracturability Mean
0.584904	44.52053	4462.815	10024.17

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	3	46280014.0	15426671.3	0.77	0.5493
sample	3	122105419.6	40701806.5	2.04	0.2093

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	3	44444696.4	14814898.8	0.74	0.5639
sample	3	122105419.6	40701806.5	2.04	0.2093

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: springiness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.09055860	0.01509310	1.71	0.2660
Error	6	0.05304017	0.00884003		
Corrected Total	12	0.14359877			

R-Square	Coeff Var	Root MSE	springiness Mean
0.630636	29.59512	0.094021	0.317692

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	3	0.01819702	0.00606567	0.69	0.5925
sample	3	0.07236158	0.02412053	2.73	0.1365

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	3	0.01818058	0.00606019	0.69	0.5928
sample	3	0.07236158	0.02412053	2.73	0.1365

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: cohesiveness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.03126827	0.00521138	2.66	0.1297
Error	6	0.01176450	0.00196075		
Corrected Total	12	0.04303277			

R-Square	Coeff Var	Root MSE	cohesiveness Mean
0.726615	16.29798	0.044280	0.271692

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	3	0.00466602	0.00155534	0.79	0.5407
sample	3	0.02660225	0.00886742	4.52	0.0553

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	3	0.00536250	0.00178750	0.91	0.4893
sample	3	0.02660225	0.00886742	4.52	0.0553

Texture of Sai-Krok-Isan

The GLM procedure

Dependent Variable: chewiness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	63005755.04	10500959.17	3.11	0.0966
Error	6	20260467.77	3376744.63		
Corrected Total	12	83266222.81			

R-Square	Coeff Var	Root MSE	chewiness Mean
0.756678	48.94922	1837.592	3754.079

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	3	3353105.85	1117701.95	0.33	0.8038
sample	3	59652649.19	19884216.40	5.89	0.0321

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	3	3062977.79	1020992.60	0.30	0.8231
sample	3	59652649.19	19884216.40	5.89	0.0321

Appendix 1.3: RCBD DUNCAN % total acidity of control, 0.6% Vit E, 1.2% Vit E and 0.5% oregano day-100

% Total acidity

The GLM procedure

Dependent Variable: %total acidity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.00206126	0.00022903	1.03	0.4604
Error	14	0.00309938	0.00022138		
Corrected Total	23	0.00516064			

R-Square	Coeff Var	Root MSE	totalacidity Mean
0.399419	21.03286	0.014879	0.070742

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	0.00001180	0.00000590	0.03	0.9737
sample	7	0.00204945	0.00029278	1.32	0.3099

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	0.00001180	0.00000590	0.03	0.9737
sample	7	0.00204945	0.00029278	1.32	0.3099

Appendix 1.3: RCBD DUNCAN % total acidity of control day-0

% Total acidity

The GLM procedure

Dependent Variable: % total acidity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00369867	0.00123289	0.10	0.9517
Error	2	0.02420208	0.01210104		
Corrected Total	5	0.02790075			

R-Square	Coeff Var	Root MSE	totalacid Mean
0.132565	47.99857	0.110005	0.229183

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	0.00368233	0.00184117	0.15	0.8679
sample	1	0.00001633	0.00001633	0.00	0.9740

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	0.00368233	0.00184117	0.15	0.8679
sample	1	0.00001634	0.00001634	0.00	0.9740

Appendix 1.4: RCBD DUNCAN color measurement L*, a*, b* of control, 0.6% Vit E, 1.2% Vit E and 0.5% oregano day-100

Color measurement

The GLM procedure

Dependent Variable: lightness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	291.2323433	13.8682068	3.23	<.0001
Error	98	420.6436267	4.2922819		
Corrected Total	119	711.8759700			

R-Square	Coeff Var	Root MSE	lightness Mean
0.409105	5.341641	2.071782	38.78550

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	32.1399200	2.2957086	0.53	0.9068
sample	7	259.0924233	37.0132033	8.62	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	32.1399200	2.2957086	0.53	0.9068
sample	7	259.0924233	37.0132033	8.62	<.0001

Color measurement

The GLM procedure

Dependent Variable: red

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	184.1538558	8.7692312	15.42	<.0001
Error	98	55.7481233	0.5688584		
Corrected Total	119	239.9019792			

R-Square	Coeff Var	Root MSE	red Mean
0.767621	9.525574	0.754227	7.917917

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	7.3081167	0.5220083	0.92	0.5428
sample	7	176.8457392	25.2636770	44.41	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	7.3081167	0.5220083	0.92	0.5428
sample	7	176.8457392	25.2636770	44.41	<.0001

Color measurement

The GLM procedure

Dependent Variable: yellow

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	266.1391292	12.6732919	9.42	<.0001
Error	98	131.8268300	1.3451717		
Corrected Total	119	397.9659592			

R-Square	Coeff Var	Root MSE	yellow Mean
0.668748	4.960486	1.159815	23.38108

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	12.3571967	0.8826569	0.66	0.8109
sample	7	253.7819325	36.2545618	26.95	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	12.3571967	0.8826569	0.66	0.8109
sample	7	253.7819325	36.2545618	26.95	<.0001

Appendix 1.5: RCBD DUNCAN color measurement L*, a*, b* of control and 1.2% Vit E day-30

Color measurement

The GLM procedure

Dependent Variable: lightness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	257.8869631	15.1698214	2.02	0.0332
Error	41	307.6369047	7.5033391		
Corrected Total	58	565.5238678			

R-Square	Coeff Var	Root MSE	lightness Mean
0.456014	8.453771	2.739222	32.40237

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	21.6713428	1.5479531	0.21	0.9987
sample	3	236.2156203	78.7385401	10.49	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	23.1061048	1.6504361	0.22	0.9982
sample	3	236.2156203	78.7385401	10.49	<.0001

Color measurement

The GLM procedure

Dependent Variable: red

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	405.976993	23.881000	1.13	0.3568
Error	42	884.438807	21.058067		
Corrected Total	59	1290.415800			

R-Square	Coeff Var	Root MSE	red Mean
0.314609	42.88698	4.588907	10.70000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	308.9819000	22.0701357	1.05	0.4286
sample	3	96.9950933	32.3316978	1.54	0.2194

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	308.9819000	22.0701357	1.05	0.4286
sample	3	96.9950933	32.3316978	1.54	0.2194

Color measurement

The GLM procedure

Dependent Variable: yellow

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	266.5785317	15.6810901	1.00	0.4722
Error	42	655.3630867	15.6038830		
Corrected Total	59	921.9416183			

R-Square	Coeff Var	Root MSE	yellow Mean
0.289149	10.90822	3.950175	36.21283

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	233.0511933	16.6465138	1.07	0.4127
sample	3	33.5273383	11.1757794	0.72	0.5479

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	233.0511933	16.6465138	1.07	0.4127
sample	3	33.5273383	11.1757794	0.72	0.5479

Appendix 1.6: RCBD DUNCAN color measurement L*, a*, b* of control, 0.6% Vit E, 1.2% Vit E and 0.5% oregano E day-0

Color measurement

The GLM procedure

Dependent Variable: lightness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	193.4837072	9.2135099	1.64	0.0569
Error	92	517.2040051	5.6217827		
Corrected Total	113	710.6877123			

R-Square	Coeff Var	Root MSE	lightness Mean
0.272249	7.167373	2.371030	33.08088

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	109.0390307	7.7885022	1.39	0.1762
sample	7	84.4446765	12.0635252	2.15	0.0464

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	108.4168249	7.7440589	1.38	0.1801
sample	7	84.4446765	12.0635252	2.15	0.0464

Color measurement

The GLM procedure

Dependent Variable: red

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	133.0378126	6.3351339	5.61	<.0001
Error	92	103.9497321	1.1298884		
Corrected Total	113	236.9875447			

R-Square	Coeff Var	Root MSE	red Mean
0.561370	12.56886	1.062962	8.457105

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	36.77606497	2.62686178	2.32	0.0086
sample	7	96.26174763	13.75167823	12.17	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	32.75762596	2.33983043	2.07	0.0205
sample	7	96.26174763	13.75167823	12.17	<.0001

Color measurement

The GLM procedure

Dependent Variable: yellow

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	625.3714734	29.7795940	13.09	<.0001
Error	92	209.2644889	2.2746140		
Corrected Total	113	834.6359623			

R-Square	Coeff Var	Root MSE	yellow Mean
0.749275	4.510907	1.508182	33.43412

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	14	58.2988593	4.1642042	1.83	0.0454
sample	7	567.0726141	81.0103734	35.61	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	14	57.1886878	4.0849063	1.80	0.0508
sample	7	567.0726141	81.0103734	35.61	<.0001

Appendix 1.7: RCBD DUNCAN oxidative rancidity acid value of control, 0.6% Vit E, 1.2% Vit E and 0.5% oregano E day-100

Oxidative rancidity acid value

The GLM procedure

Dependent Variable: acid value

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	263892.2252	29321.3584	26.58	<.0001
Error	14	15445.7952	1103.2711		
Corrected Total	23	279338.0203			

R-Square	Coeff Var	Root MSE	acidvalue Mean
0.944706	23.09151	33.21552	143.8430

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	2665.2991	1332.6496	1.21	0.3281
sample	7	261226.9260	37318.1323	33.82	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	2665.2991	1332.6496	1.21	0.3281
sample	7	261226.9260	37318.1323	33.82	<.0001

Appendix 1.7: RCBD DUNCAN oxidative rancidity acid value of control day-0

Oxidative rancidity acid value

The GLM procedure

Dependent Variable: acid value

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3793.291067	1264.430356	0.70	0.6341
Error	2	3621.918533	1810.959267		
Corrected Total	5	7415.209600			

R-Square	Coeff Var	Root MSE	acidvalue Mean
0.511555	24.54457	42.55537	173.3800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	3575.366800	1787.683400	0.99	0.5032
sample	1	217.924267	217.924267	0.12	0.7618

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	3575.366800	1787.683400	0.99	0.5032
sample	1	217.924267	217.924267	0.12	0.7618

Appendix 1.8: RCBD DUNCAN pH value of control day-100

pH

The GLM procedure

Dependent Variable: pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.14217500	0.12690833	15.19	<.0001
Error	14	0.11695833	0.00835417		
Corrected Total	23	1.25913333			

R-Square	Coeff Var	Root MSE	pH Mean
0.907112	1.714307	0.091401	5.331667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	0.01830833	0.00915417	1.10	0.3613
sample	7	1.12386667	0.16055238	19.22	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	0.01830833	0.00915417	1.10	0.3613
sample	7	1.12386667	0.16055238	19.22	<.0001

Appendix 1.9: RCBD DUNCAN pH value of control day-0

Oxidative rancidity acid value

The GLM procedure

Dependent Variable: pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.07405000	0.02468333	15.27	0.0621
Error	2	0.00323333	0.00161667		
Corrected Total	5	0.07728333			

R-Square	Coeff Var	Root MSE	pH Mean
0.958163	0.769037	0.040208	5.228333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	0.00363333	0.00181667	1.12	0.4709
sample	1	0.07041667	0.07041667	43.56	0.0222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	0.00363333	0.00181667	1.12	0.4709
sample	1	0.07041667	0.07041667	43.56	0.0222

Appendix 1.10: RCBD DUNCAN water activity of control day-0

Water activity

The GLM procedure

Dependent Variable: water activity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.18348750	0.02038750	761.13	<.0001
Error	14	0.00037500	0.00002679		
Corrected Total	23	0.18386250			

R-Square	Coeff Var	Root MSE	wateractivity Mean
0.997960	1.200114	0.005175	0.431250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
number	2	0.00002500	0.00001250	0.47	0.6365
sample	7	0.18346250	0.02620893	978.47	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
number	2	0.00002500	0.00001250	0.47	0.6365
sample	7	0.18346250	0.02620893	978.47	<.0001