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# A study of Mu Yor sausage wraps using chitosan films incorporating garlic oil, lemon grass oil and galangal oil

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**Abstract**

Chitosan films with spice oil were produced and determined their properties. Their application was to replace the plastic wrapping of meat products. The obtained chitosan films were transparent and elastic. The elongation and water vapor permeability were low (0.82-4.94% and 12.30-23.70 g.mm/m$^2$.d.kPa, respectively). The tensile strength was similar to those of plastic films. The spice oils affected film surface significantly. After cooking, Mu Yor sausages were kept in a room temperature (25-27$^o$C) and a refrigerate temperature (5-8$^o$C), it was found that the number of microorganisms in Mu Yor sausage of chitosan wrappings were lower than plastic wrapping at room temperature but they were opposite at refrigerate temperature. All treatments could be kept safely 2 days in room temperature and 35 refrigerate temperature (<6 log CFU/g). Chitosan films can be one alternative material for food preservation. It is possible to reduce the use of preservatives and plastic. Moreover, Mu Yor sausage wrapped with chitosan film was accepted by consumers.



## Introduction

Thai steamed pork sausages or Mu Yor sausages are local products of the northeastern and the northern regions of Thailand. The pork product is made from pork meat, fat, and seasonings and may include other ingredients such as milk protein, vegetable protein concentrate, and tapioca flour, which are finely blended together. To make specific types of Mu Yor sausage, some ingredients are added such as pork skin, shiitake mushroom, black pepper, and seaweed, which are mixed and completely blended. The mixtures of these ingredients are tightly wrapped and then boiled or steamed (Thai Industrial Standard, 1996). The wrap is usually made from banana leaves because they create good odor and appearance. The mixtures of the ingredients are sometimes put in a deep bottom tray and the flavor was created by food additives. Currently, plastic is often used because of its low price. However, plastic contains some chemicals that may contaminate in the food. Therefore, Mu Yor sausage with thick wrapping of banana leaves is more famous. Mu Yor sausage in a plastic wrap often contains preservatives, so it can be kept longer; it is not easily detected whether it is spoiled or not. Kietnitiprawat (2007) found, under the food product survey by the Medical Science Center of Ubon Ratchathanee province, that 17 out of 27 collected Mu Yor sausage contained benzoic acid higher than the set standard of 1 g per 1 kg of food (Thai Community Standard, 2003). Even though this substance can be discharged from the body through metabolism, it can irritate skin, eye, and epithelia and cause nausea, vomiting, stomachache, and diarrhea when receiving in a high amount. Materials for Mu Yor sausage wrapping are important. Polypropylene (PP) is good material at a temperature of lower than 140$^o$C, while High density polyethylene (HDPE) is good material at a temperature not exceeding 100oC. Polyvinyl chloride (PVC) can be used when the temperature is not over 90$^o$C. When higher than 90$^o$C, PVC decomposes and releases Vinyl Chloride Monomer (VCM) which can cause cancer. However, PVC products which are commonly used are cling wrapping films and food containers. Nevertheless, some manufacturers choose to use cheaper materials, which cannot be used in food wrapping. As consumers, we are never aware of the quality of the plastic used. Moreover, as mentioned, Mu Yor in a plastic wrap may contain preservatives that we do not know about.

Chitosan is a derivative of chitin. Through the deacetylation process, the acetamide group in chitin is transformed to be an amino group (-$NH_2$). Generally, chitin and chitosan are copolymers. Chitosan is a biopolymer which produced from

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residues of frozen sea animals such as shrimp shells, crab shells, and squid pens, which are available in the country. Currently, chitosan is a polymer consistently playing an important role in the world's economy since it is biocompatible, biodegradable, edible, and antimicrobial. Moreover, it can be casting film. Chitosan can be used to prevent the growth of microorganisms and preserving fruits and vegetables such as cucumbers, bell peppers, asparagus, baby corn, mushroom and Chinese cabbage by dipping, spraying with chitosan solution or wrapping with chitosan films (El Ghaouth *et al*., 1991; Pitak and Rakshit, 2011). However, chitosan application method affects its quality (Guilbert, 1986). Oil and spice oil were added in film solution to improve film properties such as increase barrier properties and antimicrobial properties. Chitosan seems appropriate as a Mu Yor sausage wrap which instead some part of plastic, both in terms of safety and reduction of hard decomposed plastic.

## Materials and Methods

*Production of Mu Yor sausage*

Mu Yor sausage used in the study was prepared using the recipes of a Food Institute (2003). The ingredients were pork mince (1 kg), accord (2.4 g), salt (2 table spoons), pepper power (2.5 table spoons), tapioca flour, phosphate and crushed ice. All ingredients were put in the chopper until the meat was tender. After that, the meat was wrapped with chitosan film and boiled at temperature of 100°C for 45 min. Polypropylene film was prepared for the control sample. After cooked, it was soaked in colder water.

*Formation of chitosan films*

To produce chitosan films, production methods used by Zivanovic *et al.* (2005) were adopted. The 1% w/w chitosan (85% degree of deacetylation and 65 kDa average molecular weight from NNC Product Ltd., Bangkok, Thailand) was stirred in 1% v/v acetic acid (Univar, USA) for one night. Diluted substances were then percolated. The remaining substance was sterilized at a 121°C for 12 min. After that, 0.2% spices oil (B smile Co, Ltd., Bangkok, Thailand) (Azarakhsh *et al.*, 2012) were added then were homogenized 21600 revolutions/min and were poured onto plates (Paisan Superlene Co, Ltd., Bangkok, Thailand) and were then dehydrated at 40°C for 24 hours. The thickness of film was about 0.1 mm.

*Water vapor permeability (WVP) of chitosan films*

ASTM E96 (ASTM, 2000) standard for testing WVP was adopted. The samples of chitosan film with a 6 cm diameter were prepared. Next, sealant was directed around testing cups containing distilled water. Then, the testing cups were covered with the samples. The cups were closed and knotted with the lids. They were weighted carefully and put in the chamber. The changing weight was recorded every one hour until the weight was 1% different. The following formula was used in calculating the water vapor permeability rate.

$$WVTR = \frac{G/t}{A}$$

$$WVP = \frac{WVTR \times thickness}{\Delta P}$$

Where WVTR = Water vapor transmission rate (g/ $m^2$.day)

G/t = changing weight rate per time (g/day)
A = sample surface ($m^2$)
ΔP = difference between internal and external pressure of the testing cup (kPa)

*Test of tensile strength (TS) of chitosan films*

ASTM D882 standards for testing TS (ASTM, 1997) were used. The 10x150 $mm^2$ of film pieces were stored under the temperature of 23°C with 50% relative humidity for at least 40 hours. To test the tensile strength, both ends of the samples were secured with the texture analyzer (model TA.XT plus, Stable Micro Systems Ltd., UK). The testing speed was at 50 m/min with a load cell of 0.5 KN. The distance between the two anchorages was 100 mm. The following formula was used in calculating the tensile strength and elongation.

$$TS = \frac{\max imum\ load}{width \times thickness}$$

$$E = \frac{final\ length - initial\ length}{initial\ length} \times 100$$

*Surface morphology of chitosan films*

Morphology of film surface was analyzed using a scanning electron microscope (SEM, Model 6400, JEOL, Germany). The 5x1 $mm^2$ of film pieces were mounted on bronze stubs using tape and were coated with gold allowing surface visualization. All samples were observed using an accelerating voltage of 10 kV at 400X magnifications.

*Sensory evaluation of Mu Yor sausage*

50 panelists who were regular Mu Yor sausage consumers were recruited. The sensory evaluation of the Mu Yor sausage on initial day used a 9-point Hedonic scale. A score of 1 represents dislike

extremely and a score of 9 represents like extremely. The panelists were asked about appearance, color, flavor, texture and taste as well as their overall acceptability about the products which were wrapped with plastic and chitosan. Samples were randomly coded and served individually. They were not asked to compare the ingredients between the two wrapping materials because they were produced using the same recipes.

*Microorganisms of Mu Yor sausage*

The microorganisms in the produced Mu Yor sausage, sterilized plates with a nutrient agar (Biomark Laboratories, India) were prepared. Food solution with $10^{-6}$, $10^{-5}$ and $10^{-4}$ dilution at 1 mL was collected by using a pipette and putting it on the melt nutrient agar which was on the plate. The plates were pored and left until they were harden. The plates then were inverted and incubated at 37°C for 24 hours. Then, the counting of the microorganisms was conducted which was 30-300 colonies per plate.

*Data analysis and Data presentation*

A one way analysis of variance (ANOVA) was used to determine the effect of the three spices oil on the biological characteristics of chitosan film, to compare the number of microorganisms found in Mu Yor sausage when kept at a room temperature (25-27°C) and a refrigerate temperature (5-8°C), and to evaluate the acceptance of the consumer participants on Mu Yor sausage wrapped by a chitosan film. A Duncan's multiple range test (DMRT) method will be used to determine the difference of the factors at a confidential level of 95% ($p \leq 0.05$).

## Results and Discussion

*Water vapor permeability (WVP) of chitosan films*

The WVP is the important requirement for packages because products can deteriorate by water vapor from surrounding. Moreover, the package can separate the different component foods to prolong shelf-life. The obtained chitosan films were transparent and elastic. The WVP of the chitosan film produced in this study was between 12.30-23.70 g.mm/m$^2$.d.kPa. However, there was no significant difference between with and without spices oil (Table 1). The chitosan film had a higher rate of WVP than methycellulose film and plastic film (Turhan and Sahbaz, 2004; Smith, 1986) since the chitosan film is a hydrophilic film which is edible and can be naturally decayed. Moreover, it can mix well with water. As such, the flocculation of derivatives is not as dense as that of plastic films. When added spices oil, the WVP of the chitosan film was higher but there was no significant difference between with and without spices oil. As fat molecules infiltrated into chitosan's polymers, the flocculation of chitosan molecules was lower creating more gaps between molecules. The transmission of water vapor was then greater (Rodriguez *et al*., 2006).

**Table 1.** Water vapor permeability (WVP), tensile strength (TS) and elongation (E) of chitosan films incorporating garlic oil, lemon grass oil and galangal oil

| Incorporating | WVP (g.mm/m$^2$.d.kPa) | Tensile strength (MPa) | Elongation (%) |
|---|---|---|---|
| Garlic oil | 17.71±4.67$^a$ | 9.03±1.34$^b$ | 1.39±0.29$^b$ |
| Lemon grass oil | 23.70±10.31$^a$ | 16.01±2.76$^a$ | 1.89±0.36$^b$ |
| Galangal oil | 15.33±3.43$^a$ | 9.30±2.33$^b$ | 0.95±0.16$^b$ |
| Without oil | 12.30±2.79$^a$ | 13.06±2.73$^a$ | 3.47±1.34$^a$ |

Different alphabets in the same column indicate statistical difference ($p \leq 0.05$).
Data are mean of triplicates.

*Tensile strength (TS) and elongation (E) of chitosan films*

The most films require high TS and E to make packages because it will damage during shipping, handling and sale. It is well known that edible film was more brittle and poorer elongation properties (Tapia-Blacido *et al.*, 2005). The TS and E of chitosan films were between 9.03-16.01 MPa and 0.95-3.47%, respectively. The TS value of chitosan incorporating lemon grass oil was the highest; however, it was not significantly higher than that of the film that did not add any spice oil. The TS values of chitosan incorporated with garlic and galangal oil were low and they were not significantly different from each other. In regards to the E value, it was found that chitosan films did not incorporate any spice oil had the highest E value, while the E values of the chitosan films with spice oil were low and were not significantly different between with and without spice oil. TS of chitosan without plasticizers increased with chitosan addition which leads to strong films (Rivero *et al*., 2009). The spice oil serves as plasticizer which reduced TS and E value in chitosan film due to hydroscopic and rotten polymers (Arvanitoyannis *et al*., 1997). Similar TS and E were found in gellen film, casein film, carboxymethylate starch based film and banana film (Yang and Paulson, 2000; Kim *et al*., 2002; Chambi and Gresso, 2006; Sothornvit and Pitak, 2007). However, the mechanical properties were dependent upon the property of each type of fat added.

*Surface morphology of chitosan films*

At the stretch of 400x, the surface of the chitosan with no spice oil was the smoothest which same as studied by Vargas *et al*. (2009). The smoothness was lower in the chitosan with spice oil. As seen in Figure

1, there clearly were separations between chitosan in polymeric matrix and fat (Chambi and Gresso, 2006). This characteristic of the surface had some effect on other properties of the film as found in mesquite gum–candelilla wax based edible emulsion coatings (Bosquez-Molina *et al*., 2003). When oleic oil was added, there were bumps and holes scattered all over the film. In film adding mineral oil, the oil congregated and were all blended. In smooth films, the WVP value was the lowest.

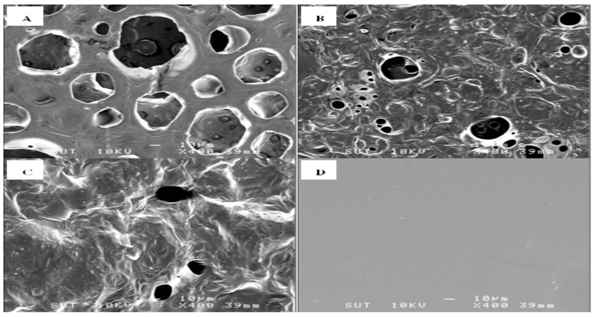


**Figure 1.** Surface morphology of chitosan films A) with garlic oil B) with lemon grass oil C) with galangal oil and D) without spice oil

*Microorganisms of Mu Yor sausage*

The antimicrobial property of chitosan films is the advantage for food packaging because it can control or reduce the growth of pathogenic bacteria. Especially, the natural materials are environmental friendly which is safety for consumer and is not cause the waste problem. The produced Mu Yor sausage was kept following the safety requirements for Agricultural Commodity and Food (National Criteria for Agricultural Products and Food, 2005), of which the microorganisms in pork exposed to heat must not exceed 6 log CFU/g. Based on the findings of the study in Day 2 at a room temperature, the number of microorganisms in most of the Mu Yor sausage wrapped with chitosan film was found to be under the set criteria, while the number of microorganisms in Mu Yor sausage wrapped with plastic (PP) was found to be higher than the set criteria (Table 2). A possible explanation would be that the WVP value was low. In such conditions, facultative bacteria could grow well and caused spoil food. Mu Yor sausage wrapped with chitosan films without spice oil could be kept for 3 days which was longer than the study of Ministry of Public Health of Thailand (2007) which found that in such condition, Mu Yor sausage could be kept for 1 day.

After 45 days in the refrigerate temperature condition, it was found that Mu Yor sausage wrapped with plastic films still had favorable smell and color, while Mu Yor sausage wrapped with chitosan films was hard and had bad smell. One characteristic of

**Table 2.** Microorganism content in Mu Yor sausage wrapped with chitosan films and plastic film in room temperature (25-27$^{o}$C) and refrigerate temperature (5-8$^{o}$C)

| Wrapping materials | Initial day (log CFU/g) | Room temperature (25-27$^{o}$C) | | | Refrigerate temperature (5-8$^{o}$C) | |
|---|---|---|---|---|---|---|
| | | Day 1 (log CFU/g) | Day 2 (log CFU/g) | Day 3 (log CFU/g) | Day 35 (log CFU/g) | Day 45 (log CFU/g) |
| Chitosan with garlic oil | nf | nf | 4.20 | 6.24 | nf | 6.44 |
| Chitosan with lemon grass oil | nf | nf | 4.85 | 6.92 | 4.11 | 5.90 |
| Chitosan with galangal oil | nf | nf | 4.38 | 6.52 | 4.00 | 5.56 |
| Chitosan without oil | nf | nf | 3.59 | 5.40 | 3.93 | 5.33 |
| Plastic | nf | nf | 6.08 | 8.15 | nf | 4.72 |

The alphabet nf means not found

a chitosan film is the humidity absorbent both from Mu Yor sausage and the environment which causes hardening in Mu Yor sausage. Then the film damped and decomposed. This is a condition appropriate of the growth of microorganism, which in turn spoils the food. Under the room and refrigerate temperatures, Mu Yor sausage with chitosan films without spices oil could be kept for 3 and 45 days, respectively, while Mu Yor with plastic films could be kept for 1 day and more than 45 days, respectively. The edible film incorporating spice oil had the antimicrobial property as same as the previous research from Dilek *et al.* (2011) who studied shelf-life of sausages and garlic-flavored sausage using logust bean gum. They found the sausage had longer shelf-life (12 days) than reference at 5$^{o}$C. Another research, alginate films with ginseng showed reduction in gram-negative bacteria cell counts significantly (Norajit and Ryu, 2011). Chitosan film without spices oil show more effective in reducing number of microbial when compare to chitosan with spices oil because the smooth surface and good water vapor barrier of chitosan film without spices oil could protect Mu yor sausage form microorganisms better than chitosan film with spices oil which had small hole on their surface.

*Sensory evaluation of Mu Yor sausage*

Based on the results of the five-element test of Mu Yor sausage, it was found that the 50 panelists could not distinguish between Mu Yor sausage wrapped with chitosan films and plastic films (Table 3). Most of the opinions were ranked as moderate or high. That means that chitosan films could be used to replace plastic films.

**Table 3.** Sensory test of Mu Yor sausage on initial day

| Characteristic | Chitosan with garlic oil | Chitosan with lemon grass oil | Chitosan with galangal oil | Chitosan without oil | Plastic |
|---|---|---|---|---|---|
| Appearance | 5.49 | 5.41 | 5.35 | 5.51 | 5.65 |
| Color | 5.64 | 5.12 | 5.40 | 5.48 | 5.56 |
| Flavor | 4.92 | 4.84 | 4.98 | 5.20 | 5.38 |
| Texture | 5.70 | 5.30 | 4.74 | 5.28 | 5.52 |
| Taste | 5.38 | 5.54 | 5.16 | 5.52 | 5.50 |
| Overall | 5.64 | 5.70 | 5.54 | 5.86 | 5.92 |

The data were not significant difference in the same row ($p \leq 0.05$), n=50.

## Conclusions

Chitosan films without spice oil had appropriate properties to be used as Mu Yor sausage wraps due to its low WVP values, high TS values, E values and tender surface compared against chitosan films with spice oil. When used as Mu Yor sausage wrapping, it was found that chitosan films could prolong the storing days of Mu Yor sausage for 3 days, three times longer than plastic films in the room temperature. However, Mu Yor with chitosan film wraps could be kept in the refrigerator for only 45 days. Consumers accepted the taste of the Mu Yor sausage wrapped with chitosan film and could not differentiate it from the one with plastic film. As such, it can be concluded that chitosan films are effective in keeping for Mu Yor sausage and can replace plastic films.

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