RESEARCH ARTICLE

EFFECTS OF TEMPERATURE AND STORAGE TIME ON FILM WITH NATURAL DYE OF “SENDUDUK” FRUIT (Melastomamalabathricum, L.) EXTRACT AS SMART PACKAGING IN DETECTING SPOILAGE ON CHICKEN MEAT.

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Abstract
This research aimed to study the effects of temperature and storage time on colour of film with natural dye of “senduduk” fruit (Melastomamalabathricum, L.) extract as smart packaging in detecting spoilage on chicken meat and the effectivity of natural dye from “senduduk” fruit extract as film for smart packaging indicator and its colour-changing indicator. This study used two factors, three and five level and three replications. First factor was temperature storage which was freezer temperature (± -20°C) and room temperature (±25°C). Second factor was storage time consisted of 0 - 28 days for ± -2°C and 0 - 6 days for ±25°C. This study showed that the most effective treatment of colour change indicator film is a natural dye “senduduk” fruit extract in room temperature storage (±25°C) with the value of colour change (ΔHue) is 351.29 (red-purple) into 41.53 (red), the pH value (4.50-7.17), total anthocyanins (7.84-1.34mg/L), and the thickness of the film used ranged from 0.11-0.23mm. The utilization of natural dye of “senduduk” fruit (Melastomamalabathricum, L.) extract as smart packaging is effective as indicator for chicken meat during storage periods. Changing of film indicator during storage temperature (±25°C) was from red-purple to red during 6 days of storage time.

Introduction:-
Smart or intelligent packaging can be stated as system that monitor the condition of the package food to provide information about the quality during transport and distribution or by simple definition, smart packaging is packaging which senses and informs the condition of the product 1. Smart packaging is one of latest packaging technology which can detect food condition inside packaging and give information about food quality inside during transportation and storage. This smart packaging can give a solution for packaging problems. There are several current researches related to smart packaging development as label or film with colour sensor to identify food quality degradation. For example, there was a research using bromothymol blue as colour indicator to detect spoilage on fish fillet 2.

Continuous development of smart packaging is related film with natural colouring as indicator. It is important to develop natural colour addition as film indicator whether temperature affects product during storage particularly ones that vulnerable to temperature and light. Colour indicator for film requires certain stability particularly on
storage temperature and sunlight. Changing colour of anthocyanin related to environment such as temperature and pH can be utilized as colour indicator on smart packaging label/film.

Anthocyanin can be obtained from “senduduk” fruit extract for making colour indicator of film can increase economical value of mangosteen peel. reported that “senduduk” fruit extract has the anthocyanin 6.85%/200g (w/w).

This research aimed to study the effects of temperature and storage time on colour of film with natural dye of “senduduk” fruit (Melastomamalabathricum, L.) extract as smart packaging in detecting spoilage on chicken meat and the affectivity of natural dye from “senduduk” fruit extract as film for smart packaging indicator and its colour-changing indicator.

Material and Methods:

Raw Materials:
The raw material had been used in this study were “senduduk” fruit (Melastomamalabathricum, L.), chicken meat, glycerol, tapioca starch, acetic acid, ethanol, aquades, pH buffer. The equipments used were oven, homogenizer, filter paper, glass plate, magnetic stirrer, stirring rod, thermometer, beaker glass, measuring cylinder, vacuum oven, rotary evaporator vacuum, pH meter (Delta Ohm, Australia), Spectrophotometer colour-Flex EZ (HunterLab Inc.: Reston, VA), Spectrophotometer UV-Vis (Biochrom, France).

Extract of “senduduk” fruit
The “senduduk” fruit was extracted by cutting it into smaller pieces and weighed about 200g, then ethanol 96% acidified with 5% acetic acid was added with comparison of “senduduk” fruit and solvent 1:2 and homogenized. Filtrate was separated from “senduduk” fruitusing filter paper until clear filtrate was obtained. Filtrate was then evaporated in rotary evaporator vacuum until solvent evaporate completely. Extract of “senduduk” fruit was obtained followed by anthocyanin total analysis, pH, colour, and residual solvent.

Film indicator preparation
Film were prepared from filmogenic consisted of tapioca starch (4.5 g), glycerol (4.5 ml) and anthocyanin (3 g). Film-forming suspension was obtained under slow and constant stirring up to 75°C and at 50 rpm for starch gelatinization (30 min). Film was poured on glass plat 26x16 cm the dried in oven vacuum at temperature 50°C for 9 hours. After dried, films were coated with anthocyanin colour from “senduduk” fruit extract then kept in freezer for 3 hours to reduce their water contents and to attach colour to films. Afterwards, bio-film thickness was measured using micrometer screw in mm (millimeter).

Chicken meat fillet preparation
Chicken meat washed and cleaned with water, the visceral part removed such as head, neck, skin, legs, lungs, kidneys and blood. In the making of chicken fillet the part used are the piece of boneless chicken meat and the size of fillet ±4x4cm.

Application of smart packaging indicator with chicken meat fillet
The smart packaging indicator with chicken meat filleting polypropylene plastic with size ±12x14cm. The indicator film is affixed to the inside of the plastic using a clear tap on the edge of the indicator film. Chicken meat fillet size ±4x4cm is inserted into the packaging that has been given indicator film. Plastic closed with sealer to close completely.

Experimental Design
Chicken meat fillet and indicator film packaging that has been vacuum closed is stored in ±25°C (freezer temp.) for 28 days and at ±2°C (room temp.) for 6 days. The Observation was conducted on total anthocyanin of “senduduk” fruit extract, pH and colour of indicator film (control) and with chicken fillet during storage period. Analytical procedures were total anthocyanin content, pH analysis and colour analysis of indicator film.

Total Anthocyanin Analysis
Peel extract of “senduduk” fruit was diluted in KCl pH 1.0 and CH3CO2Na.3H2O pH 4.5. Absorbance of those different pH solutions was measured using spectrophotometer UV-Vis (Biochrom France) at 520 nm and 700 nm of
wavelength. Total anthocyanin was calculated with molar extinction coefficient (ε) = 29.600 (based on molar extinction coefficient of cyanidin 3-glucoside) and molecular weight is 449.2.

pH
pH analysis was performed using a pH meter. After standardized on buffer pH7 and buffer pH 4, electrode was penetrated to solution after washing and drying it to obtain pH value. It was conducted three times for each sample.

Colourimetric Analysis
To evaluate the colour of film, sample was observed using Spectrophotometer ColorFlex EZ (HunterlabInc: Reston, VA). After calibration, sample was placed under sensor. The values of rectangular coordinates which are L* for lightness, +a indicates red while a- for green and +b indicates yellow and –b for blue were used to calculate based on international CIE system (Commission Internationale d’Eclairage). It was explained by ⁸ that lightness value is about 0 to 100 while chromatic parameter a* and b* are between 60 and 600. Colour range of CIELab has a uniform range thus point can be used for observing color difference. Another value named Hue (hab) indicated tonality while chroma (C) indicated intensity and its changing. The higher changing of C* or ΔC* means the higher color saturation. ⁹Hue value described visual chromatic colour value and chromatic colour range.

Result And Discussion:
Material Characteristic:-
Characteristic analysis on “senduduk” fruit extract were performed such as anthocyanin total, pH, and colourimetric analysis (L*, a*, b*) which are explained in following table 1.

Table 1: Physicochemical characteristics of “senduduk” fruit extract

| Characteristics          | Value             |
|--------------------------|-------------------|
| Anthocyanin total (mg/l) | 169.83 ± 0.13     |
| pH                       | 3.56 ± 0.05       |
| Lightness (L*)           | 3.92              |
| Redness (a*)             | 0.45              |
| Yellowness (b*)          | -0.01             |
| Thickness (mm)           | 0.11-0.23         |

Table 1 showed that anthocyanin total from “senduduk” fruit extract was about 169.83 mg/L. The result is higher compared with that anthocyanin total content from “senduduk” fruit extract was about 13.71 mg/L. The different anthocyanin anthocyanin total content might related to solvent extraction used. Anthocyanin can be utilized as pH indicator for food product related to its components such as flavilium kation that has a good response for pH changing.

pH analysis was also conducted for “senduduk” fruit extract which was 3.56 and categorized as acid. The flavonoid extraction should be performed under acid condition since acid can denaturize cell membrane thus anthocyanin pigment can be released from those cell. ¹⁰The acidic condition (pH 1-2) anthocyanins are in the most stable and most colorful conditions.

The colorimetric analysis using Spectrophotometer Color Flex (Hunter Lab Inc: Reston, VA) result noted that value for L* was 3.92 meant dark color of “senduduk” fruit extract. Then, value for a* was 0.45 showed red color and b* was -0.01 indicated yellow.

Film indicator Analysis:-
The colour analysis was performed using Hunter Lab Color Flex EZ Spectrophotometer. In this observation, there were three notations (L*, a*, and b*) as rectangular coordinated to identify color changing film indicator (control) during storage periods in two condition temperature. The colour change of film indicator (control) during storage periods showed in table 2.

Table 2: Colour change of film indicator (control) during storage

| Parameter          | Freezer temp. (±2⁰C) | Room temp. (±25⁰C) |
|--------------------|----------------------|--------------------|
| Day                |                      |                    |
In chromatic diagram, L* shows lightness or brightness while a* and b* indicates red for –a*, green for –a*, yellow for +b* and blue for –b*. Centre of achromatic with a* and b* would be increase and move out from center means increasing on color saturation. Visually, red-purple was initial film indicator colour and stay in red-purple during storage periods. During storage in room and freezer temperature, the film indicator (control) showed no colour change during storage. This condition can be used as a reference to the decline in the quality of chicken meat fillet in the packaging because without the fillet of chicken meat in the packaging does not change the color of film indicator.

The colour change of film indicator with chicken meat fillet during storage periods showed in table 3. Table 3 showed no significant colour changing on 28-days for freezer storage temperature (±2°C) based on the color range read by the instrument, but there is an increase in lightness indicated by the value of L* (lightness). According to 8, lightness ranges from 1-100. The increase in lightness occurs in film used. The change of film indicator with chicken meat fillet during storage was increased from 13.99 on the 0 day to 18.95 on the 28 day storage.

The significant colour change on 6 days at room temperature (±25°C). Based on the results conducted significant colour changes occur in the indicator film with the addition of fruit extracts. According to 11, the “senduduk” fruitextract contain high of anthocyanin. the type of anthocyanin present in the “senduduk” fruitextract is delfinidin. Colour changes of film indicator with the addition of “senduduk” fruitextract shows a red-purple color on day 0 with values of L*, a* and b* are 18.83, 2.09, and -0.32, respectively; Purple colour on the 3rd day with values of L*, a* and b* 18.71, 1.12, and -0.67, respectively; and red colour on day 6th day with values of L*, a* and b* are 18.88, 1.05 and 0.93, respectively.

The colour change of film indicator with chicken meat fillet at room temperature due to during microbial storage degrades the protein in meat and produces ammonia and amines that cause rise of pH. The colour change of indicator film does not occur in the storage of chicken meat fillet at freezer temperature because chicken fillet is stored at low temperature (± -2°C). The low temperature will decrease the kinetic energy of all molecules in the system, thereby lowering the rate of chemical reactions including bacterial cell metabolism activity. However, in refrigeration or storage in the refrigerator still allows certain bacteria to live. The low temperature can inhibit the growth of bacteria thereby inhibiting the occurrence of protein degradation during storage thus inhibiting the pH change in chicken meat fillet during storage.

The colour change of film indicator with chicken meat fillet at room temperature as the result of biochemical reaction, physicochemical, and microbial transformation and it is indicated initial spoilage on chicken meat fillet. Reaction between proteolytic activity microorganism such as Staphylococcus aureus and protein changed the components such as free amino acid. Oxidative deamination, decarboxylation, and desulphurization might be occured on amino acids as results of NH₃, CO₂, and H₂S. Protein in meat can be turned completely or partially into basic components such as CO₂, H₂O, NH₃, and H₂S.
The figure of colour change of film indicator with chicken meat fillet during storage periods shows in figure 1.

**Fig. 1:** Colour change of film indicator with chicken meat fillet during storage.

The colour change of film indicator with chicken meat fillet at room temperature (±25°C) based on visual appearance showed the red-purple on the 0 day; purple on 3rd day and red on 6th day storage. The colour change of film indicator with chicken meat fillet at freezer temperature (±2°C) based on visual appearance showed the red-purple from 0 until 28th day storage.

**Conclusion:**

The most effective treatment of colour change indicator film is a natural dye “senduduk” fruit extract in room temperature storage (±25°C) with the value of colour change (°Hue) is 351.29 (red-purple) into 41.53 (red), the pH value (4.50-7.17), total anthocyanins (7.84-1.34 mg/L), and the thickness of the film used ranged from 0.11-0.23 mm. The utilization of natural dye of “senduduk” fruit (*Melastomamalabathricum, L.*) extract as smart packaging is effective as indicator for chicken meat during storage periods. Changing of film indicator during storage temperature (±25°C) was from red-purple to red during 6 days of storage time.

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