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Effects of Temperature and Storage Time on Film with Mangosteen (*Garcinia mangostana,* L.) Peel Extract as Smart Packaging in Detecting Spoilage on Chicken Nugget.

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ABSTRACT

The purpose of this study is to obtain information about effects of temperature and storage time on color of film with extract of mangosteen peel as smart packaging to detect spoilage of chicken nugget and the effectivity of natural color from mangosteen peel extract as film for smart packaging indicator and its color-changing indicator. This study used Complete Randomized Design with two factors and three replications. First factor was temperature storage which was A1 (room temperature), A2 (chiller temperature), and A3 (freezer temperature). Second factor was storage time consisted of B1= 0 day, B2 = 7 days, B3 = 14 days, B4 = 21 days, B5 = 28 days. Observation result from each treatment then analyzed statistically using F Test and followed by DNMRT test for significant result at 5% level. This study showed that different temperature and storage time and their interaction gave significant difference on water activity and pH. On the other hand, different storage time gave insignificant difference on water activity. It can be observed that utilization of color from mangosteen peel extract is effective as indicator for nugget that susceptible to temperature and light changing. Changing of film indicator during storage was from red to yellow as ^{o}Hue value from each treatment.

Keywords: film, mangosteen peel extract, indicator, smart packaging, chicken nugget.

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INTRODUCTION

Facing globalization and free trade era, small agro-industries need to increase their added value of agriculture products. The added value is related to packaging material which is important for food products. In food industries, packaging contributes to food preservation and protecting product from contamination including food safety, maintaining quality lengthening shelf-life. Packaging becomes media for food producer to give product information such as weight, ingredients, nutrition value and expired date. Thus, food products should have excellent packaging during its marketing and distribution to work optimally. Unfortunately, sometimes good packaging cannot guarantee quality of product inside whether it is still in good quality or not. So, many researches are currently related to improve new packaging on its production or utilization.

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 color sensor to identify food quality degradation. For example, there was a research using bromothymol blue as color indicator to detect spoilage on fish fillet [9].

Continuous development of smart packaging is related film with natural coloring as indicator. It is important to develop natural color addition as film indicator whether temperature affects product during storage particularly ones that vulnerable to temperature and light.

Color indicator for film requires certain stability particularly on storage temperature and sunlight. Generally, this condition was obtained by anthocyanin. This color gives red, blue and violet including yellow and colorless except green to flowers and other part of plants. [8] stated that temperature and sunlight exposure reduced color stability of anthocyanin during storage. Changing color of anthocyanin related to environment such as temperature and pH can be utilized as color indicator on smart packaging label/film.

Anthocyanin can be obtained from mangosteen peel since its availability potency as tropical fruit from Indonesia while its peel is counted as waste. Utilization of mangosteen peel for making color indicator of film can increase economical value of mangosteen peel. It was noted by [25] that mangosteen has the highest anthocyanin content (51%). This anthocyanin component in mangosteen is higher than those in grape seed (36%) which is main anthocyanin source in Europe.

Besides detecting degradation on fish fillet from previous researcher, biofilm can be applied to product that vulnerable to temperature and light exposure such as chicken nugget (meat processed product). Chicken nugget was kept in freezing temperature during storage in order to maintain its shelf life. However, there are several factors causing degradation on product with temperature and light vulnerability besides its damage from careless handling during storage. Thus, it is important to develop smart packaging to detect chicken nugget degradation and its quality inside packaging.

The aim of this study is to obtain information about effect of temperature and storage time on color of film with mangosteen peel extract as smart packaging to detect spoilage of chicken nugget and the effectivity of natural color from mangosteen peel extract as film for smart packaging indicator and its color-changing indicator.

MATERIAL AND METHODS

Mangosteen peel, broiler chicken meat, glycerol, tapioca starch, ethanol 95%, aquades, buffer pH were used in this study. Other ingredients such as bread crumbs, spices (garlic, salt, pepper), tapioca starch, ice cubes, eggs, and corn starch were for nugget making.

Materials such as oven, cup, homogenizer, and filter paper, petri disks for film solution, magnetic stirrer, stirring rod, thermometer, beaker glass, measuring cylinder, vacuum oven, rotary evaporator vacuum, and analytical balance were used in this study. Knife, baking pan, analytical balance, blender, meat grinder, stove, frying pan, food processor, refrigerator and freezer were used for nugget making. There were also pH meter (Delta Ohm, Australia), Spectrophotometer ColorFlex EZ (HunterLab Inc.: Reston, VA) for color analysis, and Spectrophotometer UV-Vis (Biochrom, France) for anthocyanin total analysis.

Extract of Mangosteen Peel [19]

Mangosteen peel was extracted by cutting it into smaller pieces about 1x1 cm then ethanol 95% acidified with 5% acetic acid was added with comparison of mangosteen peel and solvent 1:2 and homogenized. Filtrate was separated from mangosteen peel using filter paper until clear filtrate was obtained. Filtrate was then evaporated in rotary evaporator vacuum until solvent evaporate completely. Extract of mangosteen peel was obtained followed by anthocyanin total analysis, pH, color, and residual solvent.

Color Indicator Film Preparation [21]

Film were prepared from filmnogenic 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^{o}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^{o}C for 9 hours. After dried, films were coated with anthocyanin color from mangosteen peel extract then kept in freezer for 3 hours to reduce their water contents and to attach color to films. Afterwards, biofilm thickness was measured using micrometer screw in mm (millimeter).

Chicken Nugget Sample [12]

Chicken meat was minced and added ice cubes, salt, sugar, pepper, garlic, and tapioca starch. The formula for making chicken nugget is explained chicken meat (65%), tapioca starch (19%), salt (1%), garlic (0.6%), pepper (0.4%), iced water (14%).

All ingredients were mixed until homogenized. Nugget mix was placed on aluminum tray coated with plastic then steamed until internal nugget temperature was about 60-70^{o}C for 30 minutes. After steaming process, nugget was cooled down at room temperature then put in refrigerator for 30 minutes.

Half-cooked nugget was cut for 4 x 4 cm and 1 cm of thickness then was coated by batter made from 80 g of corn starch and 100 ml water. Coating was continued with bread crumbs, eggs, and bread crumbs again. Further, nugget was deep frying for 30 seconds at temperature 180^{o}C. Nugget was packaged with film attached to polypropylene.

Experimental Design

A complete randomized design was conducted with 2 factors, three and five levels and 3 replications. There was storage temperature as first factor consisted of A1= room temperature (25°C), A2 = chiller temperature (4°C), and A3 = freezer temperature (-5°C). The second factor was storage time which were B1= 0 day, B2= 7 days, B3= 14 days, B4= 21 days, and B5=28 days. Observation was conducted on total anthocyanin of mangosteen peel extract, residual solvent, pH, and color analysis. Total plate count, pH and water activity were analyzed for nugget while color analysis and thickness were conducted for film.

Total Anthocyanin Analysis on Mangosteen Peel Extract using pH Difference Method [16]

Peel extract of mangosteen was diluted in KCl pH 1.0 and $CH_3CO_2Na.3H_2O$ 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.

Water Content [23]

A cleaned aluminum cup was dried in oven for 1 hour at temperature 110°C and cooled in desiccator then measured it weight. Further, 5 g of sample was placed on that cup then put in oven at temperature 110°C. For every one hour, cup should be removed from oven to desiccator for 10-15 minutes for measuring sample's weight. Sample should be put in oven until a stable weight obtained.

pH

pH analysis was performed using a pH meter. After standarized on buffer pH7 and buffer pH 4, electrode was penetrated to solution after washing and drying it to obtain pH value **[1]**. It was conducted three times for each sample.

Colorimetric Analysis

To evaluate the color 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 Internationaled'Eclairage). It was explained by Hunter (1958) that lightness value is about 0 to 100 while chromatic parameter a and b are between 60 and 600. Color range of CIELab has a uniform range thus point can be used for observing color difference. Another value named ^{o}Hue (h_{ab}) indicated tonality while chroma (C) indicated intensity and its changing. The higher changing of C* or ΔC* means the higher color saturation. ^{o}Hue value described visual chromatic color vaue and chromatic color range [11].

RESULT AND DISCUSSION

Material Characteristic

In this study, film was produced from mangosteen peel extract. Peel was extracted using ethanol then evaporated and later, it would be applied on film as spoilage indicator of nugget. Characteristic analysis on material were performed such as anthocyanin total, pH, remained solvent, and colorimetric analysis (including rectangular coordinates: L*, redness a* and yellowness b*) which are explained in following table 1.

Table 1. Physical and Chemical Characteristic of Mangosteen Peel Extract

Parameter	Analysis Result	Other Previous Research
Anthocyanin Total (mg/L)	0.118	0.120[a]
pH	4.44	3.95[b]
Remained Solvent (%)	5.99	-
Brightness (L*)	14.65	31.35[a]
Redness (a*)	30.00	25.17[a]
Yellowness (b*)	10.70	21.37[a]

[a] = [19], [b] = [20]

From previous table, it can be observed that anthocyanin from mangosteen peel extract was about 0.118 mg/L while **[19]** explained that anthocyanin content from mangosteen peel extract was about 0.120 mg/L. Changing of anthocyanin might related to ripeness level of mangosteen [17]. 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 mangosteen peel extract which was 4.44 and categorized as acid. Similar to this result, [20] was also obtained acid condition for her study which was 3.95. [24] explained that flavonoid extraction should be performed under acid condition since acid can denaturize cell membrane thus anthocyanin pigment can be released from those cell.

It can be found that there was 5.99% ethanol as remained solvent from this previous table. Further, colorimetric analysis using Spectrophotometer ColorFlex (HunterLabInc;Reston,VA) result noted that value for L* was 14.56 meant dark color of mangosteen peel extract. Then, value for a* was 30.00 showed red color and b* was 10.70 indicated yellow. Next, °Hue value that can be calculated from those three rectangular coordinates was 19.63. Based on °Hue value table and chromatic color, this value is related to red color. In similar to this °Hue result, visual color observation on extract was also dark red. Moreover, it can be observed from Table 3 that current color of mangosteen extract was darker compared to those from previous research.

It was also observed on a* and b* that current study had a lower a* and b* value compared to those on previous research indicated extract on current study was bright red and yellow.

Thickness of biofilm was analyzed as physical analysis. Based on the test using micrometer, thickness of biofilm with color indicator from mangosteen peel extract was 0.16 mm. It might be related to thin and easy to torn of biofilm appearance.

Film Color Analysis

Color is important in biofilm making related to its function as indicator of spoilage on nugget during storage. Color analysis was performed using HunterLabColorFlex EZ Spectrophotometer. In this observation, there were three notations as rectangular coordinated to identify color changing on biofilm during storage.

First notation is L or lightness with value between 0 indicated dark or black and 100 indicated light or white. Further, notation a* describes chromatic color between red and green with positive a* indicated red with value between 0 and 60 while negative a* with value between 0 and -60 indicated green. Next, positive b* notation indicated yellow with value between 0 and 60 while negative b* with value between 0 and -60 indicated blue. From those notation, °Hue can be calculated to classify red, yellow, blue etc. Result of color analysis can be observed on following table 2.

Table 2. Result of Film Indicator Color during Storage

Days	L*	a*	b*	h_{ab}
A1(25^{0}C)				
B1(0)	46.94	10.42	13.69	52.72
B2(7)	67.12	3.83	31.59	83.09
B3(14)	67.24	2.66	37.60	85.95
B4(21)	68.79	1.56	41.06	87.82
B5(28)	69.98	1.44	42.91	88.08
A2(4^{0}C)				
B1(0)	44.43	12.83	12.99	45.36
B2(7)	61.74	4.14	28.48	81.73
B3(14)	65.84	1.12	29.80	87.85
B4(21)	67.57	0.94	29.84	88.20
B5(28)	67.82	0.81	30.00	88.45
A3(-5^{0}C)				
B1(0)	39.87	11.17	12.07	47.22
B2(7)	57.35	8.22	22.43	69.87
B3(14)	58.81	7.62	23.09	71.74
B4(21)	60.03	6.75	24.54	74.62
B5(28)	62.04	6.36	25.08	75.77

Visually, red was initial biofilm color and gradually turn into yellow during storage. During storage in room temperature, biofilm lost its color intensity and gradually turned into yellow with higher b* that indicated yellow compared to a* that indicated red. This condition also came up at the end of chiller and freezer storage. In similar to this condition, the hue angle (h_{ab}) of room temperature, chiller, and freezer had a high value as followed 88.08, 88.45, and 75.77.

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 [1].

Moreover, changing on Total Color Difference (TCD) would be continue and consistent to various response from biofilm indicator. It can be explained that changing color can be observed easily without visual aids when TCD value is more than 5 while TCD value more than 12 indicated to a significant color [18]. TCD value from this study can be shown on following Table 3.

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Table 3. Total Color Difference (TCD) during Storage

A (Storage temp.)	B (Storage time-days)				
	B1(0)	B2(7)	B3(14)	B4(21)	B5(28)
A1(25^{0}C)	0	27.77	6.12	3.95	2.21
A2(4^{0}C)	0	24.80	5.26	1.74	0.32
A3(-5^{0}C)	0	20.53	1.71	2.09	2.12

Based on Table 3, it can be observed a significant color changing on 7-days for all three storage treatments with a higher than 12 for TCD value. On the other hand, color of biofilm from room temperature and chiller storage can be observed without visual aids on 14-days. For freezer storage, there were no significant difference on biofilm color changing after 14-days, 21-days, and 28-days since their TCD values were lower than 5. Changing color could be related to fat oxidation of nugget spoilage during storage.

Spoilage on frozen nugget is an outcome of fat oxidation. The spoilage on frozen nugget in freezer for more than 6 months is dehydration on product and rancidity as a result of fat oxidation. Theoretically, oxygen availability becomes trigger for fat oxidation. Rancidity caused of oxidation occurred when fat and oxygen meet. Oxygen molecules are attached to double bonds from unsaturated fatty acids. Double bonds on unsaturated fatty acid are oxidized and formed short fatty acid, aldehyde and ketones that caused unpleasant smell and taste.

Generally, lipid oxidation through free radical reaction consisted of three basic processes which are initiation, propagation, and termination. Initially, hydrogen was released homolytically from unsaturated fatty acid with radical alkyl as a result of initiator availability (heat, active oxygen, metal, and light). Normally, radical alkyl reacts quickly to oxygen with radical peroxy as a result. This reaction was continued with unsaturated fatty acid to create hydroperoxyde and radical alkyl then radical alkyl reacted to oxygen.

Thus, auto-oxidation means chained reaction of free radical. Since reaction rate between ra..dical alkyl and oxygen is fast, most of free radical forms are radical peroxy so main terminated double chain of fatty acid. Aldehyde, one of fat oxidation results, and ketones might affect anthocyanin stability such as pH, temperature, lightness, oxygen, metal ion, enzyme, and sugar.

Decreasing freshness of frozen nugget during storage time as the result of biochemical reaction, physicochemical, and microbial transformation and it is indicated initial spoilage on nugget. Reaction between proteolytic activity microorganism such as *Staphylococcus aureus* and protein changed smaller components such as free amino acid. Oxidative deamination, decarboxylation, and desulphurization might be occured on amino acids as results of NH_3, CO_2, and H_2S. Protein in meat can be turned completely or partially into basic components such as CO_2, H_2O, NH_3, and H_2S [3].

Relation of Color Changing on Film Indicator and Chicken Nugget Characteristic

Water Content

One of important characteristic of food product is water content since its related to quality and product shelf life. [26] stated that water content in food product affect product's freshness and shelf life. Further, [14] also explained that water is related to freshness, shelf life, ability in chemical reaction, enzyme activity and microbial growth.

From this study, water contents in 28 days during storage for three different storage temperature were about 37±2-64±2%. Initially, water content of chicken nugget was about 46-47%. SNI (Indonesia National Standard) 01-6683-2002 pointed that the maximum water content of chicken nugget product is 60%. It means that chicken nugget in room temperature storage was already out of SNI standard and indicated spoilage. This condition can be observed to moldy and slimy surface. Further, [26] also noted that high water content can cause high activity of microbes including bacteria, molds and yeast. Averagely from each temperature storage treatment, water content from sample in room temperature, chiller, and freezer during 28 days are shown in Table 4.

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Table 4. Water Content During 28 days of Storage

A (Storage temp.)	B (Storage time-days)				
	B1(0)	B2(7)	B3(14)	B4(21)	B5(28)
A1(25^{0}C)	47.48^{Aa}	50.99^{Aa}	57.37^{Aa}	60.79^{Aa}	64.85^{Aa}
A2(4^{0}C)	46.89^{Ba}	44.46^{Ba}	43.98^{Ba}	38.99^{Ba}	37.54^{Ba}
A3(-5^{0}C)	47.22^{Ba}	43.54^{Ba}	41.79^{Ba}	39.97^{Ba}	37.53^{Ba}

Means without sharing common letter were significantly different at 5% of DNMRT (Capital letter for horizontal while lower case.

It can be observed from table 4 related to storage time and storage temperature effect on water content. Water content in room temperature was increasing in longer storage time. On the other hand, water content was decreasing in chiller and freezer during storage. Thus, spoilage product from freezer might be related to dehydration and fat oxidation for rancidity. Table 4 also shows this condition where water content kept decreasing during storage in chiller and temperature in longer storage time. Product dehydration can be avoided by using neat packaging even in freezing temperature with good protection against water vapor.

Water will be freeze and separate from its solution under 0°C. Minimizing chemical changing on food product during freezing and cooling storage means prolong its quality [5]. Hence, nugget in chiller and freezer storage has longer shelf life during storage until consumption. Color of biofilm indicator and total microbe also indicated similar result since number of total microbe from total plate count analysis was accepted based on SNI.

pH

Information related pH can be used to monitor shelf life of meat. pH for chicken nugget is affected by treatment during storage and storage temperature. This following table explained pH from chicken nugget in different storage temperature during 28 days which were about 5.8±0.5 – 6.9±0.2.

Table 5. pH of Chicken Nugget during Storage

A (Storage temp.)	B (Storage time-days)				
	B1 (0)	B2 (70	B3 (14)	B4 (21)	B5 (28)
A1(25^{0}C)	6.97^{Ba}	6.69^{Ac}	5.72^{Be}	6.03^{Bd}	6.13^{Bc}
A2(4^{0}C)	6.98^{Aa}	6.93^{Ac}	6.44^{Ae}	6.92^{Ad}	6.94^{Ac}
A3(-5^{0}C)	6.96^{Aa}	6.92^{Ab}	6.39^{Ae}	6.91^{Ad}	6.92^{Ac}

Means without sharing common letter were significantly different at 5% of DNMRT (Capital letter for horizontal while lower case.

From analysis of variance (α=5%), storage temperature, storage time and interaction between two those factors have significant effect on pH. This condition was explained by [15] that meat pH might be affected by storage time. It can be noticed from table 5 that declining pH were found in all storage temperature until 14-days. However, pH were increasing after 21-days and 28-days related to decreasing activity of acid-producing microbe. [13] explained that endogenous enzyme and microbe degraded protein in meat and produced ammonia and amines causing higher pH value.

Further, [4] stated that accumulation of lactic acid will stop after reserved muscle glycogen exhausted or low pH condition causing glycolytic enzyme stop working. On the contrary, [22] noted that increasing on pH condition is result of opening miofibril filaments so thus water keep entering and increasing water holding capacity (WHC).

Total Plate Count

Total plate count informs spoilage on chicken nugget based on number of bacterial colonies in sample with certain dilution as explained by Fardiaz [6]. This following Table 6 shows total plate count of nugget during storage.

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Table 6.Total Plate Count of Nugget during Storage

A (Storage temp.)	B (Storage time-days)				
	B1(0)	B2(7)	B3(14)	B4(21)	B5(28)
A1(25^{0}C)	$1.0x10^3$	$8.8x10^5$	$4.4x10^6$	$3.0x10^6$	TNTC
A2(4^{0}C)	$1.1x10^3$	$1.9x10^4$	$1.4x10^5$	$2.0x10^5$	$2.8x10^5$
A3(-5^{0}C)	$2.5x10^3$	$3.5x10^3$	$3.7x10^3$	$1.4x10^4$	$3.8x10^4$

From total plate count, there was significant increasing on total number of colonies for all condition of storage temperature. Initially, number of bacteria from room temperature, chiller and freezer were $1.0x10^3$cfu/ml, $1.1x10^3$cfu/ml and $2.5x10^3$cfu/ml. Increasing total number of colonies significantly occured on 7-days which was $8.8x10^5$cfu/ml thenbecame $4.4x10^6$cfu/ml on 14-days, $3.0x10^6$cfu/ml on 21-daysand $2.9x10^7$cfu/ml at the end of storage time. In advance, increasing total number of microbes for chiller and freezer at the end of storage time were $2.8x10^5$cfu/ml and $3.8x10^4$cfu/ml. These condition were incompatible to SNI 01-6683-2002 standard where allowed total number of bacteria is maximum $5x10^4$ colonies/g.

Staphylococcus aureus was identified causing toxicity that produces enterotoxin and can be found frequently on high protein food. Enterotoxin from *S. Aureus* is heat resistant and it can survive after heating 100°C for 30 minutes. Other microorganism classified as psychrophile and survive at low temperature are *Pseudomonas*, *Alcaligenes*, and *Flavobacterium* [2].

Compared to SNI standard, chicken nugget in sthis study should not be consumed at the end of storage time since total place count result was put of allowed number. In similar to this condition, there were white mold on surface of chicken nugget on 28-days. it can be explained that microbes contamination on meat might be occured from it was alive to consumption [15]. Contamination could come from ground, peel, innards, water, processing tools, air and workers.

Most of food products were contaminated from beginning of production process. Microbial penetration can be found during raw material handling, processing, storage and distribution. Further, food in packaging might be penetrated by microorganism because of poor packaging, holes on storage building as the result of improper finishing building or microorganism from surrounding environment that entered packaging. Another possibility is packaging that has high permeabilityto gas and water vapour. Thus, microorganisms keep growing and increase the number during storage time [7].

CONCLUSION

It can be concluded from this study that different storage temperature and storage time also their interaction have significant differences on water content and pH. However, insignificant effect was detected for different storage time to water content. Color indicator from mangosteen peel exract was effective in indicating spoilage on nugget that vulnerable to light exposure and changing temperature. Changing color on biofilm indicator during storage was red to yellow based on its °Hue from each treatment.

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