DEVELOPMENT OF INDICATOR SENSING FILM FOR MONITORING THE FRESHNESS OF CHICKEN MEAT

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DECLARATIONS

I hereby declare that the material in this thesis is of my own effort, except for quotations, excerpts, equations, references, and summaries, which have been duly acknowledged and clearly cited its sources.

30 November 2023

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ABSTRACT

The quality and protection of meat is now a critical concern of the food industry worldwide, as it is related to economic development and public health. The high demand for meat processing has led the meat industry to monitor the freshness and quality of the products. Herein, a pH indicator film-based starch and anthocyanin extract from roselle, was developed to monitor the freshness of chicken meat. The morphological characteristics of the indicator films have been analysed using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), field-emission scanning electron microscopy (FESEM), and x-ray diffraction (XRD) analysis. Fourier-transform infrared (FT-IR) spectroscopy was used to determine the chemical characterization of the film. The colorimetric indicator film incorporating anthocyanin was sensitive to pH changes, and the color changes could be visualized by naked eye. This film successfully changed color from red to yellowish-green when applied to the chicken breast samples to monitor the freshness. The film showed bright red at pH 1.0-6.0, bluish-grey at pH 7.0-10.0, and yellowish-green at pH higher than 11.0. The standard calibration curve of ammonia indicated a good linearity with R^2 value is 0.9143. The detection limit and quantitation limit of the indicator film is 0.01506 mg/g and 0.0456 mg/g, respectively. Besides, the ammonia concentration in chicken meat ranged from 0.02 -1.85 mg/g, with recovery rate ranging from 66.07 – 91.22 %. Based on the research, a good potential food packaging might be created to track the freshness of poultry and meat products, determine the application of freshness indicator formulations, and influence consumer purchasing decisions.

ABSTRAK

PEMBANGUNAN FILEM PENGESAN PENUNJUK UNTUK PEMANTAUAN KESEGARAN DAGING AYAM

Kualiti dan perlindungan daging kini menjadi kebimbangan kritikal industri makanan di seluruh dunia, kerana ia berkait rapat dengan pembangunan ekonomi dan kesihatan awam. Permintaan yang tinggi untuk pemprosesan daging telah menyebabkan industri daging memantau kesegaran dan kualiti produk. Dalam kajian ini, filem pH indikator berasaskan kanji dan ekstrak antosianin daripada roselle, telah dibangunkan untuk memantau kesegaran daging ayam. Ciri-ciri morfologi bagi filem indikator telah dianalisis menggunakan mikroskop elektron pengimbasan (SEM), spektroskopi sinar-X penyebaran tenaga (EDX), mikroskop elektron penghantaran (TEM), mikroskop elektron pengimbasan pelepasan medan (FESEM), dan sinar-x. analisis pembelauan (XRD). Spektroskopi Fourier-transform inframerah (FT-IR) digunakan untuk menentukan pencirian kimia filem. Filem indikator kolorimetri yang menggabungkan dengan antosianin adalah sensitif terhadap perubahan pH, dan perubahan warna boleh divisualisasikan dengan mata kasar. Filem ini berjaya menukar warna merah kepada hijau kekuningan apabila digunakan pada sampel dada ayam untuk memantau kesegaran. Filem itu menunjukkan merah terang pada pH 1.0-6.0, kelabu kebiruan pada pH 7.0-10.0, dan hijau kekuningan pada pH lebih tinggi daripada 11.0. Keluk penentukuran piawai ammonia menunjukkan kelinearan yang baik dengan nilai R^2 ialah 0.9143. Had pengesanan dan had kuantiti filem penunjuk ialah 0.01506 mg/g dan 0.0456 mg/g, masing-masing. Selain itu, kepekatan amonia dalam daging ayam yang dikesan oleh filem indikator adalah antara 0.02 -1.85 mg/g, dengan kadar pemulihan antara 66.07 – 91.22 %. Berdasarkan penyelidikan, pembungkusan makanan berpotensi yang baik mungkin dicipta untuk mengesan kesegaran produk ayam dan daging, menentukan penggunaan formulasi penunjuk kesegaran, dan mempengaruhi keputusan pembelian pengguna.

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SEM	-	Scanning electron microscopy
TEM	-	Transmission electron microscopy
FESEM	-	Field emission scanning electron microscopy
XRD	-	X-ray diffraction
FTIR	-	Fourier transform infrared
EDX	-	Energy Dispersive X-Ray
TAC	-	Total anthocyanin content
MW	-	Molecular weight
DF	-	Dilution factor
WC	-	Moisture content
WS	-	water solubility
SI	-	Swelling index
TVC	-	Total viable counts
RT	-	Room temperature
CR	-	Cold room
Inc	-	Incubator

LOD	-	Limit of detection
LOQ	-	Limit of quantification
TVBN	-	Total volatile basic nitrogen
Hx	-	Hypoxanthine
Xn	-	Xanthine
Hm	-	Histamine
CV	-	Cadaverine
Tym	-	Tyramine
Ant	-	Anthocyanin
Ant+NH3	-	Anthocyanin + ammonia
AntFi	-	Anthocyanin film

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CHAPTER 1

INTRODUCTION

1.1 Research background

Chicken meat is acknowledged as the most popular food among customers in many nations due to its high protein content, which is easily absorbed and utilized by the human body (Tang & Yu, 2020). According to a prior study by Fu *et al.* (2019), chicken breast flesh is a significant source of phospholipids in human diets and contains phospholipids that are crucial for human growth and development. However, chicken meat has the drawback of having a very short shelf life, which makes it readily spoil after slaughter due to enzyme activity and microbial metabolism. Chicken meat has evolved into the best substrate for microbial growth and reproduction due to its high nutritional and water content (Noori *et al.,* 2018). According to a statistic, Malaysia would consume an estimated 48.7 kilograms of poultry meat and 5.3 kilograms for meat and pork per person in 2021. This places Malaysia among the top global consumers of poultry meat (Statista, 2021). Meanwhile, meat consumption has been increasing since the 1960s, especially from the 1980s decade until today (González *et al.,* 2020). Meat production is essential to meet market demand, making the freshness of meat a consumer concern (Henchion *et al.,* 2014). However, a massive portion of meat and meat products are spoiled annually. Approximately 3.5 billion kg of poultry and meat are wasted at consumer, manufacturer, and foodservice levels, which results in significant economic and environmental impact (Ajaykumar & Mandal, 2020).

However, the meat industry today faces obstacles in maintaining freshness and quality as meat can be easily spoiled and damaged during processing and storage. For over a century, meat scientists have been concerned by the variety in texture, particularly the tenderness of flesh. Furthermore, the regulation and prediction of meat texture remains a major topic (Purslow, 2017). Nowadays, one of the most critical challenges of the meat industry is detecting low meat qualities. The determination of meat freshness is a complex process where it includes the ageing phase involved the changes in muscle metabolism in the post-slaughter period or storage period that required the meat to reach the optimum consumption state and determine the tenderness of meat (Marino *et al.,* 2013; Cifuni *et al.,* 2004). Fresh meat is a highly perishable product due to its biological composition. The shelf life and freshness of meat can be influence by various factors including holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture, light, and most importantly, microorganisms (Zhou *et al.,* 2010). Meat spoilage occurs due to various microorganisms' growth and feeding of nutrients in meat, resulting in the release of undesirable metabolites or spoilage due to enzymatic actions (Zagorec & Champomier-Vergès, 2017; Casaburi *et al.,* 2015). Several chemical compounds are produced from protein decarboxylation during the meat spoilage process, including biogenic amines, ammonia gas, total volatile basic nitrogen (TVBN), trimethylamine, and hydrogen sulphide. Ajaykumar & Mandal (2020) has reported that the utilisation of free amino acids by bacteria leads to an increase in ammonia levels. The presence of chemical substances that release during the spoilage phase can act as the indicator for meat freshness.

Commonly, the meat quality is analysed via sensory evaluation and chemical experiments that involve the evaluation of microbial growth. Sensory evaluation is usually based on flavour, stickiness, elasticity, and colour of its texture. This so-called traditional method is sometimes rejected due to human errors that may come from the expert panels (Mohebi & Marquez, 2015). Several techniques have been developed to evaluate the meat quality, which is a crucial factor that affects the sensory assessment by consumers and their decision to purchase (Lv *et al.,* 2018). Chromatographic techniques such as high-performance liquid chromatography (HPLC), mass spectrometry and gas chromatography-mass spectrometry (GC-MS) have provided convincing results and proved suitable for monitoring freshness. Apart from exceptional sensitivity and precision, however, these chromatographic and mass spectral methods are time-consuming with high operating costs and require trained operators. It is therefore difficult to prepare samples for these chromatographic methods and does not follow the on-site applicability requirements. Recently, the development of certain freshness indicators has shown potential to act as promising alternatives for meat packaging applications because they can trace and assess microbial spoilage to maintain high-quality food that is fit for human consumption. Indicator sensor packaging technology has proved to be successful in real-time and on-site monitoring of food spoilage without the need for costly equipment to achieve packaged food quality and protection (Chen *et al.,* 2020b). Earlier, Lee & Shin (2019a) stated that intelligent packaging is ideal for evaluating microbial metabolites, especially the pH-sensitive indicators for food packaging based on colour. These indicators are typically derived from the natural dye, which will shift from acid to basic form due to the increase in headspace pH caused by volatile amines produced when the products are spoiled (Ezati *et al.,* 2019b).

Aside from that, natural colour pigments showed a growing demand in the research field as they are more environmentally friendly than synthetic dyes. Anthocyanins are a suitable alternative dye because they are nontoxic, safe, and friendly to the environment and the human body. Anthocyanin is a phenolic molecule where it shows visible colour changes towards different pH and responsible for the red, purple, and blue colours found in flowers, fruits, and vegetables (Khoo *et al.,* 2017). Most natural dyes are plant sources where they are obtained from various parts of plants and herbs including the stem, wood, roots, bark, leaves, flowers, fruits and skin of plants, which produce distinct pale to dark shades on both natural dyes as well as synthetic dyes (Clark, 2011). Roselle is one of the plants that contain high concentrations of anthocyanin where it can produce approximately a maximum yield of 88% of anthocyanin extract (Abdullah *et al.,* 2020). A previous study stated that dried roselle calyx contained 10% moisture content that contributes to 10 times greater total anthocyanin contents compared to fresh roselle calyx (Chumsri *et al.,* 2008). Previous research by Pedro *et al.* (2016), they state that anthocyanin-containing plant extracts can also act as a substitute for synthetic dyes because of their bright color and water solubility and can be incorporated into food, thus increasing possible beneficial health effects.

The largest obstacle to the commercialization of intelligent packaging films today is the possibility of food contamination caused by packaging materials. As a result, more and more research is concentrating on creating pH indicators using anthocyanins. For example, Huang *et al.* (2020) developed a novel indicator film incorporating roselle anthocyanin extract and hydroxypropyl methylcellulose (HPMC) modified polyvinyl alcohol (PVA) to monitor shrimp freshness. According to the data, the film shows a color change from rose-red to light green then yellow when the shrimp was severely spoilage. Thus, it indicated that the developed film could be applied as intelligent packaging for real-time shrimp freshness monitoring. Additionally, Zhang *et al.* (2019b) created an intelligent pH film based on starch, polyvinyl alcohol, and roselle anthocyanins to monitor the freshness of pork stored at 25°C. When used to monitor the freshness of pork stored at 25°C, the film showed visible changes from red to green before the TVB-N value of the pork gradually increased to the rejection limit. Similarly, Aydin & Zorlu, (2022) established a novel biodegradable film based on alginate and roselle extract as a potential intelligent packaging to prolong shelf life and preserve food safety. These alginate-anthocyanin films demonstrated strong antibacterial action against both Gram-positive and Gram-negative microorganisms. The findings imply that the films have potential for usage as a biopolymer-based composite packaging material for fresh fruits and vegetables, and they may eventually replace synthetic plastics, which have a negative impact on the environment.

Therefore, this study aimed to develop a biopolymer film incorporated with roselle anthocyanin that acts as a colorimetric indicator to monitor freshness in chicken breast samples due to the limited study of application of intelligent packages on chicken packaging in real-time. Roselle is chosen as a potential candidate for colour and pH indicator since it is rich in anthocyanins and could be used as a good source for producing a brilliant red colourant for many foods (Dayang *et al.,* 2018). The indicator film was developed using casting methods and characterised by using spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), Fourier transform infrared (FTIR) and UV-Vis spectrophotometer. The hydrophobicity properties, storage and colour stability, mechanical properties and physical properties also have been investigated. The indicator film will be applied in chicken breast samples for freshness detection. The selectivity and sensitivity of the indicator film have been tested with different concentrations of ammonia and different types of analytes, respectively. The sensitivity of anthocyanin incorporated with starch film would be of great value to the food industry as a smart packaging or pH indicator, particularly in the meat industry, to monitor meat freshness (Jiang *et al.,* 2020a).

1.2 Problem statement

Food waste and loss are two of the most important issues in the modern world. In Malaysia, about 16,688 tonnes of food waste is generated per day (Hashim *et al.,* 2021). Food tends to be wasted too if it does not meet the requirement or standardization and can be hazardous to health. Food products kept for extended storage or beyond the expiry date may jeopardise their quality and may lead to unnecessary waste (Siva Manikam, 2020). According to the United Nations Environment Programme's (UNEP) Food Waste Index report, in 2019, almost 931 million tonnes of food are wasted every year. The highest contributor is from the consumer level, representing 64% of total food waste, followed by 20% of manufacturing, 12% of distribution, and 3.5% of primary production and post-harvest (Karwowska *et al.,* 2021). Food rejection is primarily related to spoiling and any unwanted possibility by consumers (Koutsoumanis, 2009; Iulietto *et al.,* 2015). The number of food losses during production and product waste by customers in the consumer stage becomes large due to the relatively high consumption of meat products. In the meat industry, it is estimated that up to 23% of production is wasted or discarded (Karwowska *et al.,* 2021). On the other hand, poultry is the sixth most wasted food category by volume and the most lost by value, according to a recent study of food waste at the primary production stage in the United Kingdom. Worldwide increases in poultry farming have increased the quantity of poultry waste. A significant amount of waste is produced during the production of chicken, and this waste can be hazardous to human health since it can pollute the air, water, and land (Prabakaran & Valavan, 2021). According to sustainability body WRAP, about 66,000 tonnes of poultry meat, or 3.5 % of production and a value of US$106 million, are lost at the farm level each year. Previously, Clune *et al.* (2017) claimed that compared to most plant-based products, waste from animal products, particularly chicken meat, has a larger greenhouse gas impact. Given that chicken is perishable and safe storage is tricky, eliminating chicken meat waste in households may be tough (Yavas & Bilgin, 2010). In general, eating contaminated poultry is the leading cause of foodborne illness such campylobacteriosis (Strachan *et al.,* 2013).

Synthetic dyes or synthetic food colourants are now widely used in the research and food industries. Synthetic colour additives are organic pigments produced artificially, typically from aniline dyes and coal tar as a starting material. On the other hand, some synthetic dyes can be harmful to the human body and the environment if used in massive amounts. Some of these colours are hazardous, carcinogenic, and irritate the skin and eyes (Affat, 2021). Previous research was discovered that a number of synthetic dyes were very resistant to degradation and mildly harmful to both human and animal health. Numerous major health issues, such as allergic and asthmatic reactions, DNA damage, liver damage, renal failure, attention deficit hyperactivity disorder (ADHD), possible immunotoxicity, and reproductive toxicity, may result from the excessive ingestion of these substances (Rovina *et al.,* 2016). On the other hand, some synthetic dyes can be harmful to the human body and the environment if used in massive amounts. Many dyes may not be entirely safe, even if they are not yet outlawed. Due to their synthetic origin and complicated molecular structures, which reduce their capacity to biodegrade, effluents based on synthetic dyes can pose a major threat to the environment and water stream. The majority of artificial colours are not biodegradable. Therefore, they build up on land and in waterways, producing environmental issues (Affat, 2021). Before the invention of synthetic dyes, natural dyes derived from plants, animals, and minerals were widely used. There have been attempts to replace dangerous artificial colours with natural alternatives. On the other hand, natural dyes should be chosen with caution because some are neither eco-friendly nor effective. In some circumstances, the sensors are made of materials that are harmful to people's health. There have been attempts to develop sensors that can