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Physicochemical Effects of Rabbit Meat Fermentation using LAB

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A thesis submitted in fulfilment of requirement for degree of Bachelor of Applied Science (Food Security) with Honour

Faculty of Agro-Based Industry

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project titled "Physicochemical Effect of Rabbit Meat Fermentation using LAB" by Muhammad Ridzuan bin Hisham, matric number F18A0093 has been examined and all the correction recommended by the examiners have been done for the degree of Bachelor of Applied Science (Food Security) with Honour, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Physicochemical Effects of Rabbit Meat Fermentation using LAB

ABSTRACT

As rabbit has become one of the emerging markets in local region as well as the abroad market, functional food delivered from rabbit meat need to be widely figured out. Lactic acid bacteria (LAB) in natural yogurt have perks in terms of nutraceutical properties and it revamps health benefits effects of food products. Hence, introduction of LAB is recommendable to food products to enhance its nutritional and physical properties. In current study, physicochemical properties of raw rabbit meat that is immersed in natural yogurt containing *Streptococcus Thermophilus, Bifidiobacterium lactis* and *Lactobacillus acidophilus* is preserved at 4°C for seven days, were investigated. In this regards, physicochemical analysis (proximate analysis, colour stability and texture profile analysis) was conducted after fermentation time. In comparison with control samples, there is no significant difference between both control and treated rabbit meat samples in terms of proximate analysis. However, there is beneficial output of a* value colour spaces where rabbit meat was appeared redder compared to control samples. Texture profile analysis concluded that there is significant difference (P<0.05) in hardness component in which tenderness of rabbit meat is improved. It was concluded that probiotic microbial improved tenderness of rabbit meat when it is immersed in natural yogurt for seven days at 4°C.

Keywords: probiotic, lactic acid bacteria, rabbit meat, fermentation, tenderness

Kesan Fisikokimia Penapaian Daging Arnab menggunakan LAB

ABSTRAK

Memandangkan arnab telah menjadi salah satu pasaran baru muncul di rantau tempatan dan juga pasaran luar negara, makanan berfungsi yang dihantar daripada daging arnab perlu difikirkan secara meluas. Bakteria asid laktik (LAB) dalam yogurt asli mempunyai kelebihan dari segi sifat nutraseutikal dan ia mengubah kesan manfaat kesihatan produk makanan. Oleh itu, pengenalan LAB adalah disyorkan kepada produk makanan untuk meningkatkan sifat pemakanan dan fizikalnya. Dalam kajian semasa, sifat fizikokimia daging arnab mentah yang direndam dalam yogurt asli yang mengandungi *Streptococcus Thermophilus, Bifidiobacterium lactis* dan *Lactobacillus acidophilus* dipelihara pada suhu 4°C selama tujuh hari, telah disiasat. Dalam hal ini, analisis fizikokimia (analisis proksimat, kestabilan warna dan analisis profil tekstur) telah dijalankan selepas masa penapaian. Berbanding dengan sampel kawalan, tidak terdapat perbezaan yang signifikan antara kedua-dua sampel daging arnab kawalan dan dirawat dari segi analisis proksimat. Walau bagaimanapun, terdapat keluaran berfaedah bagi ruang warna nilai a* di mana daging arnab kelihatan lebih merah berbanding sampel kawalan. Analisis profil tekstur merumuskan bahawa terdapat perbezaan yang signifikan ($P<0.05$) dalam komponen kekerasan di mana kelembutan daging arnab bertambah baik. Disimpulkan bahawa mikrob probiotik meningkatkan kelembutan daging arnab apabila ia direndam dalam yogurt asli selama tujuh hari pada suhu 4°C.

Kata kunci: probiotik, bakteria asid laktik, daging arnab, penapaian, kelembutan

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LIST OF SYMBOLS

No.	Reference	Page
kJ	Kilojoule	23
%	Percentage	24
kg	Kilogram	29
mL	Milliliter	30
g	Gram	30
mg	Milligram	30
℃	Degree Celsius	31
L*	Lightness or darkness	33
a*	Greenness or redness	33
b*	Blueness or yellowness	33
ΔL*	Colour difference of lightness or darkness	33
Δa*	Colour difference of greenness or redness	33
Δb*	Colour difference of blueness or yellowness	33
ΔE*	Total Colour Difference	33

LIST OF ABBREVATIONS

No. of Reference		Page
LAB	Lactic Acid Bacteria	1
Mid-	Middle	8
DNA	Deoxyribonucleic Acid	11
G	Guanine	11
C	Cytosine	11
L.	*Lactobacillus*	11
Subsp.	Subspecies	11
W.	*Weisella*	12
FAOSTAT	Food and Agriculture Organization Corporate Database	20
AD	Anno Domini	20
PUFA	Polyunsaturated Fatty Acid	22
CLA	Conjugated Linoleic Acid	22
$H_2\ SO_4$	Sulphuric Acid	29
$K_2\ SO_4$	Potassium Sulphate	29
$CuSO_4$	Copper Sulphate	29
NaOH	Sodium Hydoxide	29
$C_2\ H_6\ O$	Ethanol	29
C_6H_{14}	Petroleum Ether	29
CIE	Commission Internationale de I'Elcairage	33
TPA	Texture Profile Analysis	34
TOH	Total of Carbohydrate	40

SD	Standard Deviation	40
FDP	Fermented Dairy Product	52

CHAPTER 1

INTRODUCTION

1.1 Research Background

Fermentation process has long been well known food product across the globe even before the prehistoric time preserve food. The preservation method by fermentation can be drastically influenced and affecting characteristics let alone properties of the fermented food product, be it physical or chemical changes. The function of fermentation is to make sure the food product can be consumed for a longer time as the lactic acid bacteria (LAB) formed in the fermentation process should longer the shelf life of the food products. Traditional fermented food products are often times made of local microorganism exists in the food by using biological technique (Utama *et al*., 2019).

In modern time, as education and knowledge are more accessible, the world has started to make up their mind to concern upon healthy food intake. For example, due to

the increasing health factor, probiotic dairy products have been accepted in the global market (Laranjo *et al*., 2017). In this manner, meat fermentation is being analysed as it is one of the vital protein sources in almost all nations in the world. Hence, the introduction of LAB in meat fermentation lies many possibilities and benefits. The LAB culture can be brimming of nutrients resulted from their fermentative capacity which improve quality as well as offers health benefits (Rakhmanova et al., 2018). The treatment of LAB from yogurt may improve the taste, texture and colour of the meat and most importantly will extend the shelf life of the product.

Rabbit meat or scientifically known as *Alternanthera ficoidea* that is being chosen in this current study is an edible meat which tastes almost similar as chicken but most likely to taste gamy and intense. Besides, the texture of the rabbit meat is more dried compared to other poultry or livestock products. While consumers across the globe are madly treasured by its taste, texture, and aroma, it is as wellbeing appreciated for its nutritional value it holds. Recent study stated that *Alternanthera ficoidea* is consist of high protein content but low in sodium content. Surprisingly, *Alternanthera ficoidea* does not contain high amount of cholesterol and fat compared to other widely consumed meat for instance chicken, beef, and turkey (Cavani *et al*., 2009). In the advancement of today's world, there are a lot of invention adopted by food industry in order to market their food products resulted from new research and findings.

1.2 Problem Statement

Food spoilage is a result of growing pathogenic bacteria on food in which it is no longer suitable to consume. If the spoilt food is continuing to be consumed, it should lead to foodborne disease. Foodborne disease often occurs in children as it became the major contributor to illness for example, diarrhea (Todd, 1987). Thus, new venture needs to be adopted to modify properties of preservation method from continuing to cause outbreaks.

While the traditional produced meat is still relevant and being acknowledged by consumers, with accessible knowledge, the spice of today's technologies and new findings, consumers are more likely prefer functional products instead of traditionally made products, in this manner, meat. In terms of knowledge wise, consumers will be more concern on health impact as they started to discover the benefits of functional food products when they perceived there are risks lie behind traditional meat products (Munekata *et al*. 2020).

On the other hand, apart from health benefits and current scientifical approach to food preservation method, meat texture and tenderness is what attract people to consume it. While undergo study regarding the nutritional value it contains post-fermentation, the texture of meat is a prime quality trait for consumer acceptability when it comes to consuming fresh meat (Bolumar *et al*. 2016). However, there is an inconsistent data presented between mechanical and sensory assessment due to consumers' perception while assessing that influenced by many factors rather than mechanical forces that applied by mechanical devices in which it focuses only by one biting forces required. Hence,

many mechanical devices have been developed to assess the meat tenderness with fixed forces applied.

1.3 Hypothesis

H0: There is change of meat properties after fermentation

H1: There is no changes of meat properties after fermentation

H0: The physicochemical properties change after fermentation

H1: The physicochemical properties do not change after fermentation

1.4 Scope of Study

In this current study, the research will start from the range of the functional properties of LAB obtained from yogurt culture assessing physicochemical effects of *Alternanthera ficoidea*. The first part of study involves marinating rabbit meat with the natural yogurt culture that contains LAB for example *Lactobacillus thermophilus* and

Streptococcus bulgaricus. As the fermentation period occured, proximate analysis of the fermented rabbit meat will be analysed in which it governs from determination of moisture content, ash content, crude protein content and crude fat content to result with value of macronutrients in the meat.

1.5 Significant of Study

Numbers of studies had been done previously analysing the benefits of food fermentation and its application towards food products. The treatment of LAB from natural yogurt may improve the taste and texture of the meat and most importantly will extend the shelf life of the product. On the other hand, the findings from this study may be an influence or insight to the food processing company as a guideline to venture on a new idea upon meat preservation method in which leads to the development of new products for example, sausage or nuggets. In addition, the usage of LAB in meat fermentation may in return will enhance the health benefits of the consumers who concern more on healthy lifestyle rather than the traditionally made meat products.

1.6 Objectives

1. To determine physicochemical effects of LAB fermented meat.
2. To improve health benefits for new food product development.

1.7 Limitations of Study

This study is going to cover upon physicochemical effect of fermented rabbit by inoculation from local yogurt brand. However, limited studies of related field and the database reported are still insufficient hence it might result with the relevancy result afterwards. On the other hand, predominant circumstance in this study is contamination of foreign microbials which perhaps would give impact on the result to be analysed and concluded. In addition, the suppliers of rabbit meat are probably hard to be found because the venture is still not as widely emerging as the other poultry products. Hence, if the suppliers are miles away from the campus, perhaps it might come out with circumstances in terms of transportation channel as well as the novelty might be disturbed.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Fermentation

Thousand's years ago, human has come out with the idea to ferment food as in shelf life is a major factor to initiate fermentation process, for example dairy products. With poor new inventions, fermentation is one of the most useful approaches to preserve food products. This eminent product in which it has a poor shelf life and holding quality makes fermentation relevant during the ancient time. It started when the fermentation of milk from livestock such as cattle, goats, camels, and sheep has been fermented naturally back then before century. Especially in subtropical region, the heat of this climate is being favoured by the thermophilic lactic acid bacteria (LAB) to undergo fermentation. The first yogurt is being produced coincidentally when goat milk was on its way to be

channelled by camels in the heat of North Africa in which the temperature of the region was almost 44°C making it an exemplary condition for fermentation to occur.

During mid-1800s, people has started to comprehend and grasp the understanding of how fermentation works on food and dairy products. Chemists who worked on these new findings have come out with his conclusion where fermentation can assist in holding quality of food product in which to extend its shelf life for a longer time range. The application of fermentation has somewhat offered benefit to human health derived from consuming fermented food products. Biological changes resulted from fermentation may influence nutritional value consequently and some of the bioactivity in terms of digestibility (Villarreal-Soto *et al*., 2018). With those studies and findings, the application of fermentation is still widely being analysed only to result to new findings for the quality enhancer of the food products.

2.2 Definition of Fermentation

In general, the word 'fermentation' was initially derived from Latin word, *fermentare* meaning '*to leaven*'. Several people believe the word fermentation also comes from Latin word but with different perspective, *fervere* which means *'to boil'*. With vast definitions, the meaning is still being applied as it aligns to the concept of fermentation and its applications.

In scientific manner, fermentation can be defined as a biological phenomenon called metabolic process involved in which organic substances are being converted into acids or gas with the absence of oxygen or any transport chain. This metabolic process occurs when beneficial bacteria exist in the food product functions to breakdown the starch and sugar molecules. When the microorganisms split away, the growth of the pathogenic bacteria that cause spoilage will be inhibited. All the actions of fermentation are assisted by enzymatic activity where energy source from carbohydrate is being extracted. The study of fermentation process is addressed as zymology initially introduced by a French chemist, Louis Pasteur as he concluded that fermentation was mainly cause by yeast activity.

Fermentation process commonly takes place naturally in which the environment will be controlled for instance with the addition of salt or sugar or soaking the food into water. Salt in this context will function to promote the formation and growth of LAB while inhibit the pathogenic bacteria from growing that should cause food spoilage. The growth of LAB during fermentation will inhibit or slow down the enzymatic reaction caused by pectinolytic and proteolytic enzyme from softening and putrefaction of fermented food products (Nuraida, 2015).

2.3 The Nature of Bacterial Starter Culture

Starter culture is a main element of producing fermented food products. It can appear as individual or mix formation of the desired bacterial strains treated with specific enzymatic reaction (Laranjo et al., 2017). Simply put, starter culture consists of microorganisms (i.e: bacteria) that are being inoculated into the food products to be fermented in which it functions to produce new changes to the food products. The application of bacterial starter culture as a preserving tool has been as wide and emerging vital elements in food processing industry. This utilizing agent will initiate fermentation process and resulted with properties alternation while allowing physicochemical effect of the fermented food. It plays a vital role in ripening process, most commonly in food preservation industry (Rakhmanova *et al.* 2018). This fermentation element aid in the processing of food product such as yogurt and dairy product, for example kefir, cheese and butter milk. About 8,000 ~ 10,000 years ago, the consumption of dairy product listed has long been human vital diet sources (Bintsis, 2018). Nowadays, the usage of starter culture that has been widely in used mainly in food manufacturing industry will enhance the preservation process compared to the conventional fermentation method while it offers enrichment of nutritional values, alternation of sensory analysis, let alone the value of economic of fermented food products in the market (Hutkins, 2003).

Bacterial starter culture are a diverse group of microorganisms containing immotile gram-positive bacteria in which it offers a capability to tolerate with acidic condition, while appear aerotolerant (Mulaw et al., 2019). The adopted LAB as a starter culture is usually in rod (*bacilli*) and spherical (*cocci*) shape type while it forms no spores

as it contains low proportion of G + C content in the DNA sequences. With facultative aerobic and strictly fermentative characteristic, LAB will produce lactic acid as a result of glucose fermentation. The properties of lactic acid bacteria as an acid tolerant microorganism helps in preservation, controlling hygiene and safety and improving nutritional value of food. It produces lactic acid as a by-product upon carbohydrate fermentation process as it should afford to hinder the growth of spoilage and pathogenic bacteria for instance, *Clostridium perfringens* and *Bacillus cereus.*

There are two groups of starter culture which are, simple and mixed compound. This very characteristic is defined by the number of strains. Simple starter culture contains of single strain or can be more than one yet countable while mixed starter culture contains more than one strain which each of them offer specific characteristics and attributes. Starter culture may be divided into two; mesophilic and thermophilic. Mesophilic starter culture consists of *Lactococcus lactis* subsp. *cremoris, L. delbrueckii* subsp. *lactis, L. lactis* subsp. *lactis, L. lactis* subsp. *lactis biovar diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris.* While thermophilic starter culture contains of *Streptococcus salivarisu* subsp. *thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, L. delbrueckii* subsp. *lactis, L. casei, L. helveticus,* and *L. plantarum.*

Figure 2.3: Lactic acid bacteria function in milk fermentation and preservation.

Species/ subspecies	Their main uses in different milk products	References
Lactococcus		
Lc. Lactis subsp. Lactis	Mesophilic starter used for many cheese types, butter and butter milk.	Broome et al. (2003) and Wouters et al. (2002)
Lc. lactis subsp. Lactis biovar diacetylactis	Used in Gouda, Edam, sour cream and lactic butter and butter milk.	Wood (1997) and Leroy and De Vuyst (2004)
Lc. Lactis subsp. cremoris	Mesophilic starter used for many cheese types, butter and butter milk.	Weerkam et al. (1996)
Streptococcus *Sc. thermophilus*	Thermophilic starter used for yogurt and many cheese types' particularly hard and semihard high-cook cheeses.	Broome et al. (2003) and Beresford et al. (2001)
Lactobacillus		
Lb. acidophilus	Probiotic adjunct culture used in cheese and yogurt.	Briggiler-Marcó et al. (2007)
Lb. delbrueckii subsp. Bulgaricus	Thermophilic starter for yogurt and many cheese types, particularly hard and semihard high-cook cheeses.	Slaterry et al. (2010)
Lb. delbrueckii subsp.lactis	Used in fermented milks and high-cook cheese.	Broome et al.(2003) and Giraffa 2010
Lb. helveticus	Thermophilic starter for fermented milks and many cheese types particularly hard and semihard high-cook cheeses	Broome et al. (2003) and Griffiths and Tellez (2013)
Lb. casei	Probiotic milk and cheese ripening adjunct culture	Briggs (2003) and Kongo (2013)
Lb. plantarum	Cheese ripening adjunct culture.	Leroy and De Vuyst (2004)
Lb. rhamnosus	Probiotic adjunct culture used in cheese	Coppola et al. (2005)
Leuconostoc *Ln. mesenteroides subsp. cremoris*	Mesophilic culture used for Edam, Gouda, fresh cheese, lactic butter and sour cream.	Weerkam et al. (1996) and Slaterry et al. (2010)

Lb. =Lactobacillus; Lc. =Lactococcus; Ln.=Leuconostoc; Sc.=Streptococcus, subsp.= subspecies.

LAB can be isolated from various kind of sources for example dairy products, fermented food and beverages, animal intestines, plants and soils. Based on Mulaw et al. (2019), these sources in which LAB can be found offer various type of probiotic LAB for instance, *Lactobacillus sp*. which are *Lb. acidophilus, Lb. johnsonii, Lb. casei, Lb. rhamnosus, Lb. gasseri and Lb. reuteri* and genus *Bifidobacteria*; *Bf. bifidum, Bf. animalis subsp. lactis, Bf. longum subsp. longum* and *Bf. longum subsp. infantis.* Potential probiotic sources in fermented food commonly contain of *Lactobacillus, Pediococcus, Enterococcus, Weisella* and *Leuconostoc*. Based on Nuraida (2015), Asian fermented food consists of *Lactobacillus planatarum, Lb. pentosus, Lb. brevis, Lb. fermentum, Lb. casei, Leuconostoc mesenteroides, Leu. kimchi, Leu. falax, Weisella confusa, W. koreenis, W. cibaria* and *Pediococcus pentosaceus.* Probiotic strains isolated from traditionally fermented food have seen to play a role as a starter culture for mass food production in which it has the specific functional properties as probiotic to combat against pathogen that causes food borne diseases for example diahrrea.

LAB are the homogeneous microorganisms which are extensive and can be found in variety of environment especially in carbohydrate. Its breakdown carbohydrates to result to almost entirely lactic acid known as homo-fermentation or to a mixture of lactic acid, carbon dioxide, acetic acid and ethanol known as hetero-fermentation (Nuraida, 2015). Its by-products often consist of diacetyl, acetaldehyde, and hydrogen peroxide. The by-products formed during breakdown of carbohydrate could initiate the flavouring and texture of the fermented food product and holds the capability to aid in the inhibition of undesired pathogenic bacteria. This type of microorganisms is grouped as essentially vital bacteria to be used as a starter culture. The bacteria are mainly appearing in plants and fermented food. The bacteria species that usually being used in fermentation are *genera Lactobacillus, Leuconostoc, Lactococcus, Streptococcus* and *Pediococcus*. These bacteria holds the fermentative ability and enriching nutrients while enhancing organoleptic factor as well as improving food safety and hygiene and offers better health benefits for its consumers (Rakhmanova et al., 2018).

As of today, the application of fermentative technologies has been industrialized and getting mass for example the production of dairy product, fermented sausage, saeukraut and so on. While the application of starter culture has been as wide, desired species of starter culture for specific purposes can be purchased at the culture bank at the research organizations. Although numbers of LAB exists in many form of sources, however, there are still insufficient reports available to characterized probiotic LAB and its specific functions (Mulaw et al., 2019). However, there are still numbers of fermented food that can be produced without the use of starter culture.

2.4 Lactic Acid Bacteria (LAB) for Meat Fermentation

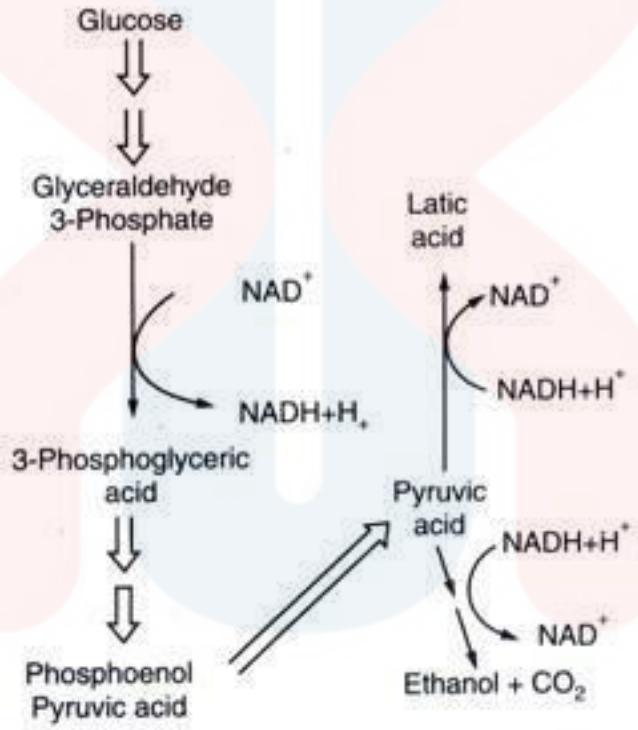
2.4.1 Chemical Changes of Fermented Meat

Starter cultures were being introduced as it functions to shorten the initial fermentation phase as well as to control the fermentation process, and up to this day, starter culture for the fermentation of meat has been widely being used across the globe. For example, the production of sausages, bacteria in variety types of combination are being applied. Of all the fermentative capacity hold by lactic acid bacteria (LAB), these are the most vital and essential species that are being used in fermented meat production; *Lactobacillus plantarum, L sake, L curvatus, Pediococcus pentosaceus, and P acidilactici.* These species of LAB are mainly involved in the activities of meat fermentation as it contributes to the fermentation process. In order to obtain LAB strains in which it is suitable as starter culture, a very detail screening is important including the study of its biological and physiological attributes to be applied in fermentation process (Hertel, 1996).

The main pathways involved in the manufacture and development of flavour in fermented food products are glycolysis in which it involves the process of sugar fermentation, lipolysis to degrade fat content and proteolysis as the medium of degradation of proteins. The product generated from the metabolism of carbohydrate and pyruvate is lactate and it can be converted into diacetyl, acetoin, acetaldehyde or acetic

acid acting as an important for typical yogurt flavours. LAB contribution to lipolysis is relatively little, however, proteolysis is the vital biological pathway as to develop flavour in fermented foods, for example meat (Bintsis, 2018).

Figure 2.4.1: Glycolysis process during fermentation.



Lactic acid bacteria (LAB) are a vital and critical agent during meat fermentation as it improves hygiene and sensory characteristics of the fermented meat product. Its fermentative capability will prevent the development of spoilage and pathogenic microflora resulted from the acidification of the meat products while it enhances colour stabilization and texture quality (Talon & Leroy, 2014). LAB influences the sensory characteristics of the fermented meat resulted from the production of acetic acid, ethanol, acetoin, pyruvic acid, carbon dioxide and its ability to initiate the production of aromatic

substances from the proteinaceous precursor. The important of the muscle proteinases in the first breakdown of sarcoplasmic and myofibrillar protein has been approved by researchers as the vital enzyme of proteolysis on the first day of fermentation process.

LAB commonly promotes the structure of the volatile and non-volatile compounds through the degradation of amino acids as well as the oxidation of unsaturated fatty acid will also be inhibited. Many non-volatile compounds for instance peptides and amino acids are also take place to promote desired flavour development during hydrolysis of meat protein. The meat with LAB treatment whether it is direct or indirect participation towards the activities, it will also enhance the sensory characteristics of the meat products (Silvina Fadda, 2010). Some LAB has also hold the capability to produce bacteriocins as it utilizations is being used as bioprotective culture to preserve meat (Zacharof & Lovitt, 2012).

Bacterial starter culture has been required during meat fermentation as it functions to lower pH value hence resulted to favourable flavour as well as it acts as non-toxic and non-pathogenic. The role of the microbial enzyme has been concluded as LAB are endowed with proteolytic activity commonly in intracellular amino, di and tri-peptidases. The intracellular enzyme of Lactobacillus is concluded as it functions and responsible for the generation of small peptides and amino acids in which it aids in the process either as direct flavour enhancer or as the precursors of the other flavour compounds during the fermentation process. The utilization of LAB has been recognized as it should improves nutritional properties and the growth of LAB in the intestinal tracts will exclude pathogenic bacteria. The structure of the meat to be fermented is dependent upon its ripening process and the survival of pathogenic bacteria. The therapeutic activities by the bacteria for example antimicrobial activities, anticancer properties and cholesterol assimilation has been the outcome of utilization of LAB in meat fermentation.

2.4.2 Physical Changes of Fermented Meat

2.4.2.1 Texture of Fermented Meat

Apart from nutritional value it holds, rabbit meat is having the advantage for the flavor, taste, aroma, and texture. In contrast with chicken, rabbit meat is considered lean or almost white. Rabbit meat is not appeared white due to low level of haemoglobin (a protein that gives colour to meat) but rather, it is more to the quantity and quality of its fat content. On the other hand, although rabbit meat tastes a little bit like chicken, it tastes gamier and more intense. In terms of texture, rabbit meat is drier on the inside compared to chicken. There are some types of rabbit that tastes great for example Californian rabbit, silver fox and Cinnamon rabbit.

Based on an article wrote by Chef's Pencil (2020), there is a difference between rabbit meat that is being raised in farm or domestic. Rabbit meat that is raised in farm are generally tastes better compared to the domestic rabbit. The meat is appeared pinkish colour, lighter and the texture is more tender, and it hardens as they age. In addition, farm breed rabbit has a milder flavour compared to the domestic rabbit. For wild or field rabbit, these breeds are lighter and finer than farm bred rabbit. The meat is harder, and they

consist various flavour due to the food source they consumed in the wild. As for the meat colour, wild rabbit meat is more reddish compared to the farm and domestic rabbit because the meat contains less fat.

Rabbit meat which addressed as white meat is suitable to be a food of choice with low calorie if cooked simply, without the addition of excess fat. It is recommendable for those who is concern on health benefit to consume rabbit meat due to its fat content, cholesterol diet and those who suffers cardiovascular disorders due to nutritional composition. If the meat is easy to chew, munch and it is soft in mouth, it will give advantage to those with difficulty to chew especially with added condiments for examples sauces and vegetables. Cooking type will also affect the texture of the rabbit meat. However, rabbit meat consists of a lot of muscle fibres making it more difficult to digest hence it is not recommendable to those with delicate stomach.

2.4.2.2 Colour of Fermented Meat

Colour measurement in food products is very crucial and essential attributes in which it is affiliated with market acceptance in a way it could attract consumers. It is a psycho-physical properties that varied to human vision. The colour measurement may be carried out by visual inspection, traditional instrument, or automated computer vision. Hence, certain colour parameters is necessary to investigate, measure and classify food products based on their colour. On the other hand, colour measurement is crucial for

quality control purposes in which it is utilized for food grading. Currently, food colour is measured in terms of CIE L*, a*, b* values, hue angle and chroma. CIELab is a colour space that is being utilized for international standard for colour measurement.

The primary sensation of food products was arisen from visuality. In this context, consumers' willingness to consume and purchase meat is often dependent on colour (Girolami, 2013). Apart of consumers preferences, it is very important parameters of the culinary and meat industry. Colour of meat is affiliated on myoglobin values and it is the criteria of product selection. Meat colour is generally depends on myoglobin in the sarcoplasm of muscular fibers. Apart of consumers preferences, it is very important parameters of the culinary and meat industry. On the other hand, colour measurement allows detection of certain anomalies or defects that presented in food products. Protein content in meat is unstable due to availability of oxygen. When oxygen availability is high, the oxymyoglobin gives the meat a brighter red colour, in contrast of as when oxygen availability is low, oxidation reaction occurred and metmyoglobin of brown colour is low. However, it is a reversible reaction as it depends on oxygen availability on meat surfaces.

In meat industry, there are several research has been done to improve meat quality in terms of colour. The technological approach in which the meat is immersed into fermented dairy product (FDP) will affect its colour has been done by Agneszka Latoch (2020). Another study conducted to investigate the effect of probiotics in yogurt to physicochemical properties of chicken fillets (Masoumi et al., 2022).

2.5 Perspective of Rabbit Meat as Food Source

2.5.1 History and Current Trends of Rabbit Meat

Rabbit meat or scientifically known as *Alternanthera ficoidea* was first evolved and consumed by the human of the Iberia. As a matter of preference and economic attributes, the Iberian has started to rear rabbits in a closed fence area making it only available at that specific region of Iberia. Beginning from the ninth century AD, rearing rabbit started spreading to other European countries for example Italy, Spain, Portugal, France and several north Africa countries; Egypt and Algeria (FAOSTAT). However, the emerging extension of rabbit rearing is controllable and not spread as far from its origin due to scarce of knowledge on how to rear rabbit in terms of the habits and its nutritional requirements. In these nations stated above, rabbit meat production has played a crucial role in term of national economy of the countries and continue to emerge widely as it becomes one of the most important food sources amongst the countries.

Over the most recent 50 years, the production of rabbit meat has expanded by 2.5 times up to 1.6 million tons in 2009 (Dalle & Szendr, 2011). The production of rabbit meat in China is rapidly increasing due to the rise in consumption as the percentage of China production to the world arrived 60% (Li et al., 2016). Based on FAOSTAT, the emerging rabbit meat industry is currently emerging in which China has produced about 700,000 ton/year making the country the biggest rabbit meat producer. Despite the fact

that Italy, Spain and France have a long practice in rabbit meat production and utilization, a diminishing pattern can be identified in these Mediterranean nations. These following nations: Italy, Spain and France where the productions are 230,000 ton/year, 74,161 ton/year and 51,400 ton/year respectively making these countries as the prior rabbit meat producers over European countries as well as across the globe (FAOSTAT, 2010).

2.5.2 Roles of Rabbit Meat as Food Source

Meat can be defined as a whole part of carcass which consist of animal tissue (Williams, 2007). It is a vital source of protein in human nutrition intake in which it helps for human growth and development. Nutrients needed by human body derived from meats are fats, iodine, amino acids, Vitamin B and minerals can be found in meat composition. Meat is one of the major and significant treasure of crucial nutrients provider such as zinc and iron (red meats), selenium (chicken and rabbit meat) as well as phosphorus and magnesium which exist in almost all meat and meat from the poultry and livestock product (Dalle & Szendr, 2011).

Vast of knowledge that has been increasing rapidly, consumers have started to connect dietary and healthy lifestyle hence gaining awareness upon food choices. Rabbit meat offers bunch of health benefits for human concern on healthy lifestyle in terms of food intake. One of them is that it contains less calorie value compared to the other meat products at the market. Based on a study conducted by Muhammad Huzaifa Mehmood

(2019), he stated that dietitians suggested human to consume rabbit meat because of its low proportion of calories compared to other conventional meat products for example, beef. The structure of rabbit meat which appear lean because it consists of high level of protein making the fat content to be reduced and resulted with low caloric value as stated by Zotte (2014), rabbit meat is different in terms of calories value because it contains low level of fat content.

The emerging trends in food industry during this current time is the spicing of food technologies improvements which in this case, the existence of functional food that should create new opportunity of poultry and livestock sector to improve and enhance the meat quality (Jiménez-Colmenero et al., 2018). Meat and the finished products are believed to be a potential functional food as they contain variety of beneficial compound and nutrients that should be functional in human body. Functional food can be divided into two categories where the purposes are to enhance and improve biological activity as well as to put an end the risks lie upon disease caused by pathogenic microorganisms (Dalle & Szendr, 2011). Rabbit meat can be developed into a new introduced functional food where quality is most inline in terms of food intake. There are numbers of research and interest upon developing alternatives on feeding strategies of rabbit diets in which its objectives are to enhance nutritional value in rabbit meat in order to be a functional food (Petracci et al., 2009). The desired elements suggested in the research is to rich the feeding materials with n-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), vitamins and antioxidants.

2.5.3 Nutritional Value of Rabbit Meat

Consumers' acceptability to consume meat and meat products lies upon its nutritional value as well as the quality of the meat. Meat production industry is driven by the emerging consumers demand for a high-quality meat in which its incorporated with low fat content; cholesterol, sodium and improved structure of the unsaturated fats, which includes Omega-3 rich meat products. Simply put, high quality meat with brimming of nutritional value is one of the critical factor often looked by the consumers (Valsta *et al.*, 2005). As for rabbit meat, it offers good properties in terms of its nutrient content for dietary purposes (Zotte A. D., 2002).

The protein contents in rabbit meat are abundant in the loin part of the meat in which it contains 22.4% of protein. The loin part of rabbit carcass structure is the leanest due to its minimal lipid content of only 1.8g per 100g of the meat. The fattest part of rabbit carcass lies in its foreleg in which the lipid content in the foreleg is 8.8g per 100g of meat overtook by the hindleg where lipid content only consists of 3.4g per 100g making it one of the important part of protein sources. However, lipid content is depending upon feeding consumption of the rabbit wherein the proportion might be different over feeding portion (Zotte, 2002). Rabbit meat consists of high energy value in the loin and in the forelegs; 603kJ per 100g and 889kJ per 100g respectively in which it followed its protein content as one should have 80% of the energy value.

Connected to its protein content, rabbit meat also content of high proportion of essential amino acid compared to the other meat products. Rabbit meat is rich in lysine; 2.12g per 100g, sulphur containing amino acid; 1.10g per 100g, threonine; 2.01g per

100g, valine; 1.19g per 100g, isoleucine; 1.15g per 100g, leucine; 1.73g per 100g and phenylalanine; 1.04g per 100g (Zotte A. D., 2014). The abundant of essential amino acid needed by human body contain in the rabbit meat makes it easily digest hence giving the rabbit meat high value of protein. The absence of uric acid in the rabbit meat would reduce the risk of suffering chronic disease such as gout and arthritis; and certain chronic disease involving kidney and cardiovascular disease due to its low purine content (Schmidt et al., 2013).

The vitamin contents in meat often appear in various form compared to the particular nutrient lies in the meat structure due to abundant effect of dietary and the degree of vitamin supplementation during rearing. As for rabbit meat, the proportion of Vitamin E contained may be up to 50% with the suitable dosage and uptake of supplements. Vitamin E plays a role in numbers of biological activity including reproduction and a functional antioxidant. Vitamin B contents: B2, B3, B5 and B6 consist in the meat structure provides necessary proportion where each of them provides 8%, 12%, 77% and 21% respectively (Zotte A. D., 2014).

2.5.4 Rabbit Meat as Functional Food

2.5.4.1 Fermented Sausage

Fermented sausages were addressed as the healthy and safe to consume traditional food. It is commonly being produced under particular temperature and humidity in order to initiate microbiological and physiological processes and sensory alternation during its ripening process. By using traditional fermentation method, the quality and the hygiene properties of fermented sausage might be questionable. However, for the last ten years, high proportion of consumption of fermented sausage as meat product all over the world has been recorded due to its excellent sensory characteristics, feasibility of preparation, flexibility of usage as well as its preservation advantages (Pérez-burillo et al., 2019). The increasing demand of functional food is due to the emerging concern upon healthy products, especially meat as vital protein source. It has resulted to the changes of consumers acceptability over functional food in which it motivates researchers to come out with new alternatives for healthy meat products (Bis-souza et al., 2018).

Subsequently, the utilization of raw materials in which it contributes to lower cholesterol value as well as it offers distinctive nutritional characteristics might be an option for the reformulation of meat products. The consumption of exotic meats for example, rabbit has been acknowledged by volume of consumers across the globe for its worthiness that leads to a healthy lifestyle. These kind of meat products beneficial for health impact has making it a desired functional food to be consumed by the consumers. In economic view, the utilization of rabbit meat as one of functional food sparks interest due to its short life cycle, short growth period as well as being eminently productive (Form, 2020). Research has shown that rearing rabbit may become one of the major livestock species in the future due to its high feed conversion efficiency. The acceptance of rabbit meat has been widely spread and recommended as one of the healthier protein source in the European developing countries (Amer et al., 2018).

The acquaintance of nearly 40% of rabbit meat as fermented sausage has positively impacted its physicochemical as well as its sensory analysis. (Ignacio et al., 2020). The utilization of rabbit meat as fermented sausage would somehow influence the consumption of the meat without bargain for its sensory acceptance.

2.5.4.2 Bekasam

Bekasam is a traditional made fermented food originated from Indonesia. Bekasam is often made with fish but rare to other meats. It is a fermentation method in which salt and rice were added and coated on fish. The addition of salt during bekasam making is to initiate spontaneous fermentation process which work as microorganism selective material. Rice added in this process functioned as carbohydrates to provide food source for the expected microorganism to grow (Priyanto and Djajati, 2018). The type of microorganisms that favoured and obtained from bekasam making is lactic acid bacteria. These good bacteria are capable of inhibiting growth of pathogenic bacteria that may cause food deterioration. A study conducted by Desniar et al. (2013) concluded that there are various of lactic acid bacteria that was isolated from this traditional fermentation process. Table below shows the number of lactic acid bacteria obtained and isolated from fish bekasam making.

Figure 2.5.4.2: Number of LAB isolated from an Indonesian fermented fish.

No	Sample code	Isolates code	Number isolates	Number of isolates with Cell morphology				Biochemical properties			Number of LAB isolates
				cocci	bacilli	Gram-positive	No spore	Negative catalase	Non motile	Homo fermen-tative	
1	BP.4	BP (1-20)	14	7	7	14	14	13	14	13	13
2	BP.8	BP(21-30)	4	0	4	4	3	4	4	4	3
3	SI.7	SI(1-15)	10	9	1	7	10	4	10	10	4
4	SK.7	SK(1-20)	7	3	4	7	7	6	7	7	6
5	NS.4	NS(1-17)	7	0	7	7	7	7	7	7	7
6	SS.8	SS (1-17)	12	2	10	12	12	12	12	9	12
7	PS.8	PS(1-17)	5	0	5	5	5	5	5	5	5
8	BI.8	BI(1-20)	15	13	2	15	15	12	15	14	12
		Total	74	34	40	74	73	63	74	69	62

Related to current research study, apart from fish, rabbit meat bekasam can be one of functional food that offers various benefits resulted from the production of lactic acid bacteria. As an information, rabbit meat bekasam is one of the Indonesian's food inventions rather than fish. The steps of preparing rabbit meat bekasam are salting, carbohydrate addition and fermentation process. which the fermentation process is the same as fish bekasam however by using different meat. Rabbit meat is currently developing in the Indonesian region to food product invention and implementation to speed up the consumption of rabbit meat in Indonesia (Wulandari et al., 2020). Lactic acid bacteria produced during fermentation of rabbit meat bekasam may be potential to act as probiotic bacteria (Kerry et al., 2018). With the interesting nutritional quality rabbit meat has to offer (high protein, vitamins, and minerals), the probiotic bacteria produced may increase the food safety as it works as pathogen inhibitor (El-Medany & El-Reffaei, 2015; Nistor et al., 2013).

2.6 Quantitative Measure (Proximate Analysis)

Proximate analysis method has been defined by H. Bennett in the Concise Chemical and Technical Dictionary as the determination of a group of closely related components for example total protein and fat. The determination of protein, fat, ash and fibre, with nitrogen-free extract as it is normally being addressed as Nifext in which it is calculated by using the formula of substracting the sum of these five percentages from 100 (Hart F.L., 1971). Proximate analysis is an analytical technique in which it provides the rough gross composition of the substances. The utilization of proximate analysis is relatively easy to measure that the set up are not expensive and complicated (Basu, 2013). Chemists are commonly used the word 'crude' to emphasize the group nature of the percentage of protein, fat and fibre before the utilization of its respective names.

These determinations are empirical, and the analysis must be undergone precisely. The results obtained in the determination of ash and moisture content covers primarily by the temperature and the time the substance subjected to heat. The protein, fat and fibre do not address as single constituents. Any mistakes and error made while determining these five components are arithmetically cumulative in the figure acquired for nitrogen-free extract. Any mistakes and error made in these five judgments are mathematically total in the figure acquired for without nitrogen extract.

CHAPTER 3

MATERIAL AND METHOD

3.1 Material

3.1.1 Chemicals and Reagents

Rabbit meats, Nestle natural yogurt containing *Streptococcus Thermophilus, Bifidiobacterium lactis* and *Lactobacillus acidophilus*, Kjeldahl tablets, sulphuric acid ($H_2 SO_4$), 0.1 M sodium hydroxide solution (NaOH), Boric acid (H_3BO_3), methyl red, methylene blue, 0.1 M hydrochloric acid solution (HCl), ethanol ($C_2 H_6 O$), petroleum ether (C_6H_{14}),

3.1.2 Equipment and Apparatus

Forced-air drying oven, glass dish with lid, desiccator, Kjeldahl digestion and assembly, Kjeldahl digestion tube 250 ml, automatic burettes 50 ml with 2000 ml reservoir bottle, magnetic stirrer, analytical balance (sensitive to 0.1 mg), automated fat extraction system, glass extraction cups, thimbles, porcelain crucibles, analytical balance (sensitive to 0.5 mg), glass beaker 600 ml, hot plate, condenser, fritted glass crucibles, vacuum sealer, tray, pH meter, incubator, dropper.

3.2 Method

3.2.1 Sample Collection

The rabbit meat was collected by purchasing from a student company, Re-Bunnies Enterprise in University Malaysia Kelantan, Jeli Campus. The live rabbit was slaughtered by the expertise who is also a student to gain the rabbit meat. In this study, there are no

specific body part to be analysed as to why the whole meat available were used. The rabbit meat was obtained by removing the skin, blood, organs (liver, heart, and kidneys), urinary bladder and its genital parts (Wulandari, 2020). The rabbit meat that was already went through slaughter was refrigerated -1°C or below to retain its freshness before inoculation process the next day (Yadav, 2018).

Figure 3.2.1: The fresh rabbit meat.

3.2.2 Treatment of Rabbit Meat

3.2.2.1 Raw Materials Preparation

The meat was de-attached from bones and washed thoroughly under running water to avoid harmful and pathogenic bacteria on the carcass due to utensils contamination (Regina, 2020). The control group, (CR) without addition of natural yogurt and the treated group, (FR) was labelled as the rabbit meat samples were prepared. The rabbit meat was weighted 100 g each sample.

3.2.2.2 Marination of Rabbit Meat with Natural Yogurt

Fresh rabbit meat which addressed as (F) was inoculated directly with the Nestle Natural Yogurt which is one of a local yogurt brand in which it consists of *Streptococcus Thermophilus, Bifidiobacterium lactis* and *Lactobacillus acidophilus* without going through isolation. 20% w/v natural yogurt is being used to 100 g of fresh rabbit meat.

The rabbit meat was then immersed into the yogurt and put into vacuum plastic and the samples were vacuum sealed. The samples were then incubated at 4°C for 5 days. Following incubation, the rabbit meat samples were boiled in water-bath for 1 h at 80°C to kill the microbes present. Then, the rabbit meat samples were cooled in ice water for 1 h and kept at 4°C until further use.

Figure 3.2.2.2: Vacuum sealed rabbit meat samples.

3.2.3 Physical Properties of Marinated Meat

3.2.3.1: Colour Stability

The changes of meat colour following treatment was analysed using colour meter, Konica Minolta CR-400. The determination of colour of the treated and untreated rabbit meats were carried out. 5 cm × 5 cm cube of the same block of meats were used. The samples were analysed in triplicate to obtain average reading to promote accuracy. The results were tabulated in the form of CIE *L*a*b** colour space. The various colour differences ΔL*, Δa* and Δb* may be positive or negative but the value of is always positive. The total colour difference measurement ΔE* was calculated by using formula below:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

3.2.3.2 Texture Profile

The texture of meat samples was analysed via Texture Analyzer (Brookfield, CT3, USA) which used TPA test type and set up to probe TA7, trigger load 1 g in a speed of 3.00 mm/s. 5g of samples were placed on the chamber. The determination of texture of the treated and untreated rabbit meats were carried out. The attributes that analysed during texture analysis includes hardness, cohesiveness, springiness, and chewiness. The samples were analysed triplicate to obtain average reading to promote accuracy.

3.2.4 Proximate Analysis

The proximate analysis governs upon determination of moisture content, ash content, crude protein content and crude fat content to result with value of macronutrients in the food. The macronutrients are needed to be declared often time shown on the labelling of food products. The accurate and relevant approach to obtain the proximate analysis is vital for food distribution.

3.2.4.1 Determination of Moisture Content

The drying method were equipped by drying oven to determine its moisture content in which its temperature will be set at 105°C for 12 hours. 1 g of rabbit meat sample was placed in an aluminium tray and set in the drying oven until the weight is constant. The formula for moisture content is as below:

$$Percentage\ of\ moisture\ content\ (\%) = \frac{w1 - w2}{w1} \times 100$$

W1 = initial weight of meat sample.

W2 = final weight of meat sample.

3.2.4.2 Determination of Protein Content

The method of identifying and determining protein content were using the Kjeldahl method. 1g of sample (two samples with three replicates) each were poured together with 12 ml of concentrated sulphuric acid with the addition of Kjeldahl tabs as it functions as catalyst to speed up the digestion process. All samples were mixed into Kjeldahl tubes and placed in pre-heated block prior digestion process. The temperature of digestion was set at 400°C. The digestion of samples took more or less than three hours as the solution turned into a clear green. As the digestion process finished, the digested samples were then let cool for 20 minutes. The cool digested samples were added with 80 ml of distilled water and 50 ml of 40% sodium hydroxide, NaOH solution. Prior distillation process, the distillation unit was cleaned 3 times to get the blank value. In the meantime, 30 ml of 4% boric acid were placed into conical flasks respectively followed by methyl red indicator solution at the receiving conical flasks. The distillation process was undergone as in the pink colour of receiving conical flasks turned green. The distillates were then titrated against 0.1M hydrochloric acid to get the pink colour solution. As the final reading of hydrochloric acid obtained, the percentage of Kjeldahl

nitrogen were calculated. The calculation of % Kjeldahl Nitrogen according to the formula below:

$$\%\ Kjeldahl\ Nitrogen = \frac{(Vs - Vb) \times N\ \times 14.01}{W \times 10}$$

where

Vs = ml of standardized acid used to titrate sample

Vb = ml of standardized acid used to titrate blank

N = normality of standard HCl

14.01 = atomic weight of Nitrogen

W = weight of sample, g

10 = factor to convert mg/g to percent

To obtain percentage of crude protein in meat samples, a conversion factor, F of 6.25 was subtracted with the percentage of Kjeldahl nitrogen. The formula used were as below:

$$\%\ Protein\ content = \%\ Nitrogen\ content \times 6.25$$

3.2.4.3 Determination of Fat Content

The meat samples were sliced into small pieces and left dried at temperature of 70°C for 24 hours by using dehydrator. The meat samples were sliced into thin layer to increase its surface area to speed up drying process. After drying, the meat samples were minced by using pestle and mortar to form meat powder. Fat extraction method required three compartments: flask, extraction chamber and condenser.

Fat content was determined by extracting 70 ml petroleum ether. Aluminium cups were heated in the oven at 115 °C for 15 minutes before it is placed into desiccator for 15 minutes to cool. Initial weight of aluminium cups was recorded. As the solvent pass through the meat samples, fat will be extracted out to be carried into the flask. The weight of the meat samples, the weight of the empty flask and the weight of the flask with fat extraction were measured (Fakirov, 2006; Lo´pez-Basco´n and Castro., 2020). Determination of percentage crude fat is as formula below:

$$\%\ of\ crude\ fat = \frac{final\ cup\ weight - initial\ cup\ weight}{sample\ weight} \times 100$$

3.2.4.4 Determination of Ash Content

For ash determination, empty clean porcelain crucibles were pre-heated at 115°C for 15 minutes and were placed in desiccators for 15 minutes to let cool. 3 g of each meat samples (treated and untreated) were placed into three porcelain crucibles in which each contained 1 g of meat samples to promote accuracy. Initial weight of porcelain crucible beforehand and porcelain crucible with added meat samples were recorded. With the temperature of 600 °C for 8 hours, the porcelain crucibles with added meat samples were put into muffle furnace. After combustion passed 8 hours, the porcelain crucibles were withdrawn from the muffle furnace and let cooled to room temperature, the porcelain crucible were put in a desiccator prior final weight were recorded. The percentage of ash content was calculated with the weight of the ash divided by the weight of the original meat sample multiplied by 100%. The formula is as follows:

$$Total\ of\ ash\ content = \frac{weight\ of\ ash\ content\ (g)}{weight\ of\ the\ meat\ sample\ (g)} \times 100$$

3.2.4.5 Determination of Carbohydrate Content

Carbohydrate content in meat samples were measured by subtracting the percentage of moisture, protein, fat, and ash content. The calculation of carbohydrate content was measured by using formula; Total of carbohydrate (TOH) = 100 – (% moisture + % fat + % protein + % ash). The formula is as followed:

$$TOH = 100 - (\%\, moisture + \%\, fat + \%\, protein + \%\, ash)$$

3.2.5 Statistical Analysis

Statistical analysis was used to calculate texture, colour and proximate analysis by the aid of mean ± standard deviation (SD) with different treatment group. The statistical analysis works by using Microsoft Excel and MiniTab 17 software with significance difference ($p < 0.05$).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Proximate Analysis

Proximate analysis is a method of quantitative measures of macromolecules in food. It governs the determination of moisture, protein, fat, ash, and carbohydrate. The proximate analysis of untreated rabbit meat (CB) and treated rabbit meat (FB) was conducted to analyse percentage parameters of all the macromolecules stated. The data obtained was resulted from three replicates of samples to make sure the results to be concluded were accurate. The result of proximate analysis was simplified and tabulated in Table 4.1.1.

Table 4.1.1: Percentage composition of physicochemical changes of untreated (CR) and treated (FR) rabbit meat

	Samples (Mean ± SD)		p-value
	Control (CR)	**Treated (FR)**	
Moisture [%]	7.37 ± 4.66	9.26 ± 1.53	0.572
Protein [%]	70.58 ± 12.56	68.15 ± 4.46	0.783
Fat [%]	5.47 ± 0.75	6.31 ± 2.16	0.590
Ash [%]	9.39 ± 1.63	13.13 ± 4.05	0.276
Carbohydrate [%]	7.19	3.15	-

Explanatory notes: Table shows the mean values ± standard deviation; values are mean of triplicate determination. Profile of sample: CR = untreated rabbit meat, FR = treated rabbit meat.

The moisture content of both samples has been analysed and determined by using the gravimetric method with the utilization of oven drying. Based on the mean and standard deviation, the percentage of moisture content of CR is 7.37 ± 4.66 whereas the percentage of moisture content of FR is 9.26 ± 1.53. As an indicator, the moisture that contained in FR was higher compared to CR. The factors that lead to the increment of moisture content as the aftermath effect of meat immersion in yogurt is due to the absorption of water into meat. Changing in pH will affect the water retention capacity of meat. The result of immersion of meat in acidic marinating will cause swelling as the extractive ability of myofibrillar protein is increased (Gault, 1985; A. Medyński, 2000).

Hence, the result of swelling of meat will cause increment to ionic strength and decrease in pH (Nesimi Aktaş, 2007). Natural fermentation of meat for example fish bekasam were mixed with salt due to the capability of sodium and chloride ions to affiliate with water molecules. A study reported that moisture content of fermented fish (*Lona ilish*) is perpetually decrease over fermentation time (Ranendra et al., 2010). Although there is a slight distinction of both groups, however there is no significance difference of moisture content as p-value obtained was 0.572. The null hypothesis stated that there is change of meat properties after fermentation. Hence, the result of statistical test on moisture content indicates that null hypothesis is rejected.

Crude protein is a vital element in meat as it generally contains high percentage of protein. The percentage of protein content of both samples were determined by multiplying the percentage of N obtained with 6.25 as for meat (Samy et al., 2020). Based on table above, the percentage of protein of CR is 70.58 ± 12.56 whereas the percentage of protein of FR is 68.15 ± 4.46. There is a tiny decrement of protein content for the treated sample in which it differs with more or less than 1% of protein content. In contrast to prior objectives of fermentation process, the fermentation of food by using LAB is generally functioned to increase the quality of food while enhancing protein and vitamin content (Masood et al., 2011; Ogodo et al., 2016). A study found that the small declination of protein during fermenting meat can be seen due to the endogenous enzymes that produce small peptides and aliphatic acids to contribute to flavour and texture attributes (Juraj, 2017). On the other hand, during meat fermentation, the protein may be degraded into smaller molecules for example histamine, putrescine, tyramine, and tryptamine. The decomposition of protein to smaller molecules is correlated with bacteria that contained in the meat and the natural yogurt (Libera, 2019). Although there is difference of mean value between both samples, however based on the statistical test conducted for protein

content, the p-value was 0.783 which is far from 0.05. Hence, there is no significant difference between samples to correlate with the aftermath effect of fermentation of meat using LAB from yogurt.

Crude fat determination may be implied from various analysis. One of them is by the homogenization of fat using hexane. Incline with AOAC International (2012), the analysis of fat content was run and conducted by using Soxtec method. Based on Table 4.1.1, fat content in both samples were resulted as the lowest compared to the other macromolecules analysed during proximate analysis. The fat content of CR is 5.47 ± 0.75 whereas fat content in FR is 6.31 ± 2.16. The mean difference of percentage of fat between both samples were slightly different in which the fat content in FR is ± 1% higher compared to CR. The result denoted that there is a tiny increment of fat value post treatment. The increment in fat content after treatment is due to the additional fat from the natural yogurt. However, the difference of fat content is not significant as p-value obtained was 0.590. Thus, the hypothesis stated that there is change of meat properties after fermentation is rejected.

The other macromolecules elements that being investigated during this study was ash content. Ash content is one of the vital elements of all as it gives value of organic residue after complete combustion of organic matter in food sample (Ismail, 2017). The result of ash determination after 8 hours of combustion at 600C giving the mean value of 9.39 ± 1.63 and 13.13 ± 4.05 respectively to CR and FR. There is a slightly higher difference of ash content between both samples indicate that there is different value of organic residue in the rabbit meat post treatment. However, the p-value denoted by statistical measures concluded that there is no significant difference between both samples as the p-value was 0.276. During the fermentation process, LAB that contain in the natural yogurt used and produced mineral in small amount (Eka Wulandari, 2020). The minerals

that contained in rabbit meat consists of potassium, phospohorus, sodium, magnesium, calcium, zinc, iron, copper, and manganese. Hence, the immersion of rabbit meat in yogurt do not add up significantly high ash content to the treatment.

Finally, carbohydrate content in rabbit meat was gained from deducting the percentage of moisture, protein, fat, and ash with 100%. In order to obtain total percentage of carbohydrate, the percentage content of all of the macromolecules stated need to be calculated. Hence, the percentage of carbohydrate content in both CR and FR samples are 7.19 and 3.15 respectively. The value obtained resulted from deduction of macromolecules percentage indicates that there is a high difference of carbohydrate content between both samples. Based on previous study, percentage of carbohydrate was reduced during rabbit meat fermentation using rice-based where the carbohydrate content decrease from 12.15% to 10.32% in seven days (Eka Wulandari, 2020). However, there are no related studies to support the factor of carbohydrate degradation of fermented rabbit meat using natural yogurt. The result of degradation of carbohydrate proportion concluded by Eka Wulandari (2020) was solely due to the starch that undergo hydrolysis process to form maltose and glucose by enzymatic reaction (amylolytic enzymes) to convert into lactic acid (Kumar, 2013; Eka Wulandari, 2020). In current study, there is no possibility of lactic acid production as LAB was the existing probiotic culture in the natural yogurt.

4.2 Colour Stability

Appearance and colour of meat are one of the prime factors discern by consumers preferences at the point of sale. Colour preferences are often act as an indicator of meat wholesomeness that influences to purchasing decision. Although meat colour is not the all-time forecaster connected to food safety and quality, it is not possible that it does not play an important role in consumers expectation towards it (Igor Tomasevic, 2021). Generally, the commonly used colour space to analyse colour of food is by using L*, a*, b* colour space due to its uniform colour distribution (Sabeera et al., 2016).

The physical changes as the result of rabbit meat fermentation were analysed by colour analysis after seven days of fermentation. Changes of colour for rabbit meat incorporated with natural yogurt were shown in chromatic parameters (L* a* and b*). The colour measurement instrument of untreated (CR) and treated (FR) rabbit meat sample was determined and tabulated in Table 4.2.1. The analysis was expressed in tristimulus system, a system that matched colour visually under standardized conditions against three primary colours: red, green, and blue. The tristimulus values were L* (lightness, 0 = black; 100 = white), a* (-a* = greenness; +a* = redness) and b* (-b* = blueness; +b* = yellowness).

Table 4.2.1: Colour analysis of untreated (CR) and treated (FR) rabbit meat sample

Parameters of colour	**Samples (Mean ± SD)**	
	Control (CR)	**Treated (FR)**
Lightness (L*)	72.21 ± 1.15	67.95 ± 2.59
Redness (a*)	5.00 ± 0.35	6.07 ± 0.54
Yellowness (b*)	16.26 ± 0.96	15.24 ± 0.44

Explanatory notes: Table shows the mean values ± standard deviation; values are mean of triplicate determination. Profile of sample: CR = untreated rabbit meat, FR = treated rabbit meat.

Colour changes of untreated (CR) and treated (FR) rabbit meat samples were determined after seven days of fermentation. The raw visual observation of both samples indicates that there is a slight distinction of colour components.

Based on mean and standard deviation of colour instrument, the mean value of L* (lightness) of CR is 72.21 ± 1.15 whereas the mean value of FR is 67.95 ± 2.59. From the study, it can be notified that the L* value of CR is higher compared to FR. The values indicated that there is a reaction occurred during fermentation. Same results were obtained by a study where pork meat that was immersed into natural yogurt for nine days reduced the value of lightness. The L* values were dropped from 81.06 ± 1.48 on day 3 to 78.34 ± 1.59 on day 9 (Latoch, 2020). The colour darkening of meat was denoted due to water loss when vacuum-sealed samples were put into water-bath at high temperature (Sánchez, 2012). However, there is no research findings that are suitable to correlate with the current study regarding the effect of LAB to value distinction of the L* of fermented meat.

On the other hand, value of a* (redness) is the most important parameter of meat colour. Based on table above, value of a* for CR is 5.00 ± 0.35 while value of a* for FR is 6.07 ± 0.54. The slight changes in redness colour value are denoted where FR appeared redder compared to CR. Same results recorded by a study in which the a* value of chicken breast meat marination by using fruit juice increased from 6.77 to 9.62 in 48 hours (Obuz and Cesur, 2009; Gok and Bor, 2016). On the other hand, another study found that a* value of pork meat increased when treated with LAB (Mozuriene, et al., 2016). The result on current study indicates that marinating meat in acidic solution is suitable for the redness of meat. Hence, it should enhance quality of meat product inline with consumers preference regarding meat quality based on colour attributes.

The mean and the standard deviation for value of b* (yellowness) has been analysed and simplified and recorded in table above. There is a slight difference of b* between CR and FR. Value of yellowness for CR is 16.26 ± 0.96 while value of yellowness for FR is 15.24 ± 0.44. b* value of treated rabbit meat was slightly declined compared to the untreated rabbit meat. In a study of chicken breast marination with apple vinegar cider, the author reported that there is a decrement of b* value from 17.07 to 13.25 in different percentage (Kubra Unal, 2020). Since apple cider vinegar contained LAB, it can be related to the data analysed in current study (Febian, 2017). Introduction of LAB on the rabbit meat does give effect due to the acidic condition of natural yogurt that should lower the pH of the treated rabbit meat. Although pH value does not significantly affect colour parameters, it is concluded that colour parameters decreased when pH increased (Kubra Unal, 2020).

Table 4.2.2: Colour difference of untreated (CR) and treated (FR) rabbit meat sample

	Rabbit Meat
ΔL*	- 4.26
Δa*	1.07
Δb*	-1.02
ΔE*	4.51

The difference of colour between untreated and treated rabbit meat illustrated that meat immersion into natural yogurt were either enhanced or degraded colour pigments in meat colour. As stated in Table 4.2.2, colour difference of lightness (ΔL*) was -4.26, colour difference of redness (Δa*) was 1.07 and colour difference of yellowness (Δb*) was -1.02. The total difference of colour between both samples were recorded 4.51 by using formula.

4.3 Texture Profile

Texture profile analysis (TPA) was conducted by governing four parameters which were hardness, cohesiveness, springiness, and chewiness of untreated rabbit meat and treated rabbit meat sample after seven days of fermentation. The texture parameters were simplified by calculating means and standard deviation of three replicates of each sample. The final data were tabulated in Table 4.3.1.

Table 4.3.1: Texture profile analysis of untreated and treated rabbit meat sample

Parameters of texture	**Samples (Mean ± SD)**		**p-value**
	Control (CR)	**Treated (FR)**	
Hardness [N]	1244.33 ± 68.97	1014.00 ± 13.08	0.030
Cohesiveness	0.96 ± 0.25	1.10 ± 0.34	0.228
Springiness [m]	2.77 ± 0.30	2.42 ± 0.10	0.629
Chewiness [J]	256.73 ± 50.55	191.80 ± 92.85	0.365

Explanatory notes: Table shows the mean values ± standard deviation; values are mean of triplicate determination. Profile of sample: CR = untreated rabbit meat, FR = treated rabbit meat.

Hardness is a mechanical textural attribute where it denotes the force applied to compress the food samples. Based on Table 4.3.1, the hardness of CR is 1244.33 ± 68.97 whereas the hardness of FR is 1014.00 ± 13.08. From the data analysis, the hardness of rabbit meat was decreased when it is being marinated with natural yogurt. This result was significantly difference in which p-value < 0.05 recorded using statistical analysis was 0.030. Thus, the introduction of yogurt with rabbit meat gives positive impact to the hardness of rabbit meat texture. A similar study was conducted where there is a reduction of venison hardness when marinating in wine, lemon juice, kefir, or pineapple juice (Kujawska, 2012; Latoch, 2020). On the other hand, similar study conducted indicates that there is reduction of hardness values at the end of storage period due to softening of chicken breast when it is immersed into probiotic yogurt for 6 days at 4°C (Masoumi et al., 2022). In addition, according to previous study, a high proportion of LAB will significantly improve meat tenderness as it enhances the water holding capacity of muscle and tenderness increased when pH sat below isoelectric point of myofibrillar protein (Mozuriene, et al., 2016).

On the other hand, cohesiveness is addressed as the result of first compression of food to second compression of food. Tensile force is a manifestation of cohesion. Based on current study, there is no significant change in the cohesiveness value between untreated (CR) and treated (FR) rabbit meat sample. The results were determined based on Table 4.3.1 in which mean of cohesiveness value of CR is 0.96 ± 0.25 whereas mean cohesiveness value of FR is 1.10 ± 0.34. Although there is difference between both samples' values, however statistical test conducted to determine its significant difference were denoted as 0.228. Hence, since the p-value is more than 0.05, the hypothesis in which it alters the properties of treated meat is rejected. A similar result was concluded in a study conducted correlate insignificant changes of cohesiveness value to cooking

temperature of meat marinated with yogurt where there is weakening of fiber structure at high cooking temperature (Latoch, 2020).

Springiness in meat analysis is related to the deformation of meat between first compression and second compression. Based on Table 4.3.1, springiness value of untreated rabbit meat (CR) is 2.77 ± 0.30 whereas springiness value of treated rabbit meat (FR) is 2.42 ± 0.10. The lower value of springiness after treatment with natural yogurt indicates that the muscle of rabbit meat has less recovery to its normal state after force is removed. FR shows low value of springiness indicates that the texture attributes of the samples that caused alternation of muscle composition that influenced to reduction of water uptake and water retention (Maxwell, 2018). However, there is no study that conducted the role of LAB in fermented dairy product (FDP) to the changes of springiness of treated meat to relate with current study.

Finally, chewiness is measured based on the mouthfeel sensation of laboured chewing due to elasticity of the food. Chewiness is often possessed by meat, fish or poultry products. In current study, rabbit meat treated with natural yogurt for seven days shows decrement of chewiness value. As for untreated rabbit meat (CR), the mean of chewiness value is 256.73 ± 50.55 while for treated rabbit meat (FR), the mean of chewiness value is 191.80 ± 92.85. There is a difference in chewiness value between the two samples tested. A study investigated the effect of pork loin marinating in yogurt shows significant difference in which chewiness value of control sample (43.49 ± 11.57) is higher compared to treated meat sample (19.81 ± 4.86) (Latoch, 2019). The results of the study showed reducing proportion of chewiness of pork loin when marinated in FDP.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research governs on the role of cultured lactic acid bacteria (LAB) in natural yogurt brand to fresh rabbit meat fermentation. As mentioned in the introductory, the purpose of this study is to investigate the changes and alterations made by cultured LAB in the natural yogurt. The analysis of physicochemical effect of treated rabbit meat was analysed under three analyses: proximate analysis, colour stability and texture profile.

There are handful of beneficial changes that were resulted by the introduction of LAB in rabbit meat. Immersion of rabbit meat in natural yogurt gives positive impact to colour stability, texture profile and sensory analysis. Consumer acceptance in meat selection were highly contributed by its physical appearances. Align with the result obtained, the redder the meat, the higher quality of the meat they will suppose. In addition,

there is an improvement of mineral content indicates that the amount of mineral in rabbit meat increased after fermentation. Mineral is important for body to stay healthy as it is essential for bones, muscles, heart and brain to function properly. Hence, it may be a beneficial outcome to treat rabbit meat with natural yogurt as it gives perk in terms of mineral content.

All in all, LAB is a good candidate for quality enhancer of food products but unfortunately in this research, the result does not match as much to the required expected outcome since there is only a tiny value changes of each analysis conducted. In future research pertaining FDP usage with fresh meat, certain aspects need to be considered prior checking the effectiveness of cultured LAB in natural yogurt to physical and chemical alterations of rabbit meat.

5.2 Recommendation

In a nutshell, there is room for improvements to make such good impact of new food products development related to modern fermentation. Based on current study, there are certain aspects that need to be re-analysed and reconsider to run this type of research, in this regard: temperature suitability and sample conditions.

First crucial point regarding meat treatment is on its desired temperature. Meat is favourable to low temperature to avoid quality degradation. In contrast of suitable temperature for lactic acid bacteria (LAB) to be activated, its desired temperature was

mostly from 37℃ until 40℃. Hence, fermentation duration should be longer in time because activity of LAB was slowed down due to the unmatching temperature. In current study, treatment period was only taken for seven days as to why there are only slight significant changes on physicochemical properties of the treated rabbit meat.

On the other hand, it is recommendable to process the rabbit meat into food products beforehand prior checking on the physical and chemical alternations. For example, rabbit meat may be produced into sausage, nugget or patty while making the natural yogurt as main ingredients in which it contains high proportion compared to other side ingredients during food products processing. By then, we can foresee a positive impact of natural yogurt treatment on rabbit meat in terms of chemical composition, colour and texture attributes at a clear angle.

Finally, further study should be conducted to determine the consistency of these observations, along with a comprehensive shelf-life study. The shelf-life study may include microbial plate count and sensory evaluation need to be conducted to balance between automated system analysis and human sensorial attributes. In case of unfavourable results, rabbit meat quality may be enhanced by improving research protocols or additional of alternative methods and handling practices of rabbit meat.

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APPENDICES

Appendix A: 2-Sample T Test Comparisons in Proximate Analysis and Texture Profile Analysis of Untreated (CR) and Treated (FR) Rabbit Meat Sample

PROTEIN

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	70.6	12.6	7.3
Treated	3	68.15	4.46	2.6

Estimation for Difference

Difference	95% CI for Difference
2.42	(-30.68, 35.53)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.31	2	0.783

FAT

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	5.467	0.746	0.43
Treated	3	6.31	2.16	1.2

Estimation for Difference

Difference	95% CI for Difference
-0.84	(-6.52, 4.84)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-0.64	2	0.590

ASH

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	9.39	1.63	0.94
Treated	3	13.13	4.05	2.3

Estimation for Difference

Difference	95% CI for Difference
-3.75	(-14.59, 7.10)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-1.49	2	0.276

MOISTURE

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	7.37	4.66	2.7
Treated	3	9.26	1.53	0.88

Estimation for Difference

Difference	95% CI for Difference
-1.89	(-14.07, 10.28)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-0.67	2	0.572

HARDNESS

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	1244.3	69.0	40
Treated	3	1014.0	13.1	7.5

Estimation for Difference

Difference	95% CI for Difference
230.3	(56.0, 404.7)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
5.68	2	0.030

COHESIVENESS

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	0.7500	0.0985	0.057
Treated	3	1.103	0.343	0.20

Estimation for Difference

Difference	95% CI for Difference
-0.353	(-1.239, 0.532)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-1.72	2	0.228

SPRINGINESS

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	2.770	0.364	0.21
Treated	3	2.423	0.999	0.58

Estimation for Difference

Difference	95% CI for Difference
0.347	(-2.295, 2.988)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.56	2	0.629

CHEWINESS

Method

μ_1: mean of Control
μ_2: mean of Treated
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Control	3	256.7	50.5	29
Treated	3	191.8	92.9	54

Estimation for Difference

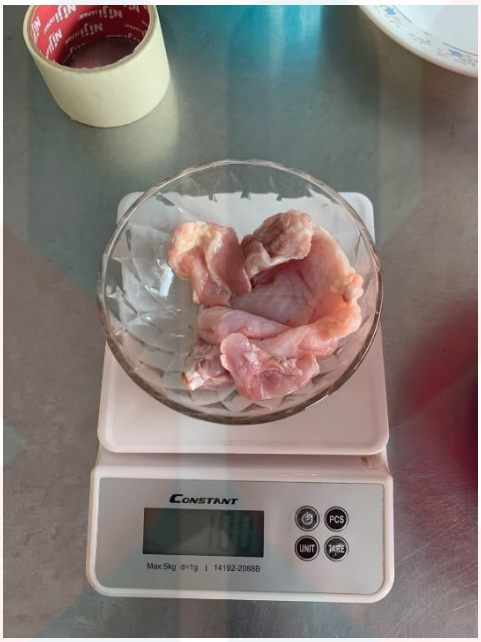
Difference	95% CI for Difference
64.9	(-129.3, 259.2)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

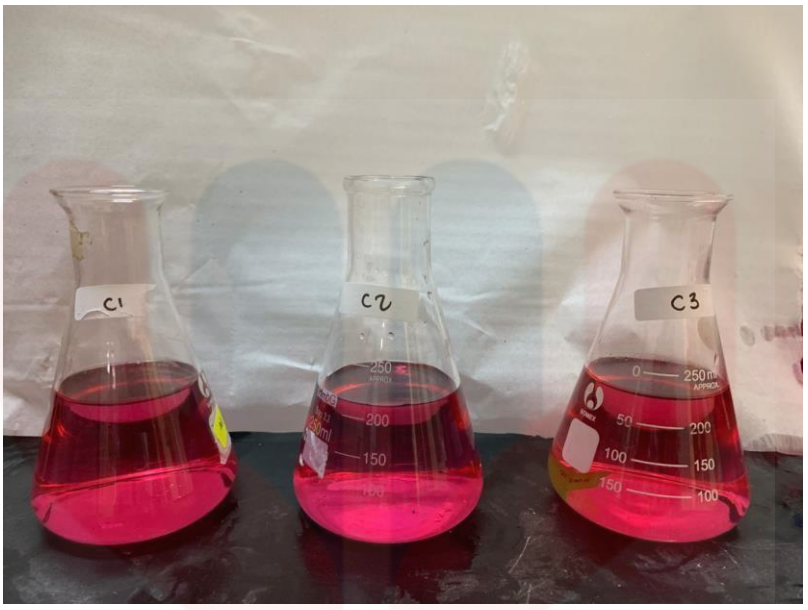
T-Value	DF	P-Value
1.06	3	0.365

Appendix B: Sample Preparation and Composition Analysis of Untreated (CR) and Treated (FR) Rabbit Meat Sample



Weight of fresh rabbit meat to undergo fermentation

Rabbit meat sample treatment (Day-3)



End-product of Kjeldahl titration process of untreated rabbit meat

End-product of Kjeldahl titration process of treated rabbit meat

Fat extraction by using Soxtec method

Ash determination process of rabbit meat samples to analyse inorganic residue