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Physicochemical Effects and Sensory Analysis of Fermented Coffee Beans by Lactic Acid Bacteria and yeast

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A thesis submitted in the fulfilment of requirements for the degree of Bachelor of Applied Science (Food Security) with Honours

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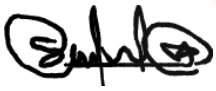
DECLARATION

I hereby declare that the work embodied in this report is the result from the original research except for the excerpts and summaries which I have just described the source.



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Physicochemical effects and sensory analysis of fermented coffee beans by Lactic Acid Bacteria and yeast

ABSTRACT

The global population increased the coffee production until coffee became one of the popular beverages due to its aroma, taste, and flavour. However, coffee processing still has flaws, challenging to control, associated with health problems, and differences in human perspectives on the final product. Thus, importantly to investigate the effects of beans by fermentation, physicochemical effects, and sensory analysis of both treated and untreated fermented coffee beans. Green coffee beans were inoculated with LAB and yeast and kept in closed container for 72 hours for fermentation. In proximate analysis, protein, fat, fibre, ash, mineral, and carbohydrate were analyse with untreated and fermented coffee beans with yeast. The sensory analysis conducted among 37 non-trained panel through hedonic scale. All analysis were calculated by Official Analytical Chemists (AOAC), Two-sample T-test in MiniTab 17 Software and Microsoft Excel. The composition nutrition of fermented coffee beans represented protein (14%), fat (2%), ash (2%), moisture (4%), carbohydrate (59%), and fibre (19%). In sensory analysis, treated coffee beans with yeast shows, colour (4%), sweetness (3%), sourness (2%), bitterness (3%), viscosity (3%). Treated coffee beans with yeast also shows higher overall acceptances in terms of delicious (35%) than treated coffee beans with LAB (30%), however both of samples represented as 5% in term of good aroma. At the end of this study, all data was obtained and produced the coffee drink with better aroma, taste and flavour.

Keywords: green coffee beans, lactic acid bacteria, yeast, mucilage layer, aroma, and taste

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Kesan fizikokimia dan analisis deria biji kopi yang ditapai oleh Bakteria Asid Laktik dan yis

ABSTRAK

Penduduk global meningkatkan pengeluaran kopi sehingga kopi menjadi salah satu minuman yang popular kerana aroma, rasa dan rasanya. Walau bagaimanapun, pemprosesan kopi masih mempunyai kelemahan, mencabar untuk dikawal, dikaitkan dengan masalah kesihatan, dan perbezaan dalam perspektif manusia terhadap produk akhir. Oleh itu, penting untuk menyiasat kesan biji melalui penapaian, kesan fizikokimia dan analisis deria kedua-dua biji kopi yang ditapai yang dirawat dan tidak dirawat. Biji kopi hijau telah disuntik dengan LAB dan yis dan disimpan dalam bekas tertutup selama 72 jam untuk penapaian. Dalam analisis proksimat, protein, lemak, serat, abu, mineral, dan karbohidrat dianalisis dengan biji kopi yang tidak dirawat dan ditapai dengan yis. Analisis deria dijalankan di kalangan 37 panel yang tidak terlatih melalui skala hedonik. Semua analisis telah dikira oleh Ahli Kimia Analitik Rasmi (AOAC), Ujian-T dua-sampel dalam Perisian MiniTab 17 dan Microsoft Excel. Komposisi nutrisi biji kopi yang ditapai mewakili protein (14%), lemak (2%), abu (2%), kelembapan (4%), karbohidrat (59%), dan serat (19%). Dalam analisis deria, biji kopi dirawat dengan yis menunjukkan, warna (4%), kemanisan (3%), masam (2%), kepahitan (3%), kelikatan (3%). Biji kopi yang dirawat dengan yis juga menunjukkan penerimaan keseluruhan yang lebih tinggi dari segi kelazatan (35%) daripada biji kopi yang dirawat dengan LAB (30%), namun kedua-dua sampel mewakili 5% dari segi aroma yang baik. Pada akhir kajian ini, semua data diperoleh dan menghasilkan minuman kopi dengan aroma, rasa dan rasa yang lebih baik.

Kata kunci: biji kopi hijau, bakteria asid laktik, yis, lapisan mucilage, aroma, dan rasa

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LIST OF ABBREVATIONS

		Pages
LAB	Lactic Acid Bacteria	1
MYR	Malaysian Ringgit	2
ICO	International Coffee Organization	9
TCS	Traditional Coffee Shops	10
GDP	Gross Domestic Product	10
MOA	Malaysia's Ministry of Agricultural and Agro-based Industries	11
DWB	Dry Weight Bases	13
SSF	Solid-State Fermentation	21
CH	Coffee Husk	21
CP	Coffee Pulp	21
SmF	Submerged Fermentation	21
EMP	Embden-Meyerhoff-Parnas	28
EPS	Exopolysaccharide Productions	29
H_2SO_4	Sulphuric acid	33
HCl	Hydrochloric acid	33
K_2SO4	Copper Sulphate	33
NaOH	Sodium Hydroxide	33

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H_3BO3	Boric acid	33
$C_{15}H_{15}N_3O_2$	Methyl red	33
$C_{21}H_{14}Br_4O_5S$	Bromocresol green	33
AOAC	Association of Official Analytical Chemists	35
ANOVA	Analysis of variance	45
CQP	Coffee Quality-Improvement Programme	48

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LIST OF SYMBOLS

		Pages
%	Percentage	8
kg	Kilogram	11
n	Variable	12
×	Multiply	12
=	Equal	12
µm	Micrometre	19
℃	Degree Celsius	23
w/w	Weight for weight	33
g	Gram	33
mm	millimetre	33
ml	Millilitre	36
mg	Milligram	36
±	Plus-Minus	43
Pa·s	Pascal	52

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CHAPTER 1

INTRODUCTION

1.0 Research Background

The global population increase the coffee production to fulfil the customer's demand. A conducted study of the perspective of global demand and found the production and supply chain of coffee increased in line with the increases the producers and exporters. It dominated by Brazil, Vietnam, Colombia, and Indonesia (Torga & Spers, 2019). The increase in the production of coffee farmers and sellers in the period 2001-2016 occurred in parallel with the global production of green coffee beans was not affected until the brand of beverages of coffee spread worldwide such as Starbucks Coffee. The rate of income and population growth rate especially in Asian countries shows an increase in the coffee demand. However, the data shows among the largest contributors of coffee enthusiasts was people between the ages of 25 to 60 in 2025 while in the range of 1990 to 2022 the percentage of people over the age of 14 increases and causes the younger person to decrease (Torga & Spers, 2019). The expected consumption

of age is important because it affects the rate of coffee consumption over the year. The population and demand of coffee force an increases of coffee quality.

Coffee became one of the popular beverages among all ages due to its aroma, taste, and flavour. It makes coffee as the second-largest producer after petroleum, with a market value of around MYR62 billion (Lanfranchi et al., 2016). The study on coffee was crucial to do by acknowledge the coffee's history, industry, types, processing, and anatomy in order to deliver a high-quality and consumer-accepted in final product. The major goal of this research was to enhance the process of removing the mucilage layer from green coffee beans which contains high amount of pectin and help in reduction the water content of the beans. This study was carried out by inoculating LAB and yeast. LAB known as probiotic food which have good impacts on consumers health and maintaining the balance of intestinal microbiota (Vasiee et al., 2018). Yeast also improved the coffee processing in fermentation. Thus, results from this study help in developing a process for producing better aroma, flavour, and taste of coffee.

1.2 Problem Statement

Despite the fact that a variety of technology available today, the coffee processing still has flaws. The way coffee is processed on the market differs, particularly in terms of the usage of starter cultures in coffee fermentation that results in a variety characteristic that includes the proteolysis and lipolysis, flavours, synthesis of volatiles such as the

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acidity or sourness in the product (Palmori & Franca, 2016). Based on Palmori and Franca (2016), the usage of starter cultures in coffee fermentation and the exploitation of industrial enzyme became one of the new novel technologies in coffee industry. It is crucial in terms of speeding up the flavour development process in coffee beans. In coffee fermentation, bacteria and yeast produce various microbial populations, and antagonistic microorganisms such as *S. cerevisiae* which control the development of mycotoxigenic fungi. Thus, outstanding coffee production methods and ways of fermenting coffee enhanced the income of coffee growers with maintaining a high standard of quality and added values to the coffee experience sector.

Coffee beans fermentation was challenges to control because it effected the quality level of final products. It can cause the development of microorganisms to create off-flavours and undesirable characteristic of fermented coffee beans due to over- and under-fermentation (Crossley & Baines, 2014). Over-fermentation happen by excessive loosen of mucilage and delayed the time during processing after the coffee cherry harvested from the farm. Under-fermentation happens when the mucilage in coffee beans is not adequately extracted and resulted of abnormally moisture content. The fermentation time can be short by pH measurement to the mucilage layer that ready to be washed from parchment coffee. Thus, the final coffee quality and performance enhanced throughout the proper fermentation process.

The consumption of coffee was associated with health problems, especially cancers (Pourshahidi et al., 2016). The association between coffee intake and blood cholesterol increases the risk of hyper-cholesterol shows a lot of reported evidence. Metabolic health effects have also been reported where coffee consumption caused the diabetes because the glucose tolerance directly and causes the diversion of sugar absorption to the intestinal tract. However, the biochemical studies conducted by

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researchers, the energy, DNA repair with gene regulation, and chronic inflammatory responses can be overcome by the coffee community (Zhao et al., 2020). Thus, the usage of LAB and yeast in the fermentation of coffee beans can improve the nutritional composition of coffee.

The differences in human perspectives on the sense of taste of food makes sensory analysis an important method to obtain the acceptances of the final product. These variations were attributable to a person's level of experience, genetic composition, and mood on that particular day (Auell, 2016). The genetic includes the amount of taste bud distribution on the tongue which gives the more taste receptor sites when taste the strongly bitterness of coffee that categorized as a taster, a non-taster and supertaster. The past memory and experience give another reaction to the new flavours and cause human struggle to describe the flavour attributes especially by language. It importantly to use hedonic scale that facilitates the human to describe the coffee flavour, aroma, and taste. Thus, the diversity of human taste throughout centuries requires improvements in coffee production for greater flavour, aroma, and overall acceptability of consumer towards the new formulation of final product.

1.3 Scope of Study

This study was focuses on coffee processing that used LAB and yeast as the starter culture to fermentation process. The pH of the samples was measured daily as observation

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of the changes in the coffee fermentation. The proximate analysis was applied to analyse the nutritional composition that includes the moisture, protein, fat, ash, fibre, and carbohydrate of fermented coffee. The sensory analysis was conducted using hedonic scale. Thus, this study improved the quality, aroma, and taste for gaining high acceptability towards the fermented coffee.

1.4 Significant of Study

In this century, many studies have been conducted recently in the field of fermentation in food production as a part of enhancing conventional methods to modern fermentation. The current research aims to inoculate LAB and yeast as a good and safety source of bacteria for the fermentation of green coffee beans. The inoculation of coffee beans with LAB and yeast may increase the fermentation rates of coffee beans by removing the mucilage layer of green coffee beans without causing excessive damage. The effects of fermented coffee beans in this research shows by the physicochemical effect with analysing the nutritional composition of both treated and untreated coffee beans. It includes the analysis of protein, fat, ash, moisture, carbohydrate, and fibre. The sensory analysis was conducted by gaining the consumer acceptability with performed hedonic scale. This study also gives the industry, retailers, or even farmers as guideline to provides proper system to produce high quality of final product. The industry scale also needs the excellent method of analyse nutritional composition for food products as well

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as help them to increase the supply and demand of their products. Thus, this study provides the innovation to the production of coffee beverages as used LAB and yeast to increase quality, aroma, and taste of the final coffee products.

1.5 Objectives

1. To investigate the effects fermentation using LAB and yeast on green coffee beans.
2. To analyse the nutritional composition and physicochemical effects on the treated coffee beans.
3. To conduct sensory analysis of both treated and non-treated fermented coffee beans.

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CHAPTER 2

LITERATURE REVIEW

2.1 History of Coffee

Initially, the name of coffee is '*kahwah*' in Arabic but the name changed because it considered as a symbolic word for wine and became the sensitivity of Muslim (Bizzo et al., 2015). The name changed to *café* (French), *caffé* (Italian), *Kaffe* (German), and coffee (English). The famous coffee's story begins by a goat with its master, Kaldi. The goat became ecstatic and energize after chewing some of cherry from the forest and it identified by Kaldi (Morris, 2018). The discovery also made a monk who investigated the tale, he roasted the cherries and attempted to drink it. He is analysed his body became fresher and peacefully to pray at night. The coffee's development began in Ethiopia and spread across the world includes Europe, Italy, and French.

The coffee's journey in Europe started with the bringing of kaffa by Venetian traders from the Turkish region to Rome and the centre of Christendom (Trobits, 2019). The coffee considered an infidel drink and as the discovered of the devil by Islam after in Europe. Pope Clement VIII (1536 to 1605) was fascinated by the aroma and taste of the coffee and made it as a Christian drink. Coffee became popular until it is appearance throughout the Italian and French peninsulas around 1644. Subsequent spread followed in Paris and coffee became their daily balance. The importation of coffee in the Netherlands began in 1616 with the production of fine koffie with French drip method.

In 1714, King Loius of France gifted a coffee factory and subsequently the improvement of a café in Paris in the 18th century. The spread of coffee happens includes in Mexico, South America, and Central America because of the suitability of the coffee plants in such places. The franchise became the largest producer and consumer of the brewed bean in 1700 in Europe. The first milk coffee named cappuccino invented by Kolschitzky who died in 1694. The coffee reached Germany in 1670 and England in the early 17th century. In the 18th century, coffee cultivation soared in Brazil and controlled 80% of the world market in 1920 followed by Mexico, the largest producer in the United States at the end of the 18th century. The Dutch known as the top consumer of coffee worldwide in 2016. Now, coffee is consumed worldwide and gained the popularity in all places.

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2.2 Coffee Industry

Based on the portion of coffee in the world's population, 25 million coffees are produced by small scale farmers in subtropical and equatorial regions with a total of over 2.25 billion coffee cups per day (Samper & Quiñones-Ruiz, 2017). In the beginning of 21st century, the coffee crisis occurred resulting in declining green coffee beans prices and food insecurity. Farmers got less assistance by local and national companies caused the rise in demand where consumers have to pay more for commercial coffees. The concept of market standards was created to practice new thinking on the process of transnational governance because it provides sustainable coffee market competitiveness both in and abroad as well as an understanding of sustainable trading and production systems (Reinecke et al., 2012).

The coffee industry has high competition among coffee traders at the global market because its raw materials are needed in international agriculture as import and export activities. The upward trend occurred with remarkable fluctuations about 65% of the world coffee production produced 87 to 143 million bags in 1995 to 2015 (Torok et al., 2018). Three largest coffee producers in the world are Brazil, Colombia, and Mexico with the production accounts for 60% of the total top 10 exporters in 2015. In 2015, Vietnam became the second largest coffee producer with producing 27.5 million coffee and more than 10 million bags per year recently. The emergence of Vietnam made other producer includes Mexico, Costa Rica, and Côte d'Ivoire to lost the popularity. In 2013 to 2015, 88% of total world coffee production was monopolized by Brazil, Vietnam, and Colombia. This competition allows exporters have more advantages especially in the

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coffee's price according to the price statistic from the first opened in 1894 by the International Coffee Organization (ICO).

The international scenario of coffee industry also effected by the climate change. Coffee cultivation became more widespread as a result of the climate suitability in a place. Nonetheless, climate change has had an impact on agricultural production all over the world (Bianco, 2020). According to survey by Lanfranchi et al. (2016), the quality of coffee is more importance to customers than the price or advertisement. This demonstrates the quality of coffee needs to improve by mitigating climate change because it alters the coffee's composition. The strategy was developed using a process-based model with connected to the soil in order to obtain soil and climate change data (Rahn et al., 2018). Thus, its controlled and improved coffee's quality.

2.3 Coffee Industry in Malaysia

The coffee industry in Asia started with the opening of small shops run by sole proprietors or families. Coffee shops in Malaysia are known as Traditional Coffee Shops (TCS) and *kopitiam* as a combination of "coffee" and "tiam" (Chinese word), and it appeared before World War II (Chongz, 2017). This culture emerged since the 1900s as a gathering place during the British occupation and history was created with the establishment of Malaysia and the Singapore Coffee Shop Owners Association in 1947. It is closely related to Hainanese Chinese immigrants. They opened a coffee shop and

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improved their skills after the arrival of Europe because it improved the standard of living by offering a unique taste with the cheaper price of coffee drink as breakfast.

Now, Malaysian's coffee industry still growth with the presence of various stores and franchises such as Starbucks, Tea leaf, and Earthlings because it has a positive effect on the increasing of the gross domestic product (GDP) per capital and total consumption (International Coffee Council, 2014). The coffee industry boosted the food and beverage industry, especially with the establishment of Starbucks in 1998 until now the brand can be found in all Malaysian shopping malls, city centres, and airports. The rapid competition continued between foreign and locals and affected the coffee consumption in this country. The growth potential attracts many locals and internationals to expand coffee shops in Malaysia because this country listed as one of the 50 countries used the most coffee, estimated at 1.3 kg of coffee consumed per capita (Rahim et al., 2019). Although the coffee production in Malaysia in 2017 resulted as less than 20% of total harvested area for food industrial crops under the preview of Malaysia's Ministry of Agricultural and Agro-based Industries (MOA)[20] and less than 1.0% of total production (Aiysyah & Ashraf, 2019), however, Malaysia became the fourth largest exporter of coffee extract that mainly to China, Thailand, and Indonesia. Thus, the coffee production helps the small coffee player to open coffee shop, grow their businesses, and became the popular one such as Kue Street Café, Kaffeinted Fish, 100 houz coffee & breakfast, Pause Café Kuala Lumpur, PastelMonoMy, Xofi, etc. (Nadzirah, 2021).

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2.4 Types of Coffee

According to Gibson and Newsham (2018), coffee has 25 major varieties in genus Coffea but *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) as the main global production for commercial. It followed by *Coffea liberica* (Liberian) with represents only 2% of the world coffee harvest. Three of the coffee genus mention have significant biological differences, however, the coffee cultivars are selected or bred with horticultural breeding techniques to increase production, volume, and characteristics of coffee.

2.4.1 Arabica

Arabica coffee or its scientific name is *Coffee arabica L.* belongs to the genus *Coffea*, in the family of *Rubiaceae* and originated from Ethiopia. Arabica has soft aromatic and low in caffeine, but it is more expensive than Robusta. The cultivation extends to 80 countries especially in Africa, Asia, and Latin America and covers up to 10.2 million hectares with a production of 9.8 million bags of coffee. The genetics of arabica coffee known as resistant to disease because the entire original genetic diversity is confined in the Afromontane rain forest (Alemayehu, 2017).

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A research was conducted by Silvarolla et al. (2004), the caffeine-free property of the wild coffee species of Madagascar to be transferred with another species. However, the research failed due to the genetic of *Coffee arabica L.* has an autogamous and allotetraploid with 95% of pollination is from itself and shows $2n=4\times=44$ (Benti et al., 2021). According to Rimlinger et al. (2020), these genetics are great for future studies because almost all of Madagascar's wild coffee is caffeine-free, but currently most of these species are endangered and disappeared from their natural habitat.

2.4.2 Robusta

Robusta coffee or its scientific name is *Coffee canephora P.* accounts for 37% of total world coffee production (International Coffee Organization, 2018). The coffee's characteristics was stronger because of the high caffeine content and the taste was bitter but it gives satisfaction to the drinker. However, the quality of Robusta is lowered than Arabica.

According to Liu et al. (2019), Robusta coffee has less colour, a muddy and spicy roast aroma, and flatter taste made it cheaper than Arabica coffee. The development of organoleptic quality of coffee is importance with the presence of sucrose but the amount was less in Robusta that shows only 2.7% of dry weight while Arabica contains 6% dwb (Liu et al., 2019). Robusta coffee was blended with arabica coffee beans in a 50/50 ratio for industrial production to reduce costs and improve crema formation. The quality of

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Robusta coffee was improved with made it taste like Arabica and made the consumers realise the principle of "it is good to buy cheaper product when the quality and taste are higher than others".

2.4.3 Liberica

Liberica Coffee known as "Barako" (*Kape Barako*) in the Philippines (Alcona & Incencio, 2019) because its represent the first harvest and sales where citizens became the sole supplier to growth the economy (District Roasters, 2019). Local farmers used their own way to identify the types of coffee between Excelsa and Liberica because both of them has inconsistent morphology markers. It causes by the coffee rust infection in the farm occurred in 1889 with an unknown transplantation of coffee seedlings. Warm tropical regions such as the lowlands of Liberia, Suriname, and Malaysia produce less commercial value cultivated for Liberica coffee (Mubarak et al., 2019). Although this coffee has importance in some parts of the world, information on the composition of chlorogenic acid, caffeine, and antioxidant activity was scarce.

Excelsa or known as *Coffea excelsa* and *Coffea liberica var. dewevrei* stated by Gibson and Newsham (2018) was technically from the Liberica family but has a different species. The cultivation of Excelsa only in Southeast Asia and represents a small fraction of the world coffee production (District Roasters, 2019). The roasted Excelsa coffee was light and dark but it producing a fresher, tastier, and unique taste.

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2.5 The Anatomy of Coffee Cherry

The coffee cherry's anatomy was importance because each part gives different effect to the production of final products (Belchior, 2021). Coffee cherry got from a plant and has different characteristics of seeds such as size, flavour, and layer as shows in the Figure 1. According to the Thiago et al. (2019), the exocarp as the outermost layer or tissue of the coffee fruit also known as the skin or peel. A layer of polygonal parenchyma cells was inside the layer as well as the colour of coffee fruit changed from green to yellow (chlorophyll pigment starts to disappear), and finally red (anthocyanins are collected by exocarp cells).

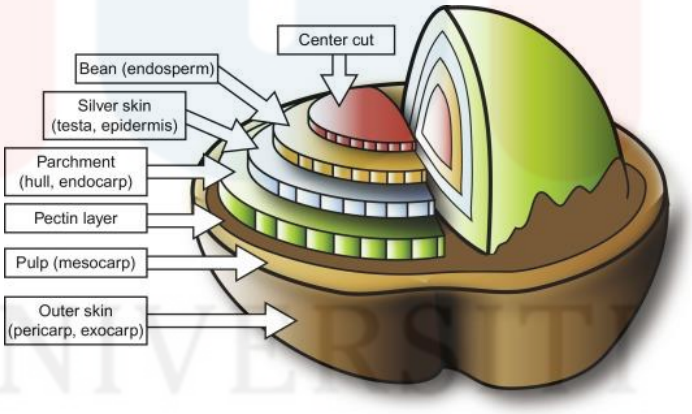

Figure 1. The anatomy of coffee cherry (Gibson & Newsham, 2018)

The second layer known as pulp (an outer mesocarp) or mucilage (inner mesocarp) and contains a layer of mucilage-type texture of pectin. The pulp or mucilage is the thinnest layer under the skin of the cherry. The pectin enzymes in hard mesocarp broken down when the coffee bean ripens by pectinolytic enzymes and produce the insoluble hydrogel with higher in sugar and pectin. This layer is removed during the

pulping or fermentation process to reduces the moisture of green coffee beans. Parchment or endocarp/hull covers the coffee beans with sclerenchyma cells until the final size of the coffee is limited because it hardens during maturation and has coated with 5 to 6 layers of fibre. This layer is maintained in green coffee beans for storage and for commercial. Basically, the pulp, mesocarp, and endocarp known as pericarp (Subba & Raju, 2020).

The silver skin known as testa or epidermis are transported the biochemical compounds from the pericarp to the endosperm. The endosperm has two seed sections about 10 mm in size and 6 mm wide. It contains small oil-rich polygonal cells located on the outside of the endosperm and larger rectangular cells with slightly thinner cell walls inside the endosperm. The silver-skin extracted during the roasting process. Therefore, the fermentation process indicates the anatomical effect on the final product.

2.6 Coffee Processing

Coffee processing has several methods includes harvesting, dry or wet method, hulling, cleaning, grading, polishing, sorting, roasting, grinding and brewing as shows in the Figure 2 (Gibson & Newsham, 2018). Harvesting was the first step after the colour of coffee cheery becomes red or deep yellow. Based on Borém (2019), variety of techniques can be performed that includes the selective manual harvesting (picking the fruit one by one with a basket tied at the waist but it less efficiency because the harvested fruit must be done several times during the growing season and requires a lot of labour), manual

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harvesting by stripping (use canvas or cloth by stripping plant, dropped the fruit, and placed it on the canvas with cleaned ground), and mechanical harvest (uses a machine to shake the plant and the fruit is taken and cleaned using a fan winnowing system, its more suitable on flat land and reduced the time and cost).

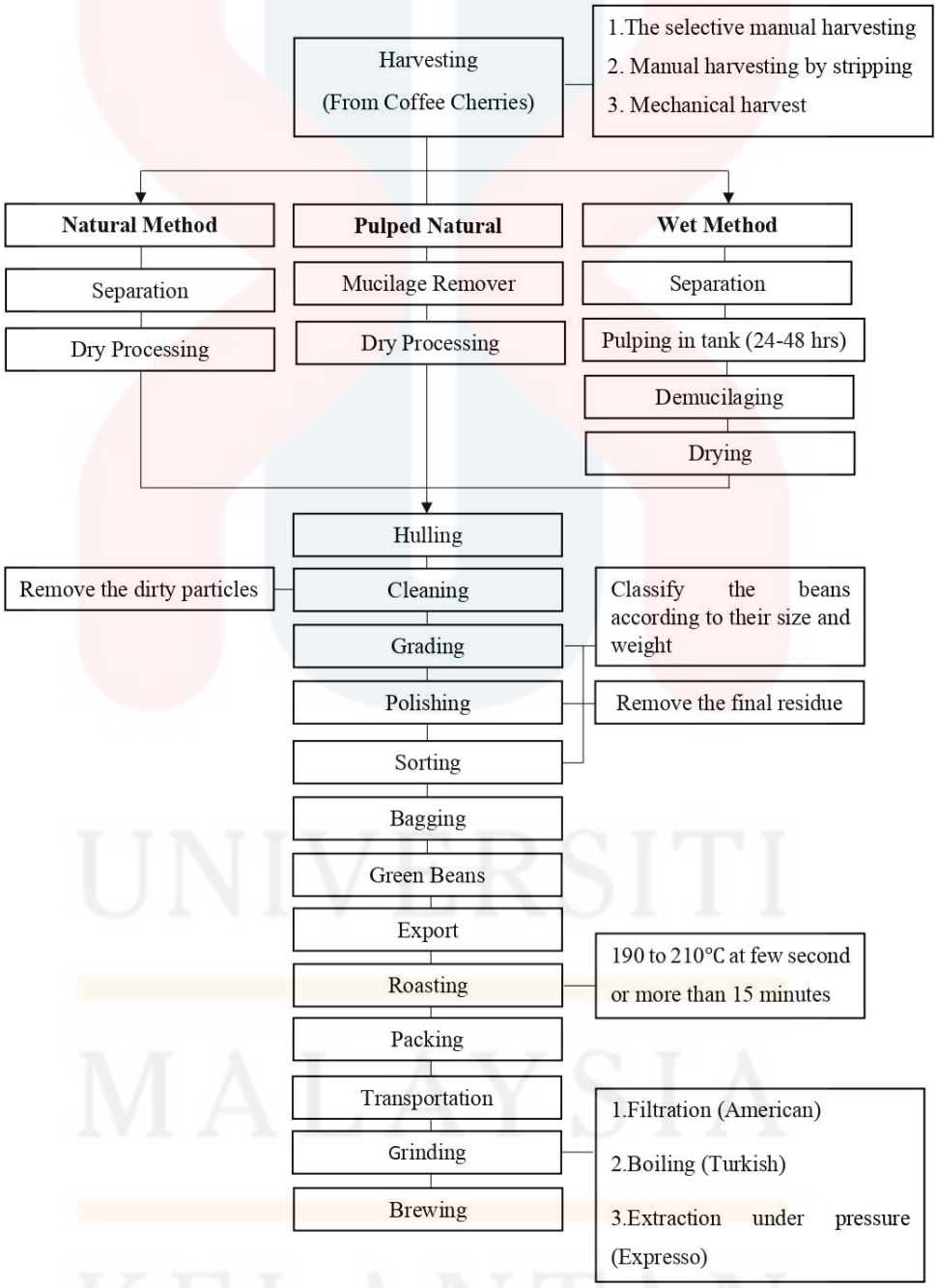

Figure 2. The coffee processing (Sucden, 2022)

The second step divided into three called natural, wet, and pulped natural (Mota et al., 2020). The newly harvested whole fruits are transferred to a drying platform and makes the fruit through fermentation and dried and occurs naturally. In wet process, the fermentation applied for 24 to 48 hours in the tank full of water after removing unwanted pulp, let the enzymes to produce naturally and removed the unwanted layer such as exocarp, mesocarp, and endocarp/parchment in surroundings the beans as demucilager and dehuller (Zhang et al., 2009). Although, the wet process reduces some minerals and sugars, increases of acid and the parchment was still presented at the end of the process, but it is used by most coffee producers to increase profit margins and quality of coffee (Said et al., 2020). The third method named natural pulped. The coffee mucilage was removed mechanically and transferred to a drying platform. The drying occurs until the moisture of the beans reaches 11%.

The drying and milling process are implemented with tumblers by rotated periodically or mechanically to reduce moisture of the green beans. Parchment coffee produced from wet processing with removing of dried husk includes the exocarp, mesocarp, and endocarp while the green beans with skin and pulp is separated with parchment by one step in dry processing (Benitez et al., 2019). It followed by a polishing process to remove the final residue of beans, grading, and sorting according to the size and weight of beans (Gibson & Newsham, 2018). Since the high humidity rate resulted in a longer drying period, the drying process must be performed properly (Valdiney et al., 2016).

Temperatures ranged for roasting process from 190 to 210°C, about few seconds or more than 15 minutes, depended on the suitability of the coffee production as softening process to increase the taste and aroma of the beans. The chemical composition is changes drastically in this phase with releasing a large of carbon dioxide. High-temperature

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roasting allowed the caramelization and condensation products and resulted in loss of dry matter such as carbon dioxide gas, water, and volatiles products of pyrolysis (Bizzo et al., 2015). The roasted beans can be declined about 66% because of the roasted process (Robert et al., 2020). Thus, the proper roasted produces sparkled and enhanced the aroma and brown pigments of coffee beans. The beans are cooled with water or cold air immediately upon reaching the desired roasting level to prevent further breakdown or molecular interaction as a final rapid cooling phase in roasting.

The coffee drink was performed in various ways includes the filtration (American), boiling (Turkish), and extraction under pressure (Espresso) where it influenced the physicochemical properties of coffee and resulted higher antioxidant activity and phenol content per cup in American coffee compared to Espresso and Turkey coffees (Benvenuto et al., 2020). Efficient process and higher quality coffee was obtained by using an automatic machine works optimally and about a few micrometres to ~1,000 μm particle sizes producing during grinding (Benvenuto et al., 2020). It affects the aroma by producing volatiles and chemicals that are easily soluble in water. Finally, brewed process increased the sensory quality and amounts of healthy compounds in a cup.

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2.7 Fermentation

Fermentation has been applied around the world for a long time ago and improved upon over a period for the various of production includes the pharmaceutical, chemical, and food industries (Singh et al., 2017). Fermentation happens either aerobic or anaerobic condition where aerobic is the state of oxygen availability but it may cause the growth of microorganisms while anaerobic is a state in absence oxygen and processing in the closed tank (Suzzanne & Mandeep, 2021). Singh et al. (2017) said, the suitability of fermentation conditions and the components of the medium such as pH, temperature, and nitrogen can maximized the parameters in fermentation.

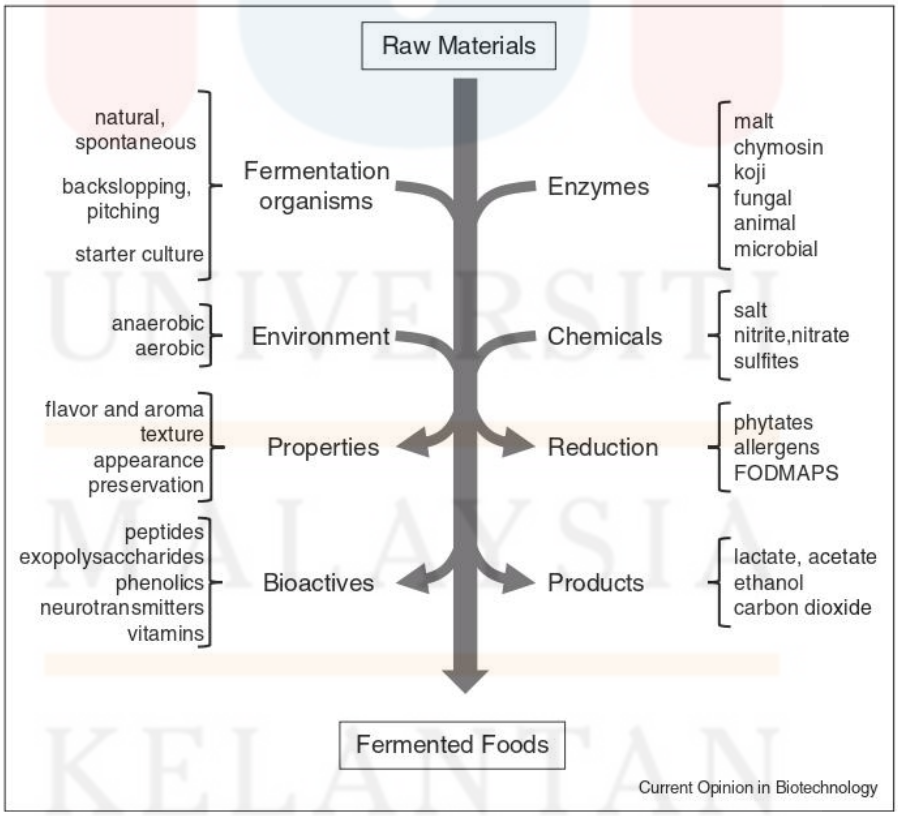

Figure 3. The elements in fermentation to produce healthy foods (Marco et al., 2017)

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2.7.1 Solid-State Fermentation (SSF)

According to Lizardi-Jiménez and Hernández-Martínez (2017), SSF occurs when there is no or almost absence of free water in the solid matrix. SSF take place in the metabolites includes the enzymes, antibiotics, organic acids, flavours, and aromas as supporter in the growth and metabolic activity of microorganisms (Catalán et al., 2019). The presence of abundant substrates such as glycerol, glucose, or other carbon sources is associated with SSF. The resistance of microorganisms to bacterial or fungal cells occurs with catabolic suppression or inhibition of enzymes. SSF help in reduction of energy used for sterilization due to less water consumption, bacterial contamination, and substrate inhibition rate (Soccol et al., 2017). Others, SSF has higher extract quality and activity, no usage of organic solvents, and reduce capital and costs during the processing.

In the industry, SSF is needed for reducing by-products from coffee fruit known as coffee husk (CH) and pulp (CP) because it accounts about 50% of the total coffee fruit production (Silveira et al., 2019). It obtained during the wet processing of coffee cherries and has a high of the sugars, proteins, minerals, and amino-acids. Based on the International Coffee Organizations (2017), the green coffee beans production worldwide in 2017 reached 9.5 million and contributed a lot of CH and CP. This indicates the importance of SSF as an alternative of submerged fermentation (SmF) because it provides a lot of benefits than SmF as shows in the Table 1 (Arora et al., 2018).

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Table 1. The comparison parameters between SSF and SmF (Arora et al., 2018)

Parameters	Solid State Fermentation (SSF)	Submerged Fermentation (SmF)
Absence of free water	Lower reactor volume required. Negligible chances of contamination. No foam formation. Lower cost of treatment of liquid effluents.	Relatively large reactor volume is required. High water activities make the process highly susceptible to bacterial contamination. Extensive foam formation and high cost for treatment of liquid effluents.
Fermentation medium	Low cost and natural.	Highly purified analytical grade chemicals are used which usually cost multiple times higher than SSF media.
Natural Environment	Solid nature of the substrate mimics the natural environment of fungi.	The dissolved nature of substrate does not provide the natural habitat for fungi.
Downstream processing	Simpler and easier since the product is highly concentrated.	Product concentration and purification costs are higher. Generally, defines the process economics.
Product quality	Heat and pH resistant products reported in few cases.	Compared to SSF, superior product quality has not been observed with SmF.

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2.7.2 Fermentation of Coffee Beans

Fermentation occurs in two condition known as wet and dry processing based on the major processing technologies in the international markets (Junqueira et al., 2019). The wet processing caused the development of microorganisms interact in fermentative metabolism while dry processing used the simple method of sun-drying to reduce moisture of coffee beans. Monsoon coffee as also a natural fermentation produced from the dry process of green coffee beans with absorption of moisture during the monsoon season (Vivien et al., 2021). Various chemical changes occurs in the beans during the fermentation and facilitate the roasting process (Pereira et al., 2016).

Coffee beans fermented in a fermentation container or sweet box produces an initial fermentation by the yeast until the production of ethanol from the sugar found in the pulp due to the increase in temperature. It contains mucilage and removed during fermentation by pectic enzymes. A decrease in pH and an increase in temperature caused the anaerobic conditions with dominated by LAB. The process of hydrolysis by dissolving the pulp seeds with a flow of water help the bean mass to penetrate by water. This process caused the ethanol to be oxidized to acetic acid by LAB. As a result, an increase in temperature up to 45-60℃ causing the yeast population to disintegrate. Beans were dried at 7% humidity after heat and acidity were combined up to 2% w/w. This is because the result of the product after baking was maintained especially for a special aroma.

The factors in fermentation such as time, temperature, and exchange of water can be initiated by wet processing to development the microorganisms spontaneously and improve coffee's quality. Fermentation by wet processing for the extracting mucilage from coffee parchment or mucilage includes polysaccharides, cellulose, and starch in order to minimise drying time, prevent mould, and preserve the final consistency of final coffee (Haile & Kang, 2019). Wet, dry, and semi-dry processing produces the green coffee beans with the production of enzymes, acids, and alcohols to improve the taste and aroma of coffee. Fermentation of coffee beans changed the sugar level and measured by pH.

Before this, one research conducted to observe the functionality and consumer acceptance of yeast fermented coffee beans. Han Sub, Yoonhwa and Missok (2018) found the use of yeast in fermented coffee has a good effect to improve the function and antioxidant activity of coffee. The measurement with oxygen radical absorbance capacity and superoxide dismutase testes resulted the total plate count in fermented coffee was higher than controlled. As a result of this study, acceptability of consumers was lower on fermented coffee than control because the green coffee beans roasting yeast did not produce a negative aroma or taste but can be enhanced with antioxidant activity. This research shows the fermentation was importantly to increase the aroma and flavour of the final product.

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2.7.3 The Flavour and Aroma Develop by Fermentated of Coffee Beans

The formation of pleasing taste resulted in coffee drink was due to the accumulation and involvement of compounds of sucrose, chlorogenic acid, caffeine, and trigonelline in mature coffee beans (Acidri et al., 2020). The caffeine produced the bitter taste as flavour determination of coffee (Wenjiang et al., 2017). The hydrozyl group from amino acids and carbonyl group produced the odorants during caramelisation in Strecker and Maillard reaction. The group performed by the converted the sucrose to fructose and glucose. However, the aroma of coffee was formed from the thermal breakdown of trigonelline into pyrroles and pyridine derivatives.

The reaction of sugars and amino acids during caramelisation and Maillard reaction by non-enzymatic browning reaction also forms melanoidin (Dybkowska et al., 2017). Melanoidins in coffee have levels associates with roasted, high temperatures produced high levels as well as lower molecular weight in coffee. The antioxidant properties found in melanoidins produced anti-mutagenic and bioavailability. Parameters on flavour effectiveness and antioxidant potential are influenced by the ratio of chlorogenic acid and caffeine. It gives the flavour to coffee. Thus, oxidants can be reduced by the Strecker and Maillard reactions as it formed the new compounds.

According to Wenjiang et al. (2017), the aroma and flavours are contributed by fats, proteins, and free amino acids to improve the quality of the coffee. The sensory quality was important for evaluating the final product such as the production of acidity and sourness of coffee brews.

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Table 2. The main acids and volatile compounds that associated with flavour identified in green or roasted coffee (Ruta & Ileana, 2021)

Yeast	**Type of Coffee**	**Main Acids Detected**	**Main Compounds Associated with Flavour Identified in Coffee Beans**	**General Aroma**	***Ref.***
S. cerevisiae UFLA YCN7 *S. cerevisiae* UFLA YCN724	*C. arabica* var. Acai	malic, lactic, acetic, butyric, propionic, citric, oxalic, succinic,	1-pentanol, 2-phenylethanol, acetaldehyde, hexanal, ethyl acetate, furfuryl alcohol, furfural,	caramel flavor in the beginning and a bitter aroma at the end	[12]
S. cerevisiae UFLACN 727	*C. arabica, var*. Acaiá	malic, isobutyrichexanoic, decanoic, nonanoic	ethanol, 2-phenylethanol, acetaldehyde,	sweet	[20]
S. cerevisiae CCMA 0543 *C. parapsilosis* CCMA 0544	*C. arabica* var. Catuaí Amarelo	citric, malic, succinic, lactic, acetic, propionic, isobutyric, chlorogenic	2,3-butanediol, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, glycerol and ethanol, hexanal,	fruit like, floral, sweet, caramel, nutty	[27]
S. cerevisiae CCMA 0200 *T. delbrueckii* CCMA 0684	*C. arabica* var. Catai Mundo Novo	malic, lactic, acetic, butyric, propionic, citric, oxalic, succinic, tartaric	2-furanmethanol propanoate, 2-ethyl-3,5-dimethylpyrazine	caramel, fruity, refreshing, walnut, cane molasses	[35]

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2.8 Lactic Acid Bacteria

Kwak et al. (2018) found the antioxidant activity can be enhanced by the use of yeast fermentation because it effects the aroma and flavour of food product. Thus, the purpose of using LAB from yogurt as a starter culture by inoculating the bacterial to ferment coffee beans for better aroma and flavour. Starter cultures was used exclusively during coffee fermentation (Tang et al., 2021). The examples of aromatic compounds released by LAB includes the benzyl alcohol, phenethyl alcohol, and ethyl hexanoate (Pereira et al., 2020).

According to Thomas (2018), inoculated food using samples from previous products is a traditional method to produce fermented food. Although it is known as a traditional method, but the quality of food produced for home-made products was high. For large-scale production, starter culture was used to control the fermentation process and divided into pure and mixed-strain cultures (contain unknown numbers of strains from the same or different species of LAB genera).

However, the usage of LAB in fermented coffee was limited because various studies used yeast to enhance the flavour. According to Haile and Kang (2019), starter culture was successfully introduced by Agate and Bhat by mixing the three species of *Saccharomyces* species namely *S. marxianus, S. bayanus,* and *S. cerevisiae var. ellipsoideus* allows the quickly degradation of the mucilage layer during the fermentation process. Studies on the microbes and physicochemical characteristics of coffee fermentation were performed with the isolated pectinolytic microbes from spontaneous fermentation. As a result, a decrease in polysaccharide compounds or modification of the

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organoleptic characteristics of the drink does not occur rapidly by microbes. LAB was recommended to get a more natural in fermentation process.

2.8.1 Metabolism of LAB in Coffee Beans Fermentation

In the formation of flavour in fermented food products, glycolysis (fermented of sugars), lipolysis (degradation of fat) and proteolysis (degradation of proteins) were involved in the pathways (Thomas, 2018). The sugar content such as pentoses, hexoses and polysaccharides in coffee pulp as the primary carbon and energy sources for the growth of LAB (Pereira et al., 2020). First, the metabolism of LAB occurs according to fermentative species includes the homofermentative LAB (*Lcc. Lactis, P. pentosacesus, E. faecalis* and *Lcb. Hirdei*) converts lactate dehydrogenase to lactic acid of ferment sugars. The conversation used the Embden-Meyerhoff-Parnas (EMP) pathways to pyruvate and to mixed-acid metabolism under coffee-related stress conditions. Second, pentose and hexoses found in coffee pulp catabolized with heterofermentative LAB such as *Leu. Mesenteroides, Leu. Citreum,* and *Lcb. Brevis* into a vast range of end-metabolites. It includes the lactate, acetate, carbon dioxide, and ethanol by the pentose phosphate pathways. Third, the different hydrolytic enzymes and the acidification process are produced from the breakdown of carbohydrate complexes from LAB. The different hydrolytic enzyme includes as pectin lyase, pectin methylesterase, and exo-

polygalacturonase. The growth of LAB used an additional carbon source from the hydrolysis of pectin and releases medium sugars.

The LAB presented during fermentation and caused the decomposition of complex carbohydrates into the production of various hydrolytic enzymes and the acidification process (Thomas, 2018). This process influences the sensory properties of coffee as well as the development of taste and flavour. Lactic acid decreasing the pH and increase the metabolism in sugars with other elements during the removal of the mucilage layer of coffee beans. LAB classified as Gram-positive bacteria, low Guanine + Cytosine (G + C), and interact with acid, non-motile, non-spore, and rod- or cocci-shaped (Thomas, 2018).

2.8.2 Types of Lactic Acid Bacteria

S. thermophilus as stater culture used frequency in fermented dairy products as the most valuable homo-fermentative LAB to improve the texture and flavour properties (Yanhua et al., 2016). Various probiotic effects of *S. thermophilus* include antioxidant activity, modulation of the intestinal microbiota and pathogen-specific inhibition. It affects fermented dairy products directly or indirectly due to its nature of acidification, proteolytic activity, rapid growth, exopolysaccharide production (EPS), bacteriocins, and flavour substances, antiphage, and host defence ability. The time and quality of fermentation of dairy products is determined by the acidification rate of *S. thermophilus*.

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High EPS values help to attract the properties of *S. thermophilus* strains and become a factor in the acceptance of dairy products. *S. salivarius* and *S. vestibularis* are under the group of *salivarius* along with *S. thermophilic* which produced Gram-positive (Uriot et al., 2017). Thermophilic bacteria exhibited ovoid cells occurring in short chains require an optimum growth temperature of 42℃ under anaerobic conditions. According to the Lorenzo (2017), *S. thermophilic* is recognized in food manufacturing because it does not give any disease after its intake in long time ago.

Lactobacillus spp. is the largest genus in group and belongs to the LAB group where it has three subspecies namely *delbrueckii*, *lactis*, and *bulgaricus* (Thomas, 2018). Based on Marhamatizadeh and Sayyadi (2019), *lactobacillus* has highest probiotic value and responsible for maintaining and promoting the microbial balance in the human gut. It also helps in the process of digestion and absorption to function better. EPS is produced to carry out natural encapsulation into bacteria where conditions such as low pH conditions from adverse environmental can be protected (Mena & Aryana, 2020).

Based on Javid et al. (2019), the number of *Streptococcus mutants* in saliva reduced with the consumption of probiotic yogurt that contains *Bifidobacterium lactic* BB-12 because it known as one of probiotic bacteria that commonly used in food products and functionally for maintaining the equilibrium of the normal gut flora. This statement was agreed by Marvin (2021), *Bifidobacterium lactis* or *B. lactics* was one of the probiotics to improve health especially for gut and oral health. Besides, *B. lactics* has been shown to reduce infection susceptibility, allergies, and lactose intolerance, as well as lower blood pressure and serum cholesterol levels. Thus, *B. lactics* gives more benefits to human health.

2.9 Baker yeast

Yeast plays an important part in the fermentation process since they have biocontrol of filamentous fungal growth (Marcelo et al., 2020). Besides, yeast helps in production of various enzymes and volatile aromatic compounds in food product. According to Kwak et al. (2018), after coffee has been roasted, bound phenolic compounds was liberated due to yeast fermentation of green coffee beans. Thus, proper inoculation of yeast as a starter culture improves the aroma and flavour of fermented coffee, reduces processing and drying time, and increases the product's economic worth (Marcelo et al., 2020). As a result, fermented coffee with yeast became more appealing on the market.

In fermentation, *Saccharomyces cerevisiae* in genus *Saccharomyces* mostly used for producing fermentation food products especially in beer, wine, and bread (Stewart, 2014). *Saccharomyces* is a single-celled fungus that reproduces vegetatively through multilateral budding and sexually through ascospores. The characteristic of vegetative cells in *saccharomyces* includes as globose, cylindrical, and has smooth and flat surface and light cream coloured. *Saccharomyces* also known as mostly used in fermented beverage production which also been isolated from unroasted coffee. The usage of yeast increased coffee's aroma. It combined approximately 800 volatile chemical compounds and the main chemical classes of chemicals during and after fermentation with yeasts (Ruta & Ileana, 2021).

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2.9.1 Metabolism of yeast in Coffee Beans Fermentation

According to Ruta and Ileana (2021), metabolism of yeast triggers the hydrolysis of macromolecules that helps in reduction of sugars, amino acids, and chlorogenic acids as well as to precursors the coffee's aroma. Furthermore, *Saccharomyces marxianus*, *S. bayanus*, *S. cerevisiae var. ellipsoideus*, and *Schizosaccharomyces spp*. contains high pectinolytic activity because this component play in a significant influence in the degradation of Robusta coffee's mucilage layer. The fermentation with yeast also produced special characteristic to the coffee such as sweet, caramel and nut characteristic that happen by the form of metabolites during biosynthesis during *S. cerevisiae* fermentations. Others, special characteristic include the Maillard reaction, which produces a unique chemical called Cicloteno as a result of fructose oxidation and/or degradation and was responsible for the maple or caramel-like odour. It also includes acids like citric, malic, succinic, chlorogenic, and quinic acid, volatile compounds such as alcohols and aldehydes, and esters, ketones, lactones, etc. All of the components were mixed together during fermentation, resulting in changes in the quality and chemical content of coffee-based beverages (Ruta & Ileana, 2021).

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CHAPTER 3

METHODOLOGY

3.1 Materials

Material used in this study were 600 g of coffee beans, 1 tablespoon of Nestle natural yogurt, 22 g of mauri-pan® bakery yeast, distilled water, 12 tablets of Kjeltabs Cu-3.5, 37 ml sulphuric acid (H_2SO_4), 8.35 ml of 1M hydrochloric acid (HCl), 7.6 g of 4% boric acid (H_3BO_3), 1.33 ml of methyl red ($C_{15}H_{15}N_3O_2$), 1.9 ml of bromocresol green ($C_{21}H_{14}Br_4O_5S$), 80 ml of petroleum ether, and 25 g (NaOH).

Apparatus used in this study were spatula, analytical balance, beaker, Philips Grinder, pestle and mortar, aluminium foils, 10 ml measuring cylinder, pipet, bowl, zipper bag, pencil, airtight jar, jug, 1 mm opening sieve, 25 oz paper cup, gloves, kjedahl tube, 50 ml measuring cylinder, 100 ml measuring cylinder, 250 ml beaker, 500 ml beaker, 250 ml conical flask, hot plate, magnetic stirrer, de-fatted cottons, 90 mm filter paper, 1000

ml measuring cylinder, 25 ml measuring cylinder, 50 ml porcelain crucibles with lid, heat resistant glove.

Equipment used in this study were,oven, Laquatwin pH Meter. Water cooler, Gerhardt, Soxtec, Fibertec™ 8000, drying oven, Desiccator, Moisture Analyser, and Muffle furnace.

3.2 Beans Preparation

2 g of natural yogurt containing *Streptococcus thermophilus, Bifidobacterium lactis* and *Lactobacillus Acidophilus* from Nestle were added to 200 g of green beans. The samples were kept into the close container and placed at room temperature. The samples were fermented for 72 hours. Samples were taken for pH analysis every 24 hours.

In separate jar, 2 g of yeast containing *Saccharomyces cerevisiae* from mauri-pan® were added dissolve in warm water and then mixed to 200 g of green beans. The samples were kept into the close container and placed at room temperature. The samples were fermented for 72 hours. Samples were taken for pH every 24 hours.

After 72 hours, both of fermented sample were rinsed with distilled. The samples treated with LAB and yeast was roasted in oven at 220℃ for 10 minutes. Finally, all samples were ground into powder form and kept in the airtight jar for further use.

0.5 g of fermented beans samples were added into 5 ml distilled water and left for 10 minutes (Adinarayana et al., 2015). The pH was measured using pH meter from Laquatwin and the data were kept.

3.3 Proximate Analysis

Based on the high acceptance of panels, 1 sample of fermented coffee beans was chosen and performed in proximate analysis to determine the nutrition composition inside both of treated and untreated coffee beans that used Association of Official Analytical Chemists (AOAC). The samples were performed by triplicate to use in each analysis.

3.3.1 Determination of Moisture Content

1 g of ground samples were weight using analytical balance. Each sample of untreated and treated coffee beans were triplicate to obtain the mean value. The moisture content's determination by moisture analyser with 120℃ for 7 minutes. The moisture content of coffee represented with 7.66% mean, 0.100% repeatability, and 1.31% CV.

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3.3.2 Protein Determination by Kjeldahl

1 g of dried sample powder were weighed using analytical balance. Each sample was put into Kjedahl tube that arranged in it insert rack. It followed by 2 Kjeltabs as catalyst and 12 ml of H_2SO_4 was measured using 100 ml beaker, dropper, and 50 ml measuring cylinder. Placed the tube inside fume manifold and attached on the preheated block before the temperature reach 400℃. The H_2O respirator was turn on. The mixture solution was boiled and digest at 420℃. The digestion takes 2 until 3 hours approximately depends on samples. Allowed the samples solution until it turns to the light green and the Kjedahl tube were removed from digestor. The tubes were cool down for 10 until 15 minutes.

After the tubes were cooled, 80 ml of distilled water was poured slowly into alkali tank and followed by 50 ml of 40% NaOH. The 30 ml of solution that contains 4% boric acid, 1.75 ml methyl red and 2.5 ml bromocresol green into receiving flask. The distillation system was pre-wash by 50 ml of distilled water and repeated after every distillation. The samples were distillate using Kjedahl auto distillation analyser. The titration began after the distillation step done with 0.1 M HCl. The amount of HCl used was recorded. The analyser tank was cleaned once every test run.

a) Calculation of % Nitrogen

$$\% \text{ Kjedahl Nitrogen} = ((Vs\text{-}V_b) \times N \times 14.01) \div (W \times 10))$$

Where, Vs was ml of standardized acid used to titrate a sample, V_b was ml of standardized acid used to titrate a reagent blank, N (0.1000) was normality of standard

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HCl, 14.01 was atomic weight of nitrogen, W was weight (g) of sample or standard, and 10 was factor to convert mg/g to percent

b) Calculation of % crude protein

$$\% \text{ Crude Protein} = \% \text{ Kjedahl Nitrogen} \times F$$

Where, F was factor to convert nitrogen to protein and 6.25 was the protein-nitrogen conversion factor.

3.3.3 Determination of Fat

The aluminium cups were heated in drying oven at 103℃ for 30 minutes and cool them in desiccators for 20 minutes. The weight of all cups was recorded by 4 decimal places. The 5g of sample was weighed and put into the 90 mm filter that inserted into the thimbles. A layer of de-fatted cotton was put on the top of sample. The thimbles were inserted into extraction unit and attached them to the magnets. 'MAINS' button was press to start the equipment. Each of the cups were inserted with 80 ml petroleum ether. The cups were inserted into the extraction unit with the cup holder. The proper program from 1 until 9 was selected and checked the timer for boiled, rinsed, recovered, and pre-drying on the control unit. The water tap was opened each time for the reflux condensers before the machine was run. This step can prevent the solvent from evaporate from the

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condensers. The RUN/STOP button was pressed, and the temperature was displayed as soon as it was reached.

Step 1 began with 'boiling'. The condenser valves were opened followed by the timer button was then pressed. In this situation, the left and right handle was at the bottom. The thimbles were moved to 'rising' position after the buzzer sound and moved the left handle to centre. Then, the timer was press and remain the position of the right handle. The left handle was moved to 'recovery' position after the buzzer sound again and the timer was press. In this part, the right handle was remain at the bottom. The cups were removed at heated at 103℃ for 30 minutes, cool in desiccators for 20 minutes, and the weight of each cup was recorded. The formula showed below,

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

3.3.4 Determination of Fibre

The sample from fat were used in determination of fibre. In preparation of solution, 1.25% H_2SO_4 and 1.25% NaOH was dissolved with distilled water until 2000 ml or represented as 2 cycles of fibre. Thus, 25 ml of H_2SO_4 and 25 g of NaOH were dissolved with distilled water until both of them reached 2000 ml. The empty crucibles were dried in drying oven at 130℃ for 2 hours and cool it in desiccator for 20 minutes. The blank crucible was weight using analytical balance. The samples were added into

each of the crucible and the weight of samples plus crucible was recorded. The weight of samples must not less than 1 g or more than 5 g. Next, half a spoon of celite was placed in each crucible containing sample because celite functions as a catalyst for the samples. The crucible inserted into Soxtec and run until the star on the Soxtec on the screen was disappears. The crucibles inserted into drying oven at 130℃ for 2 hours and cooled it in desiccator for 20 minutes. The samples were put into furnace at 525℃ for 3 hours. The crucible containing samples were cooled in desiccator for 20 minutes and the weight of each sample was recorded. The fibre determination was performed using the formula below,

$$\% \text{ Ash} = \frac{W2-W3-C}{W1}$$

Where, W_1 was the weight of sample (g), W_2 was the weight crucible and residue (g), $W_3$was the weight of crucible and ash residue (g), and C was blank.

3.3.5 Determination of Ash

The cleaned and dried porcelain crucible were weighed using analytical balance. 1 g of sample was put into each porcelain crucible. The weight of blank and the weight of sample and crucible were recorded. The crucibles were placed into the muffle furnace at 550℃ for 6 hours. The temperature was maintained until the ash turn to white, light grey or reddish, and showed the appears to be free of carbon particles. The crucibles take

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out from muffle furnace and cooled inside the desiccator. Then, the weight of crucible was record immediately. The ash determination was performed using the formula below,

$$\% \text{ Ash} = \frac{W2-W1}{Ws} \text{ x } 100$$

Where, W_1 was the weight of porcelain crucible (g), W_s was the weight samples (g), and $W_2$was the weight of porcelain crucible and ash (g).

3.3.6 Determination of Carbohydrate

Determination of carbohydrate by using the formula below,

% Carbohydrate = 100 – (% Protein + % Fat + % Moisture + % Ash)

3.4 Coffee drink preparation

15 g of ground coffee was dissolved in 200 ml hot water and leave for extraction until 5 minutes. 500 ml of room filtered water was then added for dilution. The coffee drink was filtered with 1 mm opening sieve to reduce insoluble of coffee powder. Ready-

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filtered coffee drink was put into the small cup. Each of the cup was given to non-trained panels as completed the sensory analysis.

3.5 Sensory Analysis

The sensory analysis of both treated and non-treated coffee beans was evaluated by non-trained panels using the hedonic scale to get acceptance to the final product. The scale was performed by google form. The QR Code of google form, the explanation of sensory attributes, and the procedure to do sensory were given to each non-trained panel. The test was conducted by 37 panels to analyse the colour, sweetness, sourness, bitterness, viscosity, and overall acceptance of coffee from scale 1 to 5 as represented in Table 3. The samples were given different codes provided as a way to differentiate the types of samples as represented in Table 4. The codes were generated at random to ensure that the respondents could answer the scale without bias (Tentamus, 2022).

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Table 3. The hedonic scales

Scale	Degree of Likeness
1	Dislike very much
2	Dislike
3	Neither like or dislike
4	Like
5	Like very much

Table 4. The code of samples

Code of sample	Types of samples
298	Untreated green coffee beans
761	Treated green coffee beans with yeast
124	Treated green coffee beans with LAB

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3.6 Data Collection and Statistical Analysis

Analysis of variance (ANOVA) and Two sample T-test in MiniTab 17 software (significance difference of $P < 0.05$) were used as measurement of the proximate analysis of the samples. Online standard deviation calculator was used to calculate mean and standard deviation of samples for sensory analysis. Final data were analysed by using the Microsoft Excel.

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CHAPTER 4

RESULT AND DISCUSSION

4.1 pH of Samples

Figure 4 shows the pH of both samples from 24, 48 and 72 hours. According to Graeme and Graham (2016), pH changes happen because of the warm and acidic environment presented during fermentation that help in the growth of *S. cerevisiae* strains in between 20 and 30℃ and pH 4.5 and 6.5. This condition occurs because beans was stored in closed containers and placed at the room temperature which produces anaerobic conditions during the fermentation process. Chris (2007) also said, the pH changes because of the chemical reaction in which yeast cells take in very basic ammonium ions while released the organic acids such as lactic acid. Besides, the drop of pH in both samples was proved by Han Sub, Yoonhwa and Misook (2018), which said the soluble organic acids released from green coffee beans and created during the fermentation of naturally occurring lactic acid bacteria in coffee beans.

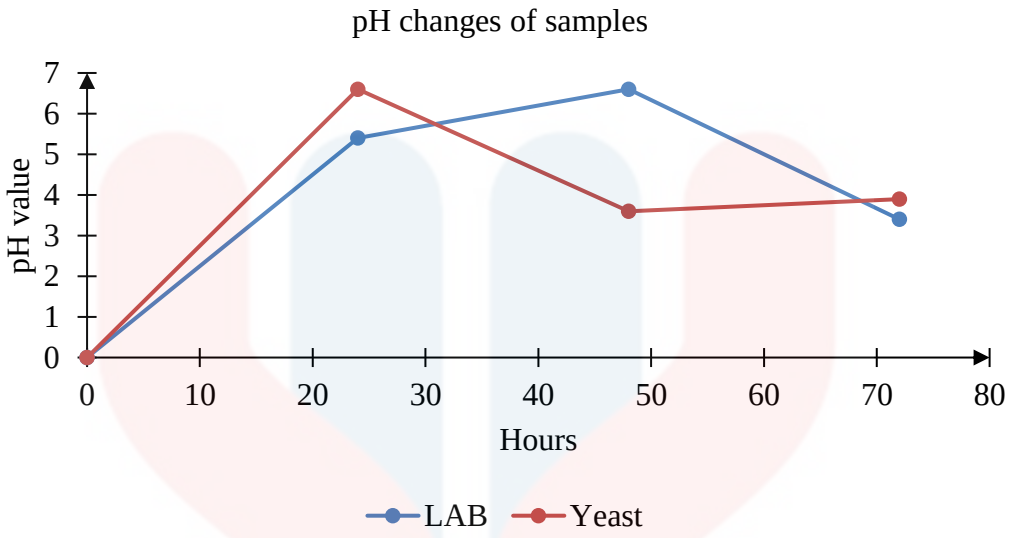

Figure 4. The pH of Samples

4.2 Proximate Analysis

Table 5 summarize the percentage of proximate analysis started from protein, fat, ash, moisture, carbohydrate, and fibre in both untreated (CB) and treated (FB) coffee beans. This table shows the percentage of protein in CB was significantly higher than FB. The green beans contain approximately one-third protein that associated with the cell wall arabinogalactan (Arya & Mohan., 2007). According to Ruta and Farcasanu (2021), both high pectinolytic activities was presented in *S. cerevisiae var.* ellipsoideus which plays a role in demucilaging coffee during the fermentation process. The breakdown of the mucilage layer of beans is subsequently increased and the pectic material was effectively removed in 7 to 8 hours. Rivera (2016) also said proteins was produced by smaller

components that called amino acids. The development of color, aroma, and flavor compound in coffee was develop with the presented of amino acids during roasted process and through Maillard reaction (Rivera, 2016). However in this experiment, the percentage of protein slighlty inaccurate readings due to the reduction machine efficiency during the analysis. The time taken during digestion in this experiment was longer than normaly which about 4 hours to digest. The digestion period was supposed around 1 or 1 and a half hours because 2 Kjedahl tablets were used in this experiment. The main function of catylst is to speed up the chemical process (Laurel, 2019). Thus, the more catalyst used the faster the reaction happened.

Table 5. The nutritional composition by mean and standard deviation of both treated and untreated coffee beans

Sample	**CB**	**FB**	***p*-value<0.05**
Protein (%)	22.35±0.84	13.89±2.15	0.02
Fat (%)	1.24±0.61	1.92±0.24	0.22
Ash (%)	3.33±0.83	1.94±1.27	0.21
Moisture (%)	5.93±1.53	4.04±0.70	0.19
Carbohydrate (%)	50.68±	59.69±	-
Fibre (%)	16.49±0.92	18.54±1.24	0.11

Samples represent, CB-Control Beans & FB-Fermented Beans, *P*-value<0.05 indicate significantly difference, & *P*-value>0.05 indicate no different significantly difference

The percentage fat in CB and FB shows no different significantly as shows in the Table 5. The higher fat percentage produced better aroma and flavours of coffee production. This statement supported by Dong et al. (2017), fats added to the scent and flavour of coffee which helped to increase its quality. Besides, the fatty acids include linolenic, palmitic, stearic, oleic, arachidic, and linolenic acids in green coffee beans plays an essential role in the coffee's quality (Tsegay, 2020). However, in the Table 5 the percentage of fat in both samples were lower because there is additional flavour such as honey, sugar, or milk. The excessive amounts of added flavours in coffee leads to the increases of fat and causes weight problem (Zamarripa, 2019). Despite this, fat enhanced the aroma, flavour and mouthfeel of coffee (Chayan et al., 2021), as well as aided in weight loss due to the low calories content of black coffee.

The percentage of ash in CB and FB shows no different significantly difference. The increases ash content contributed to the increases in mineral content because ash was represents as the total mineral content in foods (Discover Food Tech, 2017). A study done by Kwak et al (2018), shows the significantly percentage between both untreated (4.73 ± 0.05^{a} and 4.08 ± 0.06^{cd}) and treated coffee beans (4.05 ± 0.06^{d}, 4.15 ± 0.95^{c} and 4.40 ± 0.05^{b}). However, the percentage shows a strong negative correlation and this indicates that the significantly differences in percentage of ash has a negative effect on the final product particularly for nutirents contains. According to Gunnars (2018), coffee beans contains and its drink has many nutrients that includes vitamins and minerals. many nutrients contains in coffee beans and its final drink that includes the vitamins and minerals. Arya and Mohan (2007) said, the green beans basically containes close to 4% on ash basis but there is no significantly changes in the mineral content during roasting. This is because the mineral content was easily extracted during domestic brewing and commercial extraction. However, there is no statement of

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the maximum ash content in roasted ground coffee (Mariana, Flávia & Fabrícia., 2018). Thus, the absence of ash percentage differences between CB and FB samples does not adversely affect coffee production, in fact, the value of the mineral has a positive effect on consumer health.

The percentage moisture in CB and FB has no different significantly. The Coffee Quality-Improvement Programme (CQP) determined the moisture content of green coffee beans basically not fall below 8% or more than 12.5% (International Coffee Organization, 2018). This is due to the losses of aroma, freshness, and clarity of raw green beans moisture that below than 9 and 8% until its cannot be delivered (Pashley, 2017). Besides, the water content of green coffee and roasted coffee influents the water activity and stability during storage and provides a monitor of coffee extract during processing (Arya & Mohan., 2007). However, the moisture content of both sample shows the lower than range mentioned because of the roasting process. According to Wong (2020), the moisture content of the green beans of 10-12% was removed within 3 minutes after roasting process. Thus, the absence of differences significantly of moisture of both samples helped in the production of caramelization where the darker coffee has a more interesting taste, flavour and aroma.

According to Arya and Mohan (2007), the presented of carbohydrates in coffee beans was importantly as aroma binders in raw coffee beans, improved the organoleptic quality of the coffee brew/beverage, and impart to foam stability in “Espresso” coffee beverage. Fibre as carbohydrate which the body was unable to digest and cannot broken down into sugar molecules (Chan, 2019). In the Table 5, the percentage of fibre in CB and FB shows no different significantly. Fibre in coffee was slightly presented depends on the roasted and degree of grinding (Taylor, 2021). This is due to the removal of mucilage or pulp layer in green coffee beans during the fermentation. Hailer and Kang

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(2019) said, the fermentation helped in the removal of mucilage layer of green coffee beans that rich in polysaccharides (pectin) and reduced the water content of beans. Besides, the proper fermentation increased coffee's quality attributes (Hailer & Kang., 2019). However, the high fibre content of coffee mucilage, which included cellulose (49%), hemicellulose (24.5%), and lignin (7.63%) was slightly removed during the fermentation. The removal of polysaccharides (pectin), cellulose and starch in mucilage layer as the main purpose of the fermentation because its effect the moisture content of beans (Hailer & Kang., 2019). Thus, the longer time taken to dry the beans the high possibility of the mould to develop and resulted the lower final quality of coffee (Haile & Kang, 2019).

4.2 Sensory Analysis

The sensory analysis aims to get the consumer acceptability towards the new formulation of both treated and untreated fermented coffee beans by collecting the mean of respondents answered. The data kept for comparison of the sensory attributed and overall acceptances in each sample. The sensory analysis focused on respondents from Universiti Malaysia Kelantan which the total of 37 respondents answered this form. The total was more than minimum respondents of sensory analysis which is 30 respondents because the more data obtained, the more precise the results and computations to draw a conclusion from this analysis (Auell, 2016).

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Google form was used as a platform for answer the question and it distributed through QR Code whereby it printed and given to the respondents before they answer the form. The respondents answered two parts in this form called demographic information and the acceptance of respondents toward samples. The questions in the form were set up by multiple-choice questions and available in two languages, English and Malay to increase respondents understanding. The mean and standard deviation of consumers acceptances towards sample in terms of colour, sweetness, sourness, bitterness, and viscosity were shows in the Table 6 and Figure 5.

In Table 6 and Figure 5, sample 298 shows the highest mean in terms of colour followed by sample 761 and 124. This shows the panellist satisfied with the colour of sample 298 because the coffee drink looks like a black coffee drink on the market compared to sample 761 and 124 which has light colour (Appendix E). The ground bean was influenced by temperature changes because the transition of complex materials and the breaking point cannot be constant to cross the macroscopic region of the bean (Uman et al., 2016). The final colour of coffee beans also related to the roasted process (The Roasterie, 2020). The physical colour of bean changed from blue-green to brown and then produced melanoidins (Balechior, 2019). This happens when the intrinsic sucrose or sugar in the coffee bean went from sweet to caramel and became burnt during the roasting process (The Roasterie, 2020). The longer time it took to roast the beans resulted in a darker colour. Thus, colour plays a big role toward the acceptability of fermented coffee due to the attraction in choosing food and become the parameter of quality and the coffee's profile.

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Table 6. Mean and standard deviation of the samples

Code of Sample	298	761	124
Colour	3.73±0.99	3.57±1.09	3.03±1.26
Sweetness	2.97±1.26	2.65±1.11	2.57±1.07
Sourness	2.95±1.22	2.46±1.16	2.57±1.07
Bitterness	3.54±1.10	3.32±1.13	3.19±1.33
Viscosity	3.14±1.21	3.03±1.24	2.73±1.19

Samples represent, 298-Untreated green coffee beans, 761-Treated green coffee beans with yeast & 124-Treated green coffee beans with LAB.

Next, sweetness as a basic taste associated with sucrose solution (Michaela et al., 2013). In this experiment, the drink preparation prepared without the sugar as to control the samples from others interaction because the diverse metabolites of sugar were produced naturally during the coffee beans fermentation by microorganisms (Haile & Kang, 2019). The concentration of free sugars and amino acids produced from microbial activity and the degree of fermentation occurs when the beans are continued surrounded by them (Haile & Kang, 2019). Therefore, Maillard reaction compounds and volatiles during the roasting process are performed. These sugars include glucose and fructose. Besides, the diversity of coffee aroma and flavour compound also increased. The mean of sample 298 in Table 6 and Figure 5 shows the highest value of consumer acceptance followed by the samples 761 and 124. Thus, the untreated coffee has higher sugar and amino acid content compared to fermented coffee with yeast and LAB.

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The sourness of coffee beans very related to the bitterness. This happened because of the chlorogenic acids under the group of polyphenols that made themselves taste sour (Marquart, 2020). Marquart (2020) also said, the natural sourness of coffee began before it roasted or the beans in the green conditions. Besides, the usage of LAB from yogurt slightly affected the sourness attributes in this sensory analysis compared to the fermented using yeast. Thus, sample 298 has higher sourness mean compared to sample 124 and 761 as shows in Table 6 and Figure 5.

The bitter taste of coffee was a consumer's priority but when it is too concentrated or liquid, it causes the bad taste of the coffee. This is due to the differences in the level of individual coffee consumption due to the production of caffeine and ethanol metabolic process that resulted during consuming this beverage (Sheng et al., 2018). The propylthiouracil and quines were the examples of bitter compounds in coffee. According to Marquart (2020), the chlorogenic acid and initial water content of the green beans and its size effect the bitter taste of final product. The chlorogenic acid generated chlorogenic acid lactones by the intra-molecular lactonization process as it produced bitter taste of coffee that happened during the mid to mid-late stages of the roasting. Bitterness of coffee comes with the reaction and degradation of chlorogenic acid and the lactones that happens after roasted process mostly 210-220℃ and produces compound called phenylindanes, metallic, or long-lasting bitter compounds in coffee (Marquart, 2020). The grinding process also affected the bitterness of coffee which the finer the coffee powder particle size, the easier it is to dissolve in water, and the darker the colour and bitterness of the coffee (Cruz, 2021). This is because all samples have different size of coffee powder particle causes of the manual ground process. However, coffee bitterness also happened by the skilful process, experience and the ways to fix the taste. Thus, sample 298 shows the highest mean of bitterness compared to sample 761 and 124.

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The viscosity also known as sticky characteristics to palate or mucosal surface in the oral cavity (Kreumi et al., 2013). According to the Khomyakov et al. (2020), the viscosity varies of coffee extract in range of 0.0008-27.4810 Pa·s for the considered cases. The viscosity tends to be high when the values in soluble solids was high (Cruz & Cortés, 2021). The viscosity of coffee was also affected by the contain of carbohydrate during brewing (Arya & Mohan., 2007). Thus, the viscosity of samples based on Table 6 and Figure 5 shows the highest on sample 298 followed by sample 761 and 124.

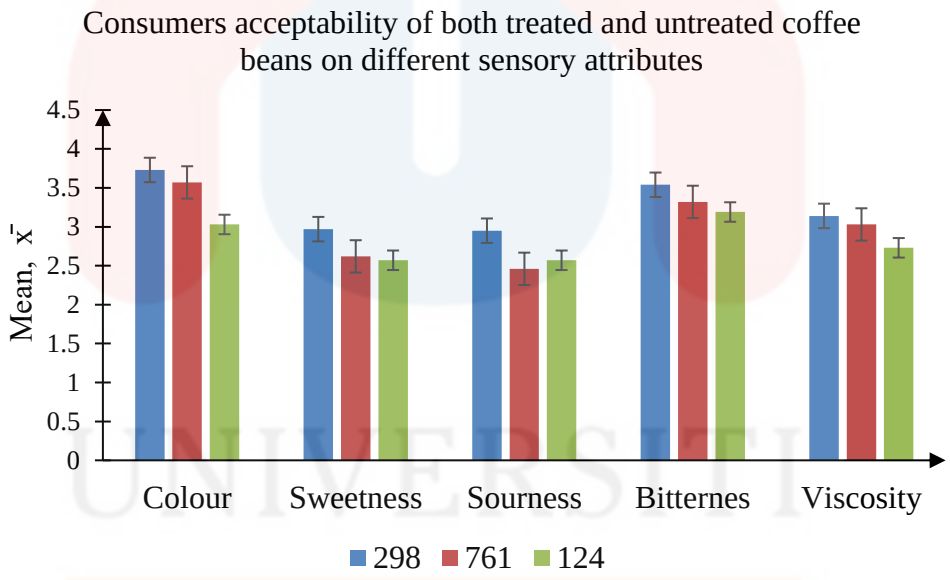

Figure 5. Consumer acceptability of both treated and untreated coffee beans on different sensory attributes

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4.3 Overall Acceptances of Samples

In Table 7, treated coffee beans with yeast shows higher overall acceptance in terms of deliciousness than treated coffee beans with LAB, but both of samples represented as 5% in term of good aroma. However, the untreated coffee beans represented as highest acceptance in terms of deliciousness and good aroma than treated coffee beans. This is because natural coffee has more complex of flavour-active compounds, a velvety tongue feels, less acidity, and a wider spectrum of fruity overtones compared to washed coffee (Palmori & France., 2016). Specialty-level naturals have a fantastic combination of clear flavours that combine nuance and complexity with tremendous intensity and body, making them able to satisfy the most discerning palates.

The treated coffee beans with yeast chosen which has more delicious and has good aroma than treated coffee beans with LAB. The usage of yeast effectively produced various flavour and aroma to fermented coffee such as caramel flavour in the beginning and a bitter aroma at the end, fruit like, floral, sweet, caramel, nutty, refreshing, walnut, cane molasses, etc. (Ruta & Ileana, 2021). This is because approximately 800 volatile of chemical compounds that includes Maillard reaction was combined during and after fermentation with yeasts to increase coffee flavour (Ruta & Ileana, 2021). All of the components were mixed together during fermentation, resulting in changes in the quality and chemical content of coffee-based beverages. The fermented coffee beans with LAB also increased aromatic compounds that includes the benzyl alcohol, phenethyl alcohol, and ethyl hexanoate (Pereira et al., 2020). However, the usage of LAB for producing fermented coffee was limited to enhance the flavour (Haile & Kang, 2019). Thus, based

on the consumers acceptances of samples in Table 7, the usage of yeast in fermented coffee beans effectively to enhance flavour and aroma of coffee.

Table 7. Overall consumers acceptances of samples

Sample	**298**	**761**	**124**
Bitterness (%)	32	33	16
Delicious (%)	46	35	30
Good aroma (%)	9	5	5
Light in colour (%)	8	22	22
No answer (%)	5	5	27

Samples represent, 298-Untreated green coffee beans, 761-Treated green coffee beans with yeast & 124-Treated green coffee beans with LAB

The overall consumers acceptances of samples in Table 7 were affected by various situations. The different perceptions and acceptance depended on individual which includes stimulus error, adaptation, and sensory threshold (Dom, 2015). Also, it affected by emotions that connected with involuntary nervous system. The taste not only depends on the tongue but it related to the smell, texture and temperature of the sample while the colouring of a taste happens through the nose (Institute for Quality and Efficiency in Health Care, 2020). The information transported from tongue to the brains happens by sending a signal from the taste of bud cells, nerves system, up to a tiny hole in the skill

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and into the brain (Lucy & Stphen, 2017). The sensed basically have 5 basic qualities of taste that includes sweet, sour, salty, bitter, and savoury that actually happens in all parts of tongue but, the certain part was more sensitive than the middle overall. The scale completed once after taste bud of panel was notified the coffee.

The sensory analysis also influenced by capriciousness, such occurs when the panellists use the sensory analysis scale's extremes (Dom, 2015). Even a small percentage of the panellists did not answer the question as shows in Table 7 causes the result or data collected to be erroneous. Besides, the unwashed mouth with mineral water before taste the sample also effected the next taste and flavour of sample. However, the results were still acceptable because the invited panellists who consist of students and lecturers do not have high expertise to analyse the taste of coffee.

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research completed the objectives of the investigation the effects fermentation using LAB and yeast on green coffee beans, the analysis of the nutritional composition and physicochemical effects on the treated coffee beans and the sensory analysis of both treated and non-treated fermented coffee beans. There has been very little study on the fermented coffee beans and this research help in improvement of the better final product of coffee. The best sample was the fermented coffee beans with yeast which received highest consumer rating over fermented coffee beans with LAB. Thus, the use of LAB and yeast for fermentation has a good impact on the end product and the processing due to high consumer acceptance of the novel fermented coffee bean formulation. This demonstrates that fermented coffee beans can be widely distributed and gain high demand in the future.

5.2 Recommendation

There are certain enhancements that can be implemented in the future study. The equipment's efficiency should be improved on a regular basis by performing routine maintenance to guarantee that each sample evaluated is properly completed. This is because equipment such as Gerhardt or Foss brand for protein analysis provided in the faculty lab was limited and the equipment has to work harder to analyse a sample. Besides, the additional equipment needs to be approved to facilitate students, lecturers, staff and researchers to analyse data easily and more efficiently.

In the preparation of ground samples, it requires additional precision where the ground coffee must be filter to get the same size. The particle size of ground coffee has an impact on the concentration of the coffee beverage. Thus, the bitterness of coffee can be control between samples because the finer the coffee powder particle size, the easier it is to dissolve in water, and the darker the colour and bitterness of the coffee (Cruz, 2021). The ground coffee should be kept in closed container at least for 3 days to increase the flavour and aroma of coffee drink.

Therefore, this research on fermented coffee beans should be conducted in detail in the future with includes any research into either fermented coffee beans with LAB and/or yeast. The improvements in each step for the production of fermented coffee beans help traders to increase product production over time.

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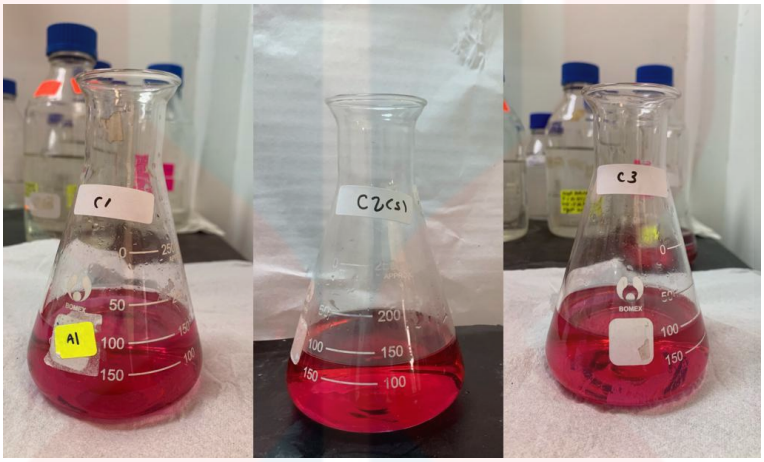
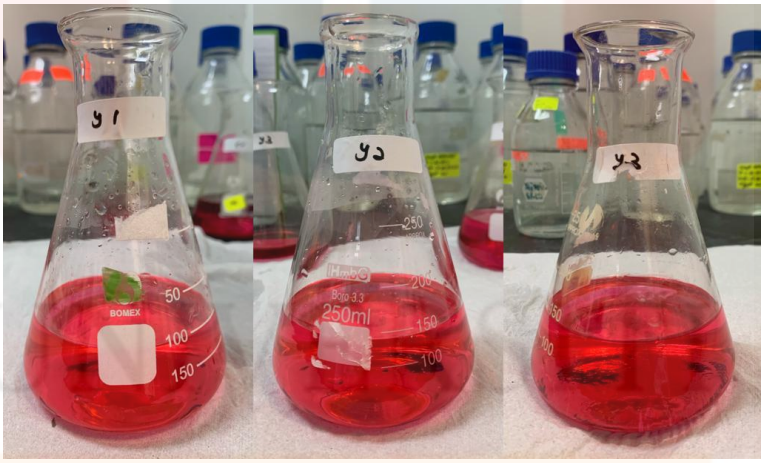
APPENDICES

Appendix A: Titration of untreated (CB) and treated (FB) coffee bean in protein content determination



Titration of untreated (CB) coffee bean in protein content determination



Titration of treated (FB) coffee bean in protein content determination (Y represents as FB)

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Appendix B: Two-Sample T-Test and CI: Control, Treated (Proximate Analysis)

Proximate Analysis	N	Mean	St Dev	SE Mean	Estimate for difference	95% CI for difference	T-Test of difference = 0 (vs ≠)		
							T-Value	P-Value	DF
Protein	Control 3	22.328	0.844	0.490	8.440	2.70, 14.17	6.33	0.024	2
	Treated 3	13.890	2.150	1.200					
Fat	Control 3	1.243	0.612	0.350	-0.674	-2.296, 0.948	-1.79	0.216	2
	Treated 3	1.917	0.227	0.130					
Ash	Control 3	3.333	0.833	0.480	-1.389	-4.184, 1.406	-1.58	0.212	3
	Treated 3	1.940	1.270	0.730					
Moisture	Control 3	5.930	1.530	0.880	1.890	-2.273, 6.053	1.95	0.190	2
	Treated 3	4.037	0.695	0.400					
Fibre	Control 3	16.486	0.925	0.530	-2.055	-4.901, 0.791	-2.30	0.105	3
	Treated 3	18.540	1.240	0.720					

Estimate for difference derived from formula, Difference = μ (Control) - μ (Treated)

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Appendix C: Sensory analysis preparation

Appendix D: Sensory analysis conducted by amateur panellist

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Appendix E: Final product which represents, sample-code of sensory analysis, 298 (untreated coffee beans), 761 (treated coffee beans with yeast), and 124 (treated coffee beans with LAB)

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