PHYSICOCHEMICAL AND ANTIOXIDANT PROFILING OF COMMERCIAL FERMENTED MILK DRINKS

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PHYSICOCHEMICAL AND ANTIOXIDANT PROFILING OF COMMERCIAL FERMENTED MILK DRINKS

By

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ABSTRACT

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Chang Yu Jie

Fermented dairy-based beverages have been consumed for centuries and continue to be widely popular in contemporary markets. Numerous brands of fermented milk drinks are commercially available, yet their physicochemical and antioxidant properties vary depending on the production process. This study aimed to analyse the physicochemical and antioxidant properties of selected commercially available natural flavoured fermented milk beverages produced by different food companies. The samples included three cultured milk drinks (Yakult®, Vitagen®, and Betagen) and three yogurt drinks (Farm Fresh®, Lactel, and Yobick). Physicochemical parameters, including pH, total titratable acidity (TTA), total soluble solids (TSS), and colour, were assessed, along with antioxidant properties, including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity (RSA). Data were analysed using nested analysis of variance (ANOVA) followed by Tukey's HSD test to determine significant differences ($p \leq 0.05$). Results indicated that Yakult® cultured milk exhibited the lowest pH (3.98±0.044) and the highest TTA (6.79±0.91), reflecting the highest acidity and a sour taste profile. Betagen cultured milk had the highest °Brix value (17.4±0.10), suggesting greatest degree of sweetness. Cultured milk drinks generally appeared darker and more yellowish, whereas yogurt drinks tended to be lighter and exhibited less yellow intensity. In terms of antioxidant properties, Farm Fresh® yogurt drink had the highest TPC (1.084±0.006) and TFC (5.148±0.071), indicating greatest phenolic and flavonoid content due to the presence of a greater number of probiotic strains. Lactel yogurt drink exhibited the highest FRAP (3012.50±12.37) and RSA (50.21±0.45), suggesting superior antioxidant activity attributed to its high protein content in fermented milk base. Fermentation conditions characterized by moderate acidity, the inclusion of acid-producing and multiple probiotic strains, and the use of a high-protein milk base were essential in order to produce fermented milk beverages with superior antioxidant quality.

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DECLARATION

I hereby declare that this final year project report entitled "**PHYSICOCHEMICAL AND ANTIOXIDANT PROFILING OF COMMERCIAL FERMENTED MILK DRINKS**" is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Tunku Abdul Rahman or other institutions.

(CHANG YU JIE)

APPROVAL SHEET

This final year project report entitled "**PHYSICOCHEMICAL AND ANTIOXIDANT PROFILING OF COMMERCIAL FERMENTED MILK DRINKS**" was prepared by CHANG YU JIE and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

Date: 21 May 2025

(Dr. Chay Shyan Yea)

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Date: 21 May 2025

PERMISSION SHEET

I, **CHANG YU JIE** (ID No: 21ADB01703) hereby certify that I have completed the final year project titled "**PHYSICOCHEMICAL AND ANTIOXIDANT PROFILING OF COMMERCIAL FERMENTED MILK DRINKS**" under the supervision of Dr. Chay Shyan Yea (Supervisor) from the Department of Agricultural and Food Science, Faculty of Science.

I understand that the University may upload the softcopy of my final year project in PDF to the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Your truly,

(CHANG YU JIE)

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LAB	Lactic acid bacteria
®	Registered
CFU/g	Colony-forming units per gram
GRAS	Generally recognized as safe
LacY	Lactose permease
PTS	Phosphotransferase system
EMP	Embden-Meyerhof-Parnas
NAD^+	Nicotinamide adenine dinucleotide
SCFAs	Short-chain fatty acids
β-galactosidase	Beta-galactosidase
TTA	Total titratable acid
NaOH	Sodium hydroxide
TSS	Total soluble solids
L*	Lightness
a*	Redness/ Red-green spectrum
b*	Yellowness/ Yellow-blue spectrum
Nrf2	Nuclear factor erythroid 2-related factor 2
GA	Gallic acid
-OH	Hydroxyl group
-COOH	Carboxyl group
C	Carbon
AA	Ascorbic acid
HAT	Hydrogen atom transfer
SET	Single electron transfer
ROS	Reactive oxygen species
IP	Ionization potential
BDE	Bond dissociation enthalpy

TPC	Total phenolic content
TFC	Total flavonoid content
FRAP	Ferric reducing antioxidant power
RSA	Radical scavenging activity
FC	Folin-Ciocalteu
$AlCl_3$	Aluminium chloride
GAE	Gallic acid equivalents
QE	Quercetin equivalents
CE	Catechin equivalents
Fe^{3+}	Ferric ions
Fe^{2+}	Ferrous ions
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
DPPH	2,2-diphenyl-1-picrylhydrazyl
A	Absorbance
w/v	Weight per volume
SD	Standard deviation
p-value	Probability
ANOVA	Analysis of variance
Tukey’s HSD	Tukey's honest significant difference
°Bx	Degree Brix
L. paracasei	*Lactobacillus paracasei*
L. acidophilus	*Lactobacillus acidophilus*
L. bulgaricus	*Lactobacillus delbrueckii*
S. thermophilus	*Streptococcus thermophilus*
EGCG	Epigallocatechin gallate
Tyr	Tyrosine
Trp	Tryptophan

CHAPTER 1

INTRODUCTION

The history of fermented dairy products dates back approximately 10,000 to 15,000 years, signifying their long-standing role in human nutrition and food preservation (Bintsis and Papademas, 2022). In recent decades, global interest in these products has surged due to growing awareness of the intricate relationship between diet, gut health, and overall well-being. Fermentation process driven by lactic acid bacteria (LAB), induces biochemical transformations that enhance the nutritional and functional profiles of dairy (Joshi et al., 2024). Dairy products serve as excellent matrices for probiotic inoculation, as their rich composition of proteins, minerals, and vitamins provides a favourable environment for the proliferation and metabolic activity of probiotic microorganisms (Andrade et al., 2019).

Among fermented dairy products, cultured milk and yogurt drinks dominate the functional food market. These beverages are prized for their probiotic content, which includes strains like *Lactobacillus casei*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*. To deliver health benefits, these products must maintain a minimum viable probiotic count of 10^6 CFU/g at consumption (Terpou et al., 2019). Regular intake is associated with improved gut health, enhanced immunity, and resistance to pathogens (Shah et al., 2024).

The commercialization of fermented milk beverages accelerated in the 1930s with Dr. Minoru Shirota's development of Yakult®, a milestone that inspired subsequent cultured milk beverage brands like Vitagen® and Betagen. Notably, Vitagen® has been Malaysia's leading cultured milk brand since 1977, reflecting the country's strong foothold in the probiotic market (Malaysia Milk Sdn Bhd, 2022; Yakult Malaysia Sdn Bhd, 2024). These products are characterized by a tangy flavour and fortification with LAB strains that support digestive and immune functions.

Similarly, yogurt drinks, which are derived from traditional milk fermentation practices have been adapted to meet the modern consumer's demand for convenience. Conventional fermented milk products such as lassi, dahi, and kefir often necessitate additional preparation prior to consumption. For instance, products may require stirring (e.g., lassi) or straining (e.g., for smoother dahi) and are typically transferred to serving containers such as bowls or glasses. Moreover, flavour enhancements such as sweeteners, salt, or spices are often added manually. In contrast, yogurt drinks are produced as ready-to-consume products that are commonly packaged in portable bottles or pouches. This design facilitates utensil-free and on-the-go consumption without any need for additional preparation or cleanup (Granato et al., 2010). These features make yogurt drinks particularly suitable for individuals with busy schedules, including during work hours, travel, or other time-constrained situations which align well with the fast-paced lifestyle of contemporary consumers.

Although yogurt drinks have been modernized, they retain the core fundamental principles of traditional milk fermentation. Produced using *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, these drinks maintain cultural significance while delivering a creamy texture, pleasant flavour, and probiotic benefits (Khurana and Kanawjia, 2007). This integration of traditional value with modern convenience highlights their growing global prominence within the functional food market.

The fermentation process significantly modifies the physicochemical properties of dairy products, such as pH, viscosity, titratable acidity, total solids, and syneresis. These changes directly affect product stability, texture, and sensory attributes such as mouthfeel and flavour. The modifications in physicochemical properties play a crucial role in achieving consistent quality and enhancing consumer appeal (Norazura et al., 2020). As a result, fermentation ensures that the final product meets expectations in terms of both structural integrity and sensory satisfaction.

A key advantage of fermentation is its enhancement of antioxidant activity. Bioactive compounds such as peptides, organic acids, and exopolysaccharides generated during fermentation combat oxidative stress by scavenging free radicals (Carvalho and Conte-Junior, 2023). Bioactive peptides, alongside vitamins and probiotic metabolites, contribute to reduced risks of chronic diseases, including metabolic disorders, cardiovascular conditions, and cancer (Pham-Huy et al., 2008).

Consequently, fermented dairy beverages are recognized as functional foods with potent nutritional and health-promoting properties. Their ability to enhance gut microbiota, deliver antioxidants, and adapt to consumer preferences solidifies their importance in modern diets. Building on these benefits, their growing role in supporting long-term wellness further reinforces their global relevance (Hadjimbei, Botsaris and Chrysostomou, 2022).

1.2 PROBLEM STATEMENTS

The commercial market is flourishing with a diverse range of cultured milk and yogurt drinks, each varying in composition, processing methods and potential health benefits. However, there is limited comparative data on their physicochemical properties and antioxidant content, which are critical indicators of their nutritional value and functional benefits. Consumers and industry stakeholders lack a standardized reference to determine which brands offer an optimal balance of physicochemical stability and antioxidant potency. This study characterizes and compares the physicochemical and antioxidant properties of six popular commercial fermented milk beverages in Malaysia, of which includes three different brands of cultured milk drinks (Yakult®, Vitagen® and Betagen) and three different brands of yogurt drinks (Lactel, Farm Fresh® and Yobick). Current findings provide insight into a better understanding of the physicochemical and antioxidant variations among these products, identifying the brands that exhibit superior physicochemical balance and higher antioxidant content.

1.3 OBJECTIVES

1. To determine the physicochemical properties of six commercial fermented milk drinks by using pH, total titratable acidity, total soluble solids, and colorimetric analysis.
2. To study the antioxidant properties of six commercial fermented milk drinks by conducting total phenolic content (TPC) analysis, total flavonoid content (TFC) analysis, ferric reducing antioxidant power (FRAP) assay and DPPH radical scavenging activity (RSA) assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) are a diverse group of Gram-positive, non-spore-forming, facultative anaerobic bacteria widely used in food fermentation, particularly in dairy products (Akpoghelie et al., 2025). The most studied LAB species include *Lactobacillus acidophilus*, *Lactococcus lactis*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*. These microorganisms play a crucial role in milk fermentation by enhancing food safety, improving sensory attributes, and providing probiotic benefits. Due to their "Generally Recognized as Safe" (GRAS) status, LAB are extensively applied in industrial dairy production (Widyastuti, Rohmatussolihat and Febrisiantosa, 2014). The fermentation process facilitated by LAB extends the shelf-life of milk, preserves its nutrients, and enhances its texture and flavour.

2.1.1 Lactic Acid Fermentation Mechanisms

The fermentation process begins with LAB transporting lactose into bacterial cells. There are two primary mechanisms for lactose uptake, including lactose permease transport (LacY System) and phosphotransferase system (PTS). For instance, *Streptococcus thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus* use the LacY system, where lactose is transported into the cell through a galactose-lactose antiport mechanism (Lorántfy et al., 2019). This process involves expelling a molecule of D-galactose in exchange for lactose uptake. Alternatively, lactose can also enter via a lactose/proton symport which driven by a proton motive force (Xu, Zhang and Wang, 2022). On the other hand, *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, and *Lactococcus lactis* utilize the PTS system, in which lactose is phosphorylated upon entry and forming lactose-6-phosphate. This modification prevents lactose from exiting the cell and directs it into glycolysis (Aleksandrzak et al., 2005).

2.1.1.1 Homolactic Fermentation

Based on **Figure 2.1**, once lactose enters the bacteria cell, enzyme β-galactosidase hydrolyses it into glucose and galactose. Glucose then undergoes glycolysis via the Embden-Meyerhof-Parnas (EMP) pathway to produce pyruvate. In homolactic fermentation, pyruvate is directly reduced to lactic acid by lactate dehydrogenase and regenerating NAD^+ to sustain glycolysis (Wolfe, 2015). This process employs of species such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus* (Arioli et al., 2017).

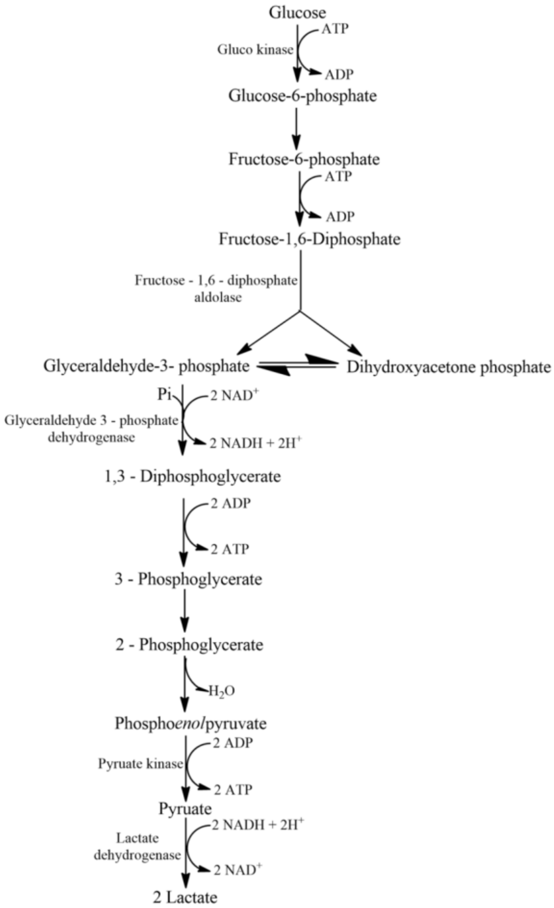

Figure 2.1: Illustration of glucose metabolism through homolactic fermentation in lactic acid bacteria (LAB) via glycolysis pathway (Vivek et al., 2019).

2.1.1.2 Heterolactic Fermentation

In heterolactic fermentation, LAB lack the enzyme aldolase, which is essential for the glycolytic pathway. Instead, these bacteria metabolize glucose via the phosphoketolase pathway (Pessione, 2012). As shown in **Figure 2.2**, this pathway involved the oxidation of glucose-6-phosphate to 6-phosphogluconate, and subsequently decarboxylated to produce ribulose-5-phosphate. This intermediate is then cleaved by phosphoketolase into glyceraldehyde-3-phosphate and acetyl phosphate (Vivek et al., 2019). These by-products are further processed to yield lactic acid, acetic acid, ethanol, and carbon dioxide, all of which contribute to the unique organoleptic properties of fermented dairy product. Microorganisms that rely exclusively on this metabolic pathway for carbohydrate consumption are known as obligate heterofermentative bacteria, including *Lactobacillus brevis, Lactobacillus fermentum*, and *Lactobacillus reuteri* (Bintsis, 2018).

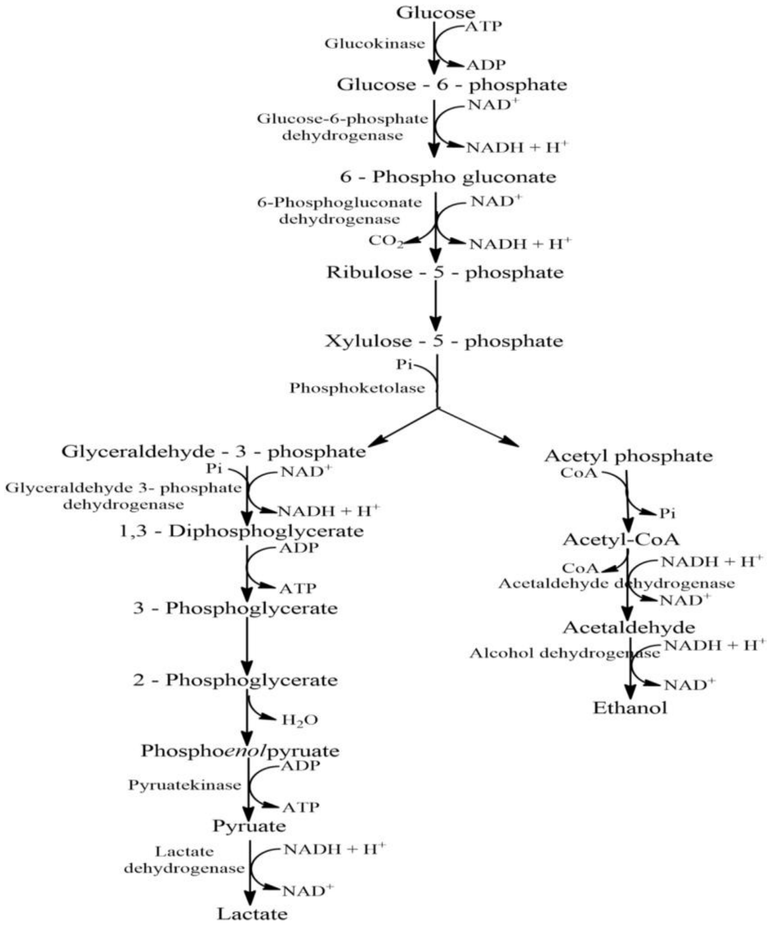

Figure 2.2: Illustration of glucose metabolism through heterolactic fermentation in lactic acid bacteria (LAB) via glycolysis pathway (Vivek et al., 2019).

2.1.2 Preservation and Antimicrobial Properties

LAB play a critical role in milk preservation through acidification and the production of antimicrobial compounds. The lactic acid lowers the pH of milk, inhibiting the growth of spoilage microorganisms and foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* (Ibrahim et al., 2021). Furthermore, LAB strains produce bacteriocins, small antimicrobial peptides that inhibit the growth of competing bacteria. For example, *Lactococcus lactis* produces nisin, a well-characterized bacteriocin widely used as a natural preservative in dairy and other food products. This is because nisin is effective against a broad spectrum of Gram-negative and Gram-positive bacteria (Sobrino-López and Martín-Belloso, 2008).

2.1.3 Flavour and Texture Development

LAB significantly influence the sensory attributes of fermented dairy products. The breakdown of lactose, proteins, and fats by LAB enzymes leads to the formation of volatile compounds such as diacetyl, acetoin, and organic acids, which contribute to characteristic dairy flavours (Smit, Smit and Engels, 2005). In yogurt production, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* work symbiotically to enhance both acidity and aroma (Yang et al., 2025). Besides, the production of exopolysaccharides (EPS) by strains like *Lactobacillus rhamnosus* and *Lactobacillus plantarum* contributes to a creamy and thick consistency in fermented milk products (Jurášková, Ribeiro and Silva, 2022).

2.1.4 Health Benefits of LAB

Fermented dairy products containing LAB provide numerous health benefits, primarily due to their probiotic properties **(Figure 2.3)**. Specific LAB strains, such as *Lactobacillus casei*, *Lactobacillus reuteri*, and *Bifidobacterium longum*, play a crucial role in modulating the intestinal microbiota and strengthening the intestinal barrier. These probiotics enhance gut integrity by promoting mucus production, reinforcing tight junction proteins, and preventing harmful bacteria and toxins from crossing into the bloodstream (Dempsey and Corr, 2022). Besides, LAB stimulate the production of short-chain fatty acids (SCFAs), such as butyrate, which serves as an energy source for intestinal cells to support epithelial regeneration and strengthens the intestinal barrier (Hodgkinson et al., 2023). This contributes to improved digestive function, enhanced nutrient absorption, and reduced gastrointestinal issues such as bloating, diarrhea, and constipation, ultimately promoting overall gut resilience (Den Besten et al., 2013).

LAB strains have been studied for their potential to alleviate lactose intolerance. For example, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* can produce maximum amount of β-galactosidase, the enzyme responsible for breaking down lactose found in milk (Ahn et al., 2023). This enzymatic activity occurs during fermentation, partially hydrolysing lactose into its simpler sugar components such as glucose and galactose, which are more easily absorbed in the intestines. When individuals with lactose intolerance consume fermented dairy products, LAB aid in lactose digestion and reduce the symptoms such as bloating, gas, and diarrhea (Ayivi et al., 2020). As a result, the presence of LAB makes fermented dairy a suitable option for those with lactose sensitivity.

Table 2.1: Functional benefit of fermented milk products using lactic acid bacteria (Widyastuti, Rohmatussolihat and Febrisiantosa, 2014).

Product name	Origin	Culture	Functional benefit
Probiotic yogurt	Ontario, Canada	*L. rhamnosus* CAN-1	Nutrition and immune function for people living with HIV
Mix ewe's and goat's milk yogurt	Antakya-Hatay, Turkey	*S. thermophilus and L. delbrueckii subsp. bulgaricus* (codes: CH-1 and YF-333)	High short chain free fatty acids
Ayran (yoghurt from goat milk)	Turkey	*L. plantarum*, *L. brevis*, *L. paracasei subsp. paracasei*, *L. casei subsp. pseudoplantarum*	High exopolysaccharide
Gioddu, traditional fermented sheep or goat milk	Sardinian, Italy	*S. thermophilus*, *L. lactis subsp. lactis*, *L. delbrueckii subsp. bulgaricus*, *L. casei subsp. casei*, *L. mesenteroides subsp. mesenteroides*	Probiotic
Tarag	Mongolia	*L. helveticus*, *L. lactis subsp. lactis*, *L.casei*	Probiotic
Fermented milk	Japan	*L. casei* strain Shirota	Maintenance treatment for myelopathy/tropical spastic paraparesis (HAM/TSP) patients
Koumiss from mare's milk	Italy	*L. delbrueckii subsp. bulgaricus*, *S. thermophilus*	Antiallergic
Lben	Marocco	Spontaneously/not identified	Low fat and high calcium traditional product

Functional fermented milk	Italy	*L. lactis* DIBCA2, *L. plantarum* PU11	Enriched of Angiotensin-I Converting Enzyme (ACE)-inhibitory peptides and G-amino butyric acid (GABA)
Kumis	West Colombia	*E. faecalis*, *E. faecium*	ACE Inhibitor
Ewe milk, traditional yoghurt	Iran	*L. brevis*	Cholesterol reduction
Maasai	Kenya	*L. plantarum*, *L. fermentum*, *L. acidophillus*, *L. paracasei*	Diarrhoea and constipation
Suusac	Kenya	*L.* curvatus, *L. plantarum*, *L. salivarius*, *L.* raffinolactis, *Leuconostoc mesenteroides subsp. mesenteroides.*	

2.2 Fermented Milk Drinks

Fermented milk beverages encompass a diverse range of dairy products that have undergone biotransformation through microbial fermentation, primarily mediated by lactic acid bacteria (LAB) and, in some cases, yeast. This category includes cultured milk drinks, yogurt drinks, buttermilk, sour cream, kefir, acidophilus milk and koumiss (Jang, Lee and Paik, 2024). The controlled fermentation process induces a series of biochemical reactions that significantly modify the milk’s physicochemical composition, sensory characteristics, and shelf life while also enhancing its nutritional and potential health benefits. These transformations involve the production of organic acids, bioactive peptides, exopolysaccharides, and various metabolites that contribute to improved digestibility, probiotic activity, and potential immunomodulatory effects (Sawant et al., 2025).

2.2.1 Cultured Milk Drinks

Cultured milk drinks, such as Yakult®, Vitagen®, and Betagen, are probiotic-rich fermented dairy beverages containing beneficial microorganisms. Yakult® primarily contains *Lactobacillus paracasei* strain Shirota, while Vitagen® includes *Lactobacillus acidophilus* and *Lactobacillus paracasei*, and Betagen features *Lactobacillus paracasei* as its dominant probiotic strain. Among these, *L. paracasei* is particularly notable for its ability to withstand gastrointestinal conditions and contribute to gut microbiota homeostasis.

Their production involves the addition of skimmed milk powder and whey powder to enhance fermentation efficiency, texture, stability, and sensory properties, contributing to a high-quality final product. Cultured milk drinks have a relatively low viscosity compared to traditional fermented dairy products like yogurt. Therefore, controlling the total solids content in the milk base to maintain a smooth and uniform consistency. Skimmed milk powder increases the non-fat solids, which improves the body and mouthfeel of the drink, while whey powder contributes to better emulsion stability and reduces whey syneresis due to its protein content (Akal and Yetisemiyen, 2016.; Arab et al., 2023). These ingredients are important for preventing sedimentation and ensuring the even distribution of probiotic bacteria.

Furthermore, the presence of milk proteins and minerals in skimmed milk powder and whey powder can enhance cell viability and probiotic stability during fermentation and storage (Alsaleem et al., 2023). The high lactose content in whey powder also provides an additional fermentable sugar source. As a result, it promotes bacterial metabolism and leads to more efficient lactic acid production (Zeng et al., 2023).

These beverages are characterized by a mildly tangy taste due to microbial fermentation as lactose is metabolized into lactic acid. To enhance palatability, these beverages often contain added sugars and fruit flavours, making them sweeter than traditional fermented dairy products such as kefir and plain yogurt.

2.2.1.1 Yakult®

Yakult® is a Japanese sweetened probiotic milk beverage fermented with bacteria strain *Lacticaseibacillus casei* Shirota. The name "Yakult" originates from the Esperanto word "jahurto", meaning "yogurt"(Yakult Honsha Co., Ltd., 2019). This reflect the brand’s global vision through the use of Esperanto which is the world’s most widely spoken constructed international auxiliary language (Li, 2003). This beverage is widely recognized for its ability to support gut health by promoting a balanced intestinal microbiota through the presence of live probiotic bacteria. Regular consumption may contribute to improved digestion, enhanced immune function, and overall gastrointestinal well-being (Chen et al., 2019).

Yakult® is formulated using ingredients, including sugar, skimmed milk powder, glucose, and vitamin D, which serve as nutrient sources for both the probiotic bacteria and the consumer. The fermentation process allows *L. paracasei* Shirota to thrive, ensuring a high concentration of live bacteria capable of surviving stomach acid and reaching the intestines, where they exert beneficial effects. Moreover, Yakult® Ace Light is available as a lower-sugar alternative, catering to consumers seeking a reduced sugar intake while still benefiting from its probiotic properties.

2.2.1.2 Vitagen®

Vitagen® is a Malaysia's No. 1 cultured milk beverage made from fermented skimmed milk and contains live probiotic cultures, primarily *Lactobacillus acidophilus* and *Lactobacillus casei* (Vitagen Malaysia, 2022). These probiotic strains are known for their ability to survive stomach acid and promote a healthy gut microbiota, contributing to improved digestion and overall gastrointestinal health (Vitagen Malaysia, 2024). Similar to Yakult®, Vitagen® is formulated to support gut health by introducing beneficial bacteria into the digestive system.

Unlike Yakult®, which comes in a single formulation, Vitagen® is available in multiple flavours, including grape, apple, orange, and original as well as less-sugar version. Besides, it also offered in both regular and less sugar versions. These variations provide consumers with more options to suit their preferences while still delivering the health benefits associated with probiotic consumption.

2.2.1.3 Betagen

Betagen is a Thai probiotic dairy drink made from fermented skimmed milk, sugar, and live probiotic cultures, primarily *Lactobacillus casei*. It is designed to support digestive health by promoting a balanced gut microbiota, aiding in digestion, nutrient absorption, and immune function (Betagen Co., Ltd., n.d.).

Unlike Yakult® and Vitagen®, which are typically sold in small bottles, Betagen comes in various bottle sizes, including larger options suitable for multiple servings. It also comes in multiple flavours, including Betagen Fat 0% (a fat-free variant), Betagen Light (a lower-sugar option), and flavoured varieties such as strawberry, pineapple, and orange. These options allow consumers to choose a product that best suits their dietary preferences while still benefiting from its probiotic properties.

2.2.2 Yogurt Drinks

Yogurt drinks are fermented dairy beverages which offering probiotic functionality, improved digestibility and convenient consumption. They are produced by fermenting milk with live cultured, including *Bifidobacterium*, *L. paracasei*, *L. acidophillus, L. bulgaricus and S. thermophilus* (Tabasco et al., 2007). During fermentation, the bacterial cultures convert lactose into lactic acid through glycolysis, leading to a reduction in pH and the coagulation of milk proteins, primarily casein. This acidification alters the rheological and textural properties of the milk, producing a naturally thick and creamy consistency characteristic of yogurt (Jaros and Rohm, 2003). Unlike conventional yogurt, where coagulation results in a semi-solid gel structure, yogurt drinks undergo mechanical agitation post-fermentation to disrupt the gel network (Lee and Lucey, 2010). This step is followed by dilution with water, milk, or fruit juice, which reduces viscosity and transforms the product into a pourable or drinkable consistency. The choice of diluent can influence the nutritional profile, flavour, and sensory attributes of the final product. The formulations may also incorporate stabilizers such as pectin to maintain homogeneity and prevent phase separation during storage.

2.2.2.1 Farm Fresh®

Farm Fresh® yogurt drink is a Malaysian probiotic dairy beverage produced from fresh yogurt, fermented with live probiotic cultures such as *Lactobacillus acidophilus* and *Bifidobacterium* spp.. It is formulated to support gut health by promoting a balanced intestinal microbiota and enhancing digestive function.

A distinguishing feature of Farm Fresh® yogurt drink is its use of fresh yogurt sourced directly from its own farms to ensure a high-quality ingredient and a natural, rich flavour profile (Farm Fresh Berhad, n.d.). Farm Fresh® yogurt drink contains no preservatives and is available in multiple flavours, including natural, mango, strawberry, and mixed berries, catering to a wide range of consumer preferences.

2.2.2.2 Lactel

Lactel yogurt drink is a flavoured probiotic dairy beverage produced by Lactalis Malaysia, formulated to deliver both digestive health benefits and a rich, indulgent taste experience. It is made from solid cow's milk and contains live probiotic cultures, including *Bifidobacterium*, *Lactobacillus acidophilus*, *L. paracasei*, *L. bulgaricus*, and *S. thermophilus*, which contribute to gut health and improved digestion. Lactel yogurt drink is positioned as a premium yogurt drink by offering a creamier texture and enriching with real fruit juice for enhanced flavour (Lactalis Group, 2023). It is available in a variety of fruit-infused flavours, including natural, passion fruit, and strawberry, catering to diverse consumer preferences.

2.2.2.3 Yobick

Yobick yogurt drink is a Japanese-style probiotic dairy beverage known for its light, smooth texture and mildly sweet taste. Produced by Pokka, it is formulated to support digestive health by incorporating live probiotic cultures, which help maintain a balanced gut microbiota and aid digestion. Unlike traditional yogurt drinks, Yobick has a lighter consistency and a refreshing, less tangy flavour, making it a popular choice among consumers who prefer a milder taste. Yobick yogurt drink is available in various flavours, including Original, Sakura and Fuji apple to cater to different taste preferences. Besides, Yobick Lite+ is marketed as a low-calorie option, appealing to health-conscious consumers looking for a refreshing yet functional yogurt drink (Yobick Philippines, n.d.).

2.2.3 Differences Between Cultured Milk Drinks and Yogurt Drinks

One key difference between cultured milk drinks and yogurt drinks is the type of milk base used. Cultured milk drinks are typically made by mixing skimmed milk powder with warm water (Shabbir et al., 2023). In contrast, yogurt drinks are produced from fresh liquid milk that undergoes direct fermentation, leading to a naturally developed texture and consistency (Aryana and Olson, 2017). This difference affects the texture and mouthfeel, with cultured milk drinks being smoother and more fluid. Meanwhile, yogurt drinks develop a thicker consistency due to the coagulation of milk proteins during fermentation.

The probiotic content also varies between these two fermented milk drinks. Cultured milk drinks, such as Yakult®, typically contain a single bacterial strain, *Lactobacillus paracasei*, with a probiotic count of at least 1×10^6 CFU per 100 mL. In comparison, yogurt drinks generally have a higher concentration of probiotics, ranging from 10^6 to 10^9 CFU/ mL, due to the presence of a diverse range of beneficial bacteria (Wang et al., 2024). The fermentation process involves multiple lactic acid bacteria (LAB) strains, including *Bifidobacterium*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (Nyanzi, Jooste and Buys, 2021).

Table 2.2: Ingredients and probiotic strains listed on the packaging nutritional facts of cultured milk and yogurt drink brands.

Fermented Drink	Brands	Ingredients	Probiotic Strains
Cultured Milk	Yakult®	Sugar, Skimmed milk powder, Glucose, Vit D, Probiotic strain	*Lactocaseibacillus paracasei* Shirota strain
	Vitagen®	Dietary fibre (Polydextrose, Inulin prebiotics), Sucrose, Fructose, Milk solids, Permitted stabiliser and Emulsifier, Glucose, Permitted flavouring, Lactic acid, Probiotics	*Lactobacillus acidophilus* and *Lacticaseibacillus casei*
	Betagen	Recombined milk 50% (Skimmed milk powder, Whey powder), Water 37.66%, Sugar 12%, Stabilizer (Pectin), Probiotic, Nature identical flavour	*Lactobacillus paracasei*
Yogurt Drink	Farm Fresh®	Cow's milk, Sugar, Live Culture & Contains Stabiliser (Pectin) as Permitted Food Conditioner	*Bifidobacterium*, *Lactobacillus acidophillus*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*
	Lactel	Milk solid (Cow's milk), Sugar, Stabilizer, Mixed live culture	*Streptococcus thermophilus* and *Lactobacillus bulgaricus*
	Yobick	Water, Sugar, Skim milk powder, Non-dairy creamer, Acidity regulator (E330, E270), Soybean polysaccharide, Flavouring, Pasteurized yogurt powder (Whey, Probiotics), Honey, Inulin (0.06g/ 100g) and Colouring (e150a)	*Streptococcus thermophilus* and *Lactobacillus bulgaricus*

2.3 Physicochemical Properties

Physicochemical properties refer to the physical and chemical attributes of a substance that influence its behaviour, structure, and interactions with the environment. These properties encompass both intrinsic characteristics, such as molecular composition and reactivity, and extrinsic factors, like temperature and pressure (Igual and Martínez-Monzó, 2022). In the context of fermented dairy products, physicochemical properties are important in determining the quality, consistency, and sensory attributes of the product. The key factors, including pH, titratable acidity (TTA), total soluble solids (TSS), and colour, directly impact product stability, texture, flavour as well as mouthfeel, which in turn influence consumer acceptance and preferences. Control over these properties is essential for ensuring product uniformity, extending shelf life, and maintaining the desired sensory characteristics throughout the distribution and consumption process.

2.3.1 pH and Total Titratable acidity (TTA)

Fermented beverages typically exhibit low pH and high total titratable acidity (TTA) due to the metabolic activities of fermentative microorganisms, primarily lactic acid bacteria, yeasts, and acetic acid bacteria. According to Romão et al. (2024), the pH of fermented milk beverages generally falls below 4.0. This is attributed to the production of acidic byproducts during fermentation, such as lactic acid, which increase the hydrogen ion concentration and subsequently lower the pH (Matela, Pillai and Thamae, 2019). The accumulation of lactic acid is also essential for the development of the characteristic thickened texture and consistency of fermented milk products, as it induces coagulation of milk proteins, particularly casein to form a gel-like structure (Chen et al., 2016).

Total titratable acidity (TTA) refers to the overall concentration of both dissociated and undissociated acids in a beverage. TTA is determined through titration with a strong base, sodium hydroxide (NaOH), to a predetermined endpoint (Tyl and Sadler, 2017). Unlike pH, which measures only the concentration of free hydrogen ions, TTA provides a comprehensive assessment of the total acid load, including weak acids that do not fully ionize. The accumulation of organic acids during fermentation increases TTA and contributes to the formation of various flavour compounds, which play a key role in defining the sensory profile of the final product.

2.3.2 Total Soluble Solids (TSS)

Total soluble solids (TSS) are a key parameter in fermented milk beverages, representing the concentration of dissolved components, primarily total sugars, with smaller contributions from soluble proteins, amino acids, and other organic compounds (Hadiwijaya et al., 2020). TSS is typically expressed in degrees Brix (°Brix), which is determined based on the refractive index of the solution, defined as the ratio of the speed of light in a vacuum to its speed as it passes through the sample (Hadiwijaya et al., 2020). A higher TSS value indicates a more concentrated solution, as the increased density of dissolved solids reduces the speed of light transmission. Besides, high TSS the enhances viscosity, texture, and creaminess of fermented milk drinks due to the greater total solids content (Bista et al., 2020).

Sugars present in TSS serve as a key energy source for lactic acid bacteria (LAB) in order to help maintain microbial activity throughout fermentation and storage (Zheng et al., 2024). During fermentation, LAB break down lactose and other sugars into organic acids, lowering the pH and creating the characteristic sour taste. To balance the acidity and improve overall taste, residual sugars or added sweeteners are often necessary to enhance sweetness perception and consumer acceptance.

2.3.3 Colour

Colour is a critical physicochemical property of fermented milk beverages as it directly influences consumer perception of taste, aroma, and overall flavour profile. It also plays a significant role in appetitive behaviours, with visual cues affecting perceptions of freshness, sweetness, and product identity (Spence, 2016). As a result, variations in colour intensity can alter consumer expectations and sensory evaluation of the product.

The objective assessment of colour in fermented milk beverages is typically conducted using colorimetry, with the CIE Lab colour system as the standard method for quantification (Milovanovic et al., 2020). This system measures colour based on three parameters: L* (lightness), a* (red-green spectrum), and b* (yellow-blue spectrum). The L* value indicates brightness, where higher values correspond to a whiter appearance, often associated with freshness and high-quality dairy products (Karlsson et al., 2019). The a* value quantifies shifts between red and green, while the b* value measures variations along the yellow-blue axis. Standardized colour measurement allows producers to maintain batch-to-batch consistency in appearance, ensuring product quality, enhancing consumer appeal, and supporting marketability (Chudy et al., 2020).

2.4 Antioxidant Properties

Antioxidants play a critical role in mitigating oxidative stress, a condition that arises due to an imbalance between the production of reactive oxygen species (ROS) and the body's capacity to neutralize their deleterious effects (Chandimali et al., 2025). Oxidative stress is implicated in cellular damage, affecting key biomolecules such as lipids, proteins, and nucleic acids. Antioxidants counteract ROS through various mechanisms, including free radical scavenging, electron donation, hydrogen atom transfer, and the inhibition of oxidative enzyme activity (Shahidi and Zhong, 2015). These protective functions are fundamental in preventing oxidative damage, which is closely linked to aging and the pathogenesis of numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer (Chaudhary et al., 2023).

Among the diverse classes of antioxidants, phenolic compounds are particularly noteworthy due to their strong redox potential. These structurally diverse compounds include flavonoids, phenolic acids, tannins, and lignans, each contributing to antioxidant activity through distinct molecular interactions (García-Pérez et al., 2023). Phenolic compounds are widely present in plant-based foods and functional ingredients. For example, they are abundant in fruits, vegetables, whole grains, tea, coffee, medicinal plants, and fermented products, making them essential constituents of a health-promoting diet (Delgado, Gonçalves and Romano, 2023). Their consumption has been associated with a reduction in systemic inflammation, improved cardiovascular function, and neuroprotective effects, further reinforcing their significance in functional foods and therapeutic applications (Tungmunnithum et al., 2018).

In addition, phenolic compounds contribute to cellular homeostasis by modulating intracellular signalling pathways involved in oxidative stress and inflammation. Notably, they activate nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of antioxidant defence mechanisms, while simultaneously suppressing pro-inflammatory mediators (Kim, Cha and Surh, 2010). Through these molecular interactions, phenolic compounds not only reduce oxidative damage but also influence cellular responses that mitigate the risk of chronic diseases.

Given their broad-spectrum health benefits and natural abundance, phenolic antioxidants have garnered increasing interest in the fields of nutrition, pharmacology, and functional food development. Ongoing research continues to explore their molecular mechanisms and therapeutic potential, highlighting their role as valuable bioactive compounds in disease prevention and health promotion.

2.4.1 Gallic Acid

Gallic acid (GA), or 3,4,5-trihydroxybenzoic acid, is one of the simplest naturally occurring phenolic compounds found in plants. In its pure form, GA appears as white or light brown needle-like crystals or powder, with a melting point between 235 and 240°C, at which it undergoes decomposition (National Center for Biotechnology Information, 2004). It has a molecular formula of $C_7H_6O_5$ and a molecular weight of 170.12 g/mol. As shown in **Figure 2.4**, GA consists of a tri-hydroxylated benzene ring, with hydroxyl (-OH) groups at the 3, 4, and 5 positions, and a carboxyl (-COOH) functional group at the 1 position (Hadidi et al., 2024). Due to its phenolic and carboxylic acid groups, GA is classified as an organic acid with a single benzene ring structure. Besides, GA is structurally unstable as it loses crystallized water when heated to 100–120 °C and releasing carbon dioxide at temperatures above 200°C (Bai et al., 2021).

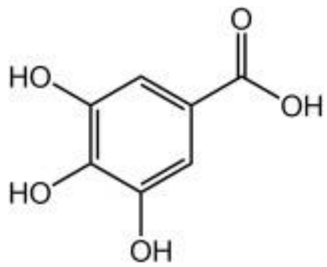

Figure 2.3: Structure of gallic acid (Hadidi et al., 2024).

Among polyphenolic compounds, GA is a low-molecular-weight tri-phenolic compound recognized for its strong antioxidant and anti-inflammatory properties (Badhani, Sharma and Kakkar, 2015). It also exhibits a wide range of pharmacological activities, including anti-tumor, antibacterial, anti-diabetic, anti-obesity, antimicrobial, and cardioprotective effects (Kahkeshani et al., 2019). These bioactive properties have contributed to increasing interest in GA's potential applications in medicine, functional foods, and therapeutic formulations.

2.4.2 Quercetin

Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a flavanol-type flavonoid that serves as the structural backbone for many other flavonoids. As shown in **Figure 2.5**, quercetin is characterized by a C6-C3-C6 skeleton, comprising two benzene rings linked by an oxygen-containing pyrone ring, a defining feature of flavonoids (Malaguti, Angeloni and Hrelia, 2013). The presence of five hydroxyl (-OH) groups contributes to its strong antioxidant, anti-inflammatory, and metal-chelating properties, which are essential to its biological activity (Qi et al., 2022).

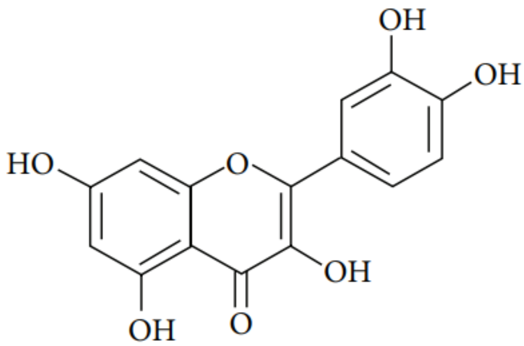

Figure 2.4: Structure of quercetin (Malaguti, Angeloni, and Hrelia, 2013).

Quercetin and its derivatives exhibit diverse pharmacological effects, making them valuable in functional foods, nutraceuticals, and therapeutic applications.

Notably, quercetin demonstrates anticancer properties by inhibiting human cathepsin B, a cysteine protease implicated in tumour progression, metastasis, and tissue invasion (Magar and Sohng, 2019). Additionally, it possesses cardioprotective, neuroprotective, and antimicrobial properties, reinforcing its potential role in preventing and managing oxidative stress-related diseases (Aghababaei and Hadidi, 2023).

2.4.3 Ascorbic Acid

Ascorbic acid (AA), commonly known as Vitamin C, is widely recognized for its potent antioxidant properties. According to the National Center for Biotechnology Information (2011), AA has a molecular formula of $C_6H_8O_6$ and a molecular weight of 176.12 g/mol. As shown in **Figure 2.6**, AA consists of a six-carbon lactone ring with enolic hydroxyl (-OH) groups at the 2 and 3 positions, which contribute to its strong reducing capacity (FEEDAP, 2013).

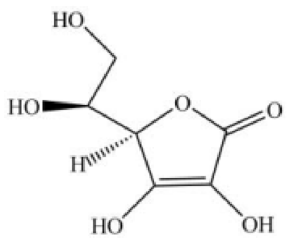

Figure 2.5: Structure of ascorbic acid (FEEDAP, 2013).

In its pure form, AA appears as a white to slightly yellow crystalline powder with a melting point of approximately 190–192 °C, at which it undergoes decomposition. It is highly sensitive to heat, light, and oxygen, which can lead to degradation and a loss of bioactivity upon prolonged exposure (FEEDAP, 2013). As a water-soluble vitamin, AA functions as a crucial antioxidant, protecting cells from oxidative stress and contributing to various metabolic processes (Gęgotek and Skrzydlewska, 2022).

As an essential nutrient, AA plays a critical role in numerous physiological functions, including collagen biosynthesis, immune defence, and iron absorption (Traber and Stevens, 2011). Furthermore, it exhibits pharmacological properties such as anti-inflammatory, neuroprotective, and wound-healing effects. Due to its ability to neutralize reactive oxygen species, AA has been extensively studied for its potential in preventing chronic diseases, particularly cardiovascular disorders and neurodegenerative conditions (Chambial et al., 2013).

2.4.4 Mechanisms Action of Antioxidants

Antioxidants function primarily through two fundamental mechanisms: (i) Hydrogen Atom Transfer (HAT) and (ii) Single Electron Transfer (SET). The HAT mechanism involves the direct donation of a hydrogen atom from the antioxidant to a free radical, neutralizing its reactivity and preventing oxidative chain reactions (Tena, Martín and Asuero, 2020). This process depends on the bond dissociation energy of the antioxidant, making it effective in lipophilic environments such as biological membranes. The HAT-based method evaluates the antioxidant potential to scavenge reactive oxygen species (ROS) by assessing its ability to donate hydrogen atom as shown in the reaction below (Siddeeg et al., 2021):

$$ROO\bullet + AH/ArOH \rightarrow ROOH + A\bullet/ArO\bullet$$

In contrast, SET mechanism involves the transfer of a single electron from the antioxidant to the free radical, resulting in the reduction of the radical species (Bibi Sadeer et al., 2020). The efficiency of this mechanism is largely influenced by the redox potential of the antioxidant and its ionization potential, which the energy required to remove an electron (Liang and Kitts, 2014). A lower ionization potential facilitates easier electron abstraction, enhancing the antioxidant's ability to donate electrons and neutralize reactive species. SET-based reactions are more common in aqueous environments such as blood plasma. The chemical reactions involved in SET mechanism in antioxidants as shown in below (Hosseinzadeh et al., 2024):

$$ROO\bullet + AH/ ArOH \rightarrow ROO^{-} + AH\bullet^{+}/ ArOH\bullet^{+}$$

$$AH\bullet^{+}/ ArOH\bullet^{+} + H_2O \rightleftharpoons A\bullet/ ArO\bullet + H_3O^{+}$$

$$ROO^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O$$

Based on **Figure 2.6**, in most situations, these two reactions occur simultaneously**.** The predominant mechanism depends on multiple factors, including the antioxidant’s structure and solubility, the partition coefficient, and the polarity of the solvent (Liang and Kitts, 2014). These physicochemical properties influence whether an antioxidant favours hydrogen atom donation or electron transfer under specific environmental conditions (Siddeeg et al., 2021).

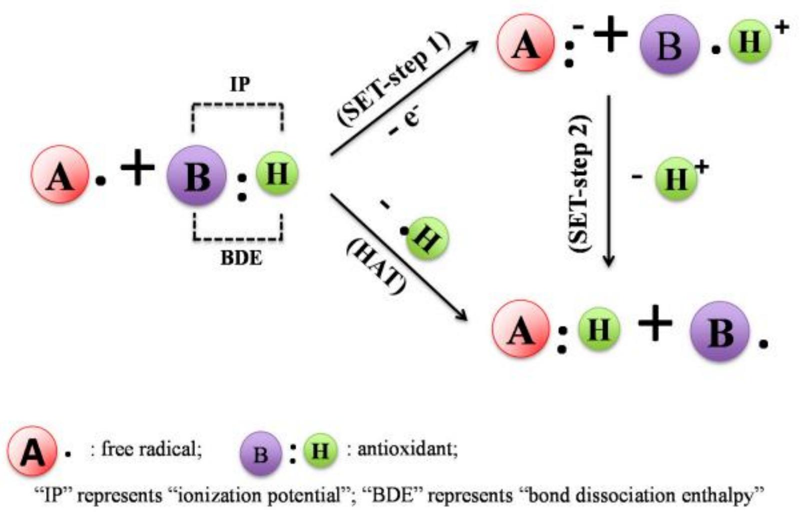

Figure 2.6: Antioxidants neutralize free radicals through SET and HAT mechanisms. In the SET mechanism, the ionization potential (IP) of the antioxidant serves as the critical energetic parameter influencing its electron-donating capacity and overall antioxidant activity. Conversely, in the HAT mechanism, the bond dissociation enthalpy (BDE) of the antioxidant is the key determinant and controlling its ability to donate a hydrogen atom and effectively scavenge free radicals (Liang and Kitts, 2014).

2.4.5 Antioxidant Analysis Assays

The antioxidant mechanisms are evaluated using various assays. The methods of evaluation of antioxidant activity must be rapid, reproducible, and require minimal sample quantities. Besides, these methods should minimize interference from the physical properties of the tested compounds to ensure accurate and reliable evaluation.

Total phenolic content (TPC) and total flavonoid content (TFC) assays measure the amount of antioxidants present in a sample to provide a quantifiable assessment of the phenolic and flavonoid compounds, respectively. In contrast, ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity assay are focus on the activity of these antioxidants. These assays evaluate the antioxidant's ability to reduce ferric ions or neutralize free radicals. The inclusion of both types of tests, amount-based (TPC and TFC) and activity-based (FRAP and DPPH) is important to ensure a comprehensive evaluation of both the concentration and functional effectiveness of antioxidants in the samples. This combination provides a balanced approach to understanding the antioxidant potential of the compounds tested.

2.4.5.1 Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) assay, commonly referred to as the Folin-Ciocalteu (FC) method, is a well-established technique for quantifying phenolic compounds in food products and beverages. The assay relies on the Folin-Ciocalteu reagent, a mixture of phosphomolybdic and phosphotungstic acids, which undergoes reduction in the presence of phenolic compounds (Martins et al., 2021). This reaction is driven by a single electron transfer (SET) mechanism, results in the formation of a blue-coloured chromophore, whose intensity is proportional to the phenolic content and is measured spectrophotometrically at 765 nm (Molole, Gure and Abdissa, 2022; Pérez, Dominguez-López and Lamuela-Raventós, 2023).

Although the TPC assay provides a useful estimation of antioxidant capacity, it is not entirely specific to phenolic compounds. Other reducing agents, such as ascorbic acid and reducing sugars (e.g., glucose, fructose, maltose), can also interact with the FC reagent, leading to potential overestimation of phenolic content (Lawag et al., 2023). Despite this limitation, the TPC assay remains a widely used, cost-effective, and reproducible method for evaluating the antioxidant potential of various food products and plant extracts.

2.4.5.2 Total Flavonoid Content (TFC)

The Total Flavonoid Content (TFC) assay is a spectrophotometric method used to determine the concentration of flavonoids in a sample, which are key contributors to antioxidant activity. This assay is commonly performed using the aluminium chloride ($AlCl_3$) colorimetric method, where flavonoids complex with Al^{3+} ions to form a yellow chromophore (Shraim et al., 2021). The reaction follows a single electron transfer (SET) mechanism, wherein flavonoids donate electrons to the aluminium complex, leading to a measurable colour change at 415 nm (Erizal et al., 2024). The results are typically expressed in quercetin equivalents (QE) or catechin equivalents (CE), depending on the standard used.

2.4.5.3 Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method for assessing the total antioxidant activity of a sample based on its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction reaction forms a blue Fe^{2+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex, which exhibits maximum absorbance at 590–700 nm, depending on assay conditions. A higher absorbance value indicates a greater reducing power, which correlates with the antioxidant potential of the sample.

According to Santos-Sánchez et al. (2019), the FRAP assay is performed under acidic conditions (pH 3.6) to maintain iron solubility and optimize redox reactions. The reducing ability of a compound depends on its degree of hydroxylation and conjugation, which influence its capacity to donate electrons. Moreover, the FRAP assay operates based on the single electron transfer (SET) mechanism. This makes it particularly useful for evaluating antioxidants that donate electrons rather than transfer hydrogen atoms through the hydrogen atom transfer (HAT) mechanism.

2.4.5.4 DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely used method for evaluating the radical scavenging activity of antioxidants. DPPH is a stable free radical with an unpaired electron delocalized across the entire molecule, which prevents dimerization and allows it to remain stable in organic solvents (Kedare and Singh, 2011). It exhibits a deep violet colour in ethanol, with a maximum absorption at 517 nm when measured using a UV-visible spectrophotometer (Baliyan et al., 2022).

When antioxidants interact with DPPH, they donate either a hydrogen atom (HAT mechanism) or an electron (SET mechanism), resulting in the reduction of DPPH to DPPH-H (Gulcin and Alwasel, 2023). This reduction results in a colour change from violet to pale yellow, with the degree of discoloration directly proportional to the antioxidant concentration. Since it incorporates both SET and

HAT mechanisms, the DPPH assay provides a comprehensive assessment of antioxidant potential across different compounds.

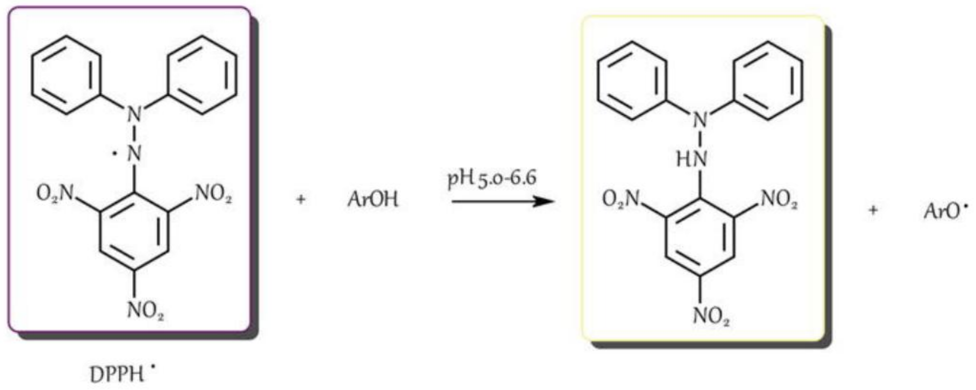

Figure 2.7: DPPH reduction by an antioxidant (Santos-Sánchez et al., 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Chemicals

Analytical-grade solvents and chemicals, including sodium hydroxide, 2% phenolphthalein, methanol, gallic acid, sodium carbonate, Folin Ciocalteu reagent, quercetin, sodium nitrate, aluminium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH) powder, L-ascorbic acid, ferrous sulphate, anhydrous sodium acetate, glacial acetic acid, ferric chloride hexahydrate, hydrochloric acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and distilled water were provided by the Department of Agricultural and Food Science, UTAR.

3.2 Samples and Storage

The two types of fermented milk, cultured milk (including brands such as Yakult, Vitagen, and Betagen) and yogurt drinks (including brands such as Farm Fresh, Lactel, and Yobick), were purchased from Lotus's Kampar. All samples were stored at 4°C in a refrigerator prior to analysis.

3.3 Analysis of Physicochemical Properties

3.3.1 pH and Total Titratable Acidity (TTA)

To determine the pH, a sample of 20 mL was placed into a beaker and the pH was determined using a calibrated pH meter.

Based on the method outlined by Yang et al. (2018), TTA was measured by diluting 5 mL of the sample with 5 mL of distilled water and titrating the mixture with 0.1 M NaOH. Phenolphthalein (2%, w/v in 96–99% ethanol) was served as the pH indicator, with the endpoint identified by the appearance of a pale pink colour. TTA was expressed as the concentration of lactic acid (g) per 100 mL of the fermented milk sample (Chaiyasut et al., 2017). The percentage of lactic acid was calculated using the equation provided below:

$$\text{Percentage of Lactic Acid (\%)} = \frac{\text{M} \times \text{V} \times \text{Eq. Wt of Lactic acid}}{\text{Sample Volume (mL)}} \times 100\%$$

Where,

M = Concentration of NaOH in M or mol/L

V = Volume of NaOH used in mL

Eq. Wt of lactic acid = 90.08 g/mol (Constant molecular weight of lactic acid)

3.3.2 Brix Analysis

The total sugar concentration in the sample was measured using a refractometer. The results were reported in °Brix and the corresponding temperature for each measurement was recorded.

3.3.3 Colour Analysis

The colour of the samples was analyzed using a chromameter with SCE mode. The measurements were based on the CIE-Lab colour space parameters, where L* represents lightness (0=black, 100=white), a* indicates the red-green spectrum (-a*=green, +a*=red), and b* reflects the blue-yellow spectrum (-b*=blue, +b*=yellow) (Andres et al., 2014). Liquid samples were placed in a petri dish for measurement. Prior to data acquisition, zero calibration was carried out using the device's baseline reflectance measurement taken from the surface, and white calibration was performed with the aid of the calibration cap to ensure measurement accuracy.

3.4 Analysis of Antioxidant Properties

3.4.1 Sample Preparation

For antioxidant analysis, the samples were mixed with methanol and subjected to centrifugation at 10,000 rpm for 10 min to obtain a clear solution (Wu et al., 2023). The mixing ratio of the samples remained consistent, but it varied for each specific test, as shown in the **Table 3.1**. The resulting clear solution was covered with aluminium foil and stored in a refrigerator in the absence of light until further testing.

Table 3.1: Mixing ratio of sample and methanol for antioxidant properties analysis.

	Antioxidant Properties Analysis			
	TPC	**TFC**	**FRAP**	**DPPH**
Sample Volume (µL)	500	1000	1000	2500
Methanol Volume (µL)	4500	4000	4000	2500
Total Volume (µL)	5000	5000	5000	5000
Dilution Factor	10	5	5	2

3.4.2 Total Phenolic Content (TPC) Analysis

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu assay with minor modifications, as described by Liang et al. (2024). A total of 0.2 mL of the sample was mixed with 0.8 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent. The mixture was allowed to react at room temperature for 3 minutes before the addition of 0.3 mL of 20% (w/v) sodium carbonate solution. After thorough mixing, the solution was incubated in the dark at room temperature for 2 hours. Distilled water served as the blank. Subsequently, 200 µL of the reaction mixture was transferred into individual wells of a 96-well microplate, and absorbance was recorded at 765 nm using a microplate reader.

A standard curve was constructed using gallic acid concentrations ranging from 0 to 50 µg/mL. Results were expressed as milligrams of gallic acid equivalents per milliliter of sample (mg GAE/mL).

3.4.3 Total Flavonoid Content (TFC) Analysis

The total flavonoid content (TFC) was assessed using the aluminium chloride colorimetric assay, with slight modifications to the method reported by Tang et al. (2023). Specifically, 0.15 mL of 5% (w/v) sodium nitrate solution was added to 0.2 mL of the sample. After a 6-minute incubation, 0.15 mL of 10% (w/v) aluminium chloride solution was introduced, followed by another 6-minute incubation period. Subsequently, 0.1 mL of 10% (w/v) sodium hydroxide solution was added, and the mixture was vortexed to ensure uniformity. The reaction mixture was then incubated in the dark at room temperature for 30 minutes. Distilled water was used as the blank control. After incubation, 200 µL of the final solution was transferred into wells of a 96-well microplate, and absorbance was measured at 510 nm using a microplate reader. A standard curve was generated using quercetin at concentrations ranging from 0 to 500 µg/mL. TFC values were expressed as milligrams of quercetin equivalents per milliliter of sample (mg QE/mL).

3.4.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was performed based on the modified procedure described by Hussein et al. (2022). The FRAP working reagent was freshly prepared by mixing 300 mM sodium acetate buffer, 10 mM

2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1 (v/v/v). A 0.2 mL aliquot of the sample was combined with 1.8 mL of the FRAP reagent, thoroughly mixed, and incubated in the dark at room temperature for 5 minutes. Distilled water was used as the blank. Following incubation, 200 µL of the reaction mixture was transferred into wells of a 96-well microplate, and the absorbance was measured at 593 nm using a microplate reader. A standard calibration curve was prepared using ferric sulphate heptahydrate at concentrations ranging from 0 to 600 mM. FRAP values were expressed as millimolar ferric sulphate equivalents per milliliter of sample (mM Fe(II)/mL).

3.4.5 DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity was evaluated following the modified protocol of Li et al. (2019). A 0.15 mM DPPH stock solution was freshly prepared by dissolving 5.915 mg of DPPH in 100 mL of 80% methanol. For the assay, 0.5 mL of sample was combined with 0.5 mL of the DPPH solution and mixed thoroughly using a vortex mixer. The mixture was then incubated in the dark at room temperature for 30 minutes. Methanol served as the blank control. Following incubation, 200 µL of the reaction mixture was transferred into individual wells of a 96-well microplate, and the absorbance was measured at 517 nm using a microplate reader. A standard calibration curve was constructed using ascorbic acid solutions at concentrations ranging from 0 to 20 µg/mL. The radical scavenging activity was calculated using the following equation:

$$\text{Radical Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where,

$A_{control}$ = Absorbance of DPPH radical solution without sample

A_{sample} = Absorbance of sample

3.5 Statistical Analysis

All results from this study were presented in triplicate as mean ± standard deviation (SD). IBM SPSS Statistics software (version 27) was used to perform a nested analysis of variance (ANOVA) to analyse the data. The nested design was necessary because the brands were different to each sample type, for instance, Brand Yakult®, Vitagen®, and Betagen were exclusive to cultured milk, while Brand Farm Fresh®, Lactel, and Yobick were only found in yogurt drinks. The lack of overlap between brands across sample types required the used of nested structure (NIST, 2021)

In this model, sample type (cultured milk drink and yogurt drink) was treated as a fixed factor, while brand was considered a random effect nested within sample type. This analysis included the main effect (sample type) and the interaction effect (Type*Brand(nested)). If the main effect of sample type was significant ($p \leq 0.05$), it indicated that cultured milk drinks and yogurt drinks differed significantly in their physicochemical and antioxidant properties. Conversely, a non-significant result ($p > 0.05$) would suggest no overall difference between the two types.

The interaction effect plays a critical role in determining how comparisons among brands should be interpreted. If interaction effect is significant ($p \leq 0.05$), it suggests that the effect of specific brand depends on the sample type, and in this case, interpreting the main effect separately becomes less meaningful. Therefore, the comparisons can be made directly across the six brands. However, if interaction effect is not significant ($p > 0.05$), the brand comparisons should be made within each sample type.

To further analyse significant differences detected in the nested ANOVA, Tukey's Honest Significant Difference (HSD) test was conducted as a post-hoc comparison. This test was applied to determine which specific brands differed significantly in their physicochemical and antioxidant properties.

To interpret the results, if Tukey’s HSD test revealed significant differences, alongside significant main and interaction effects, it suggests that a specific brand within its respective sample type exhibited notable variation in physicochemical and antioxidant properties.

CHAPTER 4

RESULTS

4.1 Physicochemical Properties Analysis

4.1.1 pH

The pH of cultured milk drinks and yogurt drinks ranged from 3.98 to 4.47, as shown in **Table 4.1**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the impact of brand on pH differs depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the pH when comparing 6 different brands. Among the tested samples, Yakult® cultured milk and Yobick yogurt drink had the lowest pH (3.98±0.044 and 4.05±0.031, respectively), with no significant difference between them. In contrast, Vitagen® cultured milk drink had the highest pH (4.47±0.006), showing a significant difference from all other samples, except Lactel yogurt drink.

Table 4.1: pH comparison in cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	Brand	pH
Cultured Milk Drink	Yakult®	3.98 ± 0.044^{A}
	Vitagen®	4.47 ± 0.006^{D}
	Betagen	4.35 ± 0.002^{C}
Yogurt Drink	Farm Fresh®	4.20 ± 0.006^{B}
	Lactel	4.40 ± 0.025^{CD}

	Yobick	4.05±0.031^{A}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

4.1.2 Total Titratable Acidity (TTA)

Total titratable acidity (TTA) of cultured milk drinks and yogurt drinks were determined and calculated. The TTA values of cultured milk drinks and yogurt drinks ranged from 4.92 g/L to 6.79 g/L, as presented in **Table 4.2**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p=0.005$), indicating that the impact of brand on TTA differs depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the TTA when comparing 6 different brands. Among the tested samples, Yakult® cultured milk had a significantly higher TTA (6.79±0.91) than Vitagen® cultured milk (4.92±0.45). However, there were no significant differences in TTA among the other four brands.

Table 4.2: Total Titratable Acidity (TTA) in cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	Brand	Total Titratable Acidity (g/L)
Cultured Milk Drink	Yakult®	6.79±0.91^{B}
	Vitagen®	4.92±0.45^{A}
	Betagen	5.37±0.45^{AB}
Yogurt Drink	Farm Fresh®	6.13±0.18^{AB}
	Lactel	6.31±0.18^{AB}
	Yobick	6.43±0.28^{AB}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

4.1.3 Total Soluble Solids (TSS)

The Brix values of cultured milk drinks and yogurt drinks ranged from 7.70 to 17.4°Bx, as shown in **Table 4.3**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the impact of brand on Brix differs depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the Brix when comparing 6 different brands. Among all tested samples, Betagen from cultured milk drink had the significantly highest Brix value (17.4±0.10). On the other hand, Yobick from yogurt drink showed the lowest Brix value (7.70±0.10), with a significant difference from other samples. Interestingly, all cultured milk samples showed Brix value that were significantly higher than yogurt drink samples.

Table 4.3: Brix level in cultured milk drinks and yogurt drinks across 6 brands at 22.2°C.

Sample Type	**Brand**	**Brix (°Bx)**
Cultured Milk Drink	Yakult®	15.3 ± 0.36^{D}
	Vitagen®	14.0 ± 0.10^{C}
	Betagen	17.4 ± 0.10^{E}
Yogurt Drink	Farm Fresh®	12.4 ± 0.12^{B}
	Lactel	12.8 ± 0.21^{B}
	Yobick	7.70 ± 0.10^{A}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of $\alpha=0.05$.

4.4 Colour

The colour attributes of cultured milk drinks and yogurt drinks were analysed based on L* (lightness), a* (red-green axis) and b* (yellow-blue axis) values showed in **Table 4.4**. A nested ANOVA revealed significant differences across all colour parameters ($p<0.001$) among the samples.

For lightness (L*), Lactel yogurt drink had the highest value (L*=62.08±0.05), making it the brightest sample, while Yakult® cultured milk was the darkest (L*=46.10±0.14). The red-green axis (a*) ranged from 2.13±0.01 (Betagen cultured milk) to -2.07±0.01 (Lactel yogurt drink). However, given the defined red-green axis limit is +128 to -127, these result vales are relatively small and do not significantly indicate pronounced redness or greenness in the samples (Liu et al., 2025). For yellowness (b*), Betagen cultured milk had a strongest yellow tone (b*=12.39±0.01), while Vitagen® cultured milk indicated the lowest yellow tone (b*=3.39±0.02). Since all b* values were positive, all samples exhibited a yellow hue with no noticeable blue tones. Overall, cultured milk drinks tend to be darker and more yellowish, whereas yogurt drinks are generally lighter and exhibit less yellow intensity.

Table 4.4: Colour analysis of cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	**Brand**	**Colour**		
		L*	**a***	**b***
Cultured Milk Drink	Yakult®	46.10±0.14^{A}	0.81±0.02^{E}	10.88±0.11^{E}
	Vitagen®	53.24±0.16^{C}	-1.14±0.01^{C}	3.39±0.02^{A}

	Betagen	50.00±0.01^{B}	2.13±0.01^{F}	12.39±0.01^{F}
Yogurt Drink	Farm Fresh®	60.10±0.04^{D}	-1.03±0.05^{D}	3.63±0.05^{B}
	Lactel	62.08±0.05^{E}	-2.07±0.01^{A}	8.16±0.01^{D}
	Yobick	53.51±0.02^{C}	-1.40±0.01^{B}	5.88±0.04^{C}

* Superscript letters denote statistically significant differences within the same column, as determined by Tukey's test at a significance level of α=0.05.

4.2 Antioxidant Properties Analysis

4.2.1 Total Phenolic Content (TPC)

The total phenolic contents of cultured milk drinks and yogurt drinks were determined using a standard curve of gallic acid (**Figure 4.1**). A satisfactory linear standard curve was obtained with regression correlation coefficient of R^2=0.9997. The equation derived from the standard curve is $y= 0.0138x + 0.1439$. The TPC of cultured milk drink and yogurt drink samples ranged from 0.234 to 1.084 mg GAE/mL, as shown in **Table 4.5**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the influence of brand on TPC varies depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the TPC when comparing 6 different brands. Among the samples tested, Farm Fresh® yogurt drink exhibited the highest TPC (1.084±0.006), followed by Lactel yogurt drink (0.874±0.002). These two yogurt drink brands contained significantly higher TPC values compared to all other brands.

Table 4.5: Total Phenolic Content (TPC) in cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	Brand	Total Phenolic Content (mg GAE/ mL sample)
Cultured Milk Drink	Yakult®	0.253±0.002^{A}
	Vitagen®	0.248±0.004^{A}
	Betagen	0.333±0.003^{B}
Yogurt Drink	Farm Fresh®	1.084±0.006^{D}
	Lactel	0.874±0.002^{C}
	Yobick	0.234±0.004^{A}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

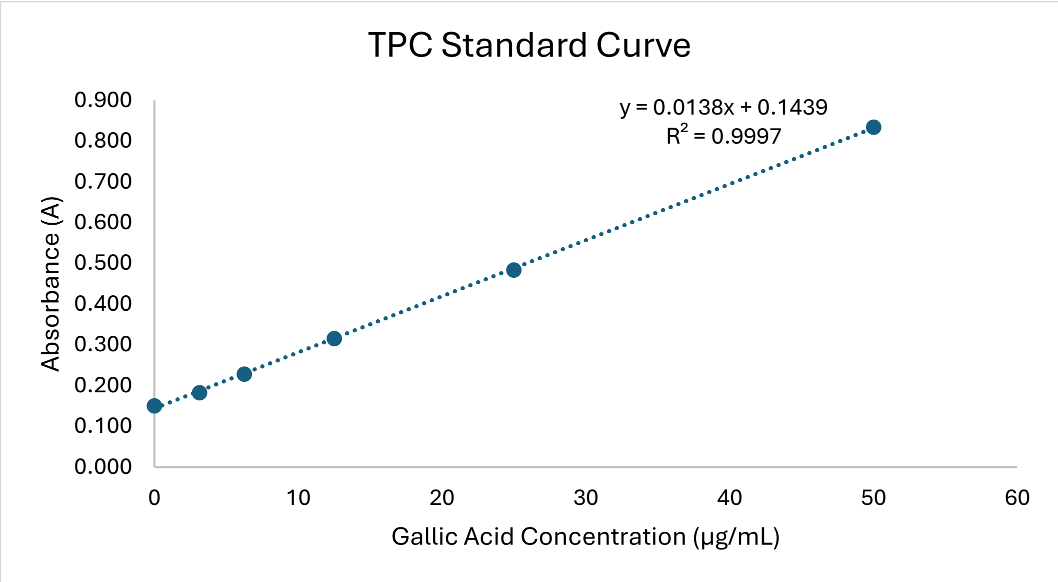

Figure 4.1: Standard curve of absorbance against gallic acid concentration (µg/mL) for TPC.

4.2.2 Total Flavonoid Content (TFC)

The total flavonoid contents of cultured milk drinks and yogurt drinks were determined using a standard curve of quercetin (**Figure 4.2**). A satisfactory linear standard curve was obtained with regression correlation coefficient of R^2=0.9913. The equation derived from the standard curve is y=0.0009x + 0.321. The TFC of cultured milk drink and yogurt drink samples ranged from 0.047 to 5.148 mg QE/mL, as shown in **Table 4.6**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the influence of brand on TFC varies depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the TFC when comparing 6 different brands. Among the samples tested, Farm Fresh® yogurt drink exhibited the highest TFC (5.148±0.071), followed by Lactel yogurt drink (4.478±0.079). These two yogurt drink brands contained significantly higher TFC values compared to all other brands. However, Yobick yogurt drink was identified as having the lowest TFC (0.047±0.047), with significance difference compared to all other sample drinks.

Table 4.6: Total Flavonoid Content (TFC) in cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	**Brand**	**Total Flavonoid Content (mg QE/ mL sample)**
Cultured Milk Drink	Yakult®	0.531 ± 0.004^{B}
	Vitagen®	0.413 ± 0.026^{B}
	Betagen	1.696 ± 0.034^{C}
Yogurt Drink	Farm Fresh®	5.148 ± 0.071^{E}
	Lactel	4.478 ± 0.079^{D}
	Yobick	0.047 ± 0.047^{A}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

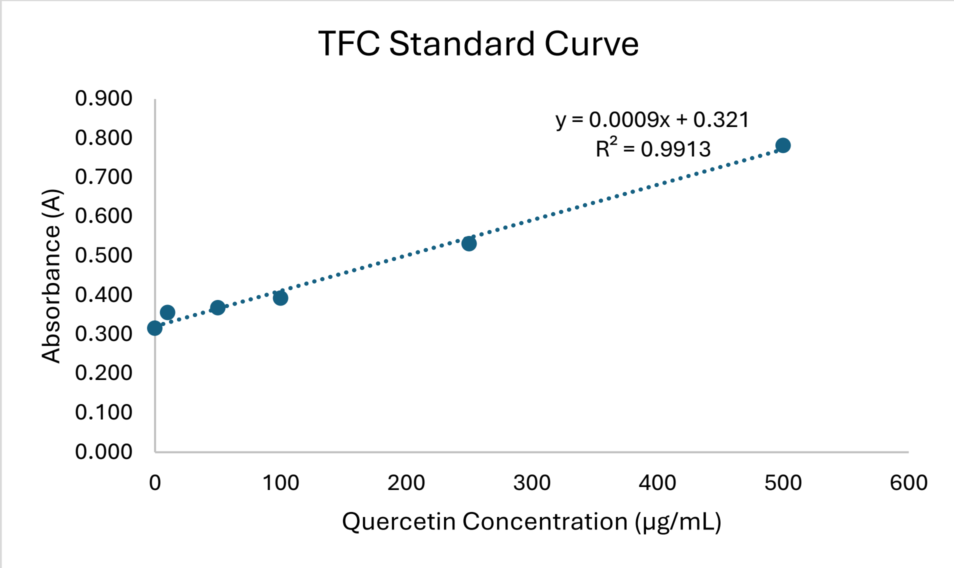

Figure 4.2: Standard curve of absorbance against quercetin concentration (µg/mL) for TFC.

4.2.3 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of cultured milk drinks and yogurt drinks were determined using a standard curve of ascorbic acid (**Figure 4.3**). A satisfactory linear standard curve was obtained with regression correlation coefficient of R^2=0.9927. The equation derived from the standard curve is y=0.001x + 0.1805. The FRAP of cultured milk drink and yogurt drink samples ranged from 308.33 to 3012.50 mM Fe (II) per mL sample, as shown in **Table 4.7**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the influence of brand on FRAP varies depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the FRAP when comparing 6 different brands. Among the samples tested, Lactel yogurt drink exhibited the significantly highest FRAP (3012.50±12.37), followed by Betagen cultured milk drink (1807.50±10.61). On the other hand, Yobick yogurt drink was determined show the lowest FRAP (308.33±52.04) among all sample drinks.

Table 4.7: Ferric reducing antioxidant power (FRAP) of cultured milk drinks and yogurt drinks across 6 brands.

Sample Type	**Brand**	**Ferric Reducing Antioxidant Power (mM Fe (II)/ mL sample)**
Cultured Milk Drink	Yakult®	1310.00 ± 14.14^{B}
	Vitagen®	1441.67 ± 10.41^{B}
	Betagen	1807.50 ± 10.61^{C}
Yogurt Drink	Farm Fresh®	1462.50 ± 17.68^{B}
	Lactel	3012.50 ± 12.37^{D}
	Yobick	308.33 ± 52.04^{A}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

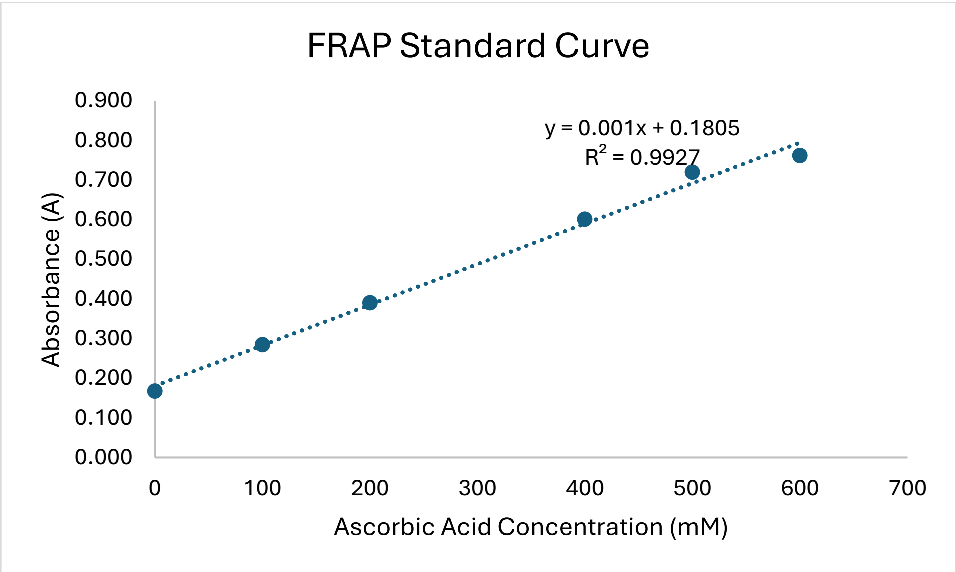

Figure 4.3: Standard curve of absorbance against ascorbic acid concentration (mM Fe (II)/mL) for FRAP.

4.2.4 DPPH Radical Scavenging Activity (RSA)

The DPPH activities of cultured milk drinks and yogurt drinks were shown in terms of radical scavenging activity (RSA), ranging from 8.644% to 50.21%, as shown in **Table 4.8**. A nested ANOVA revealed a significant interaction effect between sample type and brand ($p<0.001$), indicating that the influence of brand on RSA varies depending on the sample type. In other words, each brand, while nested within its sample type, has a significant effect on the RSA when comparing 6 different brands. Among the samples tested, Lactel yogurt drink exhibited the significantly highest RSA (50.21±0.45), followed by Yakult® cultured milk drink (35.55±0.84). However, Farm Fresh® yogurt drink was determined as having the lowest RSA (8.644±0.88) among all sample drinks.

Table 4.8: Radical scavenging activity of cultured milk drinks and yogurt drinks across 6 brands based on DPPH assay.

Sample Type	**Brand**	**Radical Scavenging Activity (%)**
Cultured Milk Drink	Yakult®	35.55 ± 0.84^{E}
	Vitagen®	20.25 ± 0.99^{B}
	Betagen	27.26 ± 0.92^{C}
Yogurt Drink	Farm Fresh®	8.644 ± 0.88^{A}
	Lactel	50.21 ± 0.45^{F}
	Yobick	30.79 ± 1.01^{D}

* Superscript letters denote statistically significant differences among 6 samples, as determined by Tukey's test at a significance level of α=0.05.

CHAPTER 5

DISCUSSIONS

5.1 Physicochemical Properties

5.1.1 pH and TTA

The pH and total titratable acidity (TTA) of fermented milk beverages serve as key indicators of acidity, influenced by the metabolic activity of lactic acid bacteria (LAB). The pH measures the concentration of free hydrogen ions (H^+), while TTA reflects the total acid content, including both dissociated and undissociated organic acids (Lobit et al., 2002). An inverse relationship can be observed between pH and TTA in fermented dairy products. A lower pH value corresponds to a higher TTA, indicating greater acidity due to a higher concentration of organic acids in the beverage.

From the results (**Table 4.1 and 4.2**), Vitagen® cultured milk exhibited the highest pH and lowest TTA among the tested samples, demonstrating a strong inverse correlation between these two parameters. This trend is expected, as a lower concentration of organic acids results in a higher pH. In contrast, Yakult® cultured milk had a significant lower pH and higher TTA than Vitagen® cultured milk, suggesting greater organic acid production and accumulation. These findings align with those of Nik Mohd Rosdy, Mohd Amin and Roslan (2023), who reported a similar trend in acidity levels, with Vitagen® having a higher pH of 4.12±0.02 and Yakult® a lower pH of 3.61±0.01. The variation in pH values between current work and that reported by literature may be attributed to differences in storage conditions and batch-to-batch variability.

Yakult® cultured milk had the highest acidity among the tested samples, reflected by lowest pH, due to the presence of *Lactobacillus paracasei* strain Shirota, which is highly efficient and active in fermenting lactose into lactic acid and other metabolites. *L. paracasei* metabolize lactose through glycolysis via the Embden-Meyerhof-Parnas (EMP) pathway, producing pyruvate, which is further converted into lactic acid (Noufeu et al., 2025). During fermentation, LAB strains also produce acetic acid, formic acid, or propionic acid, further lowering the pH (Abedi and Hashemi, 2020). In fact, fresh milk has a natural buffering system due to casein and minerals, such as calcium and phosphate, which initially resist pH changes. However, the buffering capacity is masked due to acid accumulation and leading to a steady decrease in pH.

The differences in pH of fermented milk beverages depend on fermentation parameters, including fermentation duration, temperature, and bacterial strain. For example, the extended fermentation durations and elevated temperatures, coupled with the presence of *Lactobacillus delbrueckii* subsp. *bulgaricus* T50 and *Streptococcus thermophilus* S10 can enhance acidogenesis and overall fermentation efficiency (Bai et al., 2024).

In the context of this study, Yakult® cultured milk and Yobick yogurt drink exhibited the lowest pH, with no significant different between the two. However, the fermentation parameter of these two products differs notably. For instance, Yakult® cultured milk ferments at 37°C for 6-7 days using the *Lactobacillus paracasei* strain Shirota (Shabbir et al., 2023). On the other hand, Yobick yogurt drink, which contains *S. thermophilus*, typically ferments at higher temperatures (42–45°C) for 4-6 hours (Yang et al., 2021).

The variations in fermentation conditions make Yakult® cultured milk require a longer fermentation duration to achieve the acidity levels observed in Yobick yogurt drink. For Yakult® cultured milk, the lower fermentation temperature and the absence of key acid-producing strains slow down acid production and necessitating a longer fermentation time. These differences highlight the importance of monitoring the fermentation parameters to ensure consistent pH and TTA levels for maintaining the quality of fermented milk drinks.

5.1.2 Total Soluble Solids (TSS)

Degrees Brix (°Bx) is a measure of the total soluble solids in a liquid, primarily representing sugar content, which is determined using refractive index measurements. A higher °Bx value indicates a greater concentration of dissolved solids, or a greater sugar content which then results in higher sweetness level.

In fermented milk drinks, sugar serves as an energy source for probiotics in order to maintain pH homeostasis, resist acid stress, and increase their chances of surviving the digestive process to reach the intestines alive (Corcoran et al., 2005).

Interestingly, the result show all cultured milk samples exhibited significantly higher Brix values compared to the yogurt drink samples. This can be explained by the differences in sugar content between the two types of beverages, as shown in table below:

Table 5.1: Sugar content of cultured milk drinks and yogurt drinks across 6 brands, based on nutritional information on packaging labels.

Sample Type	Brand	Sugar Content (%)
Cultured Milk Drink	Yakult®	13.3
	Vitagen®	12.6
	Betagen	14.0
Yogurt Drink	Farm Fresh®	9.10
	Lactel	10.5
	Yobick	4.80

As clearly indicated in **Table 5.1**, yogurt drinks contain lower sugar content than cultured milk drinks. This observation is consistent with the °Brix measurements presented in **Table 4.3**, where Betagen cultured milk, which had the highest sugar content, exhibited the highest °Brix value. Conversely, Yobick yogurt drink, with the lowest sugar content, recorded the lowest °Brix value among all tested samples.

The observed differences in sugar content between cultured milks and yogurt drinks can be attributed to the variations in microbial composition and fermentation strategies. Cultured milk products typically contain a single probiotic strain, which require higher sugar concentrations to support metabolic activity, maintain cellular stability, and extend product shelf life (Mendonça et al., 2022).

In contrast, yogurt drinks often incorporate multiple starter cultures, enabling synergistic interactions that enhance fermentation efficiency and reduce the need for excess sugar. In yogurt drinks, a cooperative interaction often occurs between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. For instance, *S. thermophilus* hydrolyses lactose into glucose and galactose and produces formic acid, which promotes the growth and acid production of *L. bulgaricus* (Yang et al., 2025). This mutualistic relationship enhances bacterial viability and stability, thereby reducing reliance on high sugar concentrations to sustain probiotic function and product quality.

5.1.3 Colour

The observed differences in colour parameters among the cultured milk drink and yogurt drinks due to the variations in ingredient composition, fermentation conditions, and the possible use of colour-modifying additives (Norazura Aila et al., 2020). The result in **Table 4.4** aligns with Hilali et al. (2011), who reported a temporal increase in L* (lightness) coupled with a chromatic shift toward green (reduced a*) and diminished yellowness (lower b*) in yogurt-based beverages.

One key factor influencing the lightness (L*) is milk fat content. Higher fat levels increase light scattering, resulting in a brighter, whiter appearance. From the result finding, yogurt drinks exhibited the higher L* value than cultured milk drinks, suggesting a higher fat content and effective homogenization in their formulation. The homogenization process disrupts fat globules, reducing their size and increasing the uniformity of light reflection, contributing to a brighter appearance in milk-based fermented drinks (Shao et al., 2023). Moreover, heat treatments during processing can also modify L* value by altering protein structures. Protein denaturation and interactions between β-lactoglobulin and κ-casein can further affect the drink's optical properties (Pan et al., 2022).

In terms of the b* value, which measures colour along the blue-to-yellow axis, all samples exhibited positive values, indicating a yellowish hue across the board. Overall, cultured milk drinks exhibited higher b* value than yogurt drinks. Within the cultured milk category, Vitagen® exhibited the lowest b* value, while Betagen recorded the highest b* value, reflecting a more intense yellow colour. This variation in yellowness correlates with the total sugar content in the drinks. As shown in **Table 4.3**, Betagen, which had the highest °Brix value, also exhibited the most pronounced yellowness. Higher sugar concentration in drink can alter absorption and scattering properties, intensifying warm tones like yellow (Firdausi, Sugito and Putri, 2018). Furthermore, sugar-rich dairy products subjected to heat treatment such as milk pasteurization can induce Millard reactions. This non-enzymatic browning process caused by the interactions between reducing sugars and milk proteins, ultimately contributes to slight yellowing in the final product (Shimamura and Ukeda, 2012).

Since the a* value (red-green axis) ranges from -127 to +127, the small a* values observed in the study are not considered significant in terms of redness or greenness. This is because animal milk naturally lacks red pigments. Unlike plant-based beverages, which contain anthocyanins or other red-coloured compounds, animal milk does not have significant amounts of these pigments to allow detection of redness from the samples.

The significant variation in L*, a*, and b* values across brands reflects the impact of distinct formulations and processing methods on the final product's colour characteristics. These differences highlight how manufacturing decisions, including fat content adjustments, homogenization efficiency, and ingredient selection, shaping the overall appearance of commercial cultured milk drinks and yogurt drinks.

5.2 Antioxidant Properties

5.2.1 Total Phenolic Content (TPC)

The results shown that the yogurt drink brands Farm Fresh® and Lactel exhibited significantly higher total phenolic content (TPC) compared to all other tested samples. This greater TPC in yogurt drinks compared to cultured milk drinks is due to the presence of multiple probiotic strains (Liu et al., 2019). Yogurt drinks typically contain a diverse range of bacterial cultures, whereas cultured milk drinks are generally fermented using a single strain, predominantly *Lactobacillus paracasei*.

Among the tested samples, Farm Fresh® had the highest TPC value (1.084 mg GAE/mL), which can be attributed to the presence of five live probiotic strains, including *Bifidobacterium spp.*, *Lactobacillus acidophilus*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*, contributing to the high metabolic activity that product large amount of TPC. Lactel, which exhibited the second-highest TPC (0.874 mg GAE/mL), contained mixed live cultures, such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

These diverse probiotic strains enhance metabolic activity, promoting the production of phenolic compounds through proteolysis. Fermentative microbes produce proteolytic enzymes, such as proteinases and peptidases, to hydrolyse milk proteins into peptides and free amino acids. Among these, amino acids with phenolic side chains, such as tyrosine and phenylalanine, contribute to the TPC values in fermented milk as the Folin–Ciocalteu assay reacts with phenolic hydroxyl groups present in tyrosine-containing peptides (Taşkın and Bağdatlıoğlu, 2020). Therefore, the presence of diverse probiotics in Farm Fresh® and Lactel yogurt drinks enhance proteolysis, resulting in greater release of phenolic hydroxyl groups and, consequently, higher TPC levels.

Furthermore, the acidification process that occurs during fermentation significantly influences the total phenolic content (TPC) by modifying the interactions between phenolic compounds and milk proteins. Under acidic conditions, milk proteins tend to aggregate and reduces the number of available binding sites for phenolic compounds (Anema, Lowe and Lee, 2004). This weakened interaction facilitates the release of polyphenols, thereby enhancing their extractability and bioavailability. According to Chen et al. (2022), fermentation promotes the release of epigallocatechin gallate (EGCG), a major polyphenol, from its bound state in milk. EGCG typically interacts with milk proteins such as casein through hydrogen bonding and hydrophobic interactions. However, as the pH decreases during fermentation, protein aggregation limits these binding sites, thereby disrupting protein–polyphenol complexes and enabling the liberation of EGCG into the surrounding medium. This reduction in binding not only improves the free availability of EGCG but also enhances its separation and detection during analytical procedures. In short, the TPC level is influenced by the degree of acidity as well as by the number and diversity of probiotic strains present.

5.2.2 Total Flavonoid Content (TFC)

Among the six fermented milk drinks analysed, Farm Fresh® (5.148 mg QE/mL) and Lactel (4.478 mg QE/mL) yogurt drinks exhibited significantly higher total flavonoid content (TFC), compared to the remaining four samples, consistent with their total phenolic content (TPC) results. The enhanced flavonoid concentration in yogurt drinks can be attributed to the use of multiple probiotic strains in their fermentation process, as mentioned in the TPC findings. Unlike cultured milk drinks that rely on a single strain, the presence of diverse microbial strains significantly contributes to greater enzymatic activity, facilitating the release and bioconversion of phenolic and flavonoid compounds.

Conversely, Yobick yogurt drink exhibited the lowest TFC (0.047 mg QE/mL) compared to all other tested samples. Unlike other two yogurt drinks that are made from cow's milk in solid (Farm Fresh®) and liquid (Lactel) forms with live culture strains, Yobick is produced using spray-dried yogurt powder containing probiotic cultures. Despite the fact that the yogurt powder contains two bacterial strains, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, spray drying adversely affects the survival and viability of these strains.

The dehydration or spray drying process of yogurt powder has a significant impact on both the viability and diversity of probiotic strains. Several factors influence the survival of these strains during spray drying, including inlet and outlet temperatures, nozzle pressure, material concentration, and the type of encapsulation material used (Wihansah et al., 2024).

For example, the exposure to elevated temperatures and reduced moisture content during this process leads to cell inactivation by causing structural and functional damage to essential cellular components such as DNA, RNA, proteins, lipids, membranes, and ribosomes (Russell, 2003). The loss of bound water at the bacterial cell surface is considered a major contributor to cell injury during spray drying. Among the most susceptible cellular structures is the cytoplasmic membrane, which becomes increasingly compromised under dehydration stress (Hlaing et al., 2017). Additionally, ribosomes and nucleic acids are particularly vulnerable to damage, primarily due to the leakage of Mg^{2+} ions from the heat compromised cell membrane, further impairing bacterial functionality and survival (Wihansah et al., 2024).

As a result, Yobick yogurt drink, despite containing two probiotic strains, still exhibited the lowest total flavonoid content among all 6 samples, even lower than that observed for cultured milk drinks, which use a single strain. This is because the total LAB count in rehydrated yogurt powder used for Yobick was lower than that in live cultures used to produce cultured milk drink (Yakult®, Vitagen® and Betagen) and the other two yogurt drinks (Farm Fresh® and Lactel).

5.2.3 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), indicating the reducing power of a substance. Among the tested fermented milk beverages, Lactel yogurt drink exhibited the highest FRAP value (3012.50 mM Fe^{2+}/mL), suggesting a superior antioxidant capacity. This result is attributed to the high protein content in cow's milk solids used in its formulation.

Milk solids, also referred to as non-fat dry milk, represent the dry components of milk excluding water. According to Dairy Management Inc. (2005), milk solids contain the highest protein content (35.1–36.2%) compared to other milk products used in the other sample drinks (**Table 5.2**).

Table 5.2: Average composition of milk ingredients in percentage (Dairy Management Inc (DMI), 2005).

Milk Product	Moisture	Fat	Protein	Carbohy-drate	Ash
Milk, whole	88.0	3.5	3.2	4.6	0.7
Sweetened condensed, whole milk	27.0	8.0	7.8	55.2	1.8
Evaporated, whole milk	74.0	7.5	6.5	9.8	1.4
Dry whole milk	25.0	26.7	26.3	38.4	6.1
Nonfat dry milk, noninstantized	3.2	0.8	36.2	52.0	7.9
Nonfat dry milk, instantized	4.0	0.7	35.1	52.2	8.0

During yogurt drink fermentation, lactic acid bacteria facilitate proteolysis, which break down milk proteins into smaller peptides with antioxidant activity through enzymatic hydrolysis. A higher protein concentration provides more substrate for proteolytic enzymes, resulting in a greater yield of bioactive peptides with strong electron-donating capacity to reduce ferric ions (Dhakal et al., 2024).

These peptides containing amino acids such as histidine, tyrosine, tryptophan, and cysteine exhibit strong antioxidant activity by donating electrons to reduce ferric ions (Kashung and Karuthapandian, 2025). Histidine, in particular, plays a dual role by contributing both to ferric ion reduction and overall radical scavenging activity due to its imidazole group. The imidazole group in histidine facilitates electron donation, making it a key factor in the redox reactions that improve the antioxidant potential of fermented milk products (Holeček, 2020). Furthermore, dipeptides and tripeptides, particularly those with histidine residues can also enhance FRAP values. Dipeptides with tyrosine (Tyr) and tryptophan (Trp) at the N-terminus exhibit the highest radical scavenging efficiency. Their activity is influenced by structural factors such as steric effects, hydrophobicity, and hydrogen bonding, which affect electron transfer and radical stabilization (Xu et al., 2024).

Therefore, the high protein content in Lactel's milk solids serve as a rich source of bioactive peptides. The ability of these peptides to donate electrons and neutralize reactive species enhances the overall reducing power of the beverage and contributes to the highest FRAP value among all samples.

5.2.4 DPPH Radical Scavenging Activity (RSA)

Consistent with the FRAP assay results, Lactel yogurt drink exhibited the highest radical scavenging activity (RSA) in the DPPH assay, indicating strong antioxidant potential. However, within the same category, Farm Fresh® showed the lowest RSA among the tested fermented milk beverages. This variation in

RSA can be attributed to differences in the protein content of their milk-based ingredients, as previously mentioned in the FRAP assay.

The DPPH assay is widely used method for evaluating antioxidant capacity of food product. A higher DPPH radical scavenging percentage indicates a stronger antioxidant activity. During the fermentation of yogurt drinks, lactic acid bacteria (LAB) hydrolyse milk proteins, particularly casein and whey proteins, into smaller bioactive peptides (Dhakal et al., 2024). These peptides exhibit strong antioxidant properties due to their ability to donate hydrogen atoms or electrons to neutralize free radicals such as DPPH radicals. This higher protein content provides a greater substrate for enzymatic hydrolysis, leading to an increased release of bioactive peptides.

The fermentation of Lactel yogurt drink is based on solid milk ingredients, which are known for their high protein content, whereas Farm Fresh® yogurt drink primarily uses liquid cow's milk. According to Dairy Management Inc. (2005), the protein content in liquid milk is approximately 3.2%, which is nearly ten times lower than that found in solid milk ingredients. This difference in protein concentration can influences the formation of bioactive peptides during fermentation and affect the overall RSA of the fermented milk beverages (Akbarian et al., 2022). As a result, Lactel yogurt drink exhibit stronger RSA in assays such as DPPH and FRAP than Farm Fresh®.

Besides, pH plays a critical role in determining the RSA of fermented milk beverages by influencing protein hydrolysis, peptide solubility, and stability (Tadesse and Emire, 2020). Based on **Table 4.1**, Farm Fresh® yogurt drink (pH=4.20) has a significantly lower pH compared to Lactel yogurt drink (pH=4.40). The higher pH of Lactel yogurt drink can contribute to more efficient protein hydrolysis during fermentation, as LAB exhibit high proteolytic activity within the optimal pH range of 4.8 to 5.2 (De Giori et al., 1985). In addition, most antioxidant peptides tend to remain highly soluble and stable within a moderately acidic range. Therefore, the relatively higher pH of Lactel yogurt drink supported the formation and stability of more bioactive peptides, resulting in greater antioxidant potential compared to the lower pH Farm Fresh® yogurt drink.

Interestingly, Farm Fresh® yogurt drink exhibited high TPC and TFC but demonstrated poor antioxidant activity, as indicated by its low FRAP and RSA values. This phenomenon can be attributed to the loss of optimal redox activity of polyphenolic compounds under low pH conditions of Farm Fresh® yogurt drink. For example, polyphenolic compounds, such as phenolic acids, undergo structural modifications under acidic conditions (approximately pH 4), which can reduce their antioxidant activity (Post et al., 2012). This decrease is primarily due to the protonation of phenolic hydroxyl groups, which diminishes their electron-donating capacity and weakening their ability to neutralize free radicals (Amorati et al., 2006).

As a result, although Farm Fresh® yogurt drink contains a higher abundance of phenolic and flavonoid compounds due to the presence of a greater number of probiotic strains, its low pH compromises the redox potential of these compound, thereby diminishing the antioxidant effectiveness and leading to lower RSA and FRAP values.

CHAPTER 6

CONCLUSION

In conclusion, this study successfully achieved its objectives of evaluating the physicochemical properties and antioxidant profiles of six commercially available fermented milk drinks. Based on the physicochemical analysis, Yakult® cultured milk exhibited the lowest acidity with having the highest total titratable acidity (TTA), contributing to its distinct sour taste. For total soluble solids, cultured milk drinks having the higher sugar content than yogurt drink, especially Betagen recorded the highest °Brix value, resulting in a sweeter flavour profile. Colorimetric analysis showed that cultured milk drinks appeared darker with a more yellowish hue, while yogurt drinks were comparatively lighter with less yellow intensity.

Regarding antioxidant properties, yogurt drinks generally possessed superior antioxidant content and activity compared to cultured milk drinks. Farm Fresh® yogurt drink demonstrated the highest TPC and TFC, indicating great antioxidant content. Meanwhile, Lactel yogurt drink showed the highest FRAP and RSA, suggesting superior antioxidant activity. These antioxidant properties were influenced by several key factors, including pH, the diversity and viability of probiotic strains, and the protein content of the milk base used in fermentation. Specifically, moderate acidity, the use of acid-producing and multiple probiotic strains, and a high-protein milk base were associated with enhanced antioxidant potential in fermented milk beverages.

From a consumer perspective, cultured milk drinks offer appealing sensory characteristics catering to different preferences. Yakult® may be favoured by those preferring a more acidic profile, while Betagen may appeal to consumers seeking a sweeter taste. For individuals prioritizing antioxidant intake, Farm Fresh® and Lactel yogurt drinks are recommended, as they incorporate multiple probiotic strains and demonstrate superior antioxidant activity, respectively. Besides, these yogurt drinks offer enhanced health benefits while containing comparatively lower sugar content than cultured milk drinks.

The limitation of the present study is its narrow focus on a limited number of commercial brands, all which were restricted to cultured milk drinks and yogurt drinks of natural flavour. This limited scope may constrain the generalizability of the findings as it does not capture the viability introduced by different flavour additives commonly found in the broader market.

Future research should aim to expand the range of brands and product types analysed, incorporating a wider variety of commercial fermented milk drinks. This includes the products characterized by variations in flavour, fermentation processes, milk sources, and probiotic compositions. By integrating a wider selection of offerings, the study would provide a more comprehensive understanding of the market landscape, enhanced data representativeness and facilitating nuanced comparisons across different product categories. This expanded scope would not only improve the generalizability of the findings, making them more applicable to diverse consumer preferences, but also enhance the relevance of the results for both academic research and industry applications, including product development and quality control.

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APPENDICES

APPENDIX A

Nested ANOVA and Tukey's test of physicochemical analysis results

Table A.1: Nested ANOVA of pH results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	549936.085	<.001
Type	1	12.000	20.840	<.001
Brand * Type	4	12.000	243.642	<.001

a. Dependent Variable: pH.

Table A.2: Tukey's test of pH results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	-.493*,c,d	.020	12.000	<.001	-.537	-.450
	Betagen	-.370*,c,d	.020	12.000	<.001	-.413	-.327
	Farm Fresh	-.217*,c,d	.020	12.000	<.001	-.260	-.173
	Lactel	-.417*,c,d	.020	12.000	<.001	-.460	-.373
	Yobick	-.073*,c,d	.020	12.000	.003	-.117	-.030
Vitagen	Yakult	.493*,c,d	.020	12.000	<.001	.450	.537
	Betagen	.123*,c,d	.020	12.000	<.001	.080	.167
	Farm Fresh	.277*,c,d	.020	12.000	<.001	.233	.320
	Lactel	.077*,c,d	.020	12.000	.002	.033	.120
	Yobick	.420*,c,d	.020	12.000	<.001	.377	.463
Betagen	Yakult	.370*,c,d	.020	12.000	<.001	.327	.413
	Vitagen	-.123*,c,d	.020	12.000	<.001	-.167	-.080
	Farm Fresh	.153*,c,d	.020	12.000	<.001	.110	.197
	Lactel	-.047*,c,d	.020	12.000	.036	-.090	-.003
	Yobick	.297*,c,d	.020	12.000	<.001	.253	.340
Farm Fresh	Yakult	.217*,c,d	.020	12.000	<.001	.173	.260
	Vitagen	-.277*,c,d	.020	12.000	<.001	-.320	-.233
	Betagen	-.153*,c,d	.020	12.000	<.001	-.197	-.110
	Lactel	-.200*,c,d	.020	12.000	<.001	-.243	-.157
	Yobick	.143*,c,d	.020	12.000	<.001	.100	.187
Lactel	Yakult	.417*,c,d	.020	12.000	<.001	.373	.460
	Vitagen	-.077*,c,d	.020	12.000	.002	-.120	-.033
	Betagen	.047*,c,d	.020	12.000	.036	.003	.090
	Farm Fresh	.200*,c,d	.020	12.000	<.001	.157	.243
	Yobick	.343*,c,d	.020	12.000	<.001	.300	.387
Yobick	Yakult	.073*,c,d	.020	12.000	.003	.030	.117
	Vitagen	-.420*,c,d	.020	12.000	<.001	-.463	-.377
	Betagen	-.297*,c,d	.020	12.000	<.001	-.340	-.253
	Farm Fresh	-.143*,c,d	.020	12.000	<.001	-.187	-.100
	Lactel	-.343*,c,d	.020	12.000	<.001	-.387	-.300

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: pH.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table A.3: Nested ANOVA of TTA results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12	2977.687	<.001
Type	1	12	1.332	.271
Type * Brand	4	12	6.394	.005

a. Dependent Variable: TTA.

Table A.4: Tukey's test of TTA results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	1.862[*,c,d]	.391	12	<.001	1.010	2.713
	Betagen	.420[c,d]	.391	12	.303	-.431	1.272
	Farm Fresh	.661[c,d]	.391	12	.117	-.191	1.512
	Lactel	.480[c,d]	.391	12	.243	-.371	1.332
	Yobick	.360[c,d]	.391	12	.375	-.491	1.211
Vitagen	Yakult	-1.862[*,c,d]	.391	12	<.001	-2.713	-1.010
	Betagen	-1.442[*,c,d]	.391	12	.003	-2.293	-.590
	Farm Fresh	-1.201[*,c,d]	.391	12	.010	-2.053	-.350
	Lactel	-1.381[*,c,d]	.391	12	.004	-2.233	-.530
	Yobick	-1.502[*,c,d]	.391	12	.002	-2.353	-.650
Betagen	Yakult	-.420[c,d]	.391	12	.303	-1.272	.431
	Vitagen	1.442[*,c,d]	.391	12	.003	.590	2.293
	Farm Fresh	.240[c,d]	.391	12	.550	-.611	1.092
	Lactel	.060[c,d]	.391	12	.880	-.791	.911
	Yobick	-.060[c,d]	.391	12	.880	-.912	.791
Farm Fresh	Yakult	-.661[c,d]	.391	12	.117	-1.512	.191
	Vitagen	1.201[*,c,d]	.391	12	.010	.350	2.053
	Betagen	-.240[c,d]	.391	12	.550	-1.092	.611
	Lactel	-.180[c,d]	.391	12	.653	-1.032	.671
	Yobick	-.301[c,d]	.391	12	.457	-1.152	.551
Lactel	Yakult	-.480[c,d]	.391	12	.243	-1.332	.371
	Vitagen	1.381[*,c,d]	.391	12	.004	.530	2.233
	Betagen	-.060[c,d]	.391	12	.880	-.911	.791
	Farm Fresh	.180[c,d]	.391	12	.653	-.671	1.032
	Yobick	-.120[c,d]	.391	12	.763	-.972	.731
Yobick	Yakult	-.360[c,d]	.391	12	.375	-1.211	.491
	Vitagen	1.502[*,c,d]	.391	12	.002	.650	2.353
	Betagen	.060[c,d]	.391	12	.880	-.791	.912
	Farm Fresh	.301[c,d]	.391	12	.457	-.551	1.152
	Lactel	.120[c,d]	.391	12	.763	-.731	.972

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: TTA.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table A.5: Nested ANOVA of Brix results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	91976.516	<.001
Type	1	12.000	2764.452	<.001
Type * Brand	4	12.000	477.919	<.001

a. Dependent Variable: Brix.

Table A.6: Tukey's test of Brix results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e]	
						Lower Bound	Upper Bound
Yakult	Vitagen	1.300*,c,d	.152	12.000	<.001	.970	1.630
	Betagen	-2.100*,c,d	.152	12.000	<.001	-2.430	-1.770
	Farm Fresh	2.867*,c,d	.152	12.000	<.001	2.536	3.197
	Lactel	2.533*,c,d	.152	12.000	<.001	2.203	2.864
	Yobick	7.600*,c,d	.152	12.000	<.001	7.270	7.930
Vitagen	Yakult	-1.300*,c,d	.152	12.000	<.001	-1.630	-.970
	Betagen	-3.400*,c,d	.152	12.000	<.001	-3.730	-3.070
	Farm Fresh	1.567*,c,d	.152	12.000	<.001	1.236	1.897
	Lactel	1.233*,c,d	.152	12.000	<.001	.903	1.564
	Yobick	6.300*,c,d	.152	12.000	<.001	5.970	6.630
Betagen	Yakult	2.100*,c,d	.152	12.000	<.001	1.770	2.430
	Vitagen	3.400*,c,d	.152	12.000	<.001	3.070	3.730
	Farm Fresh	4.967*,c,d	.152	12.000	<.001	4.636	5.297
	Lactel	4.633*,c,d	.152	12.000	<.001	4.303	4.964
	Yobick	9.700*,c,d	.152	12.000	<.001	9.370	10.030
Farm Fresh	Yakult	-2.867*,c,d	.152	12.000	<.001	-3.197	-2.536
	Vitagen	-1.567*,c,d	.152	12.000	<.001	-1.897	-1.236
	Betagen	-4.967*,c,d	.152	12.000	<.001	-5.297	-4.636
	Lactel	-.333*,c,d	.152	12.000	.048	-.664	-.003
	Yobick	4.733*,c,d	.152	12.000	<.001	4.403	5.064
Lactel	Yakult	-2.533*,c,d	.152	12.000	<.001	-2.864	-2.203
	Vitagen	-1.233*,c,d	.152	12.000	<.001	-1.564	-.903
	Betagen	-4.633*,c,d	.152	12.000	<.001	-4.964	-4.303
	Farm Fresh	.333*,c,d	.152	12.000	.048	.003	.664
	Yobick	5.067*,c,d	.152	12.000	<.001	4.736	5.397
Yobick	Yakult	-7.600*,c,d	.152	12.000	<.001	-7.930	-7.270
	Vitagen	-6.300*,c,d	.152	12.000	<.001	-6.630	-5.970
	Betagen	-9.700*,c,d	.152	12.000	<.001	-10.030	-9.370
	Farm Fresh	-4.733*,c,d	.152	12.000	<.001	-5.064	-4.403
	Lactel	-5.067*,c,d	.152	12.000	<.001	-5.397	-4.736

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: Brix.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table A.7: Nested ANOVA of colour (L*) results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	6218574.297	<.001
Type	1	12.000	40900.238	<.001
Type * Brand	4	12.000	5819.103	<.001

a. Dependent Variable: Colour_L.

Table A.8: Tukey's test of colour (L*) results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	-7.147*,c,d	.075	12.000	<.001	-7.311	-6.983
	Betagen	-3.900*,c,d	.075	12.000	<.001	-4.064	-3.736
	Farm Fresh	-14.007*,c,d	.075	12.000	<.001	-14.171	-13.843
	Lactel	-15.987*,c,d	.075	12.000	<.001	-16.151	-15.823
	Yobick	-7.413*,c,d	.075	12.000	<.001	-7.577	-7.249
Vitagen	Yakult	7.147*,c,d	.075	12.000	<.001	6.983	7.311
	Betagen	3.247*,c,d	.075	12.000	<.001	3.083	3.411
	Farm Fresh	-6.860*,c,d	.075	12.000	<.001	-7.024	-6.696
	Lactel	-8.840*,c,d	.075	12.000	<.001	-9.004	-8.676
	Yobick	-.267*,c,d	.075	12.000	.004	-.431	-.103
Betagen	Yakult	3.900*,c,d	.075	12.000	<.001	3.736	4.064
	Vitagen	-3.247*,c,d	.075	12.000	<.001	-3.411	-3.083
	Farm Fresh	-10.107*,c,d	.075	12.000	<.001	-10.271	-9.943
	Lactel	-12.087*,c,d	.075	12.000	<.001	-12.251	-11.923
	Yobick	-3.513*,c,d	.075	12.000	<.001	-3.677	-3.349
Farm Fresh	Yakult	14.007*,c,d	.075	12.000	<.001	13.843	14.171
	Vitagen	6.860*,c,d	.075	12.000	<.001	6.696	7.024
	Betagen	10.107*,c,d	.075	12.000	<.001	9.943	10.271
	Lactel	-1.980*,c,d	.075	12.000	<.001	-2.144	-1.816
	Yobick	6.593*,c,d	.075	12.000	<.001	6.429	6.757
Lactel	Yakult	15.987*,c,d	.075	12.000	<.001	15.823	16.151
	Vitagen	8.840*,c,d	.075	12.000	<.001	8.676	9.004
	Betagen	12.087*,c,d	.075	12.000	<.001	11.923	12.251
	Farm Fresh	1.980*,c,d	.075	12.000	<.001	1.816	2.144
	Yobick	8.573*,c,d	.075	12.000	<.001	8.409	8.737
Yobick	Yakult	7.413*,c,d	.075	12.000	<.001	7.249	7.577
	Vitagen	.267*,c,d	.075	12.000	.004	.103	.431
	Betagen	3.513*,c,d	.075	12.000	<.001	3.349	3.677
	Farm Fresh	-6.593*,c,d	.075	12.000	<.001	-6.757	-6.429
	Lactel	-8.573*,c,d	.075	12.000	<.001	-8.737	-8.409

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: Colour_L.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table A.9: Nested ANOVA of colour (a*) results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	6131.776	<.001
Type	1	12.000	33454.804	<.001
Brand * Type	4	12.000	7520.402	<.001

a. Dependent Variable: a.

Table A.10: Tukey's test of colour (a*) results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	1.947[*,c,d]	.020	12.000	<.001	1.903	1.990
	Betagen	-1.320[*,c,d]	.020	12.000	<.001	-1.363	-1.277
	Farm Fresh	1.840[*,c,d]	.020	12.000	<.001	1.797	1.883
	Lactel	2.883[*,c,d]	.020	12.000	<.001	2.840	2.927
	Yobick	2.210[*,c,d]	.020	12.000	<.001	2.167	2.253
Vitagen	Yakult	-1.947[*,c,d]	.020	12.000	<.001	-1.990	-1.903
	Betagen	-3.267[*,c,d]	.020	12.000	<.001	-3.310	-3.223
	Farm Fresh	-.107[*,c,d]	.020	12.000	<.001	-.150	-.063
	Lactel	.937[*,c,d]	.020	12.000	<.001	.893	.980
	Yobick	.263[*,c,d]	.020	12.000	<.001	.220	.307
Betagen	Yakult	1.320[*,c,d]	.020	12.000	<.001	1.277	1.363
	Vitagen	3.267[*,c,d]	.020	12.000	<.001	3.223	3.310
	Farm Fresh	3.160[*,c,d]	.020	12.000	<.001	3.117	3.203
	Lactel	4.203[*,c,d]	.020	12.000	<.001	4.160	4.247
	Yobick	3.530[*,c,d]	.020	12.000	<.001	3.487	3.573
Farm Fresh	Yakult	-1.840[*,c,d]	.020	12.000	<.001	-1.883	-1.797
	Vitagen	.107[*,c,d]	.020	12.000	<.001	.063	.150
	Betagen	-3.160[*,c,d]	.020	12.000	<.001	-3.203	-3.117
	Lactel	1.043[*,c,d]	.020	12.000	<.001	1.000	1.087
	Yobick	.370[*,c,d]	.020	12.000	<.001	.327	.413
Lactel	Yakult	-2.883[*,c,d]	.020	12.000	<.001	-2.927	-2.840
	Vitagen	-.937[*,c,d]	.020	12.000	<.001	-.980	-.893
	Betagen	-4.203[*,c,d]	.020	12.000	<.001	-4.247	-4.160
	Farm Fresh	-1.043[*,c,d]	.020	12.000	<.001	-1.087	-1.000
	Yobick	-.673[*,c,d]	.020	12.000	<.001	-.717	-.630
Yobick	Yakult	-2.210[*,c,d]	.020	12.000	<.001	-2.253	-2.167
	Vitagen	-.263[*,c,d]	.020	12.000	<.001	-.307	-.220
	Betagen	-3.530[*,c,d]	.020	12.000	<.001	-3.573	-3.487
	Farm Fresh	-.370[*,c,d]	.020	12.000	<.001	-.413	-.327
	Lactel	.673[*,c,d]	.020	12.000	<.001	.630	.717

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: a.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table A.11: Nested ANOVA of colour (b*) results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	373185.654	<.001
Type	1	12.000	15334.211	<.001
Type * Brand	4	12.000	16164.559	<.001

a. Dependent Variable: b.

Table A.12: Tukey's test of colour (b*) results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	7.497*,c,d	.042	12.000	<.001	7.405	7.588
	Betagen	-1.507*,c,d	.042	12.000	<.001	-1.598	-1.415
	Farm Fresh	7.250*,c,d	.042	12.000	<.001	7.159	7.341
	Lactel	2.723*,c,d	.042	12.000	<.001	2.632	2.815
	Yobick	5.003*,c,d	.042	12.000	<.001	4.912	5.095
Vitagen	Yakult	-7.497*,c,d	.042	12.000	<.001	-7.588	-7.405
	Betagen	-9.003*,c,d	.042	12.000	<.001	-9.095	-8.912
	Farm Fresh	-.247*,c,d	.042	12.000	<.001	-.338	-.155
	Lactel	-4.773*,c,d	.042	12.000	<.001	-4.865	-4.682
	Yobick	-2.493*,c,d	.042	12.000	<.001	-2.585	-2.402
Betagen	Yakult	1.507*,c,d	.042	12.000	<.001	1.415	1.598
	Vitagen	9.003*,c,d	.042	12.000	<.001	8.912	9.095
	Farm Fresh	8.757*,c,d	.042	12.000	<.001	8.665	8.848
	Lactel	4.230*,c,d	.042	12.000	<.001	4.139	4.321
	Yobick	6.510*,c,d	.042	12.000	<.001	6.419	6.601
Farm Fresh	Yakult	-7.250*,c,d	.042	12.000	<.001	-7.341	-7.159
	Vitagen	.247*,c,d	.042	12.000	<.001	.155	.338
	Betagen	-8.757*,c,d	.042	12.000	<.001	-8.848	-8.665
	Lactel	-4.527*,c,d	.042	12.000	<.001	-4.618	-4.435
	Yobick	-2.247*,c,d	.042	12.000	<.001	-2.338	-2.155
Lactel	Yakult	-2.723*,c,d	.042	12.000	<.001	-2.815	-2.632
	Vitagen	4.773*,c,d	.042	12.000	<.001	4.682	4.865
	Betagen	-4.230*,c,d	.042	12.000	<.001	-4.321	-4.139
	Farm Fresh	4.527*,c,d	.042	12.000	<.001	4.435	4.618
	Yobick	2.280*,c,d	.042	12.000	<.001	2.189	2.371
Yobick	Yakult	-5.003*,c,d	.042	12.000	<.001	-5.095	-4.912
	Vitagen	2.493*,c,d	.042	12.000	<.001	2.402	2.585
	Betagen	-6.510*,c,d	.042	12.000	<.001	-6.601	-6.419
	Farm Fresh	2.247*,c,d	.042	12.000	<.001	2.155	2.338
	Lactel	-2.280*,c,d	.042	12.000	<.001	-2.371	-2.189

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: b.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

APPENDIX B

Nested ANOVA and Tukey's test of antioxidant analysis results

Table B.1: Nested ANOVA of TPC results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	30467.122	<.001
Type	1	12.000	6048.890	<.001
Type * Brand	4	12.000	1974.410	<.001

a. Dependent Variable: TPC.

Table B.2: Tukey's test of TPC results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	.005[b,c]	.010	12.000	.620	-.017	.027
	Betagen	-.080[b,c,*]	.010	12.000	<.001	-.102	-.058
	Farm Fresh	-.604[b,c,*]	.010	12.000	<.001	-.626	-.583
	Lactel	-.831[b,c,*]	.010	12.000	<.001	-.852	-.809
	Yobick	.019[b,c]	.010	12.000	.084	-.003	.040
Vitagen	Yakult	-.005[b,c]	.010	12.000	.620	-.027	.017
	Betagen	-.085[b,c,*]	.010	12.000	<.001	-.107	-.063
	Farm Fresh	-.609[b,c,*]	.010	12.000	<.001	-.631	-.588
	Lactel	-.836[b,c,*]	.010	12.000	<.001	-.857	-.814
	Yobick	.014[b,c]	.010	12.000	.194	-.008	.035
Betagen	Yakult	.080[b,c,*]	.010	12.000	<.001	.058	.102
	Vitagen	.085[b,c,*]	.010	12.000	<.001	.063	.107
	Farm Fresh	-.524[b,c,*]	.010	12.000	<.001	-.546	-.503
	Lactel	-.751[b,c,*]	.010	12.000	<.001	-.772	-.729
	Yobick	.099[b,c,*]	.010	12.000	<.001	.077	.120
Farm Fresh	Yakult	.604[b,c,*]	.010	12.000	<.001	.583	.626
	Vitagen	.609[b,c,*]	.010	12.000	<.001	.588	.631
	Betagen	.524[b,c,*]	.010	12.000	<.001	.503	.546
	Lactel	-.226[b,c,*]	.010	12.000	<.001	-.248	-.204
	Yobick	.623[b,c,*]	.010	12.000	<.001	.602	.645
Lactel	Yakult	.831[b,c,*]	.010	12.000	<.001	.809	.852
	Vitagen	.836[b,c,*]	.010	12.000	<.001	.814	.857
	Betagen	.751[b,c,*]	.010	12.000	<.001	.729	.772
	Farm Fresh	.226[b,c,*]	.010	12.000	<.001	.204	.248
	Yobick	.849[b,c,*]	.010	12.000	<.001	.828	.871
Yobick	Yakult	-.019[b,c]	.010	12.000	.084	-.040	.003
	Vitagen	-.014[b,c]	.010	12.000	.194	-.035	.008
	Betagen	-.099[b,c,*]	.010	12.000	<.001	-.120	-.077
	Farm Fresh	-.623[b,c,*]	.010	12.000	<.001	-.645	-.602
	Lactel	-.849[b,c,*]	.010	12.000	<.001	-.871	-.828

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: TPC.

b. An estimate of the modified population marginal mean (I).

c. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table B.3: Nested ANOVA of TFC results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12	8813.092	<.001
Type	1	12	4326.325	<.001
Type * Brand	4	12	79.429	<.001

a. Dependent Variable: TFC.

Table B.4: Tukey's test of TFC results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	.135[b,c]	.109	12	.239	-.103	.373
	Betagen	-1.148[b,c,*]	.109	12	<.001	-1.386	-.910
	Farm Fresh	-4.600[b,c,*]	.109	12	<.001	-4.838	-4.362
	Lactel	-3.763[b,c,*]	.109	12	<.001	-4.001	-3.525
	Yobick	-5.089[b,c,*]	.109	12	<.001	-5.327	-4.851
Vitagen	Yakult	-.135[b,c]	.109	12	.239	-.373	.103
	Betagen	-1.283[b,c,*]	.109	12	<.001	-1.521	-1.045
	Farm Fresh	-4.735[b,c,*]	.109	12	<.001	-4.973	-4.497
	Lactel	-3.898[b,c,*]	.109	12	<.001	-4.136	-3.660
	Yobick	-5.224[b,c,*]	.109	12	<.001	-5.462	-4.986
Betagen	Yakult	1.148[b,c,*]	.109	12	<.001	.910	1.386
	Vitagen	1.283[b,c,*]	.109	12	<.001	1.045	1.521
	Farm Fresh	-3.452[b,c,*]	.109	12	<.001	-3.690	-3.214
	Lactel	-2.615[b,c,*]	.109	12	<.001	-2.853	-2.377
	Yobick	-3.941[b,c,*]	.109	12	<.001	-4.179	-3.703
Farm Fresh	Yakult	4.600[b,c,*]	.109	12	<.001	4.362	4.838
	Vitagen	4.735[b,c,*]	.109	12	<.001	4.497	4.973
	Betagen	3.452[b,c,*]	.109	12	<.001	3.214	3.690
	Lactel	.837[b,c,*]	.109	12	<.001	.599	1.075
	Yobick	-.489[b,c,*]	.109	12	<.001	-.727	-.251
Lactel	Yakult	3.763[b,c,*]	.109	12	<.001	3.525	4.001
	Vitagen	3.898[b,c,*]	.109	12	<.001	3.660	4.136
	Betagen	2.615[b,c,*]	.109	12	<.001	2.377	2.853
	Farm Fresh	-.837[b,c,*]	.109	12	<.001	-1.075	-.599
	Yobick	-1.326[b,c,*]	.109	12	<.001	-1.564	-1.088
Yobick	Yakult	5.089[b,c,*]	.109	12	<.001	4.851	5.327
	Vitagen	5.224[b,c,*]	.109	12	<.001	4.986	5.462
	Betagen	3.941[b,c,*]	.109	12	<.001	3.703	4.179
	Farm Fresh	.489[b,c,*]	.109	12	<.001	.251	.727
	Lactel	1.326[b,c,*]	.109	12	<.001	1.088	1.564

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: TFC.

b. An estimate of the modified population marginal mean (I).

c. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table B.5: Nested ANOVA of FRAP results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	1827.326	<.001
Type	1	12.000	.154	.702
Brand * Type	4	12.000	107.194	<.001

a. Dependent Variable: FRAP.

Table B.6: Tukey's test of FRAP results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e] Lower Bound	Upper Bound
Yakult	Vitagen	-118.333[b,c]	127.588	12.000	.372	-396.324	159.657
	Betagen	-591.667[b,c,*]	127.588	12.000	<.001	-869.657	-313.676
	Farm Fresh	-260.000[b,c]	127.588	12.000	.064	-537.990	17.990
	Lactel	-1551.67[b,c,*]	127.588	12.000	<.001	-1829.657	-1273.676
	Yobick	1015.000[b,c,*]	127.588	12.000	<.001	737.010	1292.990
Vitagen	Yakult	118.333[b,c]	127.588	12.000	.372	-159.657	396.324
	Betagen	-473.333[b,c,*]	127.588	12.000	.003	-751.324	-195.343
	Farm Fresh	-141.667[b,c]	127.588	12.000	.289	-419.657	136.324
	Lactel	-1433.33[b,c,*]	127.588	12.000	<.001	-1711.324	-1155.343
	Yobick	1133.333[b,c,*]	127.588	12.000	<.001	855.343	1411.324
Betagen	Yakult	591.667[b,c,*]	127.588	12.000	<.001	313.676	869.657
	Vitagen	473.333[b,c,*]	127.588	12.000	.003	195.343	751.324
	Farm Fresh	331.667[b,c,*]	127.588	12.000	.023	53.676	609.657
	Lactel	-960.000[b,c,*]	127.588	12.000	<.001	-1237.990	-682.010
	Yobick	1606.667[b,c,*]	127.588	12.000	<.001	1328.676	1884.657
Farm Fresh	Yakult	260.000[b,c]	127.588	12.000	.064	-17.990	537.990
	Vitagen	141.667[b,c]	127.588	12.000	.289	-136.324	419.657
	Betagen	-331.667[b,c,*]	127.588	12.000	.023	-609.657	-53.676
	Lactel	-1291.67[b,c,*]	127.588	12.000	<.001	-1569.657	-1013.676
	Yobick	1275.000[b,c,*]	127.588	12.000	<.001	997.010	1552.990
Lactel	Yakult	1551.667[b,c,*]	127.588	12.000	<.001	1273.676	1829.657
	Vitagen	1433.333[b,c,*]	127.588	12.000	<.001	1155.343	1711.324
	Betagen	960.000[b,c,*]	127.588	12.000	<.001	682.010	1237.990
	Farm Fresh	1291.667[b,c,*]	127.588	12.000	<.001	1013.676	1569.657
	Yobick	2566.667[b,c,*]	127.588	12.000	<.001	2288.676	2844.657
Yobick	Yakult	-1015.00[b,c,*]	127.588	12.000	<.001	-1292.990	-737.010
	Vitagen	-1133.33[b,c,*]	127.588	12.000	<.001	-1411.324	-855.343
	Betagen	-1606.67[b,c,*]	127.588	12.000	<.001	-1884.657	-1328.676
	Farm Fresh	-1275.00[b,c,*]	127.588	12.000	<.001	-1552.990	-997.010
	Lactel	-2566.67[b,c,*]	127.588	12.000	<.001	-2844.657	-2288.676

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: FRAP.

b. An estimate of the modified population marginal mean (I).

c. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table B.7: Nested ANOVA of DPPH results.

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12.000	19629.722	<.001
Type	1	12.000	27.442	<.001
Brand * Type	4	12.000	969.050	<.001

a. Dependent Variable: DPPH.

Table B.8: Tukey's test of DPPH results.

Pairwise Comparisons[a]

(I) Brand	(J) Brand	Mean Difference (I-J)	Std. Error	df	Sig.[e]	95% Confidence Interval for Difference[e]	
						Lower Bound	Upper Bound
Yakult	Vitagen	15.292*,c,d	.712	12.000	<.001	13.740	16.843
	Betagen	8.182*,c,d	.712	12.000	<.001	6.631	9.734
	Farm Fresh	26.915*,c,d	.712	12.000	<.001	25.364	28.466
	Lactel	-14.661*,c,d	.712	12.000	<.001	-16.212	-13.109
	Yobick	4.759*,c,d	.712	12.000	<.001	3.208	6.311
Vitagen	Yakult	-15.292*,c,d	.712	12.000	<.001	-16.843	-13.740
	Betagen	-7.109*,c,d	.712	12.000	<.001	-8.661	-5.558
	Farm Fresh	11.623*,c,d	.712	12.000	<.001	10.072	13.175
	Lactel	-29.952*,c,d	.712	12.000	<.001	-31.504	-28.401
	Yobick	-10.533*,c,d	.712	12.000	<.001	-12.084	-8.981
Betagen	Yakult	-8.182*,c,d	.712	12.000	<.001	-9.734	-6.631
	Vitagen	7.109*,c,d	.712	12.000	<.001	5.558	8.661
	Farm Fresh	18.733*,c,d	.712	12.000	<.001	17.181	20.284
	Lactel	-22.843*,c,d	.712	12.000	<.001	-24.394	-21.292
	Yobick	-3.423*,c,d	.712	12.000	<.001	-4.975	-1.872
Farm Fresh	Yakult	-26.915*,c,d	.712	12.000	<.001	-28.466	-25.364
	Vitagen	-11.623*,c,d	.712	12.000	<.001	-13.175	-10.072
	Betagen	-18.733*,c,d	.712	12.000	<.001	-20.284	-17.181
	Lactel	-41.576*,c,d	.712	12.000	<.001	-43.127	-40.024
	Yobick	-22.156*,c,d	.712	12.000	<.001	-23.707	-20.604
Lactel	Yakult	14.661*,c,d	.712	12.000	<.001	13.109	16.212
	Vitagen	29.952*,c,d	.712	12.000	<.001	28.401	31.504
	Betagen	22.843*,c,d	.712	12.000	<.001	21.292	24.394
	Farm Fresh	41.576*,c,d	.712	12.000	<.001	40.024	43.127
	Yobick	19.420*,c,d	.712	12.000	<.001	17.868	20.971
Yobick	Yakult	-4.759*,c,d	.712	12.000	<.001	-6.311	-3.208
	Vitagen	10.533*,c,d	.712	12.000	<.001	8.981	12.084
	Betagen	3.423*,c,d	.712	12.000	<.001	1.872	4.975
	Farm Fresh	22.156*,c,d	.712	12.000	<.001	20.604	23.707
	Lactel	-19.420*,c,d	.712	12.000	<.001	-20.971	-17.868

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: DPPH.

c. An estimate of the modified population marginal mean (I).

d. An estimate of the modified population marginal mean (J).

e. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

APPENDIX C

Steps to perform Nested ANOVA and pairwise comparison in SPSS

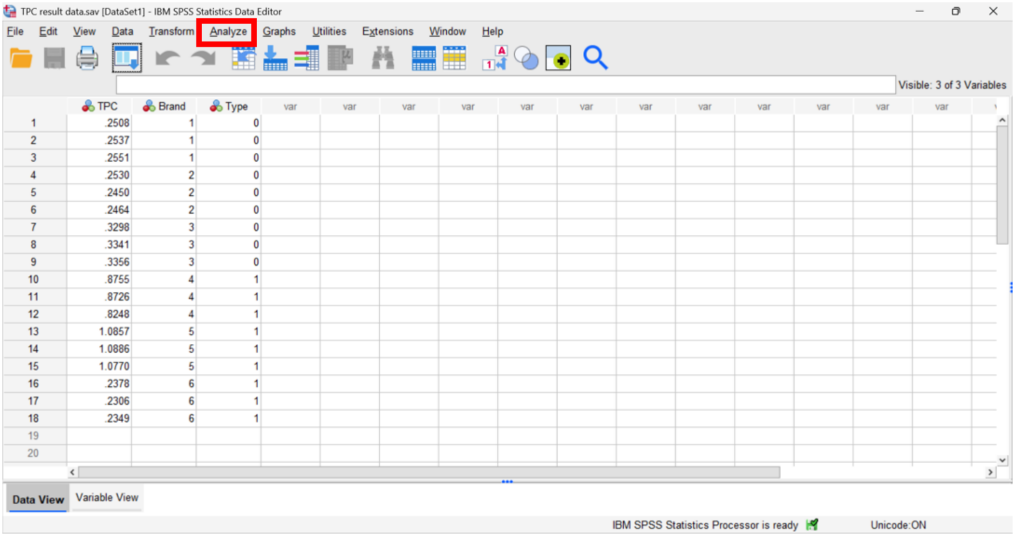

Figure C.1:
Step 1: After entering in the raw data, go to the "**Analysis**".

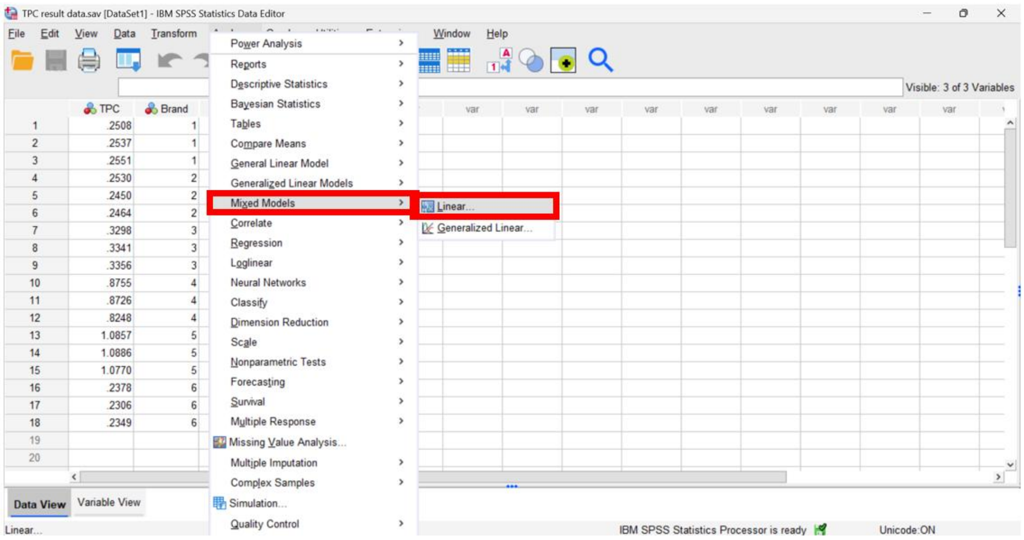

Figure C.2:
Step 2: Select "**Mixed Models**" > "**Linear**".

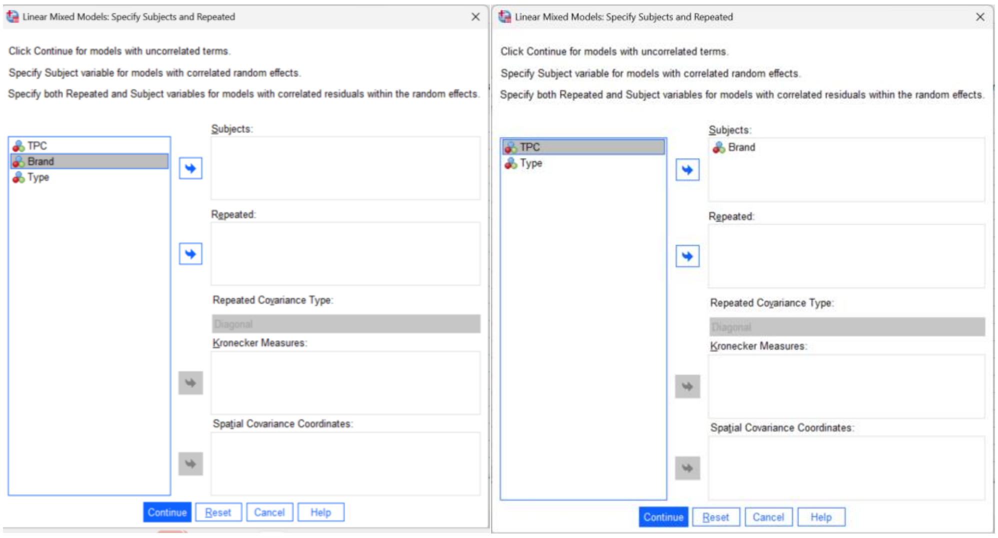

Figure C.3:
Step 3: Move the nested variable (e.g., “**Brand**”) into “**Subjects**” box.

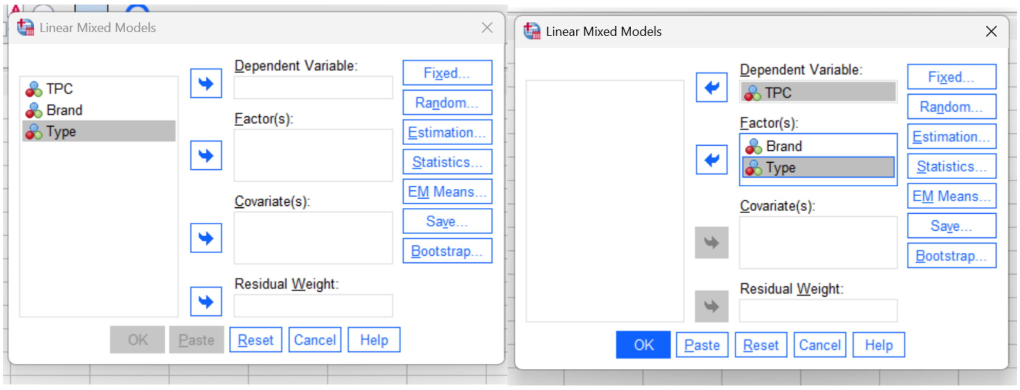

Figure C.4:
Step 4: Move the dependent variable (e.g., “**TPC**”) into “**Dependent Variable**” box and move the independent variables (e.g., “**Type**” and “**Brand**”) into the “**Factor**(s)” box.

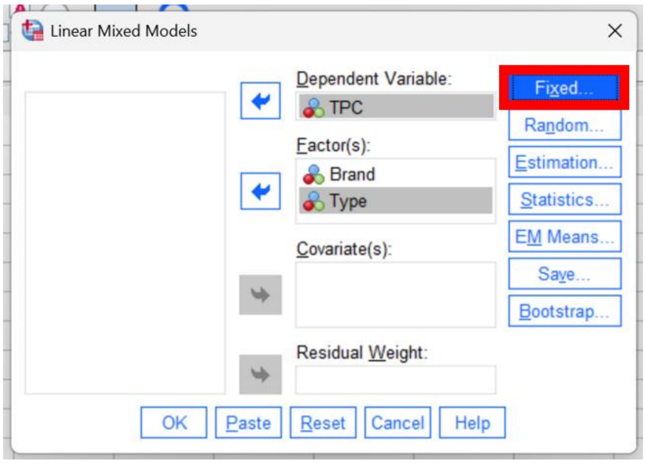

Figure C.5:
Step 5: Click the "**Fixed…**" button.

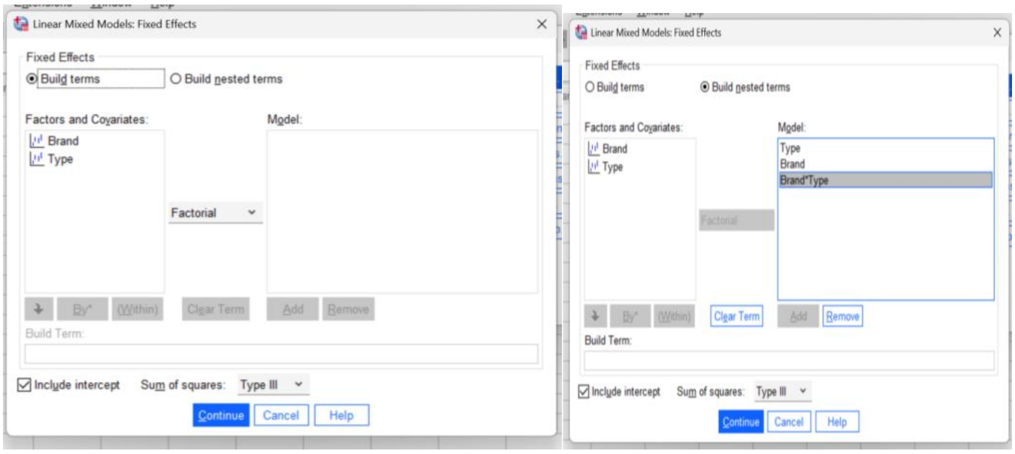

Figure C.6:
Step 6: Add the main effect and interactions (e.g., "**Type**", "**Brand**" and "**Brand*Type**") into the model, click "**Continue**".

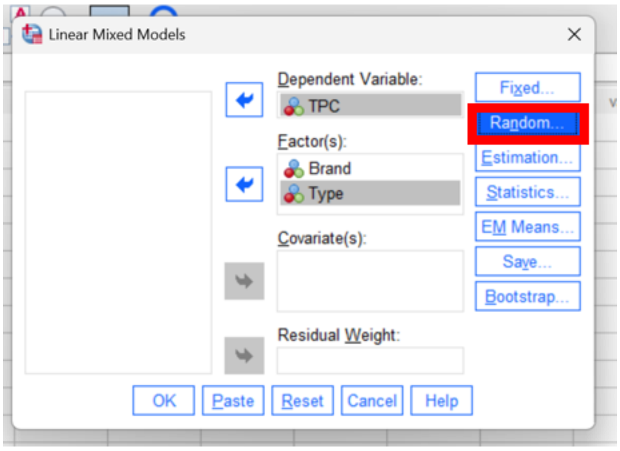

Figure C.7:
Step 7: Click the "**Random…**" button.

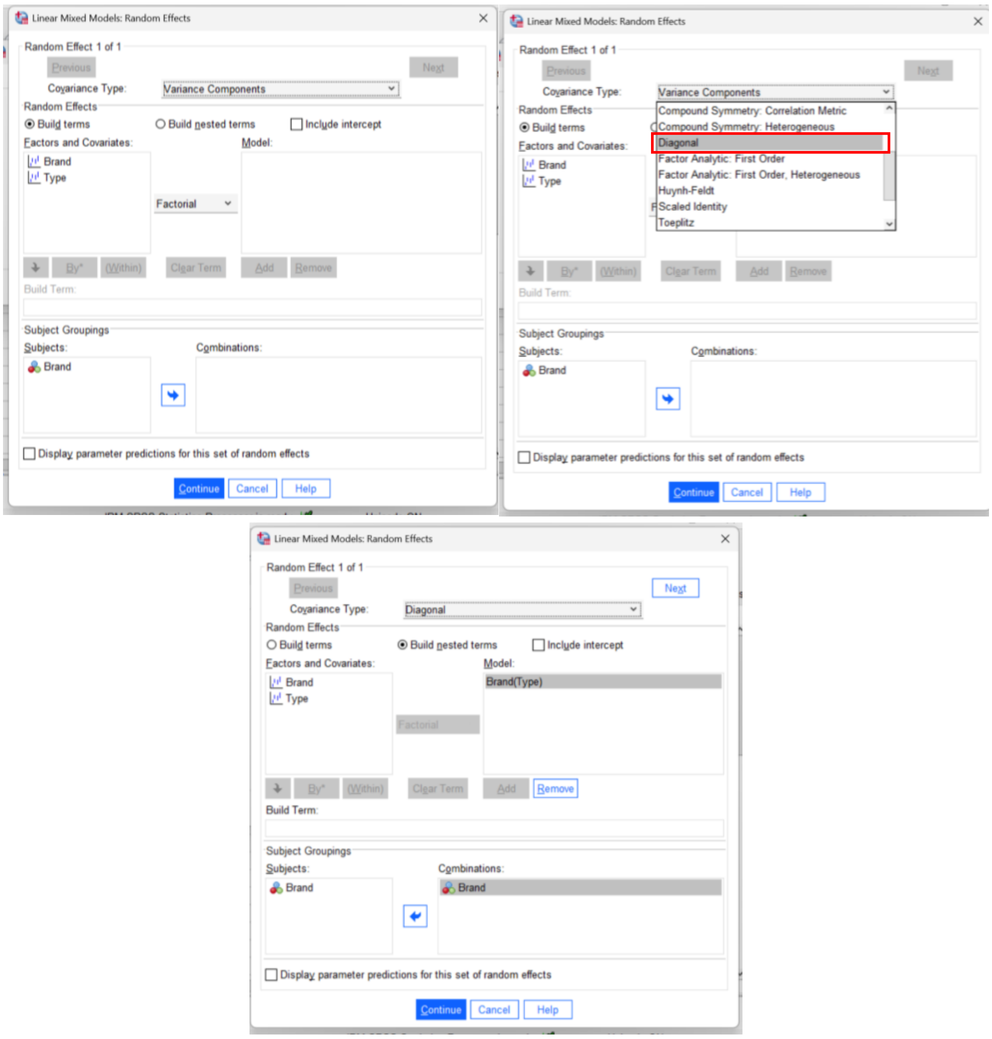

Figure C.8:

Step 8: Set **Covariance Type** to "**Diagonal**", add "**Brand(Type)**" to reflect the nesting structure into **Model**, add "**Brand**" from **Subjects** box to **Combinations** box and click "**Continue**".

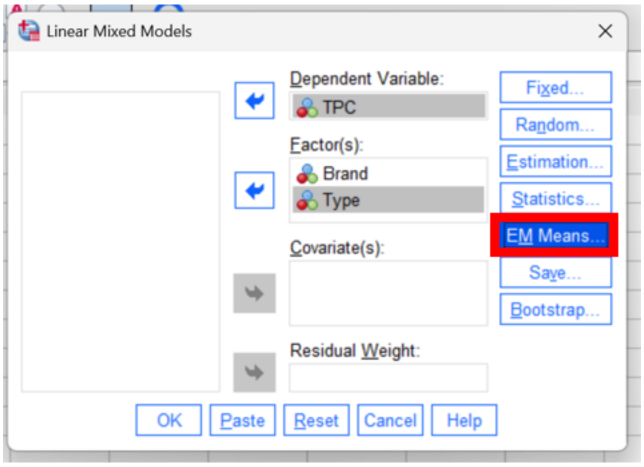

Figure C.9:
Step 9: Click the "**EM Means…**" button.

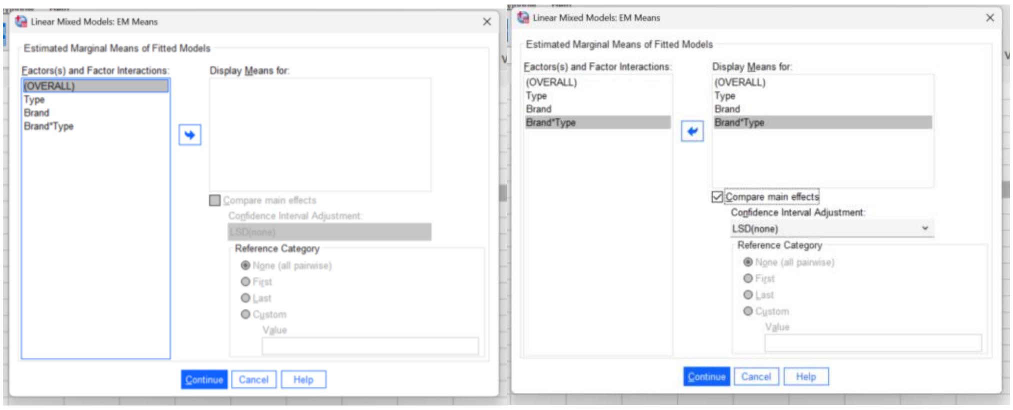

Figure C.10:
Step 10: Add all Factors(s) and Factor Interactions (e.g., "**Type**", "**Brand**", "**Type*Brand**") into "**Display Means for**" box, select the "**Compare main effect**" and choose "**LSD(none)**" and click "**Continue**".

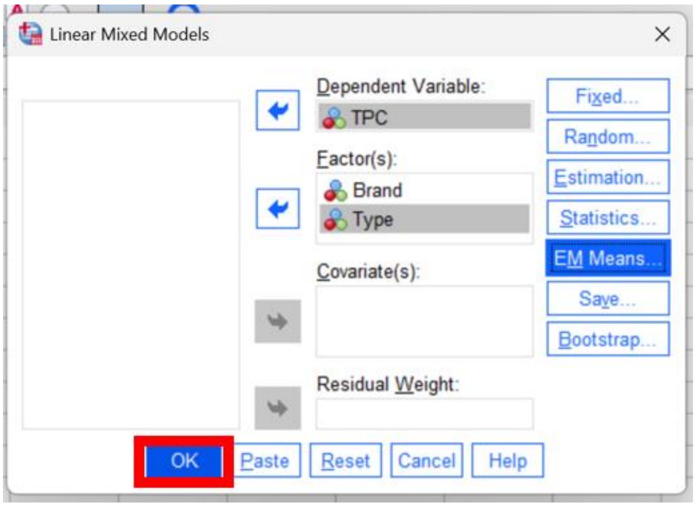

Figure C.11:
Step 11: Click "**OK**" to run the data.

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