COMPARATIVE STUDY ON ANTIOXIDANT AND SENSORY PROPERTIES OF NATTO PREPARED USING DIFFERENT BRANDS OF STARTER CULTURE

By

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ABSTRACT

COMPARATIVE STUDY ON ANTIOXIDANT AND SENSORY PROPERTIES OF NATTO PREPARED USING DIFFERENT BRANDS OF STARTER CULTURE

MONG JIA YING

Antioxidant activity and sensory properties of natto placed great significance to consumers, which were influenced by the brands of natto starter. This study was to investigate the total phenolic, total flavonoid content, DPPH radical scavenging assay, reducing power assay and sensory profile of natto prepared using two different brands of starter cultures. The Chuan Xiu starter includes *B. subtilis* var. natto, *Lactoplantibacillus plantarum* and maltodextrin, while Kawashimaya starter only contains *B. subtilis* strain. Among the samples, natto prepared using Kawashimaya (16.6 mg GAE/100g) and Chuan Xiu (24.6 mg GAE/100g) starter cultures exhibited the highest TPC. Besides, natto prepared using Kawashimaya (3.70 mg QE/100g) starter culture had the highest TFC. In terms of DPPH radical scavenging activity, both natto prepared using Kawashimaya and Chuan Xiu starters demonstrated the lowest IC_{50} values of 52.69 mg/L and 69.28 mg/L, respectively, compared to 124.61 mg/L for soybean without starter culture. Furthermore, natto prepared using Kawashimaya (8.41 mg/L) and Chuan Xiu (6.21 mg/L) starters exhibited lower EC_{50} values in

reducing power than soybean without starter culture (17.09 mg/L). Sensory evaluation revealed no significant differences ($p > 0.05$) in aroma, taste chewiness and overall acceptability. However, natto prepared using Kawashimaya starter received a lower score for appearance compared to control natto, whereas natto prepared using Kawashimaya starter showed lower stringiness score compared to both the Chuan Xiu starter and the control natto. In general, natto prepared using Kawashimaya and Chuan Xiu starter cultures has the potential to improve the antioxidant ability of natto.

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Thank you all.

DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

MONG JIA YING

APPROVAL SHEET

This final year project entitled "**COMPARATIVE STUDY ON ANTIOXIDANT AND SENSORY PROPERTIES OF NATTO PREPARED USING DIFFERENT BRANDS OF STARTER CULTURE**" was prepared by MONG JIA YING 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:

Lye Huey Shi

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(Dr. Lye Huey Shi) Date: 29/8/2024

Supervisor

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UNIVERSITI TUNKU ABDUL RAHMAN

FACULTY OF SCIENCE

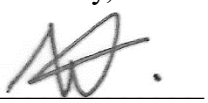
Date: 30 August 2024

SUBMISSION OF FINAL YEAR PROJECT

I, **MONG JIA YING** (ID No: **21ADB06793**) hereby certify that I have completed the final year project titled Title "**COMPARATIVE STUDY ON ANTIOXIDANT AND SENSORY PROPERTIES OF NATTO PREPARED USING DIFFERENT BRANDS OF STARTER CULTURE**" under the supervision of Dr. Lye Huey Shi from the Department of Agriculture 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.

Yours truly,



MONG JIA YING

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LIST OF ABREVIATIONS

B. subtilis	*Bacillus subtilis*
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
RP	Reducing power
RSA	Radical scavenging activity
IC_{50}	Half maximal inhibitory concentration
EC_{50}	Half maximal effective concentration
GAE	Gallic acid equivalent
QE	Quercetin equivalent
DE	Dextrose equivalent
LAB	Lactic acid bacteria
γ-PGA	Poly-γ-glutamic acid
F-C	Folin-Ciocalteu
Mo(VI)	Molybdenum(VI) oxide
Mo(V)	Molybdenum(V) oxide
UV-vis	Ultraviolet–visible spectroscopy
Fe^{3+}	Ferric ions
Fe^{2+}	Ferrous ions
LDL	Low-density lipoprotein
DNA	Deoxyribonucleic acid
w/v	Weigt per volume
TCA	Trichloroacetic acid
SPSS	Statistical Package for Social Sciences
ANOVA	Analysis of variance
MK-7	Menaquinone-7
MK-4	Menoquinone-4
MK-8	Menoquinone-8
MK-9	Menoquinone-9

CHAPTER 1

INTRODUCTION

1.1 Research Background

Natto is a traditional Japanese-fermented food produced by bacterial fermentation of soybean using natto starter strains of *B. subtilis* var. natto. According to Shurhleff and Aoyagi (2012), stringy natto was first discovered in northeast Japan in A.D. 1083 by Minamoto Yoshiie. The legend goes that Minamoto cultured natto by placing cooked soybean in a rice straw bundle on the back of a horse. It was believed that the *B. subtilis* in rice straw and the warmth from the horse aided in the fermentation process. Traditionally, natto fermentation involved wrapping boiled soybeans in rice straw. However, at the beginning of the 20th century, scientist identified and isolated *B. subtilis* and directly added to the cooked soybeans to initiate fermentation. Using a natto starter culture yields consistent and reliable results due to the well-balanced and optimized combination of microbes.

Natto is recognized for its sticky, stringiness, distinct aroma and flavour, which consumers either love or hate. The flavour of natto is often described as earthy, nutty, with a mild ammonia tang due to the breakdown of soy protein into amino acids. Additionally, the umami flavour in the natto makes it an ideal topping and complement for other food. In Japan, natto is typically served with soy sauce (tare) and mustard (karashi) and is commonly enjoyed with cooked rice. Similar

fermented soy products made using *Bacillus* strains include as thua nao from Thailand, hawaijar from India and douchi from China (Li et al., 2023).

Aside from its distinctive palatability, natto is renowned for its nutritional value and health benefits. An imbalance between free radicals and antioxidant defenses in the body leads to oxidative stress, a condition that can trigger and advance various diseases, including cancer, atherosclerosis, inflammatory disorders, neurodegenerative diseases and cardiovascular conditions (Pham-Huy, He and Pham-Huy, 2008). A recent study demonstrated that fermented soy products enhance their antioxidant profile, isoflavone content, TPC and TFC (Juan and Chou, 2010). Thus, it is clear that the consumption of natto can help mitigate this oxidative stress. Natto is acknowledged for its strong antioxidant activities due to its rich bioactive compounds, including isoflavones, vitamin K2 and bioactive compounds.

1.2 Problem Statement

Natto's distinct aroma and taste, which are not universally appealing, particularly in regions like Malaysia, have limited its broader acceptance. Recent research by Yang et al. (2021) has highlighted the potential of incorporating various strains of bacteria, such as *Lactobacillus* and *Bifidobacterium*, in dual-bacteria fermentation to enhance both the antioxidant activity and sensory attributes of natto. Despite these findings, there is a lack of comprehensive studies evaluating the impact of different commercial natto starter cultures, especially those utilizing single versus dual-strain fermentations, on the

antioxidant properties and sensory profiles of natto. This gap in research presents a challenge in optimizing natto production to meet diverse consumer preferences while maintaining its health benefits.

1.3 Significance of Study

This study aimed to study the effects of two different brands of natto starter cultures, Kawashimaya and Chuan Xiu, on the fermentation process, antioxidant activity and sensory profile of natto. The Kawashimaya culture employs a single strain of *B. subtilis* var. natto, while the Chuan Xiu culture uses a combination of *B. subtilis* var. natto and *Lactiplantibacillus plantarum*. By exploring how these different strain combinations and ingredients influence natto's properties, this study focused to provide valuable insights into optimizing natto production for improved antioxidant activity and enhanced organoleptic qualities. The findings could significantly impact the food industry by broadening natto's appeal, particularly in markets where its traditional flavour is less popular, thereby increasing its global demand and consumption.

1.4 Objectives

The objectives of this study were:

i. to prepare natto through fermentation of soybean using different brands of starter cultures.

ii. to determine the total phenolic, total flavonoid contents and antioxidant activities (DPPH and reducing power assays) of natto prepared using different brands of starter cultures.

iii. to evaluate the sensory attributes of natto prepared using different brands of starter cultures.

CHAPTER 2

LITERATURE REVIEW

2.1 Natto

2.1.1 Physical Characteristics and Sensory Attributes

Natto is a traditional Japanese fermented food made from fermenting soybeans with *B. subtilis* var. natto. This *Bacillus* strain contributes to natto's distinct taste, along with its sticky, stringy, slimy texture and pungent odour. The sticky and stringy strand of natto is due to the production of poly-glutamic acid by the probiotic cultures during fermentation and maturation (Rocchi et al., 2024). High-quality natto features a distinct flavour, intact beans with a viscous appearance and soft texture. The texture should not be too firm or soft, allowing the beans to stick and form a silky, sticky mass when stirred with chopsticks (Guo and Yang, 2015).

In contrast, lactic acid bacteria strains produce lactic and acetic acids, which help to reduce the strong aroma and create a milder, more palatable flavour. The enzymes produced by these bacteria, such as proteases, break down proteins into savoury peptides, enhancing both flavour and texture, while esterase and lipase contribute to the development of complex flavours by breaking down fats (Lan et al., 2020). Additionally, the aromatic compounds generated during fermentation, along with the production of polysaccharides, play key roles in shaping natto's aroma and texture (Liu, Song and Luo, 2018).

2.1.2 Processing

Natto fermentation begins with a soaking process to attain the desired moisture content. The hydrated soybeans facilitate the steaming and boiling processes, ensuring consistent flavour and softness, which create ideal conditions for bacteria to access nutrients and grow (Mani and Ming, 2017). Additionally, steaming effectively kills surface bacteria and enhances enzyme penetration to break down soybean components. The steamed beans are then cooled before being inoculated with the bacterial strain, followed by 18 to 20 hours of fermentation at a temperature between 40℃ to 45℃ (JNCSF, n.d.). After fermentation, the natto is matured at 5℃ to suppress further fermentation and reduce ammonia production.

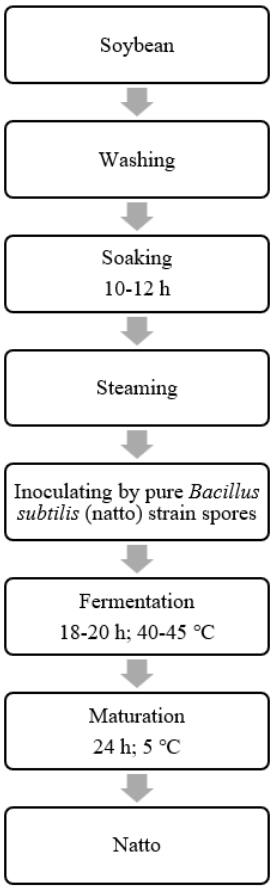

Figure 2.1: Natto processing flow chart.

2.2.1 *Bacillus subtilis*

Bacillus subtilis was recognized by the United States Food and Drug Administration (FDA) and European Food Safety Authority as generally regarded as safe (GRAS) and as having a Qualified Presumption of Safety (QPS) (Martinez, 2013).

In unfavourable conditions, *B. subtilis* can remain dormant as spores. These spores are highly resilient, capable of surviving for extended periods. When conditions such as heat, moisture and an optimal environment are met during fermentation, the spores germinate into active vegetative cells. These cells rapidly multiply and colonize the substrate, driving the fermentation process (Luu et al., 2015).

During fermentation, *Bacillus* species produces a wide range of enzyme, including amylase, β-glucosidases, phytase, α-galactosidase, protease and glutaminase. Amylases break down starches into simpler sugars, while β-Glucosidases hydrolyse β-glycosidic bonds, detoxify harmful compounds like cyanogenic glycosides and convert isoflavone glycosides to their active forms (Li et al., 2023) Also, phytases degrade phytic acid in grains and legumes, enhancing the availability of minerals. Furthermore, proteases break down proteins into peptides and amino acids, influencing the flavour, texture and nutritional value of fermented foods. Lastly, glutaminases convert glutamine into glutamate, contributing to umami flavour and aiding in the production of poly-γ-glutamic acid (Li et al., 2023).

Additionally, *Bacillus* species produce exopolysaccharides that impart the sticky, viscous texture characteristic of natto. The bacterium also generates ammonia as a byproduct of amino acid metabolism, which contributes to the strong, distinct odour of the fermentation product (KADA et al., 2008)

2.2.2 *Lactiplantibacillus plantarum*

While *B. subtilis* is the primary strain, different strains within this subspecies can be used as starter culture. *Lactiplantibacillus. plantarum* is a Gram-positive, acid-tolerant and non-spore forming. Like *Bacillus* species, *L. plantarum* is recognised as GRAS status by the U.S FDA and listed under QPS by European Food Safety Authorities (EFSA) (Echegaray et al., 2023).

Lactiplantibacillus. plantarum is notable for its ability to produce organic acids as major metabolites (Arasu et al., 2016). This bacterium is capable of thriving in various challenging environments, such as low pH conditions, exposure to lytic enzymes and bile salts found in the upper gastrointestinal tract. Additionally, *L. plantarum* efficiently utilizes a diverse range of carbohydrates, including monosaccharides and disaccharides, through extracellular enzymes, which further enhances its effectiveness in fermentation processes. Its adaptability and metabolic versatility make it a valuable asset in both industrial and food fermentation applications (Arasu et al., 2016).

2.2.3 Maltodextrin

Maltodextrin is made up of glucose units joined by α-1,4 glycosidic bonds. It is less sweet than sugar with a similar glycemic index to glucose and a dextrose equivalent (DE) < 20 (Chavan, Khedkar and Bhatt, 2016). It is classified based on its DE, which reflects the degree of hydrolysis. Higher DE are sweeter and more soluble, while lower DE correspond to more complex carbohydrates (Aidoo et al., 2013). Raw materials for maltodextrin production can include corn, wheat, rice, tapioca, potato, barley and sorghum (Chavan, Khedkar and Bhatt, 2016).

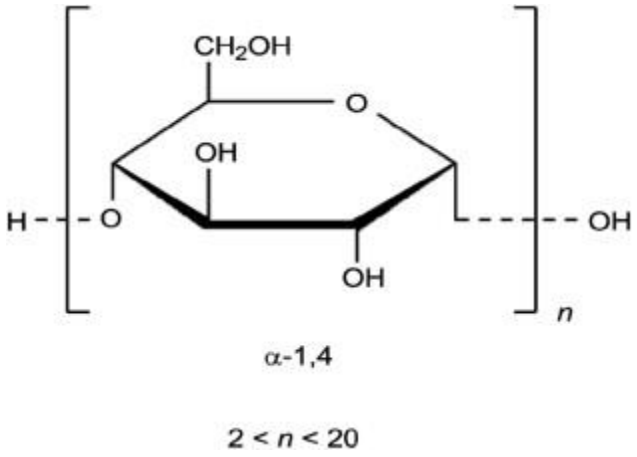

Figure 2.2: Chemical structure of maltodextrin (American Chemical Society, 2018).

The hydrogenated form of maltodextrin has been shown to decrease reactivity, while increase solubility and heat stability, making it ideal for confectionery products (Aidoo et al., 2013). Besides, it is commonly used in dairy product due to its bulking, gelling, binding properties and its ability to prevent crystallization and serves as a fat replacement (C.G. Harshitha, Sharma and Rajput, 2023).

Encapsulation is a valuable technique for protecting antioxidant phenolic compounds by covering them with a coating material. This technique enhances shelf-life, stability during food processing and digestion and bioavailability of phenolic compounds while masking any undesirable odour, taste or colour. Studies have shown that encapsulation technology can improve the intestinal uptake and bioactivity of phenolic compounds (Abdel-Aty, Barakat and Mohamed, 2022).

Maltodextrin serves as a protective wall material for sensitive antioxidant compounds such as polyphenols or isoflavones from degradation during processing and storage (Li et al., 2021b). Research by Abdel-Aty, Barakat and Mohamed (2022) demonstrated that combining maltodextrin with gum Arabic as encapsulation materials increased antioxidant activity in natto. Furthermore, the use of whey proteins in combination with either gum Arabic or maltodextrin as wall materials has been shown to enhance the stability and antioxidant capacity of polyphenols during storage (Li et al., 2021b).

2.3 Antioxidant Compounds in Natto

Antioxidants can donate electrons to neutralize unstable free radicals. This donation acts as a natural termination mechanism disrupting the initiation of chain reaction that can affect cells in the body. Thus, antioxidant is believed to be effective in maintaining the balance between radicals and antioxidants. Natto

is recognized for its potent antioxidant activities due to its rich bioactive compounds, such as phenolic compounds, isoflavones, vitamin K2, saponins, protease inhibitors and bioactive peptides.

2.3.1 Phenolic Acids

Phenolic acids are largely attribute for the antioxidant properties of natto and typically features a single carboxylic acid group. They are mainly categorized into two subgroups: hydroxybenzoic acid and hydroxycinnamic acid. These phenolic acids exert their antioxidant effects through multiple mechanisms, including hydrogen atom transfer, single electron transfer, transition metal chelation and sequential proton loss electron transfer (Zeb, 2020). However, the main mechanism is radical scavenging via hydrogen atom donation (Kumar and Goel, 2019).

The number and position of hydroxyl and methoxy groups in their molecular structures affects their free radical scavenging ability (Gao et al., 2022). The galloyl group in gallic acid makes it the most potent antioxidant among phenolic acids (Pérez, Inés Domínguez-López and Lamuela-Raventós, 2023). Similarly, the placement of hydroxyl groups in compounds like catechol can also boost their ability to neutralize free radicals. Besides, hydroxycinnamic acids show stronger reducing abilities than hydroxybenzoic acids, which is attributed to their greater resonance stabilization. It has also been observed that replacing a hydroxyl group with a methoxy group in hydroxycinnamic acids can further improve radical scavenging activity and increase reducing capacity.

2.3.2 Flavonoids

The flavonoid family includes flavones, flavonols, flavanones, anthocyanins and isoflavones, represents some of the most potent plant antioxidants. Isoflavones are primarily found in legumes, particularly soybeans (Josipovic et al., 2016). According to findings by Kimira et al. (1998), natto, miso and tofu are the main foods rich in isoflavones. The average daily intake of total aglycones was 39.46 mg, with regular consumption of natto contributing to 14.7% of genistein intake.

Isoflavones are secondary metabolites that belong to subclass of the flavonoid family of polyphenols (Engelhardt and Winterhalter, 2007). There are 12 types of isoflavones, which can be grouped into aglycones, glycosides, acetylglucosides and malonyl-glucosides. Isoflavones are often found as glycosides and further converted to free isoflavones or aglycones by β-glucosidase in the microflora. Aglycones are more lipophilic and can easily penetrate cell membranes, enhancing bioavailability. Three aglycones- genistein, daidzein and glycitein are prominent in natto for antioxidant properties (Kim, 2021). Phenolic compounds perform antioxidant activity both directly, as free radical scavenger sand indirectly, by influencing intracellular pro- and antioxidant enzymes. Both genistein and daidzein function as direct antioxidants by donating a hydrogen atom.

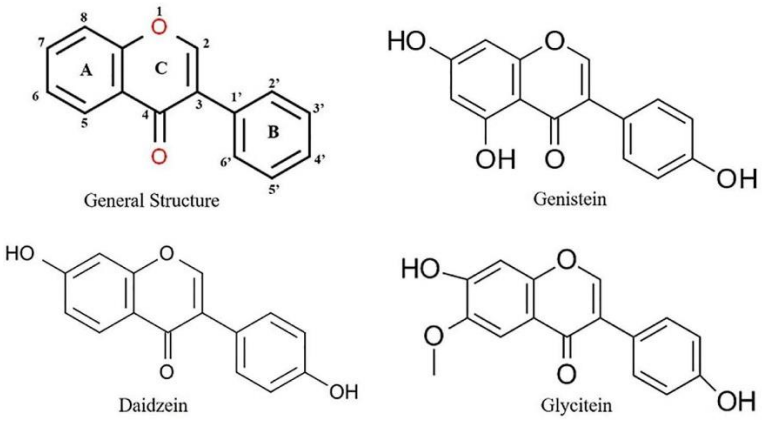

Figure 2.3: Chemical structure of isoflavones (Sohn et al., 2021).

During fermentation, microorganism such as *B. subtilis* produce enzymes like β-glucosidase. These enzymes hydrolyse the isoflavone glucosides into their corresponding aglycones. A similar finding is observed in soybeans fermentation process during tempeh production, which isoflavone glycosides are hydrolysed into aglycone forms (Andriani et al., 2014). Moreover, research has shown the isoflavones levels in natto are 2.2 times higher in natto compared to soybean (Li et al., 2021). Furthermore, fermentation reduces anti-nutritional factors such as phytic acid and protease inhibitors which can bind to isoflavones and other nutrients, making them less bioavailable (Samtiya, Aluko and Dhewa, 2020). The reduction of these compounds during fermentation helps increase the overall bioavailability of isoflavones.

2.3.3 Vitamin K2 (Menaquinone)

Vitamin K is features by a 2-methyl-1,4-naphthoquinone ring attached to isoprenoid residues. It exists primarily in two forms as vitamin K1 and vitamin

K2 (Kaźmierczak-Barańska and Karwowski, 2022). Vitamin K1 is naturally abundant in green leafy vegetables, while vitamin K2 is primarily found in fermented dairy products and produced in the intestine by LAB. The chemical structure of vitamin K2 varies depending on the number of isoprenoid groups in the side chain, with the unsaturated isoprenoid groups ranging from 1 to 13 (Słowik-Borowiec et al., 2021).

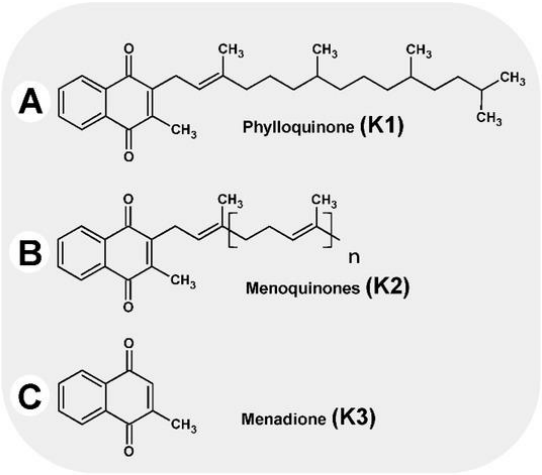

Figure 2.4: Chemical structure of vitamin K (Kaźmierczak-Barańska and Karwowski, 2022).

Natto is recognized as the richest source of vitamin K2, due to its fermentation process involving *B. subtilis*. The vitamin K2 content in natto exceeds 880 mg/100 g, which is 124 times higher than that found in unfermented soybeans (Chan et al., 2021). According to Słowik-Borowiec et al. (2021), the recommended daily intake of vitamin K2 is 65 g for men and 55 g for women, adjusted based on body weight.

MK-7 is more biologically active compared to MK-4. Research by Słowik-Borowiec et al. (2021) has shown that intestinal bacteria also produce menaquinones. For instance, *Propionobacteria* generate MK-9, whereas *L. lactis* ssp. *Lactis* and *L. lactis* ssp. *Cremoris* produce menaquinones from the MK-8 and MK-9 groups. The fermentation process that enhances MK-7 content not only boosts the levels of this potent form of vitamin K2 but also improves the bioavailability of other beneficial compounds, such as bioactive peptides, vitamins and exopolysaccharides, thereby amplifying the antioxidant properties of natto.

2.3.4 Polyglutamic acid

γ-PGA is responsible for the sticky texture of natto. It is a naturally occurring biopolymer made up of D- or L-glutamic acid units linked by γ-amide bonds. Its molecular weights ranges from 10 to 1000 kDa (Song et al., 2019). It is primarily produced through microbial fermentation and is valued for its multifunctional properties, including thickening, gelation, emulsification, film formation, moisturizing and adhesion.

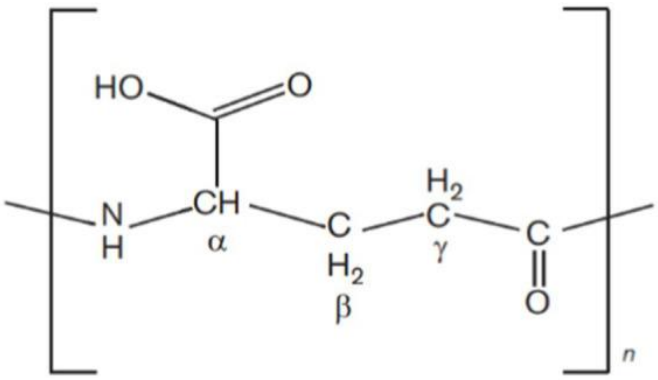

Figure 2.5: Chemical structure of γ-PGA (Elbanna et al., 2024).

In natto, γ-PGA plays a crucial role in enhancing nutrient utilization, retaining moisture and stimulating the production of nattokinase during solid-state fermentation. During this process, *B. subtilis* catalyses complex biochemical reactions that break down 50 – 60 % of proteins into peptides and amino acids. This breakdown leads to the formation of new active compounds like nattokinase, antioxidant peptides and antibacterial peptides (Wang et al., 2023).

Among these amino acids, glutamic acid polymerizes to form γ-PGA, which is a key component of natto's characteristic mucilage (Chan et al., 2021). Beyond its functional properties, γ-PGA also exhibits notable antioxidant activity. It chelates metal ions, inhibiting the formation of reactive oxygen species catalysed by metals and directly scavenges free radicals. This dual mechanism contributes to natto's overall antioxidant capacity, offering protection to cellular components and enhancing its health-promoting properties.

2.4 Methods to Study Antioxidant Activity

Antioxidant activity involving the studying the ability of compounds to neutralize reactive oxygen species (Ren et al., 2023). The phenolic compounds act as antioxidants by reacting with a variety of free radicals and are major contributors to antioxidant properties of natto. Both phenolic compounds and their subgroup flavonoids are evaluated in terms of TPC and TFC to provide an indication of potential antioxidant properties. These assessments are often complemented by specific antioxidant assays, such as DPPH scavenging activity and reducing power assay.

2.4.1 Total Phenolic Content (Folin-Ciocalteu Assay)

The Folin-Ciocalteu (F-C) assay is a reference method for quantifying the TPC in food. The assay employs the F-C reagent consists of a mixture of phosphomolybdate and phosphotungstate acid. This reagent reacts with phenolic compounds, causing a colour change from yellow to blue. This colour shift results from the antioxidant species donate an electron to F-C reagent, reducing the molybdenum centre from Mo(VI) to Mo(V) (Pérez, Inés Domínguez-López and Lamuela-Raventós, 2023). The intensity of the colour shift corresponds directly to the reducing activity of the phenolic compounds.

However, the F-C reagent can also react with non-phenolic substances, potentially leading to an overestimation of phenolic content. In natto, non-phenolic substances such as nattokinase, vitamin K2, polyglutamic acid,

biogenic amines, superoxide dismutase and small polypeptides might contribute to this overestimation (Liu et al., 2021).

Gallic acid is a reference standard used to quantify gallic acid equivalents (GAE) per unit sample. In the F-C assay, the sample is incubated with the reagent for 1 hour. The blue colour remains stable at room temperature and measurements taken after 6 hours are similar to those taken after 1 hour, though with slightly higher standard deviation. The colour develops more quickly at warmer temperatures, but temperatures above 40°C can cause the colour to fade more rapidly.

2.4.2 Total Flavonoid Content (Aluminium Chloride Assay)

The aluminium chloride colorimetric assay is commonly used quantify total flavonoids content. This assay relies on the interaction between aluminium chloride and flavonoid compounds, resulting in the formation of coloured complexes that can be quantitatively measured (Ahmed and Iqbal, 2018).

The aluminium chloride assay operates on the principle that aluminium chloride interacts with the ortho-dihydroxyl group of flavonoids to form acid-labile complexes. This interaction is specific to certain structural features of flavonoids, allowing for the differentiation and quantification based on these structural characteristics (Ahmed and Iqbal, 2018). The formation of aluminium chloride and flavonoid complexes results in coloured complexes which is directly proportional to the concentration of flavonoid compounds. When contaminated

with iron chloride, the complex often displays a yellow colour compared to the white pure compound (Kasprzak, Erxleben, & Ochocki, 2015; Pyrzynska & Pękal, 2011).

Several reagents, including sodium nitrite, acetic acid and sodium acetate, are added to promote the complex formation. Sodium nitrate serves as a nitrating agent selective, while acetic acid and sodium acetate create favourable conditions by maintaining a slightly acidic pH (Shraim et al., 2021).

Quercetin, catechin and rutin are used as reference standard for determining TFC, similar to how gallic acid is used in TPC assays. This practise ensures the accuracy of the analytical results. Although various methods exist for determining TFC, they all rely on the ability to form chelate complexes with aluminium ions. For natto or soybean analysis, a wavelength of 510 nm is selected for measurement as the aluminium-flavonoid complex formed with soybeans exhibits a strong and measurable absorbance at this wavelength (Josipovic et al., 2016; Juan and Chou, 2010).

2.4.3 DPPH Radical Scavenging Assay

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is employed to assess antioxidant activity by measuring the capacity of antioxidants to inhibit lipid oxidation and neutralize free radicals. DPPH possess a maximum UV-vis absorption at 517 nm (Škrovánková, Mišurcová and Machů,

2012). It reacts with hydrogen-donating compounds, leading to a reduction reaction that causes the violet DPPH methanol solution to turn yellow. This colour change indicates scavenging activity and is proportional to the number of electrons accepted (Cos et al., 2000).

The UV-vis spectrum of DPPH shows two prominent bands due to π–π* transitions, with the unpaired electron contributing significantly to the visible band. When an antioxidant is introduced, it donates hydrogen atoms to DPPH, reducing it to its hydrazine form. This reduction causes the visible band to disappear, resulting in a colour shift (Gülçin and Alwasel, 2023). DPPH remains a stable free radical due to the delocalization of its unpaired electron, which prevents dimerization. While DPPH is is almost insoluble in water at room temperature (Gülçin and Alwasel, 2023). To measure antioxidant capacity, various antioxidant concentrations are tested to determine the concentration required to scavenge 50% of the initial DPPH radicals within a given time frame, known as the EC_{50} (effective concentration) or IC_{50} (inhibitory concentration).

2.4.4 Reducing Power Assay

The reducing power assay assesses the antioxidant ability by measuring their ability to reduce oxidized intermediates through electron donation. This reflects both their reducing power and overall antioxidant activity. The formation of Prussian blue, an indicator of reducing power, can occur through two distinct pathways that ultimately produce the same end product.

In this assay, antioxidants convert Fe^{3+} into Fe^{2+}. Alternatively, antioxidants with reduction potential can interact with potassium ferricyanide (Fe^{3+}) to generate potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride, forming a ferric–ferrous complex that produces a blue colour (Bhalodia et al., 2013). The intensity of this blue colour, determined through spectrophotometric analysis, correlates directly with the antioxidant capacity of the sample. A more intense blue hue signifies a higher reducing power and a stronger ability of the compounds to donate electrons and reduce oxidized intermediates in the solution (Zhong and Shahidi, 2015).

2.5 Therapeutic Potential

Imbalance between free radicals and antioxidant activity in body leads to a condition known as oxidative stress, which can induce the onset and progression of diseases such as cancer, diabetes, atherosclerosis, inflammatory disorder, neurodegenerative and cardiovascular disease (Pham-Huy, He and Pham-Huy, 2008). Recent study demonstrated that fermented soy products, including natto, enhance their antioxidant profile, isoflavone content, TPC and TFC. Therefore, it is clear that the consumption of natto help mitigate oxidative stress.

2.5.1 Cardiovascular Health

The development of atherosclerosis involves the oxidation of LDL particles by reactive oxygen species, which stimulates an inflammatory response. Kawamata

et al. (2023) reported that high concentration of vitamin K in natto suppress plasma LDL, indicating that natto possesses anti-atherosclerotic effects.

Natto fractions have been found to lower plasma LDL levels, a key factor associated with arteriosclerosis. *In vitro* studies involving hypercholesterolemic mice revealed that components of natto significantly inhibit LDL oxidation. Additionally, a natto supplement containing bacillopeptidase F, known for its antithrombotic, fibrinolytic and antihypertensive properties, underscores its potential benefits in managing serious health conditions (Afzaal et al., 2022).

2.5.2 Anticarcinogenic Activity

The National Cancer Centre has reported that consuming large amounts of natto may reduce the risk of colorectal, breast and prostate cancers. Oxidative stress can damage DNA, proteins and lipids, leading to mutations and cellular changes that may promote cancer development. Natto contains anticancer compounds such as flavonoids, isoflavones, phytoestrogens, protease inhibitors and phytic acids. The fermentation process boosts the levels of these anticancer substances. Wang et al. (2023) discovered that natto lipopeptides can modify estrogen expression, damage DNA in human breast cancer cells, significantly inhibit cell proliferation and induce apoptosis.

2.6 Comparison with other soybean fermented food

A diverse variety of fermented soy food are found in Asian cuisine. The differences between these fermented soybeans products can be attributed to several factors, primarily the microorganism used in the fermentation. These of microorganisms contribute to distinct characteristics of the fermented soybean, including their aroma, texture, therapeutic and nutraceutical values. Fermentation involves the use of beneficial microorganisms such as bacteria, yeast, or a combination of both (do Prado et al., 2022).

Table 2.1: Characteristics of fermented soybean products.

Name	Description	Origin	Microorganism
Miso	Fermented paste of soybeans, rice, or barley with salt and koji.	Japan	*Aspergillus oryzae*
Tempeh	Cake-like texture, entirely covered with white mycelium, emitting clean, yeasty odour.	Indonesia	*Rhizopus oligoporus*
Cheonggukjang	Fermented soybeans paste	Korea	*B. subtilis, B. amyloliquefaciens* and *Rhizopus oligosporus*

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Framework

The research was conducted as outline in Figure 3.1.

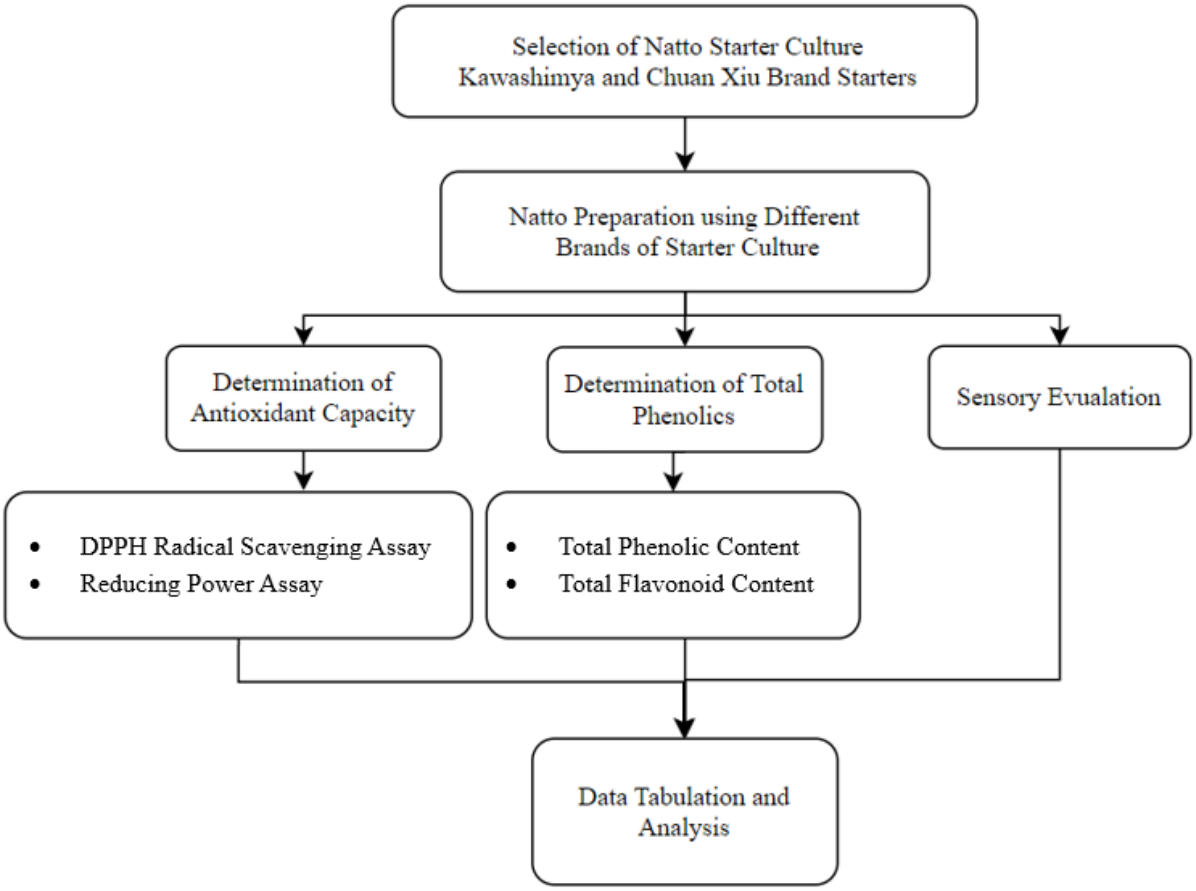

Figure 3.1: Flowchart of the research design.

3.2 Chemicals and Equipment

The chemicals used in the experiment include methanol, sodium carbonate, gallic acid, Folin-Ciocalteu reagent, sodium nitrate, aluminium chloride hexahydrate, sodium hydroxide, quercetin, DPPH reagent, ascorbic acid, phosphate buffered saline, potassium ferricyanide (III), trichloroacetic acid and ferric chloride. The equipment utilized includes a refrigerator, autoclave, electronic balance, freeze dryer, grinder, incubator, centrifuge, microplate reader, micropipette and vortex.

3.3 Natto Preparation

3.3.1 Natto Starter Selection

Two brands of natto starter culture, Kawashimaya and Chuan Xiu, were purchased from the online platform Shopee. The origin and main ingredients of these natto starter are shown in Table 3.1.

Figure 3.2: Kawashimaya (left) and Chuan Xiu (right) starter cultures.

Table 3.1: Brands of natto starter and its bacteria strain, origin and main ingredients.

Brands	Bacteria Strain	Origin	Ingredients
Kawashimaya	*Bacteria subtilis* var. natto	Japan	-
Chuan Xiu	*Bacteria subtilis* var. natto and *Lactiplantibacillus plantarum*	China	Maltodextrin

3.3.2 Soybeans Selection

1 kg of soybeans were purchased from the online platform, Shopee. The origin, brand and type of soybean was shown in Table 3.2.

Table 3.2: Soybeans and its origin, brand and type.

Category	Details
Origin	Canada
Brand	Thompsons Hyland
Type	Yellow soybean

3.3.3 Preparation of Fermented Soybean (Natto)

Natto fermentations were performed, as described by Kawashimaya the Japan Store (2024) for natto prepared using Kawashimaya starter culture with some modifications. Firstly, 200 g soybeans were washed. The soybeans were soaked in distilled water in the ratio of 1:4 (w/v) for 24 h to avoid acidification. Next, the soaked soybeans were steamed in autoclave at 121 ℃ for 15 mins. Following that, the steamed soybeans were cooled to 38℃. The natto starter solution will

be seeded on soybean and stir well. The starter culture added accordance with the recommended dosage, Kawashimaya (0.1 g/100 g) and Chuan Xiu (0.15 g/100 g). The seeded soybeans were then transferred in container with a depth of about 3 cm and small holes on the wall. The soybeans were fermented in incubator at 40 ℃ for 24 h. For the last step, the fermented soybeans were further fermented in chiller for 24 h.

3.3.4 Preparation of Natto Powder

To prepare natto powder for analysis, the fermented natto was first frozen for 24 h. The frozen natto was then transferred to containers covered with aluminium foil with small holes. These containers were placed in a freeze dryer chamber for 48 h. Once freeze-dried, the natto was ground into powder using a blender.

3.3.5 Preparation of Natto Extract

One gram of powder natto was extracted with 10 mL of 80% (w/v) methanol for 5 mins at room temperature. The extracts were centrifuged at 1000 *x g* for 15 mins. The supernatants were collected and subjected for subsequent analysis.

3.4 Determination of Antioxidant Properties

The antioxidant properties of the natto produced with different brands of starter culture were investigated by analysing phenolic compounds, including TPC and

TFC, as well as through antioxidant capacity assays such as DPPH and reducing power.

3.4.1 Determination of Total Phenolic Content by Folin-Ciocalteu Method

The TPC was determined using the Folin-Ciocalteu colorimetric method. To prepare the standard gallic acid solution, 5 mg of gallic acid powder was dissolved in 10 mL of distilled water to create a 500 mg/L stock solution. This stock solution was then diluted to produce concentrations of 100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L and 20 mg/L.

For the assay, 50 µL of the extract 0.5 was mixed with mL of 10% (w/v) Folin-Ciocalteu reagent and incubated in the dark at room temperature for 3 mins. Then, 400 µL of 7.5% (w/v) sodium carbonate was added and the mixture was left in the dark at room temperature for 30 mins. Distilled water was used as a blank. The absorbance was measured at a wavelength of 765 nm using a microplate reader. The TPC was expressed as gallic acid equivalents (mg GAE/100 g).

3.4.2 Determination of Total Flavonoid Content by Aluminium Chloride Colorimetric Method

The TFC was measured using the aluminium chloride colorimetric method. A standard quercetin solution was prepared by dissolving 5 mg of quercetin in 10 mL of distilled water to create a 500 mg/L stock solution. This stock solution

was then diluted to obtain concentrations of 100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L and 20 mg/L.

For the assay, 50 µL of the extract was mixed with 200 µL of distilled water and 15 µL of 5% (w/v) sodium nitrate. Afterward, 30 µL of 10% (w/v) aluminium chloride hexahydrate was added and the mixture was left in the dark at room temperature for 6 mins to allow the reaction to complete. Subsequently, 100 µL of 1M sodium hydroxide was added and the solution was incubated at room temperature for another 15 mins. Distilled water was used as a blank. The absorbance was then measured at a wavelength of 510 nm using a microplate reader. The TFC was expressed as quercetin equivalents (mg QE/100 g).

3.4.3 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The radical scavenging activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described by Nguyen et al. (2022), with slight modifications. A standard ascorbic acid solution was prepared by dissolving 10 g of ascorbic acid in 100 mL of distilled water to obtain a 100 mg/L stock solution, which was then serially diluted with distilled water to achieve concentrations of 25 mg/L, 20 mg/L, 15 mg/L, 10 mg/L and 5 mg/L. Similarly, the sample extracts were prepared at concentrations of 100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L and 20 mg/L. As a negative control, 80% (w/v)

methanol was used, while the blank control consisted solely of DPPH reagent and methano

To perform the assay, 1 mL of each extract was mixed with 250 µL of a 0.2 mM methanolic solution of DPPH and incubated in the dark at room temperature for 30 mins. The mixtures were covered with aluminium foil throughout the incubation period to protect them from light. Following incubation, the absorbance was measured at 517 nm using a microplate reader. The percentage of RSA was calculated using the formula below. Graphs were then generated by plotting the percentage of DPPH free radical scavenging activity against the concentration and the IC_{50} values (mg/mL) were determined from these plots.

$$\text{Percentage of DPPH Radical Scavenging Activity} = [(A_C - A_S)/ A_C] \times 100\%$$

A_C: Control absorbance (blank absorbance); A_S: the testing specimen absorbance (sample absorbance).

3.4.4 Determination of Reducing Power Assay

The reducing capacity was measured using reducing power assay, following a method modified from Lee, Lai and Wu (2015). The chemical preparations for the assay included the preparation of a 1% (w/v) Potassium ferricyanide (III) by dissolving 1 g of potassium ferricyanide in 100 mL of distilled water. Similarly, a 10% (w/v) trichloroacetic acid (TCA) solution was prepared by dissolving 10

g of TCA in 100 mL of distilled water and a 1% (w/v) ferric chloride solution was prepared by dissolving 1 g of ferric chloride in 100 mL of distilled water.

The standard ascorbic acid was prepared by dissolving 10 g of ascorbic acid in 100 mL of distilled water, resulting in a 100 mg/L stock solution, which was then serially diluted to create solutions of 25 mg/L, 20 mg/L, 15 mg/L, 10 mg/L and 5 mg/L. Similarly, sample extracts were prepared at concentrations of 100 mg/L, 80 mg/L, 60 mg/L, 40 mg/L and 20 mg/L. For the negative control, 80% (w/v)methanol was used, while the blank control consisted of all the reagents used in the assay except the sample extract.

In this test, 0.4 mL of the extract was mixed with 1 mL of phosphate buffer (0.3 M, pH 6.6) and 1 mL of potassium ferricyanide (III). The mixture was incubated at 50°C in a water bath for 20 mins. Following incubation, 1 mL of 10% TCA solution was added and the sample was centrifuged at 1000 *x g* for 10 mins. After centrifugation, 2 mL of the supernatant was collected and added with 2 mL of distilled water and 0.4 mL of 1% (w/v) ferric chloride solution. The mixture was then incubated at room temperature for 30 mins. Lastly, the absorbance was measured at 700 nm using a microplate reader. The percentage of RP was calculated using the following formula. Graphs plotting the percentage of reducing power against the concentration were generated and the EC_{50} values (mg/mL) were determined from these plots.

$$\text{Percentage of reducing power} = [(A_S - A_C)/ A_C] \times 100\%$$

A_C: Control absorbance (blank absorbance); A_S: the testing specimen absorbance (sample absorbance).

3.5 Sensory Evaluation

The consumer responses to natto made from two different starter culture, Kawashimaya and Chuan Xiu, as well as commercial natto were evaluated by 50 untrained panellists at Universiti Tunku Abdul Rahman. The commercial natto prepared was Yamada Hokkaido Natto Bean. The sensory evaluation was determined with 9 hedonic scaling tests ranging from dislike extremely (1) to like extremely (9) in term of appearance, aroma, stringiness, taste, chewiness and overall acceptability. The master sheet and score sheets were prepared as illustrated in the Appendix A. Subsequently, the paper cups were labelled with assigned random codes and arranged in the order of presentation on the tray. Each panellist was provided with 3 natto samples in paper cup, disposable plastic spoons, pencil, score sheet, tissue paper and a glass of water for cleansing their palate.

Figure 3.3: Yamada Hokkaido Natto Bean (Commercial natto).

3.6 Statistical Analysis

All tests were performed in triplicate for 2 separate runs (n = 6). The collected data was analysed using Statistical Package for the Social Sciences (SPSS) and Microsoft Excel. Each experimental condition was presented as mean ± standard deviation, with statistical significance determined at $p < 0.05$. A one-way analysis of variance (ANOVA), followed by Tukey post-hoc test to assess the significant difference in TPC, TFC, DPPH radical scavenging activity, reducing power and sensory evaluation of natto prepared with different starter culture brands.

CHAPTER 4

RESULTS

4.1 Total Phenolic Content

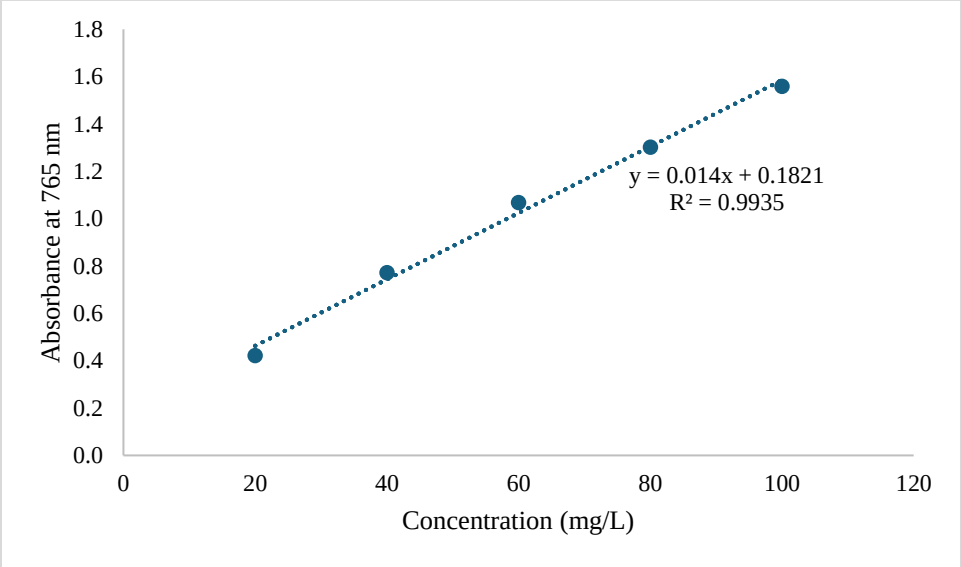

Figure 4.1: Standard curve of gallic acid.

Table 4.1: TPC of natto prepared with different starter cultures and control.

Starter Culture	Total Phenolic Content (mg GAE/100g extract)
Kawashimaya	16.6 ± 0.06^{a}
Chuan Xiu	24.6 ± 0.08^{a}
Control	3.9 ± 0.01^{b}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Figure 4.1 illustrated the gallic acid calibration curve used for determining the TPC of control and natto samples prepared with different starter cultures. A linear equation of y = 0.014x + 0.1821 with a regression coefficient of R^2 = 0.9935 was obtained using 20, 40, 60, 80 and 100 mg/L gallic acid . A higher TPC was observed for natto prepared using the Kawashimaya (16.6 ± 0.06 mg GAE/100g) and Chuan Xiu (24.6 ± 0.08 mg GAE/100g) starter cultures as compared to the control natto (0.39 ± 0.01 mg GAE/100g) in Table 4.1. However, no significant difference ($p > 0.05$) was found among the natto prepared using the Kawashimaya and the Chuan Xiu starter cultures.

4.2 Total Flavonoid Content

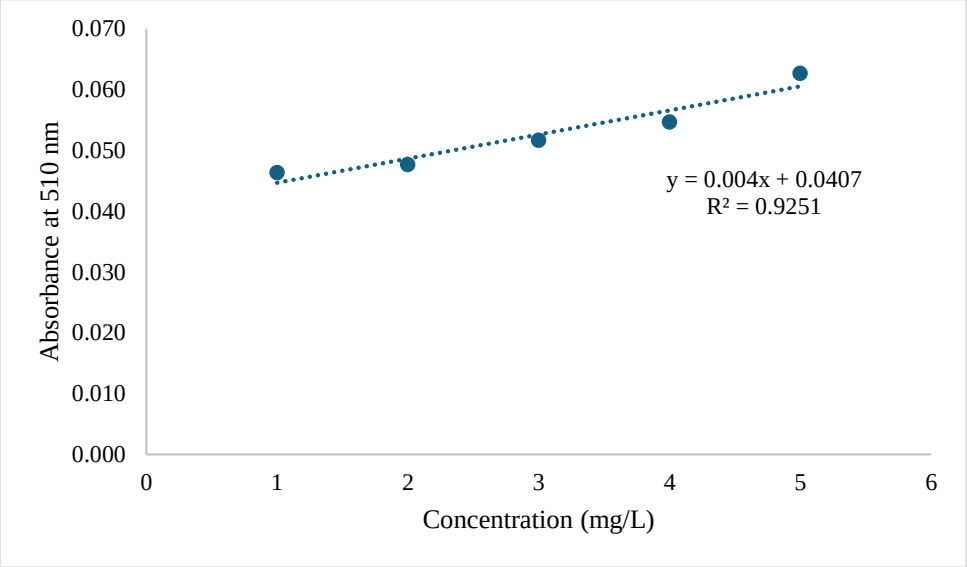

Figure 4.2: Standard curve of quercetin.

Table 4.2: TFC of natto prepared using different starter cultures and control.

Starter Culture	Total Flavonoid Content (mg QE/100g extract)
Kawashimaya	3.70 ± 0.005^{a}
Chuan Xiu	3.23 ± 0.019^{ab}
Control	1.87 ± 0.007^{b}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Figure 4.2 illustrated the quercetin calibration curve for determining the TFC of control and natto samples prepared using different starter cultures. A linear equation of $y = 0.04x + 0.0407$ with a regression coefficient of $R^2 = 0.9251$ was obtained using 20, 40, 60, 80 and 100 mg/L gallic acid. Referring to Table 4.2, the natto prepared using Kawashimaya starter culture (3.70 ± 0.005 mg QE/g) showed the highest TFC, while the control natto (1.87 ± 0.007 mg QE/g) had the lowest TFC. Besides, TFC of Kawashimaya and Chuan Xiu exhibited no significant difference ($p > 0.05$) but a significant difference ($p < 0.05$) from the control natto.

4.3 DPPH (2,2- diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

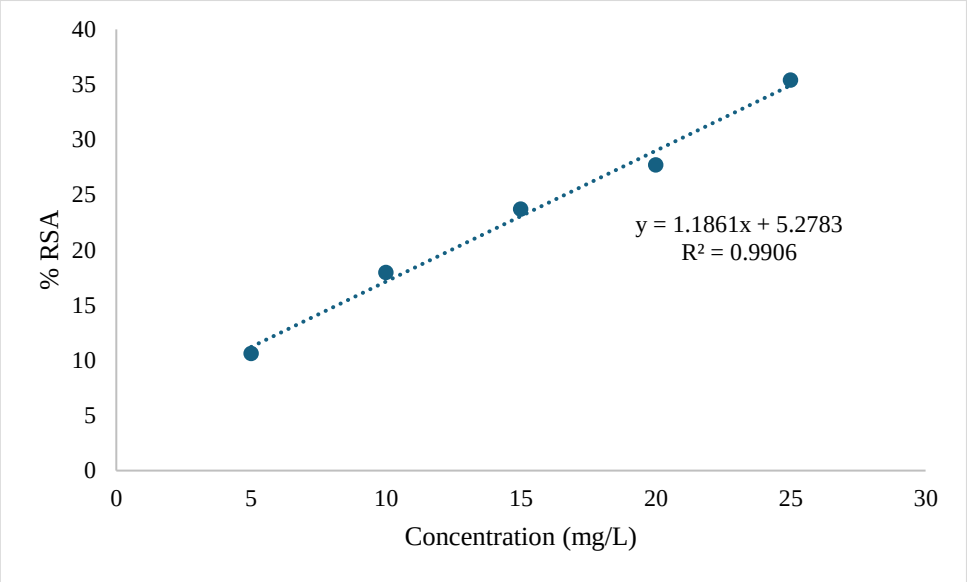

Figure 4.3: Standard curve of ascorbic acid.

Table 4.3: IC_{50} of natto prepared using different starter cultures and control.

Sample	IC_{50} (mg/L)
Kawashimaya	52.69 ± 19.35^{b}
Chuan Xiu	69.28 ± 22.70^{b}
Control	124.61 ± 33.42^{a}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

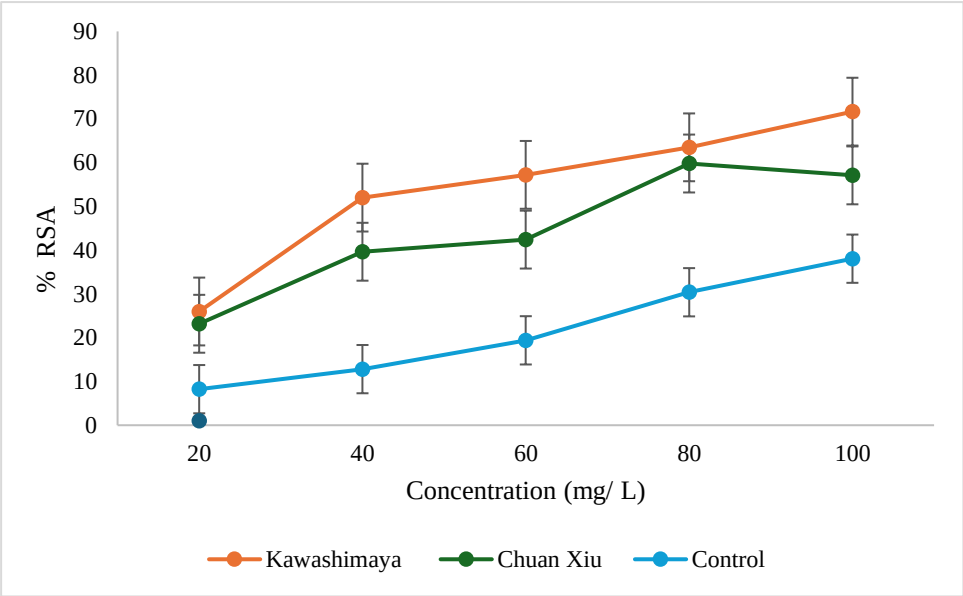

Figure 4.4: DPPH scavenging radical activity of Kawashimaya, Chuan Xiu and control.

The DPPH activities of natto were expressed in terms of radical scavenging activity (% RSA) and IC_{50} in Table 4.3. Figure 4.3 showed the ascorbic acid calibration curve used to determine the radical scavenging activity of natto samples. A linear equation of $y = 1.1861x + 5.2783$ with a regression coefficient of $R^2 = 0.9906$ was obtained using 5, 10, 15, 20 and 25 mg/L ascorbic acid. The RSA values ranged from 8.24% to 71.66%, with natto prepared using the Kawashimaya starter culture having the highest % RSA. The RSA was linearly proportional to the concentration.

The lowest IC_{50} was observed in natto prepared using Kawashimaya starter culture (52.69 ± 19.35 mg/L) and natto prepared using Chuan Xiu starter culture

(69.28 ± 22.70 mg/L). Besides, control natto with the highest IC_{50} (124.61 ± 33.42 mg/L) differed significantly ($p < 0.05$) from the other sample.

4.4 Reducing Power Assay

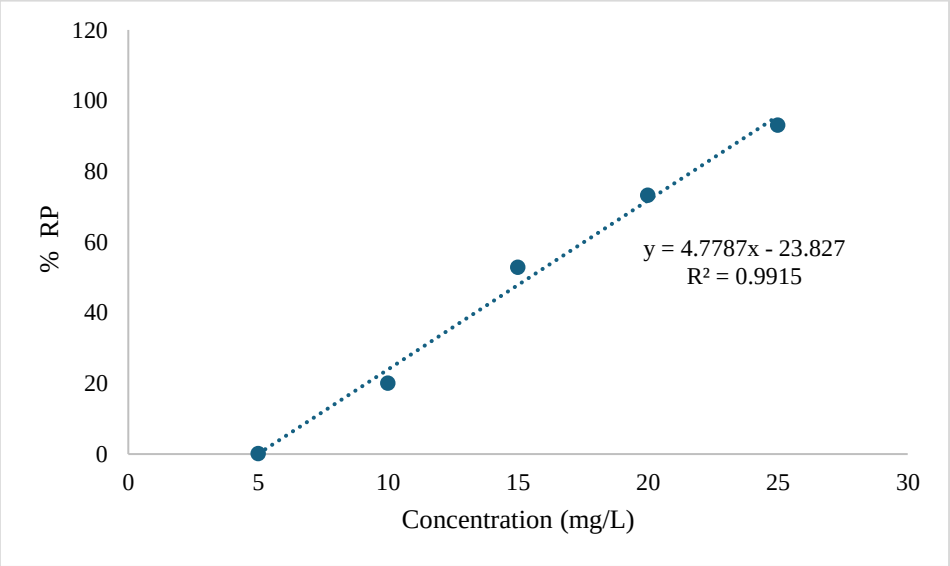

Figure 4.5: Standard curve for ascorbic acid.

Table 4.4: EC_{50} of natto prepared using different starter cultures and control.

Sample	EC_{50} (mg/L)
Kawashimaya	8.41 ± 2.4^{b}
Chuan Xiu	6.21 ± 2.74^{b}
Control	17.09 ± 5.16^{a}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significantly differences at $p < 0.05$.

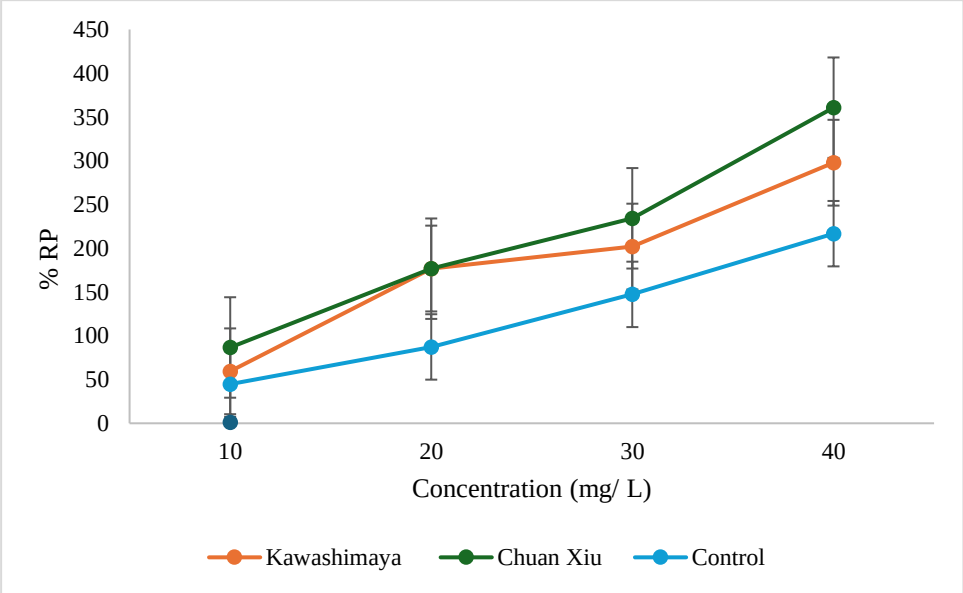

Figure 4.5: Reducing power of natto prepared using Kawashimaya, Chuan Xiu starter cultures and control.

The reducing power assay was evaluated in terms of reducing power (% RP) and EC_{50} in Table 4.4. Figure 4.4 demonstrated the ascorbic acid calibration curve used to assess the reducing ability of natto samples. A linear equation of y = 4.7787x + 523.827 with a regression coefficient of $R^2 = 0.9915$ was obtained using 5, 10, 15, 20 and 25 mg/L ascorbic acid. The RP values were ranged from 44.75% to 360.63%, with natto prepared using the Chuan Xiu starter culture having the highest % RP. Besides, the % RP was found to increase along with increasing concentration.

There was no significant difference ($p > 0.05$) between Kawashimaya (8.41 ± 2.47 mg/L) and Chuan Xiu (6.21 ± 2.74 mg/L) natto. In contrast, control had the

highest EC_{50} (17.09 ± 5.16 mg/L) differed significantly ($p < 0.05$) from the other samples.

4.5 Sensory Evaluation

Table 4.5: Sensory attributes of natto prepared using Kawashimaya and Chuan Xiu starter cultures and a control using 9-point hedonic scaling.

Attribute	Kawashimaya	Chuan Xiu	Control
Appearance	4.92 ± 1.68^{b}	5.66 ± 1.76^{ab}	6.32 ± 1.70^{a}
Aroma	5.32 ± 1.75^{a}	5.48 ± 1.80^{a}	5.86 ± 1.69^{a}
Stringiness	4.78 ± 1.67^{b}	5.80 ± 1.94^{a}	5.86 ± 1.83^{a}
Taste	4.42 ± 1.98^{a}	4.58 ± 1.88^{a}	4.62 ± 2.10^{a}
Chewiness	4.80 ± 1.68^{a}	5.38 ± 1.64^{a}	5.50 ± 1.58^{a}
Overall acceptability	4.64 ± 1.74^{a}	5.06 ± 1.78^{a}	5.06 ± 1.83^{a}

Data are expressed as mean ± standard deviation. Means within a row with different lowercase superscripts indicate significantly differences at $p < 0.05$.

Figure 4.6: Schematic representation of preference of natto for sensory attributes.

Table 4.5 described sensory profiles of the two natto samples prepared using the Kawashimaya and Chuan Xiu starter cultures and a commercial natto (control) were evaluated in terms of appearance, aroma, stringiness, taste, chewiness and overall acceptability using a 9-point hedonic scaling. Referring to the schematic representation in spider plot (Figure 4.6), control natto exhibited the highest sensory scores in all aspects, while natto prepared using the Chuan Xiu ranked second and Kawashimaya natto ranked third. There were no differences ($p > 0.05$) among Kawashimaya, Chuan Xiu and control natto in the sensory evaluation of the aroma, taste, chewiness and overall acceptability. However, in terms of appearance, natto prepared using the Kawashimaya starter culture (4.92

± 1.68) was significantly lower ($p < 0.05$) compared to the control natto (6.32 ± 1.70), with natto prepared using Chuan Xiu starter culture (5.66 ± 1.76) being intermediate. Notably, natto prepared using the Kawashimaya (4.78 ± 1.67) had a significantly lower ($p < 0.05$) stringiness compared to both natto prepared using the Chuan Xiu (5.80 ± 1.94) and control natto (5.86 ± 1.83).

CHAPTER 5

DISCUSSION

In this study, the antioxidant activities of natto samples prepared with two different starter cultures, including Kawashimaya and Chuan Xiu, were evaluated in terms of their TPC, TFC, DPPH radical scavenging activity and reducing power compared to a control sample, soybeans without starter culture addition. According to the ingredient lists on the starter packages, both Kawashimaya and Chuan Xiu starter cultures used *B. subtilis* var. natto as starter bacteria. However, Chuan Xiu also contains *Lactoplantibacillus plantarum* and maltodextrin.

5.1 Total Phenolic Content

Phenolic compounds, which are among the largest phytochemical molecules derived from plants, are well-known for their antioxidant properties. They are primarily divided into two main categories: flavonoids and phenolic acids. These compounds are powerful antioxidants due to their ability to neutralize free radicals, transfer electrons and hydrogen atoms and bind metal cations (Hassanpour and Doroudi, 2023). Given their significant role in antioxidant activity, the TPC and TFC were measured.

As presented in Table 4.1, the TPC of natto prepared using the Kawashimaya and Chuan Xiu starter cultures was 16.6 mg GAE/100g and 24.6 mg GAE/100g,

respectively, both significantly higher than the control natto, which had a TPC of 0.39 mg GAE/100g. This upward trend in TPC was consistent with the findings of Juan and Chou (2010), where natto fermented with Kawashimaya and Chuan Xiu starter cultures exhibited higher phenolic content compared to natto without starter culture. This increase can be assigned to the fermentation process, which releases bound phenolic compounds and enhances the bioavailability of free hydroxyl groups.

Soybeans contain various groups of phenolic compounds, with phenolic acids being the predominant components. These phenolic acids are often bound to the cell wall in glycosylated or polymeric form, which limits their bioaccessibility. According to Yin et al. (2020), *B. subtilis* can produce esterase enzymes during fermentation. These enzymes facilitate the release of bound phenolic compounds from soy glycinin, increasing the availability of phenolic acids in their free, bioactive forms. This increase aligns with the findings of Melini and Melini (2021), who reported a fourfold increase in free phenolics and a 1.5-fold decrease in bound phenolic content in fermented quinoa seeds.

The increase in TPC following fermentation can be attributed to the enzyme degradation of biomacromolecules and cell walls. Esterase enzymes hydrolyse ester bonds, which often link phenolic compounds to cellular components, thereby releasing these compounds from their bound forms. The increase in polyphenol levels in fermented legumes is also associated with a reduction in tannin content, facilitated by the enzyme tannase. Tannase activity leads to the

release of gallic acid and glucose, as reported by Neelam et al. (2012). Concurrently, hydrolysing enzymes such as cellulases, xylanases and proteases act on the structural components of soybeans, including cellulose, hemicellulose and proteins (Zhang et al., 2022b).

Furthermore, the biotransformation of phenolic acids also contributes to the increase in TPC. Biotransformation processes produce phenolic acid decarboxylase, which converts conjugated hydroxycinnamic acids into volatile phenols during lactic acid bacteria fermentation (Yang et al., 2023). This synthesis of phenolic compounds adds to the overall TPC. This finding aligns with the research of Svensson et al. (2010), who showed that *Lactobacillus* could metabolize ferulic acid, caffeic acid and naringenin-7-O-glucoside in sorghum dough into free phenolic acids and smaller metabolites like dihydroferulic acid and naringenin.

Additionally, the degradation of anti-nutritional factors in legumes, such as phytic acid and polyphenols, which bind to phenolic compounds, plays a role. The presence of *B. subtilis* and *L. plantarum* specifically degrades phytic acid (myoinositol hexakisphosphate) into inositol and free phosphate through the action of phytase enzyme, leading to an increase in TPC (Rasane et al., 2014).

5.2 Total Flavonoid Content

As shown in Tables 4.1 and 4.2, TPC values for natto prepared using Kawashimaya and Chuan Xiu starter cultures were consistently higher than the TFC values. This supports the observation by Mutha, Tatiya and Surana (2021) that flavonoids represent a major subgroup of phenolic compounds. Additionally, Table 4.2 indicates that natto produced with the Kawashimaya starter culture had the highest TFC, while the control natto exhibited the lowest TFC. These results were consistent with our findings, which suggest that fermentation by *B. subtilis* enhances flavonoid content (Leonard et al., 2021).

The higher TFC in natto prepared using the Kawashimaya starter culture, compared to control natto, can be attributed to the fermentation process by *B. subtilis*, which enhances the bioavailability of flavonoid compounds. This was consistent with Liu et al. (2023), who reported a 20-fold increase in isoflavone aglycone content following tempeh fermentation. Flavonoids, a class of phenolic compounds, possess the highest antioxidant capability due to their chemical structure. Isoflavone aglycones like daidzein and genistein are more bioactive and exhibit higher antioxidant capacity than their glycoside counterparts. During fermentation, *B. subtilis* hydrolyses isoflavone glucosides through deglycosylation, converting them into aglycones, thereby increasing TFC (Islam et al., 2015).

The increase in TFC in natto prepared using Kawashimaya and Chuan Xiu starter cultures was due to fermentation, which releases bound flavonoids and

enhances their bioavailability. *B. subtilis* enzymes break down cell wall components and reduce anti-nutritional factors, similar to how phenolic acids are released during fermentation, thereby boosting TFC alongside TPC.

In contrast, the TFC in natto prepared with the Chuan Xiu starter culture showed no significant difference ($p > 0.05$) compared to the control natto. Although both *B. subtilis* and *L. plantarum* can produce enzymes during fermentation that release bound flavonoid compounds or metabolize these phenolic compounds, the increase in TFC may not be substantial enough to create a measurable difference when compared to the control natto.

5.3 Antioxidant Capacity

DPPH is a commercially available radical used as the oxidizing radical and an indicator for the antioxidant activity. The colour change from purple to pale yellow which indicates a decrease in absorbance, allows the determination of antioxidant scavenging capability (Jamuna et al., 2010). Referring to Figure 4.4, the data showed that the interaction between RSA and DPPH was concentration dependent. Results were reported as IC_{50}, which higher antioxidant power corresponds to a lower IC_{50}. In DPPH radical scavenging activity assay, the IC_{50} values were lowest in natto prepared using Kawashimaya and Chuan Xiu starter, measuring 52.69 mg/L and 69.28 mg/L, whereas the control natto showed the highest IC_{50} value at 124.61 mg/L.

The reducing capacity serves as a significant indicator of its potential antioxidant activity. The reduction potential is measured by observing the colour change from yellow to green due to the Fe^{3+} to Fe^{2+} reduction. The reducing power often depends on the ability of antioxidants to interrupt the free radical chain by providing a hydrogen atom (Jamuna et al., 2010). The natto prepared using Kawashimaya and Chuan Xiu starter showed higher EC_{50} as compared to the control natto.

The higher antioxidant activity in natto prepared using Kawashimaya and Chuan Xiu starter cultures compared to control natto may be the result of biosynthesis of menaquinones during fermentation. Vitamin K2 is known to be the most active in MK-7 form, characterized by its superior bioavailability and longer half-life in human blood. The biosynthesis of MK-7 was found by fermentation, majorly using the *B. subtilis* strain (Zhao et al., 2021). This fermentation not only increase concentrations of MK-7 but also influenced the bioavailability of various compounds, including bioactive peptides, vitamins and exopolysaccharides, enabling further reinforce of antioxidant activity.

Besides, the EC_{50} and IC_{50} increased by 55.33 mg/L and 10.88 mg/L, respectively, compared to the control natto, indicating that the natto prepared using Chuan Xiu starter cultures enhances the antioxidant abilities of natto. Studies on the co-fermentation of *B. subtilis* GUTU09 with *Lactobacillus*, *Bifidobacterium* and *Mucor* showed higher nattokinase and protease activity compared to single-strain fermentation (Lan et al., 2020). Both *B. subtilis* and *L.*

plantarum were discovered to improve proteolytic activity, facilitating the breakdown of proteins into their constituent peptides and amino acids. These encrypted peptides can scavenge free radicals, inhibit lipid peroxidation and possess metal ion chelating activity (Visessanguan et al., 2005). Furthermore, the inclusion of LAB may increase the levels of menaquinones in natto. As reported by Beulens et al. (2013), lactic acid bacteria such as *Lactococcuslactis* ssp. Lactis and L. *lactisssp. Cremoris* were able to synthesis MK-7 from the MK-8and MK-9 groups. This double-strain combination synergistically increases the antioxidant activity of natto. In addition, maltodextrin's role in encapsulation significantly enhances antioxidant properties. By acting as a protective coating, maltodextrin prevents the degradation of antioxidant-phenolic compounds, thereby maintaining their stability and effectiveness (Abdel-Aty, Barakatand Mohamed, 2022).

In general, natto with higher phenolic compounds exhibits greater antioxidant activity. Natto prepared using Chuan Xiu starter culture with the highest TPC exhibited the highest reducing power, whereas natto prepared using Kawashimaya starter culture with the highest TFC showed the highest radical scavenging activity. The obtained results are in accordance with studies by Slađana Žilić et al. (2011) which describe a high correlation between TPC and reducing power, whereas TFC was strongly correlated with radical scavenging activity.

Meanwhile, the DPPH and reducing power tests yielded different antioxidant capabilities for the same starter. This contributed to each antioxidant assay offers only a rough estimate of antioxidant capacity and relies on its specific reagents and conditions, so results can be subjective (Liu et al., 2014). The DPPH and reducing power assays provided a comprehensive review of the antioxidant abilities of the natto. The consistent results across various assays improve the credibility and highlight the roles of TPC and TFC in antioxidant mechanisms.

5.4 Sensory Attributes

The sensory attributes of natto prepared using two different starter cultures (Kawashimaya and Chuan Xiu) and a control natto (Yamada Hokkaido Natto Bean) were evaluated in aspect of appearance, aroma, stringiness, taste, chewiness and overall acceptability. The control natto was originated in Japan with *B. subtilis* var. natto as bacterial fermentation. As shown in Table 4.5, there were no differences among natto prepared using Kawashimaya and Chuan Xiu starter cultures as well as control natto in the sensory evaluation of the aroma, taste, chewiness and overall acceptability.

However, natto prepared using the Kawashimaya had a significantly lower stringiness as compared to both natto prepared using Chuan Xiu starter culture and control natto. The combination of *B. subtilis* and *L. plantarum* in natto prepared using Chuan Xiu starter led to more effective protein breakdown, including glutamic acid, contributing to a greater degree of stringiness. Besides, maltodextrin, a hydrolysed starch that required to be converted into glucose by

enzymatic reactions before it can be utilized for fermentation. Its presence in Chuan Xiu starter culture promotes fermentation by acting as a readily fermentable glucose source as well as a bulking agent, which helps evenly distribute bacteria throughout the soybeans (Tiefenbacher, 2017). Also, the addition of maltodextrin leads to increase the production of glutamic acid. Yan et al. (2022) studies are consistent with the result, which indicates that optimizing carbon source such as glucose, sucrose and fructose effectively leads to increase the production of γ-PGA.

Notably, natto prepared using the Kawashimaya starter culture exhibited a significantly lower appearance score compared to the control natto. The superior appearance of the control natto may involves more precisely controlled and standardized fermentation conditions, including optimal fermentation time and temperature, as well as soybeans varieties, microbial strains used and the amount of inoculation (Yang et al., 2021).

5.5 Limitations of the Study

This study's limitations include the use of a fixed set of fermentation conditions, which may not fully capture the optimal parameters for enhancing antioxidant activity and sensory qualities. The study was also limited by its focus on only two starter cultures, potentially overlooking other strains that could have different impacts on the results.

5.6 Recommendations for Future Studies

Future research should focus on conducting correlation analysis to examine the relationships between TPC, TFC, DPPH radical scavenging activity, and RP activity. Additionally, a broader range of fermentation conditions, including variations in temperature, pH, oxygen levels, and fermentation time, should be explored to determine the optimal parameters for each starter culture. This approach will offer a more comprehensive understanding of how these factors influence antioxidant activity and sensory characteristics, potentially leading to enhanced formulations and improved fermentation processes.

CHAPTER 6

CONCLUSION

In this study, both DPPH radical scavenging and reducing power assays were employed alongside TPC and TFC to comprehensively evaluate the antioxidant activity of natto prepared with Kawashimaya and Chuan Xiu starter cultures. Both starter cultures significantly enhanced the antioxidant properties of natto, as demonstrated by higher DPPH radical scavenging activity and reducing power compared to natto prepared without any starter culture. While TPC was similar for both starter cultures, Kawashimaya starter culture produced natto with significantly higher TFC compared to the control but no significant difference ($p > 0.05$) with Chuan Xiu starter. The improved antioxidant activity can be attributed to the fermentation process induced by these starter cultures, which increases the bioavailability of antioxidant compounds and promotes the production of beneficial metabolites during fermentation.

Sensory evaluation revealed no significant differences ($p > 0.05$) in aroma, taste, chewiness and overall acceptability between natto prepared with Kawashimaya and Chuan Xiu starter cultures. However, natto prepared with Kawashimaya exhibited lower scores for appearance compared to the control and a lower stringiness score compared to the Chuan Xiu starter and the control.

In short, the application of Kawashimaya and Chuan Xiu starter cultures effectively enhances the antioxidant properties of natto, with Kawashimaya offering higher TFC. However, Kawashimaya starter exhibited a lower acceptance score in stringiness than Chuan Xiu starter. Thus, while both starter cultures are beneficial for improving antioxidant properties, the choice of starter culture may depend on the desired balance between antioxidant benefits and sensory attributes.

REFERENCES

Abdel-Aty, A.M., Barakat, A.Z. and Mohamed, S.A., 2022. Garden cress gum and maltodextrin as microencapsulation coats for entrapment of garden cress phenolic-rich extract: improved thermal stability, storage stability, antioxidant and antibacterial activities. *Food Science and Biotechnology*, 32(1), pp.47–58.

Afzaal, M., Saeed, F., Islam, F., Ateeq, H., Asghar, A., Shah, Y.A., Ofoedu, C.E. and Chacha, J.S., 2022. Nutritional health perspective of natto: A critical review. *Biochemistry Research International*, 2022, pp.1–9.

Ahmed, F. and Iqbal, M., 2018. Antioxidant activity of Ricinus Communis. *Organic & Medicinal Chemistry International Journal*, 5(4).

Aidoo, R.P., Depypere, F., Afoakwa, E.O. and Dewettinck, K., 2013. Industrial manufacture of sugar-free chocolates – Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends in Food Science and Technology*, 32(2), pp.84–96.

American Chemical Society., 2018. *Maltodextrin*. [online] Available at: <https://www.acs.org/molecule-of-the-week/archive/m/maltodextrin.html> [Accessed: 26 July 2024].

Arasu, M.V., Al-Dhabi, N.A., Ilavenil, S., Choi, K.C. and Srigopalram, S., 2016. In vitro importance of probiotic Lactobacillus plantarum related to medical field. *Saudi Journal of Biological Sciences*, [online] 23(1), pp.S6–S10.

Bhalodia, N., Nariya, P., Shukla, V. and Acharya, R., 2013. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. *AYU (An International Quarterly Journal of Research in Ayurveda)*, 34(2), pp.209.

Harshitha, G.C., Sharma, R. and Rajput, Y.S., 2023. Enzyme-based analytical methods pertinent to dairy industry. *Enzymes Beyond Traditional Applications in Dairy Science and Technology*, pp.445–469.

Chan, E.W.C., Wong, S.K., Kezuka, M., Oshiro, N. and Chan, H.T., 2021. Natto and miso: an overview on their preparation, bioactive components and health-promoting effects. *Food Research*, 5(3), pp.446–452.

Chavan, R.S., Khedkar, C.D. and Bhatt, S., 2016. Fat Replacer. *Encyclopedia of Food and Health*, pp.589–595.

Cos, P., M. Calomme, Pieters, L., A.J. Vlietinck and D. Vanden Berghe, 2000. Structure-Activity Relationship of Flavonoids as Antioxidant and Pro-Oxidant Compounds. *Studies in natural products chemistry*, 22, pp.307–341.

do Prado, F.G., Pagnoncelli, M.G.B., de Melo Pereira, G.V., Karp, S.G. and Soccol, C.R., 2022. Fermented Soy Products and Their Potential Health Benefits: A Review. *Microorganisms*, 10(8), pp.1606.

Echegaray, N., Yilmaz, B., Sharma, H., Kumar, M., Pateiro, M., Ozogul, F. and Lorenzo, J.M., 2023. A novel approach to Lactiplantibacillus plantarum: From probiotic properties to the omics insights. *Microbiological Research*, 268, pp.127289.

Elbanna, K., Alsulami, F.S., Neyaz, L.A. and Abulreesh, H.H., 2024. Poly (γ) glutamic acid: a unique microbial biopolymer with diverse commercial applicability. *Frontiers in Microbiology*, 15.

Engelhardt, U. and Winterhalter, P., 2007. Countercurrent chromatography | Flavonoids. *Elsevier eBooks*, pp.1–9.

Gao, Q., Li, Y., Li, Y., Zhang, Z. and Liang, Y., 2022. Antioxidant and prooxidant activities of phenolic acids commonly existed in vegetables and their relationship with structures. *Food Science and Technology*, 42.

Gülçin, İ. and Alwasel, S., 2023. DPPH radical scavenging assay. *Processes*, 11(8), pp.2248–2248.

Guo, J. and Yang, X.-Q., 2015. Texture modification of soy-based products. *Modifying Food Texture*, pp.237–255.

Jamuna, K.S., Ramesh, C.K., Srinivasa, T.R. and Raghu, K.L., 2010. Comparative studies on DPPH and reducing power antioxidant properties in aqueous extracts of some common fruits. *Journal of pharmacy research*, 3(10), pp.2378–2380.

JNCSF (n.d.). *How natto is made/Japan natto cooperative society federation Natto PR Center*. [online] www.natto.or.jp. Available at: <https://www.natto.or.jp/english/make/index.html> [Accessed: 1 August 2024].

Josipovic, A., Sudar, R., Sudaric, A., Jurkovic, V., Matosa Kocar, M. and Markulj Kulundzic, A., 2016. Total phenolic and total flavonoid content variability of soybean genotypes in eastern Croatia. *Croatian Journal of Food Science and Technology*, 8(2), pp.60–65.

Juan, M.-Y. and Chou, C.-C., 2010. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715. *Food Microbiology*, 27(5), pp.586–591.

Kawamata, T., Arata Wakimoto, Nishikawa, T., Masaya Ikezawa, Hamada, M., Inoue, Y., Kaushalya Kulathunga, Filiani Natalia Salim, Kanai, M., Nishino, T., Gentleman, K., Liu, C., Mathis, B.J., Obana, N., Fukuda, S., Takahashi, S., Taya, Y., Sakai, S. and Hiramatsu, Y., 2023. Natto consumption suppresses atherosclerotic plaque progression in LDL receptor-deficient mice transplanted with iRFP-expressing hematopoietic cells. *Scientific reports*, 13(1).

Kawashimaya The Japan Store, 2024. *【Made in Japan】Natto Starter Spores Powder 3gr - 100% Organic Soybean Extract*. [online] kawashimaya the japanstore. Available at: <https://thejapanstore.jp/products/natto_001> [Accessed: 26 July 2024].

Kawashimaya The Japan Store Blog., 2024. *Natto recipe with starter versus without starter*. [online] Available at: <https://thejapanstore.us/natto/natto-recipe/#:~:text=Reliable%20Fermentation%3A%20The%20starter%20is> [Accessed: 19 July 2024].

Kaźmierczak-Barańska, J. and Karwowski, B.T., 2022. Vitamin K contribution to DNA damage—Advantage or disadvantage? A human health response. *Nutrients*, 14(20), pp.4219.

Kim, I.S., 2021. Current perspectives on the beneficial effects of soybean isoflavones and their metabolites for humans. *Antioxidants*, [online] 10(7), pp.1064.

Kimira, M., Arai, Y., Shimoi, K. and Watanabe, S., 1998. Japanese intake of flavonoids and isoflavonoids from foods. *Journal of Epidemiology*, 8(3), pp.168–175.

Kumar, N. and Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24(e00370), pp.e00370.

Lan, G., Li, C., He, L., Zeng, X. and Zhu, Q., 2020. Effects of different strains and fermentation method on nattokinase activity, biogenic amines, and sensory characteristics of natto. *Journal of Food Science and Technology*, 57(12), pp.4414–4423.

Leonard, W., Zhang, P., Ying, D., Adhikari, B. and Fang, Z., 2021. Fermentation transforms the phenolic profiles and bioactivities of plant-based foods. *Biotechnology Advances*, 49, pp.107763.

Li, C., Xu, T., Liu, X.W., Wang, X. and Xia, T., 2021a. The expression of β-glucosidase during natto fermentation increased the active isoflavone content. *Food Bioscience*, 43, pp.101286.

Li, G., Li, T., He, F., Chen, C., Xu, X., Tian, W., Yang, Y., He, X., Li, H., Chen, K., Hao, N. and Ouyang, P., 2021b. Microencapsulation of nattokinase from fermentation by spray drying: Optimization, comprehensive score, and stability. *Food Science & Nutrition*, 9(7), pp.3906–3916.

Li, Z., Zheng, M., Zheng, J. and Gänzle, M.G., 2023. Bacillus species in food fermentations: an underappreciated group of organisms for safe use in food fermentations. *Current Opinion in Food Science*, 50, pp.101007.

Liu, C., Zhao, Y., Li, X., Jia, J., Chen, Y. and Hua, Z., 2014. Antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China. *Food and nutrition sciences*, 05(04), pp.293–307.

Liu, K., 2008. Food use of whole soybeans. *Soybeans*, pp.441–481.

Liu, W.-T., Huang, C.-L., Liu, R., Yang, T.-C., Lee, C.-L., Tsao, R. and Yang, W.-J., 2023. Changes in isoflavone profile, antioxidant activity, and phenolic

contents in Taiwanese and Canadian soybeans during tempeh processing. *LWT*, 186, pp.115207–115207.

Liu, Y., Han, Y., Cao, L., Wang, X. and Dou, S., 2021. Analysis of main components and prospects of natto. *Advances in Enzyme Research*, [online] 09(01), pp.1–9.

Liu, Y., Song, H. and Luo, H., 2018. Correlation between the key aroma compounds and gDNA copies of *Bacillus* during fermentation and maturation of natto. Food Research International, 112, pp.175–183.

Luu, S., Cruz-Mora, J., Setlow, B., Feeherry, F.E., Doona, C.J. and Setlow, P., 2015. The effects of heat activation on Bacillus spore germination, with nutrients or under high pressure, with or without various germination proteins. *Applied and Environmental Microbiology*, 81(8), pp.2927–2938.

Malenčić, D., Cvejić, J. and Miladinović, J., 2012. Polyphenol content and antioxidant properties of colored soybean seeds from Central Europe. *Journal of Medicinal Food*, [online] 15(1), pp.89–95.

Mani, V. and Ming, L.C., 2017. Tempeh and other fermented soybean products rich in isoflavones. *Fermented Foods in Health and Disease Prevention*, pp.453–474.

Martinez, R.M., 2013. Bacillus subtilis. *Brenner's Encyclopedia of Genetics*, pp.246–248.

Melini, F. and Melini, V., 2021. Impact of fermentation on phenolic compounds and antioxidant capacity of Quinoa. *Fermentation*, [online] 7(1), pp.20.

Mutha, R.E., Tatiya, A.U. and Surana, S.J., 2021. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future Journal of Pharmaceutical Sciences*, 7(1).

Pabich, M., Marciniak, B. and Kontek, R., 2021. Phenolic compound composition and biological activities of fractionated soybean pod extract. *Applied Sciences*, 11(21), pp.10233.

Pérez, M., Inés Domínguez-López and Lamuela-Raventós, R.M., 2023. The chemistry behind the Folin–Ciocalteu method for the sstimation of (poly)phenol content in food: Total phenolic intake in a Mediterranean dietary pattern. *Journal of Agricultural and Food Chemistry*, [online] 71(46).

Pham-Huy, L.A., He, H. and Pham-Huy, C., 2008. Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, [online] 4(2), pp.89–96. Available at: <https://pubmed.ncbi.nlm.nih.gov/23675073> [Accessed: 10 July 2024].

Rasane, P., Jha, A., Kumar, A. and Sharma, N., 2014. Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food. *Journal of Food Science and Technology*.

Ren, G., Teng, C., Fan, X., Guo, S., Zhao, G., Zhang, L., Liang, Z. and Qin, P., 2023. Nutrient composition, functional activity and industrial applications of quinoa (Chenopodium quinoa Willd.). *Food Chemistry*, 410, pp.135290.

Rocchi, R., Zwinkels, J., Merit Kooijman, Alberto Garre Perez and Smid, E.J., 2024. Development of novel natto using legumes produced in Europe. *Heliyon*, pp.e26849–e26849.

Saeed, F., Afzaal, M., Shah, Y.A., Khan, M.H., Hussain, M., Ikram, A., Ateeq, H., Noman, M., Saewan, S.A. and Khashroum, A.O., 2022. Miso: A traditional nutritious & health-endorsing fermented product. *Food Science & Nutrition*, 10(12).

Samtiya, M., Aluko, R.E. and Dhewa, T., 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, [online] 2(1).

Shraim, A.M., Ahmed, T.A., Rahman, M.M. and Hijji, Y.M., 2021. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT*, [online] 150, pp.111932.

Shurtleff, W. and Aoyagi, A., 2012. *History of nattō and its relatives (1405-2012) : extensively annotated bibliography and sourcebook*. Lafayette, CA: Soyinfo Center.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Oxidants and Antioxidants Part A*, 299, pp.152–178.

Škrovánková, S., Mišurcová, L. and Machů, L., 2012. Antioxidant Activity and Protecting Health Effects of Common Medicinal Plants. *Advances in Food and Nutrition Research Volume 67*, pp.75–139.

Slađana Žilić, Vesna Hadži Tašković Šukalović, Vuk Maksimović, Maksimović, M., Zorica Basić, Vesna Perić and Jelena Dragišić Maksimović, 2011. Antioxidant properties of soybean with black and yellow kernel coat. *46th Croatian and 6th International Symposium on Agriculture, Opatija, Croatia, 14-18 February 2011. Proceedings*, pp.686–689.

Sohn, S.I., Pandian, S., Oh, Y.J., Kang, H.J., Cho, W.S. and Cho, Y.S., 2021. Metabolic engineering of isoflavones: An updated overview. *Frontiers in Plant Science*, 12.

Song, D.-Y., Reddy, L.V., Charalampopoulos, D. and Wee, Y.-J., 2019. Poly-(γ-glutamic acid) production and optimization from agro-Industrial bioresources as renewable substrates by Bacillus sp. FBL-2 through response surface methodology. *Biomolecules*, 9(12), pp.754.

Svensson, L., Sekwati-Monang, B., Lutz, D.L., Schieber, A. and Gänzle, M.G., 2010. Phenolic acids and flavonoids in nonfermented and fermented Red Sorghum (Sorghum bicolor (L.) Moench). *Journal of Agricultural and Food Chemistry*, 58(16), pp.9214–9220.

Tiefenbacher, K.F., 2017. Technology of main ingredients—Water and flours. *Wafer and Waffle*, pp.15–121.

USDA, 2019. *FoodData Central*. [online] fdc.nal.usda.gov. Available at: <https://fdc.nal.usda.gov/fdc-app.html#/food-details/172443/nutrients> [Accessed: 16 August 2024].

Visessanguan, W., Benjakul, S., Potachareon, W., Panya, A. and Rieboy, S., 2005. Accelerated proteolysis of soy proteins during fermentation of Thua-nua innoculated with Bacillus subtilis. *Journal of Food Biochemistry*, 29(4), pp.349–366.

Wang, C., Chen, J., Tian, W., Han, Y., Xu, X., Ren, T., Tian, C. and Chen, C., 2023. Natto: A medicinal and edible food with health function. *Chinese Herbal Medicines*, [online] 15(3), pp.349–359.

Yadav, N., Sharma, S., Singh, A. and Tiwari, S., T., 2012. Effect of fermentation on the anti-nutritional factors, antiradical activity and in vitro protein digestibility of *Cicer arietinum* L. *International Journal of Current Research and Review*, 4 (13).

Yan, D., Lin, Y., Qiu, J., Lin, L., Yang, C. and Gao, X., 2022. Correlation analysis between soluble sugars of soybean and mucus drawing length of fermented natto. *Food Science and Technology*, 42.

Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., Chen, S., Ye, X. and Wang, L., 2023. Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. *Foods*, [online] 12(17), pp.3315–3315.

Yang, Y., Lan, G., Tian, X., He, L., Li, C., Zeng, X. and Wang, X., 2021. Effect of fermentation parameters on natto and its thrombolytic property. *Foods*, 10(11), pp.2547.

Yin, L., Zhang, Y., Wu, H., Wang, Z., Dai, Y., Zhou, J., Liu, X., Dong, M. and Xia, X., 2020. Improvement of the phenolic content, antioxidant activity, and nutritional quality of tofu fermented with Actinomucor elegans. *LWT*, [online] 133, pp.110087.

Yu, Y., Jing, X., Li, H., Zhao, X. and Wang, D., 2016. Soy isoflavone consumption and colorectal cancer risk: a systematic review and meta-analysis. *Scientific Reports*, 6(1).

Zeb, A., 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, 44(9).

Zhang, Q., Lan, G., Tian, X., He, L., Li, C., Tao, H., Zeng, X. and Wang, X., 2022. Effect of adding Bifidobacterium animalis B25 on the flavor, functional components and biogenic amines of natto by Bacillus subtilis GUTU09. *Foods*, [online] 11(17), pp.2674.

Zhao, C., Wan, Y., Tang, G., Jin, Q., Zhang, H. and Xu, Z., 2021. Comparison of different fermentation processes for the vitamin K2 (Menaquinone-7) production by a novel Bacillus velezensis ND strain. *Process biochemistry*, 102, pp.33–41.

Zhong, Y. and Shahidi, F., 2015. Methods for the assessment of antioxidant activity in foods11This chapter is reproduced to a large extent from an article in press by the authors in the Journal of Functional Foods. *Handbook of Antioxidants for Food Preservation*, pp.287–333.

APPENDICES

APPENDIX A

Master Sheet (9-Point Hedonic Scaling Test)

Panel No.	Sample Codes and Order of Presentation				
	Permutation	A	B	C	
1	ABC	1 *002*	2 *975*	3 *820*	
2	ACB	1 *914*	3 *237*	2 *530*	
3	CBA	3 *128*	2 *111*	1 *724*	
4	CAB	2 *677*	3 *893*	1 *003*	
5	BAC	2 *756*	1 *911*	3 *817*	
6	BCA	3 *222*	1 *724*	2 *314*	

APPENDIX B

Hedonic Scaling Test Questionnaire for Natto

Name: _____

Date: _____

Stir and taste the three natto samples to assess your preference for each. Please rinse your mouth with water before testing each sample.

Grade	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Sample Code			
Appearance			
Aroma			
Stringiness			
Taste			
Chewiness			
Overall acceptability			

Comments:

Thank you.

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	MONG JIA YING
ID Number(s)	21ADB06793
Programme / Course	Bachelor of Science (Honours) Food Science
Title of Final Year Project	Comparative Study on Antioxidant and Sensory Properties of Natto Prepared using Different Brands of Starter Culture

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Lye Huey Shi

_____

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Name: Lye Huey Shi
Date: 6/10/2024

Thesis

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