Available online: journal.ipb.ac.id/index.php/jphpi

SYNBIOTIC YOGURT BASED ON LOCAL *Lactobacillus plantarum* AND HYDROLYSATE OF *Kappaphycus alvarezii*: EFFECT OF DIFFERENT STARTERS AND FERMENTATION TIME

Aulia Hidayati, Desniar*, Joko Santoso*
Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University
Agatis Street, IPB Darmaga Campus, Bogor 16680, Indonesia

Submitted: 30 May 2025/Accepted: 10 September 2025
*Correspondence: desniar@apps.ipb.ac.id

How to cite (APA Style 7th): Hidayati, A., Desniar, & Santoso, J. (2025). Synbiotic yogurt based on local *Lactobacillus plantarum* and hydrolysate of *Kappaphycus alvarezii*: Effect of different starters and fermentation time. *Jurnal Pengolahan Hasil Perikanan Indonesia,* 28(9), 789-814. http://dx.doi.org/10.17844/wz4w4v64

Abstract

Synbiotic yogurt is a fermented milk product that is enriched with probiotics and prebiotics. This combination enhances probiotic viability, promotes bioactive metabolite production, and improves gut health by generating short-chain fatty acids (SCFA). Selecting an appropriate starter culture and fermentation time is essential for obtaining yogurt with desirable functional and physicochemical characteristics. This study aimed to determine the optimal *L. plantarum* starter treatment and fermentation duration of synbiotic yogurt with *K. alvarezii* hydrolysate as a prebiotic based on total lactic acid bacteria, pH, total titratable acidity, and reducing sugar con-tent. The factors included fermentation time (0, 12, and 24 h) and starter type (*L. plantarum* SK(5), NS(5), or their combination). The parameters analyzed were lactic acid bacteria (LAB) counts, total titratable acidity (TTA), pH, and reducing sugar. The proximate composition of *K. alvarezii* was as follows: 21.18% moisture, 28.91% ash, <0.02% fat, 3.81% protein, 46.11% carbohydrates, and 49.11% Hydrolysis produced 0.20 g/100 mL of reducing sugar with 42.98% recovery. The results revealed that starter type, fermentation time, and their interaction significantly influenced LAB counts, TTA, pH, and reducing sugar (p<0.05). The best treatment was yogurt produced with *L. plantarum* SK(5) for 24 h, yielding 9.27 log10 CFU/mL LAB, 0.74% TTA, pH 4.33, and 0.25% reducing sugar. Synbiotic yogurt formulated with *L. plantarum* SK(5) and *K. alvarezii* hydrolysate showed strong potential as an innovative functional food with greater health benefits than conventional yogurt.

Keywords: fermentation, functional food, gut health, lactic acid

Yogurt Sinbiotik Berbasis *Lactobacillus plantarum* Lokal dan Hidrolisat *Kappaphycus alvarezii*: *Starter* Berbeda dan Lama Fermentasi

Abstrak

Yogurt sinbiotik adalah produk susu fermentasi yang diperkaya dengan probiotik dan prebiotik. Kombinasi ini meningkatkan viabilitas probiotik, merangsang produksi metabolit bioaktif, serta memperbaiki kesehatan usus melalui pembentukan asam lemak rantai pendek (SCFA). Pemilihan kultur starter dan lama fermentasi yang tepat sangat penting untuk memperoleh yogurt dengan karakteristik fungsional dan fisikokimia yang diinginkan. Penelitian ini bertujuan untuk menentukan perlakuan *starter* dan waktu fermentasi terbaik yogurt synbiotik dengan prebiotik hidrolisat *K. alvarezii* berdasarkan total bakteri asam laktat, pH, total asam tertitrasi, dan gula reduksi. Perlakuan pembuatan yogurt meliputi lama fermentasi (0, 12, dan 24 jam) serta jenis starter (*L. plantarum* SK(5), NS(5), atau kombinasinya). Parameter yang dianalisis meliputi jumlah bakteri asam laktat (BAL), total asam tertitrasi (TAT), pH, dan gula reduksi. Komposisi proksimat *K. alvarezii* menunjukkan kadar air 21,18%, abu 28,91%, lemak <0,02%, protein 3,81%, karbohidrat 46,11%, dan serat pangan 49,11%. Proses hidrolisis menghasilkan gula reduksi sebesar 0,20 g/100 mL dengan rendemen 42,98%. Hasil penelitian menunjukkan bahwa jenis *starter*, lama fermentasi, serta interaksinya berpengaruh nyata terhadap BAL, TAT, pH, dan gula reduksi. Perlakuan terbaik diperoleh

http://dx.doi.org/10.17844/wz4w4v64

pada yogurt dengan starter tunggal *L. plantarum* SK(5) selama 24 jam, dengan karakteristik BAL 9,27 log10 CFU/mL, TAT 0,74%, pH 4,33, dan gula reduksi 0,25%. Yogurt sinbiotik yang diformulasi dengan *L. plantarum* SK(5) dan hidrolisat *K. alvarezii* memiliki potensi besar sebagai pangan fungsional inovatif dengan manfaat kesehatan yang lebih unggul dibandingkan yogurt konvensional.

Kata kunci: asam laktat, fermentasi, kesehatan usus, pangan fungsional

INTRODUCTION

The increasing awareness of healthy lifestyles within society serves as the foundation for the developing functional foods. Functional foods provide not only basic nutrition but also specific functions and bioactive compounds (Mahmoudi, 2022). The aim of functional foods is to reduce the risk of diseases or promote optimal health (Birch & Bonwick, 2019). The health benefits of functional foods are derived from various bioactive substances such as omega-3 fatty acids, antioxidants, probiotics, and prebiotics (Peng *et al.,* 2020). Synbiotic products (a combination of prebiotics and probiotics) are a popular type of functional food. Prebiotics selectively support the growth and production of probiotic metabolites (Yadav *et al.,* 2024). This synergistic property prevents the growth of pathogenic bacteria and enhances probiotic effectiveness (Rahman *et al.,* 2024). The effectiveness and mechanisms of synbiotics in metabolic diseases have been studied, and they have been found to address obesity (Kobyliak *et al.,* 2017; Thiennimitr *et al.,* 2018; Oh *et al.,* 2019), reduce blood sugar levels (Ebrahimi *et al.,* 2017; Djaja *et al.,* 2019), and treat kidney disorders (Anand *et al.,* 2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) classifies synbiotics into two categories: complementary and synergistic. Complementary synbiotics are combinations of probiotics and prebiotics that work independently to achieve one or more benefits, whereas synergistic synbiotics involve probiotics and prebiotics working together to achieve health benefits (Swanson *et al.,* 2020).

Probiotics are live microorganisms that provide health benefits to their hosts when administered in adequate amounts (Minj & Vij, 2025). Probiotics lower cholesterol levels (Puttarat *et al.,* 2023), prevent Alzheimer's disease (Dhami *et al.,* 2023), alleviate lactose intolerance (Maldonado *et al.,* 2019), and reduce hyperglycemia (Arriaga *et al.,* 2024). Lactic acid bacteria (LAB), such as *Lactobacillus, Bifidobacterium*, and *Enterococcus*, are considered potential probiotics. The sources for isolating these LAB vary; for example, *Lactobacillus plantarum* C16 from fresh cow milk contains bacteriocin-producing plantaricin (Reuben *et al.,* 2020), *Lacticaseibacillus rhamnosus* LHL7 from breast milk exhibits antimicrobial activity that can withstand low pH (Kang *et al.,* 2020), *L. plantarum* from fermented vegetables has probiotic potential with functional properties as antioxidants, α-glucosidase inhibitors, and antimicrobial agents (Liu *et al.,* 2022), and *Lactobacillus* sp. from the traditional saraboung food demonstrates antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Saryono *et al.,* 2023).

Local probiotics derived from aquatic food products have the advantage of producing bioactive compounds, one of which is fish fermentation products such as *bekasam*. Desniar (2012) successfully isolated *Lactobacillus plantarum* SK(5) from *bekasam* of seluang fish from Kayu Agung, Ogan Komering Ilir, South Sumatra. The bacterium *L. plantarum* SK(5) exhibits antimicrobial activity (Desniar *et al.*, 2013) and produces proteolytic enzymes (Kurniati *et al.,* 2015). This bacterium showed 93% genetic similarity to *L. plantarum* subsp. *plantarum* NC 8, indicating its potential as a food biopreservative (Desniar *et al.,* 2020). *L. plantarum* SK(5), administered at a dose of 30 mg/kg body weight in an in vivo test, demonstrated an 86.22% reduction in blood glucose levels in diabetic-induced rats, induced regeneration of pancreatic beta cells, and was found to be safe over a 14 days treatment period (Syafiqoh, 2016). Another lactic acid bacterium derived from *bekasam* is *Lactobacillus plantarum* NS(5), isolated from *bekasam* of tilapia fish of Sungai Pasir Village,

Ogan Komering Ilir, South Sumatra (Desniar, 2012). This bacterium was classified as *L. plantarum* 1 with 99.9% genetic similarity. *L. plantarum* NS(5) showed significant survival rates at stomach pH (90.49%), intestinal pH (88.99%), and bile salt concentrations (84.26-87.10%), was non-pathogenic, and exhibited antimicrobial activity against *Escherichia coli* (7.0 mm) and *Salmonella typhimurium* ATCC 14028 (6.8 mm) (Nurnaafi *et al.,* 2015). The role of prebiotics is essential for achieving the expected synbiotic condition.

Prebiotics are substrates selectively utilized by microorganisms that stimulate the growth of non-pathogenic bacteria, such as *Bifidobacterium* and *Lactobacillus* (Gibson *et al.,* 2017). These bacteria ferment polysaccharides into short-chain fatty acids (SCFAs) and produce butyrate, acetate, and propionate (Bajury *et al.,* 2017). Butyrate serves as an energy source for colonocytes and enterocytes, whereas propionate diffuses to the liver, where it is used in gluconeogenesis (Ashaolu *et al.,* 2021). One promising polysaccharide from aquatic sources that has garnered attention for use as a prebiotic is *Kappaphycus alvarezii*. This seaweed belongs to the class Rhodophyceae. According to FAO data, in 2021, seaweed production in Indonesia was dominated by *K. alvarezii*, with a volume of 7.05 million tons, accounting for 82.7% of the global production (KKP, 2023). The red algae *K. alvarezii* contains the high-value hydrocolloid carrageenan, which is used as a gelling agent, emulsifier, thickener, and stabilizer. Several diversified products have been derived from *K. alvarezii*, including bread (Mamat *et al.,* 2023; Sasue *et al.,* 2023), ice cream (Seo & Oh, 2022; Ro *et al.,* 2024), yogurt (Basroni et al., 2018; Skryplonek et al., 2019; Zhang et al., 2025), capsule shells (Soraya *et al.,* 2025; Tarman *et al.,* 2024), edible films (Bharti *et al.,* 2021; Huang *et al.,* 2024; Mahajan *et al.,* 2022), and food packaging (Mathew et al., 2024; Wu et al., 2023).

K. alvarezii is not only a source of carrageenan but also contains other components, such as fiber, carbohydrates, proteins, alkaloids, and flavonoids (Yulianti *et al.,* 2022). The fiber carbohydrate content of *K. alvarezii* is 69.3% dry weight (Santoso *et al.,* 2006) and 15.8 %, respectively (Maharany *et al.,* 2017). This polysaccharide content holds potential as a prebiotic, as it can be utilized by lactic acid bacteria (LAB). *K. alvarezii* has a cell wall composed of cellulose and galactan. These two compounds can be converted into fermentable sugars, namely, glucose and galactose (Rudke *et al.,* 2020). The energy source for *Lactobacillus* is carbohydrates, with lactic acid being the primary end product of its metabolism (Setyaningsih *et al.,* 2019). Polysaccharides act as prebiotics when their polymers are in simple forms. Complex compounds are broken down into simpler forms by hydrolysis. The hydrothermal hydrolysis of *K. alvarezii* produces prebiotics that can be utilized by *Lactobacillus plantarum* IFO 3074, offering the potential for development as a functional food with low production costs (Mutmainnah *et al.,* 2023). The principle of hydrothermal treatment involves the use of high temperature and pressure to break polysaccharide chains, with the final product being reduced sugars. Hydrolysis of *Ulva* sp. using water solvents and catalysts (at temperatures of 100–374°C) results in glucose monosaccharides and is environmentally friendly (Steinbruch *et al.,* 2020).

The utilization of *K. alvarezii* hydrolysate presents an opportunity for diversification into functional foods such as synbiotic yogurt. Extensive research has been conducted on synbiotic yogurt interventions based on macroalgae. Among them are studies involving Ulvan polysaccharides combined with commercial probiotics, *Lactobacillus acidophilus* LA-5, *Streptococcus thermophilus* TH-4, and *Bifidobacterium* sp. Bb-12 (Shalaby & Amin, 2019), *Laminaria japonica* with commercial yogurt starter cultures (Bioprox, France) (Wu *et al.,* 2025), *Saccharina japonica* (Nuñez & Picon, 2017), *Undaria pinnatifida*, *Saccharina japonica* with commercial yogurt starter cultures (Wang *et al.*, 2025), and *Laurencia capsica* with commercial freeze-dried bacterial cultures *L. delbrueckii*, *L. acidophilus La-5*, and *S. thermophilus* ssp. *bulgaricus* (Tahmasebi & Mofid, 2021). The use of yogurt starters derived from local probiotics has also been reported, such as

http://dx.doi.org/10.17844/wz4w4v64

L. plantarum 2C12 and *L. acidophilus* 2b4 isolated from beef (Astawan *et al.,* 2012), *L. plantarum* VP-3.3 isolated from soybean epidermis, a solid waste (Riftyan *et al.,* 2022; Rossi *et al.,* 2021), and LAB TP 12 isolated from *tempoyak* (Rahayu & Qurbaniah, 2019). The difference in fermentation time also affects the quality of yogurt. For example, Betancourt *et al*. (2025) reported that fermentation times of 8, 16, and 24 h had a significant effect on the pH, viscosity, and water holding capacity (WHC) of soy yogurt, while Anwar *et al*. (2025) showed that different fermentation times (4, 5, 6, 7, and 8 h) influenced the characteristics of probiotic yogurt made from sheep milk, cow milk, and their combination. Previous studies have predominantly used macroalgae-derived prebiotic sources in combination with commercial probiotic starter cultures. However, the use of local probiotics isolated from fermented aquatic fish (*Bekasam*), specifically *Lactobacillus plantarum* SK(5) and *L. plantarum* NS(5) (either individually or in combination), along with *K. alvarezii* hydrolysate to create synbiotic yogurt, has not been investigated. This study aimed to determine the optimal *L. plantarum* starter treatment and fermentation duration of synbiotic yogurt based on total lactic acid bacteria, pH, total titratable acidity, and reducing sugar content. This research is expected to contribute a novel approach by developing synbiotic yogurt based on local food resources, utilizing *L. plantarum* SK(5), *L. plantarum* NS(5), or a combination of both with the addition of *K. alvarezii* hydrolysate.ifestyles within society serves as the foundation for the developing functional foods. Functional foods produce products that not only provide basic nutrition but also offer specific functions and bioactive compounds (Mahmoudi, 2022). The aim of functional foods is to reduce the risk of diseases or promote optimal health (Birch & Bonwick, 2019). The health benefits of functional foods are derived from various bioactive substances, such as omega-3 fatty acids, antioxidants, probiotics, and prebiotics (Peng *et al.,* 2020). One popular type of functional food is synbiotic products (a combination of prebiotics and probiotics). Prebiotics selectively support the growth and production of probiotic metabolites (Yadav *et al.,* 2024). This synergistic property prevents the growth of pathogenic bacteria and enhances the effectiveness of probiotics (Rahman *et al.,* 2024). The effectiveness and mechanisms of synbiotics in metabolic diseases have been studied and found to address obesity (Kobyliak *et al.,* 2017; Thiennimitr *et al.,* 2018; Oh *et al.,* 2019), reducing blood sugar levels (Ebrahimi *et al.,* 2017; Djaja *et al.,* 2019) and kidney disorders (Anand *et al.,* 2021). The International Scientific Association for Probiotics and Prebiotics (ISAPP) classifies synbiotics into two approaches: complementary synbiotics and synergistic synbiotics. Complementary synbiotics are combinations of probiotics and prebiotics that work independently to achieve one or more benefits, while synergistic synbiotics involve probiotics and prebiotics working together to achieve health benefits (Swanson *et al.,* 2020).

Probiotics are live microorganisms that provide health benefits to their host when administered in adequate amounts (Minj & Vij, 2025). The roles of probiotics include lowering cholesterol levels (Puttarat *et al.,* 2023), preventing Alzheimer's disease (Dhami *et al.,* 2023), alleviating lactose intolerance (Maldonado *et al.,* 2019), and reducing hyperglycemia (Arriaga *et al.,* 2024). Lactic acid bacteria (LAB), such as *Lactobacillus, Bifidobacterium*, and *Enterococcus*, are considered to be potential probiotics. The sources for isolating these LAB vary; for example, *Lactobacillus plantarum* C16 from fresh cow milk contains bacteriocin-producing plantaricin (Reuben *et al.,* 2020), *Lacticaseibacillus rhamnosus* LHL7 from breast milk exhibits antimicrobial activity that can withstand low pH (Kang *et al.,* 2020), *L. plantarum* from fermented vegetables has probiotic potential with functional properties as antioxidants, α-glucosidase inhibitors, and antimicrobial agents (Liu *et al.,* 2022), and *Lactobacillus* sp. from the traditional saraboung food demonstrates antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Saryono *et al.,* 2023).

Local probiotics derived from aquatic food products have the advantage of producing bioactive compounds, one of

which is fish fermentation products, such as *bekasam*. Desniar (2012) successfully isolated *Lactobacillus plantarum* SK(5) from *bekasam* of seluang fish from Kayu Agung, Ogan Komering Ilir, South Sumatra. The bacterium *L. plantarum* SK(5) exhibits antimicrobial activity (Desniar *et al.*, 2013) and produces protease enzymes (Kurniati *et al.,* 2015). This bacterium shows a 93% genetic similarity to *L. plantarum* subsp. *plantarum* NC 8, indicating its potential as a food biopreservative (Desniar *et al.,* 2020). *L. plantarum* SK(5), administered at a dose of 30 mg/kg body weight in an in-vivo test, demonstrated an 86.22% reduction in blood glucose levels in diabetic-induced rats, induced regeneration of pancreatic beta cells, and was found to be safe over a 14 days treatment period (Syafiqoh, 2016). Another lactic acid bacterium derived from *bekasam* is *Lactobacillus plantarum* NS(5), isolated from *bekasam* of tilapia fish from Sungai Pasir Village, Ogan Komering Ilir, South Sumatra (Desniar, 2012). This bacterium is classified as *L. plantarum* 1 with a 99.9% genetic similarity. *L. plantarum* NS(5) shows significant survival rates at stomach pH (90.49%), intestinal pH (88.99%), and bile salts (84.26-87.10%), is non-pathogenic, and exhibits antimicrobial activity against *Escherichia coli* (7.0 mm) and *Salmonella typhimurium* ATCC 14028 (6.8 mm) (Nurnaafi *et al.,* 2015). The role of prebiotics is essential to achieve the expected synbiotic condition.

Prebiotics are substrates selectively utilized by microorganisms, stimulating the growth of non-pathogenic bacteria such as *Bifidobacterium* and *Lactobacillus* (Gibson *et al.,* 2017). These bacteria ferment polysaccharides into short-chain fatty acids (SCFAs), producing butyrate, acetate, and propionate (Bajury *et al.,* 2017). Butyrate serves as an energy source for colonocytes and enterocytes, while propionate diffuses to the liver, where it is used in the process of gluconeogenesis (Ashaolu *et al.,* 2021). One promising polysaccharide from aquatic sources that has garnered attention for use as a prebiotic is *Kappaphycus alvarezii* seaweed. This seaweed belongs to the Rhodophyceae class. According to FAO data, in 2021, seaweed production in Indonesia was dominated by *K. alvarezii*, with a volume of 7.05 million tons, accounting for 82.7% of global production (KKP, 2023). The red algae *K. alvarezii* contains high-value hydrocolloid carrageenan, which is applied as a gelling agent, emulsifier, thickener, and stabilizer. Several diversified products are derived from *K. alvarezii*, including bread (Mamat *et al.,* 2023; Sasue *et al.,* 2023), ice cream (Seo & Oh, 2022; Ro *et al.,* 2024), yogurt (Basroni et al., 2018; Skryplonek et al., 2019; Zhang et al., 2025), capsule shells (Soraya *et al.,* 2025; Tarman *et al.,* 2024), edible films (Bharti *et al.,* 2021; Huang *et al.,* 2024; Mahajan *et al.,* 2022), dan food packaging (Mathew et al., 2024; X. Wu et al., 2023).

K. alvarezii is not only a source of carrageenan but also contains other components such as fiber, carbohydrates, proteins, alkaloids, and flavonoids (Yulianti *et al.,* 2022). The fiber content of *K. alvarezii* is 69.3% dry weight (Santoso *et al.,* 2006), and the carbohydrate content is 15.8% (Maharany *et al.,* 2017). This polysaccharide content holds potential as a prebiotic, as it can be utilized by lactic acid bacteria (LAB). *K. alvarezii* has a cell wall composed of cellulose and galactan. These two compounds can be converted into fermentable sugars, namely glucose and galactose (Rudke *et al.,* 2020). The energy source for *Lactobacillus* is carbohydrates, with lactic acid being the primary end product of its metabolism (Setyaningsih *et al.,* 2019). Polysaccharides act as prebiotics when their polymers are in a simple form. Complex compounds are broken down into simpler forms through hydrolysis. Hydrothermal hydrolysis of *K. alvarezii* produces prebiotics that can be utilized by *Lactobacillus plantarum* IFO 3074, offering potential for development as a functional food with low production costs (Mutmainnah *et al.,* 2023). The principle of hydrothermal treatment involves the use of high temperature and pressure to break polysaccharide chains, with the final product being reducing sugars. Hydrolysis of *Ulva* sp. using water solvents and catalysts (at temperatures of 100–374°C) results in glucose monosaccharides and is environmentally friendly (Steinbruch *et al.,* 2020).

http://dx.doi.org/10.17844/wz4w4v64

The utilization of *K. alvarezii* hydrolysate presents an opportunity for diversification into functional foods, such as synbiotic yogurt. Research on synbiotic yogurt interventions based on macroalgae has been extensively conducted. Among them are studies involving Ulvan polysaccharides combined with commercial probiotics *Lactobacillus acidophilus* LA-5, *Streptococcus thermophilus* TH-4, and *Bifidobacterium* sp. Bb-12 (Shalaby & Amin, 2019), *Laminaria japonica* with commercial yogurt starter cultures (Bioprox, France) (Wu *et al.,* 2025), *Saccharina japonica* (Nuñez & Picon, 2017), *Undaria pinnatifida* and *Saccharina japonica* with commercial yogurt starter cultures (Wang *et al*., 2025) and *Laurencia capsica* with commercial freeze-dried bacterial cultures *L. delbrueckii*, *L. acidophilus La-5*, and *S. thermophilus* ssp. *bulgaricus* (Tahmasebi & Mofid, 2021). The use of yogurt starters derived from local probiotics has also been reported, such as *L. plantarum* 2C12 and *L. acidophilus* 2b4 isolated from beef (Astawan *et al.,* 2012), *L. plantarum* VP-3.3 isolated from soybean epidermis was a solid waste (Riftyan *et al.,* 2022; Rossi *et al.,* 2021), and LAB TP 12 isolated from *tempoyak* (Rahayu & Qurbaniah, 2019). The difference in fermentation time also affects the quality of the yogurt produced. For example, the study by Betancourt *et al*. (2025) reported that fermentation times of 8, 16, and 24 hours had a significant effect on the pH, viscosity, and water holding capacity (WHC) of soy yogurt; while the study by (Anwar *et al*., 2025) showed that different fermentation times (4, 5, 6, 7, and 8 hours) influenced the characteristics of probiotic yogurt made from sheep's milk, cow's milk, and their combination. Previous studies have predominantly used macroalgae derived prebiotic sources in combination with commercial probiotic starter cultures. However, the utilisation of local probiotics isolated from aquatic fermented fish (*Bekasam*), namely *Lactobacillus plantarum* SK(5) and *L. plantarum* NS(5) (either individually or in combination), with the addition of *K. alvarezii* hydrolysate as a synbiotic yogurt, has not yet been explored. The study aimed to determine the optimal *L. plantarum* starter treatment and fermentation duration of synbiotic yogurt based on total lactic acid bacteria, pH value, total titratable acidity, and reducing sugar. This research is expected to contribute a novel approach by developing synbiotic yogurt based on local food resources, utilizing *L. plantarum* SK(5), *L. plantarum* NS(5), or a combination of both with the addition *K. alvarezii* hydrolysate.

MATERIALS AND METHODS

Hydrolysate Production of *K. alvarezii*

Hydrolysis of *K. alvarezii* was performed as described by Mutmainnah *et al.* (2023). The raw material of dried *K. alvarezii* was obtained from a seaweed cultivation area in Moro District, Karimun Regency, Riau Islands Province, Indonesia. *K. alvarezii* samples were analyzed for their proximate composition and dietary fiber content. The hydrolysis process began with the preparation of *K. alvarezii*, which included sorting, washing, and size reduction. The seaweed was soaked in distilled water for 2 h (1:40, b/v ratio). The seaweed was then hydrolyzed using an autoclave (GEA LS-75HD, Indonesia) at 100°C for 3 h. The hydrolysis product was filtered and dried using a dehydrator (GETRA FD-30, Indonesia) at 50°C for 24 h. The filtrate was further processed by milling using a grinder machine (Orion MG-100B, Indonesia) and sieved through an 80 mesh sieve. The hydrolysate was stored in a tightly sealed container protected from direct sunlight. The analysis parameters for the *K. alvarezii* hydrolysate included the reducing sugar content, which was measured using the dinitrosalicylic acid (DNS) method (Miller, 1959).

Preparation of *L. plantarum* SK(5) and *L. plantarum* NS(5)

The *L. plantarum* SK(5) dan *L. plantarum* NS(5) starters are bacteria strains collected by Desniar (2012), isolated from *bekasam* of seluang fish and tilapia fish. Bacterial verification was performed as an initial step to ensure the identity of *L. plantarum*. The verification process included Gram staining, motility, catalase, and homofermentative testing. Starter preparation followed the method described by Desniar

(2012), beginning with the inoculation of the stock cultures of *L. plantarum* SK (5) and *L. plantarum* NS(5) into de Man Rogosa Sharpe Agar (MRSA) (Oxoid, United Kingdom) and incubating under anaerobic conditions using an incubator (Thermocline type 4200, United States) at 37°C for 48 h. The bacteria that grew on the MRSA were then inoculated into 10 mL of de Man Rogosa Sharpe Broth (MRSB) (Oxoid, United Kingdom) and incubated at 37°C for 24 h. The *L. plantarum* inoculum was added at a concentration of 10% (v/v) to fresh pasteurized cow's milk, including the starter for the combination of LAB treatments. The mixture was incubated at 37°C for 24 h to obtain the mother culture. The total LAB count in the mother culture was determined using the pour-plate method (BSN, 2009).

Preparation of Synbiotic Yogurt

Synbiotic yogurt was prepared with modifications in the type of prebiotic. Commercial fresh cow milk was heated to 45°C and transferred into sterilized Scott bottles. Scott bottles were sterilized using an autoclave (Yamato SM 52, Japan). K. alvarezii hydrolysate was added to warm milk. At 37°C, the *L. plantarum* starter was added to the milk according to the formulation (Table 1). The milk was then incubated at 37°C for 0, 12, and 24 h. The parameters for testing the fermented synbiotic yogurt included total LAB count, pH, total titratable acidity (TTA), and reducing sugar analysis, to determine the best synbiotic yogurt based on fermentation time and starter type (single and combined).

Proximate Analysis and Dietary Fiber Analysis

Proximate analysis included moisture, ash, protein, and fat content, according to the method outlined by the BSN (1992). Dietary fiber analysis was conducted using the enzymatic gravimetric method (AOAC, 1994) with three replicates per sample. The carbohydrate content was calculated by the difference method.

Yield Calculation

The yield of *K. alvarezii* hydrolysate was calculated using the following formula:

$$\text{Hydrolysate yield (\%)} = \frac{A}{B} \times 100$$

Information:
A = weight of *K.alvarezii* hydrolysate (g)
B = weight of dried *K.alvarezii* (g)

Total Lactic Acid Bacteria (LAB) analysis

Total lactic acid bacteria (LAB) were analyzed using the total plate count method (BSN, 2009). A 10 mL sample was serially diluted up to 10^{-8} in saline solution, and each dilution was vortexed (Thermolyne). A 1 mL sample from the 10^{-6} to 10^{-8} dilutions was incubated in sterile Petri dishes with MRSA media and $CaCO_3$ at 37°C for 48 h in an inverted position. The total LAB count was calculated using the following formula:

$$N = \frac{\Sigma C}{[(1\times n1)+(0{,}1+n2)\times(d)]}$$

Information:
N = number of product colonies per mL or colonies per gram;

Table 1 Synbiotic yogurt formulation

Materials (mL)	Sample treatment		
	L. plantarum SK(5)	*L. plantarum* NS(5)	Combination
Commercial cow's milk (mL)	84.6	84.6	84.6
Hydrolysate K. alvarezii (g)	0.4	0.4	0.4
Starter *L. plantarum* SK(5) (mL)	15	-	-
Starter *L. plantarum* NS(5) (mL)	-	15	-
Starter *L. plantarum* SK(5) and *L. plantarum* NS(5) (mL)	-	-	15

http://dx.doi.org/10.17844/wz4w4v64

ΣC = number of colonies on all plates counted;
n1 = number of plates in the first dilution counted;
n2 = number of plates in the second dilution counted;
d = first dilution counted

pH Measurement

The acidity of the sample was measured using a Pen Lutron pH-220 electronic pH meter, according to the method outlined by the BSN (2004). The pH meter was calibrated with standard buffer solutions of pH 4 and 7 for 30 min each. The meter was rinsed with distilled water and wiped with tissue. The pH electrode was immersed in the sample solution until the pH meter stabilized. The electrode was rinsed with distilled water after each sample was changed.

Total Titratable Acidity (TTA)

The total titratable acidity was determined by titration (BSN, 2009). A 5 g sample was added to 45 mL of distilled water and homogenized using a homogenizer. The homogenized sample was dissolved in distilled water in a volumetric flask to a final volume of 50 mL. The sample was filtered using filter paper, and 5 mL of the filtrate was pipetted into a beaker (Iwaki, Pyrex). A 1% phenolphthalein indicator (Merck) was added (2 drops), and titration was performed using 0.1 N NaOH until the solution turned pink in color. The percentage of total acidity was calculated using the following formula:

$$\text{TTA} = \frac{\text{V NaOH} \times \text{N NaOH} \times 90 \times \text{FP}}{\text{W}} \times 100\%$$

Information:
V NaOH= volume of NaOH used
N NaOH = normality of NaOH measured
W = sample weight
90 = molecular weight of lactic acid
FP = dilution factor

Reducing Sugar Analysis by DNS Method

Reducing sugar analysis was conducted using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The DNS reagent was prepared by mixing 1.06 g of 3,5-dinitrosalicylic acid and 1.98 g of NaOH in 141 mL distilled water. The solution was stirred until homogeneous, and then 30.6 g of K-Na tartrate, 0.76 g of liquid phenol, and 0.83 g of Na-metabisulfite were added. The solution was stirred until homogeneous and heated at 50°C. A standard glucose solution of 1,000 ppm was diluted to 500 ppm and then further diluted to create standard solutions with concentrations of 50, 100, 150, 200, 250, and 300 ppm in separate test tubes. Next, 1 mL of each standard solution was mixed with 3 mL of DNS reagent, and 1 mL of the sample solution was mixed with 3 mL of DNS. The standard solutions and samples were placed in boiling water for 10 min until the color changed to dark brown-black. The absorbance of the samples was measured using a UV-Vis spectrophotometer (Spectrostar Omega BMG Labtech) at 512 nm wavelength. The absorbance values obtained from the glucose standard solutions were used to create a standard curve for glucose quantification. The concentration of reducing sugars in the samples was calculated using a regression equation derived from the standard curve.

Data Analysis

This study used an experimental method with a completely randomized factorial design (CRFD) with two factors. The first factor was the type of starter, with three levels: *L. plantarum* SK (5), *L. plantarum* NS(5), and a combination of both (mix). The second factor was fermentation duration, with three levels: 0, 12, and 24 h of incubation. The study was conducted in triplicate, and the data were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level (α=0.05). When a significant effect (p<0.05) was observed, Duncan's Multiple Range Test (DMRT) was performed as a post-hoc analysis. Data processing was performed using Microsoft Office Excel and SPSS version 27.

RESULTS AND DISCUSSION

Chemical Composition of *K. alvarezii*

The primary raw material used in this study was dried *K. alvarezii* obtained from the waters of Karimun Regency, Riau

Islands Province. This seaweed is classified as a *carrageenophyte* macroalga that contains κ-carrageenan, a polysaccharide composed of sulfated linear galactan, and is soluble in water. Its repeating main chain consists of α(1-4)-3,6-anhydrogalactopyranose and ß(1-3)-galactopyranose-4-sulfate (Meinita *et al.,* 2022). *K. alvarezii* exhibits a range of colors, including red, yellow, brown, and green, depending on the phycoerythrin concentration (Rudke *et al.,* 2020). The physical appearance of *K. alvarezii* has short, branched, and dark brown thalli that are not easily broken between the branches. The comparison of the physical characteristics of *K. alvarezii* with several other aquatic regions shows that *K. alvarezii* from the waters of Gorontalo has branched, shiny, light green thalli. In Banten waters, the thalli are medium-sized, smooth, shiny, and brown, whereas in Kupang waters, the thalli are large, dark brown, with many newly growing apical tips (Simatupang *et al.,* 2021). The composition of the *K. alvarezii* raw material is presented in Table 2.

Table 2 shows a comparison of the proximate composition of *K. alvarezii* used in this study with that of *K. alvarezii* from the research of Kawaroe *et al.* (2017) which was sourced from Puntondo Village, Takalar, South Sulawesi Province. The moisture content of *K. alvarezii* used as a raw material for hydrolysate was 21.18%, which is higher compared to *K. alvarezii* from Kawaroe *et al.* (2017) which had a moisture content of 16.39%. This moisture content still meets the quality standard for dried seaweed according to SNI 2690:2023, which stipulates a maximum of 38% moisture content, which had a moisture content of 16.39%. This moisture content meets the quality standard for dried seaweed according to SNI 2690:2023, which stipulates a maximum of 38% moisture content (BSN, 2023). The differences in moisture content in seaweed are influenced by factors such as drying methods and storage of raw materials. Drying seaweed is crucial before processing, as a high moisture content can affect its texture, taste, and nutritional value (Suwati *et al.,* 2021). In general, fishermen in coastal areas dry seaweed naturally by spreading it on woven media under direct sunlight (Kamaruddin *et al.,* 2017). Other drying methods include wind drying, as used by Dolorosa *et al.* (2017), which resulted in a moisture content of 40.50%, while oven drying at 50°C, 60°C, and 70°C yielded moisture contents of 15.87%, 11.09%, and 10.69%, respectively (Orilda *et al.,* 2021). Variations in moisture content can also be attributed to differences in environmental conditions, storage duration, temperature, and humidity (Yanuarti *et al.,* 2017). Dried seaweed with a moisture content of 20-30% can be stored for 2-3 years, while inadequate air circulation during storage can increase the moisture content to 50-55% (Al Wazzan *et al.,* 2021). *K. alvarezii* stored in bunkers had a moisture content of 11.58%, which was lower than the 23.98% moisture content of seaweed stored in bags (Al Wazzan *et al.,* 2021).

The ash content represents the inorganic residue left after the combustion of an organic material. The ash content of *K. alvarezii* from Takalar was lower (14.39%) than that of the seaweed used in this study (28.91%). Ash content is closely related to the mineral composition of seaweed substrates. The ash content in red algae consists of salts and other minerals that adhere to the

Table 2 Comparison of the proximate composition of *K. alvarezii* (wet basis)

Parameters (%)	Research	Kawaroe *et al.* (2017)	Santoso *et al.* (2006)
Moisture	21.18±0.07	16.39±0.23	-
Ash	28.91±0.09	14.39±0.04	-
Protein	3.81±0.05	5.33±0.05	-
Lipid	<0.02	1.03±0.01	-
Carbohydrate (by difference)	46.11±0.04	63.17±1.61	-
Dietary fiber	49.11±0.11	-	69.3±1.8

http://dx.doi.org/10.17844/wz4w4v64

thallus, predominantly comprising sodium, potassium, and calcium salts (Panjaitan *et al.,* 2024; Syaharuddin 2019). This content typically ranges from 15-40%. Santoso *et al.* (2006) reported that *K. alvarezii* from the Seribu Islands, Jakarta Province, contains various minerals, including magnesium (Mg) at 2.88 mg/g, calcium (Ca) at 2.80 mg/g, potassium (K) at 87.10 mg/g, and sodium (Na) at 11.93 mg/g.

The protein content of *K. alvarezii* in this study was 3.81%, compared to 5.33% in the waters of Takalar. The protein content of seaweed is also influenced by seasonal factors. Rajaram *et al.* (2021) evaluated the proximate composition of *K. alvarezii* in four different seasons. The protein content based on these seasons was as follows: rainy season (16.26%), post-rainy season (13.06%), summer (9.59%), and pre-rainy season (9.90%). Photosynthesis encourages seaweed to absorb nutrients, such as nitrates and phosphates, which play a key role in protein formation. Safia *et al.* (2020) reported protein contents at depths of 0.5 m (3.73 %), 1 m (4.16 %), and 2 m (3.29 %). During the exponential growth phase of algae, more protein is synthesized, and cell wall and food reserve formation are minimal. This condition results in a limited nitrogen supply, and part of the protein synthesis process from photosynthetic activity is redirected to carbohydrate synthesis (Syaharuddin, 2019).

The fat content of seaweed is considered to be low. *K. alvarezii* has a low lipid content and can be considered a very good diet for humans (Rajaram *et al*., 2021). The carbohydrate content of *K. alvarezii* from Takalar waters (63.17%) was higher than that of the seaweed used in this study (46.11%). The carbohydrate content of dried *K. alvarezii* was reported as 47.36% by Lumbessy *et al.* (2020) and 38.3% by Wanyonyi *et al.* (2017). Carbohydrates in algae are produced by an increase in fluoridated starch via photosynthesis. Fluoridated starch is a compound consisting of galactose and glycerol bonded through glycosidic links (Syaharuddin, 2019). *K. alvarezii* contains dietary fiber in the form of both water-soluble and insoluble fiber. The dietary fiber content of *K. alvarezii* in this study was 49.11%, which is lower than the fiber content of *K. alvarezii* reported by Santoso *et al. (*2006), which was 69.3%. This difference suggests that geographic and environmental factors influence the nutritional properties of seaweed. The growth rate and chemical composition of *K. alvarezii* are affected by cultivation techniques, seasons, geographic location, environmental conditions, and post-harvest drying methods (Adharini *et al.,* 2020). The main component of *K. alvarezii* hydrolysates is polysaccharides.

Reducing Sugar and Yield of Hydrolysate *K. alvarezii*

Hydrolysis of *K. alvarezii* using a hydrothermal method with an autoclave (at 100°C for 3 h) resulted in a reducing sugar content of 0.20 g/100 mL. This reducing sugar content is relatively low compared to the findings of Mutmainnah *et al.* (2023), who reported 0.31 g/100 mL. This difference is attributed to the condition of the raw material used, as Mutmainnah *et al.* (2023) used fresh *K. alvarezii*. The reducing sugar content produced by hydrolysis depends on the initial carbohydrate content and duration of the hydrolysis process (Khusniati *et al.,* 2023). Autoclave heating is a hydrothermal extraction method that combines temperature and pressure effects. High temperature accelerates the hydrolysis reaction, while pressure enhances extraction efficiency by breaking open the cell structure, making it easier to release the contained compounds. Peerakietkhajorn *et al.* (2024) performed hydrothermal extraction using Milli-Q water in an autoclave for at. This hydrolysis was more effective in breaking down the cell walls of *Caulerpa lentillifera* than microwave-assisted extraction, owing to the high pressure and temperature in the autoclave. Kim *et al.* (2014) evaluated the effectiveness of the macroalga *Enteromorpha intestinalis* as a potential bioenergy source for reducing sugar production through hydrothermal hydrolysis using an autoclave at 190°C for 30 minutes, yielding 7.16 g/L; hydrolysis for 60 minutes at 170°C resulted in 7.3 g/L; and at 190°C, the reducing sugar content decreased from 7.16 g/L (30 minutes) to 4.37 g/L (60 minutes).

Excessive decomposition of reducing sugars occurs due to exposure to high temperatures for long durations.

Reducing sugars function as prebiotics because they are easily fermentable substrates of probiotic bacteria. This fermentation process produces short-chain fatty acids (SCFAs), which are beneficial for gut health and support synbiotic effects on the host. The longer the hydrolysis process, the more carbohydrates are degraded into reducing sugars (glucose) (Poespowati & Mahmudi, 2018). This leads to an increase in the reducing sugar content, which can affect the quality of the final product. The effectiveness and efficiency of the production process were assessed based on the obtained yield value. Yield was calculated by comparing the final weight percentage to the initial weight in a process, which reflects the effectiveness of the method or processing used (Sulistiana *et al.,* 2024). The high yield obtained indicates that the method used is efficient for producing the desired product. The hydrolysis of *K. alvarezii* in this study resulted in a yield of 42.98% (w/w). Extraction of *Caulerpa lentillifera* using the autoclave-assisted method produced a yield of 41.16%, whereas extraction using microwaves resulted in a yield of 40.71% (Peerakietkhajorn *et al.,* 2024).

The polysaccharides of *K. alvarezii* are classified as prebiotics because they are broken down into simpler forms with low molecular weights (Setyaningsih *et al.,* 2019). Polysaccharides that are indigestible by humans and low-molecular-weight oligosaccharides that have been hydrolyzed can support the growth of beneficial gut microbiota, such as *Lactobacillus* and *Bifidobacterium* (Padam *et al.,* 2023). Polysaccharide depolymerization occurs via various chemical, physical, and enzymatic processes. Chemical hydrolysis is inexpensive, rapid, and scalable; however, it has disadvantages, such as low sensitivity and the production of byproducts that are harmful to the environment (Cheong *et al.,* 2018; Yao *et al.,* 2014). Enzymatic depolymerization is environmentally friendly and advantageous because the oligosaccharides produced show higher homogeneity and lower polydispersity. However, this process requires a long reaction time and incurs high costs (Cheong *et al.,* 2018).

L. plantarum SK(5) and *L. plantarum* NS(5) Starters

The lactic acid bacteria used in this study were *L. plantarum* SK(5) and *L. plantarum* NS(5), isolated from fermented *bekasam* of tilapia fish from South Sumatra (Desniar, 2012). The verification of the starter culture included gram staining, catalase testing, motility testing, and homofermentative testing. The results of gram staining are presented in Figure 1. Gram staining of *L. plantarum* SK(5) and *L. plantarum* NS(5) revealed Gram-positive bacilli. The catalase test for both bacterial isolates showed negative results, with no bubbles observed on the solution surface. Lactic acid bacteria are facultative anaerobes that can break down H_2O_2 into organic compounds and H_2O, thereby preventing the formation of gas bubbles.

Starters play a crucial role in the production of synbiotic yogurt by supporting the fermentation process, resulting in characteristic yogurt products. A starter culture consists of a population of microorganisms deliberately added to the raw material of fermented products to accelerate and control fermentation (Desniar *et al.,* 2023). The bacterial characteristics tested were consistent with those described for *L. plantarum* SK(5) and *L. plantarum* NS(5) by Desniar (2012), including being gram-positive, rod-shaped, catalase-negative, non-motile, homofermentative, non-spore-forming, and producing no gas from glucose. *L. plantarum* is a microorganism measuring 0.9-1.2 × 1.0-8.0 μm, found either as single cells or in short chains (Todorov & Franco, 2010). *L. plantarum* is a lactic acid bacterium that does not produce gas and is categorized as Generally Recognized As Safe (GRAS) with Qualified Presumption of Safety (QPS) status (Liu *et al.,* 2018). A key characteristic of this bacterium is its production of lactic acid, which is crucial for fermentation (Aguirre-Garcia *et al.,* 2024). Tolerance to acid is a characteristic of both *L. plantarum* SK(5)

http://dx.doi.org/10.17844/wz4w4v64

(A) *L. plantarum* SK(5) (B) *L. plantarum* NS(5)

Figure 1 Appearance of bacteria (magnification 10x100)

and NS(5) strains. The total LAB count in the starter culture of *L. plantarum* SK(5) was 9.36 log CFU/mL (2.3×10^9 CFU/mL), and in the mother culture, it was 9.33 log CFU/mL (2.1×10^9 CFU/mL). The total LAB count in the starter culture of *L. plantarum* NS(5) was 9.22 log CFU/mL (1.6×10^9 CFU/mL), and in the mother culture, it was 9.27 log CFU/mL (1.8×10^9 CFU/mL). The total LAB count in the *L. plantarum* combination starter culture was 9.29 log CFU/mL (1.9×10^9 CFU/mL), and in the mother culture, it was 9.20 log CFU/mL (1.5×10^9 CFU/mL). The total LAB count in the *L. plantarum* starters met the probiotic bacteria count requirements for food products based on the FAO guidelines, which specify a minimum of 10^7 CFU/mL (FAO, 2022).

Microbiological and Chemical Characteristics of Synbiotic Yogurt

Total lactic acid bacteria

Yogurt must contain sufficient LAB probiotics to provide health benefits (Shori *et al.,* 2022). The total probiotic bacteria in fermented products should be a minimum of 10^7 CFU/mL (BSN, 2009; FAO, 2022). The total LAB count in the synbiotic yogurt was tested to evaluate the quality and effectiveness of fermentation. The total LAB count during synbiotic yogurt fermentation is presented in Figure 2.

Analysis of variance showed that the total LAB count was influenced by fermentation time, starter type, and the interaction between both (p<0.05). Based on

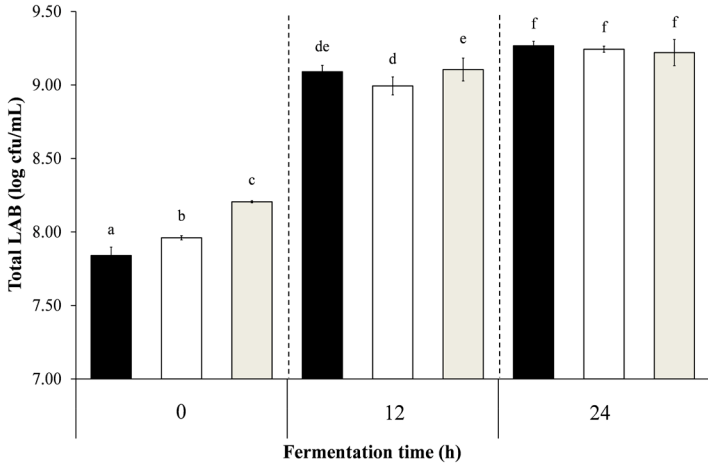

Figure 2 Total LAB of synbiotic yogurt with different strains of *L. plantarum;* (■): *L. plantarum* SK(5); (□): *L. plantarum* NS(5); (▭): combination during fermentation. Different superscript indicate differences in interaction factors (p<0.05)

Duncan's Multiple Range Test, all fermentation treatments were significantly different from the 0 hour fermentation treatment. Optimal growth occurred at 24 h of fermentation, with total LAB counts ranging from 9.22 $\log_{10}$ CFU/mL to 9.27 $\log_{10}$ CFU/mL. The total LAB counts for all treatments remained above 10^7 CFU/mL during the 24 h fermentation period. Neither single *L. plantarum* nor the combination of synbiotic yogurt treatments at 24 h of fermentation showed significant effects. The significant difference confirms that the fermentation process for 12 and 24 h effectively triggered the logarithmic growth phase of the bacteria, during which nutrients from the milk and prebiotic hydrolysate were successfully utilized for bacterial cell proliferation. The synbiotic yogurt fermented for 24 h with *L. plantarum* SK(5) as the starter culture showed a total LAB count of 9.27 $\log_{10}$ CFU/mL. The high number of LAB in this treatment indicates that *L. plantarum* SK(5) has good adaptive and metabolic capabilities in utilizing *K. alvarezii* as a prebiotic, suggesting a strong synergism between the local probiotic source and the macroalgae-derived prebiotic. This result contrasts with the findings of Dewi dan Purnamayati (2021), who studied the combination of *S. thermophilus* FNCC 0040 and *L. bulgaricus* FNCC 0041 in synbiotic yogurt, which resulted in a mutualistic symbiotic relationship. *Streptococcus thermophilus* is more active in breaking down amino acids and micronutrients than *L. bulgaricus*. *Streptococcus thermophilus* uses more oxygen, which is beneficial for *L. bulgaricus,* as it is sensitive to high levels of oxygen (Sieuwerts, 2016).

Factors supporting LAB growth in synbiotic yogurt include glucose and prebiotics. Fresh cow milk contains a total sugar and lactose content of 9 g/200 mL. The nutritional content of milk, particularly lactose, plays a role in the growth of lactic acid bacteria (Agustine *et al.,* 2018). Lactose is the primary carbon source, which can be broken down by LAB into glucose and galactose, which are then converted into lactic acid through metabolic activity (Parasthi *et al.,* 2020). LAB also utilize prebiotics as a carbohydrate source, containing many hydroxyl groups that form hydrogen bonds with polar groups on proteins to enhance their stability (Shalaby & Amin, 2019). Fermentation occurs in three stages: lag, logarithmic, and stationary stages. The lag phase occurs during the first 6 h of yogurt incubation as the bacteria adjust to their growth period. The logarithmic growth phase of the LAB occurred 12 h after incubation. Bacterial cells utilize nutrients in the medium to undergo cell division (Nelfa *et al.,* 2015). Before entering the decline phase, the stationary phase (optimal growth) of LAB occurred at 24 h of incubation. The total LAB decreased during the death phase, which occurred during fermentation from 48 to 72 h. This is due to a decrease in the available substrates for bacteria (Nahdiyah & Wikandari, 2022). Based on these observations, the total LAB in the strain combination (YMIX) showed a higher initial count than the single strains. At 12 h of fermentation, the growth of LAB in the combination was comparable to that of the best single strain, while at 24 h, all treatments reached their peak with relatively similar values. However, the single strains were slightly higher than the combined strains. This indicates that the strain combination is capable of consistently supporting LAB growth and is almost comparable to the single strains.

Enhancing the growth medium with *K. alvarezii* hydrolysate is an effective method for increasing the proliferation of lactic acid bacteria (LAB). *Lactobacillus plantarum* SK(5) and *Lactobacillus plantarum* NS(5) were homoferment. Homofermentative bacteria utilize available carbohydrates, which are broken down into pyruvic acid via the Embden-Meyerhof-Parnas (EMP) pathway and then reduced by $NADH_2$ to lactic acid ($C_3H_6O_3$) (Irdianty *et al.,* 2023). Lactic acid is produced through the breakdown of sugars, such as glucose, lactose, sucrose, raffinose, and stachyose, in the fermentation medium via glycolysis. The total LAB count was indicated by the formation of clear zones in the growth medium. The clear zone around the colonies growing on the medium represents a reaction between the lactic acid produced by the LAB and CaCO3 during the incubation period, resulting in the formation of calcium lactate (Setiawan & Agustini, 2024). The intensity of

http://dx.doi.org/10.17844/wz4w4v64

the clear zone reflects the metabolic activity of LAB in degrading carbohydrates to lactic acid via the EMP pathway. The reaction occurs when lactic acid ($C_3H_6O_3$) reacts with calcium carbonate ($CaCO_3$) to form calcium lactate ($Ca(C_3H_6O_3)$), water (H_2O), and carbon dioxide (CO_2).

Total titratable acidity (TTA)

The total titratable acidity (TTA) was expressed as the percentage concentration of lactic acid. Acidity can be measured using a titration apparatus or by assessing the buffering capacity of a liquid or solution. Lactic acid is produced when lactic acid bacteria break down lactose and other carbohydrates into lactic acid (Susmiati *et al.,* 2022). An increase in the number of lactic acid bacteria and a decrease in pH resulted in an increase in TTA in the fermentation product. The total titratable acidity (TTA) of the synbiotic yogurt is shown in Figure 3.

The results of the analysis of variance (ANOVA) showed that the fermentation time, *L. plantarum* starter type (single and combined), and their interaction significantly affected (p<0.05) the total titratable acidity (TTA) of synbiotic yogurt. Duncan's multiple range test revealed that the fermentation treatment for 24 hours for all types of starters was significantly different from the 0 hour and 12 hour fermentation treatments for all starter types. This significance reflects the successful metabolism of carbohydrate substrates into lactic acid by LAB, as indicated by the increase in TAT values during fermentation. Synbiotic yogurt fermented for 24 h with the *L. plantarum* SK(5) starter had the highest TTA value (0.74%) compared to the synbiotic yogurt with *L. plantarum* NS(5) (0.62%) and the combined *L. plantarum* starter (0.62%) after 24 h of fermentation. This advantage implies that the SK(5) strain has the most superior acid-producing capability, consistent with its nature as a homofermentative bacterium. High acid production was directly correlated with an increase in the total LAB count and a decrease in the pH value, confirming the functional relationship between bacterial growth and acid formation. The LAB in this study were homofermentative. The increase in lactic acid is inversely proportional to the decrease in pH, leading to a reduction in the total titratable acidity as the pH increases (Andriani *et al.,* 2024). *L. plantarum* SK(5) can produce 90% lactic acid from glucose breakdown. Previous studies have reported that *L. plantarum* SK(5) is a homofermentative bacterium capable of producing 778.26 ppm lactic acid after 48 h and has a TTA value of 21.60 g/L after 44 h of incubation (Desniar *et al.,* 2020). The TTA values of synbiotic yogurt fermented for 24 h (0.62%–0.74%) met the quality standards for yogurt based on SNI 2981:2009, which requires an acidity percentage, calculated as lactic acid, ranging from 0.5% to 2.0% (BSN, 2009).

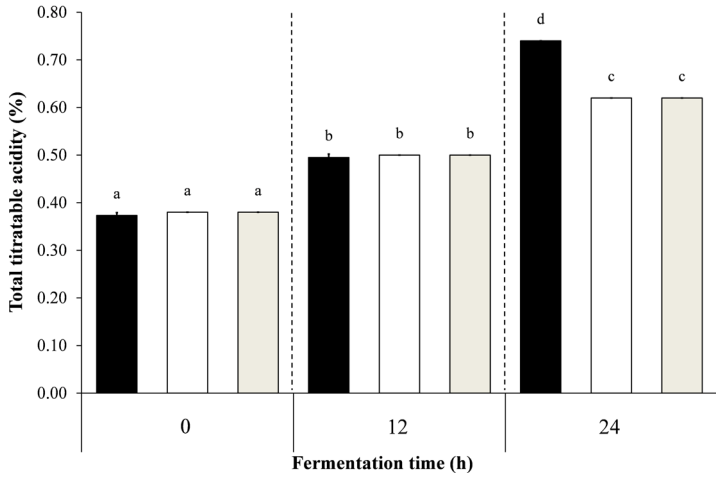

Figure 3 Total titratable acidity of synbiotic yogurt with different strains of *L. plantarum;* (■): SK(5); (□): *L. plantarum* NS(5); (▭): combination during fermentation. Different superscript indicate differences in interaction factors (p<0.05)

The carbohydrate source derived from *K. alvarezii* hydrolysate, utilized by lactic acid bacteria (LAB), is glucose. Glucose can be metabolized through two main fermentation pathways. In homofermentative LAB, the glycolytic pathway (Embden–Meyerhof pathway) converts one molecule of glucose and two inorganic phosphates (Pi) into two molecules of lactate, two molecules of ATP, and one molecule of water (H_2O). In contrast, heterofermentative LAB employ the 6-phosphogluconate/phosphoketolase (6PG/PK) pathway, in which one molecule each of glucose, inorganic phosphate (Pi), and ADP are converted into one molecule each of lactate, one molecule of acetate, one molecule of ATP, and one molecule of carbon dioxide (CO_2). Glucose substrates produce lactic acid, which is the main end product of both metabolic pathways (Desniar *et al.*, 2020). The incubation temperature also affects the growth of lactic acid bacteria in producing lactic acid. *L. plantarum* SK(5) and *L. plantarum* NS(5) grow well at 37°C (Desniar, 2012; Nurnaafi *et al.*, 2015). In this study, synbiotic yogurt was incubated at 37°C. Dewi and Purnamayati (2021) reported that 37°C is the optimal incubation temperature for *L. plantarum* and *S. thermophilus* to produce acetaldehyde, which contributes to the flavor of yogurt. The incubation temperature used in this study was consistent with that of Dewi & Purnamayati (2021), who found that synbiotic yogurt from *C. racemosa* with *L. bulgaricus* and *S. thermophilus* at 37°C produced a TTA value of 0.64% and a pH value of 5.04. A comparison with the previous study by Adhani (2021) on the diversification of yogurt using *L. plantarum* SK(5) starter and the addition of 1.5% wet biomass of *Spirulina* with a 24-hour fermentation time resulted in a pH value of 3.9 and a TTA value of 1.195%. This indicates that the use of different probiotics and prebiotics affects the acid production and viability of yogurt.

Acidity (pH)

The pH of synbiotic yogurt is a parameter used to determine the acidity and alkalinity of the products. The optimal pH affects not only the taste but also the stability of probiotic microorganisms in synbiotic yogurt. The pH values of the fermented synbiotic yogurt are presented in Figure 4.

The pH value of synbiotic yogurt was significantly influenced by fermentation time, type of *L. plantarum* (single and combined strains), and the interaction between these factors ($p<0.05$). Duncan's Multiple Range Test showed that all fermentation treatments

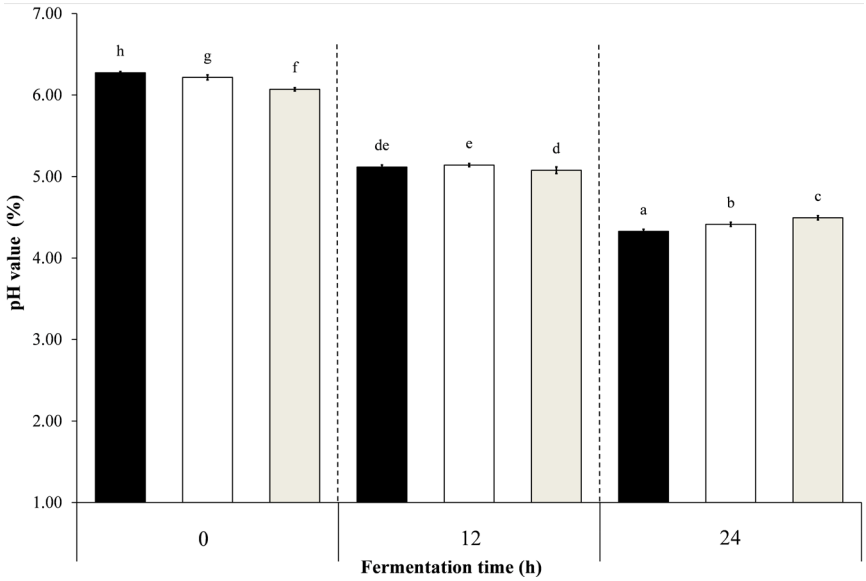

Figure 4 pH of synbiotic yogurt with different strains of *L. plantarum;* (■): *L. plantarum* SK(5); (□): *L. plantarum* NS(5); (□): combination during fermentation.
Different superscript indicate differences in interaction factors ($p<0.05$)

http://dx.doi.org/10.17844/wz4w4v64

were significantly different from the 0-hour fermentation treatment. The pH value tended to decrease from 0 h to 24 h of fermentation, ranging from 6.27 to 4.33, respectively. The pH values of all synbiotic yogurt treatments after 24 h of fermentation met the yogurt standards based on SNI 2981:2009 (BSN, 2009), which specifies a pH range of 3.80-4.50. The lowest pH value was obtained from the *L. plantarum* SK(5) treatment after 24 h of fermentation (4.33). The significant decrease in pH observed in the *L. plantarum* SK(5) treatment reflected a more efficient fermentation process. This is correlated with higher organic acid production and a potential improvement in the microbiological stability of synbiotic yogurt, attributed to lactic acid produced through LAB metabolism. This is in line with the study by Anwar *et al.* (2025), who found that fermentation time significantly affected the pH, acidity, and total LAB in cow milk yogurt. The single-strain L. plantarum SK (5) treatment in this study was more effective in lowering pH during the fermentation process than the single-strain *L. plantarum* NS(5) and the combination strain. *L. plantarum* can convert carbohydrates or sugars into lactic acid (Sudibyo *et al.,* 2024). Desniar *et al.* (2012) explained that during fermentation, *L. plantarum* SK(5) produces lactic and acetic acids as the dominant organic acids, thereby lowering the pH of the environment.

The low pH of yogurt is also caused by the presence of lactose in milk, which is converted into pyruvic acid and further converted into lactic acid, propionate, and butyrate (Setiarto *et al.,* 2022). The organic acids formed dissociate into H^+ ions. The higher the acidity, the more H^+ ions are formed, which leads to a decrease in pH, as measured using a pH meter (Andriani *et al.,* 2024). The pH value in this study was higher compared to the synbiotic yogurt study by Amaro *et al.* (2021), which used a combination of *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* starters with the addition of corn extract and the *Eucheuma spinossum* carrageenan stabilizer, resulting in a pH of 4.28. Setiadi and Husni (2024) reported that cow's milk yogurt, which was added with *L. bulgaricus* and *S. thermophilus* starters and *Caulerpa lentillifera* powder at 0.5%, 10%, and 15%, had a pH range of 3.93-4.02. The difference in these results is due to the type of lactic acid bacteria starter used. Yogurt fermented with a monoculture of *S. thermophilus* synthesizes $_{L}$-lactic acid, whereas $_{D}$-lactic acid is the main product of *L. bulgaricus* (Ge *et al.,* 2024). Co-culture fermentation produces a combination of $_{L}$- and $_{D}$-lactic acid isomers, which demonstrates a stronger ability to produce lactic acid, thus influencing yogurt pH. The decrease in pH of the synbiotic yogurt correlated with an increase in total titratable acidity (TTA). As the TTA value increased, the pH value decreased from 0 to 24 h of fermentation. This trend was in line with the increase in the total LAB count in synbiotic yogurt.

Reducing Sugar

Reducing sugars that react with the 3,5-dinitrosalicylic acid (DNS) reagent form a yellow-brown compound, 3-amino-5-nitrosalicylic acid, after heating, which is reducing sugars capable of reduction owing to the presence of free keto groups, such as glucose and fructose (Puspitarini & Susilowati, 2020). The concentrations of reducing sugars in the fermented synbiotic yogurt are presented in Figure 5.

The reducing sugar content in the fermented synbiotic yogurt was significantly influenced by fermentation time and the interaction between fermentation time and the type of *L. plantarum* starter used. According to Duncan's multiple range test, the interaction between fermentation time and starter type had a significant effect ($p<0.05$). Synbiotic yogurt with each type of *L. plantarum* starter showed a decrease in reducing sugar levels during the fermentation process. The reducing sugar content increased during the initial fermentation stage (0 h) and then decreased. The synbiotic yogurt treatment with *L. plantarum* SK(5) produced the lowest reducing sugar content after 24 h, amounting to 0.25 g/mL of reducing sugar. The highest reducing sugar content at 12 h of fermentation was observed in the *L. plantarum* NS(5) treatment, at 0.32 g/mL, whereas at 24 h, the highest reducing sugar content was observed in the *L. plantarum* combination treatment,

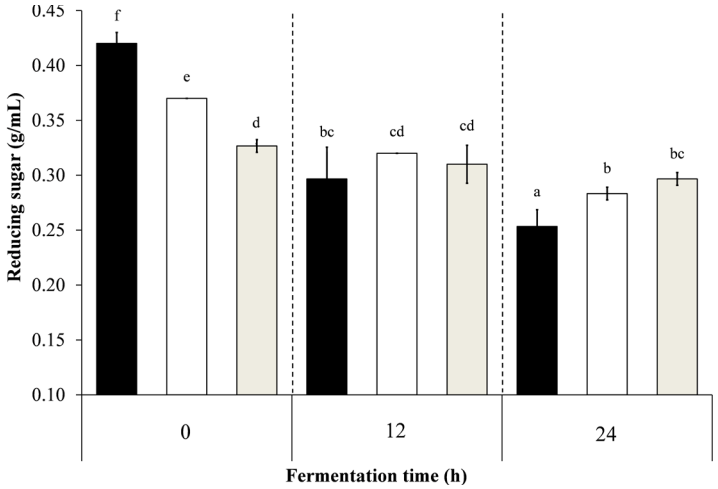

Figure 5 Reducing sugar of synbiotic yogurt with different strains of *L. plantarum;* (■): *L. plantarum* SK(5); (□): *L. plantarum* NS(5); (▢): combination during fermentation. Different superscript indicate differences in interaction factors (p<0.05)

at 0.30 g/mL. The decrease in reducing sugar content after 24 h of fermentation suggests that LAB, especially *L. plantarum* SK(5), actively utilized sugars derived from the *K. alvarezii* hydrolysate as the primary metabolic substrate. The decrease in reducing sugar content during fermentation is due to the consumption of prebiotics, which contain simple sugar structures that serve as carbon sources for bacterial cells to synthesize energy (Sofyan *et al.,* 2022). This is evidenced by the inverse correlation between the decrease in reducing sugars and the increase in total lactic acid bacteria (LAB) and titratable acidity. Lactic acid bacteria also derive their energy from milk, which contains lactose.

These results are consistent with the study by Li *et al.* (2022), on prebiotic potato (33%) and blueberry (22%) yogurt with inoculum *Bifidobacterium animalis* subsp. *lactis* BZ11, *L. plantarum* LB12, and *S. thermophilus* Q-1, where the reducing sugar content was relatively high at 0 hours of fermentation due to the saccharification solution from potatoes containing 62.38 mg/mL of reducing sugars. After fermentation, the reducing sugar content decreased to 50.56 mg/mL, accompanied by an increase in the number of lactic acid bacteria. This finding is in accordance with the increase in total LAB in synbiotic yogurt as the fermentation time. LAB also derive their energy from milk, which contains lactose. The analysis of reducing sugars was expressed as the percentage of lactose in the product, as lactose is the main reducing sugar naturally present in milk (de Morais *et al.,* 2023). Kim & Han (2019) reported that D-allulose is not metabolized by LAB; however, the sucrose it contains can be utilized by LAB to produce lactic acid. This process continues to degrade until short-chain fatty acids are produced. Fermentation times of 0, 12, and 24 h at a fermentation temperature of 37°C influenced the reduction of reducing sugars in synbiotic yogurt. The longer the fermentation time, the greater the reduction in reducing sugar content, which is caused by the conversion of carbohydrates into monosaccharides, particularly the conversion of reducing sugars into organic acids (Khusniati *et al.,* 2023).

CONCLUSION

The optimal fermentation time for synbiotic yogurt was 24 h with a single starter of *L. plantarum* SK(5), resulting in a total LAB count of 9.27 $\log_{10}$ CFU/mL, TTA of 0.74%, pH of 4.33, and a reduction in sugar content of 0.25%.

REFERENCES

Adhani, S. W. (2021). Karakteristik yoghurt *Spirulina* dengan starter *Lactobacillus plantarum* SK (5) pada perbedaan

konsentrasi biomassa dan waktu inkubasi. [Skripsi]. Institut Pertanian Bogor.

Adharini, R. I., Setyawan, A. R., Suadi, & Jayanti, A. D. (2020). Comparison of nutritional composition in red and green strains of *Kappaphycus alvarezii* cultivated in Gorontalo Province, Indonesia. *E3S Web of Conferences*, 147, 03029. https://doi.org/10.1051/e3sconf/202014703029

Aguirre-Garcia, Y. L., Nery-Flores, S. D., Campos-Muzquiz, L. G., Flores-Gallegos, A. C., Palomo-Ligas, L., Ascacio-Valdés, J. A., Sepúlveda-Torres, L., & Rodríguez-Herrera, R. (2024). Lactic acid fermentation in the food industry and bio-preservation of food. *Fermentation*, 10(3), 168. https://doi.org/10.3390/fermentation10030168

Agustine, L., Okfrianti, Y., & Jum, J. (2018). Identifikasi total bakteri asam laktat (BAL) pada yoghurt dengan variasi sukrosa dan susu skim. *Jurnal Dunia Gizi*, 1(2), 79. https://doi.org/10.33085/jdg.v1i2.2972

Al Wazzan, I. M., Wullandari, P., & Fauzi, A. (2021). Effect of dried *Eucheuma cottonii* stored in seaweed storage device in its quality. *Jurnal Perikanan Universitas Gadjah Mada*, 23(2), 137. https://doi.org/10.22146/jfs.66232

Amaro, M., Ariyana, M. D., Handayani, B. R., Nazaruddin, Widyastuti, S., & Rahayu, T. I. (2021). Yogurt as a functional drink development from various local raw materials using Eucheuma spinosum as natural stabilizer. *IOP Conference Series: Earth and Environmental Science*, 913(1), 012035. https://doi.org/10.1088/1755-1315/913/1/012035

Anand, A., Yoshida, S., & Aoyagi, H. (2021). Tailored synbiotic powder (functional food) to prevent hyperphosphataemia (kidney disorder). *Scientific Reports*, 11(1), 16485. https://doi.org/10.1038/s41598-021-95176-3

Andriani, R. D., Sawitri, M. E., Widayanti, V. T., Manab, A., Rahayu, P. P., Prasasti, F., & Saleha, R. A. (2024). Physicochemical properties of yogurt synbiotic enriched with bawang Dayak (*Eleutherine palmifolia* (L) Merr) extract. *Jurnal Ilmu-Ilmu Peternakan*, 34(2), 142–150. https://doi.org/10.21776/ub.jiip.2024.034.02.1

Anwar, A., Faiz, M. A., Badar, I. H., Jaspal, M. H., & Hou, J. (2025). Influence of fermentation time and storage conditions on the physicochemical properties of different yogurt varieties using starter cultures and probiotic *Lactobacillus rhamnosus* GG. *Processes*, 13(3), 759. https://doi.org/10.3390/pr13030759

Arriaga-Morales, J. J., Ordaz-Pichardo, C., Castro-Muñoz, R., & Durán-Páramo, E. (2024). Attenuation of hyperglycemia in diabetic rats assisted by immobilized probiotic in sodium alginate. *Probiotics and Antimicrobial Proteins*, 16(6), 2218–2228. https://doi.org/10.1007/s12602-023-10166-3

Ashaolu, T. J., Ashaolu, J. O., & Adeyeye, S. A. O. (2021). Fermentation of prebiotics by human colonic microbiota in vitro and short-chain fatty acids production: a critical review. *Journal of Applied Microbiology*, 130(3), 677–687. https://doi.org/10.1111/jam.14843

Astawan, M., Wresdiyati, T., Suliantari, S., Arief, I. I., & Septiawan, R. (2012). Production of Synbiotic Yogurt-Like Using Indigenous Lactic Acid Bacteria as Functional Food. *Media Peternakan*, 35(1), 9–14. https://doi.org/10.5398/medpet.2012.35.1.9

[AOAC] Association of Official Analytical Chemists. (1994). AOAC official method 993.21: total dietary fiber in foods and food products with 2% starch. https://academic.oup.com/aoac-publications

Bajury, D. M., Rawi, M. H., Sazali, I. H., Abdullah, A., & Sarbini, S. R. (2017). Prebiotic evaluation of red seaweed (*Kappaphycus alvarezii*) using in vitro colon model. *International Journal of Food Sciences and Nutrition*, 68(7), 821–828. https://doi.org/10.1080/09637486.2017.1309522

Basroni, A. T., Al-Baarri, A. N., & Legowo, A. M. (2018). Viability lactic acid bacteria of yogurt powder with carrageenan addition. *Journal of Applied*

Food Technology, 5(1). https://doi.org/10.17728/jaft.61

Betancourt, M. M., Guevara García, J., Luna, L., Maldonado, L., Cardona, J., & Torrico, D. D. (2025). Effects of fermentation conditions on the physicochemical and sensory properties of plant-based yogurts. *LWT-Food Science and Technology*, 230, 118242. https://doi.org/10.1016/j.lwt.2025.118242

Bharti, S. K., Pathak, V., Arya, A., Alam, T., Rajkumar, V., & Verma, A. K. (2021). Packaging potential of *Ipomoea batatas* and κ-carrageenan biobased composite edible film: Its rheological, physicomechanical, barrier and optical characterization. *Journal of Food Processing and Preservation*, 45(2). https://doi.org/10.1111/jfpp.15153

Birch, C. S., & Bonwick, G. A. (2019). Ensuring the future of functional foods. *International Journal of Food Science and Technology*, 54(5), 1467–1485. https://doi.org/10.1111/ijfs.14060

[BSN] Badan Standardisasi Nasional. (1992). SNI 01-2891-1992: cara uji makanan dan minuman. https://akses-sni.bsn.go.id/viewsni/baca/957

[BSN] Badan Standardisasi Nasional. (2004). SNI 06-6989.11-2004: cara uji derajat keasaman (pH) dengan menggunakan alat pH meter. https://akses-sni.bsn.go.id/viewsni/baca/2824

[BSN] Badan Standardisasi Nasional. (2023). SNI 2690-2023: rumput laut kering (syarat mutu dan pengolahan). https://akses-sni.bsn.go.id/viewsni/baca/9939

[BSN] Badan Standardisasi Nasional. (2009). SNI 2981-2009: yogurt. https://akses-sni.bsn.go.id/viewsni/baca/3822

Cheong, K.-L., Qiu, H.-M., Du, H., Liu, Y., & Khan, B. M. (2018). Oligosaccharides derived from red seaweed: production, properties, and potential health and cosmetic applications. *Molecules*, 23(10), 2451. https://doi.org/10.3390/molecules23102451

Desniar. (2012). Karakterisasi bakteri asam laktat dari produk fermentasi ikan (bekasam) [Tesis]. Institut Pertanian Bogor.

Desniar, D., Setyaningsih, I., & Fransiska, I. M. (2023). Perubahan kimiawi dan mikrobiologis selama fermentasi bekasam ikan nila menggunakan starter tunggal dan campuran. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 26(3), 414–424. https://doi.org/10.17844/jphpi.v26i3.50664

Desniar, Rusmana, I., Suwanto, A., & Mubarik, N. R. (2012). Senyawa antimikroba yang dihasilkan oleh bakteri asam laktat asal bekasam. *Jurnal Akuatika*, III(2), 135–145.

Desniar, Rusmana, I., Suwanto, A., & Mubarik, N. R. (2013). Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. *Emirates Journal of Food and Agriculture*, 25(6), 489. https://doi.org/10.9755/ejfa.v25i6.12478

Desniar, Rusmana, I., Suwanto, A., & Mubarik, N. R. (2020). Organic acid produced by lactic acid bacteria from bekasam as food biopreservatives. *IOP Conference Series: Earth and Environmental Science*, 414(1), 012003. https://doi.org/10.1088/1755-1315/414/1/012003

Dewi, E. N., & Purnamayati, L. (2021). Characterization of Caulerpa racemosa yogurt processed using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Food Research*, 5(S3), 54–61. https://doi.org/10.26656/fr.2017.5(S3).008

Dhami, M., Raj, K., & Singh, S. (2023). Relevance of gut microbiota to alzheimer's disease (AD): potential effects of probiotic in management of AD. *Aging and Health Research*, 3(1), 100128. https://doi.org/10.1016/j.ahr.2023.100128

Djaja, N., Permadi, I., Witjaksono, F., Soewondo, P., Abdullah, M., Agustina, R., & Ali, S. (2019). The effect of Job's tears-enriched yoghurt on GLP-1, calprotectin, blood glucose levels and weight of patients with type 2 diabetes mellitus. *Mediterranean Journal of Nutrition and Metabolism*, 12(2), 163–171. https://doi.org/10.3233/MNM-

180258

Dolorosa, M. T., Nurjanah, N., Purwaningsih, S., Anwar, E., & Hidayat, T. (2017). Bioactive compounds of seaweed *Sargassum plagyophyllum* and *Eucheuma cottonii* as lightening raw materials. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(3), 632. https://doi.org/10.17844/jphpi.v20i3.19820

de Morais, F. A., Cavalcanti, M. T., Olegario, L. S., Santos, T. Q., Evangelista, M. E. M., de Oliveira, G. B., Santana, A. G., Souto, M. V., da Fonseca, S. B., & Gonçalves, M. C. (2023). Impact of sugar reduction on physicochemical and nutritional quality of yogurt and sensory response with label stimulation. *Journal of Food Processing and Preservation*, 2023, 1–10. https://doi.org/10.1155/2023/1439953

Ebrahimi, Z. sadat, Nasli-Esfahani, E., Nadjarzade, A., & Mozaffari-khosravi, H. (2017). Effect of symbiotic supplementation on glycemic control, lipid profiles and microalbuminuria in patients with non-obese type 2 diabetes: a randomized, double-blind, clinical trial. *Journal of Diabetes and Metabolic Disorders*, 16(1), 23. https://doi.org/10.1186/s40200-017-0304-8

[FAO] Food and Agriculture Organization. (2022). Standard for fermented milks: Cxs 243-2003.

Ge, Y., Yu, X., Zhao, X., Liu, C., Li, T., Mu, S., Zhang, L., Chen, Z., Zhang, Z., Song, Z., Zhao, H., Yao, S., & Zhang, B. (2024). Fermentation characteristics and postacidification of yogurt by *Streptococcus thermophilus* CICC 6038 and *Lactobacillus delbrueckii* ssp. bulgaricus CICC 6047 at optimal inoculum ratio. *Journal of Dairy Science*, 107(1), 123–140. https://doi.org/10.3168/jds.2023-23817

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Scott, K., Stanton, C., Swanson, K. S., Cani, P. D., Verbeke, K., & Reid, G. (2017). Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, 14(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Huang, P.-H., Chen, Y.-J., Lin, Y.-W., & Huang, D.-W. (2024). Gelatin *kappa*-carrageenan based edible film incorporated with ethanol extracts from *Premna microphylla* turcz leaves for preservation of sailfish fillets. *LWT-Food Science and Technology*, 208, 116710. https://doi.org/10.1016/j.lwt.2024.116710

Irdianty, M. S., Harti, A. S., & Windyastuti, E. (2023). Synbiotic soygurt tempe extract as a functional drink for stunting prevention. *International Journal of Public Health Excellence (IJPHE)*, 3(1), 288–299. https://doi.org/10.55299/ijphe.v3i1.629

Kamaruddin, A., Uyun, A. S., Rahman, H. N., Suherman, E., & Lumbessi, S. Y. (2017). Using renewable energy to process seaweed. In *Mediterranean Green Buildings & Renewable Energy* (pp. 835–843). *Springer International Publishing*. https://doi.org/10.1007/978-3-319-30746-6_65

Kang, W., Pan, L., Peng, C., Dong, L., Cao, S., Cheng, H., Wang, Y., Zhang, C., Gu, R., Wang, J., & Zhou, H. (2020). Isolation and characterization of lactic acid bacteria from human milk. *Journal of Dairy Science*, 103(11), 9980–9991. https://doi.org/10.3168/jds.2020-18704

Kawaroe, M., Salundik, Wahyudi, R., & Lestari, D. F. (2017). Comparison of biogas production from macroalgae *Eucheuma cottonii* in anaerobic degradation under different salinity conditions. *World Applied Sciences Journal*, 35(3), 344–351. https://doi.org/10.5829/idosi.wasj.2017.344.351

Khusniati, T., Rahmaulidin, F., Yuningtyas, S., & Sulistiani. (2023). Chemical properties of vegetable yogurt with addition of amyloproteolytic enzyme *Lactobacillus fermentum* EN17-2 on storage time. *IOP Conference Series: Earth and Environmental Science*, 1200(1), 012019. https://doi.org/10.1088/1755-1315/1200/1/012019

Kim, D.-H., Lee, S.-B., & Jeong, G.-T. (2014). Production of reducing sugar from *Enteromorpha intestinalis* by hydrothermal and enzymatic hydrolysis. *Bioresource Technology*, 161, 348–353. https://doi.org/10.1016/j.biortech.2014.03.078

Kim, H. J., & Han, M. J. (2019). The fermentation characteristics of soy yogurt with different content of D-allulose and sucrose fermented by lactic acid bacteria from Kimchi. *Food Science and Technology*, 28, 1155–1161. https://doi.org/https://doi.org/10.1007/s10068-019-00560-5

Kobyliak, N., Falalyeyeva, T., Bodnar, P., & Beregova, T. (2017). Probiotics supplemented with omega-3 fatty acids are more effective for hepatic steatosis reduction in an animal model of obesity. *Probiotics and Antimicrobial Proteins*, 9(2), 123–130. https://doi.org/10.1007/s12602-016-9230-1

Kurniati, N., Mubarik, N. R., & Desniar. (2015). Optimization of production of protease by *Lactobacillus plantarum* SK(5) from bekasam with response surface methodology. *Pakistan Journal of Biotechnology*, 12(2), 123–130. https://pjbt.org/index.php/pjbt/article/view/155/151

[KKP] Kementerian Kelautan dan Perikanan. (2023). Profil Pasar Rumput Laut. Direktorat Jenderal Penguatan Daya Saing Produk Kelautan dan Perikanan Kementerian Kelautan dan Perikanan Republik Indonesia. https://kkp.go.id/storage/Materi/profil-pasar-rumput-laut66753a465fe09/materi-66753a4696928.pdf

Li, M., He, Z., He, L., Li, C., Tao, H., Ye, C., Liu, L., Zeng, X., & Ran, G. (2022). Effect of fermentation parameters on the anthocyanin content, sensory properties, and physicochemical parameters of potato blueberry yogurt. *Fermentation*, 8(10), 489. https://doi.org/10.3390/fermentation8100489

Liu, C., Xue, W., Ding, H., An, C., Ma, S., & Liu, Y. (2022). Probiotic potential of *Lactobacillus* strains isolated from fermented vegetables in Shaanxi, China. *Frontiers in Microbiology*, 12. https://doi.org/10.3389/fmicb.2021.774903

Liu, Y.-W., Liong, M.-T., & Tsai, Y.-C. (2018). New perspectives of *Lactobacillus plantarum* as a probiotic: the gut-heart-brain axis. *Journal of Microbiology*, 56(9), 601–613. https://doi.org/10.1007/s12275-018-8079-2

Lumbessy, S. Y., Setyowati, D. N., Mukhlis, A., Lestari, D. P., & Azhar, F. (2020). Komposisi nutrisi dan kandungan pigmen fotosintesis tiga spesies alga merah (*Rhodophyta* sp.) hasil budidaya. *Journal of Marine Research*, 9(4), 431–438. https://doi.org/10.14710/jmr.v9i4.28688

Mahajan, K., Kumar, S., Bhat, Z. F., Singh, M., Bhat, H. F., Bhatti, M. A., & Bekhit, A. E.-D. A. (2022). *Aloe vera* and carrageenan based edible film improves storage stability of ice-cream. *Applied Food Research*, 2(1), 100128. https://doi.org/10.1016/j.afres.2022.100128

Maharany, F., Nurjanah, Suwandi, R., Anwar, E., & Hidayat, T. (2017). Kandungan senyawa bioaktif rumput laut *Padina australis* dan *Eucheuma cottonii* sebagai bahan baku krim tabir surya. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(1), 10–17.

Mahmoudi, I. (2022). Novel probiotic camel milk yoghurt supplemented with inulin: antibacterial, antioxidant and antidiabetic effects. *Mljekarstvo*, 72(4), 201–212. https://doi.org/10.15567/mljekarstvo.2022.0402

Maldonado Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E., & Perdigón, G. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition and Metabolism*, 74(2), 115–124. https://doi.org/10.1159/000496426

Mamat, H., Ling, Y. Y., Abdul Aziz, A. H., Wahab, N. A., Mohd Rosli, R. G., Sarjadi, M. S., Zainol, M. K., Putra, N. R., & Yunus, M. A. C. (2023). Utilization of seaweed composite flour (*Kappaphycus alvarezii*) in the development of steamed bun. *Journal of Applied Phycology*, 35(4),

1911–1919. https://doi.org/10.1007/s10811-023-02989-y

Mathew, S. S., Jaiswal, A. K., & Jaiswal, S. (2024). Carrageenan-based sustainable biomaterials for intelligent food packaging: a review. *Carbohydrate Polymers*, 342, 122267. https://doi.org/10.1016/j.carbpol.2024.122267

Meinita, M. D. N., Amron, A., Harwanto, D., Trianto, A., & Jeong, G.-T. (2022). The production of levulinic acid and formic acid from red macroalga *Kappaphycus alvarezii* using methanesulfonic acid. *Bioresource Technology Reports*, 17, 100954. https://doi.org/10.1016/j.biteb.2022.100954

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3), 426–428. https://doi.org/https://doi.org/10.1021/ac60147a030

Minj, J., & Vij, S. (2025). Determination of synbiotic mango fruit yogurt and its bioactive peptides for biofunctional properties. *Frontiers in Chemistry*, 12. https://doi.org/10.3389/fchem.2024.1470704

Mutmainnah, M., Desniar, D., & Santoso, J. (2023). Degradasi hidrotermal *Kappaphycus alvarezii*: karakter hidrolisat dan kapabilitas sebagai prebiotik. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 26(1), 13–24. https://doi.org/10.17844/jphpi.v26i1.43568

Nahdiyah, T. A., & Wikandari, P. R. (2022). The effect of fermentation time on the quality of probiotic products from jackfruit seed extract with *Lactobacillus plantarum* b1765 as the starter culture bacteria. *International Journal of Progressive Sciences and Technologies (IJPSAT)*, 33(1), 456–463.

Nelfa C. Gil, Jocelyn G. Daclag, & Allen Glen C. Gil. (2015). Growth pattern of lactic acid bacteria in probiotic rice washed water. *Journal of Science, Engineering and Technology (JSET)*, 3(1), 126–138. https://doi.org/10.61569/zxe9gg95

Nuñez, M., & Picon, A. (2017). Seaweeds in yogurt and quark supplementation: influence of five dehydrated edible seaweeds on sensory characteristics. *International Journal of Food Science and Technology*, 52(2), 431–438. https://doi.org/10.1111/ijfs.13298

Nurnaafi, A., Setyaningsih, I., & Desniar. (2015). Probiotic potential of bekasam lactic acid bacteria of tilapia fish. *Jurnal Teknologi Dan Industri Pangan*, 26(1), 109–114. https://doi.org/10.6066/jtip.2015.26.1.109

Oh, Y. J., Kim, H. J., Kim, T. S., Yeo, I. H., & Ji, G. E. (2019). Effects of *Lactobacillus plantarum* PMO 08 alone and combined with chia seeds on metabolic syndrome and parameters related to gut health in high-fat diet-induced obese mice. *Journal of Medicinal Food*, 22(12), 1199–1207. https://doi.org/10.1089/jmf.2018.4349

Orilda, R., Ibrahim, B., & Uju. (2021). Pengeringan rumput laut *Eucheuma cottonii* menggunakan oven dengan suhu yang berbeda. *Jurnal Perikanan Terpadu*, 2(2), 11–23.

Padam, B. S., Siew, C. K., & Chye, F. Y. (2023). Optimization of an innovative hydrothermal processing on prebiotic properties of *Eucheuma denticulatum*, a tropical red seaweed. *Applied Sciences*, 13(3), 1517. https://doi.org/10.3390/app13031517

Panjaitan, K. V., Suryono, S., & Pramesti, R. (2024). Pengaruh perbedaan suhu pengeringan terhadap kualitas kadar air dan kadar abu karagenan rumput laut *Kappaphycus alvarezii*. *Journal of Marine Research*, 13(2), 195–202. https://doi.org/10.14710/jmr.v13i2.40257

Parasthi, L. Y. E., Afifah, D. N., Nissa, C., & Panunggal, B. (2020). Total lactic acid bacteria and antibacterial activity in yoghurt with addition of *Ananas comosus* Merr. and *Cinnamomum burmannii*. *Amerta Nutrition*, 4(4), 257. https://doi.org/10.20473/amnt.v4i4.2020.257-264

Peerakietkhajorn, S., Worakit, W., Moukamnerd, C., & Tipbunjong, C. (2024). Proximate composition, phytochemical analysis and toxicity assessment of extracts of *Caulerpa lentillifera* using autoclave- and

microwave-assisted extractions. *Sains Malaysiana*, 53(2), 335–345. https://doi.org/10.17576/jsm-2024-5302-08

Peng, M., Tabashsum, Z., Anderson, M., Truong, A., Houser, A. K., Padilla, J., Akmel, A., Bhatti, J., Rahaman, S. O., & Biswas, D. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1908–1933. https://doi.org/10.1111/1541-4337.12565

Poespowati, T., & Mahmudi, A. (2018). Optimization of acid hydrolysis process on macro-alga *Ulva lactuca* for reducing sugar production as feedstock of bioethanol. *International Journal of Renewable Energy Research*, 8(1). https://doi.org/10.20508/ijrer.v8i1.7069.g7322

Puspitarini, O. R., & Susilowati, S. (2020). Aktivitas antioksidan, kadar protein, dan gula reduksi yoghurt susu kambing dengan penambahan sari apel manalagi (*Malus sylvestris*). *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, 22(2), 236. https://doi.org/10.25077/jpi.22.2.236-241.2020

Puttarat, N., Kasorn, A., Vitheejongjaroen, P., Chantarangkul, C., Tangwattanachuleeporn, M., & Taweechotipatr, M. (2023). Beneficial effects of indigenous probiotics in high-cholesterol diet-induced hypercholesterolemic rats. *Nutrients*, 15(12), 2710. https://doi.org/10.3390/nu15122710

Rahayu, H. M., & Qurbaniah, M. (2019). Selection of Tempoyak lactic acid bacteria as candidate strain for yoghurt starter culture. *Biosaintifika: Journal of Biology & Biology Education*, 11(1), 39–46. https://doi.org/10.15294/biosaintifika.v11i1.16769

Rahman, M. S., Emon, D. Das, Nupur, A. H., Mazumder, M. A. R., Iqbal, A., & Alim, M. A. (2024). Isolation and characterization of probiotic lactic acid bacteria from local yogurt and development of inulin-based synbiotic yogurt with the isolated bacteria. *Applied Food Research*, 4(2), 100457. https://doi.org/10.1016/j.afres.2024.100457

Rajaram, R., Muralisankar, T., Paray, B. A., & Al-Sadoon, M. K. (2021). Phytochemical profiling and antioxidant capacity of *Kappaphycus alvarezii* (Doty) Doty collected from seaweed farming sites of tropical coastal environment. *Aquaculture Research*, 52(7), 3438–3448. https://doi.org/10.1111/are.15188

Reuben, R. C., Roy, P. C., Sarkar, S. L., Rubayet Ul Alam, A. S. M., & Jahid, I. K. (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *Journal of Dairy Science*, 103(2), 1223–1237. https://doi.org/10.3168/jds.2019-17092

Riftyan, E., Rossi, E., Efendi, R., & Marcellina, Z. (2022). The producing of fermented milk as an application of alternative halal culture medium for the growth of *Lactobacillus plantarum* TMW 1.1623 and *Streptococcus thermophilus*. *IOP Conference Series: Earth and Environmental Science*, 1059. https://doi.org/10.1088/1755-1315/1059/1/012052

Rossi, E., Restuhadi, F., Efendi, R., & Dewi, Y. K. (2021). Physicochemical and microbiological properties of yogurt made with microencapsulation probiotic starter during cold storage. *Biodiversitas*, 22(4), 2012–2018. https://doi.org/10.13057/biodiv/d220450

Roy, S., Hussain, S. A., Prasad, W. G., & Khetra, Y. (2024). Effect of kappa-carrageenan inclusion in the stabilizer blend on rheology, texture, and physical properties of high protein ice cream. *International Dairy Journal*, 159, 106052. https://doi.org/10.1016/j.idairyj.2024.106052

Rudke, A. R., de Andrade, C. J., & Ferreira, S. R. S. (2020). *Kappaphycus alvarezii* macroalgae: an unexplored and valuable biomass for green biorefinery conversion. *Trends in Food Science and Technology*, 103, 214–224. https://doi.org/10.1016/j.tifs.2020.07.018

Safia, W., Budiyanti, & Musrif. (2020). Kandungan nutrisi dan bioaktif rumput laut (*Euchema cottonii*) dengan metode

rakit gantung pada kedalaman berbeda. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 23(2), 261–271. https://doi.org/10.17844/jphpi.v23i2.29460

Santoso, J., Yoshie-Stark, Y., & Suzuki, T. (2006). Comparative contents of minerals and dietary fibers in several tropical seaweeds. *Buletin Teknologi Hasil Perikanan*, IX(1).

Saryono, S., Ismawati, I., Pratiwi, N. W., Devi, S., Sipayung, M. Y., & Suraya, N. (2023). Isolation and identification of lactic acid bacteria from traditional food sarobuong of Kuantan Singingi District, Riau, Indonesia. *Biodiversitas Journal of Biological Diversity*, *24*(4). https://doi.org/10.13057/biodiv/d240432

Sasue, A., Mohd Kasim, Z., & Zubairi, S. I. (2023). Evaluation of phytochemical, nutritional and sensory properties of high fibre bun developed by utilization of *Kappaphycus alvarezii* seaweed powder as a functional ingredient. *Arabian Journal of Chemistry*, 16(8), 104953. https://doi.org/10.1016/j.arabjc.2023.104953

Seo, C. W., & Oh, N. S. (2022). Functional application of Maillard conjugate derived from a κ-carrageenan/milk protein isolate mixture as a stabilizer in ice cream. *LWT-Food Science and Technology*, 161, 113406. https://doi.org/10.1016/j.lwt.2022.113406

Setiadi, M. K., & Husni, A. (2024). Aktivitas antioksidan dan tingkat penerimaan konsumen yoghurt yang diperkaya rumput laut *Caulerpa lentillifera*. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 27(5), 417–430. https://doi.org/10.17844/jphpi.v27i5.53538

Setiarto, R. H. B., Widhyastuti, N., & Risty, A. R. (2022). The effect of variation concentration white oyster mushroom flour for quality yogurt mushroom taro synbiotic during storage. *IOP Conference Series: Earth and Environmental Science*, 978(1), 012048. https://doi.org/10.1088/1755-1315/978/1/012048

Setiawan, A. L. T. P., & Agustini, R. (2024). Effect of the fermentation time of purple sweet potato (*Ipomea batatas* L) synbiotic yoghurt with probiotic starter on product quality and antioxidant activity. *Jurnal Pijar Mipa*, 19(5), 870–874. https://doi.org/10.29303/jpm.v19i5.7297

Setyaningsih, D., Musdaniaty, D., & Muna, N. (2019). Produksi bubuk sinbiotik dari hidrolisat *Eucheuma cottonii* menggunakan spray drying. *Jurnal Teknologi Industri Pertanian*, 29(3), 233–239. https://doi.org/http://dx.doi.org/10.24961/j.tek.ind.pert.2017.27.1.1

Shalaby M, S., & Amin H, H. (2019). Potential using of *Ulvan* polysaccharide from Ulva lactuca as a prebiotic in synbiotic yogurt production. *Journal of Probiotics and Health*, 7(1). https://doi.org/10.35248/2329-8901.7.1.208

Shori, A. B., Aljohani, G. S., Al-zahrani, A. J., Al-sulbi, O. S., & Baba, A. S. (2022). Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic *Lactobacillus* spp. *LWT-Food Science and Technology*, *153*, 112482. https://doi.org/10.1016/j.lwt.2021.112482

Sieuwerts, S. (2016). Microbial interactions in the yoghurt consortium: current status and product implications. *SOJ Microbiology and Infectious Diseases*, 4(2), 01–05. https://doi.org/10.15226/sojmid/4/2/00150

Simatupang, N. F., Pong-Masak, P. R., Ratnawati, P., Agusman, Paul, N. A., & Rimmer, M. A. (2021). Growth and product quality of the seaweed *Kappaphycus alvarezii* from different farming locations in Indonesia. *Aquaculture Reports*, 20, 100685. https://doi.org/10.1016/j.aqrep.2021.100685

Skryplonek, K., Henriques, M., Gomes, D., Viegas, J., Fonseca, C., Pereira, C., Dmytrów, I., & Mituniewicz-Małek, A. (2019). Characteristics of lactose-free frozen yogurt with κ-carrageenan and corn starch as stabilizers. *Journal of Dairy Science*, 102(9), 7838–7848. https://doi.org/10.3168/jds.2019-16556

Sofyan, A., Ikhsani, A. Y., Purwani, E., Hasanah, L. E. N., & Febriyadin, F. (2022). The effect of suweg (*Amorphophallus paeoniifolius*) flour and incubation

temperature on characteristics of yogurt with the addition of *Bifidobacterium bifidum* as probiotic. *Materials Today: Proceedings*, 63, S507–S512. https://doi.org/10.1016/j.matpr.2022.04.538

Soraya, M., Laksono, H., Putri, R. P. G., Royanti, I., Perwatasari, D. D., Dewi, R. A. ., & Purwoto, H. (2025). Exploring disintegration and swelling dynamics in *kappa*-carrageenan-based seaweed capsule shells. *South African Journal of Chemical Engineering*, 53, 96–102. https://doi.org/10.1016/j.sajce.2025.04.015

Steinbruch, E., Drabik, D., Epstein, M., Ghosh, S., Prabhu, M. S., Gozin, M., Kribus, A., & Golberg, A. (2020). Hydrothermal processing of a green seaweed *Ulva* sp. for the production of monosaccharides, polyhydroxyalkanoates, and hydrochar. *Bioresource Technology*, 318, 124263. https://doi.org/10.1016/j.biortech.2020.124263

Sudibyo, M. F., Santoso, J., & Desniar, D. (2024). Hydrolysis of polysaccharide *Caulerpa racemosa* seaweed with fermentation *Lactobacillus plantarum* SK (5). *Jurnal Perikanan Universitas Gadjah Mada*, 26(1). https://doi.org/10.22146/jfs.91045

Sulistiana, Z., Ridlo, A., & Sedjati, S. (2024). Karakteristik biodegradable film refined carrageenan dari *Kappaphycus alvarezii* dengan pemlastis gliserol. *Journal of Marine Research*, 13(3), 493–501. https://doi.org/10.14710/jmr.v13i3.39209

Susmiati, S., Melia, S., Purwati, E., & Alzahra, H. (2022). Physicochemical and microbiological fermented buffalo milk produced by probiotic *Lactiplantibacillus pentosus* HBUAS53657 and sweet orange juice (*Citrus nobilis*). *Biodiversitas Journal of Biological Diversity*, 23(8). https://doi.org/10.13057/biodiv/d230858

Suwati, S., Romansyah, E., Syarifudin, S., Jani, Y., Purnomo, A. H., Damat, D., & Yandri, E. (2021). Comparison between natural and cabinet drying on weight loss of seaweed *Euchuema cottonii* weber-van bosse. *Sarhad Journal of Agriculture*, 37(s1). https://doi.org/10.17582/journal.sja/2021/37.s1.01.08

Swanson, K. S., Gibson, G. R., Hutkins, R., Reimer, R. A., Reid, G., Verbeke, K., Scott, K. P., Holscher, H. D., Azad, M. B., Delzenne, N. M., & Sanders, M. E. (2020). The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nature Reviews Gastroenterology and Hepatology*, 17(11), 687–701. https://doi.org/10.1038/s41575-020-0344-2

Syafiqoh, N. (2016). Aktivitas antioksidan dan efek antidiabetes probiotik *Lactobacillus plantarum* SK(5) asal bekasam [Tesis]. Institut Pertanian Bogor.

Syaharuddin. (2019). Optimization of extraction and quality assessment based on physicochemical properties of *Carrageenan* from red algae (*Kappaphycus alvarezii*) origin of South Sulawesi Indonesia. *Journal of Physics: Conference Series*, 1341(7), 072013. https://doi.org/10.1088/1742-6596/1341/7/072013

Tahmasebi, M., & Mofid, V. (2021). Innovative synbiotic fat-free yogurts enriched with bioactive extracts of the red macroalgae *Laurencia caspica*: formulation optimization, probiotic viability, and critical quality characteristics. *Journal of Food Measurement and Characterization*, 15(6), 4876–4887. https://doi.org/10.1007/s11694-021-01061-y

Tarman, K., Supinah, P., Dewanti, E. W., Santoso, J., & Nurjanah, N. (2024). Characteristics of carrageenan from seaweed hydrolysis using marine fungi as hard-shell capsule material. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 27(8), 642–653. https://doi.org/10.17844/jphpi.v27i8.51946

Thiennimitr, P., Yasom, S., Tunapong, W., Chunchai, T., Wanchai, K., Pongchaidecha, A., Lungkaphin, A., Sirilun, S., Chaiyasut, C., Chattipakorn, N., & Chattipakorn, S. C. (2018). *Lactobacillus paracasei* HII01, xylooligosaccharides, and synbiotics reduce gut disturbance in obese rats. *Nutrition*, 54, 40–47. https://doi.org/10.1016/j.nut.2018.03.005

Todorov, S. D., & Franco, B. D. G. D. M.

(2010). *Lactobacillus Plantarum* : characterization of the species and application in food production. *Food Reviews International*, 26(3), 205–229. https://doi.org/10.1080/87559129.2010.484113

Wang, Y., Wang, Y., Huang, X., Guo, S., Jin, J., & Qi, H. (2025). Unlocking the potential of seaweed in functional yogurt: a comparative study on the impact of *Undaria pinnatifida* and *Saccharina japonica* on quality, flavor, and microbial communities. *Food Bioscience*, 68, 106627. https://doi.org/10.1016/j.fbio.2025.106627

Wanyonyi, S., Du Preez, R., Brown, L., Paul, N., & Panchal, S. (2017). *Kappaphycus alvarezii* as a food supplement prevents diet-induced metabolic syndrome in rats. *Nutrients*, 9(11), 1261. https://doi.org/10.3390/nu9111261

Wu, J., Lin, Y., Zeng, Z., Wang, L., Zhang, F., Huang, H., Zheng, B., Zhang, Y., & Pan, L. (2025). Steam-exploded *Laminaria japonica* polysaccharide improved set yogurt quality: based on flavor, storage stability and dynamic in vitro digestive properties. *Innovative Food Science & Emerging Technologies*, 100, 103933. https://doi.org/10.1016/j.ifset.2025.103933

Wu, X., Zhou, J., Liu, Z., Liu, J., He, S., & Shao, W. (2023). Constructing a biodegradable carrageenan based food packaging film according to the synergistic strategies between peppermint essential oil and thymol. *International Journal of Biological Macromolecules*, 253, 127537. https://doi.org/10.1016/j.ijbiomac.2023.127537

Yadav, M., Sehrawat, N., Sharma, A. K., Kumar, S., Singh, R., Kumar, A., & Kumar, A. (2024). Synbiotics as potent functional food: recent updates on therapeutic potential and mechanistic insight. *Journal of Food Science and Technology*, 61(1), 1–15. https://doi.org/10.1007/s13197-022-05621-y

Yanuarti, R., Nurjanah, N., Anwar, E., & Pratama, G. (2017). Kandungan senyawa penangkal sinar ultra violet dari ekstrak rumput laut *Eucheuma cottonii* dan *Turbinaria conoides*. *Majalah Ilmiah Biologi Biosfera: A Scientific Journal*, 34(2), 51. https://doi.org/10.20884/1.mib.2017.34.2.467

Yao, Z., Wu, H., Zhang, S., & Du, Y. (2014). Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity. *Carbohydrate Polymers*, 101, 359–367. https://doi.org/10.1016/j.carbpol.2013.09.055

Yulianti, E., Sunarti, & Wahyuningsih, M. S. H. (2022). The effect of *Kappaphycus alvarezii* active fraction on oxidative stress and inflammation in streptozotocin and nicotinamide-induced diabetic rats. *BMC Complementary Medicine and Therapies*, 22(1), 15. https://doi.org/10.1186/s12906-021-03496-8

Zhang, H., Goff, H. D., Liu, C., Luo, S., & Hu, X. (2025). Blending pectin and κ-carrageenan converted the liquid yogurt induced by pectin into the solid yogurt. *Carbohydrate Polymers*, 348, 122869. https://doi.org/10.1016/j.carbpol.2024.122869