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Research Article

Enhancing Jackfruit's Bioactive Properties Through SCOBY Fermentation: Implications For Cosmeceuticals

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ABSTRACT

This study investigated the bio-processing technique of fermenting jackfruit pulp (JP) and leaves (JL) using a symbiotic culture of bacteria and yeast (SCOBY) to enhance their bioactive properties. To assess the nutritional value of the jackfruit extracts, the phenolic and organic acid compositions were determined. The extracts were also evaluated for their anti-inflammatory properties by measuring their ability to suppress the production of nitric oxide (NO) in response to bacterial lipopolysaccharide (LPS) stimulation in RAW 264.7 macrophage cell line. Furthermore, the inhibitory effects of the extracts on elastase and tyrosinase, enzymes associated with skin aging, were assessed. The fermentation process led to increased phenolic content. This included vitexin, salicylic acid, and benzoic acid. Acetic acid was the most abundant organic acid detected after fermentation, with concentrations ranging from 16.0 to 16.1 mg/mL. Additionally, the fermented extracts exhibited elevated levels of other beneficial organic acids such as citric and quinic acid. The study demonstrated significant reductions in nitrite formation in LPS-stimulated RAW 264.7 cells treated with jackfruit extracts. This finding suggests that the fermented extracts can effectively suppress NO production in a concentration-dependent manner. Among the fermented extracts, JL exhibited the highest inhibition of NO production at a concentration of 19.5%, resulting in a 42.23% reduction. Moreover, fermentation enhanced the inhibitory effects of the jackfruit extracts on elastase and tyrosinase, with inhibition rates ranging from 82.3% to 95.4%. Overall, the findings suggest that fermented jackfruit exhibits increased levels of phytochemical compounds and holds promise as a natural and beneficial ingredient in cosmeceutical products, offering anti-inflammatory and skin-aging benefits.

Key words: Anti-inflammatory, fermentation, jackfruit, phytochemical, SCOBY

INTRODUCTION

Jackfruit is a non-seasonal fruit in Malaysia known by the botanical name *Artocarpus heterophyllus* and belongs to the *Moraceae* family. In Malaysia, approximately 4674 hectares of jackfruit were farmed, with an annual production of 35 624 metric tonnes. The most commonly grown kinds were J32 (Mantin), J33 (Tekam Yellow), and J37 (Mastura) (Yacob, 2022). Jackfruit's resilience to climate change has made it popular. Rising temperatures and irregular rainfall threaten major crops, but jackfruit thrives in tropical and subtropical regions. It is a nutritious and calorie-dense food option, with high fiber, antioxidants, and beneficial compounds. Recent studies have shown that its phenolics, flavonoids, sterols, and prenylflavonoids may offer various health benefits, like treating diabetes, inflammation, wounds, and fungal infections (Ong *et al*., 2006; Arung *et al*., 2007; Jagtap *et al*., 2010; Baliga *et al*., 2011; Biworo *et al*., 2015; Swami & Kalse, 2019).

The potential of jackfruit waste, especially its leaves, in product development has gained attention. These leaves have been commonly used in traditional folk medicine to treat various conditions like asthma, diarrhea, and dermatitis (Vázquez-González *et al*., 2020). The leaves can be processed to extract bioactive components that can then be added to food compositions to boost nutritional and health benefits. The nutritional, vitamin, and phytochemical content of jackfruit leaves, which includes carbs, proteins, minerals, and fiber, may contribute to their beneficial effects. Furthermore, preclinical and clinical research demonstrates that jackfruit leaves have wound healing and anti-inflammatory properties. This research has shown that jackfruit leaves have antioxidant, antibacterial, antifungal, and anti-inflammatory characteristics, which make them useful for wound healing and inflammation control. The leaves include phytochemical compounds such as flavonoids, sterols, and prenylflavones (Swami & Kalse, 2019; Banerjee *et al*., 2022). These compounds contribute to the health-promoting characteristics of jackfruit leaves and provide several advantages, including anti-cancer and anti-diabetic properties, as proven in earlier studies (Omar *et al*., 2011; Thapa *et al*., 2016; Wang *et al*., 2017). Since there hasn't been enough research conducted regarding producing products from jackfruit leaves, a lot of them end up being wasted and disposed of as agricultural waste.

The demand for cosmetic products made from natural ingredients has surged in recent years. Manufacturers are now prioritizing bio-based components derived from plants and microorganisms, employing advanced extraction techniques to create products with beneficial attributes such as antioxidant, anti-inflammatory, and anti-aging properties. These products are particularly appealing for skincare, as they promote skin health without the adverse effects associated with synthetic chemicals

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(Liu, 2022). According to projections from Future Market Insights (FMI), the global market for natural cosmetics is expected to reach $79.6 billion in revenue by 2033, growing at a compound annual growth rate (CAGR) of 5.1% from 2023 to 2033 (Sudip, 2022)

Fermentation is a natural process that uses microorganisms to improve the nutritional content of foods for better absorption. A key benefit of microbial fermentation is its ability to enhance the nutritional value of foods. Fermentation with a symbiotic culture of bacteria and yeast, known as SCOBY, involves prominent acetic acid bacteria genera like *Acetobacter, Gluconacetobacter, Gluconobacter*, and *Komagataiebacter* along with yeast groups such as Schizosaccharomyces, Saccharomyces, and *Brettanomyces*/*Dekkera* (Kruk *et al*., 2021). The SCOBY fermentation process has several advantages, including the creation of probiotics, which are beneficial bacteria that can support a balanced gut microbiota and may aid in digestion, immunity, and overall well-being. Certain types of fermentation, including SCOBY, have been shown to boost antioxidant profiles that aid in the battle against oxidative stress in the body, which can lead to a range of health issues. Several research has also been conducted to understand the influence of SCOBY fermentation and reveal that the biological activities, including antimicrobial, anti-inflammatory, anti-diabetic, and anti-carcinogenic potential, have been improved (Laavanya *et al*., 2021; de Miranda *et al*., 2022).

Aside from food, the fermentation process can provide beneficial ingredients for the cosmetics industry. The SCOBY fermentation has also been proven to successfully hinder the synthesis of anti-aging skin enzymes. Ferments derived from green coffee beans and Yerba Mate after various fermentation periods with SCOBY strains showed increased inhibition of collagenase and elastase enzymes, indicating possible anti-aging capabilities (Zofia *et al*., 2020; Ziemlewska *et al*., 2021). Furthermore, fermented agricultural by-products, including rice bran, soybean cake, and sesame seed cake, have demonstrated significant inhibition of matrix metalloproteinases (MMPs) and reduction in wrinkle formation, indicating their potential anti-photoaging properties (Abd Razak *et al*., 2017; Choi *et al*., 2019). Studies have indicated that SCOBY fermentation can result in the formation of bioactive compounds, such as polyphenols and organic acids. This process also enhances the availability of antioxidants, which contributes to its anti-inflammatory properties (Villarreal-Soto *et al*., 2018). In a study by Jakubczyk *et al*. (2020), fermenting green tea with SCOBY showed a significant increase in levels of anti-inflammatory compounds, particularly flavonoids. Similarly, de Noronha *et al*. (2022) researched black tea and found similar results, suggesting SCOBY fermentation's potential to enhance the anti-inflammatory properties of various tea varieties. SCOBY fermentation extends beyond tea-based beverages and has been explored in the production of other functional foods with potential anti-inflammatory effects. For example, fermenting fruits and vegetables with SCOBY generates various bioactive compounds with anti-inflammatory potential (Anantachoke *et al*., 2023; Sornkayasit *et al*., 2024). These findings highlight the promising role of SCOBY fermentation in developing functional foods with enhanced anti-inflammatory properties.

The Malaysian Agricultural Research and Development Institute (MARDI) has come up with a method for fermenting jackfruit extract using a controlled SCOBY fermentation process. This newly developed process has proven to be beneficial, as previous research has demonstrated that the extract possesses remarkable antioxidant, anti-bacteria, and along with the ability to combat diabetes (Aziz *et al*., 2017; Aziz *et al*., 2018; Koh *et al*., 2020). This research aimed to examine how fermentation influences the bioactive properties of jackfruit in cosmetic attributes. Specifically, it investigated the fermented extract's potential to alleviate inflammation, and its ability to inhibit enzymes linked to skin aging, and compared the phenolic and organic acid compositions of fermented extracts with those of non-fermented extracts.

MATERIALS AND METHODS

Preparation of fermented jackfruit extracts

The jackfruit (*Artocarpus heterophyllus* L.) cultivar Tekam Yellow was derived from a local jackfruit plantation in Lanchang, Pahang. The matured jackfruit pulp was separated from the seeds before being chopped into smaller pieces and oven-dried at 40°C. The leaves were properly washed to eliminate dirt before drying in a vented oven at 50°C for 24 hr. The dried leaves were milled to a particle size of 0.5 mm (Retsch ZM200, Germany) and stored vacuum-packed at 4°C. The jackfruit pulp and leaves were used as the foundation for preparing a 5% suspension, which was then inoculated with a mixture of SCOBY strains at a concentration of 10^8/mL. These SCOBY strains, sourced from MARDI's Collection of Functional Food Cultures, comprised *Dekkera* sp. and *Komagataiebacter* sp., added to the suspension at a 4:1 ratio. Fermentation occurred at 37°C for 8 days with continuous agitation at 200 rpm. After the fermentation period, residual biomass was separated from the supernatant through centrifugation at 10,000 rpm for 5 min. The resulting liquid underwent pasteurization at 90°C for 30 min and was stored under chilled conditions for further analysis (Aziz *et al*., 2017). The non-fermented samples were prepared using the same method but without inoculation. These extracts were labeled as JP and JL, signifying their origin from jackfruit pulp and leaves, respectively.

Determination of Phenolic compounds using UPLC

The extract's phenolic compound content was separated using an Acquity Ultra Performance Liquid Chromatography (UPLC) system (Waters, Milford, MA, USA) with a flow rate of 0.4 ml/min. Kinetex C18 100 A column (100 mm × 2.1 mm; 1.7µm) was employed, and the oven temperature was maintained at 30°C. The UV spectrum of 280, 330, and 360 nm was utilized. Mobile phase A (acetic acid: water = 3:97) and mobile phase B (methanol) were employed for gradient elution. The gradient elution program consisted of the following steps: 0-1 min at 100% A, 1-10 min at 100% A, 10-12 min at 40% A, and 12-14 min at 100% A. The quantification was conducted by generating calibration curves using known quantities of targeted phenolics as external standards with known retention times under the specified UV spectrum (Koh *et al*., 2020).

Determination of organic acid using HPLC

The organic acids profiles of fermented jackfruit pulp and leaf extracts were analyzed using a High-Performance Liquid Chromatography (HPLC), Alliance Separation Module (Waters, 2695) coupled with a diode array detector (Waters, 2996). The

running parameters and column used were conducted as previously described by Koh *et al*. (2019). The concentration of each organic acid was determined using a calibration curve established by injecting a known amount of organic acid as an external standard. All analyses were conducted in triplicate.

Cell culture

RAW 264.7 murine macrophage cell lines were obtained from Elabscience (CL-0190) and used in the cytotoxicity evaluation. The cells were grown in DMEM (Dulbecco's Modified Eagle Medium, Nacalai Tesque) with added l-glutamine, 4.5 g/L-glucose, and sodium pyruvate. The culture media was supplemented with 10% (v/v) fetal bovine serum (FBS, Tico Europe) and 1% (v/v) antibiotics (100 U/mL penicillin and 1000 µg/mL streptomycin, Sigma Aldrich) for optimal cell growth. The cells were incubated at 37°C in a humidified environment with 95-98% air and 5% carbon dioxide (CO_2) until they achieved 90-95% confluence. (Ryu *et al*., 2003). Subsequently, the cells were detached using cell scrapper and then seeded onto plates for the assay (Taciak *et al*., 2018).

Determination of cell viability

3-(4,5-dimethylthiazol-2yl)-2,5-dimethyltetrazolium bromide (MTT) assay was used to measure cell viability based on the method described by Shao *et al*. (2015) with some modifications. RAW 264.7 cells ($5x10^4$ cells/well) were seeded in a 96-well plate overnight at 37°C until 90% confluent. The cells were treated with JP and JL extracts in a series of two-fold dilutions using DMEM (without serum) at concentrations ranging from 0.781% to 100%, followed by incubation for 24 hr. Treatments were aspirated after incubation, and 20 µL of MTT solution (5mg/mL) was added to all wells, followed by a 4-hr incubation at 37°C in the presence of 5% CO_2. After discarding the MTT solution, DMSO was added to extract the insoluble formazan. Untreated cells were used as a control. Subsequently, the non-cytotoxic concentration with at least 50% cell viability (CC_{50}) was further selected for the anti-inflammatory assay using LPS-induced RAW 264.7 cells. The percentage of cell viability was calculated based on Equation 1 using the absorbance values obtained at 570 nm.

Equation 1:

$$Cell\ viability\ (\%) = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

Determination of nitric oxide (NO) production

The NO production was assessed by measuring the nitrite levels in the culture media using the Griess reagent assay (Liu *et al*., 2010). In a 96-well plate, RAW 264.7 cells were seeded overnight at a density of 5 x 10^4 cells per well. Then, the cells were induced with 5 µg/mL LPS followed by treatment with different concentrations of jackfruit extracts. The treated cells were incubated for 24 hr at 37°C at 5% CO_2 atmosphere. The inhibitory effect of the tested extracts on NO production was measured by Griess reagent (0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride in distilled water and 1% sulphanilamide in 5% phosphoric acid). After 24 hr of incubation, 80 µL of media from the plate was transferred carefully into a new 96-well plate whereby an equal volume of Griess reagent was added into each well, and the plate was left in dark condition for 10 min. In this assay, sodium nitrite ($NaNO_2$) with different concentrations (0-100 µM) was used as a standard control. The absorbance was then measured using a microplate reader at 540 nm.

Determination of elastase inhibition

A modified version of the procedure outlined by Liyanaarachchi *et al*. (2018) was used to evaluate the inhibitory action of fermented extracts on elastase. The enzyme utilized was porcine pancreatic elastase (PPE), with oleanolic acid serving as the standard and epigallocatechin-3-gallate (EGCG) serving as the positive control. After mixing 50 µL of the sample with 50 µL of elastase in 0.2 mM Tris HCl buffer (pH 8), the mixture was incubated for 10 min at 25°C. Subsequently, 50 µL of N-Succinyl-Ala-Ala-Ala-p-Nitroanilide (AAAPVN) solution was introduced, and the mixture was allowed to incubate for 30 min at 25°C in the dark. Next, using the spectrophotometer (Varian Cary, USA), the absorbance at 410 nm was determined.

Determination of tyrosinase inhibition

To measure the tyrosinase activity inhibition of jackfruit extracts, a modified version of the method described by Suganya *et al*. (2015) was used. Tyrosinase served as the enzyme, while L-DOPA was employed as the substrate. In a 96-well microtiter plate, 40 µL of 5 mM L-DOPA was mixed with 100 µL of 0.1 M phosphate buffer at pH 6.8. The mixture was thoroughly combined and incubated at 37°C for 10 min. Subsequently, 120 µL of jackfruit extract and 40 µL of tyrosine (62 U/mL in 0.1 M phosphate buffer at pH 6.8) were added and incubated at 37°C for 10 min before measuring the absorbance at 475 nm using the spectrophotometer. The elastase and tyrosinase inhibitory activities were measured and expressed as a percentage of inhibition using Equatio 2:

$$Enzyme\ inhibitaion\ activity\ (\%) = \frac{Abs_{control} - (Abs_{sample} - Abs_{blank})}{Abs_{control}} \times 100$$

Table 1. Phenolics and organic acid content of fermented and non-fermented jackfruit pulp (JP) and leaves (JL). Values are means ± standard deviation (SD) of triplicate experiments.

	Concentration (µg/mL)			
	JL		JP	
	Non -fermented	Fermented	Non- fermented	Fermented
Phenolics				
4 -Hydroxybenzoic acid	4.95 ± 0.25	2.99 ± 0.18	-	1.36 ± 0.13
Ferulic acid	0.94 ± 0.06	3.03 ± 1.03	-	-
Benzoic acid	28.59 ± 1.44	23.04 ± 1.18	-	0.07 ± 0.03
Salicylic acid	60.68 ± 0.88	62.72 ± 0.68	-	0.52 ± 0.06
Vitexin	25.11 ± 1.26	91.02 ± 2.45	-	-
Catechin	15.30 ± 1.94	9.13 ± 0.68	11.07 ± 0.12	7.09 ± 0.61
Organic acids				
Citric acid	222.50 ± 6.73	288.05 ± 21.20	757.96 ±7.00	871.63 ± 14.04
Malic acid	216.51 ± 12.73	79.58 ± 3.29	355.76 ± 25.03	84.92 ± 8.24
Quinic acid	455.60 ± 19.83	404.22 ± 22.43	73.22 ± 5.18	201.21 ± 6.29
Oxalic acid	18.91 ± 2.00	17.38 ± 1.23	15.02 ± 2.05	17.33 ± 0.74
Acetic acid	-	16118.80 ± 169.00	-	16012.58 ± 3576.00

Statistical analysis

The results from the triplicate experiment were analyzed using the MINITAB Version 17 software. A one-way analysis of variance (ANOVA) was performed, utilizing Tukey's test as the significance threshold ($p<0.05$). The data were presented in the form of a mean ± standard deviation.

RESULTS AND DISCUSSION

Phenolic acid analysis

Phenolic compounds, which are secondary metabolites present in plants, possess significant biological functionalities. Within plants, these compounds play a crucial role in enhancing resistance against microbial pathogens and act as inhibitors or toxic agents against harmful nematodes, insects, and herbivores The antioxidative attributes of phenolic compounds, including their ability to scavenge free radicals, contribute to the prevention of chronic ailments and oxidative stress-induced disorders such as cancer, cardiovascular diseases, and neurodegenerative conditions. Moreover, phenolics exhibit various other biological effects associated with their antioxidative capacity, such as antimicrobial, anti-inflammatory, anticancer, and cardioprotective activities (Cosme *et al*., 2020).

Phenolic compounds can change concentration and composition when subjected to fermentation, as they are susceptible to breakdown by yeast and bacteria. The effects of fermentation on phytochemicals are contingent upon several factors, including the specific type of phytochemical, the presence of fermenting microorganisms, and the conditions under which fermentation takes place (Leonard *et al*., 2021). It is important to note that fermentation can induce both increments and decrements in phytochemical concentrations, while simultaneously giving rise to the formation of new phytochemical compounds (*Villarreal-Soto et al*., 2019). The phenolics and organic acids content of fermented and non-fermented jackfruit extracts (JP & JL) are summarised in Table 1.

The analysis of the results indicated that the fermentation of JL led to the enhancement of certain phenolic acids, including vitexin, ferulic acid, and salicylic acid. Particularly, a significant increase in vitexin content (91.02 µg/mL) was observed following the fermentation process in comparison to the non-fermented JL (25.11 µg/mL). Vitexin, a well-known flavonoid compound that has been extensively investigated in scientific studies, is recognized for its diverse biological benefits. Previous research has already identified the presence of 2″-O-β-D-xylosylvitexin in jackfruit leaves, exhibiting exceptional cellular antioxidant activity as evidenced by its high ORAC values. Furthermore, the study unveiled that the hydroxyl radical scavenging activity of 2″-O-β-D-xylosylvitexin is concentration-dependent, comparable to that of quercetin and Trolox (Wen *et al*., 2017). In the field of neuroprotection, vitexin has garnered attention due to its antioxidative properties. It can directly scavenge reactive oxygen species (ROS) or enhance the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and augment the activities of antioxidant enzymes. Furthermore, it activates survival-promoting signaling pathways like ERK1/1 and phosphatidylinositol-3 kinase/Akt (PI3K/Akt), resulting in the release of anti-apoptotic proteins and the prevention of protein misfolding and aggregation (Duan *et al*., 2020). Additionally, a study by Jin *et al*. (2005) demonstrated that vitexin, derived from *Acer palmatum* leaves, can inhibit up to 70% of superoxide radicals and 60% of DPPH free radicals. These findings also suggest that vitexin can be effectively employed for preventing UV-induced adverse skin reactions by reducing free radical production and protecting skin cells from damage. Considering the various favorable impacts of vitexin, it is worth highlighting that the utilization of the SCOBY fermentation method in this research amplifies the vitexin concentration in jackfruit leaves.

Ferulic acid and salicylic acid are both bioactive compounds that have been extensively studied for their potential benefits in various applications. In the present study, it was observed that, in addition to vitexin, both phenolics showed an increase following fermentation. Ferulic acid, a common phenolic acid found in plants, possesses remarkable antioxidant properties. It

serves as a precursor to essential chemical compounds and contributes to the structural integrity of cell walls. Its antioxidant attributes enable it to protect cells against oxidative stress and damage (Srinivasan *et al*., 2007). Studies by Pluemsamran *et al*. (2012) have highlighted that ferulic acid exhibits protective effects against damage induced by ultraviolet A. This type of damage can trigger matrix metalloprotease-1, a crucial enzyme involved in collagen degradation. The protective mechanism of ferulic acid operates through the restoration of the cell's antioxidant defense mechanisms at both cellular and molecular levels. Salicylic acid, on the other hand, exerts anti-inflammatory effects by suppressing the transcription of genes responsible for cyclooxygenase, the enzyme involved in prostaglandin synthesis. Furthermore, salicylic acid acts as an inhibitor of nuclear factor kappa B (NF-κB), a crucial mechanism responsible for its anti-inflammatory properties (Choi *et al*., 2015). Aside from its anti-inflammatory properties, salicylic acid has antioxidative action and is well known for its capacity to prevent oxidative stress. Notably, salicylic acid can bind to iron, which is especially significant for its antioxidative properties, as iron plays a role in lipid peroxidation (Lapenna *et al*., 2009).

Typically, fermentation is known to enhance the phenolic content and antioxidant capacity of various substrates. However, it is worth noting that in certain cases, fermentation can lead to a decline in phenolic levels. In the current study, the fermentation of JL resulted in a reduction in specific phenolic compounds, namely 4-hydroxybenzoic acid, benzoic acid, and catechin. Previous studies have demonstrated that catechin is sensitive to acidic conditions but remains stable within the pH range of 5.0-8.0, coinciding with the pH values of the human stomach and intestine (Zhu *et al*., 1997). Consistent with these findings, our study discovered that the fermented JP and JL extracts exhibited higher acidity, with a pH below 4.0 after the fermentation process (Aziz *et al*., 2017). It is noteworthy that these results are in agreement with the findings reported by Tu *et al*. (2005) and Xie *et al*. (2009) where a decrease in catechin levels during tea fermentation was observed.

HPLC analysis was employed to determine the quantity and presence of primary organic acids in JP and JL, as outlined in Table 1. Both samples exhibited the presence of various organic acids, including citric acid, malic acid, quinic acid, acetic acid, and oxalic acid. Notably, acetic acid emerged as the most abundant organic acid in the JL and JP extracts following fermentation in this study with concentrations of 16118.80 µg/mL and 16012.58 µg/mL respectively. The significant increase in acetic acid concentration can be attributed to the activity of acetic acid bacteria, indicating the adaptability and efficient growth of this SCOBY strain on these substrates. In addition, acetic acid bacteria play a crucial role in the conversion of alcohol to acetic acid, imparting the distinct tangy flavor observed in fermented products such as vinegar. In contrast, non-fermented JL or JP contained no acetic acid.

The high content of acetic acid in the JP and JL extracts can provide different health benefits due to its antibacterial and anti-inflammatory capabilities. For example, the acetic acid present in vinegar has been shown to possess antimicrobial effects, effectively inhibiting the growth of specific pathogens. Its antimicrobial properties extend to various pathogens, encompassing both Gram-positive and Gram-negative bacteria. The wide-ranging antimicrobial activity of acetic acid makes it an invaluable constituent of fermented products contributing to their preservation, safety, and overall microbiological quality (Nagoba *et al*., 2013). These inherent properties of acetic acid render it a valuable component in fermented extracts, thereby enhancing their potential for health promotion.

During fermentation, various organic acids, including citric, malic, and quinic acid, increased alongside acetic acid. Among these organic acids, citric acid stands out as an excellent agent for wound care due to its antibacterial properties. As a natural substance, citric acid is safe to use in treating wound infections caused by Pseudomonas aeruginosa without any negative effects (Nagoba *et al*., 2017). Additionally, the combination of citric acid and zinc oxide has demonstrated a synergistic effect in the treatment of acne, resulting in a higher inhibition rate of *Propionibacterium acnes* compared to using zinc oxide alone (Bae & Park, 2016). Quinic acid, another natural organic acid, has been found to exhibit antibacterial activity against *Staphylococcus aureus* by reducing succinate dehydrogenase activity (Bai *et al*., 2018). In a study conducted by Muthamil *et al*. (2018), quinic acid was identified as one of the main compounds present in *S.cumini* leaves through in vitro assays, they demonstrated that combinations of quinic acid and undecanoic acid effectively inhibited the virulence features of *Candida sp*. Due to the synergistic effect of these multiple organic acids, the fermented JP and JL have the potential to be used as functional ingredients for application in food, medicinal, and cosmeceutical industries.

Cytotoxicity of RAW 264.7 cells treated with fermented and non-fermented jackfruit extracts.

In cell culture, the CC_{50} (50% cytotoxic concentration) is determined by exposing cells to proportionally increasing concentrations of a test compound to study which concentration causes 50% of host cell death (Terefe *et al*., 2021). Thus, before the determination of the inhibition of NO production, the non-cytotoxic concentration of the extracts must be identified.

RAW 264.7 cells are important in the research of nitric oxide (NO) inhibition because of their features and capacity for investigating immune responses and inflammatory processes. These macrophage-like cells are frequently utilized in research to evaluate the impact of different substances on NO generation and inflammatory responses. Researchers can stimulate NO generation in RAW 264.7 cells using lipopolysaccharide (LPS) and investigate how different compounds hinder this process. The use of RAW 264.7 cells in NO inhibition studies allows researchers to assess the efficiency of substances in modifying immune responses and inflammatory pathways in a controlled laboratory setting. (Taciak *et al*., 2018, Ghafari *et al*. 2022)

In this study, the cytotoxicity test of JP and JL extracts at different concentrations from 0.781% to 100% was conducted on RAW 264.7 macrophage cells to evaluate the level of toxicity of the extract and determine the CC_{50} value (Figure 1). After

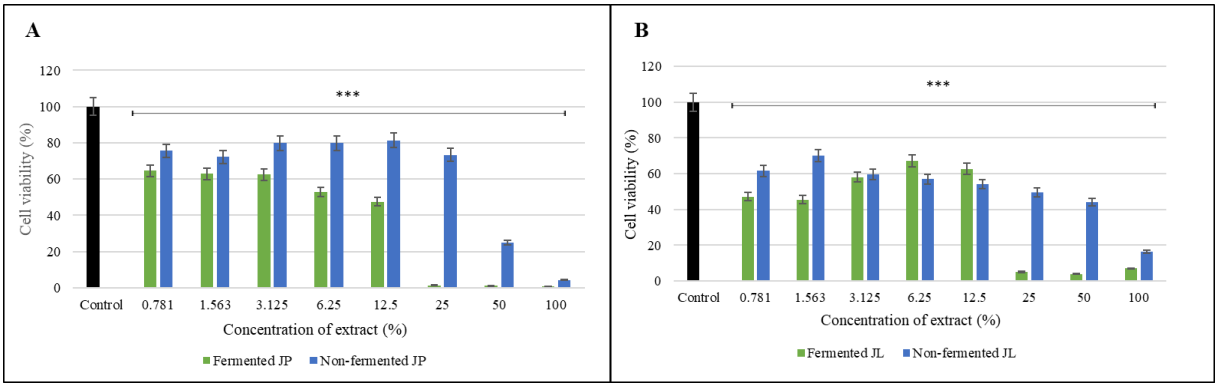

Fig. 1. Effects of fermented and non-fermented JP (A) and JL (B) on RAW 264.7 cell viability. Cells were incubated with the indicated concentration for 24 hr. Each value represents the mean ±SD of triplicate experiments. Significant differences were compared to the control group using a one-way ANOVA test, followed by Tukey's multiple comparison post-test. (*** $p<0.05$)

incubation of extracts with cells for 24 hr it was found that JP and JL extracts showed different toxicity depending on the concentration used whereby non-fermented extracts showed a lower level of toxicity than fermented extracts. This is due to the higher acid content and lower pH in the fermentation extract as explained in the previous section. According to the findings, the JL extract displayed an unpredictable and non-dose-dependent cytotoxic effect. This is because leaf extracts typically contain a wide variety of bioactive compounds such as phenolics, flavonoids, and other secondary metabolites. The differing concentrations and interactions of these compounds can result in inconsistent cellular responses. For example, individual phenolic compounds may have unique cytotoxic effects that do not necessarily align with their concentration levels (Ghimire *et al*., 2021)

Acidic pH levels can significantly impact cell viability in cell culture. Research suggests that acidic conditions can lead to decreased cell growth, with cell numbers decreasing notably at lower pH levels (Cialdai *et al*., 2022). The fermentation process with the SCOBY strain has produced an extract with a high organic acid content. The symbiotic combination of yeast and acetic acid bacteria produces an acidic extract due to the metabolic activity of these microorganisms. The combined action of the two microorganisms creates an environment conducive to the production of organic acids, especially acetic acid, which gives an acidic nature to the extract (Laavanya *et al*., 2021). Therefore, in this toxicity test, JP and JL extracts became toxic to RAW 264.7 cells at high concentrations, especially for extracts that had been fermented. In this study, the CC_{50} values for fermented and unfermented JP extracts were 12.04% and 38.80% while for fermented and unfermented JL were 19.54% and 28.38% respectively.

However, the results of this in-vitro screening do not reflect the actual level of toxicity in the human or animal body system. This is because the body system consists of a complex network of tissues and organs rather than cells. Therefore, this CC_{50} value is to be used as a reference to determine the maximum extract concentration that is non-toxic to RAW 264.7 cells for the subsequent nitric oxide inhibition test. Therefore, the concentration value that can be used for the test is lower than the CC_{50} value that has been obtained.

The effect of fermentation on nitric oxide (NO) inhibition varies between extracts.

Nitric oxide (NO) has been identified as a mediator and regulator in pathological responses, particularly acute inflammatory responses. Macrophages are essential components of the inflammatory response as they provide immediate protection against foreign pathogens by producing pro-inflammatory mediators such as NO. Excessive and unregulated NO generation can be hazardous, resulting in a variety of inflammatory illnesses. Thus, inhibiting NO generation is critical for treating a variety of disorders caused by excessive and prolonged macrophage activation (Ryu *et al*., 2003). In NO inhibition experiments on RAW 264.7 cells, lipopolysaccharide (LPS) a potent macrophage activator from the component of gram-negative bacteria's cell walls was used to stimulate the generation of NO. When RAW 264.7 cells are exposed to LPS, it activates inducible nitric oxide synthase (iNOS), which produces NO. The inhibitory effects of various substances on NO production can be assessed by monitoring the quantities of NO generated in response to LPS stimulation (Lin & Tang, 2008). Thus, the NO inhibition assay in this present study was performed to evaluate the anti-inflammatory activities of JP and JL on the RAW 264.7 cells that have been induced by LPS.

As shown in Figure 3, both JP and JL extracts demonstrated the capacity to reduce NO production, especially at increased amounts. Remarkably, the fermentation boosted the NO production suppression compared to the non-fermented sample. In this investigation, it was observed that JP extract concentrations ranging from 3% to 12% significantly inhibited the formation of NO. The highest inhibition, reaching 32.36%, was achieved with the 12% extract concentration compared to the control sample. Conversely, a decrease in inhibitory effect was noticed when lower extract concentrations were utilized. Similar trends were observed with JL extract, whereby the highest inhibition of NO production occurred with a concentration of 19.5% resulting in 75.52 ± 1.64 µM of NO compared to the control sample (130.71 ± 0.62 µM). These findings suggest that fermented JP and JL extracts possess significant potential as anti-inflammatory agents due to their ability to reduce the generation of NO. This may

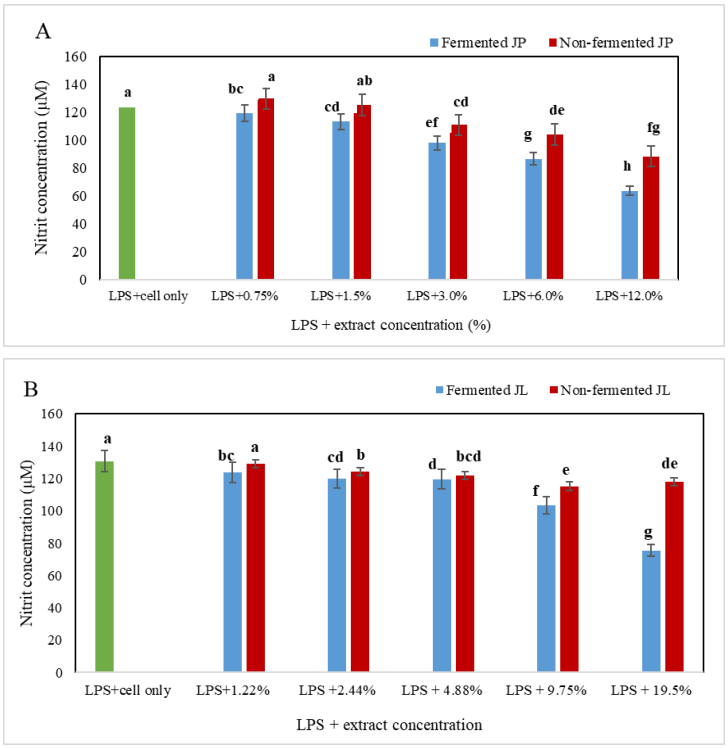

Fig. 2. Inhibitory effects of jackfruit pulp (A) and leaves (B) extracts on LPS-induced NO formation in RAW 264.7 cells. The cells and LPS in the control group received no treatment. Values correspond to mean ± std. Bars that do not share a letter differ significantly (p<0.05).

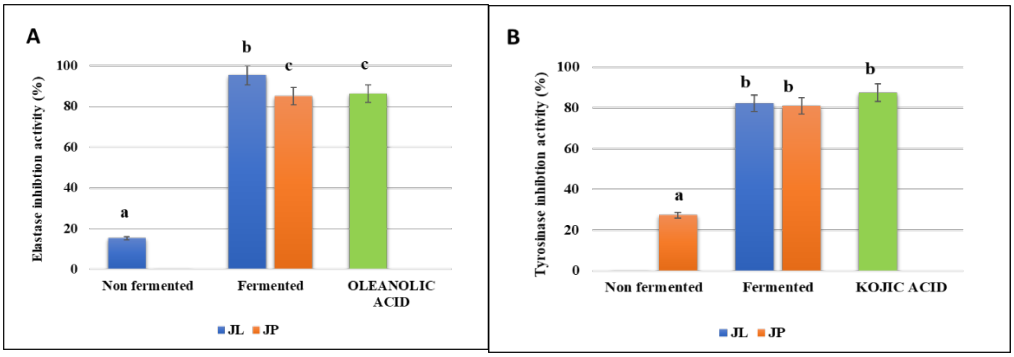

Fig. 3. Elastase (A) and tyrosinase (B) inhibitory activity of fermented and non-fermented extract of JL and JP. Data were presented as the mean ± standard deviation of triplicate analyses. Bars marked with the same letter indicate no significant differences between extracts (p<0.05).

be attributed to the presence of bioactive components such as polyphenols, which are known for their natural anti-inflammatory properties. These properties likely contribute to the jackfruit's ability to regulate the inflammatory process.

SCOBY fermentation can have effects on polyphenols enhancement that lead to improvement of anti-inflammatory properties. (Wójciak *et al*., 2023) Conducted studies on the fermentation of *Sambucus nigra* fruit extract with kombucha tea fungus. This fermentation process yielded various phenolic compounds, such as protocatechuic acid, anthocyanins, chlorogenic acid, rutin, gallic acid, dihydroxybenzoic and hydroxybenzoic glucoside, and gluconic acid. These compounds, generated during fermentation, contribute to the anti-inflammatory properties of the fermented extract.

Vitexin, the most dominant flavonoid compound in the fermented JL has demonstrated significant anti-inflammatory effects in various studies. It has been shown to reduce inflammatory responses by downregulating chemokines, adhesion molecules, and proinflammatory cytokines while increasing anti-inflammatory cytokines (Ranjan *et al*., 2023) As reported by (Borghi *et al*., 2013) they discovered that vitexin has an anti-inflammatory impact by lowering pro-inflammatory cytokines such as IL-1ß, IL-6, IL-33, and TNF-α. Benzoic and salicylic acids are also recognized for their anti-inflammatory effects. They are thought to achieve these effects via a variety of methods, including the suppression of cyclooxygenase (COX) enzymes. These acids are also known to regulate cell function, which could contribute to their anti-inflammatory properties (Tjahjono *et al*., 2021). Furthermore, salicylic acid derivatives have been proven to reduce pain, fever, and inflammation, demonstrating their strong anti-inflammatory properties (Singh *et al*., 2004). Catechins, another type of flavonoid, have been linked to oxidative stress inhibition, immune cell function regulation, and gut microbiota stability, all of which contribute to their anti-inflammatory characteristics (Zhu *et al*., 1997).

The potential anti-inflammatory effects of organic acids, namely acetic, citric, and quinic acid, which are abundant in the fermented JP and JL, have garnered significant attention in various scientific investigations. These organic acids have displayed promising characteristics in terms of their ability to combat inflammation and offer potential therapeutic advantages for individuals affected by inflammatory conditions. Acetic acid, typically found in vinegar, has been found to effectively suppress inflammatory mediators and impede the production of pro-inflammatory cytokines (Nagoba *et al*., 2013). Likewise, citric acid, which is commonly present in citrus fruits, has demonstrated anti-inflammatory properties by modulating the immune response and reducing the activity of inflammatory enzymes (Jeong *et al*., 2017). Additionally, quinic acid, prevalent in both coffee and certain fruits, actively inhibits the activation of inflammatory pathways, thus showcasing its anti-inflammatory efficacy (Muthamil *et al*., 2018). In combination, these organic acids show promising potential for inflammation reduction and may serve as natural compounds in the development of effective anti-inflammatory therapies. Hence, we hypothesized that the presence of phenolics and organic acids in jackfruit could contribute to their anti-inflammatory capabilities.

Jackfruit fermentation enhances its ability to inhibit elastase and tyrosinase.

Elastase and tyrosinase are enzymes that play significant roles in human skin physiology and appearance. Elastase is an enzyme that breaks down elastin, a protein responsible for maintaining skin elasticity. Excessive activity of elastase can lead to the degradation of elastin fibers, resulting in loss of skin elasticity, wrinkles, and sagging (Liyanaarachchi *et al*., 2018). In contrast, tyrosinase is an enzyme involved in melanin production, the pigment responsible for skin, hair, and eye color. Overactivity of tyrosinase can lead to hyperpigmentation disorders like age spots and melasma (Kanteev *et al*., 2015). Therefore, elastase inhibitors are valuable for preserving skin elasticity and reducing wrinkles, while tyrosinase inhibitors are used for skin-whitening purposes and to address hyperpigmentation issues.

The impact of fermentation on the cosmeceutical functionalities of jackfruit was examined, specifically focusing on its tyrosinase and elastase inhibitory activity. The findings as illustrated in Figure 3A showed that fermentation positively influenced the inhibitory action of elastase on both JP and JL. Following fermentation, the JL extract exhibited the highest rate of elastase inhibition at 95.41%, surpassing the inhibition rate of oleanolic acid, the standard inhibitor used in the study. On the other hand, JP displayed a slightly lower inhibition rate of 85.24%, comparable to that of oleanolic acid. This suggests that distinct active metabolites formed during the fermentation phase can induce the desired elastase inhibition effect.

The fermentation process can enhance the substrate's elastase inhibition capabilities through various mechanisms, one of which involves the production of bioactive compounds that possess increased inhibitory potency. Notable research has demonstrated that fermenting natural products, such as red ginseng, can generate bioactive compounds with more potent elastase inhibition properties. For instance, a study investigating the use of fermented red ginseng (FRG) as a skin-care anti-aging ingredient discovered that FRGs exhibited robust elastase inhibitory potency, underscoring the potential of fermentation to enhance the anti-aging characteristics of natural products (Lee *et al*., 2012). In addition, investigations by (Abd Razak *et al*., 2017) focusing on the effects of fungal fermentation on the cosmeceutical functionalities of rice bran have revealed improvements in elastase inhibition activity following the fermentation process. These studies collectively highlight the beneficial impact of fermentation in augmenting elastase inhibition capabilities, potentially achieved through the generation of bioactive compounds with greater potency, ultimately contributing to enhanced anti-aging and skin health benefits.

Numerous tyrosinase inhibitors, both synthetic and naturally occurring, have been explored for skin whitening. However, natural agents are becoming significantly important due to synthetic agent disadvantages such as high cytotoxicity, lack of penetrating power, and limited range of action (Hassan *et al*., 2023). As a result, several tyrosinase inhibition investigations based on bioprocessing from natural products have been conducted in recent years. Tyrosinase inhibitor compounds or extracts from natural sources such as plants are preferred for food, cosmetics, and medicinal applications because of their bioavailability and relatively lower toxicity. Some common tyrosinase inhibitors include hydroquinone, vanillin, kojic acid, quercetin, arbutin, kaempferol, and cumic acid (Chiocchio *et al*., 2018).

Fermented jackfruit has a higher percentage of tyrosinase inhibitory activity than non-fermented jackfruit, similar to elastase inhibitory activity (Figure 3B). The inhibition rate (80.97-82.26%) showed no significant difference with the reference inhibitor, kojic acid, utilized in this investigation. Tyrosinase inhibition activity in jackfruit extract was also observed by (Rayendra *et al*., 2016) and according to their findings, the ethanol extract of young jackfruit leaves contained a high concentration of flavonoid, which can be linked to the potential as a whitening agent, as artocarpin and artocarpanone are components of the flavonoid compound. In summary, fermented jackfruit extract inhibited more tyrosinase and elastase than non-fermented jackfruit, indicating that fermented jackfruit has the potential to be a new tyrosinase and elastase inhibitor that can be incorporated in food or cosmetic products as an alternative ingredient for anti-aging and skin whitening applications.

CONCLUSION

The SCOBY fermentation process exhibited the ability to modify the profile of phenolics and organic acids while simultaneously increasing the levels of various beneficial compounds, such as vitexin, ferulic acid, salicylic acid, and quinic acid. These alterations in the chemical composition of the fermented extracts have the potential to enhance their bioactivities. Moreover, the anti-inflammatory effects of the fermented extracts surpassed those of the non-fermented counterparts. Particularly, the fermentation process significantly improved the inhibitory capabilities of elastase and tyrosinase in all extracts. The observed results were comparable to the effects of well-documented skin-protective compounds such as kojic and oleanolic acid. The significant improvement in the inhibitory properties of these enzymes suggests that fermented jackfruit extracts have the potential to be a valuable natural source of bio-ingredients for functional food and cosmetic applications. Nevertheless, this research only shows benefits *in vitro*, and performing clinical trials on humans could confirm the effectiveness and safety of fermented jackfruit extracts for use in cosmeceuticals, particularly regarding anti-inflammatory and anti-aging effects.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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