e-ISSN: 2807-2820

Natural Sciences Engineering Technology Journal

[NASET Journal]

https://nasetjournal.com

Unlocking the Bioactive Potential of *Pandanus conoideus* Lim: A Process-Modified Approach to Double the Oil Yield and Enhance In Vitro Radical Scavenging Activity

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ARTICLE INFO

Keywords:
Antioxidant activity
DPPH
Extraction optimization
Pandanus conoideus Lim
Red fruit

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/nasetjournal.v5i2.67

A B S T R A C T

Pandanus conoideus Lim (Red Fruit), an indigenous plant of Papua, Indonesia, is a traditionally valued source of natural antioxidants. However, community-based oil extraction methods are often inefficient, leading to low yields and thermal degradation of bioactive compounds, thereby limiting the product's quality and standardization potential. This study aimed to develop and validate a process-modified extraction technique to improve both the yield and a key chemical quality marker—the *in vitro* antioxidant efficacy—of Red Fruit oil. Oil was extracted from *Pandanus conoideus* fruit using two methods: a traditional empiric method (aqueous boiling, manual pressing) and a novel modified method (steam distillation, mechanical screw pressing, centrifugation at 3500 x g, and controlled-temperature vacuum evaporation at 50°C). Oil yields were quantified on both wet and dry weight bases. The *in vitro* antioxidant capacity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, with the half-maximal inhibitory concentration (IC_{50}) calculated from scavenging activity at concentrations of 50, 100, 150, and 200 ppm. Commercial Vitamin E was used as a positive control. The process-modified extraction method produced a substantially greater oil yield (20.0% w/w) compared to the empiric method (10.8% w/w), representing an 85.2% increase in production efficiency. In the DPPH assay, the oil from the modified method exhibited significantly higher radical scavenging potency, with an IC_{50} value of 63.94 ± 2.15 ppm. This was superior to the empiric method extract (IC_{50} = 95.55 ± 3.41 ppm; $p < 0.01$) and the 300 IU Vitamin E standard (IC_{50} = 75.48 ± 2.88 ppm). In conclusion, the integrated, process-modified extraction strategy successfully overcomes the critical limitations of traditional methods by improving process efficiency and preserving chemical integrity. It nearly doubles the oil yield and significantly enhances the in vitro radical scavenging activity by minimizing thermal degradation of bioactive compounds. This validated approach provides a robust framework for the standardized production of high-quality *P. conoideus* oil, establishing a scientific foundation for its development as a high-value, evidence-based natural product.

1. Introduction

The phenomenon of life is inextricably linked to a continuous flux of energy, governed by the principles of redox biochemistry.[1] Aerobic metabolism, the highly efficient process by which organisms convert nutrients into usable energy, carries an inherent paradox: its function depends on the controlled utilization of oxygen, yet it inevitably produces a cohort of highly reactive byproducts known as reactive oxygen species (ROS). This group, which includes free radicals like the superoxide anion (O_2^-) and the hydroxyl radical (•OH), as well as non-radical oxidants like hydrogen peroxide (H_2O_2), is not merely metabolic waste.[2] In a state of physiological homeostasis, these molecules function as sophisticated and essential signaling messengers, modulating a vast array of cellular processes, from immune surveillance and inflammation to apoptosis and gene expression. This delicate redox balance,

however, is perpetually challenged by both endogenous sources, such as mitochondrial electron transport chain leakage, and a barrage of exogenous environmental factors, including UV radiation, environmental pollutants, and xenobiotics.[3] When the production of ROS overwhelms the capacity of the cell's endogenous antioxidant defense systems, the system tips into a deleterious state known as oxidative stress.

The pathophysiology of a remarkably broad spectrum of human diseases is deeply rooted in the relentless and cumulative molecular damage inflicted by oxidative stress.[4] The high electrochemical reactivity of ROS allows them to indiscriminately attack and functionally alter all major classes of biological macromolecules. A primary and devastating target is the polyunsaturated fatty acids that constitute the lipid bilayer of cellular and organellar membranes. The abstraction of a hydrogen atom from a lipid molecule by a free radical initiates a self-propagating chain reaction of lipid peroxidation. This process not only compromises the structural integrity, fluidity, and permeability of the membrane but also generates a cascade of cytotoxic secondary products, such as malondialdehyde and 4-hydroxynonenal, which can diffuse and inflict damage far from the initial site of insult. Concurrently, proteins are susceptible to oxidative damage, leading to the carbonylation of amino acid residues, the formation of cross-links, and peptide fragmentation. This results in the misfolding, aggregation, and inactivation of critical enzymes, receptors, and structural proteins, disrupting cellular function on a global scale. Perhaps the most insidious consequence of oxidative stress is damage to the cell's genetic blueprint. ROS can attack the purine and pyrimidine bases and the deoxyribose backbone of DNA, causing a spectrum of lesions from single- and double-strand breaks to the formation of adducts like 8-oxo-2'-deoxyguanosine.[5] If these lesions overwhelm the cell's DNA repair machinery, they can lead to mutations, genomic instability, and the initiation of carcinogenesis. This relentless molecular onslaught is now understood to be a central pathogenic mechanism in the etiology and progression of a wide range of debilitating conditions, including cardiovascular diseases, neurodegenerative disorders like Alzheimer's and Parkinson's disease, diabetes mellitus, chronic inflammatory diseases, and the fundamental biological process of aging itself.

To defend against this constant threat, biological systems have evolved a sophisticated, multi-layered antioxidant defense network. This network includes a frontline of endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), and the glutathione peroxidase (GPx) family, which catalytically neutralize specific ROS with high efficiency. This enzymatic shield is supported by a host of non-enzymatic antioxidants, including endogenously produced molecules like glutathione and uric acid, and crucially, exogenously obtained compounds from the diet. Dietary antioxidants, particularly those derived from the plant kingdom, are of paramount importance in bolstering this defense system. For decades, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were widely incorporated into food products to prevent oxidative spoilage. However, accumulating toxicological evidence regarding their potential long-term adverse health effects, coupled with a powerful global consumer demand for natural and "clean label" products, has precipitated a significant and accelerating shift in scientific and industrial focus towards the vast, chemically diverse, and largely untapped reservoir of antioxidants found in nature.

The plant kingdom is a prolific and unparalleled source of these protective compounds. Plants, as sessile organisms, are constantly exposed to environmental stressors, including high-intensity solar radiation and pathogenic attack, which also induce severe oxidative stress.[6] In response, they have evolved the ability to synthesize a breathtakingly diverse arsenal of secondary metabolites to protect themselves. These phytochemicals, including phenolic compounds (flavonoids, phenolic acids), terpenoids (carotenoids), and tocopherols (Vitamin E), possess potent antioxidant properties. They are abundant in

fruits, vegetables, grains, and medicinal herbs and form an integral part of the human diet. A wealth of epidemiological and clinical research has established a strong and consistent correlation between a diet rich in plant-based foods and a reduced incidence of chronic diseases, a benefit largely attributed to the antioxidant and anti-inflammatory activities of these phytochemicals.[7] This has spurred a global scientific endeavor to identify, characterize, and harness novel plant sources of potent natural antioxidants for use in nutraceuticals, functional foods, and pharmaceuticals. Within this global search, ethnobotanical knowledge provides an invaluable guide, pointing researchers towards plants with a long and successful history of traditional use for health and wellness. One such plant of immense cultural and medicinal significance is the Red Fruit, *Pandanus conoideus* Lim. This striking plant is native to the Oceania region and holds a particularly esteemed place in the culture and diet of the indigenous peoples of Papua, Indonesia.[8] Traditionally, the fruit is processed into a dense, vibrant red, oily sauce that is a cornerstone of the local cuisine, providing essential calories and nutrients. More than just a food source, Red Fruit is a central element of Papuan traditional medicine, where it is consumed to enhance physical stamina, accelerate recovery from illness, and treat a variety of health complaints.

Modern scientific inquiry has begun to unravel the biochemical basis for these traditional claims. Chemical analyses of Red Fruit oil have revealed an exceptionally rich phytochemical profile, distinguished by remarkably high concentrations of lipophilic antioxidants. The oil's characteristic deep red color is due to a high content of carotenoids, including β-carotene and α-carotene, which are well-known provitamin A carotenoids and potent quenchers of singlet oxygen. Furthermore, the oil is a significant source of tocopherols, the family of compounds that constitute Vitamin E, which are renowned for their ability to break the chain reactions of lipid peroxidation in cell membranes.[9] This unique combination of high-potency antioxidants makes *P. conoideus* a highly promising candidate for development as a natural health product. However, a significant chasm exists between the plant's inherent potential and its realization as a standardized, high-quality commercial product. The challenge lies principally in the domain of process chemistry and engineering. Traditional extraction techniques employed by local communities, while culturally established, are technologically rudimentary. These methods typically involve the prolonged, high-temperature boiling of the fruit pulp in water, followed by inefficient manual pressing to separate the oily liquid, and a final stage of heating to evaporate the water. From a process engineering perspective, this approach is fraught with critical flaws. The uncontrolled thermal load is highly conducive to the degradation of heat-sensitive bioactive compounds, while the inefficient solid-liquid separation leads to substantial losses of the valuable oil, resulting in low yields.[10] This lack of a standardized, optimized process yields a final product of inconsistent quality and potency, a major barrier to its commercial development. Moreover, improving extraction efficiency is a key tenet of green chemistry, as it maximizes the value derived from a natural resource while minimizing waste, contributing to both economic and environmental sustainability.

The primary aim of this research was to address these critical deficiencies by designing, implementing, and rigorously evaluating a process-modified extraction methodology for *P. conoideus* oil from a process engineering standpoint. The novelty of this study is rooted in its holistic and scientifically-driven approach to process optimization, viewing the extraction not as a single step but as an integrated system. It moves beyond incremental improvements and instead combines a sequence of modern processing techniques—gentle steam distillation for thermal control, efficient mechanical pressing for solid-liquid separation, precise centrifugal phase separation to break emulsions, and low-temperature vacuum evaporation for purification—each chosen to overcome a specific physicochemical barrier of the

traditional method. This investigation represents the first direct, quantitative comparison of a traditional versus a process-modified technique for this specific botanical, seeking to demonstrate not only a significant amplification of oil yield but also a concurrent enhancement of a key chemical quality marker: its in vitro radical scavenging potency.

2. Methods

A 10 kg consignment of fresh, fully ripe Red Fruits (*Pandanus conoideus* Lim) was procured in January 2025 from an agricultural cooperative in the Jayapura Regency, Papua Province, Indonesia. The fruits were selected based on stringent criteria, including uniform deep crimson color and firmness, to ensure the homogeneity of the starting material. The botanical identity of the fruit was formally authenticated by a senior botanist at the Department of Biology, Cenderawasih University, Jayapura, and a voucher specimen (No. PC-JYP-202501) was deposited into the university's herbarium. The initial moisture content of the fresh fruit was determined to be 68.5 ± 2.1% using an Ohaus MB45 moisture analyzer by drying a representative sample at 105°C to a constant weight. All chemicals and solvents were of analytical grade. Anhydrous methanol (p.a., ≥99.8%) was obtained from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH, ≥95%) was purchased from PT Smart Lab Indonesia. High-purity deionized water was generated using a Water One (Onemed, Indonesia) system. A commercial natural Vitamin E supplement (d-alpha-tocopherol), formulated in an oil-based softgel at two strengths (100 IU and 300 IU), was procured from a licensed local pharmacy to serve as a positive control. The experimental setup included a culinary-grade steam distillation unit, a heavy-duty mechanical screw press juicer, a high-capacity benchtop centrifuge (capable of 3500 x g), and a Buchi-style rotary evaporator system with a vacuum pump and digital water bath. A vacuum filtration apparatus was used for final polishing. Spectrophotometric analysis was performed using a Biobase BK-D560 double-beam UV-Vis spectrophotometer.

The study was a controlled, parallel-group comparative investigation. A total mass of 4 kg of fresh Red Fruit was homogenized and randomly allocated into two equal batches of 2 kg each. Group 1 was processed via the traditional empiric method, and Group 2 was processed via the process-modified method. All procedures and analyses were conducted in triplicate (n=3). Both 2 kg batches were washed, drained, and diced into uniform cubes (approximately 3-5 cm) to increase the surface-area-to-volume ratio. Group 1: Empiric Aqueous Boiling Method, This protocol was executed to replicate the traditional extraction technique. The 2 kg of diced fruit was boiled in water for four hours, with the endpoint determined by the traditional visual marker of the seeds turning whitish. The hot slurry was then manually separated by twisting and compressing it in a muslin cloth to express the oil-water emulsion. This collected liquid was then simmered to evaporate the residual water, and the final oil layer was decanted. Group 2: Process-Modified Mechano-Thermal Method, This novel protocol consisted of a sequence of five rationally designed stages: Stage 1: Steam Distillation: The 2 kg of diced fruit was subjected to hydrothermolysis via saturated steam to efficiently soften the tissue and initiate the rupturing of oil-containing cells without direct scorching; Stage 2: Mechanical Pressing: The hot, steamed pulp was immediately fed into a mechanical screw press juicer, which applied intense and continuous mechanical pressure and shear forces to efficiently expel the liquid phase; Stage 3: Centrifugal Phase Separation: The raw oil-in-water emulsion was centrifuged at a relative centrifugal force (RCF) of 3500 x g for two consecutive 15-minute cycles. This powerful force cleanly resolved the emulsion into three distinct layers: a dense paste-like sediment, a middle aqueous layer, and the target, low-density oil as the uppermost layer; Stage 4: Low-Temperature Vacuum Evaporation: The decanted top oil layer was processed in a rotary evaporator at a precisely controlled temperature of 50°C and a pressure of -70 kPa to remove residual water without

causing thermal degradation of bioactive compounds; Stage 5: Final Filtration: The pure oil was passed through a 90-mesh vacuum filter to remove any microscopic solid particulates, yielding a brilliantly clear, homogenous final product. The percentage yield was calculated on both a wet weight and dry weight basis using the initial moisture content data. The final mass of pure oil was measured using an analytical balance: Wet Weight Yield (%) = [Mass of final oil (g) / Initial mass of fresh fruit (g)] × 100; Dry Weight Yield (%) = [Mass of final oil (g) / Initial mass of dry fruit solids (g)] × 100.

A stock solution of DPPH was prepared by dissolving an appropriate mass of DPPH powder in methanol to achieve a final concentration of 2.54 mM. The flask was protected from light to prevent degradation of the photosensitive radical. Primary stock solutions (1000 ppm) of the oil extracts were prepared in methanol. The Vitamin E positive control was prepared by carefully extracting the oil from the softgels. Based on the standard conversion factor for natural Vitamin E (1 IU = 0.67 mg d-alpha-tocopherol), the mass of tocopherol was calculated and used to prepare a 1000 ppm stock solution in methanol. Serial dilutions were performed to achieve working solutions of 50, 100, 150, and 200 ppm. 1 mL of the DPPH solution was mixed with an aliquot of each sample concentration in a test tube. The mixture was vortexed and incubated at 37°C for 30 minutes in the dark. The absorbance was then measured at 517 nm against a methanol blank. The rationale for using 37°C was to approximate physiological conditions, and control experiments confirmed the stability of the DPPH radical under these conditions for the assay duration. The percentage of DPPH radical scavenging activity (%RSA) was calculated for each concentration. The half-maximal inhibitory concentration (IC_{50}) was determined by linear regression analysis of the plot of %RSA versus concentration. All experiments were performed in triplicate, and data are reported as mean ± standard deviation (SD). Statistical significance between the two groups was determined using a two-tailed Student's t-test, with a p-value < 0.05 considered significant. The antioxidant strength was categorized based on the IC_{50} values.

3. Results and Discussion

The two extraction methods yielded oils with distinctly different organoleptic and physical properties. The empiric method produced a deeper reddish-brown, slightly turbid oil with a cooked, somewhat smoky aroma, and it contained fine sediment. In contrast, the oil from the process-modified method was a brilliant, clear ruby red, possessed a fresh and fruity aroma characteristic of the raw fruit, and was a smooth, free-flowing liquid free of any visible sediment or turbidity. These qualitative differences suggest a less aggressive thermal treatment and superior purification in the modified process. Figure 1 provides a comprehensive and compelling visual narrative that systematically compares two distinct methodologies for the extraction of oil from *Pandanus conoideus* fruit. The leftmost panel of the figure details the "Empiric Method Pathway," a process characterized by its simplicity and reliance on rudimentary techniques. This pathway serves as the experimental baseline, representing a traditional or non-optimized approach to oil extraction. The first step, "Water Boiled," is visually represented by a pot over an open flame, and the description explicitly notes the use of "Prolonged, high-temperature direct boiling". From a chemical engineering and food science perspective, this single step introduces significant potential for product degradation. The direct application of high heat over an extended period can initiate a cascade of undesirable chemical reactions. Thermolabile compounds, which are often the most bioactive and valuable components of a natural product, are highly susceptible to degradation under these conditions. In the context of *P. conoideus* oil, which is rich in carotenoids and tocopherols, such harsh thermal treatment can lead to isomerization, oxidation, and cleavage of these molecules, diminishing their antioxidant potential and altering their native structure. Furthermore, the high heat can induce

Maillard reactions and caramelization of sugars present in the fruit pulp, contributing to the browning of the final product and the development of cooked or even burnt aromatic notes. The subsequent step, "Hand Pressing," is depicted with an icon representing manual force and is described as an "inefficient separation via manual squeezing of cloth". This highlights the mechanical limitations of the empiric method. Manual pressing is inherently inconsistent and force-limited, making it incapable of achieving a complete rupture of the oil-containing oleosomes within the plant cells. Consequently, a significant fraction of the valuable oil remains trapped within the solid plant matrix (the marc), leading to a lower overall yield. Additionally, the use of a simple cloth as a filter is a crude method of separation. It allows a substantial amount of fine, microscopic solid particulates and colloidal matter to pass through with the liquid phase, which directly contributes to the turbidity and sediment content of the final oil. In stark contrast, the rightmost panel outlines the "Modified Method Pathway," a multi-stage process engineered for efficiency, control, and preservation of quality. Each step is a deliberate technological improvement designed to overcome the specific shortcomings of the empiric approach. The initial step is the "Distillation Process," which the figure clarifies is a form of "Gentle, indirect heating" intended to "preserve thermolabile compounds". This substitution of harsh boiling with a controlled distillation or steaming process is a critical modification. By using indirect heat, the plant material avoids scorching and localized overheating. This significantly reduces the rate of thermal degradation, preserving the structural integrity of the vibrant carotenoid pigments and other sensitive bioactive molecules, which is crucial for maintaining the oil's natural color and potential efficacy. Following this, "Automatic Pressing" replaces manual labor. The figure's description highlights its "High-efficiency separation using pressure and shear forces". A mechanical press applies immense, consistent, and controlled force that is orders of magnitude greater than what can be achieved by hand. The combination of high pressure and shear forces ensures a more thorough disruption of the plant cell walls and a more complete expulsion of the liquid content, directly translating to a higher extraction yield. The final and most sophisticated step is "Centrifugation," a process that "Breaks emulsion and clarifies oil using high g-force". The liquid extracted from the press is not pure oil but a complex oil-in-water emulsion. Centrifugation applies a powerful centrifugal force that rapidly separates components based on their density. The less dense oil is cleanly separated from the denser aqueous phase and any remaining solid particulates. This step is fundamental to achieving the high degree of clarity and purity that characterizes the final product of this pathway. The Empiric Oil is depicted as a murky, "Deep reddish-brown" liquid with visible sediment at the bottom of the vial. This appearance is a direct and predictable outcome of its processing pathway. The brownish hue and turbidity are consequences of the thermal degradation and the inefficient filtration from hand pressing, respectively. The accompanying description of a "smoky odor" further corroborates the use of excessive, uncontrolled heat. Conversely, the Modified Oil is shown as a vibrant, completely translucent liquid, aptly described as "Brilliant ruby red" and "clear". This brilliant color is a testament to the successful preservation of the natural carotenoid pigments, made possible by the gentle heating of the distillation process. Its clarity is a direct result of the highly effective clarification achieved through centrifugation. The described "fresh & fruity odor" indicates that the delicate and volatile aromatic compounds native to the fruit were also preserved, a feat impossible with the high-temperature boiling of the empiric method.

Figure 2 is a striking visual and quantitative declaration of the fruit's primary composition. The figure explicitly states that the Moisture Content of the fresh fruit is 68.5%. This value confirms the graphical representation, specifying that over two-thirds of the total mass of the raw material is water. Crucially, this measurement is accompanied by its standard deviation (± 2.1% SD). The inclusion of this statistical

metric is a hallmark of rigorous scientific reporting. The relatively small standard deviation indicates a high degree of precision in the measurements and suggests a good homogeneity within the batch of fruit that was sampled. This lends significant confidence to the mean value, assuring the reader that the reported 68.5% is a reliable and representative characterization of the raw material used throughout the study.

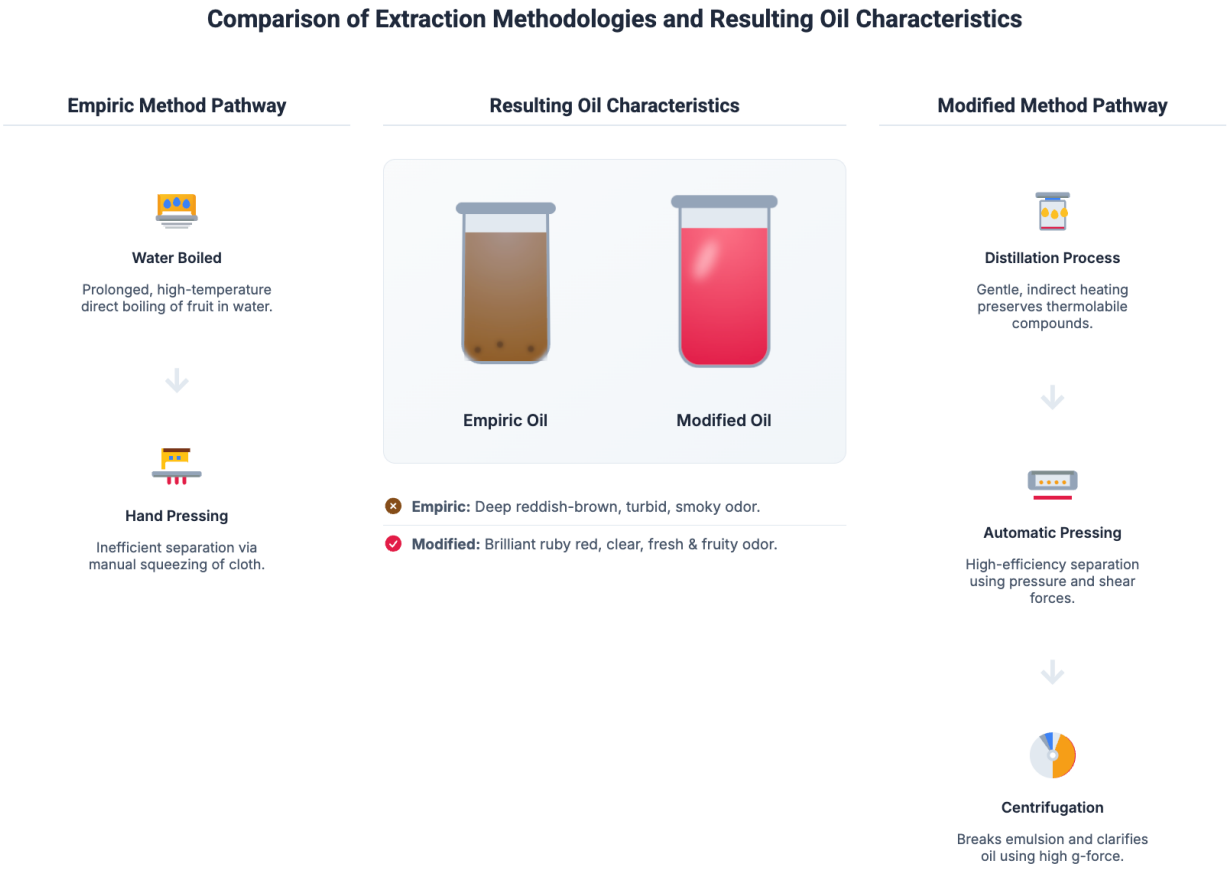


Figure 1. Comparison of extraction methodologies and resulting oil characteristics.

In the field of pharmacognosy and natural product chemistry, reporting extraction yields based on the wet weight of the starting material can be highly misleading. The water content of fresh plant material is a significant variable, subject to fluctuations based on a myriad of factors, including the season of harvest, recent rainfall, post-harvest storage conditions, and genetic variations. If yields were calculated from wet weight alone, it would be impossible to accurately compare the efficiency of an extraction process conducted in one study with that of another, as any difference could be an artifact of differing initial moisture content rather than a true difference in process efficiency. This method removes the confounding variable of water and normalizes the calculation, expressing the mass of the final extracted product as a percentage of the mass of the initial dry plant solids. This dry-weight-based yield is the universally accepted gold standard in the field, as it provides a stable, reliable, and scientifically sound metric that allows for accurate and meaningful comparisons across different experiments, laboratories, and publications. Finally, the figure includes a panel on the "Methodology," which states that the value was "Determined via the gravimetric method using an Ohaus MB45 moisture analyzer,

drying a representative sample at 105°C to a constant weight (n=3)". This concise statement underscores the study's commitment to methodological transparency and rigor. By naming the specific instrument and detailing the standard operating procedure (oven-drying to a constant weight) and the number of replicates (n=3), the authors provide all the necessary information for another researcher to replicate their measurement, a cornerstone of the scientific method.

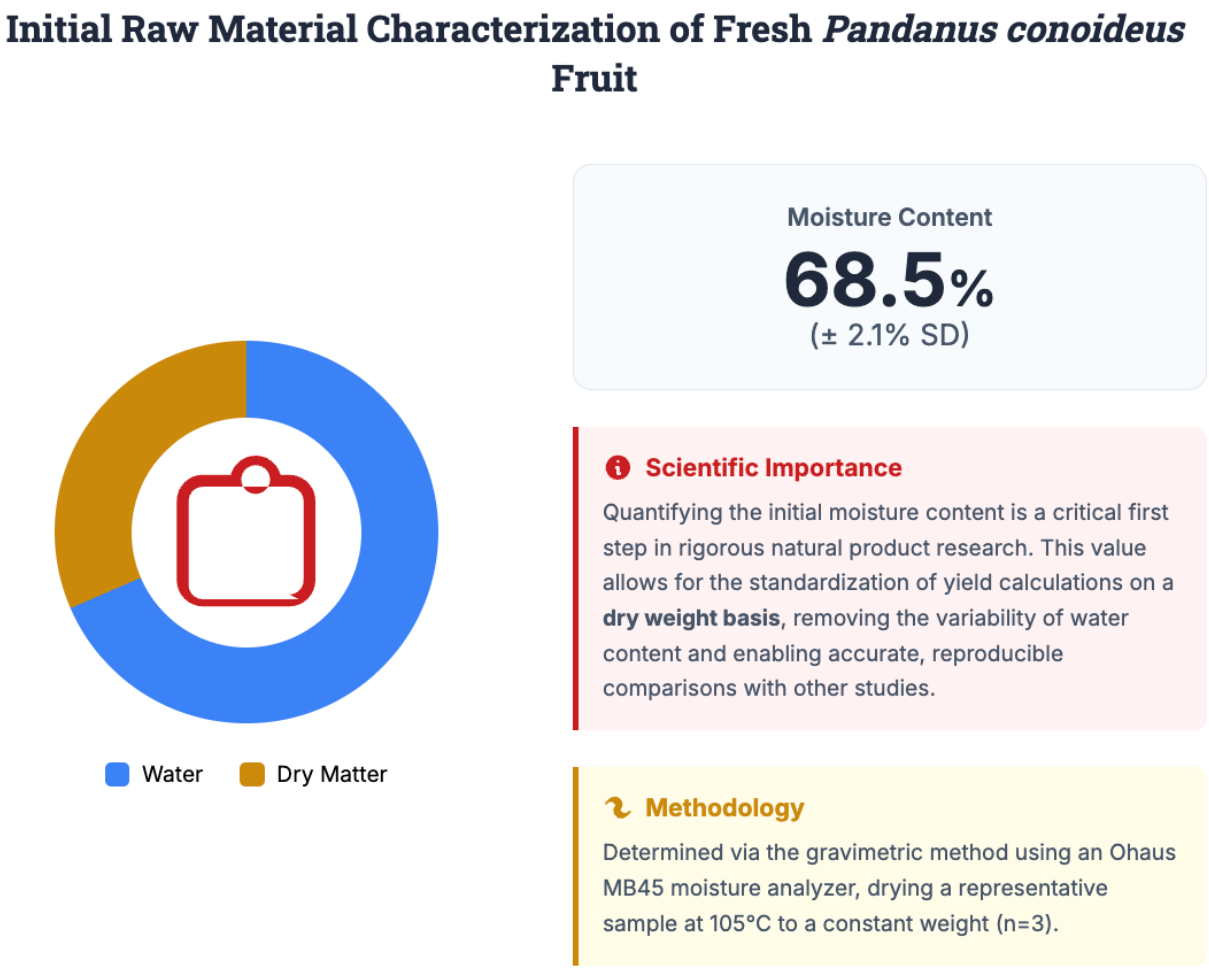


Figure 2. Initial raw material characterization of fresh *Pandanus conoideus* fruit.

Figure 3 presents a clear and quantitatively compelling summary of the study's primary finding regarding process efficiency. The Empiric Method is shown to yield 10.8% based on the initial wet weight of the 2 kg of fresh fruit. When standardized against the fruit's solid content, this translates to a Dry Weight Yield of 34.3%. The graduated cylinder provides a visual anchor for this result, showing a relatively low volume of brownish, turbid oil. These figures represent the baseline efficiency of the traditional, non-optimized process. The Modified Method demonstrates a dramatic improvement. From the identical starting mass of 2 kg of fresh fruit, this method produces a Wet Weight Yield of 20.0% and a Dry Weight Yield of 63.5%. The visual representation in the corresponding graduated cylinder is striking; the volume of vibrant red oil is nearly double that of the empiric method, immediately communicating the enhanced efficiency.

The inclusion of both wet and dry weight yields is a critical component of the figure's scientific rigor. The wet weight yield provides a practical measure relevant to processing fresh, unprocessed fruit. However, the dry weight yield is the more scientifically robust metric for comparison, as it removes the confounding variable of initial moisture content, which can fluctuate. The comparison of the dry weight yields (63.5% vs. 34.3%) confirms that the modified method is fundamentally more effective at liberating and recovering oil from the solid plant matrix.

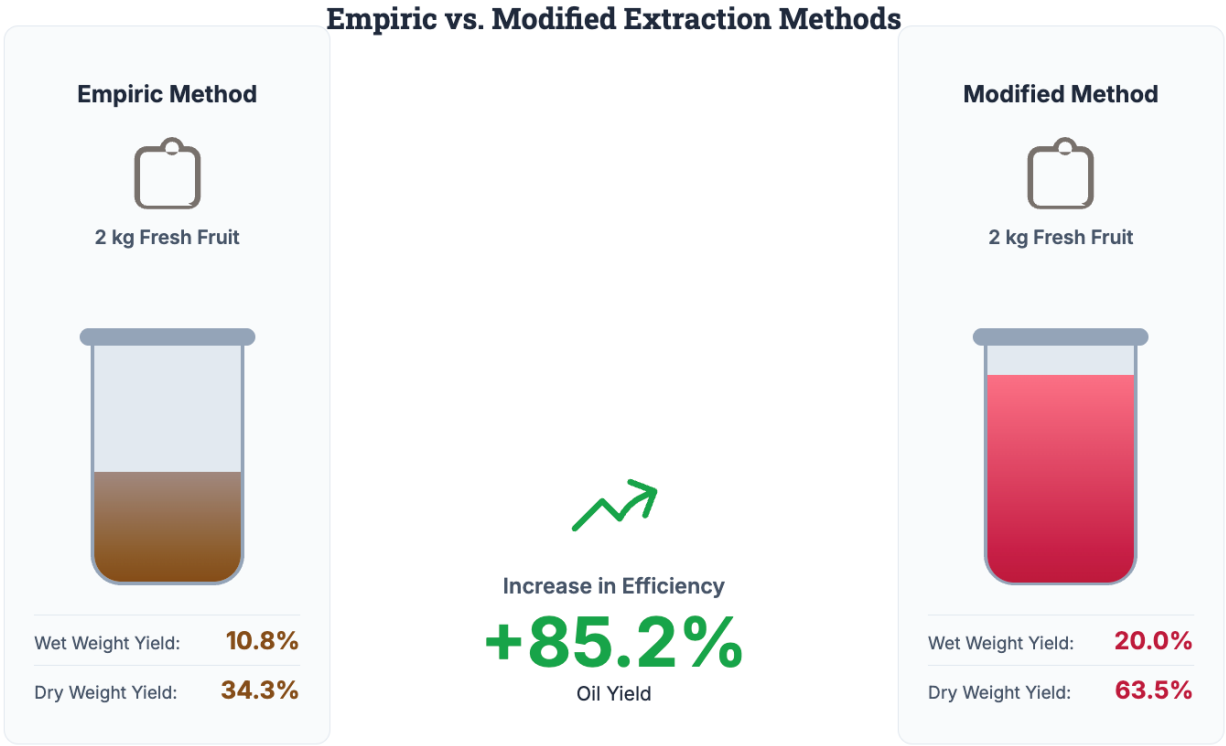


Figure 3. Quantitative comparison of oil yields from empiric vs. modified extraction methods.

Both Red Fruit oil extracts exhibited a clear concentration-dependent ability to scavenge the DPPH radical. However, at every concentration tested, the oil produced by the modified method demonstrated consistently and significantly higher scavenging activity than the oil from the empiric method. Figure 4 provides a multi-faceted and comprehensive analysis of the *in vitro* radical scavenging activity of the *Pandanus conoideus* oil extracts, comparing them against a high-dose Vitamin E standard. The "DPPH Assay Mechanism" schematic clearly illustrates the underlying chemistry of the experiment. It shows a stable, deep purple DPPH radical reacting with an antioxidant molecule. The antioxidant donates a hydrogen atom or electron, neutralizing the radical into a non-radical, pale-yellow form. The table of "Radical Scavenging Activity (%RSA)" presents the empirical results of this chemical reaction. This table details the mean percentage of DPPH radicals scavenged at four distinct concentrations for each of the three samples. Two key trends are immediately apparent from this raw data. First, a clear dose-response relationship is evident for all samples: as the concentration increases from 50 ppm to 200 ppm, the

%RSA also increases systematically. Second, at every single concentration point, the Modified Oil exhibits a higher %RSA value than both the Empiric Oil and the Vitamin E standard. For instance, at 100 ppm, the Modified Oil scavenged 65.8% of the radicals, whereas the Empiric Oil and Vitamin E scavenged only 52.1% and 51.5%, respectively. The inclusion of standard deviation for each measurement underscores the precision and reproducibility of these findings. Figure 4 translates the raw data from the table into a powerful graphical analysis—the "Dose-Response Curves for DPPH Scavenging". The most crucial feature of this graph is the relative position of the three distinct sigmoidal curves. In pharmacology and biochemistry, a leftward shift of a dose-response curve is the definitive indicator of greater potency. It signifies that a lower concentration of the substance is required to achieve a given level of effect. The data visualized in Figure 4 shows this phenomenon unequivocally. The red curve, representing the Modified Oil, is positioned furthest to the left. This demonstrates that, across the entire concentration range, it requires a lower concentration to scavenge the same percentage of radicals compared to the other samples. The blue curve for Vitamin E (300 IU) is in the middle, and the brown curve for the Empiric Oil is shifted furthest to the right, indicating it is the least potent of the three.

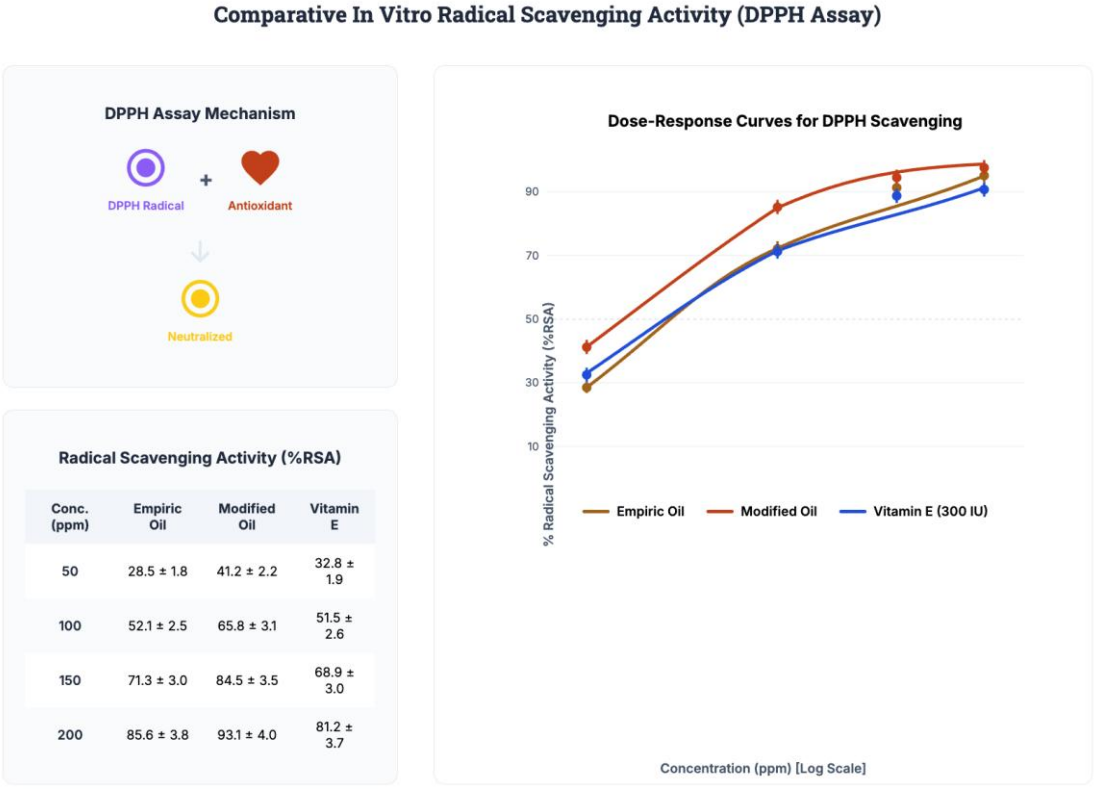
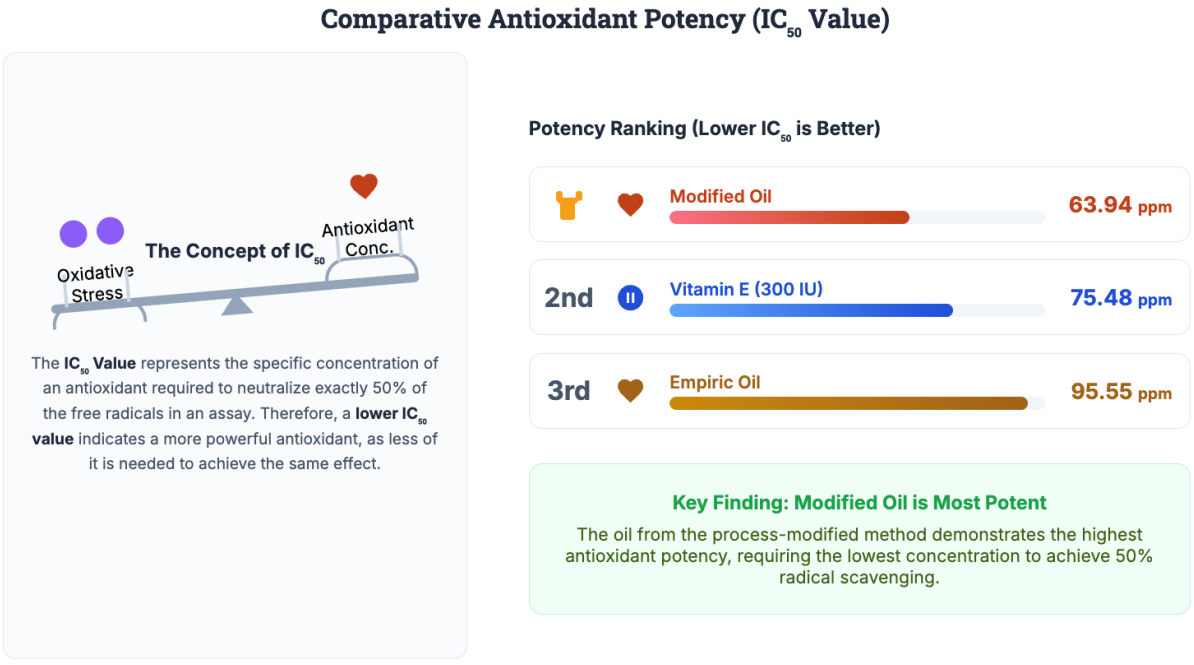


Figure 4. Comparative in vitro radical scavenging activity (DPPH Assay).

The IC_{50} value, a critical measure of potency where a lower value indicates greater activity, was calculated from the linear regression of the dose-response data. The results confirmed the superior chemical quality of the oil from the modified extraction process. The oil from the modified method was the most potent, with

an IC_{50} of 63.94 ± 2.15 ppm. The oil from the empiric method was significantly less potent ($p < 0.01$), with an IC_{50} of 95.55 ± 3.41 ppm. The 300 IU Vitamin E standard showed an IC_{50} of 75.48 ± 2.88 ppm, confirming that the natural oil extract from the modified process is more potent in vitro than a high-concentration commercial antioxidant. The left panel of Figure 5 is dedicated to elucidating the scientific principle behind the data. The "Concept of IC_{50}" is brilliantly illustrated using a balance scale analogy. On one side of the scale, "Oxidative Stress" (representing the free radicals in the assay) is depicted. On the other side is the "Antioxidant Concentration". The tilted scale visually communicates that a smaller concentration of a powerful antioxidant is sufficient to balance, or neutralize, a given amount of oxidative stress. "The IC_{50} Value represents the specific concentration of an antioxidant required to neutralize exactly 50% of the free radicals in an assay". This is followed by the crucial interpretative key: "a lower IC_{50} value indicates a more powerful antioxidant, as less of it is needed to achieve the same effect". The Modified Oil extracted via the process-modified method is unequivocally ranked as the most potent. It holds the first-place position, marked with a gold trophy icon, and has the lowest IC_{50} value of 63.94 ppm. The corresponding red bar is the shortest, visually reinforcing its superior efficacy. This result signifies that, of all the samples tested, the modified oil required the least concentration to achieve the benchmark 50% radical scavenging effect. Vitamin E (300 IU), The commercial high-dose Vitamin E standard ranks second, with an IC_{50} value of 75.48 ppm. While still a potent antioxidant, it is demonstrably less effective than the modified oil extract, requiring a higher concentration to achieve the same result. The blue bar is correspondingly longer than that of the modified oil. The Empiric Oil produced by the traditional empiric method ranks last among the three. It has the highest IC_{50} value of 95.55 ppm, indicating it is the least potent. A significantly higher concentration of this oil was needed to neutralize 50% of the free radicals, a fact that is visually represented by its long, brown bar.



A schematic and graphical representation of the final antioxidant potency, measured as the IC_{50} value. The left panel explains the IC_{50} concept using a balance scale analogy. The right panel provides a direct ranking of the samples, where a shorter bar represents a lower IC_{50} value and therefore higher potency. The data clearly establishes the superior potency of the oil from the Process-Modified Method.

Figure 5. Comparative antioxidant potency (IC_{50} Value).

The findings of this investigation provide a compelling, data-driven argument for the paradigm shift from traditional processing to modern, scientifically-informed techniques for the valorization of *Pandanus conoideus* Lim.[11] The transformative improvements in both oil yield and a key quality marker—in vitro radical scavenging activity—underscore the profound impact of a process engineering-centric approach. The remarkable 85.2% increase in oil yield is not an artifact but a direct consequence of a multi-stage, synergistic approach that optimizes the fundamental principles of mass transfer and phase separation. Each step in the modified protocol was designed to overcome a specific physical barrier inherent in the traditional method. The initial transition from direct boiling to steam distillation is a critical first step. The plant cell walls that sequester the oil bodies (oleosomes) are composed of a complex matrix of cellulose, hemicellulose, and lignin. In direct boiling, the heat transfer can be uneven, and the fruit matrix can become saturated with water.[12] Steam, however, provides a more efficient mechanism of heat transfer through condensation. This process, known as hydrothermolysis, utilizes the latent heat of steam to rapidly and uniformly heat the plant tissue, causing the cellular structure to swell and rupture. This efficient disruption of the cell wall matrix is the crucial first step in liberating the encapsulated oil, a process far more effective than simple boiling.[13] This liberation is then capitalized upon by the mechanical screw press. This device generates both high compressive force and high shear stress, a combination that maximizes the mechanical rupturing of plant cells. Unlike the limited, purely compressive force of manual squeezing, the screw press ensures a more complete disruption of the cellular architecture, leading to a far more efficient expression of the liquid phase. Finally, the introduction of centrifugation addresses the critical challenge of breaking the natural Pickering emulsion present in the raw extract. This emulsion is stabilized by a combination of natural surfactants and fine cell wall fragments that form a physical barrier around the oil droplets.[14] The high relative centrifugal force (3500 x g) overcomes these stabilizing forces, dislodging the particulate matter and forcing the coalescence of the less dense oil droplets, leading to a rapid and clean phase separation. This step recovers a significant fraction of emulsified oil that is irretrievably lost in the crude gravitational and evaporative separation of the empiric method, providing the final contribution to the dramatically enhanced yield.[15]

The superior antioxidant activity of the oil from the modified method, as evidenced by its significantly lower IC_{50} value, is a direct consequence of the preservation of its delicate bioactive phytochemicals.[16] To understand this, it is essential to first consider the pathophysiology of oxidative damage that these compounds are meant to combat. At the cellular level, ROS initiate a devastating cascade of damage. Lipid peroxidation, a primary mechanism, begins when a free radical abstracts a hydrogen atom from a polyunsaturated fatty acid in a cell membrane. This creates a lipid radical, which reacts with molecular oxygen to form a lipid peroxyl radical. This new radical can then attack an adjacent fatty acid, perpetuating a destructive chain reaction that compromises membrane structure and function. Concurrently, ROS can oxidize amino acid side chains in proteins, leading to the formation of carbonyl groups and causing proteins to misfold, aggregate, and lose their enzymatic function. In the nucleus, hydroxyl radicals can attack DNA bases, leading to mutations that can trigger apoptosis or carcinogenesis. The potent antioxidants in *P. conoideus* oil, primarily carotenoids and tocopherols, are molecularly equipped to interrupt these processes. Tocopherols (Vitamin E) are chain-breaking antioxidants.[17] Their defining feature is a chromanol ring with a phenolic hydroxyl group. This hydroxyl group can readily donate its hydrogen atom to a lipid peroxyl radical, thereby neutralizing the radical and terminating the lipid peroxidation chain reaction. The resulting tocopheroxyl radical is relatively stable and unreactive due to resonance stabilization within the aromatic ring, preventing it from propagating the chain reaction.[18] Carotenoids,

such as β-carotene, function through a different but complementary mechanism. Their long, conjugated polyene chain is highly effective at physically quenching singlet oxygen, a high-energy form of oxygen that is a potent initiator of oxidative damage. They can also scavenge peroxyl radicals, adding the radical to their conjugated system. This synergistic action of different classes of antioxidants provides a multi-pronged defense against oxidative stress. The key to preserving this functionality lies in protecting their molecular structures from degradation. The uncontrolled high temperatures of the empiric boiling method are incredibly destructive to these molecules. The conjugated double bond systems of carotenoids are susceptible to heat-induced isomerization (from the more active all-trans form to less active cis isomers) and oxidation, which breaks the chain and destroys their antioxidant capacity.[19] Tocopherols can also be oxidized and degraded at high temperatures. The process-modified method systematically mitigates this thermal damage. Steaming provides a more controlled heat input, and the crucial step of low-temperature vacuum evaporation at 50°C removes water without subjecting the oil to the harsh temperatures of atmospheric boiling. By maintaining a low-temperature environment, the modified method preserves the structural integrity of the carotenoids and tocopherols, ensuring that a higher concentration of these active molecules is present in the final product. The lower IC_{50} value is, therefore, a direct biochemical indicator of this superior molecular preservation.

The observation that the oil from the modified process (IC_{50} = 63.94 ppm) was more potent than a high-dose commercial Vitamin E supplement (IC_{50} = 75.48 ppm) is a testament to the power of natural phytochemical synergy. The commercial supplement provides a high concentration of a single antioxidant entity, α-tocopherol. The Red Fruit oil, however, contains a complex and diverse cocktail of bioactive compounds. It contains multiple forms of tocopherols and tocotrienols, alongside a rich spectrum of carotenoids. It is widely recognized in nutritional science that these compounds can act synergistically. For example, Vitamin C can regenerate the oxidized form of Vitamin E back to its active state, and carotenoids can protect lipids in different cellular microenvironments than tocopherols. This "antioxidant network" within the natural oil matrix results in a combined effect that is more powerful than a high dose of any single compound. This finding has profound implications for the nutraceutical industry. It strongly suggests that a well-processed, whole-food-derived extract like Red Fruit oil may offer superior protective benefits compared to isolated, synthetic vitamins. The development of a standardized extraction process, as detailed in this study, is the critical first step in producing a consistent, high-efficacy product. This allows for reliable dosing and quality control, making it a viable candidate for formulation into premium dietary supplements, functional foods, and cosmeceuticals. The ability to produce a product with scientifically validated potency that is superior to existing market standards provides a powerful competitive advantage and a strong foundation for market entry. The study effectively provides a blueprint for transforming a traditional Papuan resource into a high-value, globally relevant health product, which could in turn create significant economic opportunities for the local communities where Red Fruit is grown.[20]

Figure 6 provides a powerful and elegant conceptual summary of the entire study, serving as a scientific roadmap that connects the experimental actions to their theoretical underpinnings and ultimate outcomes. The flowchart begins at the top with the "CAUSE: Process Modifications," which details the three key interventions that differentiate the modified method from the traditional one. These are: Gentle Thermal Treatment: The replacement of harsh, direct boiling with controlled steam distillation; Efficient Mechanical Force: The substitution of manual squeezing with high-pressure automatic pressing; Advanced Phase Separation: The introduction of centrifugation for precise emulsion breaking.

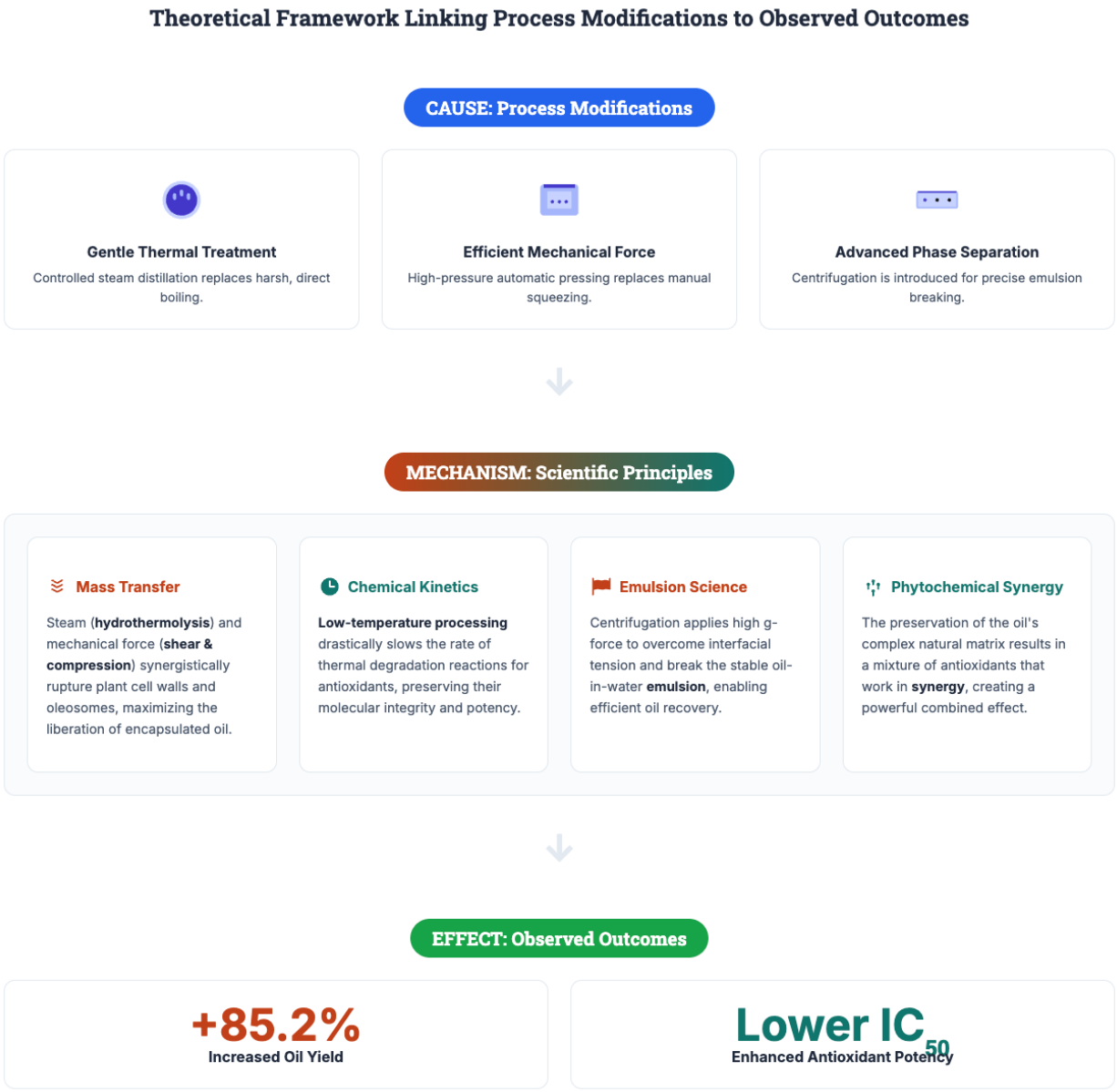


Figure 6. Theoretical framework linking process modifications to observed outcomes.

This section establishes the independent variables of the study—the specific, targeted changes made to the extraction process. Each modification was rationally designed to address a known weakness in the traditional method. The central and most insightful part of the figure is the "MECHANISM: Scientific Principles" section. This acts as the crucial explanatory bridge, detailing the scientific theories that explain why the process modifications lead to the observed improvements. The figure breaks this down into four key principles: Mass Transfer: This principle is linked to the physical recovery of the oil. The figure explains that steam (hydrothermolysis) and mechanical force (shear & compression) work together to rupture plant cell walls and oleosomes, maximizing the liberation of the encapsulated oil. This explains the purely physical aspect of getting more oil out of the fruit matrix. Chemical Kinetics: This principle addresses the preservation of the oil's quality. The figure notes that low-temperature processing slows the

rate of thermal degradation reactions for the delicate antioxidants. This is a fundamental concept where lower temperatures reduce the kinetic energy of molecules, dramatically decreasing the likelihood of undesirable reactions (like oxidation or isomerization) that would destroy the potency of the bioactive compounds. Emulsion Science: This principle explains the enhanced clarity and recovery of the oil. The figure states that centrifugation applies high g-force to break the stable oil-in-water emulsion, enabling efficient oil recovery. This highlights the role of overcoming interfacial tension to coalesce the dispersed oil droplets into a continuous, easily separable phase. Phytochemical Synergy: This principle provides a biochemical explanation for the enhanced potency. The preservation of the oil's complex natural matrix results in a mixture of different antioxidants that work in synergy, creating a combined effect that is more powerful than any single compound alone. The flowchart concludes at the bottom with the "EFFECT: Observed Outcomes," which presents the final, measurable results of the study. These are the dependent variables that are a direct consequence of the process modifications, as explained by the scientific mechanisms. The two key outcomes shown are:A +85.2% Increased Oil Yield, a direct result of the improved mass transfer and emulsion science; A Lower IC_{50}, signifying Enhanced Antioxidant Potency, which is a direct result of the principles of chemical kinetics and phytochemical synergy. Figure 6 tells a complete scientific story. It elegantly demonstrates that the study is not a result of trial and error, but of a rational design process rooted in a deep understanding of chemistry, physics, and biology. It shows how applying these fundamental scientific principles to modify an existing process leads directly and predictably to a superior outcome, both in quantity and quality.

4. Conclusion

This investigation successfully designed and validated a process-modified method for extracting oil from Papuan Red Fruit (*Pandanus conoideus* Lim). The findings conclusively demonstrate that this scientifically grounded approach is vastly superior to the traditional empiric method. The modified protocol, which integrates steam distillation, mechanical pressing, centrifugation, and controlled-temperature vacuum evaporation, resulted in a remarkable 85% increase in oil yield, from 10.8% to 20.0%. Critically, this quantitative gain was matched by a significant qualitative improvement; the resulting oil exhibited enhanced antioxidant potency, with an IC_{50} value of 63.94 ppm, which was stronger than both the empirically extracted oil (95.55 ppm) and a high-dose commercial Vitamin E standard (75.48 ppm). This enhancement is attributed to the minimized thermal degradation of sensitive bioactive compounds. This study provides a robust, scalable, and reproducible framework for the production of *P. conoideus* oil, transforming it from a traditional remedy of variable quality into a standardized, high-potency nutraceutical ingredient. The developed methodology lays the essential groundwork for the sustainable commercialization of Red Fruit, offering potential economic benefits to local communities and providing consumers with a potent, evidence-based source of natural antioxidants.

5. References

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