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Research Article

Synergetic Effect of Fermented Coconut Inflorescence Sap for the Production of Virgin Coconut Oil

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The study aimed to optimize the process parameters for the production of virgin coconut oil (VCO) by exploring the use of aerobic fermented neera (coconut inflorescence sap) at different concentrations (ranging from 5% to 25%). Fermented neera can be prepared from fresh neera without incurring additional expenses, presenting a potential advantage over commercially available cultures in the market. Coconut milk was extracted from a coconut kernel using a hydraulic coconut milk expeller. The extracted coconut milk was then fermented with varied concentrations of fermented neera ranging from 5% to 25%, and then, the oil layer was separated. Fermented neera as a starter at a concentration of 10% resulted in the highest VCO yield of 19.8% following 14 hours of fermentation. The study also found that the fermentation duration of the starter (6-hour, 8-hour, and 10-hour fermented neera) and its concentration had significant effects on the yield of VCO. However, the effects of starter on the physical properties (moisture content, viscosity, specific gravity, and refractive index) and biochemical profile (free fatty acid, peroxide value, acid value, saponification value, iodine value, and total phenolic content) of the resultant VCO were not significant. The physicochemical properties of the VCO produced using the fermented neera-based coconut milk fermentation method met the standards stipulated by the Food Safety and Standards Authority of India (FSSAI) and the Asian and Pacific Coconut Community (APCC). Thus, fermented neera offers a novel economical alternative to enhance the fermentation rate during VCO production.

1. Introduction

Coconut (*Cocos nucifera* L.) assumes great significance in India as a cultural and economic crop. Various value-added products such as virgin coconut oil (VCO), coconut chips, coconut milk, coconut flakes, and desiccated coconut powder have gained substantial market potential worldwide [1]. Recently, VCO has been recognized as a super food due to its functional and nutraceutical properties, as well as its antimicrobial and antiviral characteristics [2, 3].

Virgin coconut oil (VCO) can be produced using different methods, including the hot process, fermentation process, centrifugal process, and extraction from dried gratings. Among these methods, fermentation and centrifugal processes are referred to as cold processes since these processes do not involve the generation of heat. The fermentation method is particularly attractive for VCO production due to its simplicity, minimal machinery requirements, and the preservation of bioactive compounds such as vitamin E, tocotrienols, polyphenols, and phytosterols [3, 4].

Two fermentation methods are commonly used for VCO production: natural fermentation and induced fermentation techniques. Natural fermentation is more popular than induced fermentation, mainly because the latter requires higher initial costs in the development of inoculums/

cultures. Lactic acid bacteria plays a significant role in both the fermentation methods and the presence of bacteriocin in these bacteria offers additional advantages in terms of antibacterial properties, a distinguishing feature from other VCO production methods [5].

Different cultures, such as bacteria (*Lactobacillus plantarum*), yeast (*Saccharomyces cerevisiae*), and mold (*Rhizopus oryzae, Rhizopus oligosporus,* and *Rhizopus stolonifer*), can be utilized while producing VCO from the fermentation method [6–8]. For instance, Purba et al. [8] demonstrated that the addition of baker's yeast at a concentration of 15%, during fermentation, resulted in a VCO yield of 13.6% over 24 hours. Another recent study reveal that using 5% *Saccharomyces cerevisiae* in VCO production led to a yield of 25.74% after 24 hours of fermentation [6]. Similarly, the addition of 5% *Lactobacillus plantarum* and 5% *Rhizopus oligosporus* resulted in 24.15% and 22.01% yields, respectively. Additionally, the use of crude papain enzyme at a 1.5% concentration and an incubation temperature of 40°C yielded 18.80% VCO [9]. It was documented that the microorganism exhibits varied performances in terms of yield, fermentation time, and physicochemical properties of VCO.

However, the use of a pure bacterial strain would escalate the production cost of VCO. As a result, VCO industries warrant an effective culture and or starter that can enhance yield and reduce fermentation time without increasing production costs. The retention of minor nutrients and bioactive components is crucial during VCO production, alongside the objective of increased yield. In this study, fermented neera (FN) was used to enhance the VCO yield. Microorganisms such as yeasts and bacteria in neera obtained from the spadix of coconut palm initiate fermentation, leading to the production of ethyl alcohol. The duration of fermentation and the amount of alcohol produced depend on the storage time following neera collection [10]. Somashekaraiah et al. [11] analyzed lactic acid bacteria (LAB) isolates from neera and demonstrated that the LAB exhibited potential probiotic properties, suggesting their utility in the production of functional fermented foods. However, no literature is available regarding the application of FN in the process of VCO production adopting the fermentation process. Furthermore, fermented neera can be prepared from fresh neera without additional cost. In this context, this work aims to evaluate the effects of FN as a starter during the fermentation process on VCO yield, fermentation time, and physical and chemical properties.

2. Materials and Methods

2.1. Raw Materials. VCO production involved the use of matured fresh coconuts (Variety: WCT; Maturity: 12 months) sourced from the farm section of ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI) located in Kasaragod, India.

2.2. Preparation of Coconut Milk. The coconut dehusking process was carried out using a power-operated machine, while the deshelling process was done with a machine developed by ICAR-Central Plantation Crops Research Institute (CPCRI). A testa-removing machine was then used to eliminate the outer covering, or testa, of the coconut kernel. Afterward, the testa-free coconut kernel underwent a washing procedure before being fed into a mechanical grating machine that could process up to 250 nuts per hour. The resultant grated coconut meat was then pressed using an in-house developed manually operated hydraulic coconut milk expeller to extract the coconut milk.

2.3. Preparation of Starter. The neera-based starter was prepared through the natural fermentation process of neera obtained from CPCRI, Kasaragod, India (Figure 1). In this experiment, 600 mL of neera was distributed into three separate beakers and allowed to ferment for 6 hours, 8 hours, and 10 hours, respectively, at a room temperature of 27 ± 2°C with a relative humidity of 78 ± 2% (Figure 1).

2.4. Fermentation Procedure. Beakers of 250 mL capacity were used to hold approximately 200 mL of coconut milk. Different starters obtained from FN, which underwent the fermentation process for 6, 8, and 10 hours at varied concentrations ranging from 5% to 25%, were added to the beakers. The mixtures were stirred gently with a spatula until they were uniformly blended. To prevent the introduction of aerobic contaminants such as bacteria, molds, or yeast, the beakers were covered with muslin cloth. The static fermentation process was carried out in a controlled environment inside the laminar airflow. Throughout the fermentation period, the temperature, relative humidity, and duration of fermentation were monitored and recorded.

2.5. Oil Separation. Once the fermentation process was complete, three distinct layers were visible: water, oil, and a curd layer that floats on top (Figure 2). The oil layer was separated using a pipette and then centrifuged at 10,000 rpm for 10 minutes to remove any sediments and to obtain pure oil. The weight of the resulting oil was measured and stored in a PET bottle for further quality analysis.

2.6. Yield Estimation. The process yield was determined using equation (1), which involved the measurement of the weight of the extracted VCO and the weight of the coconut grating utilized.

$$\text{Yield}\,(\%) = \frac{\text{Weight of oil}\ (g) \times 100}{\text{Weight of coconut grating}\ (\text{kg})}. \quad (1)$$

2.7. Estimation of pH. The pH of the samples was measured using a digital pH meter (ECPHWP15002K, Eutech, Singapore) that was calibrated with buffers of pH 4.7 and 10.

2.8. Estimation of Titratable Acidity. The titratable acidity was determined through acid-base titration, employing a standardized solution of 1N sodium hydroxide and phenolphthalein as an indicator. The results were expressed as

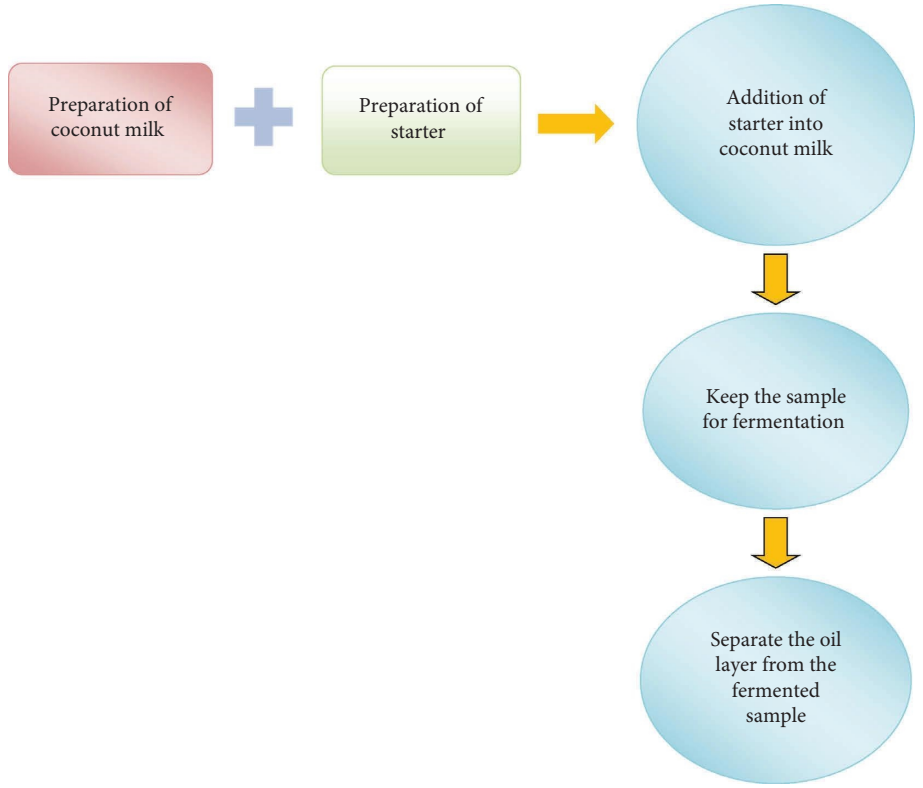

Figure 1: Process flowchart of the preparation of the VCO.

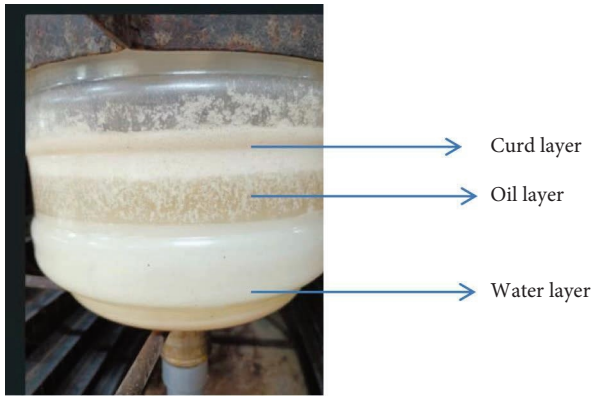

Figure 2: Formation of three different layers (curd, oil, and water) in the fermentation process of VCO production.

lactic acid equivalent, and the percentage of lactic acid content (w/v) was calculated using the standard method outlined as follows:

$$\text{Titratable acidity, \%} = \frac{N \times V \times E \times 100}{W \times 1000}, \quad (2)$$

where N = normality of NaOH. V = volume of NaOH (mL). E = equivalent weight of lactic acid. W = volume of sample (g).

2.9. Estimation of Turbidity. The Eutech TN-100 turbidimeter was employed to measure the turbidity of the samples. The sample was carefully filled in a vial until it reached the designated mark, after which it was left undisturbed to settle for a certain period. Subsequently, the vial was positioned in the sample holder of the instrument, and the measurement was taken. The constant value displayed on the instrument's screen was recorded.

2.10. Determination of Total Soluble Solids. The measurement of total soluble solids (TSS) was conducted using a handheld refractometer manufactured by M/S Erma, Japan. The TSS value was expressed in degrees, Brix.

2.11. Estimation of Moisture Content. The moisture content of the VCO samples was analyzed using an A&D MX-50 moisture analyzer, following the procedure outlined by Ramesh et al. [3].

2.12. Estimation of Viscosity. The viscosity of VCO samples was measured using a Brookfield viscometer (DVNXLVMJG, AMETEK). It works by detecting the torque required to rotate a spindle that remains at a constant speed while being submerged in the sample, allowing for viscosity measurement. To conduct the test, a beaker containing around 200 mL of oil sample was used, and the spindle number 61 was set to rotate at a speed of 150 rpm. The viscometer was run for approximately 5 minutes, and the constant value was recorded.

2.13. Estimation of Specific Gravity. To determine the specific gravity of different VCO samples, their mass and volume were measured. First, a preweighed 1 mL pipette tip was used

to transfer the VCO samples into a tube. The combined weight of the sample and the tip was then measured using a weighing balance. Finally, the specific gravity was calculated using equation (3) [12].

$$\text{Specific gravity} = \frac{\text{Density of oil}}{\text{Density of water}}. \quad (3)$$

2.14. Estimation of Refractive Index. The refractive index of VCO samples was determined by utilizing a pocket refractometer (M/S Erma, Japan) based on the principle of refraction. The reading was subsequently converted into refractive index values using a conversion table [13].

2.15. Estimation of Free Fatty Acid. Precisely, 2 grams of an oil sample was measured into a 50 mL conical flask. Then, 10 mL of a mixture comprising ethanol and diethyl ether (1 : 1 ratio) was added. A few drops of phenolphthalein acted as an indicator. The resulting solution was titrated with 0.01N KOH solution until a pale pink color emerged, which remained stable for a minimum of 15 seconds. The recorded titration value was used to calculate the percentage of free fatty acid as oleic acid equivalent, employing the following equation:

$$\text{FFA (\% oleic acid)} = \frac{\text{VNM}}{10W}, \quad (4)$$

where V = titre value. N = normality of KOH. M = molecular weight of oleic acid. W = weight of sample (g).

2.16. Estimation of Peroxide Value. Approximately, 5 g of oil was taken in a 50 mL conical flask. To dissolve the oil, a mixture of about 10 mL of acetic acid and chloroform was added and shaken vigorously. Next, 0.5 mL of saturated potassium iodide solution was added and the mixture was left undisturbed for 1 minute until a yellow color appeared. Then, 30 mL of distilled water was added to 1 mL of 1% starch solution.

To determine the endpoint, a gradual addition of a 0.01 N Na_2SO_3 solution was performed, while using a starch solution as an indicator. The addition was continued until the color changed from its original hue to a colorless state. The titer value was measured and PV was calculated using equation (6) [14].

$$\text{Peroxide Value} = \frac{\text{Titre value} \times \text{Normality of } Na_2SO_3 \times 1000}{\text{Weight of sample}}. \quad (5)$$

2.17. Estimation of Acid Value. In a 25 mL conical flask, approximately, 2 grams of oil sample was added. Next, a mixture of ethanol and diethyl ether, previously neutralized, was added to the flask and made up to 10 mL. Two drops of phenolphthalein were included as an indicator. The resulting solution was titrated against a 0.01N solution of KOH until a pale pink color appeared which remained for 15 seconds. The volume of KOH solution required to reach this endpoint, known as the titer value, was recorded. Using equation (6), the acid value was then calculated as the number of milligrams of KOH per gram of the oil sample [15].

$$\text{Acid value (\%)} = \frac{\text{Titre value (mL)} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample } (g)}. \quad (6)$$

2.18. Estimation of Saponification Value. Approximately, 1 gram of oil sample was added to a 250 mL round bottom flask. A measured 25 mL of alcoholic KOH from a burette was added slowly over a specific period while thoroughly shaking the flask. For blank, 25 mL of KOH was also allowed to drain from the burette for the same duration. An air condenser was connected to the flask, and the mixture was gently refluxed for approximately 30 minutes. Afterward, the saponified oil was cooled, and a small amount of distilled water was used to rinse the inside of the condenser. Then, around 1 mL of phenolphthalein indicator was added, and the mixture was titrated against 0.5 N HCl until the pink color disappeared [16].

2.19. Estimation of Total Polyphenol Content. The Folin–Ciocalteau method with slight modifications was used to estimate the total polyphenol content [3]. Briefly, 1 mL of oil extract was taken in a 10 mL centrifuge tube and the volume was adjusted to 3 mL using distilled water. A blank sample containing 3 mL of distilled water was also prepared. To this mixture, 100 μL of FCR reagent was added, followed by the addition of 0.5 mL of 20% Na_2CO_3. The solution was vortexed for 2 minutes and then incubated for 45 minutes. After incubation, it was centrifuged at 10,000 rpm for 15 minutes, and the absorbance was measured at a wavelength of 745 nm using a UV-visible recording spectrophotometer (Shimadzu UV-160 A).

2.20. Estimation of Iodine Value. 2 g of oil sample was added to a 500 mL iodine flask. To dissolve the sample, 10 mL of carbon tetrachloride (CCl_4) was added. Subsequently, 20 mL of iodine monochloride (ICl) solution was introduced. The resulting mixture was left undisturbed in a light-free environment at a temperature ranging from 15° to 25°C for 30 minutes, with a topper securely in place. Afterward, 15 mL of potassium iodide solution was added. To ensure thorough mixing, the flask and stopper were gently swirled with 100 mL of water, followed by agitation. The resulting solution was titrated against 0.1 M sodium thiosulphate solution until the endpoint of the titration, using a freshly prepared starch solution as an indicator [17].

2.21. Estimation of Fatty Acid Profile. Gas chromatography (GC) was employed to determine the fatty acid composition of the oils after they had been converted to fatty acid methyl esters (FAMEs). Methyl esters are frequently examined derivatives that are produced through methylation. This method involves the hydrolysis of ester bonds in complex

lipids to release free fatty acids, which are then transmethylated to form FAMEs. The procedure outlined by Supriya et al. [18] and Shunmugiah Veluchamy et al. [19] was used to estimate the fatty acid composition.

2.22. Statistical Analysis. Three replications were made for analyzing each parameter. The statistical analysis was conducted using two-way ANOVA at the significance level of $p \leq 0.05$. The relationship between the parameters was analyzed by the principal component analysis (PCA) technique. The summary statistic and correlation among parameters using Pearson correlation were also performed. The statistical analysis was performed by using Minitab 21.

3. Results and Discussion

3.1. Physicochemical Profile of Fermented Neera. The physicochemical composition of neera fermented for 6 hours, 8 hours, and 10 hours was analyzed, and the findings are presented in Table 1. Throughout the fermentation process, neera exhibited increased turbidity and a lighter color (golden brown to white). The pH of neera decreased from 6.4 to 4 during fermentation. The decrease in pH is often a result of the accumulation of specific acids, such as lactic acid or acetic acid, depending on the microorganisms involved in the fermentation process [12]. Gopal et al. [20] reported that the fermentation of neera due to the symbiotic culture of bacteria and yeasts. The titratable acidity increased from 0.024% to 0.367%. However, there were no notable alterations in the overall soluble solids content across the different fermentation durations.

3.2. Effect of Starter Incubation Period and Concentration on VCO Yield. A significant yield of VCO (19.8%) was obtained from 8 h fermented neera (FN) at a concentration of 10% (Figure 3). Similarly, the utilization of 6 h FN at a 10% concentration resulted in the highest yield. In our previous study [18], it was observed that the 6-hour FN contains too high population of yeasts colonies than 2 and 4 h FN. Shetty et al. [22] found that the lactic acid bacteria were predominant up to 7-8 h of fermentation and then the bacteria count decreased. Hence, 6 and 8 h FN offered appreciable results than 10 h FN. Increasing the concentration of fermented neera beyond this led to a decrease in VCO yield. These findings highlight the substantial influence of both fermentation duration and FN concentration on the production of VCO. The optimal conditions for achieving the highest VCO yield appear to be a 24-hour fermentation period with a 10% FN concentration. This indicates that a specific duration of fermentation is indispensable to facilitate the metabolic processes responsible for VCO synthesis effectively. Furthermore, the concentration of FN plays a critical role in determining the yield, with a 10% concentration proving to be effective. The lactic acid bacteria were predominantly found in FN [23]. The presence of sucrose content in neera could increase the log phase of lactic acid bacteria and *Saccharomyces cerevisiae* during fermentation [21]. The presence of lactic acid bacteria and *Saccharomyces cerevisiae* separates the fat globules from the protein layer during the fermentation of coconut milk. Satheesh and Prasad [24] examined the impact of temperature, pH, *Lactobacillus plantarum* concentration, and fermentation time on the yield of VCO. It was optimized that specific treatment conditions, including a temperature of $45 \pm 1^{\circ}$C, pH of 5.0 ± 0.1, inoculum concentration of 2%, incubation time of 48 hours, and anaerobic conditions, could enhance the yield by up to 34.68% (wet basis).

The temperature range employed in this study, $27 \pm 2^{\circ}$C, was found to be below the optimal range recommended by previous research. This suggests that the metabolic activity of *Lactobacillus* species in the current study may not have been at its peak when compared to their performance at higher temperatures. Nevertheless, it is important to observe that *Lactobacillus* species possess adaptability to various environmental conditions and can still exhibit metabolic activity and survive at lower temperatures, albeit potentially with reduced efficiency [25, 26].

3.3. Effect of Starter Incubation Period and Concentration on Fermentation Time. The objective of this study is to shorten the fermentation time by increasing the growth rate of fermentative bacteria cells. Studies have indicated that aerobic conditions can enhance the growth rate of *Lactobacillus* spp. [24]. To facilitate aerobic fermentation, the upper part of the fermentation chamber was covered with muslin cloth. Somashekaraiah et al. [12] isolated LAB from fresh and naturally fermented coconut palm sap. Satheesh and Prasad [24] also found that a fermentation time of 48 hours is necessary for proper separation of the oil and water layers when 2% *Lactobacillus plantarum* is added.

Table 1: Physiochemical profile of the fermented neera.

Starter	pH	Acidity (%)	Turbidity (NTU)	TSS (%)
Fresh neera	6.4 ± 0.2	0.024 ± 0.0	80 ± 5	15.7 ± 0.1
6 h fermented neera	4.3 ± 0.3	0.297 ± 0.0	238 ± 12	15.9 ± 0.1
8 h fermented neera	4.1 ± 0.2	0.361 ± 0.0	298 ± 18	15.6 ± 0.1
10 h fermented neera	4.0 ± 0.2	0.367 ± 0.0	349 ± 24	15.7 ± 0.0

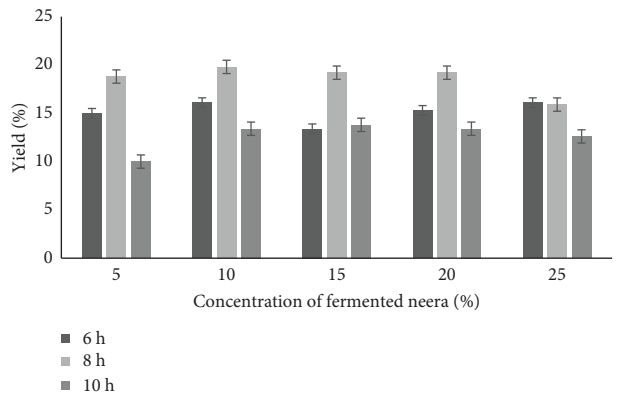

Figure 3: Yield of the virgin coconut oil (VCO) prepared from different concentrations of fermented neera.

However, the addition of FN resulted in the separation of oil and water layers within 14 hours. This difference could be attributed to the fermentation method (aerobic/anaerobic) and the strength of the starter culture employed.

3.4. Effect of Starter Incubation Period and Concentration on VCO Moisture. Table 2 presents the moisture content of VCO prepared using various cultures and concentrations (5–25%). Moisture content plays a crucial role in the formation of free fatty acids in VCO, contributing to hydrolytic rancidity. Moreover, high moisture levels in VCO can lead to the hydrolysis of fat molecules and/or oxidation, reducing its shelf life [6]. The highest VCO moisture content was observed when 10-hour FN ($0.156 \pm 0.005\%$) of 25% concentration was applied. Conversely, the lowest moisture content ($0.143 \pm 0.005\%$) was found in 6 h FN (5% concentration) and 8 h FN (20% concentration). The moisture content of VCO was not significantly affected by different starters and concentrations. These findings comply with previous findings [3] and comply with the moisture content standards stipulated by FSSAI (not exceeding 0.5%) and APCC (0.1 to 0.5%) for VCO. Similarly, Purba et al. [8] reported a moisture content of 0.113–0.136% in baker's yeast fermented VCO. The authors also observed that an increase in starter concentration would lead to higher moisture accumulation in the VCO.

3.5. Effect of Starter Incubation Period and Concentration on VCO Viscosity. Viscosity refers to the resistance to flow exhibited by a fluid [6]. It is influenced by factors such as temperature, molecular shape, size, and molecular weight. In this study, all the samples exhibited a viscosity value of 37 Pa s. The viscosity of oil samples is higher when they contain a larger proportion of fatty acids with double bonds, while samples with a higher content of saturated fatty acids have lower viscosity [6]. Furthermore, the presence of proteins and total soluble solids also contributes to an increase in oil viscosity. Similarly, Asiah et al. [6] emphasized that VCO has a low viscosity due to its composition of 90% medium-chain saturated fatty acids and 10% unsaturated fatty acids. In comparison to the reported value (48.73) by Mansor et al. [27], the viscosity of VCO samples in this study showed a significantly low value. These differences in viscosity could be attributed to variations in temperature.

3.6. Effect of Starter Incubation Period and Concentration on VCO Specific Gravity. Specific gravity refers to the ratio of the density of oil to the density of an equal volume of water at a specific temperature. Determining the specific gravity is crucial as it impacts oil storage, rancidity, and other related factors. The influence of different starters and their concentrations on the specific gravity of VCO is presented in Table 2. The specific gravity of VCO ranges from 0.90 to 0.92, with the highest value (i.e., 0.92) observed in VCO prepared using 6- and 8-hour FN and a 10% concentration of the starter. These values are in accordance with the standard specific gravity range for VCO set by CODEX (0.908–0.921) and the APCC standards (0.915–0.920) and are consistent with previous studies [28, 29].

3.7. Effect of Starter Incubation Period and Concentration on VCO Refractive Index (RI). The refractive index (RI) is a measure of how fast light travels in a vacuum compared to its speed in a fat sample. It is an indicator of the oil's saturation level. In the case of VCOs extracted using FN, the observed refractive index was 1.449. This value falls within the range of the Indian standard (1.448–1.449) and the APCC standard (1.448–1.449). Changing the starter concentration and fermentation time did not cause any significant variations in the RI values. Srivastava et al. [29] also reported a similar refractive index of 1.448. These findings support the results reported by Satheeshan et al. [28]. Overall, the results suggest that the fermentation process was stable and suitable for reliable industrial production, as the RI values remained consistent.

3.8. Effect of Starter Incubation Period and Concentration on Free Fatty Acids. The range of free fatty acid (FFA) content in VCO prepared using fermented neera was found between 0.164% and 0.197% (Figure 4). VCO samples prepared with 24 hours of FN, at both 5% and 15% concentration levels, showed the lowest FFA value of 0.164%. All the samples had significantly lower FFA content confining to the specifications set by the Asia Pacific Coconut Community (≤0.2%) for VCO and the CODEX oil standards (≤0.3%). This suggests that the VCO produced met the requirements of Codex and APCC and is unlikely to exhibit signs of rancidity. Lower FFA values indicate reduced susceptibility to rancidity [30]. The fermentation process involves the activity of different microorganisms, including bacteria and yeast, which can produce extracellular lipases. These lipases hydrolyze triglycerides into free fatty acids. The microbial lipases are released into the fermentation medium and contribute to the increase in FFA content during the fermentation process. Factors such as fermentation time, temperature, microbial strain, and moisture content influence the generation of FFAs [31].

Ghani et al. [16] examining the physicochemical properties, antioxidant capacities, and metal contents of VCO produced through wet and dry processes found that the free fatty acid (FFA) values of VCO were similar between the two processes. However, the FFA value obtained in this study was low compared to the FFA value ($0.293 \pm 0.025\%$) reported by Ramesh et al. [3] for VCO. The FFA content of VCO can serve as an indicator of its flavor and aroma. Thus, the lower FFA value observed in this study suggests that the VCO produced meets the sensory quality requirements of VCO in terms of flavor and aroma.

3.9. Effect of Starter Incubation Period and Concentration on Peroxide Value. The peroxide number is an important parameter that has a serious implication on the deterioration of oil, serving as an early indicator of rancidity [32]. All the samples analyzed showed a consistent peroxide number of 0.01 m eq. peroxide oxygen/Kg of oil. These results indicate that the VCO meets the quality standards of APCC (maximum 0.3 m eq. peroxide oxygen/Kg of oil).

Table 2: Physical characteristic features of VCO samples prepared by fermentation method.

Starter	Percentage of starter (%)	Moisture (%)	Viscosity* (Pa.s)	Specific gravity	Refractive Index*
6 h fermented neera	5	0.143 ± 0.005	37	0.910 ± 0.005	1.449
	10	0.150 ± 0.005	37	0.916 ± 0.005	1.449
	15	0.146 ± 0.005	37	0.913 ± 0.005	1.449
	20	0.153 ± 0.005	37	0.910 ± 0.005	1.449
	25	0.156 ± 0.005	37	0.906 ± 0.005	1.449
8 h fermented neera	5	0.153 ± 0.005	37	0.913 ± 0.005	1.449
	10	0.153 ± 0.005	37	0.916 ± 0.005	1.449
	15	0.143 ± 0.005	37	0.906 ± 0.005	1.449
	20	0.146 ± 0.005	37	0.906 ± 0.005	1.449
	25	0.156 ± 0.005	37	0.910 ± 0.005	1.449
10 h fermented neera	5	0.156 ± 0.005	37	0.913 ± 0.005	1.449
	10	0.150 ± 0.005	37	0.910 ± 0.005	1.449
	15	0.146 ± 0.005	37	0.913 ± 0.005	1.449
	20	0.143 ± 0.005	37	0.910 ± 0.005	1.449
	25	0.153 ± 0.005	37	0.906 ± 0.005	1.449

* ± 0.

Water content in the oil can influence the level of peroxide number as moisture in the oil can act as a catalyst for the formation of peroxides by peroxide enzymes. Additionally, it can oxidize saturated fatty acids, generating methyl ketones, which contribute to oil rancidity [33]. This observation was in accordance with the findings of Teguh Isworo Program Studi [34], which suggests that high water content in the sample or starter may be responsible for the elevated peroxide number.

Furthermore, the low peroxide values obtained can be attributed to the immediate analysis of the oil samples without resorting to storage. This prevents any interaction between oxygen and the oil sample, which could lead to increased peroxide formation.

3.10. Effect of Starter Incubation Period and Concentration on Acid Value. The acid number is one of the crucial factors in assessing the quality of oil, as it indicates the presence of free fatty acids resulting from the hydrolysis process in VCO. Higher acid values signify greater oil quality deterioration [35]. Free fatty acids, being more reactive to factors like light and temperature, make the oil more susceptible to quick deterioration. Figure 4 demonstrates that the acid values of the produced VCO meet the APCC standards, with values being below 6 mg KOH·g^{-1}. The VCO with the lowest acid number is obtained from 20% concentrations of 6-hour FN, while the highest acid number is observed in VCO derived from 10-hour FN (Figure 5). The low acid values in the VCOs from 6-hour FN indicate high quality as they exhibit increased resistance to rancidity. During fermentation, microorganisms like bacteria and yeast convert carbohydrates in the coconut milk into various compounds, including organic acids. The acid number reflects the presence of acidic compounds and indicates the level of acidity. The lowest acid number observed in the 6-hour FN samples when used at 20% concentration can be attributed to the initial stages of fermentation, where microbial activity and metabolism are still developing. Consequently, the production of organic acids, which contribute to the acid number, may be relatively lower than at the later stages of fermentation [36].

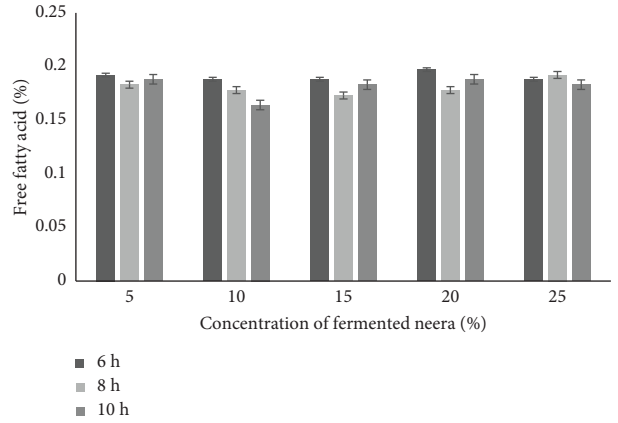

Figure 4: Free fatty acid (FFAs) content of VCO produced by the induced fermentation method.

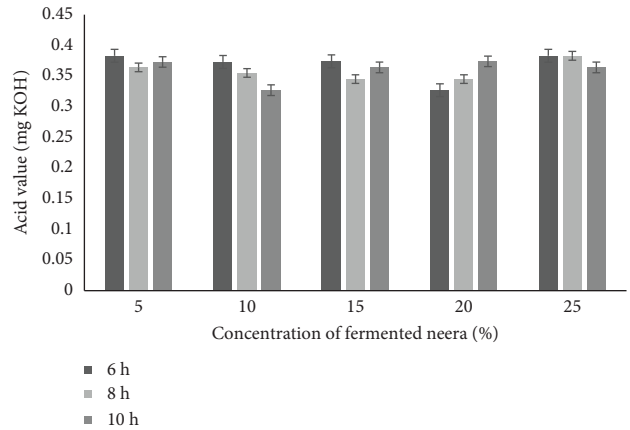

Figure 5: The acid value of VCO produced with different concentrations of fermented neera.

3.11. Effect of Starter Incubation Period and Concentration on VCO Saponification Value. The saponification value (SV) represents the average molecular weight of all fatty acids present in the oil. When the SV is higher, it indicates that the fatty acids are shorter in length. According to Figure 6, all samples exhibited high SV values ranging from 262 to 266 mg KOH/g of fat. The concentration of the starter had a significant impact on the SV, as shown in Table 2. The VCO prepared using 8-hour FN with a 10% concentration of starter recorded the highest SV.

The SV of the given samples was slightly higher than the specifications of the APCC (250–260 mg KOH/g). However, they complied with the Indian standard, which recommends an SV greater than 250 mg KOH/g. The standard value for SV in fats is typically within the range of 248–265 mg KOH/g, as stated by the Commission [37]. A little deviation in the SV could be attributed to the presence of a large amount of lauric acid, which is a medium-chain fatty acid (MCFA). It is important to note that MCFA contributes to a higher saponification number [38].

The SV of VCO samples from this study was higher than the (250.07 to 260.67 mg KOH) values reported by Marina et al. [25]. The SV depends on the average molecular mass of fats and oils and is inversely proportional to the length of the fatty acid chains within them. A higher SV indicates a shorter average chain length of fatty acids. A high SV serves as an indicator of the suitability of vegetable oil for various industrial applications, including the production of soaps, shampoos, pharmaceutical products, and food processing [39, 40].

3.12. Effect of Starter Incubation Period and Concentration on Total Phenolic Content. The total phenol content in oil serves as an indicator of its bioactive potential, such as antioxidants, antimicrobial agents, and anti-inflammatory compounds. Figure 7 displays the total phenolic content (TPC) of VCO. The analysis revealed that the TPC of VCO ranged between 1-2 mg GAE/100 g oil. Notably, VCOs produced using 6 hours of FN at a 10% concentration exhibited the highest TPC. This outcome can be attributed to the extended interaction between the oil and phenolic solution within the fermentation system, resulting in a greater incorporation of phenolic compounds into the oil [25]. According to Figure 6, though 6 h FN at 10% concentration resulted in maximum TPC, the 8 h FN at 15% concentration resulted in an equal value of TPC.

Our study yielded low total phenolic content (TPC) compared to the TPC ($401.23 \pm 20.11\ \mu g/g$) reported by Srivastava et al. [29] for VCO; however, it was in accordance with the findings of Ramesh et al. [3]. The impact of processing methods on TPC in oil has been widely recognized [25], and it is possible that the extraction process or the formation of insoluble complexes with other compounds could have contributed to the lower phenolic compound levels [41].

Studies indicate significant variation in TPC even when gallic acid is used as the standard, suggesting that different processing methods and standards utilized may influence the TPC in VCO. Furthermore, temperature fluctuations could have also affected the TPC of the oil [21, 29].

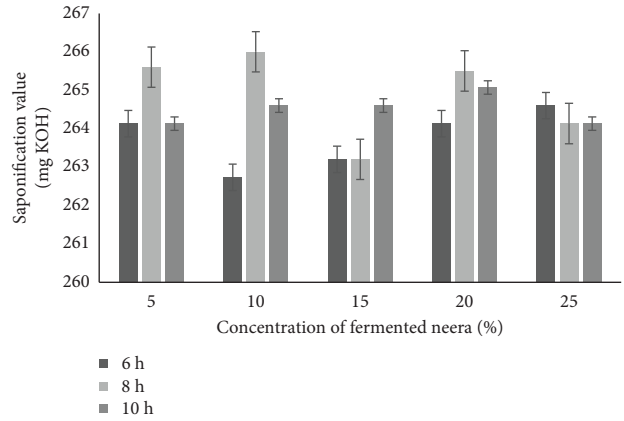

Figure 6: Saponification value of VCO prepared with different concentrations of fermented neera.

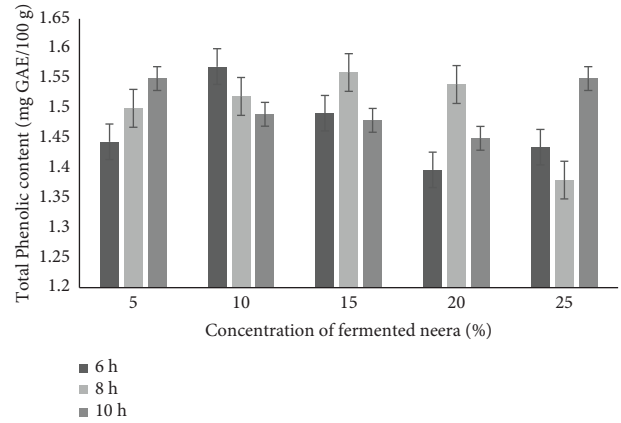

Figure 7: Total phenolic content of VCO prepared with different concentrations of fermented neera.

Interestingly, our study revealed that VCO prepared using 6-hour FN at 10% concentration exhibited the highest TPC. This suggests that the longer fermentation time and higher neera concentration allowed for a greater release of phenolic compounds into the oil. The extended contact between the oil and phenolic solution during fermentation may have facilitated the extraction and incorporation of these compounds into the VCO. The ANOVA studies for quality parameters of VCO at the significance level of $p \leq 0.05$ are presented in Table 3. There is a significant difference between the starter and the percentage of starter with the yield, IV, SV, FFA, and acid value while results of TPC are not significant at $p \leq 0.05$.

3.13. Effect of Starter Incubation Period and Concentration on Iodine Value. The iodine value is a measure of the quality of oil, indicating the relative degree of unsaturation in its components by measuring the uptake of halogen [42]. Higher iodine values signify a greater proportion of unsaturated and fewer saturated fatty acids, which are considered beneficial for health [25, 43]. However, oils with high iodine values tend to oxidize more quickly due to their higher level of unsaturation [44].

Table 3: Analysis of variance for quality parameters of VCO.

Source	DF	Adj SS	Adj MS	F value	p value
Analysis of variance for yield					
Starter types	2	266.476	133.238	6447.00	0.001
Starter percentage	4	20.660	5.165	249.92	0.001
Starter types × starter percentage	8	50.944	6.368	308.13	0.001
Error	30	0.620	0.021		
Total	44	338.700			
Analysis of variance for IV					
Starter percentage	4	0.01949	0.004873	3.31	0.023
Starter types	2	0.09599	0.047997	32.58	0.001
Starter types × starter percentage	8	0.04509	0.005637	3.83	0.003
Error	30	0.04420	0.001473		
Total	44	0.20478			
Analysis of variance for SV					
Starter percentage	4	7.817	1.9544	2.19	0.094
Starter types	2	10.064	5.0319	5.64	0.008
Starter types × starter percentage	8	17.995	2.2494	2.52	0.032
Error	30	26.780	0.8927		
Total	44	62.657			
Analysis of variance for FFA					
Starter percentage	4	0.000822	0.000205	4.23	0.008
Starter types	2	0.000981	0.000490	10.09	0.001
Starter types × starter percentage	8	0.001272	0.000159	3.27	0.008
Error	30	0.001458	0.000049		
Total	44	0.004533			
Analysis of variance for acid value					
Starter percentage	4	0.003743	0.000936	4.86	0.004
Starter types	2	0.004647	0.002324	12.06	0.001
Starter types × starter percentage	8	0.004442	0.000555	2.88	0.017
Error	30	0.005779	0.000193		
Total	44	0.018611			
Analysis of variance for TPC					
Starter percentage	4	0.03694	0.009234	0.92	0.464
Starter types	2	0.01333	0.006667	0.67	0.521
Starter types × starter percentage	8	0.10461	0.013076	1.31	0.278
Error	30	0.30009	0.010003		
Total	44	0.45496			

The Asian and Pacific Coconut Community (APCC) has specified the iodine value of coconut oils in the range of 4.1–11. This range sets acceptable limits for the iodine value in fats or oils. The observed values in the samples (6-7 g iodine/100 g oil) fall within this limit, indicating that the samples possess an acceptable level of degree of unsaturation of fatty acids characteristic of coconut oil (Figure 8). Among the samples, the highest iodine value was exhibited by the VCO prepared using 10 hours FN (6.59 ± 0.036 g/100 g oil). This suggests that the VCO obtained through this particular process has a relatively higher degree of fatty acid unsaturation than other samples. Similar results (6.300 ± 0.200 g/100 g oil) were also reported by Ramesh et al. [3].

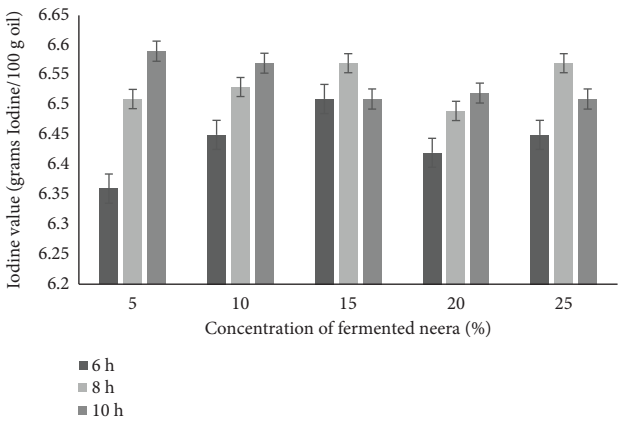

Figure 8: The iodine value of the VCOs prepared with different concentrations of fermented neera.

3.14. Fatty Acid Profile of VCO. The fatty acid composition of VCO prepared using 6 h, 8 h, and 10 h fermented neera was analyzed using gas chromatography-mass spectrometry (GC-MS). The results revealed that VCO is predominantly composed of medium-chain fatty acids (MCFAs), with lauric acid (C12) being the most abundant fatty acid. The total lauric acid content ranged from 52% to 54%, which is consistent with the APCC and CODEX standard of 43% to 53%. The highest lauric acid content (54.206%) was found in

Table 4: Fatty acid profile of VCO prepared using fermented neera.

Culture	Percentage of culture	C8	C10	C12	C14	C16	C18	C18: 1n9c	C18: 2n6C
APCC standard (%)		5–10	4.5–8	43–53	16–21	7.5–10	2–4	5–10	1–2.5
	5	5.184	5.974	52.815	19.712	7.518	3.274	4.703	0.742
6 h fermented neera	10	5.257	5.426	53.058	20.412	7.602	3.373	4.328	0.686
	15	4.851	5.53	53.845	20.277	7.565	3.177	4.28	0.575
	20	5.228	5.63	53.119	19.914	7.624	3.205	4.379	0.685
	25	3.984	5.381	53.856	20.512	7.837	3.278	4.441	0.711
	5	5.418	5.776	53.354	19.712	7.4747	3.185	4.3	0.675
8 h fermented neera	10	5.742	5.886	53.1	19.558	7.452	3.221	4.284	0.68
	15	5.435	5.628	54.206	20.106	7.579	3.14	4.303	0.694
	20	4.865	5.729	53.463	19.842	7.544	3.392	4.309	0.67
	25	5.24	5.729	53.413	19.874	7.59	3.197	4.307	0.67
	5	5.495	5.768	53.128	19.718	7.536	3.315	4.354	0.69
10 h fermented neera	10	5.719	5.807	52.967	19.633	7.525	3.33	4.342	0.683
	15	5.769	5.847	53.041	19.591	7.49	3.184	4.279	0.671
	20	5.007	5.676	53.729	19.913	7.537	3.165	4.296	0.676
	25	5.23	5.68	53.075	19.83	7.588	3.327	4.366	0.699

*All fatty acid values are in percentage. **Mean values are represented in the table.

Table 5: Correlation analysis of variables.

	Yield	Culture (%)	IV	SV	FFA	Acid value	CFP
Culture (%)	0.003						
IV	−0.075	0.034					
SV	0.262	−0.026	−0.060				
FFA	−0.226	0.132	−0.334	−0.132			
Acid value	−0.214	0.174	−0.373	−0.111	0.979		
CFP	−0.378	−0.001	0.597	0.259	−0.382	−0.413	
TPC	0.055	−0.220	0.193	−0.097	−0.242	−0.264	0.155

VCO produced using 8 h fermented neera culture. There was no significant difference in lauric acid content among the different VCO samples.

Myristic acid (C14), the second most abundant MCFA after lauric acid, showed significant variation among the samples (19%–22%). The highest myristic acid content was found in VCO prepared using 8 h fermented neera culture.

These findings suggest that the duration of fermentation of neera significantly affects the fatty acid composition of VCO. VCO produced using 8 h fermented neera has the highest lauric acid and myristic acid content, which may contribute to its potential health benefits (Table 4). The obtained values of VCO were similar to the fatty acid content of samples reported by Raghavendra and Raghavarao [26].

The high percentage of MCFAs, particularly lauric acid and myristic acid, in the VCO samples makes them promising ingredients for nutraceuticals and functional foods. MCFAs are readily absorbed and metabolized by the body, providing a rapid source of energy [45].

3.15. Correlation Analysis. The correlation analysis of variables is presented in Table 5. This analysis shows the statistical relationship between dependent and independent variables. The percentage of starters (5–25%) showed a positive correlation with acid value, FFA, IV, and yield in that order, while a negative correlation was observed with TPC and SV. The acid value and FFA increase with the addition of a starter, which contains more lactic acid bacteria, into the sample. The culture fermentation period (6, 8, and 10 h) showed a positive correlation with IV, SV, and TPC, while a negative correlation with acid value, FFA, and yield.

3.16. Principal Component Analysis. The principal component analysis of two major contributing components is shown in Figure 9. Acid value and FFA value are strongly correlated with each other followed by the percentage of starters. The starter fermentation period shows a strong positive correlation with IV followed by TPC. There is a negative correlation between yield and the percentage of starter, FFA, and acid value. The SV and percentage of culture have a weak influence on the components.

The feature that contributes the most to the x-axis, and thus for dimension 1, is TPC followed by FFA and acid value; whereas, for dimension 2 (the y-axis), the largest contributor is from the yield. Features such as SV, IV, starter fermentation period, and percentage of starter are contribute moderately to both dimensions.

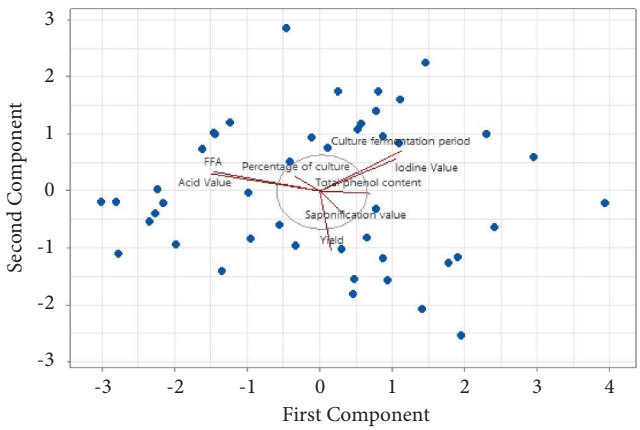

Figure 9: Biplot principal component analysis of variables.

4. Conclusion

The induced fermentation method to produce VCO warrants high costs due to the initial expense of obtaining pure microbial strain cultures. However, an alternative and potentially cheaper option for improving the fermentation rate in VCO production entails the utilization of fermented neera. The use of this starter culture resulted in minimal fermentation time of the sample (14–17 h), which was lesser than the conventional practice. When 8-hour-old fermented neera at a concentration of 10% was used as a starter, the VCO yield recorded the highest value of 19.8%, while the lowest levels of free fatty acids were observed with 10-hour-old fermented neera at a concentration of 10%. Overall, the treatment condition involving 8-hour fermented neera at a concentration of 10% yielded VCO with the desired characteristics and also complied with FSSAI standards. It would be invaluable to investigate the impact of coconut milk pH, temperature, and fermentation conditions (aerobic/anaerobic) on VCO yield as a future line of work.

Data Availability

The data used to support the findings of the study are available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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