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Production of the Virgin Coconut Oil from Induced Fermentation with Lactic Acid Bacteria

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Abstract

Virgin Coconut Oil (VCO) is a value-added coconut product with many applications in cosmetics and pharmaceuticals. Lactic acid bacteria (LAB) can be employed to generate VCO through a fermentative process. Such a bacteria group can contribute to forming desirable sensory characteristics in the final product and its bioactivity. The present work aimed to isolate LAB strains from the natural fermentation of coconut cream and develop a process to produce VCO mediating by fermentation method. The highest oil yield at 25% (v/v) was obtained from the coconut meat and water mixture fermentation at the ratio of 1:2 with 10% (v/v) inoculum of M01strain sourced from pickle fermentation after 10 h at pH 5.5. The oil satisfied the requirements according to national standard TCVN 6311 and exhibited antibacterial activity against two food-borne pathogens, *Salmonella* and *Shigella*. This study proposed the production of coconut oil at a large scale that can substantially supply VCO to the food and cosmetic industry with remarkable benefits.

Keywords: Virgin Coconut Oil (VCO), lactic acid bacteria (LAB), antibacterial activity, fermentation

Introduction

Coconut oil (CO) or Virgin coconut oil (VCO) has received public attention in recent years, and its consumption by the global population has risen owing to its health advantages. Furthermore, it has been encouraged the use of these oils as a cooking medium instead of ordinary cooking oil and as additional components in food or personal care products. VCO are the source of antioxidants and antibacterial compounds, including polyphenols, phytosterols, alkaloids, terpenoids and organosulphur compounds due to the presence of hydroxyl (–OH) group; VCO also contains the richest source of phytonutrients such as medium chain fatty acid (MCFA), phenolics, and phytosterols (Illam et al., 2017). It has been demonstrated that VCO contains 90% saturated fatty acids and 12% MCFA. Lauric acid, an MCFA that dominates the fatty acid composition with a proportion ranging from 46 to 48 percent, is one of the pharmacologically active components in the VCO. In the human body, oral ingestion of VCO stimulates the conversion of lauric acid to monolaurin (Nitbani et al.), which is a key component of breast milk that boosts baby immune systems and has the potential to damage bacteria's lipid membranes. Thus, VCO is a natural food preservative and exhibits antibacterial acitivity. VCO is frequently used in the flavoring industry due to their increased hydrophilicity when compared to normal fats and oils, allowing them to dissolve a broad range of polar compounds. VCO has a large market in South East Asia as a result of these advantages (Boateng et al., 2016).

According to documents from the Asia-Pacific Coconut Association, Codex, Philippine National Standards (PNS), and Vietnamese standards, virgin coconut oil (VCO) has antibacterial and anti-fungi effects, rich in Zinc, enhance the patient's immunity (Dayrit et al., 2007). Many studies have proven the ability of coconut oil to cure psoriasis, speed up wound healing. Results obtained from studies in obese experimental animals show that VCO has anti-inflammatory, analgesic, and antipyretic effects (Intahphuak et al., 2010). The antioxidant capacity of VCO can be attributed to

the presence of phenolic compounds such as ferulic acid and p-coumaric acid in fairly high concentrations (Marina et al., 2009). Apart from that, VCO is also food ingredient or uses to create detergents, soaps or shampoo or replace lipid in milk with high nutritional value in cream products with coconut flavor (Ng et al., 2021). Studies on soaking chicken in VCO aim to significantly reduce moisture and microbial contamination and increase the shelf life of chicken. CO has been used as a solvent to dissolve essential oils (lemon, eucalyptus, lavender) in massage oil preparations (Songkro et al., 2010). VCO is more effective and safer than mineral oil when used as a moisturizer (Agero & Verallo-Rowell, 2004).

Coconut milk, a natural oil-in-water emulsion, is commonly extracted from endosperms of coconuts. Due to it's singularity as a relatively stabilized emulsion, coconut milk required some agents to destabilize the emulsion (Lad et al., 2012). There are many daily life methods of extraction and processing of coconut oil such as: dry process, wet process, the Vietnamese traditional method, hot-dried and hot-pressed, pH method, cold-dried and cold-pressed, using enzyme, etc (Canapi et al., 2005). In addition, one of the methods of applying biotechnology is using lactic acid bacteria or *Lactobacillus* sp. group fermentation ability. Here, we would like to shed new light on industrial effieciency in producing coconut oil, which most of the previous ones didn't address. The process using probiotic to ferment and collect coconut oil will be controlled in multiple devices to optimize maximum specifications to increase efficiency. Mix copra with water in an appropriate ratio to squeeze for coconut cream, then sterilize using UV ray. In harvest phase, coconut cream get centrifuged to collect coconut oil. After that, the final coconut oil will go through several examinations for quality check before finally industrialized.

Materials and Methods

Microorganisms

The samples for natural fermented coconut oil and pickles were collected from the local market in Ho Chi Minh. Bacteria and fungi strains for antibacterial and antifungal activities experiment were received from Microbiology and Parasitology Department, School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 (MSSA), *Methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 43300, *Salmonella enterica ATCC* 14028, *Shigella*, *Candida albicans*, *Malassezia furfur*.

Isolating LAB

Estimating LAB from natural fermented coconut oil and pickles began with a dilution of samples using PBS buffer up to 10^{-3} dilutions. Next, 100 µL of suspension from each dilution was evenly added and spread onto MRS agar (MRSA) and Sabouraud dextrose agar (SDA). Bacteria developed when they were incubated at 37 ºC for 24 - 48 hours in candle jars. Identification was carried out by morphologically and biochemical tests. Carbonate dissolution ability: 3 - 4 separate colonies of each strain obtained from MRSA and SDA above were selected to inoculate on MRSA medium that supplemented with $CaCO_3$, continued to incubating at 37 ºC for 24 - 48 hours in candle jars. The initial isolation and identification were based on morphological appearance and preliminary physiological tests. Moreover, the bacteriocin was isolated. After all, they were increased in MRS liquid medium and mid-seed in MRS medium supplemented with Glycerol at the final concentration of 15%(w/v), reserved at -80 °C.

General process of producing coconut oil

Firstly, we pureed copra and water 1:1 ratio, squeezed for coconut milk, and got rid of the coconut carcasses (instantly use after pressing). Secondly, the coconut milk was sterilized by UV.

Thirdly, we inoculated the isolated LABs to the sterilized coconut milk under the laminar airflow cabinet and incubated at 37 ºC for 48 hours. Then, the carcasses and big impurities were filtered by a sieve and harvested the oil in the upper layer. In order to remove the water from the oil, anhydrous NaCl should also be added to the oil that was extracted from the decantation glass. The oil should be covered tightly and left to stand at room temperature for 24 hours. The hydrated sand below was taken out while the oil on top was decanted. Similarly, if the oil was not transparent, anhydrous NaCl would be added again and repeated the previous procedure until the coconut oil was completely pure (no water inside). Finally, the product was gathered, the volume was measured, and other parameters were tested. The whole procedure can be summarized in Figure 1 below

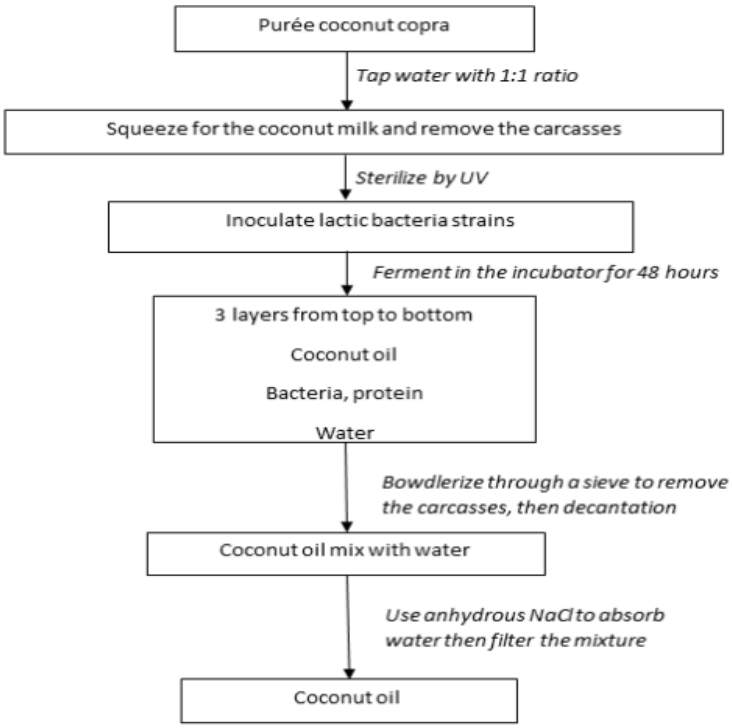

Figure 1. Diagram of coconut oil production by fermentation method

Investigating fermentation factors

We performed experiments to investigate factors including: bacterial strains, copra and water ratio, inoculate ratio, initial pH of fermentation. Bacterial strains: Obtaining the most effective bacterial strains was the aim of this investigation. Utilizing the general procedure that we have described above. During the fermentation of LABs, we fixed the ratio of copra – water was 1:1 (400g:400g). In order to pick the optimal strain, performance (the amount of coconut oil produced after fermentation) and sensory evaluation (color, taste) should be used. Additionally, we compared the fermentation yield using LABs with a second natural fermentation sample, in which the process of combining copra with water and pressing coconut milk continued as usual. This sample, however, will not be UV irradiated to prevent losing the bacteria present in it.

Copra and water ratio: To determine how much water was required to efficiently extract oil, we looked at copra-water (Weight/Weight) in a range of ratios, including 1:0,5, 1:2, 1:4, and 1:8. The fixed weight of copra is 400g; mix with the appropriate amount of water to achieve the above

ratio. The scale was only altered in the first step; all remaining steps followed the same pattern as previously. The strain used for this ratio study was the one we had previously selected that had the highest production of coconut oil. To select the ideal ratio, we evaluated the yield of coconut oil that was obtained.

Inoculating ratio: With the top strain of LABs that was screened, we conducted to do experiment with various inoculating ratios, including 2,5%, 5%, 10%, 20% and 50%. On MRSA, bacteria were initially stimulated before the subsequent steps in the procedure. Next, we transferred 2 colonies of the strains from MRSA that would produce the best yield and highest-quality coconut oil into 10mL of sterile brine. The solution was inoculated into coconut milk with a best copra – water that was surveyed above. Then, we researched in 5 bacterial fluid – coconut milk ratios: 0,25mL – 9,75mL; 0,5mL – 9,5mL; 1mL – 9mL; 2mL – 8mL; 5mL – 5mL; and put it an anaerobic tank, incubated at 37 ºC for 24 – 48 hours in the incubator. Oil was then decanted in order to extract. On the other hand, using anhydrous NaCl to entirely eliminate any water that had combined with the oil. The other steps followed the same procedure as performed.

Initial pH of fermentation: Using Hydrochloric acid and Sodium hydroxide, numerous initial coconut milk pH was obtained: 4,5; 5,0; 5,5; 6,0; 6,5. Applying the aforementioned procedure to bacterial strains which obtained from expurgating tests. To analyze the proper initial pH of fermentation, we used the following metrics: efficiency, color, degree of clarity, ease of decanting, and the ability to get coconut oil.

Investigate indicators of finished product quality

After expurgating and obtaining high efficiency strains and essential statistics for fermentation, we investigated the quality standards of the product. Coconut oil which was fermented from LAB need to pass the qualities for food grade coconut oil according to TCVN 6311-1997 and certified by the legal authority. We chose the highest efficient coconut oil which was produced from LAB and met the requirements, then send them to Ho Chi Minh City Oil and Oil Plant Research Institute to analyze important indexes: Density, Refraction, Saponification, Iodine, Peroxide, Acid.

Antibacterial and antifungal activities

The selected strains were further confirmed for antibacterial and antifungal activities, namely, *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcus aureus* (MSSA), *Methicillin-resistant Staphylococcus aureus* (MRSA), *Salmonella typhi, Shigella, Candida albicans, Malassezia*. Before analysis, they were enriched in Sabouraud dextrose agar (SDA), Tryptic Soy Broth (TSB) or MRS agar (MRSA). Cultures grown on solid medium (TSA) were kept stationary and incubated at 37^{o}C until colonies were form (~24 hours). Next, 4 separated colonies were taken into tubes, which contained TSB medium, continued to be incubating at 37^{o}C for 6 hours to activate. The bacterial suspension was adjusted to 1.0 x 10^6 CFU/ml by physiological saline and then diluted into 1.0 x 10^4 CFU/ml. After being activated, strains should be used within 15 minutes.

The antibacterial and antifungal analysis was carried out in Mueller-Hilton agar (MHA). To emulsify, the coconut oil and MHA ratio was 10% (10ml - 90ml) with 1% Tween. The material was melted and then poured into plates that were 3–4 mm thick. If the agar surface had ponded, dry it by opening the lid and placing it in the incubator at 35–37°C for 15–30 minutes. The same exercise was repeated with the control sample of coconut oil that obtained from a fermentation approach using LAB strains *Antibio pro*, and 1 blank plate that only contained MHA and Tween 1% (without any oil). 20 µl bacteria and fungal were dripped onto the medium surface and incubated at 37 ^{o}C for 18 hours; aside from MRSA for 24 hours.

Results and Discussion

Isolation results and preliminary identification

After culturing the samples, we isolated 6 strains (M01, M02, M03, M04, M05, M06) of bacteria on MRSA and 1 strain (D01) of fungi on SDA. The initial isolation and identification was based on morphological appearance and preliminary physiological tests. Isolation of LAB using 2 types of medium namely MRSA medium only and MRSA + $CaCO_3$ gave different results.

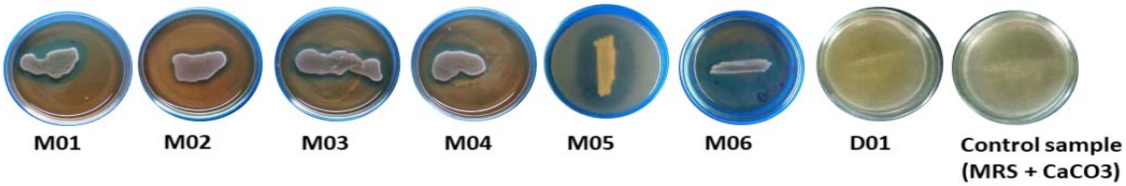

Figure 2. Carbonate dissolution among isolates

Figure 2 shows colonies of LAB grown on the selective medium MRSA + 0.3% $CaCO_3$. Colonies of LAB grown in $CaCO_3$ soluble rings, where there was a neutralization reaction between lactic acid produced by LAB and $CaCO_3$ base, so that the areas became transparent. This medium is a selective medium for these LAB, by using this medium, it can be ascertained that the one that grows in $CaCO_3$ soluble rings is LAB, so it can be directly identified. In fact, 6 strains (M01, M02, M03, M04, M05, M06) had cyclic resolution on medium with $CaCO_3$, the remaining strain (D01) had no phenomenon.

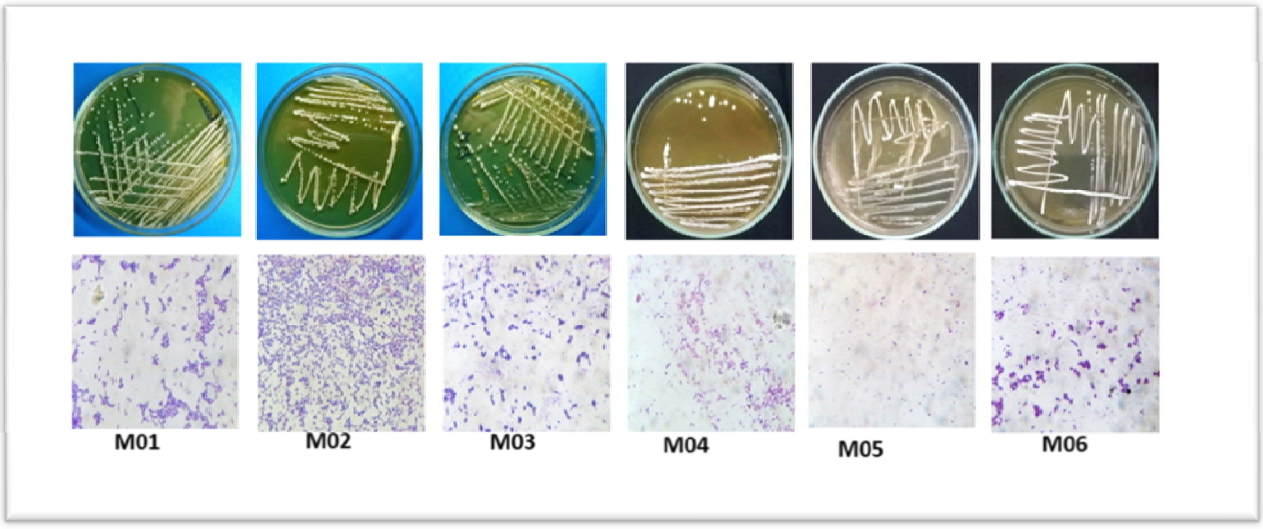

Figure 3. Colony and microscopic morphology of isolates

All the 7 strains absented of catalase activity, oxidase ability. On the contrary, only 1 strain D01 could not produce lactic acid and all the remaining 6 strains had reagent which changed into lemon yellow in identify lactic acid in the culture supernatant. Continuing to Gram staining, the procedure was based on the reaction between peptidoglycan in the cell walls of some bacteria. Gram-negative bacteria had much less peptidoglycan in their cell walls, so decolorizing step essentially

rendered them colorless, while only some of the color was removed from gram-positive cells, which had more peptidoglycan (60-90% of the cell wall). The thick cell wall of gram-positive cells was dehydrated by the decolorizing step, causing them to shrink and trapping the stain-iodine complex inside. After the decolorizing step, a counterstain was applied (fuchsine) to color the bacteria pink. Both gram-positive and gram-negative bacteria picked up the pink stain, but it was not visible over the darker purple of the gram-positive bacteria. Because of LABs were gram-positive, so the results of the Gram stain which were viewed using light microscopy, were dark-purple non-spore forming rods or cocci for 6 strains (M01, M02, M03, M04, M05, M06). At this point, based on these strains, which were preliminarily identified for further biochemical and physiological tests as described in materials and methods, we could temporarily conclude 6 strains that were LABs strains (M01, M02, M03, M04, M05, M06) which is displayed through figure 3

Coconut oil yield from strains survey

Fermentation procedure was carried out to identify and select the most optimized strains after successfully isolating 6 strains (M01, M02, M03, M04, M05, M06) in perspective of producing highest coconut oil yield with high-class quality then compare with natural fermentation samples. We fixed the ratio copra-water to 1:1 (400g:400g) during the fermentation process, followed the remaining stages as described above, and the results were presented in Table 1.

Table 1. Coconut oil yield from strains survey

No.	Bacteria Strains	Coconut oil collected (mL)	Coconut oil collected description
1	M01	56.0	-Transparent -Colorless -Signature smell
2	M02	51.0	-Transparent -Colorless -Signature smell
3	M03	38.5	-Transparent -Colorless -Signature smell
4	M04	18.0	-Transparent -Colorless -Signature smell
5	M05	24.0	-Transparent -Colorless -Signature smell
6	M06	25.0	-Transparent -Colorless -Signature smell
7	Natural fermentations strains	23.0	-Transparent -Colorless -Signature smell

The experiment demonstrated clearly that utilizing LABs strains that were isolated would result in a substantially larger yield than the naturally fermented sample (23mL coconut oil extracted from 400g copra). Especially, strains M01 and M02 gave outstanding yields, respectively 56mL and

51mL per 400 g of copra. All fermentation samples produced the distinct aroma of coconut oil, which was clear and colorless. Due to having the highest efficiency in producing coconut oil, M01 strains will be chosen to be examined other surveys to maximized the efficiency of coconut oil fermentation procedures.

Result of survey copra-water ratios

We used strain M01 for this fermentation survey based on the results of the previous survey. The water was mixed in turn with 400g of copra according to the ratios (1:0,5; 1:1; 1:2; 1:4; 1:8) and fermented following to the process. All obtained coconut oil products were clear, colorless, and have a distinct flavor.

Table 2. Result of the coconut oil-water ratio

No.	Oil/water ratio	Amount of Coconut oil/water	Coconut oil collected (mL)	Coconut oil collected description
1	1:0.5	400g : 200g	18	-Transparent -Colorless -Signature smell
2	1:1	400g : 400g	40	-Transparent -Colorless -Signature smell
3	1:2	400g : 800g	48	-Transparent -Colorless -Signature smell
4	1:4	400g : 1600g	26	-Transparent -Colorless -Signature smell
5	1:8	400g : 2000g	26	-Transparent -Colorless -Signature smell

Regarding the yield of coconut oil obtained, it can be clearly seen that the 1:2 ratio gave the highest yield of 48mL from 400g of copra.

Results of survey of different inoculation rates

To conduct this survey, we used the same strain M01 and copra-water ratio 1:2 (due to the highest coconut oil yield in the previous two surveys) to conduct fermentation. Strain M01 was inoculated into coconut milk according to ratios: 2,5%; 5%; 10%; 20%; 50%; the results were as shown in Table 3

Table 3. Results of the survey of different inoculation rates

No.	Bacteria fluid / coconut milk ratio	Amount of bacterial fluid / coconut milk (mL)	Coconut oil collected (mL)	Coconut oil collected description
1	2.5% : 97.5%	0.25 : 9.75	48	-Transparent -Colorless -Signature smell
2	5% : 95%	0.5 : 9.5	47	-Transparent

				-Colorless -Signature smell
3	10% : 90%	1.0 : 9.0	51	-Transparent -Colorless -Signature smell
4	20% : 80%	2.0 : 8.0	43	-Transparent -Colorless -Signature smell
5	50% : 50%	5.0 : 5.0	44	-Transparent -Colorless -Signature smell

The survey revealed that the yield of coconut oil obtained was not significantly impacted by the initial inoculation rate. Yields were 48, 47, 51, 43, 44 (mL) for inoculation rates of 2,5%, 5%, 10%, 20%, and 50% respectively. However, the ratio of 1ml of LAB to 9ml of coconut milk would generate the best output when the fermentation process would be optimized to produce coconut oil for the highest overall efficiency.

pH result

In progression for this research, we used the same strain M01, copra-water ratio 1:2 and the LAB fluid- coconut milk ratio 1:9 (due to the highest coconut oil yield in the previous three surveys) to proceed LABs fermentation. Using Hydrochloric acid and Sodium hydroxide, numerous initial coconut milk pH were obtained: 4.5; 5.0; 5.5; 6.0; 6.5. After fermenting to extract coconut oil according to the process described above, we got the results from the Table 4.

Table 4. pH effect

No.	pH survey	Actual pH	Coconut oil collected (mL)	Coconut oil collected description
1	4.5	4.56	42	-Transparent -Colorless -Signature smell
2	5.0	5.01	46	-Transparent -Colorless -Signature smell
3	5.5	5.55	50	-Transparent -Colorless -Signature smell
4	6.0	5.90	49	-Transparent -Colorless -Signature smell
5	6.5	6.51	41	-Transparent -Colorless -Signature smell

Similar to the inoculation rate, the initial pH had a relatively small impact on the production of coconut oil; nevertheless, at pH 5.5, a yield of 50mL was obtained, which was higher than other

pH ranges which illustrate respectable affiliations between pH and coconut oil collected despite small impact from pH to production of coconut oil

Determine coconut oil quality

Coconut oil fermented from M01 strain met the TCVN 6311-1997 several requirements like density, refraction, saponification index, iodine index and peroxide index Table 5.

Table 5. Determine coconut oil quality

No.	Targets	Result		TCVN 6311-1997 requirements
		Ho Chi Minh Oil and Oil Plant Research Institute	Pharmacognosy laboratory – Faculty of pharmacy at UMP Ho Chi Minh city	
1	Density at 20°C	0.921	0.912	0.908 to 0.921
2	Refraction at 20°C	1.450		1.448 to 1.450
3	Saponification index	258.20mg KOH/g	257.46mg KOH/g	248mg to 265mg KOH/g
4	Peroxide index	0.35 mEq/kg		≤ 10 mEq/kg
5	Iodine index	7.61g I_2/100g	8.55g I_2/100g	6g to 11g I_2/100g
6	Acid index		2.69 mg KOH/g oil	≤ 4 mg KOH/g oil
7	Color		Colorless, transparent product	Colorless, transparent
8	Smell		Signature coconut oil smell	Signature coconut oil smell

Among the investigated LAB strains, M01 strains produced to highest yield of coconut oil and met qualified all of the TCVN 6311-1997 requirements. M01 was chosen as the representative of the Lactic bacteria to investigate the optimize ratio in the coconut oil fermentation process by LAB strains. In conclusion, coconut oil fermented by M01 strains able to be consumed in household condition

Antibacterial and antifungal activities

After optimizing the fermentation process to produce coconut oil from LAB bacteria by investigating the factors affecting the fermentation process, we carried out antibacterial and antifungal analysis.

Two different coconut oil samples and one blank sample would be used to conduct this antibacterial and antifungal test. Firstly, a sample of coconut oil was produced through fermentation using strain M01 (strain selected through fermentation optimization surveys for the highest yield and quality of coconut oil). Secondly, the control sample of coconut oil was prepared through fermentation utilizing LAB *Antibio pro* strain (M05). Thirdly, blank plates were made up simply of MHA

and Tween 1% (w/v) to demonstrate that the composition of Tween 1% (w/v) had no impact on the antibacterial or antifungal activity, as well as ensured that the MHA medium remained normally.

We conducted fermentation of coconut milk using strains M01, M05 according to the factors that have been optimized above, such as: copra-water ratio 1:2, the LAB fluid-coconut milk ratio 1:9, initial pH 5,5. To make an emulsion, the ratio of coconut oil and MHA was 10% (10ml - 90ml) with 1% Tween. After many times we did research, the best success rate of coconut oil emulsion was 10%. If we chose higher ratio, MHA medium would not solidify.

The survey was done and presented in Figure 4, Figure 5 and Table 6.

Table 6. Antibacterial and antifungal activities

No.	Bacteria or Fungi	Result		
		Blank Sample	Coconut oil from antibiotic M05	Coconut oil from M01
1	*E. coli*	Grow	Grow	Grow
2	*P. aeruginosa*	Grow	Grow	Grow
3	*MRSA*	Grow	Grow	Grow
4	*MSSA*	Grow	Grow	Grow
5	*S. faecalis*	Grow	Grow	Grow
6	*Salmonella typhi.*	Grow	Grow	Can not grow
7	*Shigella*	Grow	Grow	Can not grow
8	*Candida albicans*	Grow	Grow	Can not grow
9	*Malassezia furfur*	Grow	Grow	Grow

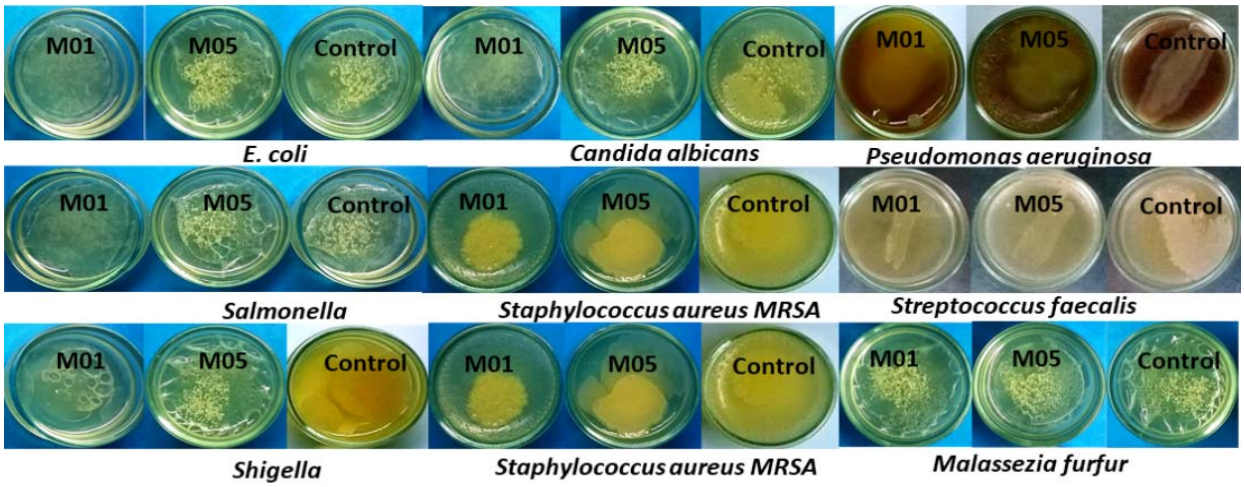

Figure 4. VCO from M01 and M05 against pathogen bacteria

For the control samples, all bacterial and fungal strains were able to grow on MHA that supplemented with Tween 1%. There were no antibacterial or antifungal effected for the fermented coconut oil samples from strain M05. Some strains, namely, *E. coli*, *Salmonella typhi*, *Shigella*, and *Candida albicans* did not grow on MHA that emulsified with coconut oil fermented by strain M01. Which can conclude that LABs strains M01 can produce coconut oil containing antibacteria and anti fungi abilities, mostly inhibiting disgestive bacteria and common dermatophytes.

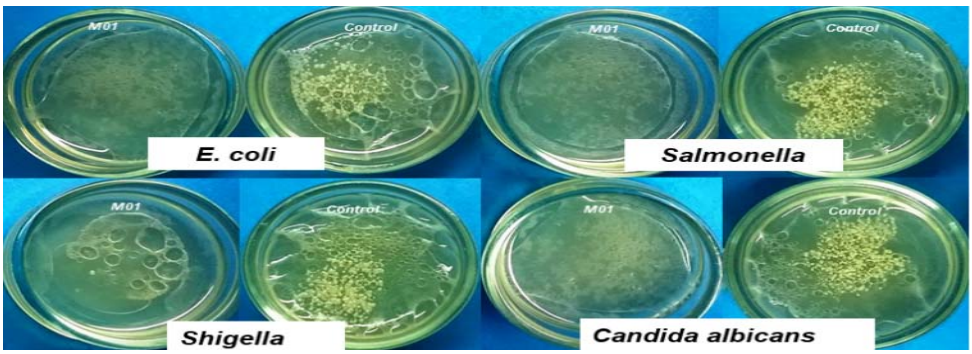

Figure 5. VCO from M01 against *E.coli*, *Salmonella*, *Shigella*, and *Candida albicans*

Discussion

According to previous results, all the 7 strains studied can ferment and produce coconut oil. Six strains belonged to the group of LAB that are potentially beneficial to humans. Among the 6 strains, M01 produce highest oil yield. Coconut oil fermented from the strains all passed the TCVN 6311-1997 for food grade coconut oil and was not contaminated by any microorganisms, however M02 showed itself to produce highest yield of coconut oil with the lowest acid index of all strains which mean the product would not degrade much comparing to other strains, it also has highest iodine index which is crucial for human health among the other coconut oil fermented. M01 strains product has also been sent to Ho Chi Minh City Oil and Oil Plant Research Institute and the quality has been confirmed. The results also implied that Oil: Water with the ratio of 1:2 produce highest yield combine with bacterial inoculum to coconut milk at ratio 1:9 and pH 5.5 created the best enviroment for LAB to grow.

Using the results received above, we can conclude that LABs strains and coconut oil : water ratio affects the coconut oil fermentation efficiency sharply due to the fact that when we adjust differents value, the coconut oil collected change dramatically. In contrary, bacteria fluid : Coconut milk and pH only contributes little to the final result of fermenting coconut milk since adjusting values only slightly changed the coconut oil received. In conclusion, when further researches is conducted for coconut oil efficiency, LABs strains and coconut oil : water ratio must be considered carefully.

We have been able to ferment coconut oil from copra using LABs, and even received a lot more positive yield than traditional methods, which open up an opportunity for a greater good in coconut oil widespread in usage and cost, which at this time is quite luxurious but still able to meet TCVN 6311-1997 requirements. In order to improve coconut oil yield, fed-batch cultivation and continuous flow cultivation are suggested. Due to the fact that this whole research is based on batch cultivation, which is one of the most basic but also one of the lowest efficient methods, applying fed-batch or continuous flow cultivation will provide unlimited copra and water, in which turning LABs strains into log phase, dramatically increase the effectiveness of the coconut oil production process so as to produce more VCO. In other case, we can co-culture the *Lactobacillus* with a Yeast-produce fungi, so we can improve both the quality of the coconut oil and fuse oil with other good fatty acid

Another way to use LABs M01 strains, which recently found out earlier that producing more high-quality VCO, in industry scale or commercially use, identification must be done. After identifying M01 strains, further experiments come in handy in order to understand about the gene expression, learning the precise ferment progression in order to rennovate the gene expression, which con-

stantly increase coconut oil production efficiency. Or we can even applying gene delivery to create even more proficient strains.

Our finding resulted that the addition of VCO to MHA could inhibit the growth of some bacteria and fungi, namely, *Escherichia coli*, *Salmonella typhi*, *Shigella*, *Candida albicans*, which is usually causing diarrhea, gastroenteritis or even typhoid fever. It isn't the pioneer of finding out antimicrobial ability since the antimicrobial effect of coconut oil was first reported by Hierholzer and Kabara in 1982 (Hierholzer et al., 1982). Apart from microorganisms above, recent researches has revealed that coconut oil has antibacterial action against a variety of gram-positive and gram-negative bacteria, including *Escherichia vulneris, Enterococcer* spp, *Staphylococcus aureus, Streptococcus mutans*, *Clostridium,…*(Peedikayil et al., 2016; Widianingrum et al., 2019). Coconut oil has a high percentage of lauric acid (LA), 40-60%, mostly in the form of free fatty acids and monoglycerides, which has antibacterial, antiviral, antifungal, antiprotozoal, and can enhance the immune system. Numerous studies have also shown that free LA may have antimicrobial effects. Because this ingredient did not cause any issues with resistance, the use of coconut oil as an alternative medicine in people has increased. These include advantages for lowering stress levels, managing cholesterol levels, losing weight, having immunomodulatory effects, treating cardiovascular issues, preventing Alzheimer's disease (Neelakantan et al., 2020). SEM observations demonstrated that LA might harm the *Staphylococcus aureus* through processes found on the bacterial cell wall surface. LA first coated the whole surface of the cell wall, penetrating and killing the bacteria. The MCFA class, particularly LA, has been altering the permeability of bacterial cell walls, upsetting metabolism, preventing key nutrients that bacteria need to survive, or interfering with links with carbohydrate metabolism (Manohar et al., 2013). As a result, it is thought that VCO should exhibit LA like antibacterial properties. However, several research have shown that VCO does not have antibacterial action against *Clostridium* or *Staphylococcus aureus*. Our antibacterial test also showed similar results, *Staphylococcus aureus* was still able to grow on MHA medium supplemented with coconut oil (Satoshi et al., 2017).

These findings raise the question of whether VCO has antibacterial capabilities and if the antimicrobial activity of VCO is the same as that of LA. There have been several research demonstrating the antibacterial properties of coconut oil. However, most studies have not specifically identified which components in coconut oil are responsible for the main task. Since LA is only one component of coconut oil, as well as caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid, more research is required to determine if other components or LA are responsible for its antibacterial property. LA was the most dominant fatty acid and ranged from 48% to 51%. Changes in the fatty acid composition has not been observed in hot extracted virgin coconut oil, cold extracted virgin coconut oil, and commercial coconut oil. This could be due to same geographical origin and ecological conditions. This finding is in the agreement with Banzon and Resurreccion, in which no changes were observed in the fatty acid distribution in samples of coconut oil obtained by heating, fermentation, freeze thawing, and solvent extraction (Banzon & Ressureccion, 1979; Dia et al., 2005). Thus, using LAB strains for fermentation to produce coconut oil does not affect the fatty acid composition of coconut oil. On the other hand, we found that VCO had antimicrobial properties against some strains of bacteria, but not *Staphylococcus aureus*. It is worth noting that the antimicrobial action of coconut oil is controversial. Therefore, we believe that VCO's antibacterial properties and mechanism are vastly different from those of LA. The reason why VCO's antibacterial action differs from that of LA is unknown. This raises the issue of whether LA in conjunction with other MCFA can modify the quality or amount of LA's antibacterial activi-

ties. Because few experiments have looked at this question, future studies should concentrate on these elements.

Conclusion

To sum up, the best conditions and materials for fermented coconut oil production process is M01 strains, the water: oil ratio is 2:1, the bacterial fluid: coconut milk ratio is 1:9 at pH 5.5 will bring highest yield of coconut oil. And the coconut oil quality is qualified according to TCVN 6311-1997 monograph on foodgrade coconut oil on following criteria: Density, refraction, smell, color, acid index, iodine index, saponification index. Futhermore, the process and method in this article can be carried out on a large scale and in mass production. As coconut oil from M01 strain exhibit significailaly antibacterial and antifungal acitivity, particularly, the common foodbonth bacteria, e.g., *Salmonella typhi*. and *Shigella*, which is very important since digestive bacteria has been a troublesome and sometimes dreadful to human health. We also recommend combining LABs coconut oil into daily life usage, especially in meals for youngsters and elderly to prevent and inhibit harmful bacteria in digestive system.

Due to unique and signature smell and it's antimicrobials ability, coconut oil can be used to develope oil essence or shampoo and body lotion alongside with food industry for commercial use, mainly due to the fact that coconut oil from M01 can inhibit the growth of *C.albicans*, a common but very annoying problems for daily life.

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