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Production of virgin coconut oil by induced fermentation with Lactobacillus plantarum NDRI strain 184.

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Summary

Present study was designed to produce the Virgin Coconut Oil in induced fermentation by Lactobacillus sp. Virgin Coconut Oil is a Value Added Product of coconut which have different applications. Natural fermentation is one of the commercial methods to produce Virgin Coconut Oil, where the natural microorganisms are playing a major role. In such process, contamination is one of the main problems; to overcome this, induced fermentation was performed in the controlled conditions by using probiotic microorganisms like Lactobacillus plantarum. Studies were conducted to determine the effect of major parameters, to produce higher yields of Virgin Coconut Oil in induced fermentation. It was conformed that the pH 5.0±0.1, temperature 45±1°C, inoculum concentration 2%, incubation time of 48 hrs and anaerobic conditions were the optimum conditions for the efficient production of Virgin Coconut Oil by induced fermentation with L. plantarum.

Key words: *Virgin Coconut Oil, Lactobacillus plantarum, Coconut Milk, Natural Fermentation, Induced Fermentation*

Introduction

Coconut oil is extensively used for food, industrial applications, health promotion and disease prevention (Manisha and Shyamapada, 2011). Blanca et. al., (2007), Fabian et. al., (2007) reported that VCO is produced by wet processing where the minimal processing conditions should involve preserving natural components of coconut according to Asian Pacific Coconut Community (APCC), Codex, The Philippines National standards (PNS), Bureau of Product Standards (BPS)-2004. In VCO production process determined that natural fermentation is a method where the less proceeding conditions were involved. In the natural fermentation process extracted milk from wet coconut was allowed for microbial fermentation (Divina, 2003). Fermentation is a microbiological process, where the microorganisms are involved and produced the valuable products for mankind (Primary and Secondary metabolites of fermentation process). In the environment various kinds of microorganisms are present; they are connecting in the both the cases of useful and harmful.

In natural fermentation process of VCO production, normal flora of micro organisms will ferment the milk and separates the coconut oil on the top portion within 24 - 48 hours as shown in figure 1.A and B. The separated oil can collect and use. But there was a chance of contamination with microorganisms because the coconut milk is the rich source of proteins, carbohydrates and moisture (Chee and Choon, 1997) which can attracts the microorganisms, some may spoil the coconut milk resulting the production of poor quality VCO (generally in yellow color) as shown in figure 1C and D.

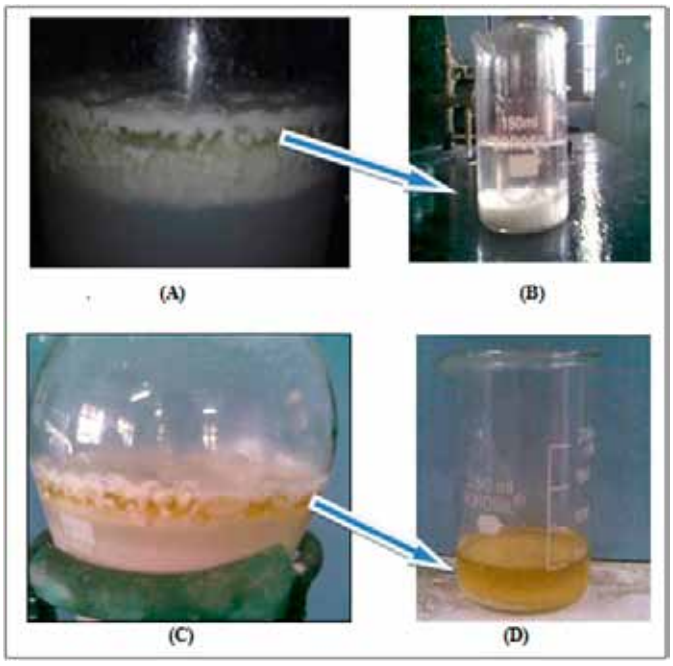

Figure 1. *(A).Separated oil layer in natural fermentation of coconut milk (water white VCO without contamination). (B). VCO separated and placed in a beaker (C). Separated oil layer in natural fermentation of coconut milk (yellow color due to contamination). (D). yellow color VCO separated and placed in another beaker.*

Anti-microbial property of VCO was reported by Handayani (2009) against both Gram positive and negative bacteria. Laboratory animals when fed with the VCO reported that

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it reduces High Density Lipoproteins (HDL) (Nevin and Rajamohan, 2004). Researchers were proven the ability of VCO to cure the psoriasis (Tepeng and Rivera, 2007). Sarunyoo *et. al.,* 2007 reported that the prospective usage of VCO as the massage oils with combination of different essential oils in aroma therapy. VCO is reported as high quality raw material for drinks, cooking lotions, soaps, shampoos (Foale, 2007).

In literature, different processes were reported for the production of VCO, in wet methods using of enzymes, chilling and centrifugation (Raghavendra and Raghavarao, 2011), super critical extraction methods were available (Nik Norulani *et. al.,* 2009). In low temperature method the coconut milk was heated to the 45°C and centrifuged to separate VCO (Hanid *et. al.,* 2003). Fabian *et. al.,* (2007) proposed that the natural fermentation method is a familiar process for production of VCO in house hold level and also industrial scale.

Different researchers were reported that *L. plantarum* have many Immune and probiotic benefits. A study on laboratory animals reported that some strains of heat-killed *L. plantarum* has potential positive effect on immune system and stimulates the macrophages and dendritic cells to produce T-helper (T_H) cells, which are useful in protection form influenza infection (Naoyoshi and Risa, 2009). *L. plantarum* is the species which has the probiotic capacity and commonly found in the Italian and Argentinean cheese (Miriam *et. al.,* 2011). Carmen *et. al.,* (1970) proposed that *L. plantarum* enhances the yield of coconut oil in wet process than other *Lactobacillus* sp.

Literature on the fermentative production of VCO from coconut by using probiotic organisms is relatively low, which instigated us to carry out this problem. The major objective of present work is to develop a process for the production of VCO mediating probiotic organism (*L. plantarum*) by fermentation method using computer control bioreactor and optimization of different parameters to obtain higher yields.

Materials and Methods

Coconut Sample

Randomly selected uniformly sized, 12 months old (matured) nuts were collected from local commercial market.

Microbial Culture

Pure culture of the *L. plantarum* was collected from the NDRI-NCDC (National Dairy Research Institute-National Center for Dairy Cultures) in the lyophilized form in a glass vial, as per the instructions given by the NCDC catalogue. After two sub-culturing, organism was used for the seed culture preparation and one slant was stored as the stock culture on MRS medium for further studies.

Coconut milk Extraction

Coconut milk was extracted from solid endosperm; coconut milk is oil in water emulsion, stabilizing by proteins and phospholipids. In literature, coconut milk was extracted by using different equipments, at different processing conditions. But the conditions adapted were not suitable for the production of VCO, hence followed the short and simple method.

Fresh coconuts were dehusked and water was collected from the pore in separate container, further used in the fermentative production of coconut water alcoholic beverage and vinegar. Coconuts were broken and solid endosperm was collected, testa was removed by using kitchen peeler, white coconut balls were disintegrated into small pieces and grind with 1:2 ratio of water for 10 min. Ground mass was transferred to the cheese cloth, pressed manually for coconut milk extraction; the same process was repeated twice and coconut milk was pooled up. Extracted coconut powder was dried and preserved for another application.

Coconut milk sterilization

In coconut milk extraction microbes may enters through water, environment and utensils in to coconut milk. Exposing of coconut milk to Ultra Violet light (Dose of 40 mW/Cm^2/Sec.) in laminar air flow was done for 20 min per liter in glass beaker.

Seed culture preparation

Seed culture was prepared by using of Nutrient broth medium, culture flasks were incubated at 37°C for 36 hours at 100 RPM in orbital shaker and same was maintained for entire the study. By the serial dilution and spread plate method approximately amount of microbes were calculated by colony count using the following formula (1).

$$\frac{\text{Number of Microorganisams present in sample}}{} = \frac{\text{Number of Colonies present on plate}}{\text{Dilution Factor}}$$

Fermenter scale-up process (upstream processing)

According to the Spectrochem-India Biotron model bioreactor user manual probes of dissolved oxygen (DO), pH were standardized, they were fixed to the fermenter vessel lid, closed the fermenter and sterilized at 121°C for 15 min. in autoclave. Sterilized coconut milk was poured in to bioreactor vessel at aseptic conditions. Further, the parameters were arranged according to the designed study.

VCO recovery (Downstream processing)

After successful completion of bioreactor runtime, the fermented milk was centrifuged in temperature controlled centrifuge at 27°C and 6000G for 10 min. Separated VCO was collected; pooled VCO of all batches were finally centrifuged for clear oil at above conditions.

Calculation of recovery and process efficiency

For coconut sample, moisture was determined by hot air oven (BIS) method (BIS, 1994) and oil content by soxhlet (AOCS) method (AOCS, 1969). Oil yield and efficiency of the method was calculated by using following formulas 2&3 respectively.

$$\text{Oil yield}(\%) = \frac{\text{Weight of VCO obtained}}{\text{Weight of Coconut Kernal taken for milk Extraction}}$$

$$\frac{\text{Efficiency of the Method}(\%)}{} = \frac{\text{Yield Percentage by dry bases}}{\text{Oil Content percentage estimated by soxhelt method}}$$

Table 1. *Effect of temperature on VCO production*

Variable parameter (temperature)	No. of microbes present/ml	Moisture content of coconut (%)	Oil content of coconut (%)	Oil yield on wet basis (%)	Efficiency of process (%)
30 ±1°C	$5.2X10^5$	41.95	44.77	30.10	67.23^{e}
37±1°C	$5.1x10^5$	40.88	42.11	33.82	80.31^{b}
40 ±1°C	$5.5x10^5$	41.00	42.69	34.99	81.96^{ba}
45±1°C	$5.7X10^5$	41.12	43.91	37.02	84.30^{de}

Values followed by different letters differ significantly from each other at $P \leq 0.05$, based on SAS software

Studies on Effect of Parameters on VCO yield

Different major parameters such as temperatures, pH, concentrations of inoculum and oxygen, fermentation end time were studied. All the parameters (temperature 37 ±1°C, pH 6±0.1, 2% inoculum concentration, 48 hours fermentation end time, in aerobic conditions) remained same during the entire study except the particular parameter to be studied. All the values of study were represented as the mean of two observations.

Effect of temperature

Temperature is one of the parameter which can influence the microorganism's metabolic actions. The temperatures at 30, 37, 40, and 45°C were used with ±1°C as dead band.

Effect of pH

pH is one of the parameter which can influence the microorganism's metabolic actions. Fermentation pH range of 5.0 to 9.0 was used with ±0.1 as dead band.

Effect of Inoculum concentration

Inoculum concentration of 1%, 2% and 5% were used.

Effect of fermentation end time

Fermentation end time or fermentation time was maintained for the duration of 24, 48, 72 hours.

Effect of Oxygen concentration

Oxygen concentration also a one of the influencing factor for bacterial metabolism, aerobic with 100% oxygen, microaerophilic condition with 10% oxygen, anaerobic without oxygen fermentation process was maintained.

Coconut milk is emulsion of fat, protein, water. If it allows for some time the oil portion with protein is floats on water, it leads to improper mixing of acids, bases and microbes in submerged conditions. So, a constant stirrer rotation of 200 rpm was used in entire study.

Statistical Analysis

All parameters were carried out in triplicate. Statistical mean of three values were presented in the study. Significant differences between means were determined by Duncan's multiple range tests and were considered to be significant when ≤P0.05, based on SAS software (procedure followed is PROC ANOVA)

Results and Discussion

In coconut milk around 5.5-8.5 % of different carbohydrates are present; amongst the major are sucrose and starch (Chee and Choon, 1997). *L. plantarum* has the capacity to convert the sugars in to lactic acid; it decreases the pH of fermented milk to acidic which leads to denaturation and destabilization of proteins, causing the release of water and clustering the oil droplets (Man *et al*., 1992, 1997).

Temperature

Robert *et. al.,* (1957) were reported that the *L. plantarum* can resist temperature up to 45^0 C, average temperature for the growth was at 37°C. It is also reported that *L. plantarum* was well metabolize at 40-50°C (Marina *et. al.,* 2009). In the present study, at 45±1°C the process efficiency was highest (84.30%). According to the earlier studies (Raghavendra and Raghavarao, 2010) the yields were reported that at 40, 50°C 60, 70% respectively, but, at the temperature of 75-80°C of the reported study they have achieved the highest yield which is approximately equal to the highest yields obtained in this study. In a study reported by Raghavendra and Raghavarao, 2010 where the temperature of 40°C was used as the maximum limit and yields were reported in high at this temperature. The present study better yields were achieved by the combination of two parameters for destabilization of coconut milk. They are the acidic condition (pH 6±0.1) and the temperature which was maintained at 45±1°C. There is significant difference ($P \leq 0.05$) in process efficiency between temperatures at 40 ±1,

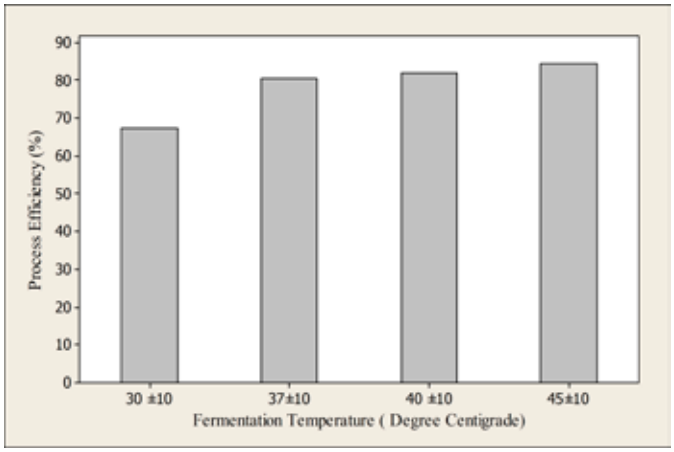

Figure 2. *Effect of temperature on VCO yield*

Table 2. *Effect of pH on VCO production*

Variable parameter (pH)	No. of microbes present/ml	Moisture content of coconut (%)	Oil content of coconut (%)	Oil yield on wet basis (%)	Efficiency of process (%)
5±0.1	$5.5x10^5$	42.11	40.44	33.80	83.58^{dca}
6±0.1	$5.6x10^5$	42.01	40.49	32.74	80.85^{c}
7±0.1	$5.5x10^5$	40.32	41.25	31.20	75.63^{b}
8±0.1	$5.8x10^5$	39.55	40.65	32.17	79.13^{e}
9±0.1	$5.4x10^5$	40.44	41.22	33.91	82.26^{f}

Values followed by different letters differ significantly from each other at $P \leq 0.05$, based on SAS software

45±1°C, in the present study and lowest yields (67.23%) were reported at 30±1°C. The data is presented in the table 1 and effect of temperature is shown in figure 2.

pH values

Range of pH from 5± 0.1 to 9±0.1 was used in the present study. It is reported in a study that the pH resistance ability of *L. plantarum* which was actively metabolized at the high acidic pH from 3.2-4.0 (Sawaminee and Dimitris, 2011). In the present study, the percentage of process efficiency was more (83.19%) at pH 5± 0.1. Generally, protein gets destabilize at the acidic pH. According to the study reported by Raghavendra and Raghavarao (2010) at pH 5.0 the yields were around 78%, where as in the present study more process efficiency was achieved by using *L. plantarum* at pH 5± 0.1.

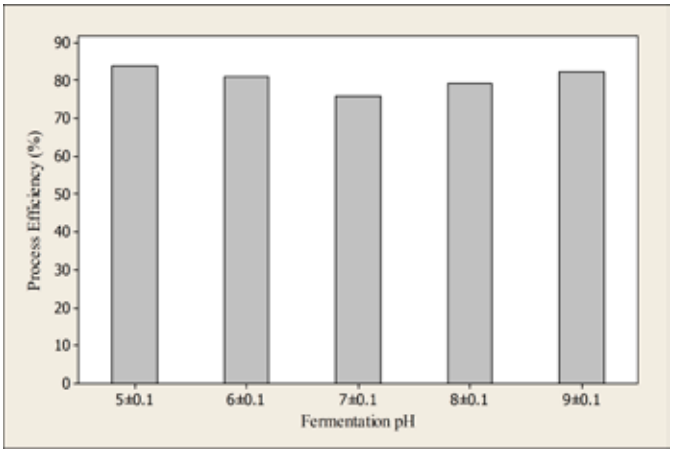

Figure 3. *Effect of pH on VCO yield*

In the case of basic conditions, the process efficiency (82.26%) was achieved at 9.0± 0.1, where as the oil yield reported (Raghavendra and Raghavarao, 2010) was approximately 80% at pH 9; the yield was decreased at higher pH because *Lactobacillus* may not metabolize properly at the pH of 9 ± 0.1. But the coagulation of proteins was reported well at the basic pH it may be one of the causes to produce higher yields (Belle, 1943). Fermentation process carried in the acidic conditions was easy when compared to the basic conditions because lactic acid was produced continuously by organism. The yields are depicted in table 2 and effect of pH is shown in figure 3.

Inoculum concentration

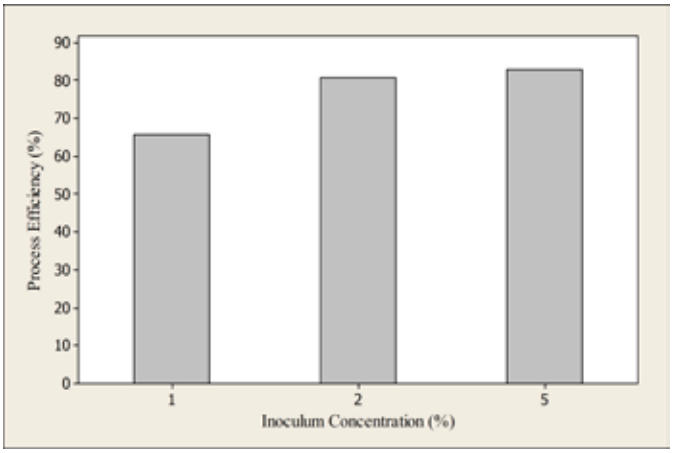

Figure 4. *Effect of inoculum conc., on VCO yield*

Inoculum concentration of 1, 2, and 5% were studied to determine its effect on yield. Inoculum concentration of 5% was shown more efficient with 82.91% and poor with the 1% with 65.67%, it shows that the efficiency was directly proportional to the inoculum concentration. Some supportive studies (Handayani *et. al.,* 2009*;* Man *et. al.,* 1997*,* Carmen *et. al.,* 1970) also reported that the inoculum concentration of 5% was suitable for the production of higher yields. The contented yields (80.72) are reported at 2% inoculum. Though the concentration of inoculum between 2% and 5% is more than twice, but the difference in the yields were not significant ($P \leq 0.05$) (less

Table 3. *Effect of Inoculum concentration on VCO production*

Variable parameter (inoculum concentration)	No. of microbes present/ml	Moisture content of coconut (%)	Oil content of coconut (%)	Oil yield on wet basis (%)	Efficiency of process (%)
1%	$5.0x10^5$	41.11	43.00	28.24	65.67^{ba}
2%	$5.6x10^5$	42.05.	42.96	34.68	80.72^{b}
5%	$5.1x10^5$	39.92	41.09	34.07	82.91^{e}

Values followed by different letters differ significantly from each other at $P \leq 0.05$, based on SAS software

Table 4. *Effect of fermentation end time on VCO production*

Variable parameter (fermentation end time)	No. of microbes present/ml	Moisture content of coconut (%)	Oil content of coconut (%)	Oil yield on wet basis (%)	Efficiency of process (%)
24 h	$5.1x10^5$	41.91	44.41	22.73	51.18^{dca}
48 h	$5.9x10^5$	40.03	40.22	32.47	80.73^{b}
72 h	$4.9x10^4$	40.18	39.97	32.50	81.31^{e}

Values followed by different letters differ significantly from each other at $P \leq 0.05$, based on SAS software

than 2%) so 2 % inoculum concentration was preferable in the Fermentative production of VCO by *L. plantarum.* The values are presented in table 3 and effect of inoculum concentration shown in the figure 4.

Fermentation end time

Fermentation end time of 24, 48, 72 hours were studied. Poor separation was obtained at 24 hours with 51.18% of VCO yield, but the fermentation time 48 h and 72 h the efficiency was increased to 80.73, 81.31% respectively. The fermentation time of 24 hours increased between 48 and 72 h but the yield was increased less than 1%, by considering the time factor the optimum fermentation end time can be considered as 48 h. Less than 1% more yield was reported when compared with 48 and 72 h of fermentation end time. So 48 hours of fermentation end time is preferable to save the production time. The results are presented in the table 4 and the effect of fermentation end time is shown in figure 5.

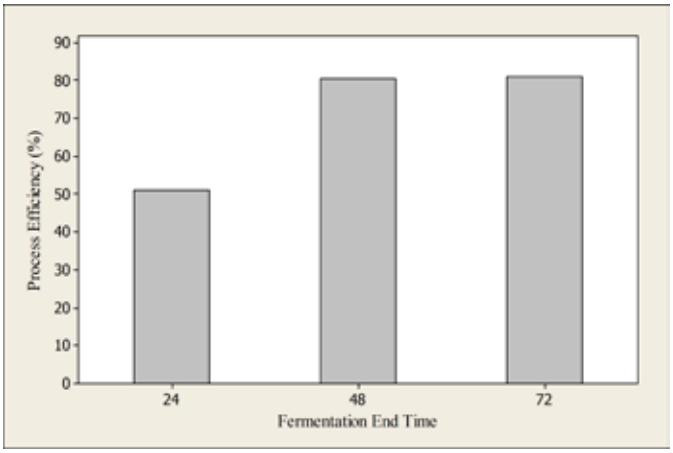

Figure 5. *Effect of fermentation end time on VCO yield*

Oxygen concentration

Oxygen concentrations at aerobic, microaerophilic, anaerobic conditions were maintained. 79.01%, 81.20% of process efficiency was obtained at aerobic and microaerophilic conditions respectively. The highest efficiency was noticed at anaerobic conditions with 83.83%. Generally *Lactobacillus sp.* was anaerobic to facultative aerobic in nature, *L. plantarum* is an aero tolerant bacteria in nature, it could resist oxygen concentration by non enzymatic super oxide reduction mediated by manganese (Mark *et. al.,*1994). It is reported that the cell growth was fast at anaerobic conditions (Man *et. al.,* 1997) and was preferable due to improved yields in the present study also. There is no extra supply of the sterilized oxygen in to the fermentation process in anaerobic conditions by using *L. plantarum.* The results were presented in table 5 and oxygen concentration on VCO production shown in figure 6.

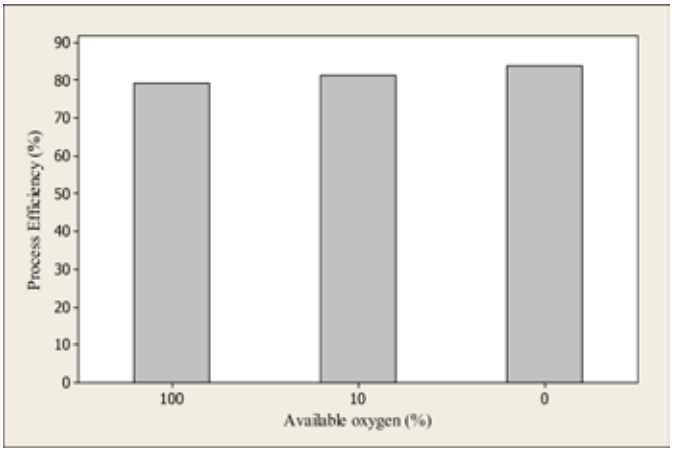

Figure 6. *Effect of oxygen condition on VCO yield.*

Conclusions

L. plantarum one of the probiotic organism, it can efficiently involves in induced fermentation of coconut milk. In present study the temperature 45±1°C, pH 5 ±0.1, inoculum concentration 2%, fermentation end time 48 h, anaerobic conditions were determined as the most preferable conditions for the induced fermentative production of VCO. Due to the probiotic properties of the organisms in VCO, we can utilize the produced VCO in both medicinal and food applications (Fig.

Table 5. *Effect of oxygen concentration on VCO production*

Variable parameter (available oxygen)	No. of microbes present/ml	Moisture content of coconut (%)	Oil content of coconut (%)	Oil yield on wet basis (%)	Efficiency of process (%)
100%	$5.3x10^5$	40.52	45.25	35.86	79.24^{a}
10%	$5.8x10^5$	39.42	42.98	34.90	81.20^{bc}
0%	$5.7x10^5$	36.45	40.21	33.71	83.83^{e}

Values followed by different letters differ significantly from each other at $P \leq 0.05$, based on SAS software

7 A). Due to the limitations in the production process of VCO, to minimize the processing conditions, the study was designed by taking the parameters in the narrow range. Coconut powder (Fig. 7 B) produced in the process as a by-product with low-fat, high- fiber which may be successfully used in foods and as confectionery ingredient. Whey (Fig. 7 C) produced as a waste may be used in the production of bioextract.

Figure 7. *(A) The VCO produced from the induced fermentation. (B) Coconut powder and (C) Whey.*

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