

Optimization of Parameters for Fermentative Production of Virgin Coconut Oil by *Lactobacillus fermentum* NDRI 141

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Abstract: Commercially, many methods are adopted for the production of the Virgin Coconut Oil (VCO). Nowadays, natural fermentation is widely employed to produce VCO in wet processing. But the problem in natural fermentation process has much contamination, due to surplus micro organisms present in natural environment, which leads to the poor quality of VCO. To overcome this, usage of probiotic organism like *Lactobacillus fermentum* is more beneficial for the fermentative production of VCO. Fermentation studies were conducted scientifically in computer controlled bioreactor to determine the effect of pH, temperature, inoculum concentration, oxygen requirement and incubation time on the yield of VCO. Yield efficiency of VCO in each parameter was determined. The pH of 5 ± 0.1 , temperature at 45 ± 0.1 °C, inoculum concentration of 2%, fermentation end time of 48 hrs and microaerophilic conditions are the most suitable parameters for the superior production of VCO.

Key words: Virgin coconut oil (VCO), coconut milk, Lactobacillus fermentum.

1. Introduction

Coconut (Cocos nucifera L.) is a tree of life, which is giving many useful products for mankind. Virgin Coconut Oil (VCO) is one of the Value Added Product (VAP) of Coconut, which has wider proven applications in medicine, food, cosmetics etc.. Anti-microbial property of VCO was reported against Pseudomonas florescence, **Bacillus** subtilis, Salmonella spp. and Escherichia coli [1]. In some animal studies of VCO, it reduces total cholesterol, triglycerides, phospholipids, Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) levels and increases the High Density Lipoproteins (HDL) in serum and tissue [2]. VCO is showing significant anti thrombotic effect, in animal studies animals fed with VCO showed increased anti-oxidant vitamin levels [3]. Researchers proved the

ability of VCO to cure Psoriasis (skin disease) [4]. VCO with the different essential oils (lemon, eucalyptus, lavender) are reported in aroma therapy [5]. VCO is reported as high quality raw material for drinks, cooking lotions, soaps, shampoos [6]. According to the specifications of Asian Pacific Coconut Community (APCC), Codex, the Philippines National standard (PNS), Bureau of Product Standard (BPS) 2004, VCO is the product of minimal processing in order to preserve the original components of the coconut [7]. VCO is obtained from the fresh, matured coconut kernel by mechanical or natural means, with or without heat, without refining, bleaching and deodorization, which do not lead to the alteration of the natural oil [8]. Traditionally many methods are there for processing of coconut in wet basis like applying of temperature, alteration of pH (extreme acidic to basic), chilling and centrifugation, incorporation of enzymes [9], microbes such as L. plantarum [10], natural fermentation [11], supercritical fluid extraction [12], using crab paste as

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traditional java method [13], using acetic acid [14] were reported. The methods which are practiced in all the cases of wet processing are not according to APCC or any other standard specifications for the production of VCO. 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 main objective of present work is to develop a process for the production of VCO mediating probiotic organism (*L. fermentum*) by fermentation method using computer control bioreactor and optimization of different parameters to obtain higher yields.

In the present work, VCO production was practicing by natural fermentation. Coconut milk was subjected to the fermentation in natural conditions. Coconut milk was destabilizing by the microbes present in the natural environment and leads to the separation of the oil. In such practice the milk was contaminated by certain type of microorganisms and producing the poor quality oil with yellow color which is shown in Fig. 1.

To overcome this problem, the production of VCO was carried out in controlled conditions by using probiotic organisms. *L. fermentum* was reported as probiotic and used in the production of sillage [15]. In many food products *L. fermentum* was used successfully.

2. Materials and Methods

2.1 Coconut Sample

Uniformly sized, 12 months old (matured) nuts were collected from local market.

2.2 Microbial Culture

Pure culture of the *L. fermentum* 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.

2.3 Coconut Milk Extraction

Coconut milk was extracted from solid endosperm; coconut milk was 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. Coconuts were broken and solid endosperm was collected, testa



Fig. 1 (A) Growth of some fungi in natural fermented milk (green color), (B) spoiled VCO (yellow color).

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.

2.4 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 (U.V) light in laminar air flow was done for 20 min per liter in glass beaker.

2.5 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.

No. of micro organisms present in sample

$$= \frac{\text{No. of Colonies present on plate}}{\text{Dilution factor}} (1)$$

2.6 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.

2.7 VCO Recovery (Downstream Processing)

After successful completion of bioreactor runtime, the fermented milk was centrifuged in temperature controlled centrifuge at 27 °C and 6,000 rpm for 10 min. Separated VCO was collected; pooled VCO of all batches were finally centrifuged for clear oil at above conditions.

2.8 Calculation of Recovery and Process Efficiency

For coconut sample, moisture was determined by hot air oven (BIS) method [16] and oil content by soxhlet (AOCS) method [17]. Oil yield and efficiency of the method was calculated by using following formulae respectively.

Oil yield (%) =	
Weight of VCO Obtained	(2)
Weight of Coconut taken for milk extraction	- (2)
Efficiency of the method (%)	
X7. 110/ (1)	

$$= \frac{4400 \text{ whet basis}}{600 \text{ of oil content in coconut}}$$
(3)

2.9 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 the same during the entire study except the particular parameter to be studied.

2.10 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 \pm 1 °C as dead band.

2.11 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 \pm 0.1 as dead band.

2.12 Effect of Inoculum Concentration

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

2.13 Effect of Fermentation End Time

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

2.14 Effect of Oxygen Concentration

Oxygen concentration, also one of the influencing factors 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.

2.15 Statistical Analysis

All parameters were carried out in duplicate. Statistical mean of two 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 $P \le 0.05$, based on SAS software (procedure followed was PROC ANOVA).

3. Results and Discussion

In coconut milk around 5.5%-8.5% of different carbohydrates are present; amongst the major are sucrose and starch [18]. *L. fermentum* has the capacity to convert the sugars in coconut milk to lactic acid; it decreases the pH of fermenting milk to acidic. In acidic conditions, coconut milk was undergoing to denaturation and destabilization of proteins, causing the release of water and clustering the oil droplets [14, 10].

3.1 Effect of Temperature

It was reported that the *L. fermentum* can resist temperature from 15 °C to 45 °C [19]. But average temperature for the growth of bacteria is at 37 °C. In the studied temperatures range, the highest process

efficiency was present at 45 ± 1 °C with 82.92% and at 40 ± 1 °C with 82.02%. According to the earlier studies [9] the yields were reported that 60%, 70% at 40, 50 °C respectively whereas in the present study yields were improved. This was 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 was no much difference in process efficiency between temperatures at 40 ± 1 , 45 ± 1 °C in present study. Effect of temperature is shown in Fig. 2.

3.2 Effect of pH

Range of pH from 5 ± 0.1 to 9 ± 0.1 was used in the present study. It is reported that L. fermentum detected in intestine can resist the high acidic conditions [20], and also reported that some strains have capacity to produce the α -amylase, α -galactosidase [21, 22]. So the process efficiency percentage is more at pH 5 \pm 0.1 with 83.19% generally proteins gets destabilize by the acidic pH. According to the earlier study [9] at pH 5.0 the yields were 78%, where as in the present study more process efficiency was achieved by using L. *fermentum* at pH 5 ± 0.1 . In the case of basic conditions, the process efficiency achieved was 81.04% at $9.0 \pm$ 0.1 where as the oil yield reported [9] 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 . Fermentation process carried in the acidic conditions was easy when compared



Fig. 2 Graph of effect of temperature.

to the basic conditions because lactic acid was produced continuously by organism. Effect of pH was shown in Fig. 3.

3.3 Effect of Inoculum Concentration

Number of microorganisms is directly proportion to inoculum concentration. In the study 1%, 2%, 5% inoculum concentrations were taken and higher yields were reported at 2% inoculum with 79.33%, and 5% with 81.07%. The efficiency of the process at 1% was very poor with 60.93%. Though the concentration of inoculum between 2% and 5% is more than twice, but the difference in the yields were not significant, so the inoculum concentration of 2% was preferred for the production. The results were shown in Fig. 4.

3.4 Effect of Fermentation End Time

The sample was collected from the sampling drain with the intervals of 24, 48, and 72 hrs and yields obtained were 51.21%, 80.20%, 80.25% respectively. There was no appreciable increase in yields in the process when compared with yields at 48 and 72 hrs. So 48 hours of fermentation end time is preferable to save the production time. The values were shown in Fig. 5.

3.5 Effect of Oxygen Concentration

Oxygen concentrations at aerobic, microaerophilic, anaerobic conditions were maintained. 79.01% and 82.09% of process efficiency were obtained at aerobic and anaerobic conditions respectively. The highest efficiency was noticed at microaerophilic condition with 83.12%. Generally lactobacillus sp. was anaerobic to facultative aerobic in nature, L. fermentum is an aero tolerant in nature, it could resist oxygen concentration by non enzymatic super oxide reduction mediated by manganese [23]. So, growth at aerobic, microaerophilic and anaerobic conditions was possible for L. fermentum. It is reported [24] that the cell growth was fast at microaerophilic conditions and was preferable due to improved yields in the present study also. The oxygen dependency of VCO production efficiency is represented in Fig. 6.



Fig. 3 Graph of effect of pH.



Fig. 4 Graph of effect of inoculum concentration.



Fig. 5 Graph of effect of fermentation end time.

4. Conclusions

It is clearly evident that the optimal parameters for the production of VCO (Fig. 7A) to obtain higher yield are: temperature 45 ± 1 °C, pH 5.0 ± 0.1, 2% of inoculum



Fig. 6 Graph of effect of oxygen concentration.

concentration, fermentation end time of 48 concentration, fermentation end time of 48 hrs and microaerophilic conditions. In the process, coconut powder (Fig. 7B) produced as a by-product with low-fat, high-fiber which may be successfully used in foods as confectionery ingredient. The whey is a waste product in the fermentation process which may be used for the bioextract production. Finally, it concludes that VCO extraction by using probiotic organisms is more beneficial and it avoids the natural contamination.

Table 1 Study of different parameters on the production of VCO.							
Parameter	Study conditions	Microbial count	Moisture content	Oil content Soxhlet	Yield % in proo by dry basis	cess % of efficiency of method*	
	30 ± 1	5.0×10^{5}	40.14	41.43	31.66	76.42 ^f	
Temperature	37 ± 1	4.9×10^{5}	39.60	42.78	33.90	79.24 ^{de}	
	40 ± 1	5.0×10^{5}	39.17	44.01	35.26	80.11 ^{dce}	
	45 ± 1	5.1×10^{5}	42.01	40.29	33.41	82.92 ^a	
рН	5 ± 0.1	4.8×10^{5}	41.68	40.82	33.96	83.19 ^{ba}	
	6 ± 0.1	5.0×10^{5}	40.02	39.92	32.01	80.18 ^{dc}	
	7 ± 0.1	4.9×10^{5}	39.74	43.10	31.52	73.13 ^g	
	8 ± 0.1	4.9×10^{5}	41.07	40.85	31.07	76.05 ^f	
	9 ± 0.1	5.0×10^{5}	40.59	41.04	33.26	80.04 ^c	
Inoculum concentration	1%	4.8×10^{5}	41.99	39.77	24.23	60.93 ^h	
	2%	5.0×10^{5}	40.02	42.08	33.39	79.34 ^{dce}	
	5%	4.9×10^{5}	39.42	40.05	32.47	81.07 ^c	
Fermentation time	24 hrs				20.60	51.21 ⁱ	
	end 48 hrs	5.1 × 105	42.01	40.22	32.23	80.13 ^{dce}	
	72 hrs				32.28	80.25 ^{dce}	
	Aerobic	4.9×10^{5}	41.47	43.03	34.00	78.01 ^e	
Oxygen require	ment Microaerophilic	4.9×10^{5}	40.56	41.46	35.77	83.12 ^b	
	Anaerobic	5.0×10^{5}	41.92	40.32	33.10	82.09 ^{ba}	

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



Fig. 7 (A) Water white virgin coconut oil produced in fermentation process, (B) By-product of process low fat contained coconut powder.

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