Annals. Food Science and Technology
2013

OPTIMIZATION OF PARAMETERS FOR FERMENTATIVE PRODUCTION OF VIRGIN COCONUT OIL BY *LACTOBACILLUS Sp.*

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Abstract
In general, different wet and dry methods were employed for the production of Virgin coconut Oil (VCO). Natural fermentation is a traditional process with industrial application and is common in commercial and domestic processing, but, this method has contamination problem. In the present study, production of VCO was carried out by using certain probiotic organisms under controlled conditions to overcome the contamination problem which was frequently encountered in natural fermentation. It was carried out in computer controlled bioreactor by using L. fermentum NDRI-141, L. plantarum NDRI-184 and L. acidophilus NDRI-II individually; by studying the effect of different parameters viz., temperature, pH, inoculum concentration, fermentation end time, oxygen requirements and were optimized and compared. L. plantarum showed highest yield of VCO than L. fermentum and L. acidophilus and there was no contamination of coconut milk. For all the three species of Lactobacillus; fermentation temperature 45±1^{0}C, pH 5±0.1, inoculum concentration 2%, fermentation end time 48 hrs were optimum. In the case of oxygen requirement, anaerobic condition was optimum for L. plantarum and microaerophilic was for the remaining two species. The results suggest that utilization of L. plantarum may be the choice of organism for induced fermentative production of VCO. VCO produced in induced fermentation process has characteristic natural coconut flavour, water white in color and sweet in aroma.

Keywords: *L. plantarum, L. fermentum, L. acidophilus*, natural fermentation, induced fermentation, computer controlled bioreactor, virgin coconut oil.

Submitted: 03.05.2013 Reviewed: 24.05.2013 Accepted: 10.06.2013

1. INTRODUCTION

VCO differs from the commercial coconut oil (CNO) in the way of its processing. VCO is a value added product from coconut with numerous applications for mankind. Asian Pacific Coconut Community (APCC), The Philippine National Standards (PNS), Bureau of Product

Standards like Bureau of Product Standards (BPS) defined VCO as it is naturally processed either from ball copra (within the nut) or fresh coconut meat derivatives (coconut milk), free from additives, which has not undergone any chemical, high temperature processing changes after extraction in order to preserve its natural qualities (Fabian MD et al. 2007 & Blance JV et al.2007). VCO has its special identity in food (Choo SY et al.2010 & Aritonang SN et al. 2009), medicine (Winarsi H et al.2008 & Nevin KG and Rajamohan T 2010), cosmetic applications (Songkro S et al.2010) and in the preparation of silver nano-particle, nano emulsion creams (Zamiri R et.al 2011 & Al-Edresi S, Saringat B 2009). Nevin KG, Rajamohan T (2006&2008) reported that VCO contains antioxidant vitamin levels were more than CNO.

In natural fermentation, coconut milk was produced and allowed for fermentation with microbes present in natural environment. In such process, coconut milk was destabilized by microbes and separates out VCO. In most of the cases, unwanted microorganisms were contaminating the process resulting spoilage of coconut milk which produces yellow colored CNO. To overcome such problems, fermentation was carried out under controlled conditions by using specific probiotic species of organisms. Kaenhammer TR (2000) was

proposed that probiotic cultures have been associated historically with different fermented food products from which there were substantial evidences for positive effects on human health and general well-being. Literature on fermentative production of VCO 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 by mediating different probiotic organisms (*L. plantarum, L. fermentum, L. acidophilus*) in induced fermentation method using computer controlled bioreactor and also study the effect of different operational parameters to obtain higher yields.

2. MATERIAL AND METHODS

Microbial Culture:

Lyophilized pure cultures in a glass vial was collected from National Dairy Research Institute-National Center for Dairy Cultures (NDRI-NCDC), Karnal, Haryana, India and sub-cultured by following the instructions given in NCDC catalogue.

Coconut milk Extraction:

Coconut milk was extracted from solid endosperm. Marasabessy A et al. (2010), Norulaini NAN et al. (2009) & Che Man Y B et al. (1997), were reported several methods were reported for the extraction of coconut milk. But, some of the conditions adapted and instruments used were not suitable for the extraction of coconut milk, hence followed short and simple method.

Fresh coconuts were dehusked and water was collected from the pore in a separate container. Coconuts were broken and solid endosperm was collected, testa was removed by using kitchen peeler, disintegrated into small pieces with knife and crushed 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 extracted coconut milk was pooled up for the production of VCO. After milk extraction, residual coconut powder was dried and preserved for other food applications.

Coconut milk sterilization:

Microbes may enter through water, environment and utensils in to coconut milk during extraction. Coconut milk was exposed to Ultra Violet (U.V.) light in laminar air flow for 20 min per liter in a glass beaker to avoid such contamination.

Seed culture preparation:

Seed culture was prepared by using nutrient broth medium; culture flasks were incubated at 37^0C for 36 hours at 100 RPM in orbital shaker and same conditions was maintained for entire study.

Fermenter scale-up process (Upstream Processing):

According to the Spectrochem-India Biotron model bioreactor user manual, Dissolved Oxygen (DO) and pH probes were standardized, they were fixed to the fermenter vessel lid, closed the fermenter and sterilized at 121^0C for 15 min. in autoclave. Sterilized coconut milk was poured in to bioreactor vessel at aseptic conditions. Further, the parameters were arranged in accordance to the designed study.

VCO recovery:

After successful completion of fermentation, the fermented material was centrifuged in a temperature controlled centrifuge at 27^0C and 6000 rpm for 10 min. Separated VCO was collected and pooled from all batches were finally centrifuged to obtain a clear VCO by maintaining the same conditions.

Calculation of recovery and Yield in method:

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 formulae (1&2) respectively.

$$\text{VCO recovery (\%)} = \frac{\textit{weight of the VCO obtained}}{\textit{weight of the Coconut taken for milk extraction}} \quad \text{---------- (1)}$$

$$\text{Yield in method (\%)} = \frac{\text{Yield \% on wet basis}}{\text{oil content present in the coconut}} \quad \text{----------(2)}$$

Studies on the Effect of Parameters on VCO yield:

Different major parameters such as temperature, pH, concentration of inoculum and oxygen, fermentation end time were studied. All the parameters (temperature 37 $\pm 1^0$C, pH 6 ± 0.1, 2% inoculum concentration, 48 hours fermentation end time and aerobic conditions) remain same during the entire process except the particular parameter to be studied.

Coconut milk is oil in water emulsion of fat stabilized by proteins, phospholipids and water. If it allows for some time the oil portion along with protein is floats on water, it leads to improper mixing of the contents in submerged conditions. Therefore, a constant rotation of stirrer with 200 RPM was used during the entire study.

Temperature and pH are the parameters which can influence the microorganism's metabolic actions.

Effect of temperature: The temperatures at 30, 37, 40 and 45^0C were used with $\pm 1^0$C as dead band.

Effect of pH: The 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 was maintained for the duration of 24, 48 and 72 hours.

Effect of Oxygen concentration: Oxygen concentration is also one of the influencing factors for bacterial metabolism, aerobic with 100% oxygen; microaerophilic condition with 10% oxygen and anaerobic without oxygen in fermentation process were maintained.

Statistical Analysis:

All parameters were carried out in quadruplicate and statistical mean was presented. Significant differences between means were determined by Duncan's multiple range tests (Duncan BB, 1955) by using PROC ANOVA and were considered to be significant when ≤ P 0.05 based on SAS software.

3. RESULTS AND DISCUSSION

In coconut milk, about 5.5-8.5% of different carbohydrates are present; amongst the major are sucrose and starch (Seow CC & Gwee CN 1997). *Lactobacillus* has the capacity to convert sugars in coconut milk as lactic acid which decreases the pH of fermenting milk to acidic. In acidic conditions, coconut milk undergoes denaturation and destabilization of proteins, causing the release of water and clusters of oil droplets (Che Man YB et al., 1997).

Effect of temperature on the yield: Production of VCO by *Lactobacillus sp.* at different temperatures was presented in fig.1.

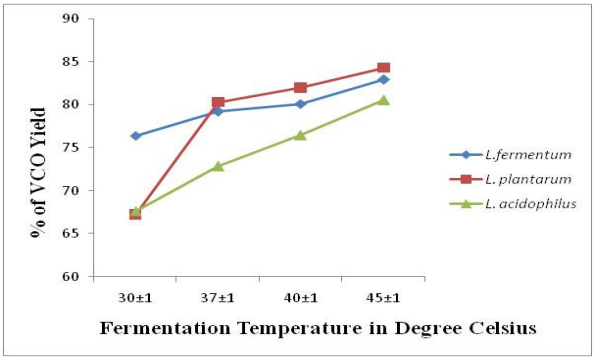

Fig.1. Effect of temperature on VCO yield

Breed RS et al. (1957) reported that normally members of *Lactobacillus* genus resist high temperatures. In the present study, highest VCO was obtained at 45 ± 1^0C by all the three organisms. However, *L. plantarum* was showed highest VCO yield (84.30%) followed by *L. fermentum* (82.92 %) and *L. acidophilus* (80.55%) at the same temperature. In a reported study by Raghavendra SN, Raghavarao KSMS (2010), VCO produced about 60, 70% at temperatures of 40 and 50^0C respectively. But the VCO yields were improved > 10 % in the present study when compared with the reported. This was achieved by the combination of two parameters for destabilization of coconut milk; they were in acidic condition (pH 6±0.1) and the

temperature which was maintained at 45±1^{0}C. Responses of three *Lactobacillus sp.* at different temperatures were depicted in the table 1.

Effect of pH on the yield: Production of VCO by three *Lactobacillus sp.* presented in fig. 2 and it was observed from figure that pH 5±0.1 is optimum for all the three organisms. *Lactobacillus* genus organisms resist acidic pH (Breed RS, 1957).

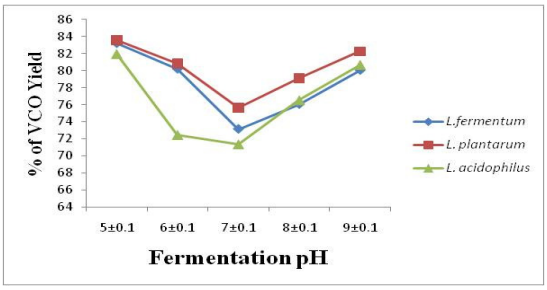

Fig. 2. Effect of pH on VCO yield

At pH 5±0.1 highest VCO yield was obtained by *L. plantarum* (83.58%) followed by *L. fermentum* (83.19 %) and *L. acidophilus* (81.49%). According to the earlier study of Raghavendra SN, Raghavarao KSMS (2010) at pH 5.0 the yields around 78%, where as in the present study more process efficiency was achieved by using *Lactobacillus sp.* at pH 5± 0.1. In basic conditions at pH 9±0.1, 82.86% of VCO yield was obtained by *L. plantarum* and remaining two organisms, *L. fermentum* (80.04%) and *L. acidophilus* (80.66%) was produced almost equal with reported study. Processing of VCO by fermentation under acidic conditions is easier than basic conditions because lactic acid was produced profusely by the organisms. The data obtained was tabulated in table 1.

Effect of inoculum concentration: Effect of inoculum concentration on VCO production by three *Lactobacillus sp.* was showed in fig.3.

It is concluded from the fig.3 that inoculum concentration of 5% was the effective for the production of VCO by three *Lactobacillus sp.* i.e., *L. plantarum* (82.91%), *L. fermentum* (81.07%) and *L. acidophilus* (74.09%). With 2% inoculum concentration VCO yields of 80.72%, 79.34% and 73.44% were obtained for *L. plantarum, L. fermentum* and *L. acidophilus* respectively.

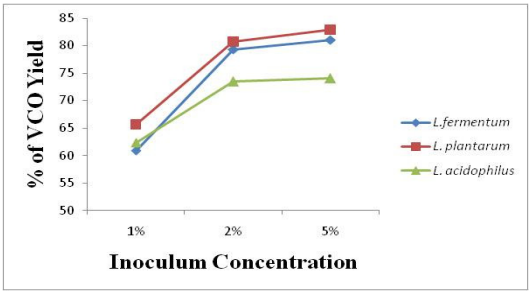

Fig. 3. Effect of pH on VCO yield

The difference between 2 and 5% of inoculum concentrations was more than twice but the yield was @ 2 % only. Based on this observation, it is more economical to fix inoculum concentration of 2% as optimum for fermentative production of VCO and the results were given in table 1.

Effect of fermentation end time: Effect of fermentation end time on VCO production by three *Lactobacillus sp.* was shown in fig.4.

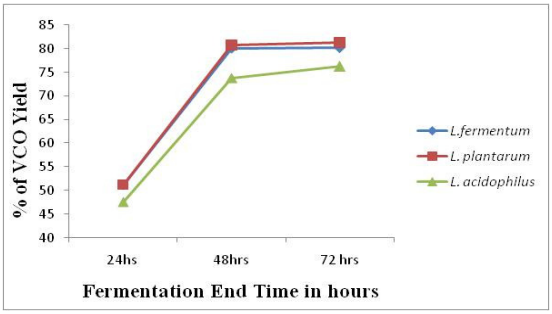

Fig. 4. Effect of fermentation end time on VCO Yield

Fermentation end time of the 24, 48 and 72 hours were maintained in the study. The highest yields of VCO were obtained at 72 hours by all the three organisms i.e., *L. plantarum* (81.31%), *L. fermentum* (80.25%) and *L. acidophilus* (76.27%). After fermentation of 48 hours the VCO yields of 80.73%, 80.13% and 73.82% were obtained for *L. plantarum, L. fermentum* and *L. acidophilus* respectively. The difference of 24 hrs of fermentation end time is present between 48 and 72 hrs but the difference in yield is < 3%. So, it is more economical to fix fermentation end time of 48 hrs as optimum by considering production cost, time and to minimize unit

operations. The results obtained were shown in table 1.

Effect of oxygen requirement: Effect of oxygen requirement on VCO production by three *Lactobacillus sp.* was drawn in fig.5.

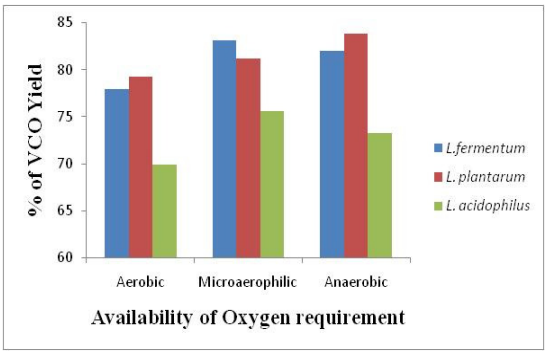

Fig. 5. Effect of oxygen requirement on VCO yield.

Most of the bacterial members of the *Lactobacillus* genus were the anaerobic to facultative anaerobic in nature but they can resist the aerobic conditions by non enzymatic super oxide reduction mediated by Mn (Mark AD& Ingolf FN 1994). In the present study, highest VCO yields were obtained by *L. plantarum* (83.83%) showed at anaerobic conditions whereas *L. fermentum* (83.12%) and *L. acidophilus* (75.60%) at microaerophilic conditions. At aerobic conditions all three organisms showed the lower yields compared to the anaerobic and microaerophilic conditions. The data obtained was tabulated in table 1.

4. CONCLUSIONS

Based on the study, it is concluded that for all the three species of *Lactobacillus;* fermentation temperature 45±1^{0}C, pH 5±0.1, inoculum concentration 2%, fermentation end time 48 hrs was optimum. In the case of oxygen requirement, anaerobic condition was optimum for *L. plantarum* and microaerophilic was for the remaining two species. At optimum conditions the *L. plantarum* showed highest production of VCO followed by *L. fermentum* and *L. acidophilus.*

Contamination of coconut milk was totally avoided in induced fermentation which was most common in natural fermentation.

Table 1: Effect of different parameters on the production of VCO by induced fermentation by using *L. fermentum, L. plantarum* and *L. acidophilus.*

Parameter		% of VCO yield		
		L. fermentum	***L. plantarum***	***L. acidophilus***
Temperature	30±1	76.42^{f}	67.23^{e}	67.67^{f}
	37±1	79.24^{de}	80.31^{b}	72.87^{dce}
	40±1	80.11^{dce}	81.96^{ba}	76.43^{bc}
	45±1	82.92^{a}	84.30^{de}	80.55^{ba}
pH	5±0.1	83.19^{ba}	83.58^{dca}	81.94^{a}
	6±0.1	80.18^{dc}	80.85^{c}	72.45^{dce}
	7±0.1	73.13^{g}	75.63^{b}	71.34^{bdf}
	8±0.1	76.05^{f}	79.13^{e}	76.51^{bc}
	9±0.1	80.04^{c}	82.26^{f}	80.66^{ba}
Inoculum concentration	1%	60.93^{h}	65.67^{ba}	62.39^{g}
	2%	79.34^{dce}	80.72^{b}	73.44^{dce}
	5%	81.07^{c}	82.91^{e}	74.09^{h}
Fermentation end time	24hs	51.21^{i}	51.18^{dca}	47.61^{dce}
	48hrs	80.13^{dce}	80.73^{b}	73.82^{dce}
	72 hrs	80.25^{dce}	81.31^{e}	76.27^{bc}
Oxygen requirement	Aerobic	78.01^{e}	79.24^{a}	69.90^{fe}
	Microaerophilic	83.12^{b}	81.20^{bc}	75.60^{dc}
	Anaerobic	82.09^{ba}	83.83^{e}	73.30^{b}

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

Utilization of *L. plantarum* is the choice of organism in induced fermentative process for the production of VCO. VCO produced by induced fermentation has characteristic natural coconut flavor, water white in color and sweet in aroma. In the process, low-oil, high-protein and fiber-rich coconut meal was obtained as a by-product which was another value added product with ample applications in the field of confectionary and food. The whey which was produced as waste product in the process was effectively used for the production of bio-extracts.

5. ACKNOWLEDGEMENTS

First author is greatly acknowledges to the JNT University authorities for providing financial support, permitting to work in Oil Technological Research Institute (OTRI) and Department of Chemical Engineering, JNTUCEA. Also, thankful to Directors OTRI and IRP JNTUA for their constant support and encouragement.

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