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Research Article

Induced fermentative production of virgin coconut oil

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Abstract

Coconut (*Cocos nucifera* L.) is one of the major commercial crops in southern India, which gives many useful products to the inhabitants. Virgin Coconut Oil (VCO) is one among them, produced from both ball copra (within the nut) and fresh coconuts. VCO differs from commercial coconut oil (CNO) the way it is processed. In wet processing, natural fermentation is well known for its traditional and industrial outlook as it is carried out by microbes present in the natural environment. However, this process has contamination problems due to the presence of unwanted microorganisms and uncontrolled conditions. It leads to the production of poor quality CNO (usually yellow in colour, rancid smell). To overcome this problem, VCO was produced by the induced fermentation method where certain species of probiotic microorganisms were used under semi-controlled conditions. Quality control parameters for VCO produced both in natural fermentation and induced fermentation (semi-controlled conditions) were studied and compared with the standards given by APCC (Asian Pacific Coconut Community).

Keywords: Natural fermentation, VCO, *L. plantarum*, India.

Introduction

According to the specifications of Asian Pacific Coconut Community (APCC), Codex Alimentarius, 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 [1]. VCO is obtained from either fresh, matured coconut kernel or ball copra (within the nut) by mechanical or natural means, with heat (< 50^0C), without the use of chemicals, which do not alter its natural qualities [2]. Traditionally, many methods have been reported for wet processing of coconut by

applying high temperature, alteration of pH (extreme acidic to basic), chilling and centrifugation, incorporation of enzymes [3], microbes such as *Lactobacillus sp.* [4], natural fermentation [5], supercritical fluid extraction [6], using crab paste as traditional java method [7], using acetic acid [8] etc. All these methods of wet processing do not meet the definition of VCO.

VCO is one of the Value Added Products (VAP) to coconut, which has numerous proven applications in medicine, food, cosmetics etc. Anti-microbial properties of VCO has been reported against *Pseudomonas florescence*, *Bacillus subtilis*, *Salmonella sp.* and *Escherichia coli* [9, 10]. In some animal studies, VCO reduces total cholesterol, triglycerides, phospholipids, Low Density Lipoproteins (LDL), Very Low Density Lipoprotein (VLDL) levels and increasing the High Density Lipoproteins (HDL) in serum and tissue were reported [11]. VCO showed significant anti thrombotic effect in animal studies, where animals fed with VCO increases antioxidant vitamin levels [12]. Researchers also proved the ability of VCO to cure Psoriasis (skin disease) [13]. VCO incorporated in different essential oils (lemon, eucalyptus, lavender) were reported in the application of aroma therapy [14]. VCO has been reported as high quality raw material for health and skin care products [15]. Researchers also reported that VCO contains more phenolic compounds and antioxidant capacity than CNO [16]. Some of the phenolic acids (protocatechic, vallinic, caffeic, syringic, ferulic and p-coumaric) were identified in VCO which confirmed its antioxidant activity [17].

Probiotic cultures have been associated historically with cultured milk and dairy products, from which there is substantial evidence for positive effects on human health and general well-being [18]. Several *in vitro* and *in vivo* experiments on antagonism of different *Lactobacillus* strains against *Helicobacter pylori* and *Clostridium difficile*, *Campylobacter jejuni*, *E. coli* were performed. All tested human *Lactobacillus* strains were able to inhibit the growth of all strains of anaerobic human gastrointestinal pathogens [19].

Literature on the fermentative production of VCO from coconut by using probiotic organisms is relatively low, which instigated this research to study this problem. The main objective of the present work is to develop a process for the production of VCO mediating probiotic organism by induced fermentation method under semi-controlled conditions to overcome the contamination problem in natural fermentation, to determine the quality control parameters according to the APCC standards and to compare the VCO produced from both the methods.

Materials and Methods

Coconut sample

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

Coconut milk extraction

Coconut milk was extracted from solid endosperm; coconut milk was oil in water emulsion, stabilized by proteins and phospholipids. In the literature [3, 4, 5, 6, 7, 8], several methods were reported for extracting coconut milk. However, some of the conditions adapted and instruments used were found to be not suitable for extraction of coconut milk, hence a short and simple method was followed.

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 a kitchen

peeler, white coconut balls were disintegrated into small pieces and crushed with 1:2 ratio of water for 10 min. Ground mass was transferred to a cheese cloth, pressed manually for coconut milk extraction; the same process was repeated twice and coconut milk was pooled up for the production of VCO. Extracted coconut powder was dried and preserved for another application.

Production of VCO from natural fermentation

Produced coconut milk was allowed to ferment naturally in a separating funnel or in a clean container with drain and kept aside for 48 hrs at room temperature. During fermentation, coconut milk was slowly destabilized into coconut oil, protein portion and whey. VCO was present on top of the vessel, in the middle non-destabilized cream with protein matter and whey water remained at the bottom. Whey water was drained off through the drain; separated oil and non-destabilized cream with protein matter was collected into a beaker and filtered by Whatman filter paper as shown in Figure1. The entire process was carried for several times to study the production yields.

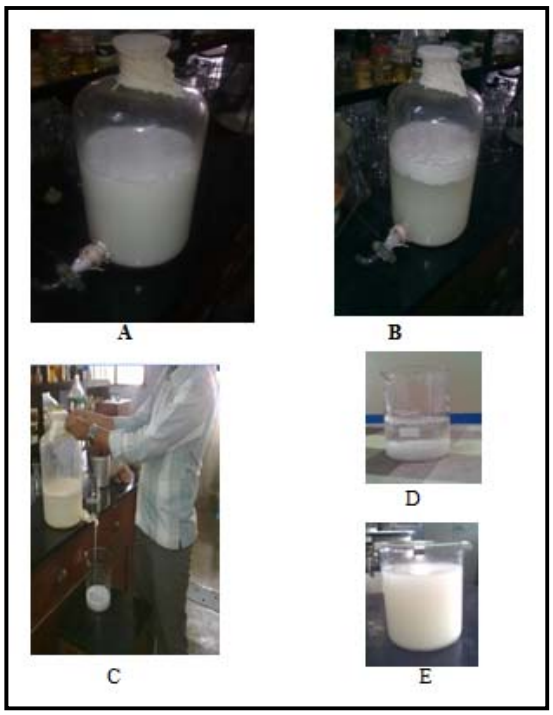

Figure 1. Natural fermentation of the coconut milk.

A. milk taken in a container having drain facility. B. Milk allowed for natural fermentation. C. Separation of whey and VCO produced. D. Water-white VCO produced in the process. E. whey produced in the process.

During the production of VCO by natural fermentation, contamination by different microbes occurred much of the time resulting in the production of yellow coloured spoiled coconut oil (Figure 2).

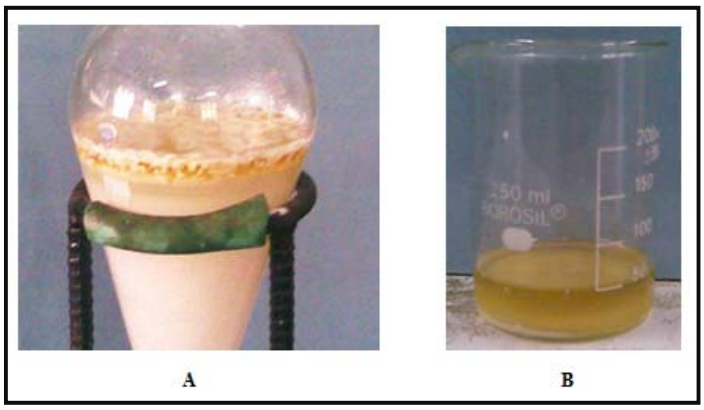

Figure 2. Natural fermentation (contaminated) process.
A. Spoiled fermented products during natural fermentation. B. Separated yellow colored oil in contaminated fermentation process.

Production of VCO from induced fermentation

Microbial culture

Lyophilized pure culture of *L. plantarum* in a glass vial was collected from National Dairy Research Institute-National Centre for Dairy Cultures (NDRI-NCDC) and sub-cultured by following the instructions given in the NCDC catalogue.

Coconut milk sterilization

Microbes may enter through water, environment and utensils into coconut milk during extraction. Coconut milk was exposed to Ultra Violet (UV) light in a laminar air flow for 20 min per litre 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 an orbital shaker and the same conditions were maintained for the entire study.

Induced fermentation

After sterilization, seed culture with 2% of broth was added to the coconut milk under aseptic conditions in laminar air flow unit and the conical flask was made airtight by closing its mouth with cotton plug and placed in shaking incubator for 48 hrs at 37^0C temperature (Fig.3). Due to fermentation the coconut milk was destabilized into VCO and protein matter.

After successful completion of fermentation, the fermented products were centrifuged in a temperature controlled centrifuge at 27^0C and 6000 rpm for 10 min. Separated VCO collected and pooled from all batches was finally centrifuged to obtain a clear VCO by maintaining the same conditions. The entire process was repeated for several times to study the production yields.

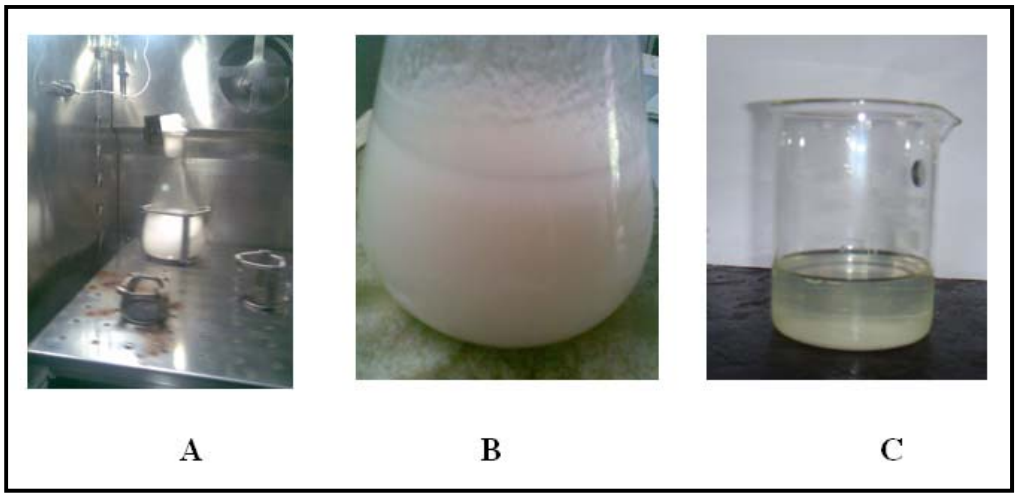

Figure 3. Production of VCO by induced fermentation (semi controlled).
A. Coconut milk with inoculum placed in orbital shaker. B. Coconut milk after fermentation and C. VCO produced from induced fermentation

Determination of quality control parameters for the produced oil
Physico-chemical [20, 21] and microbiological [22] quality parameters were analyzed for both the VCOs produced in natural and induced fermentation (semi controlled) process and the data is given in Tables 1 and 2.

Statistical analysis
All parameters were carried out quadruplicate and statistical means were determined. Significant differences between means were revealed using Duncan's multiple range test [23] and were considered to be significant when ≤P0.05 based on SAS software.

Results and Discussion

Yield
VCO yield percentage of 25.68± 0.963 (natural fermentation) and 28.47±1.070 (induced fermentation, semi-controlled) were obtained. It was observed that VCO yield percentages were improved around 3% by using probiotic organism *L. plantarum*.

Further, it was observed that the contamination of coconut milk was totally absent in the induced fermentation (semi-controlled). This was achieved by introduction of a sterilization step for coconut milk before fermentation. Induced fermentation process was done in semi-controlled conditions without the entry of any unwanted organisms. These two steps prevented contamination of the production process. It is reported that coconut milk contains 5.5 - 8.5% of different carbohydrates; amongst the major were sucrose and starch [24]. *L. plantarum* has the capacity to convert sugars to lactic acid in coconut milk; it decreases the pH of fermented milk to acidic. In acidic conditions, coconut milk underwent the destabilization of proteins, causing the release of water and clustering of oil droplets [4, 8]. The VCO produced in induced fermentation (semi-controlled) was pure, water white in colour with characteristic coconut smell.

Table 1. Quality parameters of VCO produced from both natural and induced fermentation (semi-controlled) methods.

S.No.	Parameter		VCO from NF	VCO from IF	APCC standards
1	Specific gravity at 30/30^{0}C		0.918 ± 0.0028^a	0.920±0.0055^a	0.915-0.920
2	Refractive index at 40^{0}C		1.4490 ± 0.0036^a	1.4483±0.0016^b	1.4480-1.4492
3	Moisture,%		0.52 ± 0.01^a	0.56±0.02^a	0.1-0.5
4	Insoluble impurities, %		0.034± 0.06^b	0.029±0.13^a	0.05
5	Saponification Value		252.4±0.71^a	252.5± 0.94^b	250-260 min
6	Iodine value (Wijs)		4.9±0.31^a	5.1±0.99^b	4.1-11.0
7	Unsaponifiable matter,%		0.38±0.035^a	0.40±0.031^a	0.2-0.5
8	Polenske value		13.9±0.6a	13.9±0.3^a	13 min
9	Free fatty acids,%		0.46±0.19^b	0.49±0.15^a	≤ 0.5%
10	Peroxide value (meq/kg oil)		0.86±0.02^a	0.85±0.01^a	≤ 3
11	Total plate count CFU/0.1mg		31± 3.1^a	49±2.8^a	< 10
12	Metal contaminants (mg/kg)				
	1	Iron	1.08	1.13	5
	2	Copper	0.33	0.22	4
	3	Lead	ND	ND$^{\#}$	0.1
	4	Arsenic	0.01	0.02	0.1
13	Tocopherol (mg/100gms)		6.12	6.23	NA*

Note: in the row values followed by different letters differ significantly from each other at P ≤ 0.05, based on SAS software.
$^{\#}$ND= Not Detected. * NA= Not Available.

Physico-chemical parameters

All the physico- chemical parameters except moisture for the VCOs produced from both natural and induced fermentation (semi-controlled) processes were under the normal range. According to the APCC standards acceptable moisture content is 0.1-0.5%. However, in the present study, VCO showed higher levels of moisture which may be due to wet processing.

Microbial count

Total aerobic plate count of 31±3.1 and 49±2.8 CFU/0.1mg was determined in VCO produced by the natural and induced fermentation methods respectively. In the present study, the fermentation process was mediated by the organisms and some of them may have remained in VCO even after the extraction. In case of induced fermentation (semi-controlled), it may not be a problem with the microbial number because probiotic microorganisms were used in the process, whereas in the case of

natural fermentation, it is not possible to determine which type of organisms were involved in the process.

Metals present

A certain amount of metals occurred naturally in both coconut and in water (which was used for the production of coconut milk) and this may be effect the concentration of metals in VCO. The metals present in VCO were within the standard range of APCC values. Lead was not detected in VCO samples produced by both natural and induced fermentation (semi-controlled) methods.

Fatty acid composition

The major fatty acid, (Lauric acid) was a little higher in the VCO sample produced from induced fermentation (semi-controlled) (49.2%) than natural fermentation (48.1). All other fatty acids were within standard range of APCC standards.

Table 2. Fatty acid composition of the VCO samples produced by natural and induced fermentation methods.

Type of fatty acid	Fatty acid composition in (%)		APCC standard value (%)
	NF VCO	IF VCO	
C8(Caprylic)	9.4	9.7	5.0 -10.0
C10(Capric)	6.2	6.4	4.5 -8.0
C12(Lauric)	48.1	49.2	43.0 -53.0
C14(Myristic)	19.8	19.5	16.0 -21.0
C16(Palmitic)	7.5	7	7.5 -10.0
C18(Stearic)	2.7	2.3	2.0 -4.0
C18:1(Oleic)	5.0	4.7	5.0 -10.0
C18:2(Linoleic)	1.2	1.1	1.0 -2.5

The results of the present study were in correlation with the reported by Raghavendra and Raghavarao [25] on VCO production on wet basis by enzymatic, chilling and centrifugation methods.

Conclusion

VCO quality and productivity was improved by using induced fermentation under semi-controlled conditions with *Lactobacillus sp*. which was paved the steps to produce VCO in controlled conditions by using a computer controlled bioreactor. In this process, de-oiled coconut powder was produced as a by-product, which may be used effectively as supplement in different food, confectionary industries and also serve as a fibre rich, low fat, high protein coconut powder. The whey is a waste product in the fermentation process which may be used for the production of bioextract. Finally, it is concluded that VCO extraction by induced fermentation under semi-controlled conditions using probiotic organisms gives greater yields and also avoids the natural contamination problem.

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