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Production of Virgin Coconut Oil from Coconut (*Cocos nuciferal L*) milk Through Microbial Fermentation and it's Antibacterial Property

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Abstract: Fermentation to produce Virgin Coconut Oil (VCO) through induced and spontaneous fermentation and screening for the antimicrobial property of the oil produced against *Staphylococcus aureus* was investigated in this study. Matured coconut was processed to obtain coconut milk. The milk was subjected to induced fermentation using 1.25 % of overnight broth culture of *Lactobacillus plantarum* adjusted to 0.5 and 1.0% Macfarland for 24 and 48 hours. Spontaneous fermentation of the coconut milk was carried out for the same period. The resulting oil was tested for its antimicrobial property against *S. aureus* and the physicochemical property was also determined. The results show that induced fermentation with 1 % Macfarland of *L. plantarum* for 48 hours recovered more oil, (10.6 %) as compared to other fermentation methods. Oil recovered at 48 hours from induced fermentation with 0.5 % Macfarland of *L. plantarum* was found to be moderately potent against *S. aureus* with 8 mm in diameter of zone of inhibition. The physicochemical parameters tested revealed free fatty acid content ranging from 0.14 to 0.22 %, the iodine value (IV) ranges from 4.11 to 4.18 gl2 /100g fats, the peroxide value (PV) ranges from 0.72 to 0.87 meqoxygen/kg, the saponification value ranges between 250.67 to 259.67 mg KOH/g oil and acid value ranges from 0.03 to 0.08. In conclusion, induced fermentation with *L. plantarum* at 1 % Macfarland for 48 hours yielded more oil and it is effective for VCO production. The oil produced after 48 hours through induced fermentation was effective against *S. aureus* and the physicochemical parameter of the VCO generally conformed to standard requirements.

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1. Introduction

The fermentation of food by microbes has been employed for millennia to ensure extended shelf life and improve the functionality, texture, and flavor of food products through the activities of selected and or desired microorganisms (Salqul *et al*., 2013). Coconut oil is commonly promoted worldwide for its various applications, the oil has been variously accepted in food production, pharmaceuticals, cosmetics, health promotion and disease preventing industries (Manisha and Shyamapada, 2011; Satheesh and. Prasad, 2014; Masyithah, 2017).

Coconut, *Cocos nucifera* L of the family *Arecaceae* (Palmae) is the source of virgin coconut oil (VCO). It is oil obtained from the fresh, mature kernel of coconut by mechanical or natural means, with or without the use of heat, without undergoing chemical refining, bleaching or deodorizing, which does not lead to the alteration of the nature of oil (Marina *et al.,* 2009). VCO is theproduct of minimal processing of coconut milk in order to preserve the original components of the coconut (Satheesh and. Prasad,2012).

Virgin coconut oil is extensively applicable in the food, medical, pharmaceuticals and cosmetic industries (Manisha and Shyamapada, 2011; Suryani *et al.,* 2020; Olateru *et al.,* 2020). There is a progressive increase for the demand for production of more VCO hence the need for better methods of improving quality and quantity of coconut oil (*Cocos nucifera L.*) to produce best quality of oil for industrial application. One way of meeting such demand is a method of extraction through fermentation system. Blanca *et al.* (2007); Fabian *et al.* (2007); Satheesh and. Prasad (2012) reported various methods of production; where the minimal processing conditions should involve preserving natural components of coconut according to Asian Pacific Coconut Community (APCC) (2003) Codex,

The Philippines National Standards (PNS) and Bureau of Product Standards (BPS). Some of the reported methods result in low quality products which could easily deteriorate and contain high content of free fatty acid. Microbial fermentation process using minimal energy has been considered safer and more beneficial with more yield (Satheesh and Prasad, 2014; Masyithah, 2017). The virgin coconut oil processed by fermentation system is more prefer than traditional methods, since they are often infected by insects or aflatoxin producing molds that caused potential toxicity problem during manufacturing(Soeka *et al.,* 2008).VCO produced via fermentation should have high content of saturated fatty acids, mostly lauric acid that is highly resistant to oxidative rancidity, very stable and more functional (Brien *et al.,* 2009).Desirable qualities of VCO can be identified through its physical and chemical properties(Nakatsuji *et al.,*2009; Nevin and Rajamohan, 2006;Nevin and Rajamohan, 2010). Main benefits of which include antioxidant antimutagenic, antiproliferative and anticarcinogenic activities (Marina *et al.,* 2009; Chew *et al*., 2019; Suryani *et al.,* 2020; Olateru *et al.,* 2020).

There are two types of fermentation commonly employed for VCO production. They are from wet coconut was allowed for microbial fermentation (Divina, 2003) while induced fermentation is the fermentation that is performed under controlled conditions by using probiotic microorganisms like *Lactobacillus plantarum*. Induced fermentation has several advantages over spontaneous; reduction in time, reduce production of malolactic acid, inhibits spoilage bacteria, interference by bacteriophages and bio-genic amines and unwanted biproducts (Divina, 2003).

Lactic acid bacteria (LAB) (of which *Lactobacillus plantarum* is a member) are the major bacteria used in food fermentations worldwide. *Lactobacillus plantarum* has a wide history of use in VCO production process (Naoyoshi and Risa, 2009; Satheesh and. Prasad, 2014; Olateru *et al.,* 2020). *L. plantarum* has the probiotic capacity (Miriam *et al.,* 2011; Olateru *et al.,* 2020). They also belong to the group of Generally Regarded as Safe (GRAS) organisms (EFSA, 2016).

The use of these bacteria in fermentations to produce functional foods has greatly increased in recent years due to the numerous associated benefits (Parvez *et al.,*2006; Ejtaheed*et al.*, 2016).

Staphylococcus aureus skin infections are the most common form of its infection (Tony *et al*., 2015). Though it frequently occur on the skin as a facultative anaerobe and commensal, it often become opportunistic pathogen in development of skin infections which may not require treatment with oral antibiotics (Masalha *et al.*, 2001;Birmie *et al.*, 2008; AAD, 2013). *S. aureus* is believed to exploit defects in the skin barrier to establish dermatitis although some form of atopic antibiotics could be useful ((Hon *et al*., 2012; Kobayashi *et al*., 2015; Nakatsuji *et al*., 2016; Chew, 2019).

The increase demand for the production of VCO at household and industrial level and the need to overcome the problem of contamination as associated with spontaneous fermentation justifies this research. Hence this present work seeks to produce VCO according to APCC standards through induced fermentation by probiotic organism (*Lactobacillus plantarum*) free from the challenge of contamination commonly encounter in natural fermentation and to determine the antibacterial potential and physicochemical parameters of the oil produced.

2. Materials and Methods

2.1. Collection and authentication

Uniformly sized 12 months old (matured) coconuts of the same species from the same farm were procured from Ikogosi town in Ekiti State and authenticated at Institute of Agricultural Research and Training (IART), Nigeria, with number: HSCode, 1513.

2.2. Source of microbial culture

The *Lactobacillus plantarum* and *Staphylococcus aureus* used were obtained from Microbiology and Medical Microbiology Departments of University of Ibadan and University College Teaching Hospital, Ibadan, Oyo State respectively. The cultures were subcultured twice and maintained on appropriate agar slants at 4 ^{0}C for further analysis.

2.3. Coconut Milk Extraction

Coconuts were dehusked and water was collected from the pore in plastic container, they were broken and solid endosperm was collected, testa was removed by using kitchen peeler, while coconut balls were disintegrated into small pieces and grinded with distilled water in ratio 1:2 for 10 minutes. Grinded mass was transferred into muslin cloth, pressed manually for coconut milk extraction. The same process was repeated twice and 3,600 ml of coconut milk was obtained, 800 ml of coconut milk were dispersed into 6 glass jars for fermentation process (Satheesh and Prasad, 2014).

2.4. Induced of fermentation

The Coconut milk in four jars were autoclaved at 121 ^{0}C for 15 minutes to sterilize it. Two of the jars were inoculated with10 ml (1.25 %) of overnight broth culture of *L. plantarum* which has been adjusted to 0.5 % Macfarland Standard which is equivalent adjusted to about 1.5 x 10^8 Cfu/ml and the other two jars were inoculated

with 10 ml of overnight broth culture of *L. plantarum* adjusted to 1 % Macfarland Standard which is equivalent to about 3.0 x 10^8 Cfu/ml. The jars were labeled and incubated anaerobically for 24 and 48 hours. Oil was collected after 24 and 48 hours respectively (Satheesh and Prasad, 2014).

2.5. Spontaneous of fermentation

Spontaneous fermentation was carried out in two jars. Equal volume of milk was dispensed into labelled jars as described. The milk was not autoclaved but was allowed to undergo spontaneous fermentation by the resident organisms for 24 and 48 hours under laboratory conditions. Oil was collected from the fermented milk after 24 and 48 hours respectively (Chee and Choon, 1997).

2.6. Hot Extraction Processes

Extraction of the VCO was done by heating fermented coconut milk to remove water completely (Siddalingaswamy *et al.*, 2011). The protein was coagulated by slow heating at 60 ^{0}C in VCO cooker and releasing the oil that was separated from pertinacious residue through filtering with muslin cloth and the remaining residue was further heated to remove more oil until water was completely evaporated (Srivastava *et al.*, 2016). The quantity of oil recovered was recorded.

2.7. Antibacterial susceptibility test.

The susceptibility screening of the test bacteria to VCO produced by both induced and spontaneous fermentation were done in accordance with the method of Irobi *et al.* (1994); Nguyen *et al.* (2017). Standardized culture of *Stapylococcus aureus* adjusted to 0.5 % McFarland (equivalent to about 1.5 × 10^8 CFU/ml using McFarland Nephelometer standard) was used. Sterile Mueller Hinton agar plates were seeded with 0.5 ml *Stapylococcus aureus* and allowed to stand at 37 ^{0}C for 30 mins. Sterile paper discs (Whatman filter paper) impregnated with 0.1 ml of Virgin coconut oil and labeled as Spontaneous 24 hours (STA), Spontaneous 48 hours (STB), *Lactobacillus plantarum* 0.5% 24 hours (LPA), *Lactobacillus plantarum* 0.5% 48 hours (LPB), *Lactobacillus plantarum* 1% 24 hours (LPC) or *Lactobacillus plantarum* 1 % 48 hours (LPD) were placed on uniformly inoculated plates for susceptibility test. The plates were allowed to stand on the bench for 1hour to allow proper diffusion of the VCO into the media and thereafter incubated at 37 ^{0}C for 24 hours, after which they were observed for zones of inhibition. The observed zones were recorded in millimeter.

2.8. Determination of Physicochemical Properties of VCO

2.8.1. Yield Measurement

Yield of obtaining coconut oil was determined by using the method of gravimetric (v/v) as follows: (Handayani *et al*., 2009).

$$\text{Yield} = \frac{\text{Volume of obtaining oil (mL)}}{\text{Volume of coconut cream (mL)}} \times 100\%$$

2.8.2. Moisture content

Moisture content was determined according to the method described by Handayani *et al*. (2009) using the formula:

$$\text{Moisture content} = \frac{A - B}{A} \times 100\%$$

Where, A= Weight of VCO before heated. B= Weight of VCO after heated

2.8.3. Iodine value (IV)

The analysis of iodine value was conducted using AOCS Official Method No. Cd1d-92 (Wijs Method) (AOCS, 2009).

2.8.4. Free fatty acid (FFA)

Free fatty acid content was measured according to the Official Method of AOAC, (2000).

2.8.5. Peroxide value (PV)

The peroxide value was determined using the standard method of Association of Official Analytical Chemist (AOAC, 2000).

2.8.6. Saponification value (SV)

Saponification values (SV) were determined using the international Union of Pure and Applied Chemistry (IUPAC) Method 11.D.2. 2.0 gram oil samples was added with ethanolic potassium hydroxide 0.5 N and boiled for 60 minutes in a reflux condenser. The mixture was cooled and subsequently titrated with 0.5 N hydrochloric acid until colour of the mixtures changed from pink to the original colour. All SV determinations were carried out in triplicates (Marina *et al*., 2009).

3. Results

3.1. Oil recovery

The oil recovery through the various methods used for fermentation is presented in Table 1. It is evident that the 48 hours induced fermentation of 1 % inoculum ($3.0x10^8$ CFU/ml) (LPD) recovered the most oil (10.6%)

3.2. Antimicrobial susceptibility

The diameters of zones of inhibition of the VCO obtained against the test organism is as presented in Table 2. *S. aureus* was only sensitive to virgin coconut oil recovered from LPB

3.3. Physicochemical Analysis

The results of the physicochemical analyses performed on all the VCO are shown in Table 3.

The range of iodine value of the samples were 4.11 - 4.18 gl_2/100g fats. The lowest iodine value was obtained from the LPC while the highest was obtained from STA.LPC gave the highest FFA of 0.22 g/KOH/g fats while the STA and LPD have the least value of FFA (0.14 mg/KOH/g fats).

The saponification value of all the VCO obtained range from 250.67-259.27 mgKOH/g of fats. The moisture content recorded was in the range of 0.08 - 0.12 % (w/w) the lowest was from LPC and the highest value was from LPB. The acid values ranged from 0.03 - 0.08 after 48 at hours.

Hypothesis

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$

H_0: $\mu_1 \neq \mu_2 \neq \mu_3 \neq \mu_4 \neq \mu_5 \neq \mu_6$

Table 1. Quantity of oil recovered

Parameter	STA	STB	LPA	LPB	LPC	LPD
Yield (%) of oil Recovery	$7.43^a \pm 0.02$	$9.99^a \pm 0.01$	$7.94^a \pm 0.01$	$10.00^a \pm 0.02$	$5.83^a \pm 0.01$	$10.60^a \pm 0.02$

The table above shows mean ± S.D for each of the variables

Note: Mean with same superscripts mean they are statistically significance to each other while mean with different superscript mean they are not statistically significance to each other

Keys:

STA Spontaneous 24 hours

STB Spontaneous 48 hours

LPA *Lactobacillus plantarum*0.5% 24 hours

LPB *Lactobacillus plantarum*0.5% 48 hours

LPC *Lactobacillus plantarum* 1% 24 hours

LPD *Lactobacillus plantarum*1 % 48 hours

Table 2. Antimicrobial susceptibility of *Staphylococus aureus* to VCO

VCO	Zone of inhibition (mm)
STA	no zone
STB	no zone
LPA	no zone
LPB	8
LPC	no zone
LPD	no zone

Table 3. Physicochemical analysis of VCO

Parameters	STA	STB	LPA	LPB	LPC	LPD	APCC standard
%FFA (Palmitic)	0.14	0.21	0.17	0.20	0.22	0.14	0.5
Acid Value	0.03	0.08	0.03	0.06	0.04	0.06	Max 0.5
Peroxide Value (meq O_2/kg)	0.86	0.84	0.72	0.82	0.83	0.87	3
Saponification (mg KoH/g oil)	256.49	250.67	259.27	255.06	255.07	2 52.08	250 -260
Iodine Value (gl_2/100 g fats)	4.18	4.17	4.14	4.17	4.11	4.13	4 - 11
Moisture Content (%wt)	0.11	0.10	0.09	0.12	0.09	0.08	0.1 – 0.5
pH	5.2	4.8	5.7	4.5	5.8	4.1	___
Temperature (^{0}C)	35.1	34.3	35.2	34.1	35.3	34.4	___

Key:

APCC Asian and pacific of coconut community

FFA Free fatty acid

4. Discussion

Fermentation of coconut milk to produce VCO using *Lactobacillus plantarum* and spontaneous fermentation was studied. Virgin coconut oil (VCO) or oil recovered through microbial fermentation is often rated very high because high temperature, chemicals or other physical treatment are not used in its processing. VCO in this study is naturally processed through microbial enzymatic fermentation, it is unhydrogenated, undeodorized, and unbleached. The component of the fatty acids, especially lauric acid of this coconut oil is not change, VCO is the least vulnerable of all the dietary oils to oxidation and free-radical formation, and it is therefore the safest to use in cooking. It does not become polymerized to form by-products as do other oils when heated at normal cooking temperatures (Rindengan and Novarianto, 2004; Sulistyo, 2004).

Poor separation was obtained at 24 hours, but incubation period of 48 hours was found to be more effective. The fermentation with *Lactobacillus plantarum* yielded more oil compare to the other ones. At 48 hours of fermentation more oil was recovered. Inoculums concentration of 1 % for 48 hours was more effective yielding a little more oil than others. This discovery on oil recovery shows that efficiency was directly proportional to inoculums concentration and inoculation period. This was supported by earlier studies on oil recovery (Handayani *et al*., 2009). Although the yield was low compared to the report of Satheesh and Prasad (2012; Satheesh and Prasad (2014). The difference in yield could be due to the difference in percentage of inoculum used, coconut species and experimental condition. According to Satheesh andPrasad (2012), 3 % of inoculum could improve yield.

Virgin coconut oil produced were mostly unable to inhibit the growth of *Staphylococcus aureus* except for one of the samples as shown in Table 2. *S. aureus* was only sensitive to virgin coconut oil recovered from fermented milk using 0.5 % of *Lactobacillus plantarum* as starter inoculum after 48 hours. The result was contrary to the findings of Olateru *et al.* (2020) where all the oil samples had inhibitory property but the zone of inhibition recorded was higher than their record. Difference in antimicrobial activity could be due to difference in strains of test organism used. VCO recovery and antimicrobial activity could possibly be enhanced by induced fermentation as recorded in this study.

Generally, the iodine and saponification value of the oil recovered in this study conforms to standard. The free fatty acid (FFA) of VCO is expressed as palmitic acid according to the nature of its fats. This result is similar to the report of (Marina, 2009).Thesaponification values obtained in this experiment agreed with the APCC standard (2003). Low iodine value indicates that the oil is suitable for cooking. Virgin coconut oil contains low level cholesterol and high composition of medium chain fatty acids. Although values obtained in this study is lower compared to the findings of Suryani*et al.* (2020); Olateru *et al*. (2020).

5. Conclusion

The 1 % MacFarland inoculums concentration 48 hours of incubation and anaerobic conditions was found to be the most preferable condition for the controlled fermentative production of VCO with antibacterial activity. The VCO produced in this research may find application in food and medicine.

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