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COMPARISON OF QUANTITY AND QUALITY PARAMETERS OF VIRGIN COCONUT OIL FROM TWO COCONUT VARIETIES BY EMPLOYING INDUCED FERMENTATIVE METHODOLOGIES

Agricultural Science

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ABSTRACT

Virgin Coconut Oil (VCO) was prepared from two induced fermentative methodologies viz., yeast and bacterial induced methodologies. The two varieties of coconuts like West Coast Tall (WCT) and Deejay Coconuts (DJ) were also used for the VCO production. The production methodologies were optimized by four common variable parameters like the temperature 25±1ºC, 30±1ºC, 35±1ºC, 40±1º C, PH 2.5±0.1,3.5±0.1, 4.5±0.1, 5.5±0.1, Inoculum concentration 1% (w/v), 3% (w/v) ,5% (w/v) ,7% (w/v) and the Fermentation end time ranges like 24, 36, 48 and 60hrs used for both *Saccharomyces cerevisiae* and *Lactobacillus plantarum to* estimate the yielding efficiency of VCO with using two coconut varieties. The yielding efficiencies of both processes are calculated by using total percentage of Oil extracted from Soxhlet method from WCT and DJ Coconuts .Among the two set of processes the maximum yielding efficiencies were obtained in WCT coconuts using *Lactobacillus plantarum (*9511) at 40±1ºC, 5.5±0.1 PH, 7% (w/v) Inoculum concentration and the Fermentation end time in 60 hrs. Proximate analysis for both VCO samples are estimated followed by different fatty acid compounds are also identified by ethanol extracted VCO samples under GC-MS. VCO produced from WCT Coconuts using *Lactobacillus plantarum* gave highest yield and higher fatty acid compositions than the VCO produced from DJ Coconuts. The results suggest that WCT coconuts are being the best oil yield with the interaction of Lactobacillus plantarum strain both in quantity as well as quality parameters are compared in this present study.

KEYWORDS

Virgin coconut oil, yielding efficiency, *L. Plantarum (9511), S. cerevisiae,* WCT, DJ.

INTRODUCTION

Coconut is considered as a "*Kalpavriksha*" or "the tree of heaven" which means "the tree that gives all that is necessary for living", in our daily life. It is mainly cultivated in Philippines, Indonesia, India, SriLanka, Papua New Guinea, Thailand, Malaysia and Fiji (Naveena K *et al. 2014)*. There are 50 more value-added products are obtained from coconut tree, which are used in domestic purposes as well as importing quality products (Market research planning cell report, 2008).

Coconut oil is edible oil commonly extracted from the mature coconut kernels for food and Industrial purposes. It is one of the primary sources of energy, in tropical countries like India, SriLanka, Philippines and Indonesia (Mohd. Shamim Khan *et al.*2015). According to the Shijna Kappally et *al.* (2015), coconut oil is utilized for various purposes including skin care, hair care, stress relief, weight loss, cholesterol level maintenance, immunomodulatory effects and cardiovascular uses. Commonly coconuts are obtained in two forms based on their moisture content namely; wet coconut and dry coconuts (James Abiodun Adeyanju *et al.* 2016). Wet matured coconuts are used for the extraction of Virgin Coconut Oil (VCO) (Bawalan and Chapman, 2006). According to Villarino, Dy, & Lizada, 2007 the coconut oil produced through with or without the use of heat and without undergoing chemical refining is called as Virgin Coconut Oil. The wet preparation of VCO has remedial properties to reduce the intensity of diseases like Alzheimer's, Autism, Dementia and Aids (Deepthi Nair.S.2012).

VCO commonly obtained through wet and dry methods. In dry method, the kernel was heated under controlled conditions to remove the moisture in it to prevent the microbial contamination and oil recovered through grinding method (Mansor, T. S. T. *et al*, 2012).Wet methods are commonly divided into chilling and thawing, fermentation, enzymatic and pH method or any of these in combination (Raghavendra and Raghavarao, 2010).The results revealed from the thesis of Marina Binti Abdul Manaf (2009) VCO Produced from both Fermentation and Chilling method had higher antioxidant potency than Refined Bleached Deodorized (RBD) coconut oil. The antioxidant potential is helps to fight against several diseases of peoples Marina A.M.*et.al. (2009).*

Codex gives a general definition for "Virgin Oils", which states that such oils should be suitable for human consumption (Olivia Erin M.*et.al.*2007). It provides an alternative inexpensive source of energy to neurons of humans in the case of Alzheimer's disease (AD), this was reported by Gandotra S *et al. (*2014). Also James Abiodun Adeyanju *et al.* (2016), noticed that various fractions of coconut oil are used as drugs. Almost 50% of the fatty acid in virgin coconut oil is in form of lauric acid. This fatty acid has wide application as wide spectrum of antimicrobial substances against fungi, bacteria and viruses. Moreover, the fatty acid profile of coconut oil shares the similar characteristic with breast milk (Hamid. M.A *et.al, 2011* & Deepthi Nair.S.2012*)*.

Natural microbial flora present in coconut milk can induce fermentation to produce VCO. But the quality of VCO is poor due to the presence of contaminated microorganisms in coconut milk, which have the microbial attractive sources of proteins, carbohydrates and moisture (Chee and Choon, 1997). Also destabilization of coconut milk is required to extract the VCO from the stable protein fat emulsion (Tangsuphoom and Coupland, 2008). These can be overcome by induced fermentation using probiotic micro organism *Lactobacillus plantarum* (Satheesh Neela and N.B.L. Prasad, 2012). As per the comparative study of Nurah Tijani Oseni.*et.al., 2017* on the quality of coconut oil was observed with induced fermentation using *L. plantarum* was high oil yield. Also it showed higher level of antioxidant activity compared to other methods.

Earlier studies on VCO production through commercial yeast was focused only the carbohydrate breakdown but not the proteins and fats from the substrate (Meroth, B.C.*et.al., 2003)*. According to Mansor, T. S. T. *et al*, (2012) Superior quality of VCO was obtained with low viscosity and low free fatty acids by using *Saccharomyces cerevisiae*. This organism produces two enzymes like invertase and protease. Invertase act on sucrose to produce glucose and fructose which have reducing sugar ability, while protease serves to break down proteins (Sadiah Djajasoepena. *et.al.2011*) thereby destabilization of coconut emulsion is achieved efficiently. So the time interval for the production is lesser and also quality wise is best in this production process.

According to the parametric study on the production of VCO from *Saccharomyces cerevisiae* fermentation by Zuhrina Masyithah, 2017, results showed that the maximum yields of the oil obtained at 0.3 %(w/v) inoculum concentration and the fermentation end time at 24 hrs.

Also *Saccharomyces cerevisiae* noted as the significant effect on oil yield, moisture content, free fatty acid, peroxide, and Saponification number from the study of Riko Aditiya *et al. 2014.*

Quality and Quantity of coconut oil is not only determined by the external factors but also the variety of coconut. The above research status was reported by Naresh Kumar.S, (2011) from 60 Talls, 14 Dwarfs, and 34 hybrid coconut varieties fatty acid profiles. Also reported that, the quality of VCO is determined by different maturity level of coconut (Nur Raihan Ali.*et.al,* 2014 & Prakruthi Appaiah *et al.2015*).

West coast tall variety is one of the major varieties cultivated in India (Gopalakrishnan. R., 2013 & Mannekote. J. K. and S. V. Kailas, 2013). As per the market research planning cell report (2008), WCT coconuts yields 68% of oil and it is mostly cultivating in southern states (Remany Gopalakrishnan,2013) . Also, Aslin Joshi.J, 2010 reported that, WCT coconuts yields more quantity of oil when compare to other tall coconut varieties. DJ in India is the biggest organization supplying such high quality commercial (Hybrid) coconut seedlings to the farming community (Murali.M.2013). According to the Sona John (2012), the Deejay coconut (DJ) is one of the high oil yielding (68%) hybrid variety crossed from selected parents. In Coimbatore district, Pollachi is a known major coconut cultivation area (Yamuna.S.M & Ramya.R.2016). The above two varieties are majorly cultivating in Pollachi region. In this present study the West Coast Tall (WCT) coconuts and Deejay coconuts (DJ) are taken in the study for VCO Production. Fermentation is a well-known method in cold process for the extraction of virgin coconut oil from the coconut milk (Ravindra kumar agarwal and SJD Bosco, 2017). The purpose of research is to determine which one of the induced fermentative methodology yields more in quantity as well as quality of virgin coconut oil produced from the two coconut varieties.

MATERIALS AND METHODS

Coconut samples

Two varieties of uniformly sized 11-12 months old matured brown in colour, which have high oil content (Arumuganathan.T. *et.al, 2011,* Yashi Srivastava .*et.al, 2016 &* Nur Raihan Ali.*et.al,* 2014*)* West Coast Tall (WCT) and Deejay Vishwas Coconuts (DJ) are used in this study. The coconuts were collected from South farm of Vanavarayar Institute of Agriculture (VIA), Manakkadavu and the selected nuts are transferred to lab site without any physical damage.

Microbial cultures

Two types of pure microbial cultures are used in these fermentation methodologies. Pure culture of the *Lactobacillus plantarum (*9511) was purchased from M/S.IMTECH, Chandigarh in lyophilized vial. As per the instructions from culture catalogue, two sub-culturing was done followed by; slant culture was prepared and stored in MRS (Man Rogosa and Sharpe growth Medium) Agar medium.

The strain *Saccharomyces cerevisiae (*< 2-months old culture) was collected from Biotech laboratory, VIA, Manakkadavu. The working stock plate culture was inoculated on a Yeast Extract Peptone Dextrose (YPD) broth medium and it was incubated at 30°C for 16–18 hr with shaker at 230–270 rpm. *(Yeast Protocol Hand book, 2009*). After seed culture prepared, the morphological character was determined by stained with lacto phenol-cotton blue, carbol fuchsin and seen under phase contrast microscope (Somdatta chatterjee *et al.2011).*

Coconut Milk Extraction

As per the study of Nur Raihan Ali.*et.al,* 2014 the matured brown coconuts are selected because, which determined the both quality and quantity in virgin coconut oil production. The selected West Coast tall and DJ matured de-husked coconuts are split into half cups using manual cutter and kernel was grated by manually operating coconut grater and the grated coconut was mixed with hot tap water (1:1, w/v) (Rini Handayani, *et al.* 2009 & Nurah Tijani Oseni et.al,2017). Then coconut milk was squeezed from grated kernel using a screw type coconut milk press (Pilot smith India (p) Ltd) and the milk was transferred to sterile food grade plastic tank. The machineries for VCO production was supported from DST Project on WTP at Dr.Mahalingam College of Engineering & Technology, Pollachi. According to the study of Rini Handayani *et.al,2009* & Zuhrina Masyithah*,2017* the squeezed milk was kept it for one hour to separate the skim layer from coconut milk cream, which is having rich quantity of coconut oil. After separation, only the milk cream layer was taken for fermentation process.

Coconut milk cream sterilization

As per the methodology of Neela Satheesh and N.B.L Prasad (2013 & 2014), the collected each one litre quantity of coconut milk cream was sterilized under Ultra Violet light (Dose of 40 mW/Cm2/ Sec.) in laminar air flow hood for 20 min. This practice helps to reduce the contamination in coconut milk cream.

Seed cultures preparation

a. Saccharomyces cerevisiae

Seed culture was prepared as per the earlier studies of Satheesh Neela and Prasad N.B.L, 2012 by using YPD broth medium, and they are incubated at 37°C for 36 hours at 100 rpm in orbital shaker incubator and this preparation was maintained for entire fermentation process

b. Lactobacillus plantarum

The seed culture inoculum was prepared as per the L. F. Coelho *et.al.*2011 by transferred the *2* % of stock culture to conical flasks containing growth medium (MRS). The incubation temperature was 35±1°C for 18 hrs at 150 rpm.

Calculation of Microbial Quantity

In both the above seed culture preparations, microbial quantity was estimated by serial dilution and spread plate method. The approximate amount of microbes was calculated by colony count by using the below formula.

Number of Microorganisms present in the sample

$$= \frac{\text{Number of colonies present on plate}}{\text{Dilution factor}}$$

Induced Fermentation method

The UV-Sterilized coconut milk cream emulsion was destabilized initially at 40°C as per the Raghavendra.S. N and Raghavarao. K.S.M.S. 2010. After that each 200 ml of milk emulsion was transferred to sterilized labeled fermentation flasks (500-mL Erlenmeyer containing 200 ml final volume of fermentation medium) for two types of induced fermentation series. Further the parameters were arranged according to the comparative study.

Parameters for comparative study of VCO yield

There are four different parameters like Temperature, PH, Inoculum and Fermentation End Time for take in account to estimate the yielding efficiency of VCO using *Lactobacillus* and *Saccharomyces* strains.

Temperature on study

Temperature is a key factor which influences the growth of both microbial strains used in this study. The temperature used for both organisms are 25±1ºC, 30±1ºC, 35±1ºC and 40±1º C. The study was slightly modified from Neela Satheesh and N.B.L Prasad, (2013) and it was studied by using temperature controlled with energy regulator attached Incubator from Genuine Company an Indian made instrument.

pH on study

It is a second parameter in the study which also influences the growth of microorganisms in coconut milk medium. The pH ranges from 2.5, 3.5, 4.5 and 5.5 was used for both organisms from the slight modifications of Neela Satheesh and N.B.L Prasad, (2013). In the above study were PH was adjusted by using Eutech Instrument from (thermo fisher scientific company) Singapore Company.

Inoculum concentration on study

Inoculum concentration is another one parameter to determine the growth of microorganism. It is taken in the fermentation from the ranges of (1% (w/v), 3% (w/v), 5% (w/v) and 7% (w/v) for both organisms used in the study.

Fermentation end time on study

Fermentation end time was maintained from the ranges 24, 36, 48 and 60 hrs for both organisms used in the study to estimate the yielding efficiency of VCO. The same like instrument was used in the temperature optimization study.

Recovery of VCO

After completion of the two fermentation batches, the VCO pushed up in upper layer because the density of the virgin coconut oil at room temperature is 0.903 g/ml (Patricia Janelle Ferrer. *et.al, 2018).* Then it was collected from each series of four comparative studies using sterile suction pipette followed by the remaining portion of oil with cream

layer was centrifuged two times as per Nurah Tijani Oseni *et.al, 2017* in a temperature controlled centrifuge at 27°C and 6000 rpm for 10 min.The acquired oil was prepared as triplicates and kept in refrigerated until further analysis.

Estimation of initial oil content - Soxhlet extraction

The total initial oil content of the two coconut varieties was estimated by AOAC Soxhlet method (AOAC, 2017). As per the methodology, ten grams of each grated dry coconut pieces are weighed along with extraction thimble and then it was covered with wool. The dried boiling flask was weighed and after it was filled with n-hexane. The extraction was carried out in triplicates for 30 hours. The extracted oil samples are then dried in the oven at 103 ± 1°C for 2 hours to remove the remaining solvent from the oil and cooled in desiccator before reweighing.

Calculation of Oil recovery & Process efficiency

As per the Zuhrina Masyithah (2017), the oil recovery was determined according to the initial oil content in the coconut to the oil extracted from two different fermentative methodologies. The below formulas are used to calculate the oil recovery and the efficiency of the two processes: -

% oil extraction on wet basis

$$= \frac{\text{Weight of oil extracted}}{\text{Weight of coconut milk Cream used}} \times 100$$

Efficiency of the Process (%) or yield in method

$$= \frac{\text{oil yield (\%) in wet basis}}{\text{Oil content \% by Soxhlet method}} \times 100$$

Physico-chemical characteristics

The coconut oil obtained from two fermentative methodologies was evaluated for moisture content, acid value, free fatty acid content (%), Iodine number, peroxide value and Saponification number.

Moisture content

Moisture content was determined according to the A.O.A.C (2016a) method by weighing 20g of VCO sample was placed onto a Pre-weight determined Petri dish. The sample was heated up to 110±5°C for 2hrs in a closed air ventilated oven and cooled it down in a desiccator for approximately 15 min and weighted again.

$$\text{Moisture content (\%)} = \frac{\text{Initial Wt} - \text{Final Wt}}{\text{Initial Wt.}} \times 100$$

Acid value & free fatty acids

The acid value and free fatty acids of the VCO sample was determined by the adaptation of A.O.A.C (2016b) method. The 2.5g of sample was taken in conical flask. Initially a titration reagent of 25 ml alcohol-benzene (1:1, v/v) was pre-heated on a water bath at 70° C for 10min after addition with 3 drops of phenolphthalein as indicator and the mixture was titrated with 0.01N NaOH up to the solution just turned to slight red. The solution was mixed with VCO sample and heated for 5 min and titrated again with 0.01 N NaOH at least for 10 min to get the slight red solution. Then the consumed NaOH reading was noted and acid value and free fatty acids are calculated by the below formulas.

$$\text{Acid value} = \frac{A \times N \times 40}{\text{Sample Wt (g)}}$$

A-Quantity of NaOH
N-Normality of NaOH
40- Molecular Wt of NaOH

$$\text{Free fatty acids (\%)} = \frac{A \times N \times M \times 100}{\text{Sample Wt (g)}}$$

A = Quantity of NaOH
N = Normality of NaOH
M = MW of lauric acid (214 g)

Iodine number

It was estimated by using A.O.A.C Official Method No. 920.158 (Wijs Method) (AOAC, 2016c). Approximately 3.0 g of VCO was taken in a 250 ml conical flask. Then, 20 ml cyclohexane was added into it to dissolve the fat followed by 25 ml of Wijs solution was added into the VCO solution. The flask was completely closed by parafilm or by cork and the solution was continuously shaken for about 30 minutes. After that, 20 ml of 15% potassium iodide solution (KI) and 100 ml of de-ionized water were added into the mixture. The mixture of VCO solution was titrated against 0.1 N Sodium thiosulfate solution ($Na_2S_2O_3$) until the yellow colour form has almost disappeared. Next, 2-3 drops of starch solution were added (blue colour solution will appear) and titration was continued until the blue colour has disappeared. Volume of $Na_2S_2O_3$ is represented as S. The titration step was repeated with blank sample and the volume of $Na_2S_2O_3$ is represented as B. The Iodine number was calculated using the below Equation:

Iodine Number

$$= \frac{(B - S) \times N \text{ of } Na_2S_2O_3 \times 12.69}{\text{Weight of sample (g)}}$$

Where, B = V mL of $Na_2S_2O_3$ volume for blank
S = V mL of $Na_2S_2O_3$ volume for sample
N = Normality of $Na_2S_2O_3$

Peroxide value

The peroxide value was estimated by using the standard method AOAC (2016d). A 5 g of coconut oil sample was added with 30 ml of acetic acid-chloroform (3:2) and the solution was stirred until the oil has been completely dissolved. Then, 0.5 ml of saturated potassium iodine (KI) was added and stirred for about one minute. The solution was titrated against with 0.01 N Na_2SO_3 until its colour changed to light yellow. The step of titrating can be skipped by adding 0.5 ml of 1% soluble starch as an indicator that gives a light blue colour, followed by titration with 0.01N Na_2SO_3 until the colour disappear. The volume of titration was recorded and peroxide value (PV) was calculated using the below formulae:

$$PV = \frac{N \times V}{W}$$

Where, PV unit is in Millie-equivalents (meq) of peroxide O_2 per kg of oil.

V is the titer volume of Na_2SO_3 solution (0.01 N), W is the weight of coconut oil (kg) & N is the normality of Na_2SO_3 solution (0.01N).

Saponification number

The saponification value was determined by the AOAC (2016e). Initially the sample should be filtered through a filter paper to remove any impurities. Make sure that the sample is completely dry and after that, mix the sample thoroughly and weigh about 1.5 to 2.0 gm of dry sample into a 250 mL Erlenmeyer flask. Pipette 25 ml of the alcoholic potassium hydroxide solution into the flask. Conduct a blank determination along with the sample. Connect the sample and blank flasks with air condensers; keep on the water bath, gently and steadily boiling until saponification is complete, indicated by absence of any oily matter and the appearance of a clear solution. Clarity may be achieved within one hour of boiling. After the flask and condenser have cooled, wash down the inside of the condenser with about 10 mL of hot ethyl alcohol neutral to phenolphthalein. The excess potassium hydroxide is determined by titration with 0.5N hydrochloric acid, using about 1.0 mL phenolphthalein indicator.

$$\text{Saponification Value} = \frac{56.1 (B-S) N}{W}$$

Where, B = Volume in mL of standard hydrochloric acid required for the blank. S = Volume in mL of standard hydrochloric acid required for the sample. N = Normality of the standard hydrochloric acid and W = Weight in gm of the oil/fat taken for the test.

GC-MS analysis of fatty acid composition

The extracted oil from two induced fermentative method was analyzed to determine its fatty acid compositions.

GC Programme

As per the AOCS (2000) methodology, Fifty milligrams of oil sample was dissolved in 0.95 ml of hexane in a 1.5 ml vial, and then shaken vigorously to dissolve the oil. Then, 0.05 ml sodium methoxide was added to the solution using micropipette. The vial was capped and the solution mixed vigorously for 5seconds using vortex mixer. The clear upper layer of 2µl of methyl ester was pipetted off and injected into a gas chromatography (GC) column using external standard method. The detector used in this programme is TQ Quadrupole Mass Spectrometer with Carrier gas 1 ml per minute, given as split like 10:1. Software MS Work station 8 was used to analyze.

The GC column was 30 m in length, with a 0.25 µm film coating, 0.25 mm ID, and 436-GC Brucker phase (non-polar). The column temperature was 110°C for the first 3.50 min, increased up to 200°C at the rate of 10º C / minute –no hold and then finally increased to 280º C at the rate of 5º C / minute – 12minute hold . The rate of temperature increase was 5°C/min. The injection temperature and detector temperature were maintained at 200°C and 280 °C, respectively. The total running time was 40.50 minutes.

MS Programme

The software NST Version-11 library was used to analyze. The temperature for inlet is 290º C, source temperature 250º C. Solvent delay time maintained between 0-3.5 minutes. Total running time was 40.50 minutes.

Statistical analysis

All experimental values were carried out in triplicate, and the mean values were presented. Significant differences between the means were determined by Duncan's multiple range tests (SPSS 16.0 for Win wrap basic, SPSS Inc., Polar Engineering and consulting, 2007) at a 95 % confidence level.

RESULTS AND DISCUSSION

Coconut milk contains rich quantity of nutrients, mainly 21.3% fat, 2.0% protein, 2.8% carbohydrate and 2.1% of sugar (Sri Kumalaningsih & Masdiana Padaga, 2012) together with some vitamins and trace elements. Saccharomyces cerevisiae is widely utilized food processing, for the production lipids, proteins and vitamins (Hoppe A. *et.al,* 2011). It can grow even in low nitrogen content medium and showed osmo-tolerant ability that is, it could grow on nutrient medium contains 50-60% of glucose (Bayraktar. V. N, 2013).Also *Saccharomyces* is an enzyme protease producing organism (Sadiah Djajasoepena, 2011), so fat separation from coconut milk is easier. *Lactobacillus* sp could effectively extract more virgin coconut oil than the other microbial strains from the Rini *et et.al.* (2009) observations. The bacteria have a potential to destabilize the protein from coconut milk there by fat separation is easier in coconut milk fermentation (Sri Redjeki and Ely Kurniati, 2013).

In the present study two coconut varieties especially West Coast Tall (Tall cultivar) and Dee jay Vishwas (Dwarf variety) was used for VCO Production. WCT revealed that better nut yields as well as 68% oil yielding capacity (Mubarak Ali. A 2012). Like that Deejay coconuts also have the ability to yield the same quantity of oil yielding capacity stated by Sona John (2012).

Soxhlet extraction of Coconut Oil

The initial oil content of both grated coconut pieces of WCT and DJ was determined from Soxhlet method (AOAC, 2017). The calculated total oil contents are 67.5% and 61.5% from WCT and DJ respectively. As per the Neela Satheesh and N.B.L Prasad, (2013), the result percentages take it for calculate the yielding efficiencies of two fermentative methodologies

Effect of temperature on VCO yield in WCT and DJ Coconuts using two organisms:

Production of VCO in respect with temperature by two organisms in both WCT and DJ Coconuts was furnished in table.1. Temperature is an important factor that directly affects the growth rate of the microorganisms (Charoenchai et al., 1998). From the research investigations of (Bayraktar. V. N, 2013 & Ali Jabbar Resheg AL-Saady 2014), Dac hai nam ho and Chris powel (2014) the optimal growth temperature of *Saccharomyces* referred that 28-30ºC and 25-35ºC vice versa. In this present result, the maximum process efficiency of 79.92 % was obtained at 35±1ºC (Fig.1) using

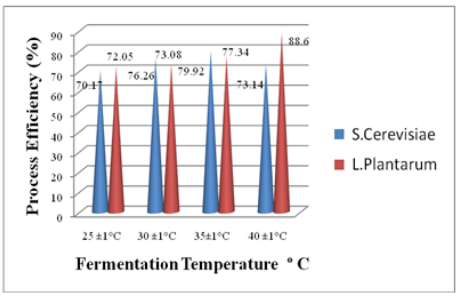

Fig.1.Effect of temperature on VCO yield in WCT

Saccharomyces cerevisiae in WCT Coconuts wherein DJ Coconuts the maximum process efficiency 74.06% was obtained at 30±1ºC (Fig.2) this is a similar result of Grosshans, B. L et.al, 2006. The yielding efficiencies are increased with increasing temperature (Fig.1) this is a similar research statement of Noe Arroyo-Lopez, F.*et al*. (2009) that is *Saccharomyces* can start the fermentation in few hours after even a low temperature. .But after optimum temperature (35±1ºC) it got decreased (Fig.1) in WCT Coconuts using *Saccharomyces* cerevisiae induced fermentation.

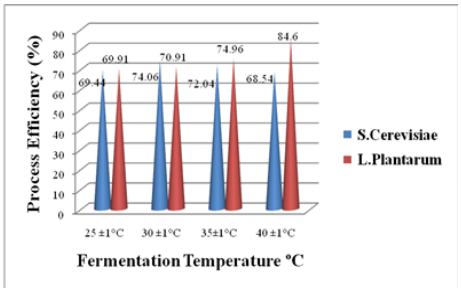

Fig.2.Effect of temperature on VCO yield in DJ

The maximum process efficiencies of 88.60% in WCT and 84.6% in DJ was noted by using *Lactobacillus plantarum* induced fermentation at 40±1ºC (Fig.1&2). This is a similar result of Raghavendra and Raghavarao, (2010). The research of Sri Redjeki and Ely Kurniati (2013) showed that the optimum temperature for the growth of *Lactobacillus plantarum* is 40ºC is now proved again. Same like in WCT the yielding efficiencies after optimum temperature decreased with the increase of temperature (Neela Satheesh and N.B.L Prasad, 2014.*)* using *Lactobacillus plantarum* in DJ Coconuts (Fig.2).

Effect of PH on VCO yield in WCT and DJ Coconuts using two organisms:

The PH dependent production of VCO by two organisms in WCT and DJ Coconuts was presented in table.1 and it was observed from Fig.3 showed that optimum PH for *Saccharomyces* is 3.5±0.1, where the maximum process efficiency 77.05% was obtained in WCT Coconuts, as like in DJ Coconuts at the same PH where the process efficiency obtained maximum that is 73.85% (Fig.4). The above results are come as like the similar study of Fleet and Heard (1993) observed that growth rate of *Saccharomyces cerevisiae* were decreased as the pH was decreased from 3.5 to 3.0. And also the report revealed from Noe Arroyo-Lopez, F.*et al*. (2009) the PH from 2.24 to 3.80 is optimum for *Saccharomyces cerevisiae* and that PH is responsible for the maximum growth of an organism. After optimum PH (3.5±0.1) the yielding efficiencies are decreased with the increase of PH Values. This is noted by Charoenchai et al. (1998) and Fleet and Heard (1993) in their research that the PH 3-4 is optimum for *Saccharomyces* growth other than affect the growth at considerable level.

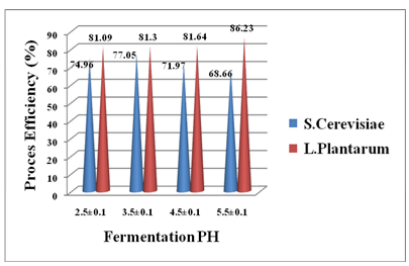

Fig.3.Effect of PH on VCO yield in WCT

The maximum process efficiencies of 86.23% in WCT and 81.90% in DJ was registered by using *Lactobacillus plantarum* induced fermentation at 5.5±0.1PH (Fig.3&4).

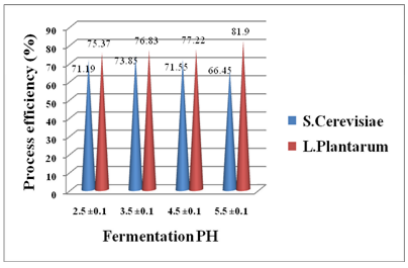

Fig.4.Effect of PH on VCO yield in DJ

This is a similar result of Raghavendra and Raghavarao, (2010) where at the PH 5.0 the maximum process efficiency (78%) was obtained. But in the present results showed that the similar maximum yield efficiencies obtained in both WCT (86.23% and DJ (81.90%).So, it is more over equal to the reported study. The other than optimum PH (5.5±0.1ºC) level was used in this study showed that decrease in PH is directly proportional to the yielding efficiencies. This is a similar note of previous research of Neela Satheesh and N.B.L Prasad, 2013.

Effect of Inoculum concentration on VCO yield in WCT and DJ Coconuts using two organisms:

Effect of Inoculum concentration on VCO production by the two induced fermentative methodology was given in Fig.5.

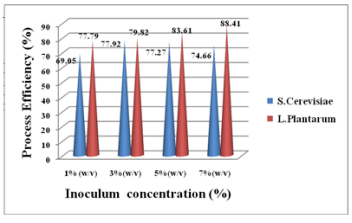

Fig.5.Effect of Inoculum concentration on VCO yield in WCT

The previous study of Riko Aditiya *et al.*2014 and (Mansor. T.S.T.*et.al, 2012),* the maximum yield of best VCO was obtained at 0.1% and 0.2% respectively using of *Saccharomyces inoculum but* in the present study inoculum concentration modified as 1%, 3%, 5% and 7% to achieve maximum destabilization. Since, this organism is a protease producing organism (Sadiah Djajasoepena, 2011), thereby destabilization in coconut milk is increased. The present result clearly noted that the optimum inoculum concentration of *Saccharomyces cerevisiae* is 3%, where the maximum process efficiency that is 77.92% was obtained using WCT Coconuts (Fig.5). In the case of DJ Coconuts at the same inoculum concentration where the maximum process efficiency 75.34% was registered (Fig.6). Other than optimum inoculum concentration (3%) the process efficiencies are decreased gradually with the increase of inoculum concentrations of both Coconut varieties.

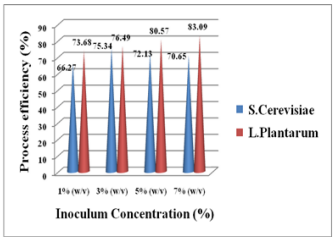

Fig.6.Effect of Inoculum concentration on VCO yield in DJ

In another process the maximum process efficiency of 88.41% was noted at 7% *Lactobacillus* inoculum with WCT Coconuts an d at the same inoculum concentration in DJ Coconuts where the maximum process efficiency 83.09% was obtained with *Lactobacillus plantarum* (Fig 5&6). In previous study of Neela Satheesh and N.B.L Prasad, (2013) the maximum process efficiency was obtained with 5% Inoculum concentration and the maximum efficiency was 82.91% with *Lactobacillus plantarum* but in this present study the process efficiency is much greater than the previous study with 7% Inoculum. Other than optimum inoculum concentration (7%) the process efficiencies are gradually decreased with the decrease of inoculum concentrations is noted in both coconut varieties using *Lactobacillus plantarum* strain.

Effect of Fermentation end time on VCO yield in WCT and DJ Coconuts using two organisms:

Fermentation end time on VCO production by two organisms in WCT Coconuts was depicted in Fig.7.

According to the earlier study of Riko Aditiya *et al.2014 the* fermentation end time selected as 24, 36, 48 and 60 hrs for conducted the study. But in the highest yields of VCO were obtained at 36 hrs in both WCT and DJ Coconuts using *Saccharomyces cerevisiae* induced fermentation that is 79.31% with WCT and 73.85% in DJ Coconuts. (Fig.7 & 8).

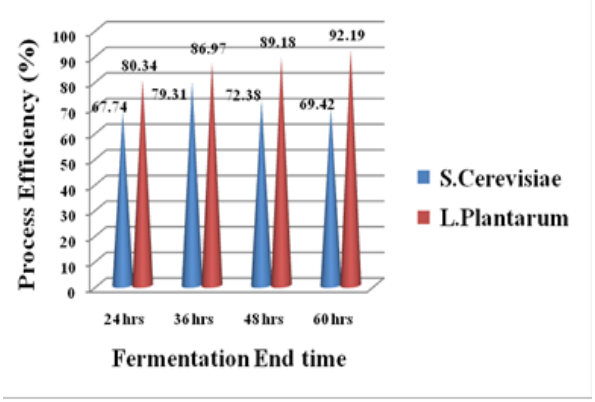

Fig.7.Effect of Fermentation end time on VCO yield in WCT

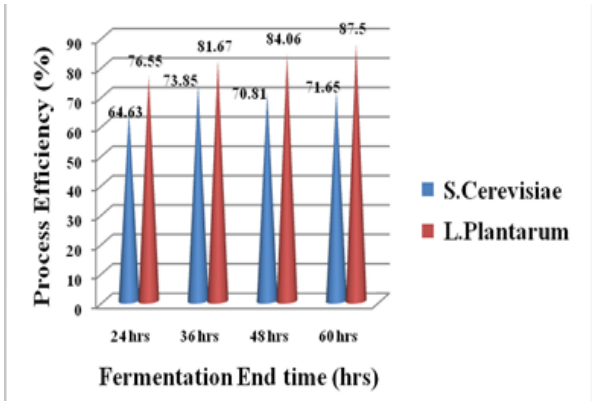

Fig.8.Effect of Fermentation end time on VCO yield in DJ

Table 1: Effect of different parameters on the production of VCO in WCT & DJ Coconuts using *Saccharomyces cerevisiae and Lactobacillus plantarum induced fermentation*

Parameters		VCO yield efficiency (%)			
		WCT Coconuts		DJ Coconuts	
		Saccharomyces	*Lactobacillus*	*Saccharomyces*	*Lactobacillus*
Temperature	25±1ºC	70.17^{b}	72.05^{a}	69.44^{a}	69.91^{b}
	30±1ºC	76.26^{b}	73.08^{a}	74.06^{ab}	70.91^{a}
	35±1ºC	79.92^{ab}	77.34^{b}	72.04^{b}	74.96^{a}
	40±1ºC	73.14^{a}	88.6^{ab}	68.54^{b}	84.6^{ab}
PH	2.5±0.1	74.96^{a}	81.09^{b}	71.19^{a}	75.37^{b}

	3.5±0.1	77.05[ab]	81.3[b]	73.85[ab]	76.83[a]
	4.5±0.1	71.97[b]	81.64[a]	71.55[b]	77.22[a]
	5.5±0.1	68.66[b]	86.23[ab]	66.45[a]	81.9[ab]
Inoculum concentration	1% (w/v)	69.05[b]	77.79[a]	66.27[b]	73.68[a]
	3% (w/v)	77.92[ab]	79.82[a]	75.34[ab]	76.49[a]
	5% (w/v)	77.27[a]	83.61[b]	72.13[b]	80.57[a]
	7% (w/v)	74.66[a]	88.41[ab]	70.65[b]	83.09[ab]
Fermentation end time	24hrs	67.74[a]	80.34[b]	64.63[b]	76.55[a]
	36hrs	79.31[ab]	86.97[b]	73.85[ab]	81.67[a]
	48hrs	72.38[a]	89.18[b]	70.81[a]	84.06[b]
	60hrs	69.42[a]	92.19[ab]	71.65[b]	87.5[ab]

Note: *values given with superscript mentioned that significantly difference from (the interactions of both organism and process parameters on VCO recovery) each other at* P≤0.05 Based on SPSS 16.0 software

After 36 hrs of fermentation the VCO yields in WCT Coconuts using *Saccharomyces* are 72.38% and 69.42% at 48 and 60 hrs respectively. Similarly in DJ Coconuts the yielding efficiencies decreased with the increase of fermentation end time that is 70.81% and 71.65% at 48 and 60 hrs respectively.

The yield efficiencies are gradually decreased after 36hrs in both coconut varieties using *Saccharomyces cerevisiae* strain fermentation. In another set of process the maximum process efficiency of 92.19% was registered with 60hrs fermentation end time using *Lactobacillus plantarum* strain with WCT Coconuts. At the same fermentation end time period the maximum process efficiency of 87.5% was registered in DJ Coconuts. In this test series, the yielding efficiencies are directly proportional to the increase of fermentation end time in both WCT and DJ Coconuts using *Lactobacillus* organism. This is similar to the study of Neela Satheesh and N.B.L Prasad, 2013, where the maximum process efficiency was obtained at 72 hrs fermentation end time and the efficiency was 81.31% with *Lactobacillus plantarum strain.*

Physical and Chemical Properties

Table 2. Showed the physical and chemical properties of VCO, which are estimated from two coconut varieties under induced fermentative methodology. Moisture content is another important quality characteristic for oils and fats. It is desirable to keep the moisture content low as it will increase the shelf life by preventing oxidation and rancidity processes (Che Man et al., (1997) & Mansor. T.S.T.*et.al, 2012).* In the present two VCO samples where, the moisture content is almost come within the range, so rancidity is not possible under storage condition.

Table. 2. The Physico-Chemical Properties of VCO from WCT and DJ Coconuts

Parameter	VCO from WCT Coconut	VCO from DJ Coconut	APCC Standard
Moisture	0.38	0.37	0.1-0.5
Acid value	2.244mg/KOH/g		6max
Free fatty acids	0.5	0.49	0.5
Iodine Number	5.9	6.0	4.1-11.0
Peroxide value	2.3millie equivalents O_2/kg	2.2 millie equivalents O_2/kg	< 3meq O_2/kg
Saponification number	252.4mg KOH/g	255.6 mg KOH/g	250-260mg KOH/g

The free fatty acid is present in the two VCO samples are equal to the Standard so it is a good indicator of oil quality such as off taste and aroma (Mansor. T.S.T.*et.al,2012).* Peroxide value gives an indication of the primary oxidation state of oil (Kamariah, A.*et.al, 2008).* It is present in both VCO Samples below the maximum value of APCC standard. Also, all the six parameters taken in the study for the VCO of the two coconut varieties are comes within the APCC Standards.

Fatty acid analysis of Virgin Coconut Oil

The GC-MS analysis of fatty acid methyl esters indicated that the virgin coconut oil prepared from two induced fermentative methodology commonly showed that the saturated fatty acids namely C6-Caproic acid, C8-Caprylic acid, C10-Capric acid, C12-Lauric acid, C14Myristic acid, C16-Palmiticacid, C18:2-Linoleic acid, C18:1-Oleic acid and C18:0-Stearic acid. The concentration of the lauric acid, the major component of the fatty acids (Probir Kumar Ghosh *et. al,2014*) in virgin coconut oil has ranged from 50.50 – 51.52% depending upon the method of preparation (Fig.9). The difference in fatty acid profile of two induced methodologies is furnished in Table.3. Among the two induced methodologies the high concentration of lauric acid (51.52 %) was obtained in the WCT VCO produced by *Lactobacillus plantarum* induced fermentation method and in the case of *Saccharomyces cerevisiae* induced fermentative methodology yields 50.50%of lauric acid concentration using DJ Coconuts (Fig.10 &11)

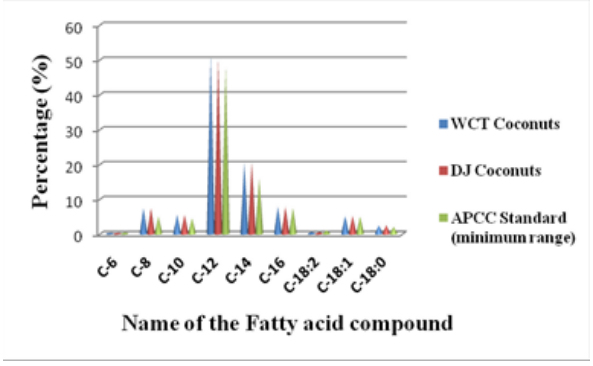

Fig.9.Comparison of fatty acid composition of VCO prepared from WCT and DJ Coconuts

The results clearly indicate that the variety of coconut (Jnanadevan.R, 2018) and also the maturity of the coconut determined the fatty acid composition (Nur Raihan Ali.et.al, 2014).In the present study both WCT and DJ Coconuts are selected as brown matured level (Ryan et al., 2002; Gucci et al., 2004; ref) so the yielding of the fatty acid profile is more over equal in proportion and also comes within the range of APCC Standards.

Table. 3. Fatty acid profile of virgin coconut oil prepared from two induced fermentation method

S.No	Name of the Fatty acid	Virgin Coconut Oil by induced fermentation		
		Saccharomyces Cerevisiae	*Lactobacillus plantarum*	Limit as per Codex (APCC)
1.	C6-Caproic acid	0.278	0.278	0.4-0.6
2.	C8-Caprylic acid	7.360	7.360	5.0-10.0
3.	C10-Capric acid	5.464	5.464	4.5-8.0
4.	C12-Lauric acid	50.505	51.525	48.0-53.0
5.	C14-Myristic acid	20.401	20.401	16.0-21.0
6.	C16-Palmitic acid	7.801	7.836	7.5-10.0
7.	C18:2-Linoleic acid	0.536	0.567	1.0-2.5
8.	C18:1-Oleic acid	5.161	5.161	5.0-10.0
9.	C18:0-Stearic acid	2.493	2.493	2.0-4.0

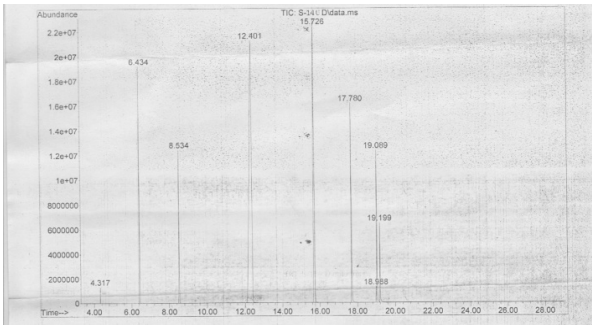

Fig. 10. GC-MS Chromatogram of Virgin Coconut Oil prepared from WCT Coconuts

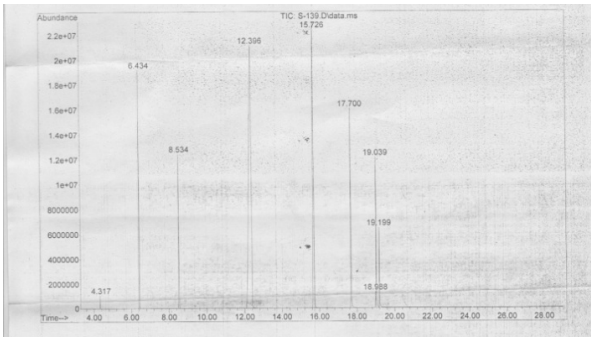

Fig. 11. GC-MS Chromatogram of Virgin Coconut Oil prepared from DJ Coconuts

CONCLUSIONS

From the above study we conclude that, extraction method, selection of coconut variety and organisms used for induced fermentation had impact on yield efficiency, physicochemical characteristics and fatty acid profile of the derived VCO. The highest oil yield efficiency was obtained at 40±1ºC fermentation temperature, PH 5.5±0.1 , Inoculum Concentration 7% and Fermentation end time 60 hrs by using WCT Coconuts with *Lactobacillus plantarum* induced fermentation. The physico chemical properties are comes within the ranges of APCC to the two induced methods. From the GC-MS results comparison, the major fatty acid present in the coconut is lauric acid, which is obtained maximum (51.525%) in *Lactobacillus plantarum fermented* oil than Saccharomyces fermented oil (50.505%). So in this research portion finally we concluded that the selection of coconut variety and strain selection for induced fermentation are determines the best quality of Virgin Coconut Oil for better life in future.

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