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Study on Induced Fermentative Methodology for the Production of Virgin Coconut Oil from two *Cocos nucifera* Varieties

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***Abstract*:** In this study, Virgin Coconut Oil (VCO) was produced from West Coast Tall (WCT) and Deejay Coconuts (DJ) under induced fermentative methodology by employing *Saccharomyces cerevisiae* and *Lactobacillus plantarum* (9511) strains. This production methodology was optimized with temperature, PH, Inoculum concentration and Fermentation end time. The VCO yielding efficiencies were compared from two strains. Among the four parameters in the study, the maximum yielding efficiencies were obtained from WCT coconuts employing *L. plantarum* (9511) at 40±1ºC temperature, 5.5±0.1 PH, 7% (w/v) Inoculum concentration and 60hrs of fermentation end time. In conclusion, it has been observed that proximate analysis of VCO showed within the standard limits and also WCT Coconuts in combination with L. plantarum offered higher yield and better fatty acid proportion than the DJ Coconuts.

Keywords: VCO, yielding efficiency, parameters, L. Plantarum (9511), S. cerevisiae, WCT, DJ.

I. INTRODUCTION

Coconut is considered as a "*Kalpavriksha*" or "the tree of heaven" which means "the tree that gives all that is essential for living", in our common life. It is chiefly cultivated in Philippines, Indonesia, India, SriLanka, Papua New Guinea, Thailand, Malaysia and Fiji (1).There are more than 50 value-added products are acquired from coconut palm are utilized in domestic purposes as well as importing quality products (2). Coconut oil is one of the edible oil commonly extracted from the matured coconut kernels for food and Industrial preparations. It is a primary source of energy, in tropical countries like India, SriLanka, Philippines and Indonesia (3). The oil is utilized for various health related concerns including skin care, hair care, stress relief, weight loss, body cool, cholesterol level maintenance, immunomodulatory effects and cardiovascular uses (4). Based on the moisture content, coconuts are classified as wet and dry coconuts (5).Wet matured coconuts are used for the extraction of VCO (6). Virgin Coconut Oil was defined as, an oil manufactured through with or without heat and chemical refining (7). VCO from wet preparation has more remedial potential to reduce the diseases intensity like Alzheimer's, Autism, Dementia and AIDS (8). VCO is commonly produced from both wet and dry methodologies. In dry method, kernel was heating under controlled conditions to remove the moisture as well as for the prevention of microbial contamination and finally oil is recovered through grinding process (9).Wet methods are commonly classified into chilling and thawing, fermentation, enzymatic and pH method or any of these in combination (10).Among the above methods, VCO Produced from both Fermentation and Chilling method had higher antioxidant potency than Refined Bleached Deodorized (RBD) coconut oil (11). This potential is helps to fight against several dreadful diseases of human beings.

As per the Codex "Virgin Oils", are defined as oils which are suitable for human consumption (12). It provides an alternative inexpensive source of energy to neurons of humans in the case of Alzheimer's disease (13). Also various fractions of coconut oil are used as drugs (14). Almost 50% of the fatty acid in VCO is available in the form of lauric acid, which has applied as wide spectrum of antimicrobial substances against fungi, bacteria and viruses. Moreover, the fatty acid profile of coconut oil shares the similar characteristic nature of breast milk (15 & 16). A Normal coconut milk have innate microbial flora which is involved in fermentation to produce VCO. Commonly VCO from natural fermentation showed poor in quality due to the entry of unwanted microbes through coconut milk, which provides rich attractive sources for the microbes (17). Also, natural fermentation is not enough to destabilize the fat protein emulsion. Since, it could be overcome by induced fermentation with probiotic micro organism like *Lactobacillus plantarum* which is actively involves in VCO production (18&19). A comparative study showed that, both quantity and quality of coconut oil was high with *L.Plantarum* induced fermentation (20). A

prior study on VCO production with commercial yeast was focused only the carbohydrate breakdown but not proteins and fats from the substrate (21*)*. In one more research proved that, superior quality of VCO was obtained in low viscosity and also low free fatty acids content by employing *Saccharomyces cerevisiae* (22). *Saccharomyces cerevisiae* secrets two enzymes like invertase and protease. Invertase act on sucrose and break into glucose and fructose while protease serves to break down proteins thereby destabilization of coconut emulsion is occurred efficiently (23). So the time interval for the production is much quicker also superior quality product was obtained. A parametric study results showed that, the maximum yields of oil obtained with 0.3 %(w/v) *Saccharomyces cerevisiae* inoculum at 24 hrs fermentation (24). Also a profound study on *Saccharomyces cerevisiae* noted that, the significant effect on oil yield, moisture content, free fatty acid, peroxide value, and Saponification number (25).

A study on fatty acid profiles of 60 Talls, 14 Dwarfs, and 34 hybrid coconut varieties research revealed that, the quality and quantity is not only determined by the external factors but also the variety of coconuts (26). Also a similar research accounted that, the quality of VCO is determined by the different maturity stages of coconuts (27 & 28).

WCT is one of an essential variety cultivated in India It has the capability to yield more oil (68%) when compare to other tall trees, since it is preferred for cultivation in southern states (29, 30 &31). DJ is the biggest commercial organization supplying of superior quality coconut seedlings to farming community (32). It is one of the higher oil yielding (68%) hybrid variety crossed from selected parents (33). Pollachi is a major coconut cultivation area in Coimbatore District (34).WCT and DJ are taken in the study for VCO Production. Now a days, the above two varieties are majorly cultivating in Pollachi region. Fermentation is a well-known cold process method for the extraction of virgin coconut oil from the coconut milk (35). Yield is considered as a prime parameter in VCO extraction. In fermentation process both yield and quality of the final product could be affected by various factors such as variety of coconuts, type of fermentable microorganism, temperature, PH, Inoculum concentration and Fermentation end time. In this present study the selected two coconut varieties (WCT, DJ) are subjected to induced fermentation with both Yeast and bacteria. The yield and quality of the end product are discussed and compared in this study.

II. MATERIALS AND METHODS

Coconuts

Coconuts such as WCT and DJ Vishwas, which have 11-12 months old equally sized matured brown in colour, high oil content nuts are selected (36, 37 & 38). The selected nuts were taken from South farm of Vanavarayar Institute of Agriculture (VIA), Manakkadavu and are transferred to lab venue without any corporal damage.

Microbes in fermentation

Two pure microbial cultures are used in this methodology. The culture *Lactobacillus plantarum (*9511) was purchased from M/s. IMTECH, Chandigarh under lyophilized condition. As per the sub-culturing instruction, two sub-culturing was done followed by; slant culture was prepared and stored in MRS (*Man*

Rogosa and Sharpe growth Medium) Agar medium for further process. The culture *Saccharomyces cerevisiae* (< 2-months old culture) was taken from Plant Biotechnology laboratory of VIA, Manakkadavu. The working stock was inoculated on a Yeast Extract Peptone Dextrose (YPD) broth medium and it was incubated at 30° C for 16−18 hr in a shaker at 230−270 rpm (39). After the preparation of seed culture, the morphological character was determined by stained with lacto phenol-cotton blue and carbol fuchsin (40*).*

Milk Extraction

The selected nuts are split into half cups using manual cutter and kernel was grated by manually operating coconut grater along with testa, which contains more phytochemical especially polyphenol content responsible for antioxidant potential of VCO (41).After that grated portion was mixed with hot water (1:1, w/v) (42&43) Then the milk was squeezed from grated kernel using a screw type coconut milk press (Pilot smith India (p) Ltd) (Fig 1) and the milk was transferred to sterile food grade plastic tank. The machineries and equipments for VCO production was supported from DST Project on RWTP at Dr.Mahalingam College of Engineering & Technology, Pollachi. The milk out from the process was kept it for one hour to separate the skim layer from coconut milk, which is having rich quantity of coconut oil (44&45). After separation, only milk cream layer was taken for the fermentation process.

Fig. 1. Coconut milk press (screw press)

Sterilization of Coconut Cream

The collected coconut milk cream (1lit) was sterilized inside the laminar air flow hood for 20 min under Ultra Violet light (Dose of 40 mW/Cm2/ Sec.) (46 & 47). This sterilization practice on liquid food leads to strong attenuation effect, which is help to reduce the contamination in cream (48).

Preparation of Seed cultures

a. Saccharomyces cerevisiae

Seed culture was prepared by using YPD broth medium in a shaker incubator (100rpm) at 37°C for 36 hours (49). This preparation was maintained for entire fermentation process.

b. Lactobacillus plantarum

The seed culture was prepared by transferring of 2 % of stock culture to the conical flask containing MRS growth medium (50). The incubation temperature was maintained at 35±1°C for 18 hrs with 150 rpm. The prepared culture was used as an inoculum for the fermentation process.

Enumeration of microorganisms

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

Number of Microorganisms present in the sample

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

Induced Fermentation method

Destabilization is a primary process, which is required to maximize the VCO yield (51). The UV-Sterilized coconut milk cream was exposed at 40°C for initial destabilization (52). After that each 200 ml quantity of milk emulsion was transferred to a series of sterilized labeled fermentation flasks (500-mL Erlenmeyer containing 200 ml final volume of fermentation medium) for to conduct the two types of induced fermentation process. Additionally, the parameters were arranged according to the comparative study.

Parameters in comparative study

The four parameters such as Temperature, PH, Inoculum concentration and Fermentation End Time were taken in account to estimate the yielding efficiency of VCO.

Temperature

Temperature is a first and key parameter, which influences the growth of microbes.This study, was slightly modified a series of common temperatures like 25±1ºC, 30±1ºC, 35±1ºC and 40±1º C are used for both organisms used in this process (53). The entire study was completed by using energy regulator enabled temperature controlled Incubator (Genuine make an Indian company).

PH

A slight modification of the previous study, the second parameter was changed as in the ranges of 2.5, 3.5, 4.5 and 5.5 for both organisms used in the fermentation process (54). In this study PH was adjusted by using Eutech Instrument of thermo fisher scientific Company (Singapore).

Inoculum concentration

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

Fermentation end time

It is maintained in the ranges of 24, 36, 48 and 60 hrs for both organisms used in the study to estimate the yielding efficiency of VCO.

Recovery of VCO

After finished of the two fermentation batches, VCO will pushed up in the upper layer of flask due to its density is 0.903g/ml at room temperature (55). Then it was collected from each series of four comparative studies using sterile suction pipette followed by the remaining oil combined with cream layer was centrifuged by two times under temperature controlled centrifuge at 27°C for 10 min in 6000 rpm (56).The acquired oil was prepared as triplicates and kept in refrigerated until further analysis.

Estimation of initial oil content

Initial oil content of two coconut variety was estimated by AOAC Soxhlet method (57).This method is adopted only for to quantify the oil from particular coconut variety but this method produce low phytochemical content in oil (58). As per the above procedure, 10 grams of pre-dried coconut (pieces) was taken in an extraction thimble and then covered with wool. In another hand, the pre-dried boiling flask was weighed and filled with n-hexane chemical. After that, the set up was assembled one by one as boiling flask, Soxhlet flask and condenser. The extraction was carried out in triplicates for 30 hours. Finally, the extracted oil sample was dried in an oven at 103 ± 1°C for 2 hours to take out the residual solvent and cooled in desiccator before reweighing.

Calculation of Oil recovery & Process efficiency

The oil recovery was determined according to the initial oil content in the coconut to the oil extracted from two different fermentative methodologies (59). The below

formulas are used to calculate the oil recovery and the efficiency of the two processes: -
% oil extraction on wet basis

$$= \frac{Weight\ of\ oil\ extracted}{\text{Weight of Coconut milk cream Taken}} \text{x}100$$

Efficiency of the Process (%) or yield in a method

$$= \frac{Oil\ yield(\%)\ on\ wet\ basis}{\text{Oil content (\%) by soxhlet method}} \text{x}100$$

Physico-chemical characteristics

Moisture content, acid value, free fatty acid content (%), Iodine number, peroxide value and Saponification number are estimated from the VCO produced.

Moisture content

Moisture content was estimated by weighing 20g of VCO sample was placed onto a Pre-weight determined beaker. 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 (60).

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

Acid value & free fatty acids

Initially 25 ml of titration reagent that is alcohol-benzene (1:1 v/v) was pre-heated in a water bath for 10min at 70° C. After cool, 3 drops of phenolphthalein indicator was added to the mixture and titrated against 0.01N NaOH up to slight red in colour end point. Then the above solution was mixed with 2.5g of VCO sample and heated for 5 min and again titrated with 0.01 N NaOH at least for 10 min to get the slight red solution. Then the consumed NaOH level was noted. The acid value and free fatty acids are calculated by the below formulas (61).
Acid value =

$$\frac{\text{A x N x 10}}{\text{Sample Wt (g)}}$$

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

Free fatty acids (%) =

$$\frac{A\ x\ Nx\ M}{\text{Sample Wt(g)}} \text{x}100$$

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

Iodine number

At the start a 3.0 g of VCO was taken in a 250 ml conical flask. Then, 20 ml cyclohexane was added to conical flask for dissolve the fat followed by 25 ml of Wijs solution was added. The flask was completely closed by parafilm or by cork and it was kept in a shaker for 30 min. After that, 20 ml of 15% potassium iodide solution (KI) and 100 ml of de-ionized water were added into the mixture. The mixture 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 by the below Equation (62).

$$\text{Iodine Number} = \frac{(B-S)x\ N\ of\ Na2S2O3\ x12.69}{\text{Weight of Sample (g)}}$$

Where, B = V ml of Na2S2O3 volume for blank
S = V ml of Na2S2O3 volume for sample
N = Normality of $Na_2S_2O_3$

Peroxide value (PV)

Principally a 5 g of VCO was mixed with 30 ml of acetic acid-chloroform (3:2) mixer and the solution was stirred to dissolve completely. Then, 0.5 ml of saturated potassium iodine (KI) was added to it and stirred for one minute. The solution mixer was titrated against with 0.01 N Na_2SO_3 until its colour changed to light yellow. The step of titration 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 by the below formulae (63).

$$PV = \frac{N\ X\ V}{\text{W}}$$

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 (SN)

Initially a 2.0 gm of filtered dried VCO sample was transferred into a 250 ml conical flask. Then 25 ml of the alcoholic potassium hydroxide solution was added into the flask. Same as, blank determination was also performing along with the sample. Then both sample and blank flasks are connected to the air condensers and switch-on the water bath until the Saponification process was completed. This was indicated by absence of oily matter. After that, the flask and condenser have cooled; wash down the inside of the condenser with about 10 ml of hot ethyl alcohol. The excess potassium hydroxide was determined by titration with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator (64).

$$SN = \frac{56.1(B - S)N}{W}$$

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

A 50 ml of VCO 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 was 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 (65).

MS Programme

The software NST Version-11 library was used to analyze. The temperature for inlet was 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 of the experimental values were carried out in triplicate manner, and the mean values were presented in this study. Significant differences between the means were determined by Duncan's multiple range tests at a 95 % confidence level (66).

III. RESULTS AND DISCUSSION

Coconut milk has higher in fat and calories than cow's milk (67). It is a rich source of fat (21.3%), carbohydrate (2.8%), sugar (2.1%), protein (2.0%) especially albumin, globulin, prolamin, glutein and also with some vitamins and trace elements (68 & 69). Food industries are widely depends upon *Saccharomyces cerevisiae* for the production of lipids, proteins and vitamins (70). This strain can grow well even in low nitrogen content also showed osmo-tolerant ability on nutrient medium contains 50-60% of glucose (71).

Coconut milk is an emulsion of fat and protein. Even though, the destabilization of these two components is a tedious process, it is possible and easy with an enzyme protease producing organism of *Saccharomyces cerevisiae* (72).Like that, *Lactobacillus. sp* could effectively extract more VCO in fermentation process (73). The bacteria have an excellent potential to destabilize the protein from coconut milk thus fat separation is simple (74). In the present study West Coast Tall (Tall cultivar) and Deejay Vishwas (Dwarf variety) was used for the Production of VCO. WCT revealed that better nut yields as well as high oil (68%) yielding capacity (75). Like that Deejay coconuts also have the ability to yield as similar as WCT (76).

Soxhlet extraction

The initial oil content of WCT and DJ was calculated as 67.5% and 61.5% respectively by the Soxhlet method (77). The results of Soxhlet estimated are taken for to calculate the yielding efficiencies of two fermentative methodologies (78).

Effect of temperature on VCO yield

Production of VCO in respect to four variable temperatures by two organisms in both Coconut varieties is furnished in table.1. Temperature is one of an environmental factor that directly or indirectly influences the growth rate of a microorganism (79). The optimal growth temperature referred for *Saccharomyces* is 25-35ºC (80 & 81). In this present methodology, the maximum process efficiency 79.92 % was obtained in WCT Coconuts by the *Saccharomyces cerevisiae* fermentation at 35±1ºC (Fig.2), Wherein DJ Coconuts the maximum process efficiency 74.06% was obtained at 30±1ºC (Fig.2) this is a similar result of Previous study (82). The yielding efficiencies are increased with increasing temperature (Fig.2) this is also a similar research statement of previous investigations mentioned that, *Saccharomyces* start the fermentation in few hours even a low temperature. But after reached the optimum temperature (35±1ºC) it showed decreased in production efficiency (83).

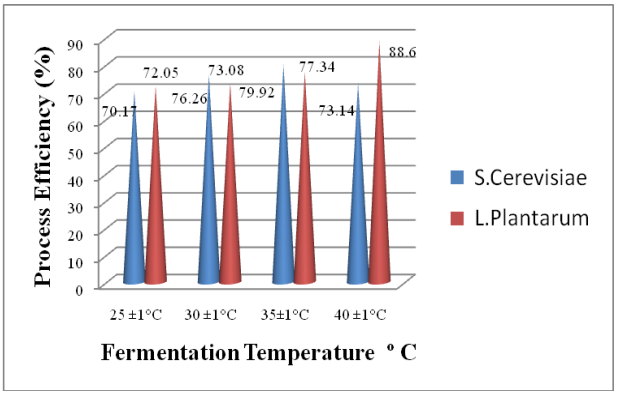

Fig .2 .Effect of temperature on VCO yield in WCT

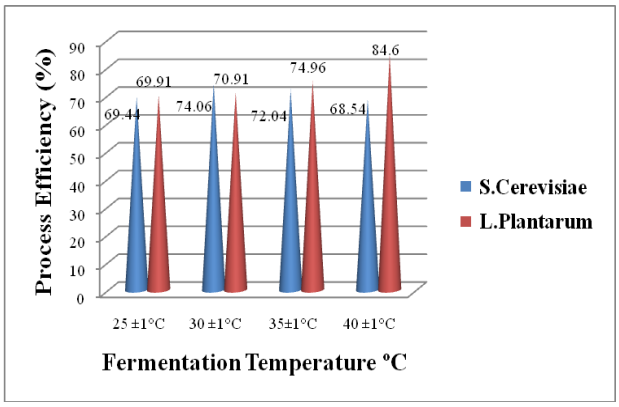

Fig.3.Effect of temperature on VCO yield in DJ

While in *Lactobacillus plantarum* induced fermentation, the maximum process efficiency that is 88.60% was obtained in WCT and 84.6% was observed in DJ at 40±1ºC (Fig.2&3). This is a similar result of the earlier study (84& 85). Same like in WCT, *Saccharomyces* induced fermentation showed the decrease of efficiency after optimum temperature (86).

Effect of PH on VCO yield

Coconut milk is an emulsion, which can be destabilized by the adjustment of PH then only VCO recovery is simple (87). The PH dependent production of VCO by two organisms in WCT and DJ Coconuts was presented in table.1.The maximum process efficiency 77.05% was obtained in WCT Coconuts by *Saccharomyces* induced fermentation with the adjustment of PH 3.5±0.1 (Fig.4). At same PH, the efficiency showed maximum that is 73.85% in DJ by *S.cerevisiae* induced fermentation (Fig.5). The above results confirmed that the growth rate of *Saccharomyces cerevisiae* were decreased as the pH was decreased from 3.5 to 3.0 value and also the PH 2.75-4.25 was an optimum for *Saccharomyces* growth (88). Similar report revealed that, PH 2.24-3.80 was optimum and it responsible for maximum growth of *Saccharomyces cerevisiae* (89). After the optimum PH (3.5±0.1) the yielding efficiencies are decreased with the increase of PH Values (90).

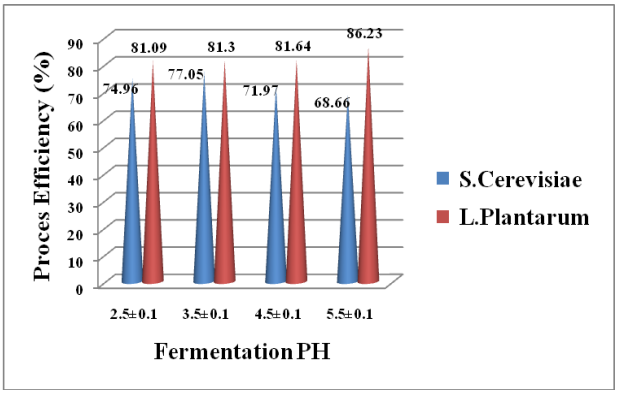

Fig.4.Effect of PH on VCO yield in WCT

The maximum process efficiencies of 86.23% in WCT and 81.90% in DJ was recorded by using *Lactobacillus plantarum* induced fermentation at 5.5±0.1PH (Fig.4&5). But below this PH level the efficiencies are showed as decreased in order (81.64, 81.30, 81.09 in WCT & 77.22, 76.83, 75.37 in DJ). The above similar result was noted at PH 5.0 (maximum efficiency 78%) with the same organism induced fermentation in coconut milk (91). The other than optimum PH (5.5±0.1ºC) level is used in this study showed that decrease in PH is directly proportional to the yielding efficiencies. This is a similar result of a of previous research (92).

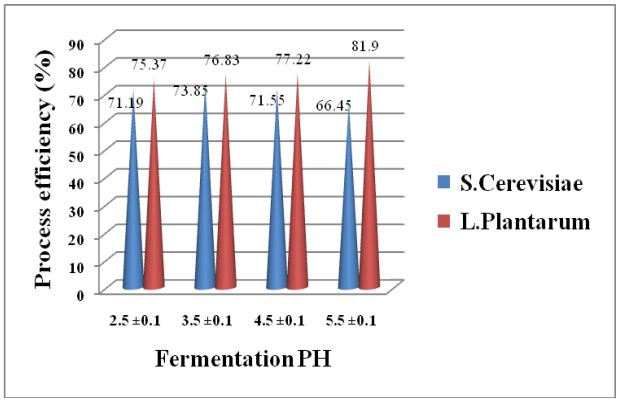

Fig.5.Effect of PH on VCO yield in DJ

Effect of Inoculum concentration on VCO yield

Effect of Inoculum concentration on VCO production by the two induced fermentative methodology was represented in Fig.6.

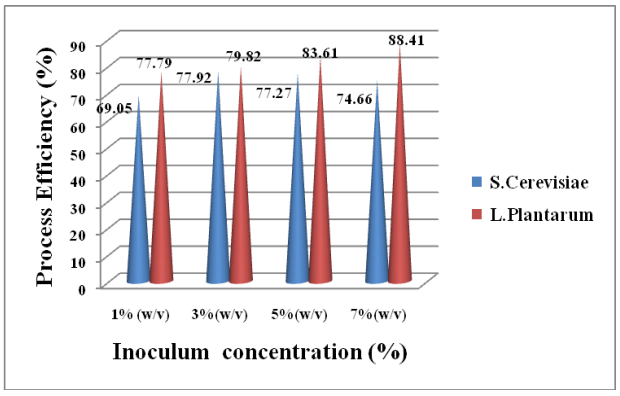

Fig.6.Effect of Inoculum concentration on VCO yield in WCT

As per the previous study the maximum yield of best VCO was obtained at 0.1% and 0.2% respectively using of *Saccharomyces inoculums* (93 &94). But in the present study inoculum concentration was modified as 1%, 3%, 5% and 7% to achieve maximum destabilization. The present result is clearly illustrated that the optimum inoculum concentration of *Saccharomyces cerevisiae* is 3% and the maximum process efficiency that is 77.92% was obtained with WCT Coconuts (Fig.6). At the same

percentage inoculum concentration the maximum process efficiency that is 75.34% was registered in DJ coconuts (Fig.7). Other than optimum inoculum concentration (3%) the rest of the process efficiencies are showed decrease in order against the increase of inoculum concentrations for the both Coconut varieties.

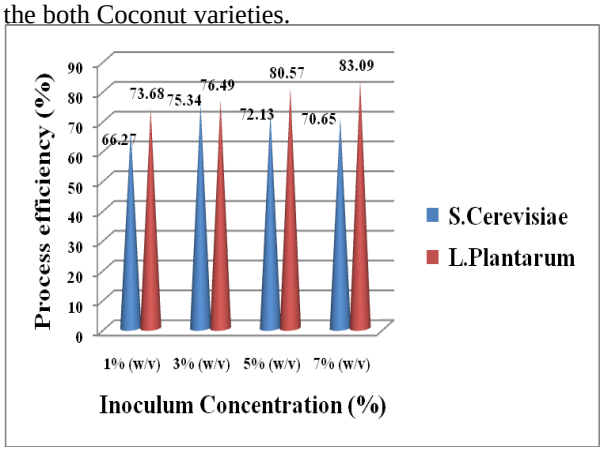

Fig.7.Effect of Inoculum concentration on VCO yield in DJ

of 88.41% was noted at 7% *Lactobacillus* inoculum concentration in WCT Coconuts and at the same concentration in DJ Coconuts where the maximum process efficiency 83.09% was obtained with *Lactobacillus plantarum* (Fig 6&7). As per the earlier study, the maximum process efficiency 82.91% was obtained with 5% *Lactobacillus plantarum* Inoculum concentration (95). But in this current study the process efficiency is little 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 was noted in both coconut varieties using *Lactobacillus plantarum* strain.

Effect of Fermentation end time on VCO yield:

Production of VCO in respect to fermentation end time by two organisms in WCT Coconuts was depicted in Fig.8. The fermentation end time was selected as 24, 36, 48 and 60 hrs for conducted the study as per the previous reference (96).

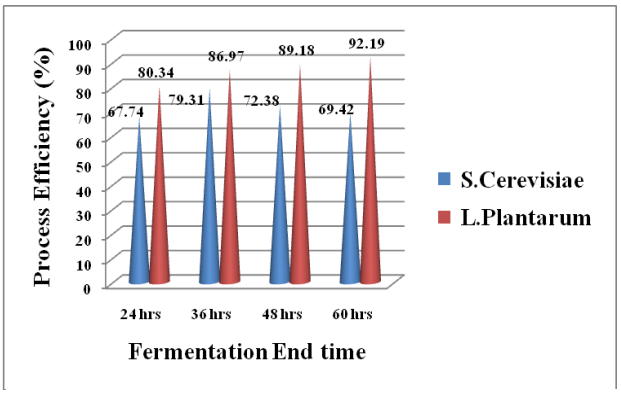

Fig.8.Effect of Fermentation end time on VCO yield in WCT

The yield was obtained maximum at 36 hrs in both Coconuts using *Saccharomyces* induced fermentation that is 79.31% with WCT and 73.85% in DJ Coconuts. (Fig.8 & 9). After 36 hrs, the efficiencies are registered in WCT coconuts as 72.38% and 69.42% at 48 and 60 hrs respectively with *S.cerevisiae.* Similarly in DJ Coconuts the yielding efficiencies are decreased with the increase of fermentation end time that is 70.81% and 71.65% at 48 and 60 hrs respectively by *S.cerevisiae fermentation*. Thus, the yield efficiencies are gradually decreased after 36hrs in both coconut varieties under *Saccharomyces cerevisiae* fermentation. While observed with *L.plantarum*, the yielding efficiencies are increase in order to the fermentation end time such as 80.34% (24hrs), 86.97% (36hrs), 89.18% (48hrs), 92.19% (60hrs) in WCT and 76.55% (24hrs), 81.67% (36hrs), 84.06% (48hrs), 87.50% (60hrs) in DJ as similar the earlier studies (97).

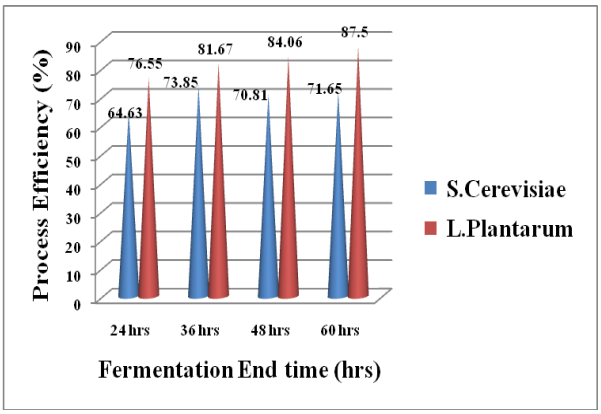

Fig.9.Effect of Fermentation end time on VCO yield in DJ

Physio-Chemical Properties

The quality of VCO is determined by the Physio-chemical properties. These properties were estimated from two coconut varieties are furnished in Table 2. Fermentation is a wet method production, which yields superior quality VCO when compared to other methods (98). Moisture content estimation is an important factor to determine the quality of oils and fats. Low moisture content in oil (0.1-0.5%) is preferable to increase the shelf life also to prevent the oxidation and rancidity processes (99). The moisture content of VCO came within the value recommended by the Asian and Pacific Coconut Community (APCC) range. So rancidity prevention is possible under prolonged storage condition (100). Acid value is defined as a measure of number of carboxyl groups in fatty acids. It will increase in oil and fats due to rancidity or conversion of triglycerides to fatty acids and glycerol (101).

Table 1: Effect of different parameters on the production of VCO in WCT & DJ Coconuts using S. cerevisiae and L. plantarum induced fermentation

(*Values are given with superscript mentioned that significantly difference from each other at P≤ 0.05, SPSS)*

Parameters		**VCO yield efficiency (%)**			
		DJ 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}

The estimated values are less than the APCC recommendation so acid damage is not occurred in this oil (102).An earlier study on VCO production through integrated wet process has a free fatty acid value was 0.13% (103). But in the present study, the free fatty acid values are equal to the standard so it guarantee for the good taste and aroma VCO (104*).* The Iodine Number is indicating that the weight percentage of VCO related to unsaturated fatty acids which can be absorbing more halogen (105). The values are estimated (5.9 and 6.0) from two VCO samples are came within the range of APCC Standard (4.1 -11).

Table. 2. Physico-Chemical Properties of VCO

Parameter	**WCT VCO**	**DJ VCO**	**APCC Standard**
Moisture	0.38	0.37	0.1-0.5
Acid value	2.244mg/KOH/g	2.238mg /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 peroxide value is defined as an indicator of the oxidation or rancidity level of VCO. The values are (2.3 and 2.2) obtained from this method is within the APCC recommendation so lipid oxidation is not possible (106). The Saponification number is related to the mean molecular mass of the fats and oils, and it is inversely related to the chain length of the fatty acids fats and oils. The present process yields higher Saponification number, which means the shorter average chain length of fatty acids presence in the VCO sample (107).

Fatty acid analysis of VCO

The GC-MS analysis of fatty acid methyl esters showed the following 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. Among them lauric acid is a key fatty acid, which is registered maximum in the Virgin Coconut Oil (108). Other than Lauric acid commonly more than 10 numbers of fatty acids are also present in VCO. The comparison of fatty acid composition of coconut varieties is depicted in Fig.10 and Table.3. In the two induced methodology, the high concentration of lauric acid (51.52 %) was noted from WCT Coconut VCO employed with *Lactobacillus plantarum* fermentation and in the case of *Saccharomyces cerevisiae* induced fermentation yields 50.50%of lauric acid from DJ Coconuts (Fig.11 &12).

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

S.No	Name of the Fatty acid	VCO Production by induced fermentation		
		DJ with S.cerevisiae	*WCT with L.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

The results are clearly showed that variety and maturity of the coconut is determined the fatty acid composition (109). In the present study both WCT and DJ Coconuts are selected in matured brown stage for VCO preparation (110 & 111). So the yielding of the fatty acid profile is more over equal in proportion and also came within the recommended level of APCC Standards.

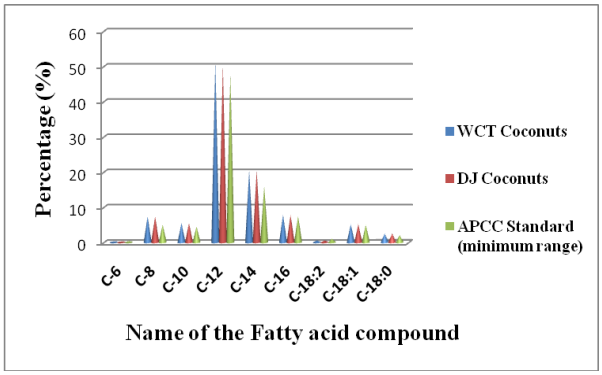

Fig.10.Comparison of fatty acid composition of VCO prepared from WCT and DJ Coconuts

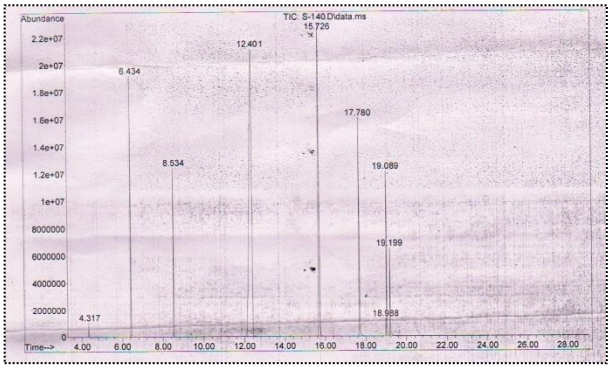

Fig. 11. GC-MS Chromatogram of VCO from WCT Coconuts

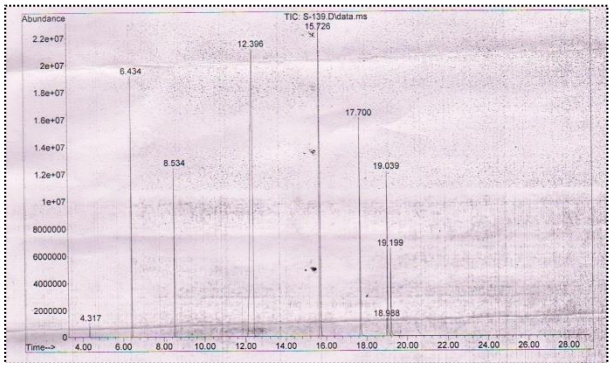

Fig. 12. GC-MS Chromatogram of VCO from DJ Coconuts

IV. CONCLUSIONS

The present study was conducted to compare both quality and quantity of VCO prepared from two fermentation process. Coconut milk emulsion is maintained stability by various intrinsic factors (112). Since, before fermentation the destabilization was carried out with four parameters such as Temperature, PH, Inoculum concentration, Fermentation end time. However four parameters optimized in this production process, the maximum yield efficiency (92.19%) was noted at 40±1ºC fermentation temperature, 5.5±0.1 PH, 7% Inoculum Concentration and 60 hrs Fermentation end time with WCT Coconuts employing *Lactobacillus plantarum* induced fermentation. The lowest efficiency record (73.85%) was registered at 3.5±0.1 PH with DJ coconuts employing Saccharomyces fermentation. In statistical view revealed that, combination of temperature with organism showed significance in WCT coconuts.

The physico chemical properties are comes within the ranges of APCC in both induced methods. The GC-MS analysis showed that 9 numbers of fatty acid combination in both VCO samples also VCO had high contents of MCFAs, especially lauric acid (113). In this present study it was registered maximum (51.525%) in *Lactobacillus plantarum fermented* oil than Saccharomyces fermented oil (50.505%). So in this research section finally we concluded that the coconut variety selection and selection

of strain for induced fermentation are determines the better quality of Virgin Coconut Oil. Furthermore this methodology not only depends upon the organism and parameters employed in this study but also both intrinsic and extrinsic factors such as age of coconut, innate microbial load, enzymes are to be taken for future studies.

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