COST EFFECTIVE TECHNOLOGY FOR HOME SCALE AND SMALL SCALE PRODUCTION OF VIRGIN COCONUT OIL

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by

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THESIS

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KERALA, INDIA

2015

DECLARATION

I, hereby declare that this thesis entitled **"COST EFFECTIVE TECHNOLOGY FOR HOME SCALE AND SMALL SCALE PRODUCTION OF VIRGIN COCONUT OIL"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"COST EFFECTIVE TECHNOLOGY FOR HOME SCALE AND SMALL SCALE PRODUCTION OF VIRGIN COCONUT OIL"** is a record of research work done independently by Ms.Thanuja T.T. (2013-12-110) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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Vellayani *Thanuja.T.T.*

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LIST OF ABBREVIATIONS

%	Per cent
µg	microgram
α	alpha
β	beta
ºC	Degree Celsius
AOAC	Association of Official Agricultural Chemists
APCC	Asian and Pacific Coconut Community
AV	Acid value
BHT	ButylatedHydroxy Toluene
B:C	Benefit : Cost
cfu	Colony forming units
CD	Critical difference
CRD	Completely randomized design
DNS	dinitrosalicylic
DPPH	2,2 diphenyl -1- picrylhydrazyl
et al.	And others
FAO	Food and Agricultural Organization
FFA	Free fatty acid
Fig.	Figure
g	gram
h	hours
IC_{50}	Inhibitory concentration 50
i.e.	that is
ISI	Indian Standards Institution
IV	Iodine value
KAU	Kerala Agricultural University
kg	Kilogram
KOH	potassium hydroxide
mg	milligram
min.	minutes
ml	millilitre
N	Normality
nm	nanometre
No.	number
ppm	parts per million
PV	Peroxide value
rpm	Revolutions per minute
s	Seconds

SE	Standard Error
SV	Saponification value
VCO	Virgin coconut oil
viz.	namely
WCT	West Cost Tall

INTRODUCTION

1. INTRODUCTION

Virgin coconut oil is considered unique in the sense that it is the only oil with multi-functional uses. Introduced onto the world market at the end of the 20th century, virgin coconut oil is one of the highest value products derived from the fresh coconut. Unlike refined, bleached and deodourized coconut oil which is tailor-made for cooking purposes, virgin coconut oil is marketed lately as functional oil. It is the only oil which one can eat as a food supplement or functional food, or neutraceutical, for cooking, apply to the hair and skin as a moisturiser and conditioner, and use as a major ingredient in skin care products or as carrier oil in aromatherapy and massage oils (Bawalan, 2011). The present fast developing and high value niche market for virgin coconut oil thus offers a good prospect for the improvement of the income of coconut farmers.

Virgin coconut oil in Kerala is traditionally prepared by boiling technique. The high temperature experienced in heating succumbs the oil to ester hydrolysis leading to a higher free fatty acid content. In addition, the traditional method utilizes higher fuel for removing the water content. Apart from the traditional boiling method, virgin coconut oil production can also be made by adopting other alternative methods like fermentation technique and centrifugation method. The virgin coconut oil produced by fermentation method is of high quality since no chemical or heat treatment is involved, water clear and is reported to have lower free fatty acid content thus fetching premium price in the market. The technique if not hygienically practiced can cause contamination. Hence to avoid unhygienic conditions, induced fermentation need to be explored and standardized which can prevent natural contamination. The virgin coconut oil produced by the fresh-dry centrifuge process has a very intense, fresh coconut aroma, viscous, greasy on the skin and is suitable as functional food. But the centrifuge technique requires high energy input for the production of virgin coconut oil. For the adoption of a suitable technology,

standardization of each technique is required and the quality, shelf life and feasibility of virgin coconut oil produced by different techniques need to be investigated. Virgin coconut oil can be easily produced at home or at cottage industry level or small scale level using any of these methods. The technology for virgin coconut oil production will thus provide an opportunity for coconut farmers to improve their income, an alternative to low value copra production.

The investigation entitled “Cost effective technology for home scale and small scale production of virgin coconut oil” was initiated at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani with the objective to standardize the cost effective technique for home scale and small scale production of virgin coconut oil through fermentation, induced fermentation and centrifugation and to compare the technology developed with the traditional boiling method.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Coconut, the "tree of heaven" is grown in the world over an area of 12.26 million hectares producing 64327 million nuts. In production and productivity, India has the number one status, with a production of 16943 million nuts (26.34 per cent) (CDB, 2015).

The major coconut products traded in the world market are coconut oil, desiccated coconut, coconut milk, coconut cream, coconut milk powder, shell charcoal, activated carbon, coir and coir products, virgin coconut oil, coconut flour, oleo chemicals *etc*. (Arancon, 2010).

Virgin coconut oil is one of the traditional products of coconut made in the households of Kerala from time immemorial. It had been used for culinary purposes as well as therapeutic uses and was described in ancient books of Ayurveda as "Urukkuvelichenna" since it was made by heating coconut milk. The present fast developing and high value niche market for virgin coconut oil offers a good prospect for the improvement of the income of coconut farmers. It is expected that virgin coconut oil will experience a dramatic growth in the market (Marina *et al*., 2009).

Virgin coconut oil (VCO) is obtained from fresh and mature kernel (12 months old from pollination) of the coconut (*Cocos nucifera L.*) by mechanical or natural means with or without the application of heat, which does not lead to alteration of the nature of the oil. Virgin coconut oil has not undergone chemical refining, bleaching or deodourizing. It can be consumed in its natural state without the need for further processing. Virgin coconut oil is colourless, free of sediment with natural fresh coconut scent. It is free from rancid odour or taste (APCC, 2003).

This present chapter reviews the literature on different methods of production of virgin coconut oil, their physical and chemical characteristics as well as the quality aspects of virgin coconut oil on storage.

2.1. FERMENTATION METHOD

According to Seow and Gwee (1997) coconut milk was the natural oil in water emulsion extracted from the endosperm of mature coconut. Divina (2002) observed that if properly diluted coconut milk was allowed to stand under favourable conditions for several hours, the oil naturally separates from the water and protein that binds them together as coconut milk emulsion. It is believed that natural enzymes in coconut may be acting as the fermentation medium. In the natural fermentation process extracted milk from wet coconut was allowed for microbial fermentation. This technology required very little investment, modest labour and low energy inputs.

McGlone *et al*. (1986) observed that freshly extracted coconut milk was a stable emulsion, which required extra energy to destabilize this emulsion. Some of the proteins present in the aqueous phase of the coconut milk interact with fat globules and act as emulsifier by surrounding its surface (Peamprasart and Chiewchan, 2006). It was naturally stabilized by coconut proteins such as globulins and albumins as well as phospholipids (Tangsuphoom and Coupland, 2008).

Bawalan and Chapman (2006) reported that virgin coconut oil can be easily produced at home by fermentation method, using a manual coconut grater and kitchen utensils. This process was very sensitive to the maturity and the freshness of the coconuts. Fully mature coconuts should be processed within three days from the time of harvesting to ensure that the oil separates naturally from the coconut milk after 16 hours.

Fabian *et al.* (2007) proposed that the natural fermentation method was a familiar process for production of virgin coconut oil in house hold level and also

in industrial scale. Virgin coconut oil, which was extracted by a wet process directly from coconut milk under controlled temperature, had more beneficial effects than copra oil since it retained most of its beneficial components (Marina *et al*., 2009). Virgin coconut oil was made from fresh coconuts, not copra. Since high temperatures and chemicals solvent were not used, the oil retained its naturally occurring phyto - chemicals which produced a distinctive coconut taste and smell. The oil was pure white when the oil was solidified or crystal clear like water when liquified (Rini *et al*., 2009).

In the traditional natural fermentation method, settling and subsequent fermentation of coconut milk lasted for 36 to 48 hours (Bawalan and Chapman, 2006). Bawalan (2011) reported that when proper operating conditions and sanitary precautions were strictly followed, five distinct layers could be seen in the fermenting container after 16 hours. The bottom layer was a gummy material. The next layer up was the watery portion which was actually fermented skim milk. Above the layer of skim milk there was a solid layer composed of spent fermented curd and above this, was the separated oil for recovery as virgin coconut oil and at the top there was another layer of fermented curd. The fermented curd, especially the topmost layer, contained a lot of oil. Premium grade virgin coconut oil could be harvested when the colour of this curd was light cream. It should not be allowed to turn brown prior to recovery.

A study was carried out by Arlee *et al*. (2013) to compare the differences in chemical components and antioxidant related substances in virgin coconut oil from coconut hybrids and their parents. In this study they produced virgin coconut oil from coconut milk, using a 1:1 ratio of coconut meat and water. They used the time period of 16 to 24 hour for the fermentation of coconut milk. In natural fermentation generally there were 3 groups of cultures; lactic acid bacteria, yeast and mold and they played a role of hydrolysis and breaking coconut milk emulsion According to Satheesh and Prasad (2014) in natural fermentation process of virgin coconut oil production, normal flora of microorganisms

fermented the milk and separated the coconut oil on the top portion within 24 - 48 hours.

2.1.1. Standardisation of drying temperature

Bawalan and Chapman (2006) recommended different drying methods after extraction of virgin coconut oil. They were dried in a double boiler and simmer for about fifteen minutes or incubated or air heated the open oil filled container at 50^0C for 12 hours until the oil had turned water clear. Bawalan (2011) was of the opinion that the presence of water in virgin coconut oil would cause it to go rancid and shorten its shelf life. Hence, entrained moisture in the oil should be reduced to as low a level as possible (not more than 0.1 per cent). Arlee *et al*. (2013) carried out drying in fermented oil at a lower temperature of 65°C.

2.1.1.1. Moisture percentage

Moisture content is an important character 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 (Mansor *et al*., 2012). Bawalan and Chapman (2006) reported that the moisture content of oil recovered from modified natural fermentation method was 0.12 per cent and below. At low moisture levels, that was at less than 0.06 per cent, virgin coconut oil was stable to microbial decomposition (Dayrit *et al*., 2011).

Moisture content of virgin coconut oil recovered by adding microbial inoculum (*L.bulgaricus*) as enzymatic starter was recorded as 0.3 per cent (Rini *et al*., 2009). Bawalan and Chapman (2006) reported that the moisture content of oil recovered from centrifuge method was 0.1 per cent. Moisture content of virgin coconut oil from centrifugation method varied from 0.88 to 1.03 percent as reported by Wong and Hartina (2014).

Vermont *et al*. (2005) observed the moisture percentage of laboratory produced virgin coconut oil as 0.06 to 0.12 per cent. The moisture content of the virgin coconut oil was more compared to refined, bleached and deodourized

coconut oil (Dayrit *et al*., 2007). Manaf *et al*. (2007) observed that the moisture content of the oil was one of the parameters which affected the shelf life. The higher the moisture content, it adversely influenced the oxidation process and thus promoted rancidity. Osawa *et al*. (2007) also observed that high moisture content assisted in hydrolysis process and observed that by keeping the moisture content as low, could increase the shelf life by preventing oxidation and rancidity processes. Satheesh and Prasad (2012) compared the quality parameters of virgin coconut oil produced from natural fermentation method and induced fermentation method. The moisture percentage of oil from natural fermentation method was 0.52 per cent and the oil from induced fermentation method was 0.56 per cent.

2.1.1.2. Free fatty acid content

Hydrolytic rancidity in coconut oil had been attributed to the presence of free fatty acids (Fernandez, 1988). A high acid value may indicate a higher tendency to become rancid (Karim, 1997). According to Shahidi and Wasundara (1998) free fatty acid value might be an important measure of rancidity of foods. Free fatty acids may be formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture.

Virgin coconut oil produced through the fermentation method would have high free fatty acid content due to the action of lipolytic enzymes, which was enhanced by the addition of water (Lalas and Tsaknis, 2002). Madhavan *et al*. (2005) reported that the acid value of the oil extracted by fermentation method as 0.263 - 0.434 mg KOH/g oil. Bawalan and Chapman (2006) reported that the free fatty acid value of oil recovered from modified natural fermentation method was 0.1 per cent.

Elizebath *et al*. (2011) observed that the oil produced by fermentation method were found to have higher levels of acetic acid and free fatty acids compared to virgin coconut oil produced using the centrifuge and expeller methods. They conducted a study to analyse the volatile organic compounds in virgin coconut oil and their sensory attributes and stated that samples produced by

fermentation were found to have higher levels of free fatty acid compared to virgin coconut oil produced using the centrifuge and expeller methods.

Free fatty acid value of virgin coconut oil recovered by adding microbial inoculum (*L. bulgaricus*) as enzymatic starter was recorded as 0.22 per cent (Rini *et al*., 2009). Mansor *et al*. (2012) conducted a study to compare the physiochemical properties of different processing methods. In his study, the free fatty acid value of virgin coconut oil extracted from fermentation method recorded the lowest free fatty acid value 0.29 ± 0.02 mg KOH/ g fats.

Vermont *et al*. (2005) reported the free fatty acid value of laboratory produced virgin coconut oil as 0.09 to 0.18 per cent lauric acid. Dayrit *et al*. (2007) observed the average free fatty acid value of virgin coconut oil samples as 0.131 per cent lauric acid. The free fatty acid values for virgin coconut oil samples ranged from 0.15 to 0.25 (Marina *et al*., 2009). A study was carried out by Arlee *et al*. (2013) to compare the differences in chemical components and antioxidant related substances in virgin coconut oil from coconut hybrids and their parents extracted by cold pressing and fermentation methods. In this study, the acid value of the virgin coconut oil after different method of processing ranged from 0.06 to 0.63 mg KOH per gram oil.

Srivastava *et al*. (2013) reported that both cold extracted virgin coconut oil and hot extracted virgin coconut oil had low free fatty acid (0.05 and 0.04 per cent lauric acid) content. During storage, free fatty acid content was found to increase in all the packaging system. Free fatty acid value increase was more pronounced in cold extracted virgin coconut oil samples as compared to hot extracted virgin coconut oil samples. The rate of hydrolysis reaction was found to be slightly higher in samples stored at 37°C than the ones stored at room temperatures.

2.1.1.3. Total Phenolic Content

Phenolic compounds plays an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), besides contributing towards the colour and sensory characteristics of fruits and vegetables (Alasalvar *et al*., 2001). The beneficial effects derived from phenolic compounds had been attributed to their antioxidant activity (Heim *et al*., 2002).

Dia *et al*. (2005) determined the total phenolic content in virgin coconut oil produced from different methods. The results revealed that virgin coconut oil contained higher total phenolic content compared to refined coconut oil. The total phenolic content in coconut oil produced by traditional and commercial methods (dry processing) were also compared by Seneviratne and Dissanayake (2008). The result indicated that the total phenolic content of traditional coconut oil was nearly seven times higher than that of commercial coconut oil.

Marina *et al*. (2009) conducted a study on commercial virgin coconut oil in Malaysian and Indonesian markets and confirmed that total phenolic contents of virgin coconut oil samples (7.78–29.18 mg GAE/100 g oil) were significantly higher than refined, bleached and deodourized coconut oil (6.14 mg GAE/100 g oil). Virgin coconut oil was as good as refined, bleached and deodourized coconut oil in chemical properties with the added benefit of being higher in phenolic content. It suggested that the refined bleached and deodourized process being applied through dry method had considerably destroyed some of the phenolic compounds in the coconut oil.

The total phenolic content of the laboratory produced virgin coconut oil ranged from 22.88 to 91.90 mg catechin equivalent per kg oil while that of the commercial virgin coconut oil was 35.26 to 49.07 mg catechin per kg oil (Vermont *et al*., 2005). In addition to the method of extraction, the components of the endosperm may also play an important role in determining the final phenol content of coconut oil (Kapila *et al*., 2009). Marina *et al*. (2009) reported the total phenolic value of virgin coconut oil samples. It ranged from 7.78 mg GAE/100 g

oil to 29.18 mg GAE/100 g oil. A study was carried out by Arlee *et al*. (2013) to compare the differences in chemical components and antioxidant related substances in virgin coconut oil from coconut hybrids and their parents extracted by cold pressing and fermentation methods. Total phenolic contents of the virgin coconut oil were 48.17 to 57.89 mg GAE/100 g oil. The results indicated that virgin coconut oil extracted by the cold pressing method was richer in phenolic substances compared with the fermentation method.

According to Nazck and Shahidi (2004), Follin-Ciocalteau reagent was not specific and could detect all phenolic groups found in the samples including those found in the extractable proteins. This method could cause interference of reducing substances such as ascorbic acid as well. According to Hodzic *et al.* (2009) the amount of total phenolic content affected the antioxidant capacity.

2.1.1.4. Oil recovery

The quality of virgin coconut oil produced by fermentation of coconut milk from freshly harvested coconuts was reported by Madhavan *et al*. (2005). The oil recovery from fermentation method was about 28-35 per cent of the volume of coconut milk. Bawalan and Chapman (2006) reported that by modified natural fermentation method about 19.8 Kg oil was recovered from 100 Kg of fresh grated meat. According to Satheesh and Prasad (2012) the oil recovery from natural fermentation method was 25.68 ± 0.963 per cent.

Carmen *et al.* (1970) proposed that *Lactobacillus plantarum* enhanced the yield of coconut oil in wet process than other *Lactobacillus* species. CheMan *et al*. (1996) discovered 74 per cent oil yield from one per cent enzyme mixture of cellulase, α-amylase, polygalacturonase and protease. Christensen (1989) also reported a very significant oil yield of more than 90 per cent by the use of galacto mannase in combination with a polysaccharide enzyme. A maximum virgin coconut oil yield of 27.2 per cent was achieved by adding 5.0 per cent microbial inoculum of *Lactobacillus bulgaricus* enzymatic starter (Rini *et al*., 2009).

Satheesh and Prasad (2012) conducted a study and reported that the yield of virgin coconut oil by using *Lactobacillus plantarum* was 28.47±1.070 per cent.

Wong (2010) reported that the highest yield of virgin coconut oil obtained by centrifugation method at 12000 rpm and 105 minutes was 37.3 per cent. For the combination of microwave and centrifugation method, the highest yield of 46.88 per cent virgin coconut oil was obtained at 720 watt of microwave power at 12000 rpm and 105 minutes. Results showed that the combination of microwave and centrifugation method gave a better yield of virgin coconut oil. The yield of virgin coconut oil by centrifugation was reported by Wong and Hartina (2014) as 13.53 per cent at 12000 rpm, at 120 minutes. The highest yield of virgin coconut oil was 13.80 per cent at centrifugation temperature of 40°C.

2.1.1.5. Sensory parameters

2.1.1.5.1. Colour

Colour of oil reflects the degree of refining and is an important criterion for its intended use in food formulation (Nasir *et al*., 2009). Villarino *et al*. (2007) observed that fermented samples had the highest colour scores among the virgin coconut oil samples. Soeka *et al*. (2008) stated that the traditional coconut oils were considered to be low quality products which was indicated by high moisture and free fatty acid content. It was therefore easily to rancid and turn to be brown and exhibit relatively short life time by sensory test.

Descriptive sensory analysis was conducted to describe and differentiate Philippine virgin coconut oil and refined, bleached and deodourized coconut oil samples. Evaluation of the samples revealed that refined, bleached and deodourized oil significantly differed in colour having a distinct yellow colour while all virgin coconut oil samples were almost colourless. Ratings on colour demonstrated that the application of heat affected the colour of the samples. Refined, bleached and deodourized oil underwent severe mechanical, chemical and heat treatments. Fermented samples received the highest colour scores among the virgin coconut oil samples (Villarino *et al*., 2007).

2.1.1.5.2. Odour

According to Villarino *et al*. (2007) sensory analysis showed that virgin coconut oil produced by fermentation (with and without heat) could be distinguished from those produced using the expeller and centrifuge methods due to their higher acid and rancid aromas. The refined, bleached and deodourized oil sample had no perceptible aroma. The virgin coconut oil samples were described to have an acid, cocojam, latik, nutty and rancid aromas which differed among samples. The differences in the aroma ratings among virgin coconut oil samples might be attributed to the processes applied. Centrifugation with heat had the lowest acid aroma rating amongst all virgin coconut oil samples while fermentation and fermentation with heat samples were described to have slightly perceptible acid aroma. The acid aroma might be attributed to the acetic acid produced during the fermentation process. Another possible explanation of the rancid aroma in the non-heated virgin coconut oil sample was that the microorganisms present in the sample degrade the oil to methyl ketones. Methyl ketones provided undesirable odours noted as perfume rancidity. The presence of moisture aggravated the release of free C_8–C_{12} fatty acids and their partial degradation to methyl ketones.

Elizebath *et al*. (2011) conducted a study to analyse the volatile organic compounds in virgin coconut oil and their sensory attributes. Five descriptors were used for the aroma attributes of virgin coconut oil: acid, cocojam, latik, nutty, and rancid aromas. The mean descriptive ratings of aroma attributes of commercial virgin coconut oil samples indicated that samples from centrifuge method tended to give lower acid and rancid aromas.

2.1.1.5.3. Taste

Virgin coconut oil produced by fermentation contained higher levels of acetic acid and octanoic acid (Dayrit *et al*., 2011). The flavour of the oil is key property, which is subjective to temperature, moisture, air in contact, light and presence of antioxidants (Nazir *et al*., 2009).

2.1.2. Influence of atmospheric temperature and relative humidity on fermentation

2.1.2.1. Atmospheric temperature

Bawalan and Chapman (2006) reported that the heart of the fermentation method was the preparation of coconut milk and the right temperature (35°–40°C) that will promote overnight separation of the milk into different layers of gum, water, proteinaceous curd and oil. Arlee *et al*. (2013) conducted a study to compare the differences in chemical components and antioxidant related substances in virgin coconut oil from coconut hybrids and their parents. They used the temperature range of 70-80°C for the fermentation of coconut milk. Srivastava *et al*. (2013) stated that in natural fermentation process the favourable atmospheric temperature needed was 35 - 40˚C. It helped to separate the oil from the water and the protein.

Kumalaningsih and Masdiana (2012) analysed the fermented coconut milk for numbers and types of Lactic Acid Bacteria (LAB) at duration of 0, 6, 12, 18 and 24 hours and incubated at 25°C, 30°C and 40°C. The isolates were then characterised based on morphology and biochemical characteristics, followed by proteases and amylase enzymes activities assayed. The results indicated that the bacteria could grow well at 25°C, 30°C and 40°C. The best temperature of growing was 30°C. According to Satheesh and Prasad (2013) for *Lactobacillus* species, the optimum fermentation temperature needed was 45 ± 1°C.

2.1.2.2. Relative Humidity

According to Srivastava *et al*. (2013) in the natural fermentation process, the oil got separated from the water and protein at 75 per cent relative humidity.

2.2. INDUCED FERMENTATION METHOD

According to Satheesh and Prasad (2012) natural fermentation was one of the commercial methods to produce virgin coconut oil, where the natural

microorganisms were playing a major role. In such processes, contamination was one of the major problem. To overcome this, induced fermentation could be done where certain species of probiotic microorganisms were used. Microbial starter or enzymes which have proteolytic, amylolytic and lipolytic capacities were required to hydrolyse protein, carbohydrate and lipid components contained in the coconut kernel.

2.2.1. Isolation and screening of microorganisms

According to Carmen *et al.* (1970) the optimum dilution range for rapid fermentation of coconut milk and separation of the oil and protein was found to be 1:1 to 1:2 (weight /volume) coconut meat per water. Pelczar and Chan (1986) described the extraction process of coconut oil *via* fermentation or enzymatic system involved microbial cell and enzymes those could solve the emulsion; however, their activities would be influenced by some conditions of substrate, enzyme, pH, temperature, and incubation period.

Basically, the purpose of the fermentation or enzymatic processes was to make the coconut emulsion into unstable condition and therefore separate into oil phase on upper layer and carbohydrate, protein and water phase on below layer (Rahayu *et al.*, 2008). Soeka *et al*. (2008) reported that the fermentation of coconut cream occurred when the enzymatic starter had been employed for processing. Crude coconut oil was formed due to a phenomenon of protein digestion that played a role to stabilize emulsion of the coconut cream into a soluble material. The enzymatic starter with high capacity of amylolytic and proteolytic activity could hydrolyze carbohydrate and protein which contained in the coconut cream as its substrate into soluble sugar and amino acid and peptide.

Rini *et al*. (2009) conducted a study and observed that the strain of *Lactobacillus bulgaricus* could effectively extract the virgin coconut oil higher than the other microbial strains like *Saccharomyces cerevisiae*, *Candida rugosa* and *Aspergillus oryzae* when it was employed into the coconut cream under the

enzymatic fermentation condition at pH 5.0, 45ºC and 5 per cent starter concentration.

Mansor *et al*. (2012) added baker's yeast (*Saccharomyces cerevisiae*) as an inoculum for the fermentation process. Fresh coconut milk was added with distilled water with 1:1 ratio. In each one litre of the mixture, two gram of Baker's yeast was added. Mixture was made homogenous by mixing it rigorously. The mixture was then left to stand for 36 hours at room temperature. As the layers of oil and water became separated, the upper oil layer was simply decanted. It resulted in a superior quality virgin coconut oil with low saponification value, free fatty acid, moisture content and acceptable range of brightness and yellowness.

According to Kumalaningsih and Masdiana (2012) lactic acid bacteria had the proteolysis and amylolytic enzyme activity and indicated that lactic acid bacteria had the potential to be used as pure culture for the production of virgin coconut oil of high quality oil.

A study was carried out by Satheesh and Prasad (2013) in computer controlled bioreactor by using *Lactobacillus fermentum* NDRI-141, *Lactobacillus plantarum* NDRI-184 and *Lactobacillus acidophilus* NDRI-II individually; by studying the effect of different parameters *viz*., temperature, pH, inoculum concentration, fermentation end time, oxygen requirements and were optimized and compared. *Lactobacillus plantarum* showed highest yield of virgin coconut oil than *Lactobacillus fermentum* and *Lactobacillus acidophilus*. Thus it was concluded that the utilisation of *Lactobacillus plantarum* was the choice of organism for induced fermentative production of virgin coconut oil.

2.2.1.1. Protease and amylase activity

According to Kumalaningsih and Masdiana (2012) lactic acid bacteria had highest proteolytic and amylolytic enzyme activity which indicated that lactic acid bacteria had the potential to be used as pure culture for the production of virgin coconut oil of high quality oil.

Rini *et al*. (2009) described that the strain of *Lactobacillus bulgaricus which* could effectively extract the virgin coconut oil, showed the highest activity for amylolytic and proteolytic enzymes by formation of colony surrounding clear zones. Diameter of clear zone was ± 2.0 cm. It illustrated that the strain of *Lactobacillus bulgaricus* was capable of producing amylase and protease those were availably important to digest protein and carbohydrate which contained in coconut cream as its substrate.

2.2.1.2. Count of Lactobacillus

Kumalaningsih and Masdiana (2012) reported that the population of lactic acid bacteria increased during the fermentation period of 18 hour reached up to 19 x 10^9 cfu /g and at 24 hour decreased to 15 x 10^9 cfu /g. According to Satheesh and Prasad (2013) for *Lactobacillus* species, the optimum fermentation end time was 48 hours. According to Satheesh and Prasad (2014) the optimum fermentation end time by using *Lactobacillus plantarum* was 48 hour.

2.3. CENTRIFUGATION METHOD

Centrifugation is indeed a potential method of producing virgin coconut oil which can be applied at industrial level. The high performance of centrifugation speed makes it ideal in producing high yield of virgin coconut oil and it was one of the alternative ways to produce virgin coconut oil instead of other traditional methods such as fermentation. Different combinations of centrifugation speed and time resulted in different oil yields with small variation in the quality of virgin coconut oil (Nour *et al.,* 2009).

Chilling was a wet milling method which had been widely used in studies to break the coconut milk emulsion in order to extract virgin coconut oil (Nevin and Rajamohan, 2004; Raghavendra and Raghavarao, 2010). It was a relatively simple technology without involving any substances other than coconut milk and shorter time was required compared to natural fermentation method. Furthermore, chilling had been proven to be effective in conserving the phenolic compounds in

virgin coconut oil (Nevin and Rajamohan, 2004; Marina *et al*., 2009). The commonly reported steps in chilling method involved subjecting the coconut milk to chilling temperature for certain hours, followed by the separation of cream phase where the cream was then heated mildly and centrifuged to separate the virgin coconut oil (Seneviratne *et al*., 2009; Raghavendra and Raghavarao, 2010).

Gunetileke and Laurentius, (1974) conducted a study on chilling temperature. Coconut milk extracted from fresh coconuts, was centrifuged to obtain cream and skim milk. The whole mass of cream was chilled to 17°C or below. At 17°C, crystallization of the oil phase was observed under the microscope. On warming to 25°C the emulsion broke with separation of oil and protein. This process differed from similar processes in that no enzymes were used and the temperature of the whole mass of cream had to be lowered only to 17°C. It was established that 17°C was the critical temperature for subsequent phase separation.

The close contact among large droplets (higher interaction time) and applied force during centrifugation led to destabilization of emulsion, resulting in the phase separation and formation of oil and aqueous layers (Chiewchan and Tansakul., 2004). Christine (2012) studied by introducing different combinations of centrifugation speed and time to the coconut milk emulsion after it had been subjected to different chilling temperatures. Mansor *et al*. (2012) conducted a study in which coconut milk was centrifuged at 3600 G for 10 minutes and the upper layer of cream was removed for chilling. Chilling was done at 5°C for 24 h and then the chilled cream was thawed slowly in water bath at 50°C to extract the oil.

2.3.1. Revolutions per minute and time required

The potential of using centrifugation in breaking the coconut milk emulsion to produce virgin coconut oil had been reported by Nour *et al.* (2009). He used different centrifugation speeds ranging from 6000 to 12000 rpm. The potential of the centrifugation method and the combination of microwave and

centrifugation method in demulsification of coconut milk emulsion was investigated by Wong (2010). For the centrifugation method, the centrifuge speeds used were varied from 6000 to 12000 rpm. Wong and Hartina (2014) also used various centrifugation speed (2000, 4000, 6000, 8000, 10000, and 12000 rpm) and temperature intervals (20°C, 25°C, 30°C, 35°C, 40°C) for the extraction of virgin coconut oil. The highest yield of virgin coconut oil was recorded at 12000 rpm and at centrifugation temperature of 40°C.

Nour *et al.* (2009) and Wong (2010) used different time periods (30-105 minutes) along with different centrifuge speeds for the extraction of virgin coconut oil. For the combination of microwave and centrifugation method, Wong (2010) used the centrifugation times varied from 60 to 105 minutes. The highest yield of virgin coconut oil by centrifugation method was recorded at 120 minutes (Wong and Hartina, 2014).

2.4. COMPARISON OF VIRGIN COCONUT OIL PRODUCED BY FERMENTATION, INDUCED FERMENTATION AND CENTRIFUGATION WITH TRADITIONAL BOILING METHOD

Carmen *et al*. (1970) reported that in the traditional method, the fresh coconut was grated and pressed to yield coconut milk. The fat rich fraction separated as a cream, and the cream was then boiled until the moisture was removed and the oil separated. The residue in this case was a very pleasant flavoured, toasted flake retaining relatively large amounts of oil on the surface.

2.4.1. Quality parameters

The quality standards for virgin coconut oil as put forward by Asian and Pacific Coconut Community Standards is as follows (APCC, 2003).

Table.1. Essential composition and quality factors of virgin coconut oil

Parameter	Desirable Amount
Moisture (per cent)	Max 0.1
Matters Volatile at 120° C (per cent)	Max 0.2
Free Fatty Acid (per cent)	Max 0.2
Peroxide Value meq/kg	Max 3
Relative density	0.915 – 0.920
Refractive index at 40° C	1.4480 – 1.4492
Insoluble impurities per cent by mass	Max 0.05
Saponification Value	250 – 260 mg
Iodine Value	4.1 -11
Unsaponifiable matter (per cent) by	0.2 - 0.5
Specific gravity at 30 deg./30 deg. C	0.915 – 0.920
Polenske Value, min	13
Total Plate Count	< 0.5
Colour	Water clean
Odour and Taste	Natural fresh coconut scent, free of
Food Additives	None permitted

2.4.1.1. Refractive index

The refractive indices of oils related to the degree of unsaturation in a linear way (Rudan and Klofutar, 1999). Madhavan *et al*. (2005) reported that the refractive index at 40°C of the oil extracted by natural fermentation method ranged from 1.4483 to 1.4491 and indicated good quality. Kamariah *et al*. (2008) conducted a study to know the physico-chemical and quality characteristics of virgin coconut oil. Refractive index of oil increased with increase in the number

of double bonds. Gopalakrishna *et al.* (2010) reported that the Indian specification for the refractive index of refined coconut oil produced by expressed method and solvent extraction was 1.4480-1.4490 .

Ten virgin coconut oil samples from Malaysian market were collected and analysed and observed that the refractive index at 40°C of the virgin coconut oil ranged from 1.4467 to 1.4472. According to Satheesh and Prasad (2012) refractive index at 40°C of the oil extracted by natural fermentation method was 1.4490 and the oil from induced fermentation method was 1.4483. Sashya and Coorey (2012) in a study reported that the refractive index of pale yellow and dark yellow coconut oil varied in the range of 1.498 −1.466 and the refractive index of white coconut oil varied in the range of 1.484 −1.466.

2.4.1.2. Specific gravity

Specific gravity at 30°C of the virgin coconut oil extracted by natural fermentation method was 0.918 and from induced fermentation method was 0.920 (Satheesh and Prasad, 2012).

2.4.1.3. Relative density

Kamariah *et al*. (2008) conducted a study to know the physico-chemical and quality characteristics of virgin coconut oil. Ten virgin coconut oil samples from Malaysian market were collected and analysed and observed that the relative density of the virgin coconut oil ranged from 0.9185 to 0.9194.

2.4.1.4. Colour

Colour is the first characteristic that distinguishes virgin coconut oil from any other type of plant derived oil (vegetable or oilseed). The colour of virgin coconut oil also indicated that it had been processed at the right temperature and with strict quality control in handling the fresh coconut. For the coconut oil to be categorised as virgin, its colour should be water clear (Bawalan, 2011). Sashya and Coorey (2012) observed that yellow coconut oil could result when virgin

coconut oil was heated to excessive temperatures where oil undergoes hydrogenation resulting in trans-fatty acids. When heated beyond smoke point of 180°C of coconut oil, it turned to dark yellow and had a strong flavour. In order to make coconut oil to be more stable and long lasting, manufacturers heat coconut oil excessively and subject to partially or fully hydrogenation which creates trans-fatty acids which are unhealthy for human consumption.

2.4.1.5. Iodine value

Dutta (1991) observed that higher the iodine number, the more will be the degree of unsaturation in the fats or oil. Iodine value, for instance, is still used as an ingredient specification in the edible oils industry (Kaylegian and Lindsay, 1995). Vermont *et al*. (2005) observed the iodine value of laboratory produced virgin coconut oil as 4.35 to 6.85 g I_2 per 100 g fats. Dayrit *et al*. (2007) reported that the iodine value of virgin coconut oil samples ranged from 5.64- 10.34g I_2 per 100 g fats. Kamariah *et al*. (2008) mentioned the iodine value of virgin coconut oil samples and recorded as 5.5 to 7.3percent. According to Marina *et al*. (2009) the iodine value of virgin coconut oil samples ranged from 4.47 to 8.55g I_2 per 100 g fats. Mansor *et al*. (2012) conducted a study to compare the physiochemical properties of virgin coconut oil extracted from different processing methods. In this study, the range of iodine value of all the samples were 4.13 – 4.33 g I_2 per 100 g fats. The lowest iodine value was obtained from the chilling and thawing method while the highest was from fermentation method.

2.4.1.6. Unsaponifiable matter

The unsaponifiable matter is defined as the substances in an oil which after saponification are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter in non-volatile at 100º C (mineral oil) which may be present (FAO, 1986). Unsaponifiable matter content of the virgin coconut oil extracted by natural

fermentation method was 0.38 per cent and from induced fermentation method was 0.4 per cent (Satheesh and Prasad, 2012). The unsaponifiable matter accounted for about one per cent of the oils and it was composed of squalene, a fraction formed by hydrocarbons and carotenoids and six classes of alcoholic compounds, such as 4 dimethylsterols, 4, 4 - dimethylsterols, isoprenoid alcohols, *n*-alkanols and tocols (Mozzon *et al*., 2015).

2.4.1.7. Polenske value

The polenske value is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil or fat. The Polenske value is the number of milliliters of 0.1 N aqueous alkali solution required to neutralise steam volatile water insoluble fatty acids distilled from 5 g of the oil /fat under the prescribed conditions (FSSAI, 2012). Polenske value of the virgin coconut oil extracted by natural fermentation method was 13.9 ± 0.6 and from induced fermentation method was 13.9 ± 0.3 minimum (Satheesh and Prasad, 2012).

2.4.1.8. Peroxide value

Off flavour resulting from peroxidation of unsaturated fatty acids was the major cause of spoilage of stored oils (Semwal and Arya, 1992). Madhavan *et al*. (2005) reported that the peroxide value of the oil extracted by fermentation method was 0.0005 - 0.0023 meq peroxide/kg oil. Dayrit *et al*. (2007) observed the peroxide value of the virgin coconut oil as1.86 meq peroxide/kg oil. The peroxide values of the virgin coconut oil ranged from 0.21 to 0.63 meq oxygen/kg oil (Marina *et al*., 2009).

Peroxide value is a good guide to judge about the quality of oil. The peroxide value of cold extracted virgin coconut oil and hot extracted virgin coconut oil was observed as 4.95 and 5.65 meq O_2/kg oil, respectively which increased as a function of storage time in all samples up to 12 months of storage (Srivastava *et al*., 2013).

According to Fritsch (1981), the determination of peroxide value was not suitable for the assessment of used frying oils. Peroxide value and oxidative stability of crude sesame oil significantly ($p \leq 0.05$) depend on moisture content of the seed, roasting duration and temperature (Akinoso *et al*., 2010).

2.4.1.9. Saponification value

The saponification value basically refers to the mean molecular mass of the fats and oils and have an inverse relationship with the chain length of the fatty acid in fats and oils. This means, the longer the average fatty acid chain length, the smaller the saponification value. Fats containing short chain fatty acids exhibit saponification value higher than those composed entirely of long chain fatty acids (Sonntag, 1982). Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fats or oil. The shorter the average chain length in the fatty acids, the higher is the saponification number (Jain, 1995). The saponification value is the number of milligram of potassium hydroxide required to saponify one gram of oil/fat (FSSAI, 2012).

Saponification value of virgin coconut oil by fermentation method was 253-280 mg KOH/g oil (Madhavan *et al*., 2005). Vermont *et al*. (2005) observed the saponification value of laboratory produced virgin coconut oil as 264 to 274 mg KOH per gram oil. Kamariah *et al*. (2008) mentioned the saponification value of virgin coconut oil samples and recorded as 258.8 to 263.7 mg KOH/g oil. Marina *et al*. (2009) reported that the saponification value of virgin coconut oil samples ranged from 250.07–258.26 mg KOH/g oil. According to the Codex standard, specification for saponification value of edible coconut oil should be between 248 and 265 mg KOH/g oil (AOAC, 1999).

2.4.1.10. Antioxidant properties

Numerous studies suggest that the consumption of foods containing dietary phenolics may significantly contribute to human health (Naczk and Shahidi, 2004). Beneficial effects resulting from phenolic antioxidants creates a

niche in finding of food worth of these phenolic compounds. Nevin and Rajamohan (2004) observed that virgin coconut oil has the capability to increase antioxidant enzymes and reduce lipid peroxidation.

The difference in antioxidant activity among virgin coconut oil samples could be due to the differences in processing methods used. Gazzani *et al*. (1998) stated that the antioxidant activity can also be affected by thermal treatment. Introduction of heat during production or extraction of virgin coconut oil could therefore decrease the antioxidant activity (Marina *et al*., 2009).

According to Moure *et al.* (2001), quality of natural extracts and antioxidant activities not only depend on storage time, geographic origin, harvesting date but also environment and technological factors as well. A study conducted to determine the effect of virgin coconut oil in comparison with copra oil and groundnut oil on both in vitro and in vivo lipid peroxidation and the levels of antioxidant enzymes in rats showed that virgin coconut oil administration increased the antioxidant enzymes and reduced the lipid peroxide content. Virgin coconut oil polyphenols were also capable of preventing in vitro lipid peroxidation than polyphenols from copra oil and groundnut oil. Study results showed that consumption of virgin coconut oil extracted from fresh coconut meat, with its high content of biologically active components was superior in antioxidant property than coconut oil extracted by dry process (Nevin and Rajamohan, 2005).

The antioxidant properties of virgin coconut oil produced through chilling and fermentation were investigated and compared with refined, bleached and deodourised coconut oil by Marina *et al*. (2009). Virgin coconut oil showed better antioxidant capacity than refined, bleached and deodourised coconut oil. The major phenolic acids detected were ferulic acid and p-coumaric acid. Very high correlations were found between the total phenolic content and scavenging activity and between the total phenolic content and reducing power. There was

also a high correlation between total phenolic acids and beta-carotene bleaching activity.

Kapila *et al*. (2009) compared the antioxidant activities of coconut oil extracted under hot and cold conditions. The coconut oil extracted under hot conditions (HECO) contained more phenolic substances than the coconut oil extracted under cold conditions (CECO). It was the common belief that virgin coconut oil extracted under cold conditions preserves several thermally unstable antioxidants and as a result, better beneficial qualities could be expected for virgin coconut oil. However, high temperatures used in the hot extraction of coconut oil favoured the incorporation of more thermally stable phenolic antioxidants into coconut oil. Therefore, the consumption of coconut oil extracted under hot conditions may result in the better improvement of antioxidant related health benefits compared with the consumption of coconut oil extracted under cold conditions.

2.4.1.10.1. DPPH radical scavenging activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale (Ferreira *et al.,* 2007). The virgin coconut oil produced through fermentation had the strongest scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl as reported by Marina *et al*. (2008).

Unlike laboratory generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Amarowicz *et al*., 2004). The free radical scavenging activity of virgin coconut oil samples ranged from the EC_{50} of 0.48 to 1.27 mg GAE/ml (Arlee *et al*., 2013).

Marina *et al.* (2009) also reported that fermented virgin coconut oil had a strong scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The phenolic extract of virgin coconut oil was able to scavenge 39.1 per cent DPPH radicals at greater than 500 μg/ml (Lim *et al*., 2014).

2.4.1.10.2. Total antioxidant activity

The antioxidant properties of phenolic substances vary significantly, depending on their functional groups (Evans *et al*., 1996). Therefore, when the antioxidant activity of a mixture of phenolic substances are evaluating, it is important to compare the effect of concentration as well as the quality of phenolic substances on the antioxidant activity. For virgin coconut oil, the phenolic substances were protocatechuic, vanillic, caffeic, syringic, ferulic and p-coumaric acids (Marina *et al*., 2008).

The antioxidant activity in virgin coconut oil was reported to be high in virgin coconut oil compared to refined coconut oil (Dia *et al*., 2005; Marina *et al*., 2009). Vermont *et al*. (2005) reported the antioxidant activity of laboratory produced virgin coconut oil as 47.4 to 78 per cent relative peroxidation. According to Marina *et al*. (2008) virgin coconut oil with the highest total phenolic content possessed the highest antioxidant activity. The virgin coconut oil produced through fermentation had the highest antioxidant activity based on beta-carotene-linoleate bleaching method. The antioxidant activity of virgin coconut oil samples ranged from 52 to 80 per cent (Marina *et al*., 2009).

2.4.1.10.3. Reducing power

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{2+}). The presence of reducers (*i.e*., antioxidants) causes the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form. According to Hodzic *et al.* (2009), ferric reducing antioxidant power assay had been used to determine antioxidant activity as it is simple and quick. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants.

Schafer and Buettner (2001) stated that FRAP assay could be used for assessment of antioxidant activity in plants materials as humans only absorb

limited amount of glutathione. Higher FRAP values gave higher antioxidant capacity because FRAP value was based on reducing ferric ion, where antioxidants were the reducing agent. However, some disadvantage was found in this method as FRAP assay did not react fast with some antioxidants such glutathione (Guo *et al*., 2003). Virgin coconut oil obtained through chilling method had the highest reducing power (Marina *et al*., 2008).

2.4.1.10.4. IC_{50} values

IC_{50} value means the effective concentration at which 50 per cent of DPPH radicals were scavenged. The free radical scavenging activity of virgin coconut oil samples ranged from the EC_{50} of 0.48 to 1.27 mg GAE/ml (Arlee *et al*., 2013).

2.4.2. Total plate count

Microbial contamination in virgin coconut oil products is due to the quality of production and not the type of process (Dayrit *et al*., 2007). Total aerobic plate count of 31± 3.1and 49 ± 2.8 cfu per 0.1mg was determined in virgin coconut oil by the natural and induced fermentation method (Satheesh and Prasad, 2012).

2.4.3. Shelf Life

There is a remarkable difference between coconuts and other seeds that are used to extract edible oils. Coconut endosperm contains a liquid portion, white coconut kernel and a thin brown outer skin of coconut kernel known as coconut testa.

The quality and shelf life of the packaged food are mainly determined by the barrier properties of the package against moisture, oxygen and interaction of food constituents with the packaging material (Sharma *et al.*, 1996). Packaging system, storage time and temperature all have significant effect on the stability of hot and cold extracted virgin coconut oil. The storage stability of virgin coconut oil was found to be one year in different flexible linear low density polyethylene

(LLDPE), low density polyethylene (LDPE), Metallised polyester (MET) and rigid packaging materials like polyethylene tetra phthalate (PET) bottle, high density polyethylene (HDPE) and amber high density polyethylene (AHDPE) at room temperature (15-35˚C) and 37°C (Srivastava *et al*., 2013).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation entitled “Cost effective technology for home scale and small scale production of virgin coconut oil” was undertaken at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during the period 2013-2015. The experiment was done with the objective of standardizing the cost effective technology for home scale and small scale production of virgin coconut oil through fermentation, induced fermentation and centrifugation and to compare the technology developed with the traditional boiling method. The materials used for virgin coconut oil production during the course of investigation and the method followed for the standardization of fermentation, induced fermentation, centrifugation and comparison of these methods with the traditional boiling method and the method followed for the quality analysis, sensory analysis, shelf life study, and antioxidant assay of different types of virgin coconut oil extraction are presented in this chapter.

The study was carried out as four experiments

1. Standardisation of fermentation method
2. Standardisation of induced fermentation
3. Standardisation of centrifugation method
4. Comparison of virgin coconut oil produced by fermentation, induced fermentation and centrifugation with traditional boiling method

3.1. STANDARDISATION OF FERMENTATION METHOD

3.1.1. Coconut milk

Mature coconuts of 12 months old were collected from WCT palms from Venniyoor located at Venganoor Panchayath, Thiruvananthapuram, dehusked,

shelled and grated. The grated coconut was mixed with coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and made into fine paste. The ground mass was then transferred to cheese cloth and pressed manually for extraction of coconut milk.

3.1.2. Preparation of coconut milk

1. Coconut milk extracted from grated coconut with coconut water in the ratio 1:1
2. Coconut milk extracted from grated coconut with water in the ratio 1:1
3. Coconut milk extracted from grated coconut with water in the ratio 1:2

The coconut milk extracted was kept for varying hours for fermentation to extract virgin coconut oil.

3.1.3. Standardisation of drying temperature

The coconut milk extracted from grated coconut with coconut water in the ratio 1:1and water in the ratio 1:1 and 1:2 during June- July was kept for fermentation at 24, 36 and 48 hours. The virgin coconut oil extracted from these treatments were subjected to drying in hot air oven at temperatures 40^0C, 45^0C and 50^0C. The best drying temperature was selected based on free fatty acid content, total phenol content and sensory parameters.

Treatments

1. T_1– Coconut gratings + coconut water (1:1) (CG+CW, 1:1) kept for 24 hours, VCO dried at 40°C
2. T_2– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 24 hours, VCO dried at 45°C
3. T_3 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 24 hours, VCO dried at 50°C

4. T_4 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours, VCO dried at 40°C
5. T_5 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours, VCO dried at 45°C
6. T_6 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours, VCO dried at 50°C
7. T_7 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 48 hours, VCO dried at 40°C
8. T_8 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 48 hours, VCO dried at 45°C
9. T_9 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 48 hours, VCO dried at 50°C
10. T_{10} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 24 hours, VCO dried at 40°C
11. T_{11} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 24 hours, VCO dried at 45°C
12. T_{12} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 24 hours, VCO dried at 50°C
13. T_{13} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 36 hours, VCO dried at 40°C
14. T_{14} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 36 hours, VCO dried at 45°C
15. T_{15} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 36 hours, VCO dried at 50°C
16. T_{16} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 48 hours, VCO dried at 40°C
17. T_{17} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 48 hours, VCO dried at 45°C

18. T_{18} – Coconut gratings + water (1:1) (CG+W 1:1) kept for 48 hours, VCO dried at 50°C
19. T_{19} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 24 hours, VCO dried at 40°C
20. T_{20} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 24 hours, VCO dried at 45°C
21. T_{21} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 24 hours, VCO dried at 50°C
22. T_{22} – Coconut gratings+ water (1:2) (CG+W 1:2) kept for 36 hours, VCO dried at 40°C
23. T_{23} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 36 hours, VCO dried at 45°C
24. T_{24} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 36 hours, VCO dried at 50°C
25. T_{25} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 48 hours, VCO dried at 40°C
26. T_{26} – Coconut gratings + water (1:2) (CG+W 1:2) kept for 48 hours, VCO dried at 45°C
27. T_{27} – Coconut gratings+ water (1:2) (CG+W 1:2) kept for 48 hours, VCO dried at 50°C

Treatments – 27

Replication – 3

Design of Experiment – Two factor CRD

The oil recovered after drying was analysed for free fatty acid content, total phenol content and sensory parameters like colour, odour and taste. The best drying

temperature was selected based on the above observations and was used as the temperature for drying for all the other experiments.

Treatments involved in fermentation method

1. T_1– Coconut gratings + coconut water (1:1) (CG+CW, 1:1) kept for 24 hours
2. T_2– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours
3. T_3 – Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 48 hours
4. T_4 – Coconut gratings + coconut water (1:1) (CG+W 1:1) kept for 24 hours
5. T_5 – Coconut gratings + coconut water (1:1) (CG+W 1:1) kept for 36 hours
6. T_6 – Coconut gratings + coconut water (1:1) (CG+W 1:1) kept for 48 hours
7. T_7 – Coconut gratings + coconut water (1:1) (CG+W 1:2) kept for 24 hours
8. T_8 – Coconut gratings + coconut water (1:1) (CG+W 1:2) kept for 36 hours
9. T_9 – Coconut gratings + coconut water (1:1) (CG+W 1:2) kept for 48 hours

Treatments – 9

Replication – 3

Design of Experiment –CRD

The oil recovered from this experiment was kept for further quality analysis.

The experiment on fermentation was repeated on March- April and November-December to standardise the atmospheric temperature and relative humidity and the virgin coconut oil was dried at the best temperature obtained from the experiment carried out during June- July.

3.2. STANDARDISATION OF INDUCED FERMENTATION

3.2.1. Coconut milk

The selection of coconut was similar to the method followed for fermentation. For the isolation of microorganism involved in induced fermentation, coconut milk was extracted from grated coconut with water in the ratio 1:1.

3.2.2. Isolation of microorganisms involved in fermentation of coconut milk

Coconut milk extracted from grated coconut with water in the ratio 1:1 was kept for fermentation for 24 and 36 hours. Microorganisms were isolated from coconut milk kept for fermentation at 24 and 36 hours by serial dilution and spread plate method.

Treatments

1. Isolation of microorganisms at 24 hours
2. Isolation of microorganisms at 36 hours

Treatments – 2

Replication- 10

Design – CRD

The microorganisms isolated at 24 and 36 hours were tested for clear zone formation and quantitatively for protease and amylase activity. Microorganisms having the highest protease and amylase activity were selected and maintained in slants. Mother culture of the selected microorganism was multiplied in broth. To study the multiplication efficiency of the best isolate, it was inoculated in sterilized coconut milk.

3.2.3. Sterilization of coconut milk

The coconut milk extracted with grated coconut and water in the ratio 1:1 was sterilized at 70°C for 30 minutes. One ml of mother culture of the best isolate was inoculated into the sterilized coconut milk and colony count was taken.

3.2.4. Induction of fermentation and comparison with fermentation

The selection of coconut was similar to the method followed for fermentation. Coconut milk was extracted with grated coconut and coconut water in the ratio 1:1, grated coconut and water in the ratio 1:1 and 1:2. One ml of mother culture of the best isolate was inoculated into 100ml of coconut milk and kept for varying hours *viz*., 18, 24 and 36 hours and the oil recovery of the induced fermentation (Group1) was compared with the fermentation (Group 2).

Treatments

1. T_1– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 18 hours
2. T_2– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 24 hours
3. T_3– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours
4. T_4– Coconut gratings + water (1:1) (CG+W 1:1) kept for 18 hours
5. T_5– Coconut gratings + water (1:1) (CG+W 1:1) kept for 24 hours
6. T_6– Coconut gratings + water (1:1) (CG+W 1:1) kept for 36 hours
7. T_7– Coconut gratings + water (1:2) (CG+W 1:2) kept for 18 hours
8. T_8– Coconut gratings + water (1:2) (CG+W 1:2) kept for 24 hours
9. T_9– Coconut gratings + water (1:2) (CG+W 1:2) kept for 36 hours

Groups- 2

Group 1- Induced fermentation

Group 2- Fermentation

Treatments - 18

Replication- 3

Design of the experiment – Two factor CRD

A comparison in oil recovery was made between induced fermentation and fermentation for each method of extraction of coconut milk and fermenting durations. The selection of coconut was similar to the method followed for fermentation. Coconut milk was extracted from grated coconut and coconut water in the ratio 1:1, grated coconut and water in the ratio 1:1 and 1:2. One ml of mother culture of the best isolate was inoculated into coconut milk and kept for varying hours *viz*., 18, 24 and 36 hours.

Treatments

1. T_1– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 18 hours
2. T_2– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 24 hours
3. T_3– Coconut gratings + coconut water (1:1) (CG+CW 1:1) kept for 36 hours
4. T_4– Coconut gratings + water (1:1) (CG+W 1:1) kept for 18 hours
5. T_5– Coconut gratings + water (1:1) (CG+W 1:1) kept for 24 hours
6. T_6– Coconut gratings + water (1:1) (CG+W 1:1) kept for 36 hours
7. T_7– Coconut gratings + water (1:2) (CG+W 1:2) kept for 18 hours
8. T_8– Coconut gratings + water (1:2) (CG+W 1:2) kept for 24 hours
9. T_9– Coconut gratings + water (1:2) (CG+W 1:2) kept for 36 hours

Treatments – 9

Replication – 3

Design of the experiment – CRD

The virgin coconut oil extracted from these treatments were subjected to drying in hot air oven at 50°C and kept for further quality analysis.

3.3. STANDARDISATION OF CENTRIFUGATION METHOD

3.3.1. Coconut milk

The selection of coconut was similar to the method followed for fermentation. Coconut milk was extracted with grated coconut and coconut water in the ratio 1:1, grated coconut and water in the ratio 1:1 and 1:2. The coconut milk was kept in refrigerator at 5-7°C for 10 hours. The refrigerated coconut milk was centrifuged at different revolutions per minute (rpm) and time and oil recovery was noted. The coconut milk was centrifuged at 6000, 8000, 10000 and 12000 rpm and time period of 5, 10, 15, 20, 25 and 30 minutes were tried. Based on the oil recovery, the experiment was further carried out using 8000, 10,000 and 12,000 rpm and 10, 15 and 20 minutes.

Treatments

1. T_1 (CG+CW 1:1 -8000rpm- 10 minutes) 5376 N
2. T_2 (CG+CW 1:1 -8000rpm- 15 minutes) 5376 N
3. T_3 (CG+CW 1:1 -8000rpm- 20 minutes) 5376 N
4. T_4 (CG+W 1:1- 8000rpm- 10 minutes) 5376 N
5. T_5 (CG+W 1:1- 8000rpm- 15 minutes) 5376 N
6. T_6 (CG+W 1:1- 8000rpm- 20 minutes) 5376 N
7. T_7 (CG+W 1:2- 8000rpm- 10 minutes) 5376 N
8. T_8 (CG+W 1:2- 8000rpm- 15 minutes) 5376 N
9. T_9 (CG+W 1:2- 8000rpm- 20 minutes) 5376 N
10. T_{10} (CG+CW 1:1 - 10000rpm- 10 minutes) 8400 N
11. T_{11} (CG+CW 1:1- 10000rpm- 15 minutes) 8400 N
12. T_{12} (CG+CW 1:1- 10000rpm- 20 minutes) 8400 N
13. T_{13} (CG+W 1:1 - 10000rpm- 10 minutes) 8400 N
14. T_{14} (CG+W 1:1 - 10000rpm- 15 minutes) 8400 N
15. T_{15} (CG+W 1:1 - 10000rpm- 20 minutes) 8400 N
16. T_{16} (CG+W 1:2 - 10000rpm- 10 minutes) 8400 N
17. T_{17} (CG+W 1:2 - 10000rpm- 15 minutes) 8400 N

18. T_{18} (CG+W 1:2 - 10000rpm- 20 minutes) 8400 N
19. T_{19} (CG+CW1:1 - 12000rpm- 10 minutes) 12096 N
20. T_{20} (CG+CW1:1 - 12000rpm- 15 minutes) 12096 N
21. T_{21} (CG+CW1:1 - 12000rpm- 20 minutes) 12096 N
22. T_{22} (CG+W1:1 - 12000rpm- 10 minutes) 12096 N
23. T_{23} (CG+W1:1 - 12000rpm- 15 minutes) 12096 N
24. T_{24} (CG+W1:1 - 12000rpm- 20 minutes) 12096 N
25. T_{25} (CG+W1:2 - 12000rpm- 10 minutes) 12096 N
26. T_{26} (CG+W1:2 - 12000rpm- 15 minutes) 12096 N
27. T_{27} (CM+W1:2 - 12000rpm- 20 minutes) 12096 N

Treatments – 27

Replication – 3

Design of the experiment – CRD

Based on the oil recovery, the rpm and time period were standardized. Thus for further studies on centrifugation, coconut milk was extracted with grated coconut and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and centrifuged at selected rpm and time period.

Treatments

T_1– Coconut milk extracted with coconut water (1:1)

T_2– Coconut milk extracted with water (1:1)

T_3– Coconut milk extracted with water (1:2)

Treatments - 3

Replication- 7

Design of the experiment – CRD

The oil recovered was subjected to drying at 50°C and kept for quality analysis.

3.4. COMPARISON OF VIRGIN COCONUT OIL PRODUCED BY FERMENTATION, INDUCED FERMENTATION AND CENTRIFUGATION WITH TRADITIONAL BOILING METHOD

The virgin coconut oil produced by fermentation, induced fermentation and centrifugation were compared with the traditional boiling method.

Treatments

1. T_1- VCO by fermentation + standardized temperature in hot air oven
2. T_2- VCO by induced fermentation + standardized temperature in hot air oven
3. T_3- VCO by centrifugation + standardized temperature in hot air oven
4. T_4- VCO by traditional boiling method + standardized temperature in hot air oven

Treatments - 4

Replication - 5

Design - CRD

The oil recovered by different extraction methods were subjected to drying and analysed for quality parameters, sensory parameters, oil recovery, microbial load, shelf life and economics of production.

3.5. OBSERVATIONS

3.5.1. Climatic parameters

The fermentation of coconut milk was carried out at three different seasons *viz*., June – July of 2014, November – December of 2014 and March – April of 2015. The atmospheric temperature and relative humidity during the fermentation period has been recorded.

3.5.1.1. Atmospheric temperature

The daily mean maximum temperature and daily mean minimum temperature for the year 2014 for the period from January to December and for the year 2015 for the period from January to April during the fermentation and induced fermentation period was recorded by Department of Agricultural Meteorology, College of Agriculture, Vellayani has been provided.

3.5.1.2. Relative Humidity

The daily mean maximum relative humidity and daily mean minimum relative humidity for the year 2014 for the period from January to December and for the year 2015 for the period from January to April during the fermentation and induced fermentation period was recorded by Department of Agricultural Meteorology , College of Agriculture, Vellayani has been provided.

3.5.2. Moisture Percentage

Moisture percentage was determined by hot air-oven method. Five gram of the sample was taken and kept in the hot and weighed crucible and kept at 40°C, 45°C and at 50°C till a constant weight was attained.

$$\text{Moisture Percentage} = \frac{W_1 \times 100}{W}$$

Where,

W_1 = Loss in g of the sample on drying

W = Weight in g of the sample taken for test

3.5.3. Fermentation Time

Coconut milk prepared by different treatments were kept for 24, 36 and 48 hours and the best time required for fermentation was recorded.

Time required for extraction of virgin coconut oil by centrifugation method was expressed in minutes.

3.5.4. Oil Recovery

The oil recovery was calculated according to the initial weight of coconut milk to the oil extracted after fermentation (AOAC, 1997).

$$\text{Oil Recovery (in percentage)} = \frac{\text{Weight of oil extracted}}{\text{Weight of coconut milk used}} \times 100$$

3.5.5. Free Fatty Acid

Free fatty acid value was determined by titrating the oil dissolved in neutral solvent (diethyl ether and ethanol in the ratio 1:1) against potassium hydroxide, using phenolphthalein as indicator (Sadasivam & Manikam, 1992) and was expressed as mg KOH/g oil. It is also known as acid number or acid value.

$$\text{Acid value (mg KOH/g of oil)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample (g)}}$$

3.5.6. Total Phenolic content

Total Phenolic content of the oil samples was determined according to the procedure of Sadasivam and Manikam (1992) with some modifications by Ramma *et al*. (2002) using catechin as standard.

3.5.7. Protease activity

Protease activity was determined by using Folin and Ciocalteus reagent. It primarily reacts with free tyrosine to produce a blue coloured chromophore, which is quantifiable and measured as an absorbance value on the spectrophotometer at 660nm (Enyard, 2008).

3.5.8. Amylase activity

Amylase activity was measured by DNS (dinitrosalicylic) method using soluble starch as its substrate and maltose solution was used as its standard following the method of Sadasivam and Manikam (1996).

3.5.9. Sensory evaluation for colour, odour and taste

Sensory evaluation of the extracted oil under different methods were organoleptically scored by 10 member semi- trained panel. The panel were asked to score the colour, odour, and taste of the sample using a nine - point hedonic scale (ISI, 1971) in the order of preference as shown below.

Like extremely – 9

Like very much – 8

Like moderately – 7

Like slightly – 6

Neither like nor dislike – 5

Dislike slightly – 4

Dislike moderately – 3

Dislike very much – 2

Dislike extremely – 1

3.5.10. Revolutions per minute

The experiment was done in HERMLE Z323K centrifuge having 7.5cm radius of rotation and the revolutions per minute was expressed in G force.

G force (Newton) $= 1.12 \times r \times (rpm/1000)^2$

Where,

r = radius of rotation

rpm = revolutions per minute

3.5.11. Quality Parameters

3.5.11.1. Refractive Index

Refractive Index of the oil at 40^0C was determined by using prism minimum deviation method. A hollow prism cell was used to contain the oil and angle of diffraction was noted and refractive index was calculated as per the following equation (Ariponnammal, 2012).

$$n = \frac{\frac{\text{Sin } A + D}{2}}{\frac{\text{Sin } A}{2}}$$

Where,

n= Refractive index

A = Angle of prism, 60°

D = Angle of diffraction

3.5.11.2. Relative Density

Relative Density of the oil was measured at 25^0C by using 50 ml pycnometer (ISO/FDIS 6883, 2000).

3.5.11.3. Specific gravity

Specific gravity of the oil at 30^0C was determined by using pycnometer with the following equation (AOAC, 2000).

$$\text{Specific gravity at } 30^0\text{C} = \frac{A - B}{C - B}$$

Where,

A = Weight of pycnometer with oil in g at 30°C

B = Weight of pycnometer in g at 30°C

C = Weight of pycnometer with water in g at 30°C

3.5.11.4. Colour

Colour of the oil was measured by using spectrophotometer CM2600D (Konica Minotta, Inc, Osaka, Japan). The colour was analysed by using ten gram of oil in a plastic petri dish and was exposed to the spectrophotometer. The colour co-ordinates (L* and b*) were measured for finding yellowness index. The yellowing index was found by using the following equation (Tijskens *et al*., 2001).

$$\text{Yellowness index} = \frac{142.86 \times b^*}{L^*}$$

Where,

b* = Yellowness/blueness value of the sample with values varying from -60 (blue) to +60 (yellow)

L* = Lightness/darkness value of the sample with values varying from 0 (black) to 100 (white)

3.5.11.5. Iodine Value

Iodine value of oil samples was determined as described by Sadasivam and Manikam (1992) and expressed as gram of iodine absorbed / 100g oil .

$$\text{Iodine Value} = \frac{(B- S) \times N \times 12.69}{\text{g sample}}$$

Where,

B = mL thiosulphate for blank

S = mL thiosulphate for sample

N = Normality of thiosulphate solution

3.5.11.6. Unsaponifiable matter

Unsaponifiable matter in the oil was determined according to AOAC method (2000) and expressed as percentage. The following equation was used for finding unsaponifiable matter.

$$\text{Unsaponifiable matter} = \frac{100\ (A - B)}{W}$$

Where,

A = Weight in g of the residue

B = Weight in g of the free fatty acids in the extract

W = Weight in g of the sample

3.5.11.7. Polenske Value

Polenske value of the oil sample was determined as per ISI (1984).

Polenske value = 10 × V × N

Where,

V = Volume in ml of standard sodium hydroxide solution required for the test

N = Normality of standard sodium hydroxide solution

3.5.11.8. Peroxide Value

Peroxide value of the oil sample was determined following the method described by (Sadasivam & Manikam, 1992) and expressed as milli equivalent peroxide / kg of oil . The peroxide value was determined by the formula

$$\text{Peroxide value} = \frac{S \times N \times 1000}{\text{g sample}}$$

Where,

S = mL of sodium thiosulphate (Test – Blank)

N = Normality of sodium thiosulphate solution

3.5.11.9. Saponification value

Saponification value was determined following the procedure mentioned by Sadasivam and Manikam (1992) and expressed as mg/g of oil. The saponification value of the oil was determined by the formula

$$\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Weight of sample (g)}}$$

3.5.11.10. Antioxidant properties

3.5.11.10.1. Free Radical Scavenging Activity (DPPH Method)

The antioxidant activity of the virgin coconut oil was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method. The method followed was as described by Seneviratne *et al*. (2009) with a slight modification. The oil was taken into various concentrations (5-35 µg/ml) and ascorbic acid was used as control instead of BHT. The inhibitory effect of DPPH radical was expressed in percentage and was calculated according to the formula

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1) \times 100}{A_0}$$

Where,

A_0 = absorbance of DPPH solution in ethanol without sample

A_1 = absorbance of DPPH solution in ethanol with sample (after 30 min of incubation)

3.5.11.10.2. Total Antioxidant Activity (β- carotene – linoleate bleaching activity / Phosphomolybdenum antioxidant assay)

The total antioxidant capacity of the oil was determined by phosphomolybdate method (Benhammou *et al*., 2013). Total antioxidant capacity was measured in terms of inhibitory percentage.

3.5.11.10.3.Reducing Power

The reducing power of the oil was measured using the ferric reducing anti-oxidant potential assay as described by Yen and Duh (1993). Reducing power was measured in terms of absorbance.

3.5.11.10.4. IC_{50} Values

IC_{50} values were calculated by linear regression.

3.5.12. Total Plate Count

Total plate count consisting of fungal, bacterial, actinomycete, present in virgin coconut oil after drying as well as the population of *Lactobacillus* and yeast were also estimated.

The microbial population in the oil was estimated by the serial dilution plate technique. 1ml of oil was taken and transferred to 9 ml of sterile water and shaken well. From this stock suspension, different dilutions of 10^{-2}, 10^{-3}, 10^{-4},10^{-5}were prepared. The *Lactobacillus* and yeast population was estimated at 10^{-2} dilution. The fungal and actinomycetes population was estimated at 10^{-3} dilution while 10^{-5} dilution was used for bacterial population.

The number of colony forming units (cfu) per ml of the sample was calculated using the formula,

$$\text{Number of colony forming units} = \frac{\text{Total number of colony formed x Dilution factor}}{\text{Weight of oil taken}}$$

3.5.13. Shelf life

Shelf life of the virgin coconut oil extracted by fermentation, induced fermentation, centrifugation and traditional boiling method was observed. The moisture content, microbial load, acid value, peroxide value, saponification value, phenolic content and sensory scores for colour, odour and taste immediately after extraction and three and six months after extraction was observed and compared with APCC (2003) standards for virgin coconut oil.

3.5.14. Economics of Production

The economics of production of virgin coconut oil was worked out for the processing of 100kg of coconut per day for one year after taking into account the cost of production including the fixed asset and variable asset and calculated as per the prevailing market price that existed during 2013-2015.
The net income was calculated as follows:
Net income = Gross income – Cost of production.

$$\text{Benefit cost ratio} = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

3.6. Statistical analysis

The analysis of sensory evaluation was done using Kruskal - Wallis one way analysis of variance technique (Kruskal and Wallis, 1952). The quality parameters of virgin coconut oil was analysed using analysis of variance technique (Gomez and Gomez, 1984).

RESULTS

4. RESULTS

The study entitled, “Cost effective technology for home scale and small scale production of virgin coconut oil” was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2013-2015. The data generated during the laboratory experiment were statistically analyzed and the results are presented in this chapter.

The study was conducted in four experiments. Standardisation of fermentation method, standardisation of induced fermentation method, standardisation of centrifugation method and comparison of virgin coconut oil produced by fermentation, induced fermentation and centrifugation with the traditional boiling method was undertaken.

4.1. STANDARDISATION OF FERMENTATION METHOD

4.1.1. Standardisation of drying temperature

4.1.1.1. Moisture percentage

The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 respectively were kept for 24, 36 and 48 hours (plate 1.) and subjected to drying at 40°C, 45°C and 50°C and the results are presented in Table 2. Virgin coconut oil produced by the extraction of coconut milk from coconut gratings with coconut water in the ratio 1:1, water in the ratio 1:1 and 1:2 and kept for 24, 36 and 48 hours irrespective of drying temperature did not show any significant difference in moisture percentage between treatments. Comparison of drying temperature 40°C, 45°C and 50°C (group 1, 2 and 3) also showed no significant difference between the temperatures on moisture content. Thus 40°C, 45°C and 50°C had same effect on moisture content (0.078 per cent). The different methods of virgin coconut oil

extraction and the different temperatures 40°C, 45°C and 50°C as well as their interaction did not show any significant difference in moisture content.

4.1.1.2. Free fatty acid content

The effect of drying temperature on free fatty acid content of virgin coconut oil produced by fermentation is presented in Table 3. The virgin coconut oil recovered from coconut milk extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 40°C, 45°C and 50°C did not show any significant difference between treatments and drying temperatures 40°C, 45°C and 50°C (group1, 2, 3). The interaction effect was also found to be non significant. The free fatty acid content of the virgin coconut oil produced by the fermentation method dried at different temperatures varied from 0.200 mg KOH/g of oil to 0.240 mg KOH/g of oil.

4.1.1.3. Total phenolic content

The effect of drying temperature on total phenolic content of virgin coconut oil produced by fermentation is presented in Table 4. The virgin coconut oil recovered (plate 2.) from coconut milk extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 40°C, 45°C and 50°C did not show any significant difference between treatments. Between the drying temperatures 40°C, 45°C and 50°C (group 1, 2, 3) also no significant difference was noted. The interaction effect also could not make any significant change in the total phenolic content of virgin coconut oil. The total phenolic content of the treatments ranged from 55.666 to 76.000 mg catechin equivalent /kg of oil.

Table: 2. Effect of drying temperature on moisture content (%) of virgin coconut oil produced by fermentation

Treatments	Moisture content (%)			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW1:1 -24 h)	0.093	0.080	0.073	0.082
T_2 (CG+CW 1:1 -36 h)	0.080	0.073	0.080	0.078
T_3 (CG+CW1:1 -48 h)	0.073	0.093	0.073	0.080
T_4 (CG+W 1:1-24 h)	0.073	0.073	0.073	0.073
T_5 (CG+W 1:1-36 h)	0.073	0.073	0.066	0.071
T_6 (CG+W 1:1-48 h)	0.086	0.080	0.080	0.082
T_7 (CG+W 1:2-24 h)	0.073	0.080	0.086	0.080
T_8 (CG+W 1:2-36 h)	0.086	0.080	0.080	0.082
T_9 (CG+W 1:2-48 h)	0.073	0.086	0.080	0.080
Mean	0.078	0.089	0.078	-
CD (0.05) (Groups)	NS			
CD (0.05) (Treatments)				NS
CD (0.05) (Treatments Vs Groups)	NS			

Plate 1. Treatments showing coconut milk kept for fermentation

Plate 2. Treatments showing fermented layers of coconut milk

Table: 3. Effect of drying temperature on free fatty acid content (mg KOH/g of oil) of virgin coconut oil produced by fermentation

Treatments	Free fatty acid content (mg KOH/g of oil)			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW 1:1- 24 h)	0.220	0.240	0.200	0.226
T_2 (CG+CW 1:1- 36 h)	0.240	0.240	0.200	0.213
T_3 (CG+CW 1:1- 48 h)	0.220	0.220	0.220	0.240
T_4 (CG+W 1:1- 24 h)	0.200	0.240	0.220	0.233
T_5 (CG+W 1:1- 36 h)	0.200	0.200	0.220	0.220
T_6 (CG+W 1:1- 48 h)	0.240	0.220	0.233	0.233
T_7 (CG+W 1:2- 24 h)	0.240	0.240	0.220	0.206
T_8 (CG+W 1:2- 36 h)	0.240	0.220	0.220	0.224
T_9 (CG+W 1:2- 48 h)	0.240	0.240	0.220	0.220
Mean	0.224	0.220	0.221	-
CD (0.05) (Groups)	NS			
CD(0.05) (Treatments)				NS
CD (0.05) (Treatments Vs Groups)	NS			

Table: 4. Effect of drying temperature on total phenolic content (mg catechin equivalent /kg of oil) of virgin coconut oil produced by fermentation

Treatments	Total phenolic content (mg catechin equivalent /kg of oil)			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW 1:1 -24 h)	69.333	59.333	61.333	61.222
T_2 (CG+CW 1:1 -36 h)	55.666	69.333	56.000	63.555
T_3 (CG+CW 1:1-48 h)	58.666	66.000	63.333	65.333
T_4 (CG+W 1:1-24 h)	68.000	60.000	72.000	64.888
T_5 (CG+W 1:1-36 h)	66.000	65.666	64.000	66.333
T_6 (CG+W 1:1-48 h)	56.666	73.333	62.666	61.888
T_7 (CG+W 1:2-24 h)	62.000	64.000	69.333	60.222
T_8 (CG+W 1:2-36 h)	76.000	61.333	59.333	66.222
T_9 (CG+W 1:2-48 h)	58.000	60.333	66.666	65.111
Mean	65.030	63.707	62.858	-
CD(0.05) (Groups)	NS			
CD (0.05) (Treatments)				NS
CD (0.05) (Treatments Vs Groups)	NS			

4.1.1.4. Oil recovery

The effect of drying temperature on oil recovery of virgin coconut oil produced by fermentation method is depicted in Table 5. The virgin coconut oil recovered (Plate 3.) from coconut milk extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 40°C, 45°C and 50°C showed significant difference among them. Significantly higher oil was recovered from T_5 *i.e.*, coconut milk extracted from grated coconut and water in the ratio 1:1 and kept for 36 hours (20.42 per cent) and was on par with T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours (20.40 per cent).

Among the groups, virgin coconut oil dried at 50°C showed maximum oil recovery (19.19 per cent) compared to other groups. The interaction effect was also significant and the oil recovery for coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours and dried at 50°C was 20.47 per cent and on par with the treatments coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours and dried at 40°C (20.37 per cent) and coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours and dried at 50°C (20.23 per cent).

Table: 5. Effect of drying temperature on oil recovery (%) of virgin coconut oil produced by fermentation

Treatments	Oil recovery (%)			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW1:1 -24 h)	18.47	18.53	18.63	18.54
T_2 (CG+CW1:1 -36 h)	19.37	19.60	19.83	19.60
T_3 (CG+CW 1:1-48 h)	20.03	20.07	20.23	20.11
T_4 (CG+W 1:1-24 h)	19.53	19.60	19.73	19.62
T_5 (CG+W 1:1-36 h)	20.37	20.43	20.47	20.42
T_6 (CG+W 1:1-48 h)	20.37	20.37	20.47	20.40
T_7 (CG+W 1:2-24 h)	16.10	16.17	16.27	16.18
T_8 (CG+W 1:2-36 h)	18.53	18.47	18.53	18.51
T_9 (CG+W 1:2-48 h)	18.23	18.37	18.53	18.38
Mean	19.00	19.07	19.19	-
CD (0.05) (Groups)	0.106			
CD (0.05) (Treatments)				0.178
CD (0.05) (Treatments Vs Groups)	0.300			

4.1.1.5. Sensory parameters

4.1.1.1.1. Colour

The effect of drying temperature on the colour of virgin coconut oil produced by fermentation is presented in Table 6. There was significant difference between treatments. The drying temperature (40°C, 45°C and 50°C) did not differ significantly. However the treatments drying temperature interaction significantly influenced the virgin coconut oil produced.

The virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours recorded the highest score (8.87). The lowest value was recorded for the virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 48 hours.

4.1.1.1.2. Odour

The effect of drying temperature on the odour of virgin coconut oil produced by fermentation is presented in Table 7. The virgin coconut oil recovered from coconut milk extracted with grated coconut and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and kept for 24, 36 and 48 hours showed significant difference among treatments, groups and their interaction.

Virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours scored maximum scores and minimum scores noticed in virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 24 hours. The oil which was dried at 50°C recorded maximum score followed by 45°C and 40°C.

Table: 6. Effect of drying temperature on sensory parameter (colour) of virgin coconut oil produced by fermentation

Treatments	Mean sensory scores			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW1:1 -24 h)	8.20	8.40	8.40	8.33
T_2 (CG+CW 1:1-36 h)	8.50	8.30	8.50	8.43
T_3 (CG+CW1:1 -48 h)	8.40	8.20	8.40	8.33
T_4 (CG+W 1:1-24 h)	8.50	8.20	8.50	8.40
T_5 (CG+W 1:1-36 h)	8.90	8.80	8.90	8.87
T_6 (CG+W 1:1-48 h)	8.30	8.30	8.30	8.30
T_7 (CG+W 1:2-24 h)	8.30	8.30	8.20	8.27
T_8 (CG+W 1:2-36 h)	8.30	8.60	8.30	8.40
T_9 (CG+W 1:2-48 h)	8.10	8.30	8.30	8.23
Mean	8.39	8.39	8.41	
CD (0.05) (Groups)	NS			
CD (0.05) (Treatments)				0.239
CD (0.05) (Treatments Vs Groups)	0.414			

Table: 7. Effect of drying temperature on sensory parameter (odour) of virgin coconut oil produced by fermentation

Treatments	Mean sensory scores			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW1:1 -24 h)	6.80	7.10	7.40	7.10
T_2 (CG+CW1:1 -36 h)	7.10	6.80	7.40	7.10
T_3 (CG+CW1:1 -48 h)	6.60	7.10	7.20	6.97
T_4 (CG+W 1:1-24 h)	7.30	7.20	6.90	7.10
T_5 (CG+W 1:1-36 h)	7.20	7.70	7.80	7.60
T_6 (CG+W 1:1-48 h)	7.10	7.30	7.20	7.20
T_7 (CG+W 1:2-24 h)	6.70	6.80	7.20	6.90
T_8 (CG+W 1:2-36 h)	6.80	7.20	7.30	7.10
T_9 (CG+W 1:2-48 h)	7.20	7.20	7.40	7.30
Mean	6.98	7.16	7.31	
CD(0.05) (Groups)	0.127			
CD (0.05) (Treatments)				0.219
CD(0.05)(Treatments Vs Groups)	0.381			

4.1.1.1.3. Taste

The effect of drying temperature on the taste of virgin coconut oil produced by fermentation is presented in Table 8. The virgin coconut oil recovered from coconut milk extracted with grated coconut and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and kept for 24, 36 and 48 hours showed significant difference among treatments, groups and their interaction.

Virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours scored maximum scores (7.20) and minimum scores (5.50) noticed in virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 36 hours. The oil which was dried at 50°C recorded maximum score followed by 45°C and 40°C.

4.1.2. Standardisation of fermentation method at different seasons

4.1.2.1. Oil recovery

The effect of different fermentation method at three different seasons on oil recovery is presented in Table 9. On June- July, the oil recovery showed significant difference among the treatments. T_5, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours recorded the higher oil recovery (20.466 per cent) which was on par with the T_3, coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours (20.100 per cent). Coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 24 hours recorded the lower oil recovery (16.266 per cent).

Table: 8. Effect of drying temperature on sensory parameter (taste) of virgin coconut oil produced by fermentation

Treatments	Mean sensory scores			Mean
	40°C Group 1	45°C Group 2	50°C Group 3	
T_1 (CG+CW1:1 -24 h)	6.10	6.60	6.30	6.33
T_2 (CG+CW1:1 -36 h)	6.30	6.30	6.30	6.30
T_3 (CG+CW1:1 -48 h)	6.20	6.40	6.10	6.23
T_4 (CG+W 1:1-24 h)	6.00	6.00	6.60	6.20
T_5 (CG+W 1:1-36 h)	6.60	6.80	7.20	6.87
T_6 (CG+W 1:1-48 h)	6.30	6.30	6.40	6.33
T_7 (CG+W 1:2-24 h)	5.70	5.80	6.30	5.93
T_8 (CG+W 1:2-36 h)	5.90	6.40	5.50	5.93
T_9 (CG+W 1:2-48 h)	6.40	5.70	6.50	6.20
Mean	6.17	6.26	6.36	
CD (0.05) (Groups)	0.133			
CD (0.05) (Treatments)				0.230
CD (0.05) (Treatments Vs Groups)	0.399			

During November – December, since the separation of oil from the coconut milk did not occur, the treatments were kept in incubator at a temperature of 28.8°C and the oil recovery was analysed (Table 14).

During March- April, maximum oil recovery was noticed in T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours (23.066 per cent) and was on par with T_5, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours (22.900 per cent) and coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours (22.666 per cent). Minimum oil recovery (17.133 per cent) was noticed inT_7, coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 24 hours.

4.1.2.2. Moisture content

The effect of different fermentation method at three different seasons on the moisture content of virgin coconut oil recovered from coconut milk extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 50°C is presented in Table 10. On June-July and March- April, the moisture percentage of virgin coconut oil extracted by different fermentation method did not show any significant difference.

4.1.2.3. Sensory parameters

The results of different fermentation method on sensory parameters like colour, odour and taste on June- July is depicted in Table 11. The sensory parameters like colour and odour of virgin coconut oil recovered from different treatments did not differ significantly in colour and odour. However there was significant difference in taste between the treatments. The treatment T_5, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours recorded the maximum scores (7.2) compared to other treatments.

Table: 9. Effect of different fermentation method at different seasons on oil recovery (%)

Treatments	Oil recovery (%)		
	June - July	November – December	March – April
T_1 (CG+CW1:1 -24 h)	18.633	Not recovered	20.600
T_2 (CG+CW1:1 -36 h)	19.800	Not recovered	22.000
T_3 (CG+CW1:1 -48 h)	20.100	Not recovered	22.666
T_4 (CG+W 1:1-24 h)	19.733	Not recovered	20.500
T_5 (CG+W 1:1-36 h)	20.466	Not recovered	22.900
T_6 (CG+W 1:1-48 h)	20.466	Not recovered	23.066
T_7 (CG+W 1:2-24 h)	16.266	Not recovered	17.133
T_8 (CG+W 1:2-36 h)	17.866	Not recovered	18.600
T_9 (CG+W 1:2-48 h)	18.200	Not recovered	19.200
SE	0.207	-	0.297
CD (0.05)	0.434	-	0.627

Plate 3. Treatments showing virgin coconut oil separated by fermentation method

Plate 4. Treatments kept at incubator for fermentation

The sensory scores for colour, odour and taste for virgin coconut oil extracted during the month of March- April by different fermentation methods did not vary significantly (Table 12).

During November – December, since there was no separation of oil from the coconut milk, the treatments were kept in incubator at a temperature of 28.8°C (Plate 4.).

4.1.3. Fermentation of coconut milk in incubator

4.1.3.1. Oil recovery

The effect of different fermentation method on moisture content and oil recovery of virgin coconut oil produced during November – December kept in incubator at 28.8°C is presented in Table 14. The oil recovery was maximum for T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours (22.733 per cent) and it was on par with T_5, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and T_3, coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours (22.333 per cent). Minimum oil recovery was noticed for T_7, coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 24 hours (16.866 per cent).

Table: 10. Effect of different fermentation method at different seasons on the moisture content (%) of virgin coconut oil

Treatments	Moisture content (%)		
	June - July	November – December	March – April
T_1 (CG+CW1:1 -24 h)	0.073	-	0.073
T_2 (CG+CW1:1 -36 h)	0.080	-	0.080
T_3 (CG+CW1:1 -48 h)	0.073	-	0.066
T_4 (CG+W 1:1-24 h)	0.073	-	0.066
T_5 (CG+W 1:1-36 h)	0.066	-	0.066
T_6 (CG+W 1:1-48 h)	0.080	-	0.073
T_7 (CG+W 1:2-24 h)	0.086	-	0.073
T_8 (CG+W 1:2-36 h)	0.080	-	0.073
T_9 (CG+W 1:2-48 h)	0.080	-	0.073
SE	0.026	-	0.026
CD (0.05)	NS	-	NS

Table: 11. Effect of different fermentation method on sensory parameters of virgin coconut oil produced during June- July

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (CG+CW1:1 -24 h)	8.4	7.4	6.3
T_2 (CG+CW1:1 -36 h)	8.5	7.4	6.3
T_3 (CG+CW1:1 -48 h)	8.4	7.2	6.1
T_4 (CG+W 1:1-24 h)	8.5	6.9	6.6
T_5 (CG+W 1:1-36 h)	8.8	7.8	7.2
T_6 (CG+W 1:1-48 h)	8.3	7.2	6.4
T_7 (CG+W 1:2-24 h)	8.2	7.2	6.3
T_8 (CG+W 1:2-36 h)	8.3	7.3	6.5
T_9 (CG+W 1:2-48 h)	8.3	7.4	5.5
Kruskal Wallis H	8.6	8.6	5.4***
	NS	NS	S

Table: 12. Effect of different fermentation method on sensory parameters of virgin coconut oil produced during March- April

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (CG+CW1:1 -24 h)	8.70	7.50	6.10
T_2 (CG+CW1:1 -36 h)	9.00	7.60	6.50
T_3 (CG+CW1:1 -48 h)	8.70	7.60	6.50
T_4 (CG+W 1:1-24 h)	8.90	7.80	6.80
T_5 (CG+W 1:1-36 h)	9.00	8.00	6.90
T_6 (CG+W 1:1-48 h)	8.90	8.00	6.60
T_7 (CG+W 1:2-24 h)	8.90	7.70	6.20
T_8 (CG+W 1:2-36 h)	8.70	7.70	6.20
T_9 (CG+W 1:2-48 h)	8.60	7.50	6.30
Kruskal Wallis H	2.28	5.28	13.74
	NS	NS	NS

4.1.2.4. Atmospheric temperature

Observations on maximum and minimum atmospheric temperature during the fermentation period is presented in Table 13. The maximum atmospheric temperature during the fermentation period when oil was recovered in the month of June- July and March - April ranged from 31.05°C to 32.60°C. The minimum atmospheric temperature during the fermentation period when oil was recovered in the month of March- April and June- July ranged from 23.25°C - 25.80°C. The oil did not recover at maximum atmospheric temperature below 31.05°C. The oil did not recover at atmospheric minimum temperature of 24.60°C, 23.75°C, 22.80°C, 23.25°C, 22.90°C, 23.55°C and 22.40°C. The atmospheric maximum temperature during those periods were 29.20°C, 29.50°C, 26.50°C, 28.35°C, 29.25°C, 26.50°C and 30.00°C respectively. The atmospheric maximum and minimum temperature during the month of November – December ranged from 26.50°C to 30.00°C and 22.40°C - 23.75°C, during which separation of oil did not take place and have zero recovery during this period.

4.1.2.5. Relative humidity

The relative humidity had an effect on the separation of virgin coconut oil recovered from coconut milk extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours (Table 13). The maximum relative humidity during the period when the oil was separated ranged from 97 per cent to 90.50 per cent. The minimum relative humidity during the period when the oil was separated ranged from 67 per cent to 83 per cent. At maximum relative humidity of 97.50 and a minimum relative humidity of 92.50 per cent oil was not separated. Similarly at maximum relative humidity of 90.00, 92.50, 97.50, 94.50, 90.50, 89.50 and 93.00 per cent with corresponding minimum relative humidity of 82.50, 83.50, 92.50, 84.00, 89.00, 77.00 and 69.50 per cent oil was not separated.

4.1.3.2. Moisture percentage

The moisture percentage of virgin coconut oil extracted by different fermentation method during November- December kept in incubator at 28.8°C (Table 14) did not show any significant difference between the treatments.

4.1.3.3. Sensory parameters

The effect of different fermentation method on sensory parameters of virgin coconut oil produced during November – December and kept in incubator at 28.8°C is depicted in Table 15. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours did not show any significant difference for the sensory parameters like colour, odour and taste.

Table 13. Observations on maximum, minimum temperature (°C) and relative humidity (%) during the fermentation period

Month and Date	Atmospheric temperature (°C)		Room temperature (°C)	Relative humidity (%)			Oil recovery
	Max. temp.	Min. temp.	Average	Max.	Min.	Average	
June 16-17	31.40	25.80	27.57	91.00	83.00	87.00	recovered
June 23-24	31.05	25.50	28.50	92.00	81.50	86.75	recovered
July 6-7	29.20	24.60	26.70	90.00	82.50	86.25	Not recovered
November 3-4	29.50	23.75	26.40	92.50	83.50	88.00	Not recovered
November 22- 23	26.50	22.80	25.95	97.50	92.50	95.00	Not recovered
November 27-28	28.35	23.25	25.50	94.50	84.00	89.25	Not recovered
December 1-2	29.25	22.90	26.00	90.50	89.00	89.75	Not recovered

Table 13. Observations on maximum, minimum temperature (°C) and relative humidity (%) during the fermentation period (contd.)

December 14-15	26.50	23.55	25.00	89.50	77.00	83.25	Not recovered
December 20-21	30.00	22.40	26.07	93.00	69.50	81.25	Not recovered
March 9-10	32.25	23.25	30.14	92.50	72.00	82.25	recovered
April 3-4	32.60	24.70	30.90	97.00	67.00	82.00	recovered
April 23-24	32.25	24.65	30.50	90.50	78.00	84.25	recovered

Table: 14. Effect of different fermentation method on oil recovery (percent) and moisture content (percent) of virgin coconut oil produced during Nov-Dec (in incubator at 28.8°C)

Treatments	Oil recovery (percent)	Moisture (percent)
T_1 (CG+CW1:1 -24 h)	20.000	0.073
T_2 (CG+CW1:1 -36 h)	21.666	0.086
T_3 (CG+CW1:1 -48 h)	22.333	0.073
T_4 (CG+W 1:1-24 h)	20.200	0.073
T_5 (CG+W 1:1-36 h)	22.333	0.066
T_6 (CG+W 1:1-48 h)	22.733	0.080
T_7 (CG+W 1:2-24 h)	16.866	0.073
T_8 (CG+W 1:2-36 h)	18.600	0.073
T_9 (CG+W 1:2-48 h)	19.000	0.086
SE	0.327	0.026
CD (0.05)	0.698	NS

Table: 15. Effect of fermentation method on sensory parameters of virgin coconut oil produced during Nov- Dec (in incubator at 28.8°C)

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (CG+CW1:1 -24 h)	8.30	7.30	6.00
T_2 (CG+CW1:1 -36 h)	8.40	7.40	6.10
T_3 (CG+CW1:1 -48 h)	8.40	7.20	6.40
T_4 (CG+W 1:1-24 h)	8.70	7.60	6.60
T_5 (CG+W 1:1-36 h)	8.60	7.70	6.70
T_6 (CG+W 1:1-48 h)	8.70	7.60	6.50
T_7 (CG+W 1:2-24 h)	8.40	7.20	6.20
T_8 (CG+W 1:2-36 h)	8.70	7.20	6.00
T_9 (CG+W 1:2-48 h)	8.60	7.00	6.00
Kruskal Wallis H	4.43	9.02	9.97
	NS	NS	NS

4.2. STANDARDISATION OF INDUCED FERMENTATION

4.2.1. Isolation and screening of microorganisms

Table 16 represents the microorganisms isolated from the fermented coconut milk extracted from coconut gratings and water in the ratio 1:1 and kept at 24 and 36 hours. There was a significant difference in the population of isolated microorganisms from coconut milk extracted with coconut gratings and water in the ratio 1: 1 and kept for fermentation at 24 and 36 hours after fermentation. The maximum bacterial population of 44.60×10^6 per ml was observed at 24 hours after fermentation compared to 36 hours. However there was no significant difference in colony forming units of *Lactobacillus* (plate 5.) and yeast (plate 6.) isolated.

4.2.1.1. Protease and amylase activity

Protease and amylase activity of microorganisms isolated is presented in Table 17. *Lactobacillus* (plate 7.) isolated at 24 hours showed significantly superior protease (184.200µg tyrosine released in 2 hour) and amylase (26715.000µg of maltose produced during 5 minutes) activity compared to other treatments. The lower protease and amylase activity was observed for yeast (plate 8.) isolated at 36 hours.

4.2.1.2. Count of Lactobacillus

Count of *Lactobacillus* in coconut milk at different hours of fermentation under sterile conditions is presented in Table 18. The *Lactobacillus* count was increased with time and maximum population of 94×10^4 cfu per ml was noticed after 36 hours.

Plate 5. Plate showing growth of *Lactobacillus*

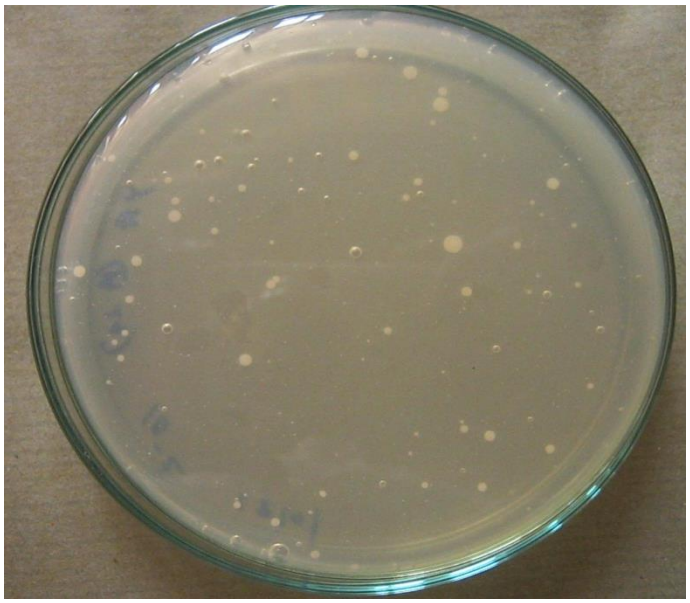

Plate 6. Plate showing growth of yeast

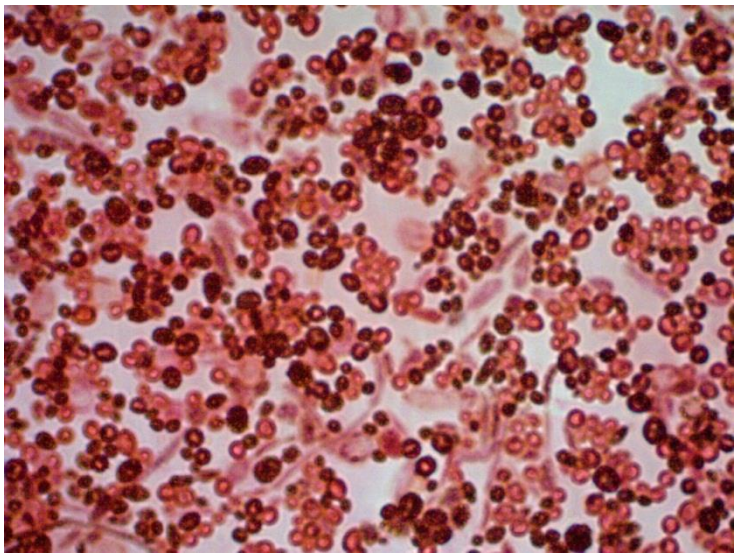

Plate 7. Microscopic view of stained *Lactobacillus* separated from fermented coconut milk

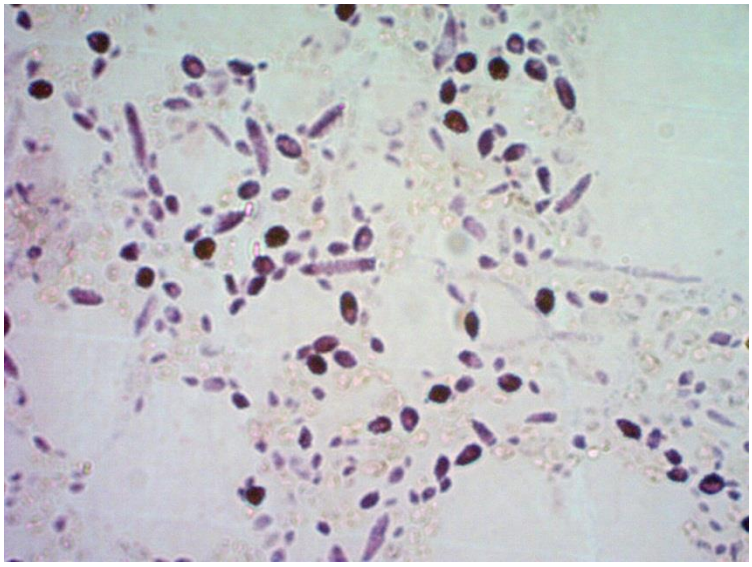

Plate 8. Microscopic view of stained yeast separated from fermented coconut milk

Table: 16. Microorganisms isolated from coconut milk fermented at 24 and 36 hours

Time	Bacteria $\times 10^6$ cfu ml^{-1}	*Lactobacillus* $\times 10^3$ cfu ml^{-1}	Yeast $\times 10^3$ cfu ml^{-1}
24h	44.60	33.12	5.41
36h	38.10	37.00	5.96
SE	2.25	2.24	0.48
CD(0.05)	4.74	NS	NS

Table: 17. Protease and amylase activity of microorganisms isolated

Microorganisms isolated	Protease activity (µg tyrosine released in 2 hour)	Amylase activity (µg of maltose produced during 5 minutes)
Isolate1 (*Lactobacillus* isolated at 24 hours)	184.200	26715.000
Isolate2 (*Lactobacillus* isolated at 36 hours)	163.230	19770.000
Isolate3 (Yeast 1 isolated at 24 hours)	84.085	3925.000
Isolate4 (Yeast 2 isolated at 24 hours	71.435	3235.000
Isolate 5 (Yeast isolated at 36 hours)	70.975	3305.000
SE	7.689	904.905
CD(0.05)	16.385	1928.358

Table 18. Count of *Lactobacillus* in coconut milk at different hours of fermentation under sterile conditions

Treatments	Count (cfu /ml x 10^4)
After 18 hours	18
After 24 hours	86
After 36 hours	94

4.2.1.3. Oil recovery

The effect of induced fermentation and fermentation on oil recovery is presented in Table 19. Oil recovery by induced fermentation and fermentation method showed significant difference among treatments, groups and their interaction. The virgin coconut oil recovered from induced fermentation method was significantly superior (17.733 per cent) compared to fermentation method (17.3410 per cent). Among treatments T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours (21.780 per cent) showed significantly superior oil recovery compared to other treatments. There was significantly superior interaction between treatments and groups. Oil recovery was more for T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours by induced fermentation method and it was on par with the same treatment by fermentation method.

4.2.2. Induced fermentation

4.2.2.1. Free fatty acid

The effect of induced fermentation on free fatty acid content of virgin coconut oil is presented in Table 20. Free fatty acid content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with 1 per cent of *Lactobacillus* culture and kept for fermentation for 18, 24 and 36 hours and dried at 50°C did not show any significant difference between treatments.

Table: 19. Effect of induced fermentation and fermentation on oil recovery (%)

Treatments	Oil recovery (percent)		
	Induced fermentation (Group1)	Fermentation (Group 2, Control)	Mean
T_1 (CG +CW 1:1+ 18h)	16.000	15.567	15.780
T_2 (CG +CW 1:1+ 24h)	18.833	18.467	18.650
T_3 (CG+ CW 1:1+ 36h)	21.300	21.067	21.180
T_4 (CG +W 1:1 + 18h)	16.567	15.733	16.150
T_5 (CG + W 1:1 + 24h)	19.167	18.933	19.050
T_6 (CG+W 1:1 + 36h)	21.967	21.600	21.780
T_7 (CG+W 1:2 + 18h)	13.633	12.933	13.280
T_8 (CG +W 1:2 + 24h)	15.200	15.400	15.300
T_9 (CG +W 1:2 + 36h)	16.933	16.367	16.650
Mean	17.733	17.341	-
CD (Groups)	0.322		
CD (Treatments)			0.152
CD (Induced fermentation Vs fermentation)	0.456		

4.2.2.2. Total phenolic content

The effect of induced fermentation on the total phenolic content of virgin coconut oil is presented in Table 20. The phenolic content of virgin coconut oil did not differ significantly among treatments which showed that the coconut oil recovered from coconut milk extracted by different methods inoculated with one per cent *Lactobacillus* culture could not influence total phenolic content content of oil.

4.2.2.3. Moisture content

The effect of induced fermentation on the moisture content of virgin coconut oil is presented in Table 20. The moisture content of virgin coconut oil did not differ significantly among treatments which showed that the coconut oil recovered from coconut milk extracted by different methods inoculated with one per cent *Lactobacillus* culture could not influence the moisture content of oil.

4.2.2.4. Oil recovery

The effect of induced fermentation on oil recovery is presented in Table 20. Virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with one per cent of *Lactobacillus* culture and kept for 18, 24 and 36 hours showed significant difference between the treatments. The treatment T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours recorded maximum oil recovery (21.966 per cent) followed by T_3, coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 36 hours (21.300 per cent).

4.2.2.5. Atmospheric temperature

The observations on maximum, minimum temperature (°C) during the period when induced fermentation was undertaken is presented in Table 21. Virgin coconut

oil was recovered by induced fermentation method in November 10 and 11of 2014 and March 7 and 8 of 2015. On January 5 and 6 of 2015, the oil was not separated from the *Lactobacillus* inoculated coconut milk. During November 10 and 11 of 2014, virgin coconut oil was recovered from the *Lactobacillus* inoculated coconut milk when the maximum temperature was 30.55°C and minimum temperature was 23.40°C. On March 7 and 8 of 2015, oil was recovered when the maximum temperature was 32.20°C and minimum temperature was 23.95°C. On January 5 and 6 of 2015, the maximum temperature was 30.40°C and the minimum temperature was 22.15°C and the oil did not separate from the coconut milk.

4.2.2.6. Relative humidity

The observations on maximum and minimum relative humidity during the period of induced fermentation is presented in Table 21. During November 10 and 11 of 2014, the virgin coconut oil was recovered from the induced coconut milk when the average relative humidity was 85.50 per cent and on March 7 and 8 of 2015, virgin coconut oil was recovered when the average relative humidity was 73 per cent (Table 20). On January 5and 6 of 2015, the average relative humidity was 84.50 per cent.

4.2.2.7. Sensory parameters

The effect of induced fermentation method on sensory parameters of virgin coconut oil is depicted in Table 22. The sensory parameters like colour, odour and taste of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2, inoculated with one per cent *Lactobacillus* and kept for 18, 24 and 36 hours did not show any significant difference between treatments.

Table 20. Effect of induced fermentation on free fatty acid (mg KOH/g of oil), total phenolic content (mg catechin equivalent/kg of oil), moisture content (%) and oil recovery (%) of virgin coconut oil

Treatments	Free fatty acid (mg KOH/g of oil)	Total phenolic content (mg catechin equivalent /kg of oil)	Moisture content (%)	Oil recovery (%)
T_1 (CG +CW1:1 + 18h)	0.220	57.666	0.066	16.000
T_2 (CG +CW1:1 + 24h)	0.226	66.000	0.080	18.833
T_3 (CG+ CW1:1 + 36h)	0.220	60.000	0.080	21.300
T_4 (CG +W 1:1 + 18h)	0.200	65.000	0.073	16.566
T_5 (CG + W 1:1 + 24h)	0.226	48.333	0.053	19.166
T_6 (CG+W 1:1 + 36h)	0.200	62.333	0.073	21.966
T_7 (CG+W 1:2 + 18h)	0.220	63.666	0.073	13.633
T_8 (CG +W 1:2 + 24h)	0.233	71.000	0.073	15.200
T_9 (CG +W 1:2 + 36h)	0.226	67.333	0.073	16.933
SE	0.045	7.816	0.025	0.263
CD(0.05)	NS	NS	NS	0.553

Table: 21. Observations on maximum, minimum temperature (°C) and relative humidity (%) during induced fermentation

Month & Date	Atmospheric Temperature (°C)		Average Room temperature (°C)	Relative humidity (%)			Oil recovery
	Max.	Min.	Average	Max.	Min.	Average	
Nov 10,11	30.55	23.40	27.66	89.00	82.00	85.5	Recovered
Jan 5,6	30.40	22.15	26.50	96.00	73.00	84.5	Not recovered
March 7,8	32.20	23.95	29.50	83.00	63.00	73.0	Recovered

Table: 22. Effect of induced fermentation on sensory parameters of virgin coconut oil

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (CG +CW1:1 + 18h)	8.80	7.40	6.20
T_2 (CG +CW1:1 + 24h)	8.70	7.30	6.50
T_3 (CG+ CW1:1 + 36h)	8.80	7.70	6.20
T_4 (CG +W 1:1 + 18h)	8.90	7.40	6.60
T_5 (CG + W 1:1 + 24h)	9.00	7.70	6.30
T_6 (CG+W 1:1 + 36h)	9.00	7.80	6.70
T_7 (CG+W 1:2 + 18h)	8.60	7.50	6.20
T_8 (CG +W 1:2 + 24h)	8.30	7.50	6.20
T_9 (CG +W 1:2 + 36h)	8.30	7.50	6.20
Kruskal Wallis H	10.40	4.87	6.37
	NS	NS	NS

4.3. STANDARDISATION OF CENTRIFUGATION METHOD

4.3.1. Revolutions per minute and time required

The effect of revolutions per minute (rpm) which was expressed in terms of gravitational force (N) for different time on oil recovery is presented in Table 23. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 showed significant difference among the treatments at different rpm. Oil recovery was more for the treatment, T_{21}, coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm with gravitational force of 12096 N for 20 minutes (27.806 per cent) and it was on par with the treatment, T_{20}, coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 15 minutes (27.800 per cent). This was followed by the treatment, T_{12}, coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 10000 rpm for 20 minutes with an oil recovery of 26.933 per cent. Oil recovery was minimum for coconut milk extracted from grated coconut and water in the ratio 1:2 centrifuged at 8000 rpm for 10 min (6.966 per cent).

For further continuation of the experiments with centrifugation, the revolutions per minute was standardised as 12000 rpm with 12096 N gravitational force for 15 minutes.

Table: 23. Effect of rpm (gravitational force - N) and duration (minutes) on oil recovery (%) under centrifugation

Treatments	Oil recovery (%)
T_1 (CG+CW1:1 -8000rpm- 10 minutes) 5376 N	10.066
T_2 (CG+CW1:1 -8000rpm- 15 minutes) 5376 N	18.133
T_3 (CG+CW1:1 - 8000rpm- 20 minutes) 5376 N	21.533
T_4 (CG+W 1:1- 8000rpm- 10 minutes) 5376 N	7.933
T_5 (CG+W 1:1- 8000rpm- 15 minutes) 5376 N	15.900
T_6 (CG+W 1:1- 8000rpm- 20 minutes) 5376 N	19.866
T_7 (CG+W 1:2- 8000rpm- 10 minutes) 5376 N	6.966
T_8 (CG+W 1:2- 8000rpm- 15 minutes) 5376 N	14.166
T_9 (CG+W 1:2- 8000rpm- 20 minutes) 5376 N	15.466
T_{10} (CG+CW1:1 - 10000rpm- 10 minutes) 8400 N	16.566
T_{11} (CG+CW1:1 - 10000rpm- 15 minutes) 8400 N	23.866
T_{12} (CG+CW1:1 - 10000rpm- 20 minutes) 8400 N	26.933
T_{13} (CG+W 1:1- 10000rpm- 10 minutes) 8400 N	10.200

Table: 23. Effect of rpm (gravitational force - N) and duration (minutes) on oil recovery (%) under centrifugation (contd.)

T_{14} (CG+W 1:1- 10000rpm- 15 minutes) 8400 N	19.566
T_{15} (CG+W 1:1- 10000rpm- 20 minutes) 8400 N	22.500
T_{16} (CG+W 1:2- 10000rpm- 10 minutes) 8400 N	8.000
T_{17} (CG+W 1:2- 10000rpm- 15 minutes) 8400 N	16.966
T_{18} (CG+W 1:2- 10000rpm- 20 minutes) 8400 N	21.366
T_{19} (CG+CW1:1 - 12000rpm- 10 minutes) 12096 N	15.600
T_{20} (CG+CW1:1 - 12000rpm- 15 minutes) 12096 N	27.800
T_{21} (CG+CW1:1 - 12000rpm- 20 minutes) 12096 N	27.806
T_{22} (CG+W 1:1- 12000rpm- 10 minutes) 12096 N	17.666
T_{23} (CG+W 1:1- 12000rpm- 15 minutes) 12096 N	22.400
T_{24} (CG+W 1:1- 12000rpm- 20 minutes) 12096 N	22.433
T_{25} (CG+W 1:2- 12000rpm- 10 minutes) 12096 N	16.766
T_{26} (CG+W 1:2- 12000rpm- 15 minutes) 12096 N	21.133
T_{27} (CG+W 1:2- 12000rpm- 20 minutes) 12096 N	21.133
SE	0.293
CD(0.05)	0.582

4.3.2. Free fatty acid

The effect of centrifugation on free fatty acid content (mg KOH/g of oil) of virgin coconut oil recovered is depicted in Table 24. The results showed that there was no significant difference between the treatments on free fatty acid content (mg KOH/g of oil) of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and was dried at 50°C.

4.3.3. Total phenolic content

Table 24 presents the total phenolic content (mg catechin equivalent /kg of oil) of virgin coconut oil recovered by centrifugation method. The treatments did not differ significantly on total phenolic content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and dried at 50°C.

4.3.4. Moisture content

The moisture content of the virgin coconut oil recovered from different treatments by centrifugation is presented in Table 24. The moisture content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and centrifuged at 12000 rpm for 15 minutes and dried at 50°C did not differ significantly between treatments.

4.3.5. Oil recovery

The effect of centrifugation on virgin coconut oil recovery (%) is presented in Table 24. The results showed that there was significant difference on virgin coconut

oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which is centrifuged at 12000 rpm for 15 minutes (plate 12.). The treatment T_1 (CG+ CW 1:1 12000 rpm – 12096 G force) at 15 minutes had maximum oil recovery (28.087 per cent) followed by treatment,T_2 (CG+ W 1:1 12000rpm – 12096 G force) at 15 minutes (22.474 per cent).

4.3.6. Sensory parameters

The effect of centrifugation on sensory parameters of virgin coconut oil is presented in Table 25. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and was dried at 50°Cdid not show any significant difference between the treatments on sensory parameters like colour, odour and taste.

4.4. COMPARISON OF VIRGIN COCONUT OIL PRODUCED BY FERMENTATION, INDUCED FERMENTATION AND CENTRIFUGATION WITH TRADITIONAL BOILING METHOD

4.4.1. Quality parameters

4.4.1.1. Oil recovery

The effect of different methods of extraction on oil recovery is compared and presented in Table 26. A significant variation in oil recovery was noticed between various methods of extraction. Among the treatments virgin coconut oil recovered from centrifugation method was found to be significantly superior (28.180 per cent) compared to other methods. However, oil recovered from fermentation method also gave better oil recovery (22.960 per cent) followed by induced fermentation (22.340 per cent) and traditional boiling method (22.080 per cent).

Table: 24. Effect of centrifugation on free fatty acid content (mg KOH/g of oil), total phenolic content (mg catechin equivalent /kg of oil), moisture content (%) and oil recovery (%).

Treatments	Free fatty acid (mg KOH/g of oil)	Total Phenolic content (mg catechin equivalent /kg of oil)	Moisture content (%)	Oil recovery (%)
T_1 (CG+ CW 1:1 12000rpm – 12096 G force at 15 mints)	0.208	56.800	0.065	28.087
T_2 (CG+ W 1:1 12000rpm – 12096 G force at 15 mints)	0.208	62.000	0.072	22.474
T_3 (CG+ W 1:2 12000rpm – 12096 G force at 15 mints)	0.224	59.200	0.072	19.687
SE	0.045	5.850	0.020	0.330
CD(0.05)	NS	NS	NS	0.810

Table: 25. Effect of centrifugation on sensory parameters of virgin coconut oil

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (CG + CW 1:1)	8.9	8	7.9
T_2 (CG + W 1:1)	8.9	7.8	7.7
T_3 (CG + W 1:2)	8.9	7.8	7.5
Kruskal Wallis H	0.00	0.27	1.46
	NS	NS	NS

Table: 26. Effect of different methods of extraction on oil recovery (%)

Treatments	Oil recovery (%)
T_1 (Fermentation)	22.960
T_2 (Induced fermentation)	22.340
T_3 (Centrifugation)	28.180
T_4 (Traditional boiling method)	22.080
SE	0.158
CD (0.05)	0.341

4.4.1.2. Moisture content

The effect of different methods of extraction of virgin coconut oil on moisture content (per cent) of virgin coconut oil is depicted in Table 27. The mean value for moisture content of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method did not show any significant difference among the treatments. The lower mean value of moisture content was noticed for virgin coconut oil recovered from traditional boiling method (0.052) and the higher value was noticed for virgin coconut oil recovered from fermentation and induced fermentation method (0.068).

4.4.1.3. Refractive index

The effect of different methods of extraction of virgin coconut oil on refractive index of virgin coconut oil is presented in Table 27. There was a significant difference in refractive index of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. The higher mean value was noticed for the treatment T_4, traditional boiling method (1.453) and lowest refractive index was obtained for the treatment centrifugation (1.421).

4.4.1.4. Specific gravity

The effect of different methods of extraction of virgin coconut oil on specific gravity at 30°C of virgin coconut oil is presented in Table 27. No significant difference was found in specific gravity of virgin coconut oil recovered by fermentation, induced fermentation, centrifugation and traditional boiling method. However, the higher mean value was noticed for the treatment T_2, induced fermentation method (0.917) and lowest specific gravity was obtained for the treatment T_4, traditional boiling method (0.912).

Table 27. Effect of different methods of extraction on quality parameters of virgin coconut oil

Treatments	Moisture content (%)	Refractive index at 40°C	Specific gravity at 30°C
T_1 (Fermentation)	0.068	1.446	0.916
T_2 (Induced fermentation)	0.068	1.448	0.917
T_3 (Centrifugation)	0.064	1.421	0.916
T_4 (Traditional boiling method)	0.052	1.453	0.912
SE	0.020	0.755	0.727
CD (0.05)	NS	0.006	NS

4.4.1.5. Relative density

Relative density of virgin coconut oil produced by different methods of extraction is presented in Table 28. Virgin coconut oil extracted by different methods of extraction did not show any significant difference between fermentation, induced fermentation, centrifugation and traditional boiling method. The value of relative density ranged from 0.912 to 0.917.

4.4.1.6. Colour

The effect of different methods of extraction of virgin coconut oil on colour (yellowing index) is presented in Table 28. A significant difference in colour of virgin coconut oil as represented as yellowing index was noticed among different methods of extraction like fermentation, induced fermentation, centrifugation and traditional boiling method. The yellowing index was highest for the traditional method (7.810) and lowest for the treatment, T_1, fermentation (1.616) and was on par with T_3, centrifugation (1.620) and T_2, induced fermentation (1.650).

4.4.1.7. Iodine value

Iodine value of virgin coconut oil recovered by different methods of extraction is presented in Table 28. There was no significant difference in iodine value of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. However, higher iodine value was noticed for the treatment, T_4, traditional boiling method (6.140g of iodine/ 100g of oil) and lower mean value was noticed for the treatment, T_2, induced fermentation method (5.532g of iodine/ 100g of oil).

4.4.1.8. Unsaponifiable matter

The effect of different methods of extraction of virgin coconut oil on unsaponifiable matter (per cent by weight) is presented in Table 28. Virgin coconut

oil recovered from fermentation method (0.360 per cent) showed significant superiority and was on par with centrifugation compared to virgin coconut oil recovered by traditional boiling method and induced fermentation method.

4.4.1.9. Acid value

Acid value of virgin coconut oil produced by different methods of extraction is presented in Table 29. A significant difference in acid value of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method was noticed in the present experiment. Virgin coconut oil recovered from centrifugation (0.200mg KOH/g of oil) showed significantly superior mean value and was on par with induced and fermentation method compared to virgin coconut oil recovered from traditional boiling method.

4.4.1.10. Polenske value

The effect of different methods of extraction on polenske value of virgin coconut oil produced by different methods is presented in Table 29. Virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method did not show any significant difference in polenske value.

4.4.1.11. Peroxide value

The effect of different methods of extraction on peroxide value of virgin coconut oil produced by different methods is presented in Table 29. There was a significant difference in peroxide value of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. The peroxide value of virgin coconut oil recovered from centrifugation (0.196 meq/ kg of oil) was on par with fermentation method 0.208 meq/ kg of oil and significantly differed from other two methods.

Table: 28. Effect of different methods of extraction on quality parameters of virgin coconut oil (contd.)

Treatments	Relative density	Colour (Yellowing index)	Iodine value (g of iodine/ 100g of oil)	Unsaponifiable matter (percent by weight)
T_1 (Fermentation)	0.916	1.616	5.582	0.360
T_2 (Induced fermentation)	0.917	1.650	5.532	0.398
T_3 (Centrifugation)	0.916	1.620	5.736	0.368
T_4 (Traditional boiling method)	0.912	7.810	6.140	0.404
SE	0.727	0.040	0.286	0.035
CD (0.05)	NS	0.095	NS	0.023

4.4.1.12. Saponification value

Saponification valueof virgin coconut oil produced by different methods of extraction is presented in the Table 29. No significant difference in the saponification value was noted among the treatments.

4.4.1.13. Sensory parameters

The effect of different methods of extraction on sensory parameters of virgin coconut oil is presented in Table 30. The sensory parameters like colour, odour and taste showed significant difference for the virgin coconut oil recovered by different methods.

4.4.1.13.1 Colour

Among the treatments, virgin coconut oil recovered from induced fermentation and centrifugation method showed higher score (9.00) and the treatment, T_4, traditional boiling method showed lowest score (6.30) for the sensory parameter colour (Table 30).

4.4.1.13.2. Odour

Virgin coconut oil recovered from traditional boiling method showed highest taste scores (9.00) and the treatment induced fermentation method showed the lowest taste score (7.80) (Table 30).

4.4.1.13.3. Taste

Virgin coconut oil recovered from traditional boiling method showed significantly superior mean value score (8.90) for taste. The lowest score was noticed for the virgin coconut oil recovered from fermentation and induced fermentation (6.80) method (Table 30).

Table: 29. Effect of different methods of extraction on quality parameters of virgin coconut oil (contd.)

Treatments	Acid value (mg KOH/g of oil)	Polenske value	Peroxide value (meq/ kg of oil)	Saponification value (mg/g of oil)
T_1 (Fermentation)	0.220	13.0	0.208	262.428
T_2 (Induced fermentation)	0.220	13.0	0.220	262.428
T_3 (Centrifugation)	0.200	13.2	0.196	262.428
T_4 (Traditional boiling method)	0.380	13.2	0.240	262.652
SE	0.045	0.2	0.020	0.386
CD (0.05)	0.059	NS	0.014	NS

Table: 30. Effect of different methods of extraction on sensory parameters of virgin coconut oil

Treatments	Mean sensory scores		
	Colour	Odour	Taste
T_1 (Fermentation)	8.90	8.00	6.80
T_2 (Induced fermentation)	9.00	7.80	6.80
T_3 (Centrifugation)	9.00	8.00	8.00
T_4 (Traditional boiling method)	6.30	9.00	8.90
Kruskal Wallis H	13.53***	14.37***	29.12***

4.4.1.14. Antioxidant properties

4.4.1.14.1. Total phenolic content

The effect of different methods of extraction on total phenolic content of virgin coconut oil is presented in Table 31. A significantly superior difference was noticed in the virgin coconut oil produced by centrifugation method (71.0mg catechin equivalent /kg of oil) and it was on par with virgin coconut oil produced by fermentation and induced fermentation method. The lower value was obtained for virgin coconut oil produced by traditional boiling method (54.0mg catechin equivalent /kg of oil).

4.4.1.14.2. DPPH radical scavenging activity

The effect of different methods of extraction on DPPH radical scavenging activity of virgin coconut oil is presented in Table 32. Inhibition per cent was more for the virgin coconut oil recovered from fermentation method followed by induced fermentation and centrifugation method. Virgin coconut oil recovered from traditional boiling method showed the lower value of DPPH radical scavenging activity.

4.4.1.14.3. Total antioxidant activity

The effect of different methods of extraction on total antioxidant activity (per cent) of virgin coconut oil is presented in Table 33. Total antioxidant activity was more for the virgin coconut oil recovered from fermentation method followed by induced fermentation and centrifugation. Virgin coconut oil recovered from traditional boiling method showed the lower value for total antioxidant activity.

Table: 31. Effect of different methods of extraction on antioxidant properties of virgin coconut oil - Total phenolic content

Treatments	Total phenolic content(mg catechin equivalent /kg of oil)
T_1 (Fermentation)	70.2
T_2 (Induced fermentation)	70.4
T_3 (Centrifugation)	71.0
T_4 (Traditional boiling method)	54.0
SE	3.21
CD (0.05)	6.82

Table: 32. Effect of different methods of extraction on DPPH radical scavenging activity (%) of virgin coconut oil

Concentration	Inhibition Percentage (%)			
	Fermentation method	Induced fermentation method	Centrifugation method	Traditional boiling method
5	40.5	36.6	35.3	34
10	43.2	38	37.9	37.4
15	44.9	41.8	41.3	40.5
20	45.6	43.5	44.1	43.3
25	48.9	46.4	46.2	45.8
30	51.5	50.4	49.3	47.3
35	54.6	53.8	52	50.9

Table: 33. Effect of different methods of extraction on Total antioxidant activity (%) of virgin coconut oil

Concentration	Inhibition Percentage			
	Fermentation method	Induced fermentation method	Centrifugation method	Traditional boiling method
5	42.6	37.6	35.3	33.1
10	45.2	41.4	38.2	36.5
15	47.6	44.4	42.7	38.9
20	50.9	47.7	45.1	42.6
25	56.6	49.3	48.9	45.6
30	59.0	52.1	50.1	48.8
35	62.3	55.8	53.4	52.3

4.4.1.14.4. Reducing power

Reducing power is more for the virgin coconut oil recovered from fermentation method followed by induced fermentation and centrifugation method (Table 34). Virgin coconut oil recovered from traditional boiling method showed the lower absorbance value for reducing power. As the absorbance value increases, reducing power also increases.

4.4.1.14.5. IC_{50} value

The IC_{50} value of virgin coconut oil recovered from different extraction methods is presented in Table 35. The value was minimum for virgin coconut oil recovered from fermentation method and had the best antioxidant property followed by virgin coconut oil recovered from induced fermentation, centrifugation and traditional boiling method.

4.4.2. Total plate count

Microbial load present in the virgin coconut oil produced by different methods of extraction after drying is found to be nil in all the media (Table 36). However, virgin coconut oil recovered from induced fermentation method showed 2×10^2 colony forming units of *Lactobacillus in Lactobacillus* selection agar media.

4.4.3. Shelf life

4.4.3.1. Moisture percentage

The effect of different methods of extraction on moisture content of virgin coconut oil under storage is presented in Table 37. No significant difference was noticed in moisture content of virgin coconut oil under storage. However, moisture content increased with the time period. The lower moisture content was noticed in virgin coconut oil recovered from traditional boiling method for the initial period (0.052 per cent) and it was increased after 3 months (0.272 per cent) and 6 months

(0.352 per cent). Virgin coconut oil recovered from other methods also moisture content increased with the time period.

4.4.3.2. Total plate count

The effect of different methods of extraction on microbial load (cfu/ml) of virgin coconut oil under storage is presented in Table 38 and Table 39. Microbial load of virgin coconut oil recovered from different methods under storage showed an increasing rate with the time period. However in virgin coconut oil recovered from fermentation method, 9 $\times 10^2$ cfu ml^{-1} *Lactobacillus* colony forming units which was increased to 22×10^2 cfu ml^{-1} after 3 months (Table 38). Also in virgin coconut oil recovered from traditional boiling method, bacterial colony forming units (7×10^5 cfu ml^{-1}) was found to be less after 3 months (Table 39).

4.4.3.3. Acid value

The effect of different methods of extraction on acid value (mg KOH/g of oil) of virgin coconut oil under storage is presented in Table 40. Acid value of virgin coconut oil recovered from different methods showed significant difference among the treatments under storage. Acid value increased with the time period. The lower acid value was noticed in virgin coconut oil recovered from centrifugation method for the initial period (0.200mg KOH/g of oil) and it was increased after 3 months (0.320mg KOH/g of oil) and 6 months (0.400 mg KOH/g of oil). For other treatments also acid value increased with the time period.

Table: 34. Effect of different methods of extraction on reducing power of virgin coconut oil

Concentration	Absorbance			
	Fermentation method	Induced fermentation method	Centrifugation method	Traditional boiling method
25	0.047	0.043	0.038	0.032
50	0.052	0.049	0.043	0.035
75	0.06	0.052	0.047	0.038
100	0.065	0.059	0.051	0.041
125	0.073	0.063	0.055	0.044
150	0.079	0.068	0.059	0.048

Table: 35. Effect of different methods of extraction of virgin coconut oil on Inhibitory Concentration 50 (IC 50) values

Treatments	IC 50 Values (µg/ml)
T_1 (Fermentation)	27
T_2 (Induced fermentation)	30
T_3 (Centrifugation)	31
T_4 (Traditional boiling method)	33

Table: 36. Microbial load (cfu/ml) of virgin coconut oil produced by different methods of extraction

Treatments	Bacteria (x 10^5)	Fungi (x 10^3)	Actinomycetes (x 10^3)	*Lactobacillus* (x 10^2)	Yeast (x 10^2)	Coliforms(x 10^3)
T_1 (Fermentation)	0	0	0	0	0	0
T_2 (Induced fermentation)	0	0	0	2	0	0
T_3 (Centrifugation)	0	0	0	0	0	0
T_4 (Traditional boiling method)	0	0	0	0	0	0

Table: 37. Effect of different methods of extraction on moisture content (%) of virgin coconut oil under storage

Treatments	Moisture content (%) Initial	Moisture content (%) - After 3 months	Moisture content (%) - After 6 months
T_1 (Fermentation)	0.068	0.296	0.388
T_2 (Induced fermentation)	0.068	0.292	0.384
T_3 (Centrifugation)	0.064	0.284	0.364
T_4 (Traditional boiling method)	0.052	0.272	0.352
SE	0.020	0.040	0.045
CD (0.05)	NS	NS	NS

Table: 38. Observations on microbial load (cfu/ml) of virgin coconut oil produced by fermentation and induced fermentation under storage

Microorganisms (cfu/ml)	Microbial load in fermentation method (cfu/ml)			Microbial load in induced fermentation method (cfu/ml)		
	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months
Bacteria ($x 10^5$)	0	48	121	0	63	133
Fungi ($x 10^3$)	0	28	49	0	35	84
Actinomycetes ($x 10^3$)	0	15	41	0	27	38
Lactobacillus ($x 10^2$)	0	9	22	2	72	156
Yeast ($x 10^2$)	0	27	53	0	76	167

Table: 39. Microbial load (cfu/ml) of virgin coconut oil produced by centrifugation and traditional method under storage

Microorganisms (cfu/ml)	Microbial load in centrifugation method (cfu/ml)			Microbial load in traditional method (cfu/ml)		
	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months
Bacteria ($x 10^5$)	0	11	25	0	7	17
Fungi ($x 10^3$)	0	25	58	0	31	65
Actinomycetes ($x 10^3$)	0	38	72	0	32	69
Lactobacillus ($x 10^2$)	0	12	29	0	18	48
Yeast ($x 10^2$)	0	23	51	0	78	153

Table: 40. Effect of different methods of extraction on the acid value (mg KOH/g of oil) of virgin coconut oil under storage

Treatments	Acid value (mg KOH/g of oil) Initial	Acid value (mg KOH/g of oil) - After 3 months	Acid value (mg KOH/g of oil) After 6 months
T_1 (Fermentation)	0.220	0.340	0.440
T_2 (Induced fermentation)	0.220	0.340	0.440
T_3 (Centrifugation)	0.200	0.320	0.400
T_4 (Traditional boiling method)	0.380	0.440	0.600
SE	0.045	0.028	0.02
CD (0.05)	0.059	0.019	0.014

4.4.3.4. Peroxide value

The effect of different methods of extraction on peroxide value (meq/ kg of oil) of virgin coconut oil under storage is presented in Table 41. Peroxide value of virgin coconut oil recovered from different methods showed significant difference among the treatments under storage. Peroxide value increased with the time period. The lower peroxide value was noticed in centrifugation method for the initial period (0.196meq/ kg of oil) and it was increased after 3 months (0.560meq/ kg of oil) and 6 months (0.840meq/ kg of oil). For virgin coconut oil recovered from other methods also peroxide value increased with the time period.

4.4.3.5. Saponification value

The effect of different methods of extraction on saponification value (mg/g of oil) of virgin coconut oil under storage is presented in Table 42. No significant difference was noticed in saponification value of virgin coconut oil under storage. The higher saponification value was noticed in traditional boiling method for the initial period (262.652mg/g of oil) and it was decreased after 3 months (242.798mg/g of oil) and 6 months (222.592mg/g of oil). For other treatments also saponification value decreased with the time period.

4.4.3.6. Total phenolic content

The effect of different methods of extraction on total phenolic content (mg catechin equivalent /kg of oil) of virgin coconut oil under storage is presented in Table 43. Total phenolic content showed significant difference among the treatments. However, total phenolic content decreased with the time period. The maximum total phenolic content was noticed in virgin coconut oil recovered from centrifugation method for the initial period (71.0mg catechin equivalent /kg of oil) and it was decreased after 3 months (68.4mg catechin equivalent /kg of oil) and 6

months (60.0mg catechin equivalent /kg of oil). For other treatments also total phenolic content decreased with the time period.

4.4.3.7. Sensory parameters

The sensory parameters like colour, odour and taste of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method is presented in Table 44, 45 and 46. The observation showed significant difference among the treatments.

4.4.3.7.1. Colour

Virgin coconut oil recovered from induced fermentation and centrifugation method showed maximum score (9.00) and the treatment traditional boiling method showed the minimum score (6.30) for the parameter colour (Table 44).

4.4.3.7.2. Odour

Virgin coconut oil recovered from traditional boiling method showed maximum score (9.00) and the treatment induced fermentation method showed the minimum score (7.80) (Table 45).

4.4.3.7.3. Taste

Virgin coconut oil recovered from traditional boiling method showed maximum score (8.90) for taste. The minimum score for taste was obtained for virgin coconut oil recovered from the treatment fermentation and induced fermentation (6.80) method. All the sensory parameters decreased with the time period (Table 46).

Table: 41. Effect of different methods of extraction on the peroxide value (meq/ kg of oil) of virgin coconut oil under storage

Treatments	Peroxide value (meq/ kg of oil) Initial	Peroxide value (meq/ kg of oil) After 3 months	Peroxide value (meq/ kg of oil) After 6 months
T_1 (Fermentation)	0.208	0.582	0.850
T_2 (Induced fermentation)	0.220	0.604	0.860
T_3 (Centrifugation)	0.196	0.560	0.840
T_4 (Traditional boiling method)	0.240	0.670	0.890
SE	0.020	0.045	0.034
CD(0.05)	0.014	0.051	0.027

Table: 42. Effect of different methods of extraction on the saponification value (mg/g of oil) of virgin coconut oil under storage

Treatments	Saponification value (mg/g of oil) - Initial	Saponification value (mg/g of oil) - After 3 months	Saponification value (mg/g of oil) - After 6 months
T_1 (Fermentation)	262.428	242.462	222.268
T_2 (Induced fermentation)	262.428	242.574	222.376
T_3 (Centrifugation)	262.428	242.462	222.268
T_4 (Traditional boiling method)	262.652	242.798	222.592
SE	0.386	0.173	0.161
CD (0.05)	NS	NS	NS

Table: 43. Effect of different methods of extraction on the total phenolic content (mg catechin equivalent /kg of oil) of virgin coconut oil under storage

Treatments	Phenolic content(mg catechin equivalent /kg of oil) - Initial	Phenolic content (mg catechin equivalent /kg of oil) - After 3 months	Phenolic content (mg catechin equivalent /kg of oil) - After 6 months
T_1 (Fermentation)	70.2	65.6	59.0
T_2 (Induced fermentation)	70.4	66.4	59.2
T_3 (Centrifugation)	71.0	68.4	60.0
T_4 (Traditional boiling method)	54.4	49.0	44.6
SE	3.21	3.92	3.32
CD (0.05)	6.82	8.32	7.04

Table: 44. Effect of different methods of extraction on sensory parameter (colour) of virgin coconut oil under storage

Treatments	Mean sensory scores for colour		
	Initial	After 3 months	After 6 months
T_1 (Fermentation)	8.90	8.40	8.40
T_2 (Induced fermentation)	9.00	8.20	8.30
T_3 (Centrifugation)	9.00	8.40	8.40
T_4 (Traditional boiling method)	6.30	4.70	4.40
Kruskal Wallis H	13.53***	19.12***	19.15***

Table: 45. Effect of different methods of extraction on sensory parameter (odour) of virgin coconut oil under storage

Treatments	Mean sensory scores for Odour		
	Initial	After 3 months	After 6 months
T_1 (Fermentation)	8.00	4.70	4.30
T_2 (Induced fermentation)	7.80	4.60	4.30
T_3 (Centrifugation)	8.00	6.20	4.90
T_4 (Traditional boiling method)	9.00	6.80	5.90
Kruskal Wallis H	14.37***	28.47***	20.96***

Table 46. Effect of different methods of extraction on sensory parameter (taste) of virgin coconut oil under storage

Treatments	Mean sensory scores for taste		
	Initial	After 3 months	After 6 months
T_1 (Fermentation)	6.80	4.70	4.20
T_2 (Induced fermentation)	6.80	4.90	4.50
T_3 (Centrifugation)	8.00	5.90	5.10
T_4 (Traditional boiling method)	8.90	7.20	6.30
Kruskal Wallis H	29.12***	28.37***	24.26***

4.4.4. Economics of production

Economics of production of virgin coconut oil by different methods is depicted in Table 47. For the processing of 100kg coconut per day for one year, the cost of production was Rs. 22,65,190 for the centrifugation method. The income calculated comes to Rs. 57,96,900 and accordingly net profit Rs.35,31,710 was expected more from the centrifugation method. The benefit cost ratio was more (2.55) for centrifugation method compared to other methods.

Table: 47. Economics of production of virgin coconut oil by different methods

Treatments	Total cost (Rupees)	Income (Rupees)	Net profit	BC Ratio
T1 (Fermentation)	9,46,090	23,70,000	14,23,910	2.50
T2 (Induced fermentation)	11,28,690	22,97,700	11,69,010	2.03
T3 (Centrifugation)	22,65,190	57,96,900	35,31,710	2.55
T4 (Traditional boiling method)	19,86,240	36,20,549	16,34,309	1.82

DISCUSSION

5. DISCUSSION

The study entitled as “Cost effective technology for home scale and small scale production of virgin coconut oil” which included standardisation of fermentation method, induced fermentation method, centrifugation method and comparison of virgin coconut oil produced by fermentation, induced fermentation and centrifugation with traditional boiling method, the results of which are discussed here based on the experiments conducted during the year 2013- 2015.

5.1. STANDARDISATION OF FERMENTATION METHOD

5.1.1. Standardisation of drying temperature

5.1.1.1. Moisture percentage

The effect of drying temperature on moisture content of virgin coconut oil produced by fermentation method is presented in Table 2. The results showed that virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 did not show any significant difference in moisture percentage. There were no significant difference in moisture content of virgin coconut oil dried at 40°C, 45°C and 50°C (group 1,2,3). This might be because drying of virgin coconut at 40°C, 45°C and 50°C (group 1,2,3) were carried out till constant weight of oil was attained and hence there was not much difference in the moisture content. The interaction effect of different treatments with different drying temperatures also did not make any significant change in the moisture percentage.

Moisture content is an important character 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 (Mansor *et al*., 2012). In the present experiment, the moisture content of the treatments ranged from 0.066 to 0.093 percentage and was with in the limit of 0.1 per cent as prescribed by the APCC standards for virgin coconut oil (APCC, 2003). Bawalan and Chapman (2006) reported that the

moisture content of oil recovered from modified natural fermentation method as 0.12 per cent and below. According to Dayrit *et al*. (2011) at low moisture levels less than 0.06 per cent, virgin coconut oil was stable to microbial decomposition.

5.1.1.2. Free fatty acid content

The virgin coconut oil extracted from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours did not show any significant difference (Table 3). The treatment in which coconut milk extracted from grated coconut with water in the ratio 1:2 which was kept for 24 hours for fermentation showed lower free fatty acid content (0.206mg KOH/g oil). The free fatty acid content of virgin coconut oil produced by different fermentation methods and dried at 40°C, 45°C and 50°C did not show any significant difference. This might be because of the less variation in temperature ranging from 40°C- 50° C. The interaction of treatments and drying temperature also did not significantly affect the free fatty acid content.

According to Madhavan *et al*. (2005) the acid value of the oil extracted by fermentation method ranged from 0.263-0.434 mg KOH/g oil. Virgin coconut oil produced by the fermentation method would have higher free fatty acid content due to the action of lipolytic enzymes, which was enhanced by the addition of water (Lalas and Tsaknis, 2002). Elizebath *et al*. (2011) observed that the samples produced by fermentation method were found to have higher levels of acetic acid and free fatty acids compared to virgin coconut oil produced using the centrifuge and expeller methods. The free fatty acid value of virgin coconut oil extracted by fermentation method recorded the lowest free fatty acid value 0.29 ± 0.02 mg KOH/1g fat (Mansor *et al*., 2012).

5.1.1.3. Total phenolic content

The effect of drying temperature on total phenolic content of virgin coconut oil produced by fermentation is presented in Table 4. The total phenolic content of virgin coconut oil recovered from coconut gratings and coconut water

in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours did not show any significant difference. Total phenolic content of virgin coconut oil dried at 40°C, 45°C and 50°C also did not differ significantly in the total phenolic content between the temperatures. The interaction effect of treatments and different temperature also could not make any significant difference on the total phenolic content. This showed that the virgin coconut oil extracted by different methods resulted in the same content of soluble total phenols. The drying temperatures 40°C,45°C and 50°C were within a small range and comparatively lower. The nonsignificance of drying temperatures might be because the temperature was not higher enough to make any significant effect on total phenolic content.

According to Vermont *et al*. (2005) the total phenolic content of the laboratory produced virgin coconut oil ranged from 22.88 to 91.90 mg catechin equivalent per kg oil while that of the commercial virgin coconut oil was 35.26 to 49.07 mg catechin per kg oil. Arlee *et al*. (2013) reported that total phenolic contents of the virgin coconut oil were 48.17 to 57.89 mg GAE/100 g oil.

Marina *et al*. (2009) also conducted a study on commercial virgin coconut oil in Malaysian and Indonesian markets and confirmed that total phenolic contents of virgin coconut oil samples (7.78–29.18 mg GAE/100 g oil) were significantly higher than refined, bleached and deodourized coconut oil (6.14 mg GAE/100 g oil) and concluded that virgin coconut oil was as good as refined, bleached and deodorized coconut oil in chemical properties with the added benefit of being higher in phenolic content. The refined bleached and deodourized process being applied through dry method had considerably destroyed some of the phenolic compound in the coconut oil.

Kapila *et al*. (2009) was of the view that in addition to the method of extraction, the components of the endosperm may also play an important role in determining the final phenol content of coconut oil.

5.1.1.4. Oil recovery

The effect of drying temperature on oil recovery of virgin coconut oil produced by fermentation is depicted in Fig.1. The oil recovery of virgin coconut oil from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours showed significant difference among treatments, groups and their interaction. T_5 (CG+W 1:1-36 hours) showed maximum oil recovery (20.42 per cent) and it was on par with T_6 (CG+W 1:1-48 hours) which had 20.40 per cent. The more recovery for these treatments might be due to effective destabilisation of the coconut emulsion that happened from 36- 48 hours.

Madhavan *et al*. (2005) reported the quality of virgin coconut oil produced by the fermentation of coconut milk from freshly harvested coconuts. The oil recovery from fermentation method was about 28-35 per cent of the volume of coconut milk. Bawalan and Chapman (2006) reported that by modified natural fermentation method about 19.8 Kg oil recovered from 100 Kg of fresh grated meat. According to Satheesh and Prasad (2012) the oil recovery from natural fermentation method was 25.68± 0.963 per cent.

5.1.1.5. Sensory parameters

5.1.1.1.1. Colour

The effect of drying temperature on the colour of virgin coconut oil produced by fermentation is presented in Table 6. The sensory evaluation for colour showed significant difference among different treatments but the colour did not vary among temperatures (groups). The treatment drying temperature interaction significantly influenced the colour of virgin coconut oil produced. The virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours recorded the highest score (8.87). The colour measurement is important because it reflects the quality, consistency and safety of the virgin coconut oil. Villarino *et al*. (2007) observed that fermented samples had the highest colour scores among the virgin coconut oil samples.

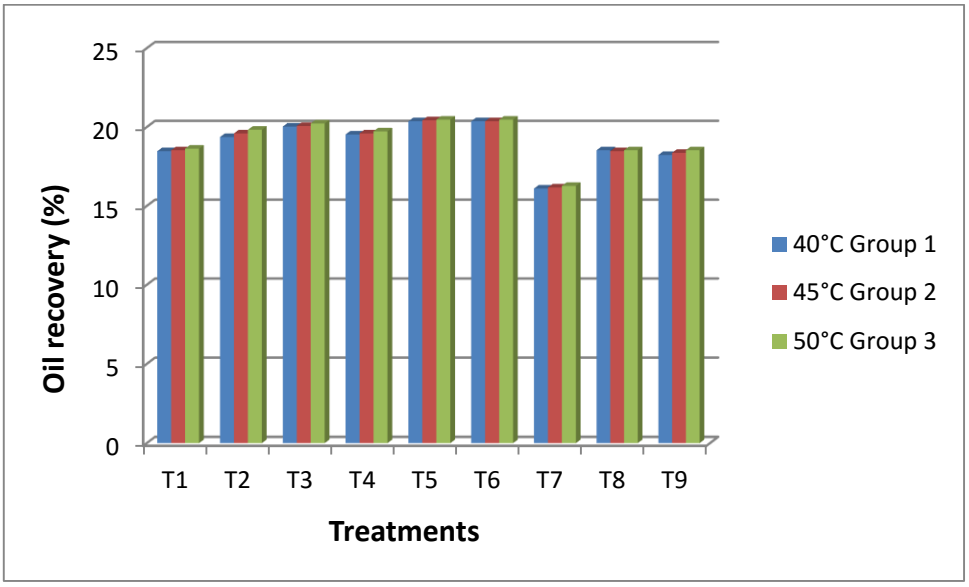

Fig.1. Effect of drying temperature on oil recovery (%) of virgin coconut oil produced by fermentation

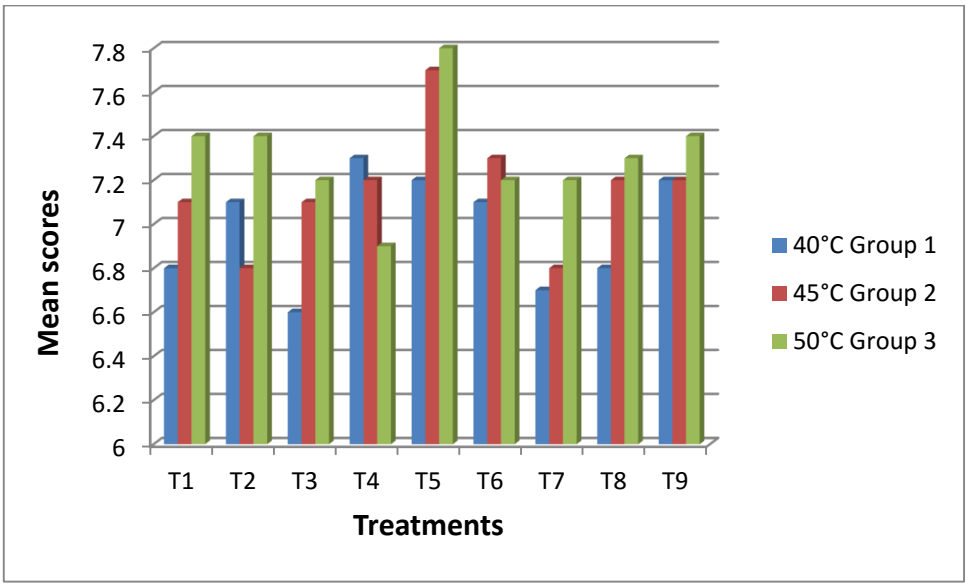

Fig.2. Effect of drying temperature on sensory parameter (odour) of virgin coconut oil produced by fermentation

5.1.1.1.2. Odour

Significant difference among odour was noticed in the virgin coconut oil recovered from coconut milk extracted with grated coconut and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and kept for 24, 36 and 48 hours among treatments, groups and their interaction (Fig.2). Virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours scored maximum scores (7.60) and minimum scores (6.90) noticed in virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:2 and kept for 24 hours. The difference in odour rating samples might be attributed to the processes applied. At 36 hours much of the fermentation might have occurred and many of the constituents might have been extracted which resulted in better odour from that treatment compared to others.

Among the drying temperature, the virgin coconut oil dried at 50°C recorded the highest odour rating of 7.31 compared to the virgin coconut oil dried at 40°C (6.98). The drying temperature 50°C might have killed the microorganisms in virgin coconut oil and prevented further processes which resulted in producing better odour in that temperature. According to Villarino *et al*. (2007), sensory analysis showed that virgin coconut oil produced by fermentation (with and without heat) could be distinguished from those produced using the expeller and centrifuge methods due to their higher acid and rancid aromas.

5.1.1.1.3. Taste

The effect of drying temperature on the taste of virgin coconut oil produced by fermentation method is presented in Table 8. The sensory evaluation for taste showed significant difference among different treatments, groups and their interaction. Virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours showed maximum scores (6.87). The virgin coconut oil dried at a temperature of 50°C recorded

maximum scores (6.36) and it was on par with the drying temperature 45°C (6.26). The better taste might be due to the better drying at these temperatures. According to Villarino *et al*. (2007) the virgin coconut oil samples produced by fermentation, fermentation with heat and centrifugation with heat had detectable sweet taste and nuty flavour compared to refined, bleached and deodourised coconut oil which had slight salty taste with no perceivable flavour.

From the above experiment drying temperature of 50°C in hot air oven was found to be the best and was recommended for the further experiments. The moisture percentage, free fatty acid content and total phenolic content did not vary significantly between the virgin coconut oil extracted by different fermentation methods and dried at 40°C, 45°C and 50°C. However the oil recovery and the sensory parameters like odour and taste was significantly higher for virgin coconut oil recovered from coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and dried at 50°C. Since the different temperatures could not make any significant change in physico chemical characteristics like moisture content, free fatty acid and total phenolic content and the drying temperature of 50°C in hot air oven produced superior odour, taste and higher oil recovery, drying temperature of 50°C was selected for further experiments.

5.1.2. Standardisation of fermentation method at different seasons

5.1.2.1. Oil recovery

The effect of fermentation methods at different season on oil recovery is presented in Table 9. On June- July, the oil recovery showed significant difference among the treatments. T_5, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours recorded the higher oil recovery (20.466 per cent) which was on par with T_3, coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours (20.100 per cent).

During March- April, maximum oil recovery was noticed in T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 48 hours (23.066 per cent) and was on par with T_5 treatment coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours and T_3 coconut milk extracted from grated coconut with coconut water in the ratio 1:1 and kept for 48 hours.

The oil recovered from coconut milk extracted using grated coconut and water in the ratio 1:1 and coconut water in the ratio 1:1 required 36-48 hours for the total destabilisation of coconut milk and for the complete separation of oil by the breakdown of coconut emulsion.

The absence of separation of oil from coconut milk during November-December might be due to the climatic factors prevailed during that period. According to Carmen *et al.* (1970) approximately 60 per cent of the milk produced from individual coconuts showed a breaking of emulsion when fermented under controlled conditions. Fourty per cent failed to break, indicating that some factors responsible for coconut emulsion stability remained uncontrolled during fermentation. The optimum dilution range for rapid fermentation of coconut milk and separation of oil and protein was found to be 1:1 to 1:2 (w/v) coconut meat/water. According to Bawalan (2011) in the fresh-wet modified natural fermentation method the coconut milk mixture produced premium grade virgin coconut oil when the coconut milk mixture was kept for 12–16 hours in a place where the temperature was maintained at 35°– 40°C.

According to Satheesh and Prasad (2014) in natural fermentation process of virgin coconut oil production, normal flora of microorganisms will ferment the milk and separates the coconut oil on the top portion within 24 - 48 hours.

5.1.2.2. Moisture content

The effect of different fermentation method at different season on the moisture content of virgin coconut oil recovered from coconut milk extracted

from coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours is presented in Table 10. On June- July and March- April, the moisture percentage of virgin coconut oil extracted by fermentation method did not show any significant difference between the treatments. The virgin coconut oil recovered by different methods of extraction was dried at a constant temperature of 50° C till constant weight was obtained and found that it was not influenced by different methods of extraction. Bawalan and Chapman (2006) reported that the moisture content of oil recovered from modified natural fermentation method is 0.12 per cent and below.

5.1.2.3. Sensory parameters

The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours did not show any significant difference in colour and odour for the oil recovered during June- July (Table 11). There was significant difference in the taste of virgin coconut oil extracted by different methods of fermentation in June - July. The treatment, T_5 (CG+ W 1:1- 36 hours) recorded the highest score for taste of virgin coconut oil recovered during June-July (7.2) compared to other treatments.

The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 50°C during March- April did not show any significant difference in sensory parameters like colour, odour and taste (Table 12).

5.1.2.4. Atmospheric temperature

The observations on maximum and minimum atmospheric temperature during the fermentation period in different season is shown in Table 13. The maximum atmospheric temperature during the fermentation period when the oil was recovered in the month of June- July and March- April ranged from 31.05°C

to 32.60°C. The minimum atmospheric temperature during the fermentation period when oil was recovered in the month of June- July and March- April ranged from 23.25°C - 25.80°C. The oil did not recover at maximum atmospheric temperature below 31.05°C. The oil did not recover at atmospheric minimum temperature of 24.60°C, 23.75°C, 22.80°C, 23.25°C, 22.90°C, 23.55°C and 22.40°C. The atmospheric maximum temperature during those periods were 29.20°C, 29.50°C, 26.50°C, 28.35°C, 29.25°C, 26.50°C and 30.00°C respectively. Thus the critical maximum atmospheric temperature was 31.05°C below which could not separate the oil from the coconut milk. The oil was recovered when the minimum atmospheric temperature was 23.25°C but the maximum atmospheric temperature during the day was 32.25°C. The minimum atmospheric temperature along with maximum atmospheric temperature determines the separation of oil as observed from the data.

The coconut milk when kept in incubator at 28.8°C during November – December showed separation of oil since the temperature was constant during the whole period of fermentation unlike natural fermentation. Under natural conditions the separation of oil takes place when the protein bonds are broken, mostly due to the air borne bacteria which has the capability to break protein bonds. These bacteria require a particular atmospheric temperature and relative humidity for its multiplication which result in the separation of oil. Hence the absence of sufficient population of these bacteria, which did not multiply fastly under the lower atmospheric temperature might have resulted in no separation of oil from the coconut milk. The fermented coconut milk analysed by Kumalaningsih and Masdiana (2012) reported that the best temperature of growing lactic acid bacteria was 30°Cand the inoculation of Lactic Acid Bacteria (LAB) with CO No B 64 on to fresh coconut milk and incubated at 30°C for 13 hours could produce virgin coconut oil.

Bawalan and Chapman (2006) reported that the heart of the fermentation method was the preparation of coconut milk and the right temperature (35°- 40°C) that promote overnight separation of the milk into different layers of gum, water,

proteinaceous curd and oil. Arlee *et al*. (2013) conducted a study to compare the differences in chemical components and antioxidant related substances in virgin coconut oil produced by two different methods from coconut hybrids and their parents. Coconut milk from the extraction was placed into a fermentation container which controlled the temperature at 70°C to 80°C and allowed for 16 to 24 hours for natural fermentation. Srivastava *et al*. (2013) stated that in natural fermentation process the favourable atmospheric temperature needed was 35°C - 40°C for the separation of the oil from the water and protein.

5.1.2.5. Relative humidity

The relative humidity affected the separation of virgin coconut oil from the coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours (Table 13). The maximum relative humidity during the period when the oil was separated ranged from 90.50 per cent to 97.00 per cent. The minimum relative humidity during the period when the oil was separated ranged from 67.00 per cent to 83.00 per cent. At maximum relative humidity of 90.00, 92.50, 97.50, 94.50, 90.50, 89.50 and 93.00 per cent with corresponding minimum relative humidity of 82.50, 83.50, 92.50, 84.00, 89.00, 77.00 and 69.50 per cent oil was not separated. The data showed that maximum relative humidity, minimum relative humidity along with maximum and minimum atmospheric temperature had influence on the virgin coconut oil separation.

According to Srivastava *et al*. (2013) in the natural fermentation process, the oil gets separated from the water and protein under 35-40°C and at 75 percentage relative humidity.

5.1.3. Fermentation of coconut milk in incubator

5.1.3.1. Oil recovery

The effect of different fermentation method on oil recovery and moisture content (per cent) of virgin coconut oil produced during November - December kept in incubator at 28.8°C is presented in Fig.3.

The oil recovery was maximum for the treatment T_6, (CG+ W 1:1- 48 hours) which was 22.733 per cent and it was on par with T_5, (CG+ W 1:1- 36 hours) and T_3, (CG+ CW 1:1- 48 hours) which was 22.333 per cent. In incubator, at a temperature of 28.8°C, the maximum virgin coconut oil recovery was noticed at 36-48 hours from the coconut milk extracted with water and coconut water in the ratio 1:1. The temperature was effective in bringing about separation of oil.

Handayani *et al*. (2009) studied the effect of using four different starter cultures incubated at 25°C, 30°C, 35°C, 40°C and 45°C temperatures to the coconut cream and further incubated at 40°C for overnight for virgin coconut oil production. It was observed that highest yield of oil could be obtained for *Lactobacillus bulgaricus* culture after incubating the starter at 45°C. The binding of enzyme to its substrate and rise in temperature up to a certain degree has increased kinetic energy and promoted movements of reacted molecules which was therefore increasing bumping occurance between enzyme and its substrate optimally. The enzyme exhibited its activity at certain optimal condition of temperature and therefore when the temperature was over than its optimal condition, the enzyme would certainly be denatured.

5.1.3.2. Moisture percentage

The moisture percentage of virgin coconut oil extracted by different fermentation method during November- December kept in incubator at 28.8°C did not show any significant difference between the treatments (Table 14).

4.1.3.3. Sensory parameters

The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 kept for 24, 36 and 48 hours and dried at 50°C did not show any significant

difference for the sensory parameters like colour, odour and taste (Table 15). The different treatments did not differ in colour, odour and taste of the virgin coconut oil.

5.2. STANDARDISATION OF INDUCED FERMENTATION METHOD

5.2.1. Isolation and screening of microorganisms

The microorganisms isolated and screened from the coconut milk extracted with coconut gratings and water in the ratio 1:1 and kept for fermentation at 24 and 36 hours is presented in Table 16. There was significant difference among the bacterial growth isolated at 24 and 36 hours after fermentation. Bacterial population was maximum at 24 hours after fermentation (44.60×10^6). This showed that the bacterial population increased upto 24 hours after fermentation and it decreased further. However there was no significant difference in colony forming units of *Lactobacillus* and yeast isolated. The fermented coconut milk was analysed by Kumalaningsih and Masdiana (2012) for the numbers and types of lactic acid bacteria at duration of 0,6,12, 18 and 24 hours and incubated at 25°C, 30°C and 40° C. Fresh coconut milk harboured 64×10^6cfu/g LAB and the population of LAB increased during the fermentation period of 18 hours, reached upto 19×10^6cfu/g and then decreased to 15×10^6 cfu/g at 24hours.

Soeka *et al*. (2008) reported that the fermentation of coconut cream occured when the enzymatic starter had been employed for processing. Crude coconut oil was formed due to a phenomenon of protein digestion that played a role to stabilize emulsion of the coconut cream into a soluble material. The enzymatic starter with high capacity of amylolytic and proteolytic activity could hydrolyse carbohydrate and protein which contained in the coconut cream as its substrate into soluble sugar and amino acid and peptide.

5.2.1.1. Protease and amylase activity

Plate 9. Treatments representing protease activity of microorganisms isolated

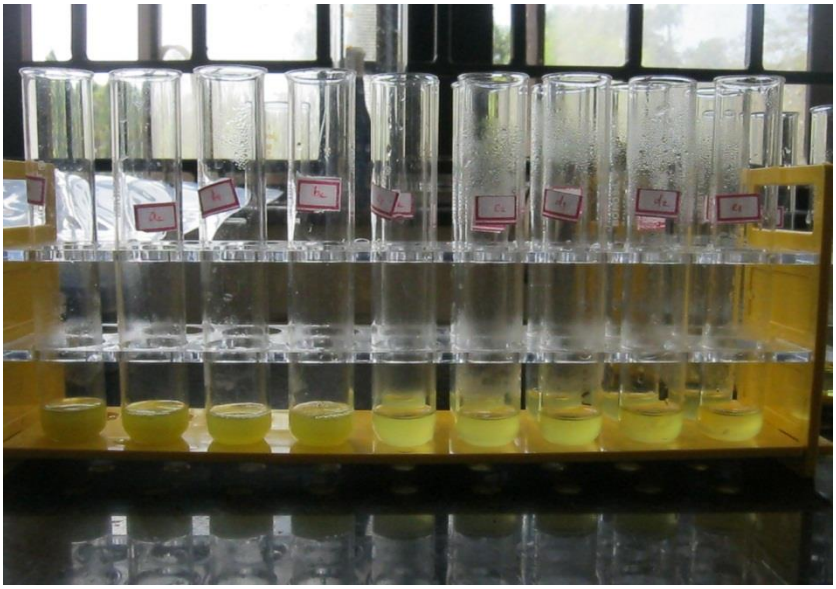

Plate 10. Treatments representing amylase activity of microorganisms isolated

Plate 11. Plate showing broth culture of *Lactobacillus* and yeast isolated

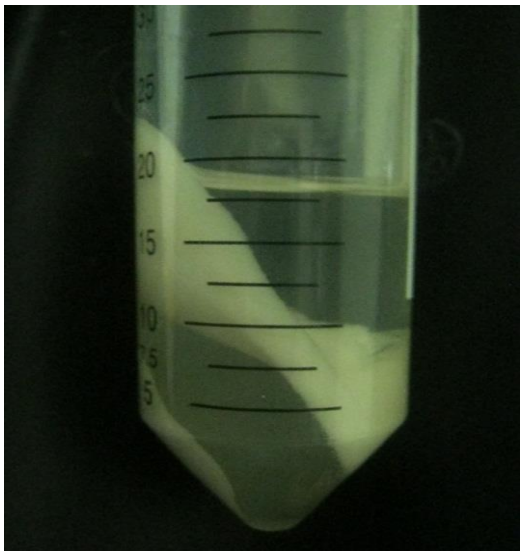

Plate 12. Plate showing separated virgin coconut oil by centrifugation method

protease and amylase activity of microorganisms isolated is presented in Fig.4 and Fig.5. *Lactobacillus* isolated at 24 hours showed significantly superior protease (184.200) and amylase (26715.000) activity compared to other treatments (plate 9. and 10.). According to Kumalaningsih and Masdiana (2012) lactic acid bacteria have highest proteolysis and amylytic enzyme activity which indicated that lactic acid bacteria has the potential to be used as pure culture for the production of virgin coconut oil of high quality oil.

Rini *et al*. (2009) in their study observed that the strain of *Lactobacillus bulgaricus* can effectively extracted higher virgin coconut oil compared to other microbial strains like *Saccharomyces cerevisiae*, *Candida rugosa* and *Aspergillus oryzae* when it was employed into the coconut cream under the enzymatic fermentation condition at pH 5.0, 45ºC and 5 per cent starter concentration.

5.2.1.2. Count of Lactobacillus

The culture of *Lactobacillus* (plate 11.) multiplied was added to the coconut milk partially sterilized at 70°C and the count of the *Lactobacillus* at 18, 24 and 36 hours were estimated (Table 18). The *Lactobacillus* count was increased with time and maximum population was noticed after 36 hours. A higher population at 36 hours showed that the activity was maximum at that period compared to 18 and 24 hours and hence the maximum recovery of oil at that period can be attributed to its higher population at that period.

5.2.1.3. Oil Recovery

The effect of induced fermentation and fermentation on oil recovery is presented in Fig.6. Oil recovery by induced fermentation and fermentation method showed significant difference among treatments, groups (induced fermentation and fermentation) and their interaction. Virgin coconut oil recovered from induced fermentation method was significantly superior (17.733 per cent) compared to fermentation method. There was only an increase of two per cent in oil recovery by induced fermentation compared to the fermentation method. The

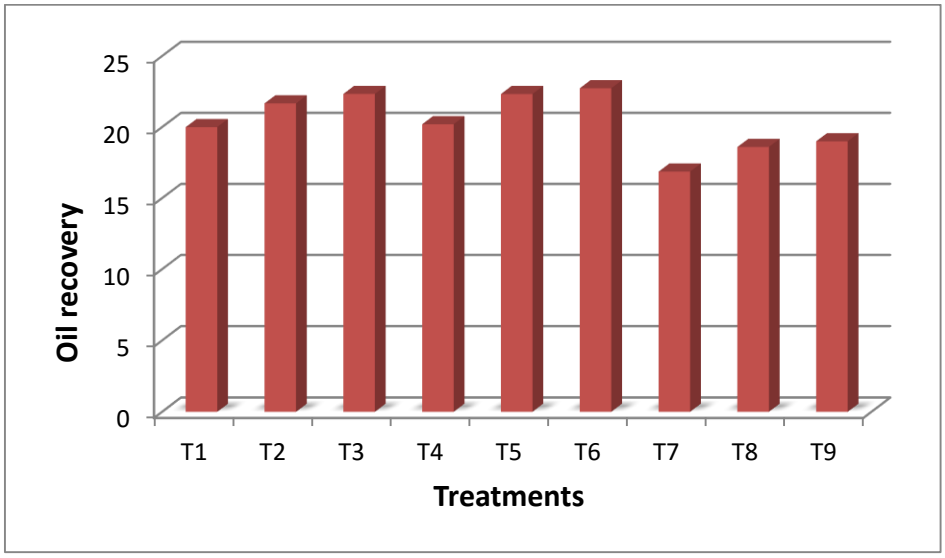

Fig.3. Effect of different fermentation method on oil recovery (%) of virgin coconut oil produced during Nov- Dec (in incubator at 28.8°C)

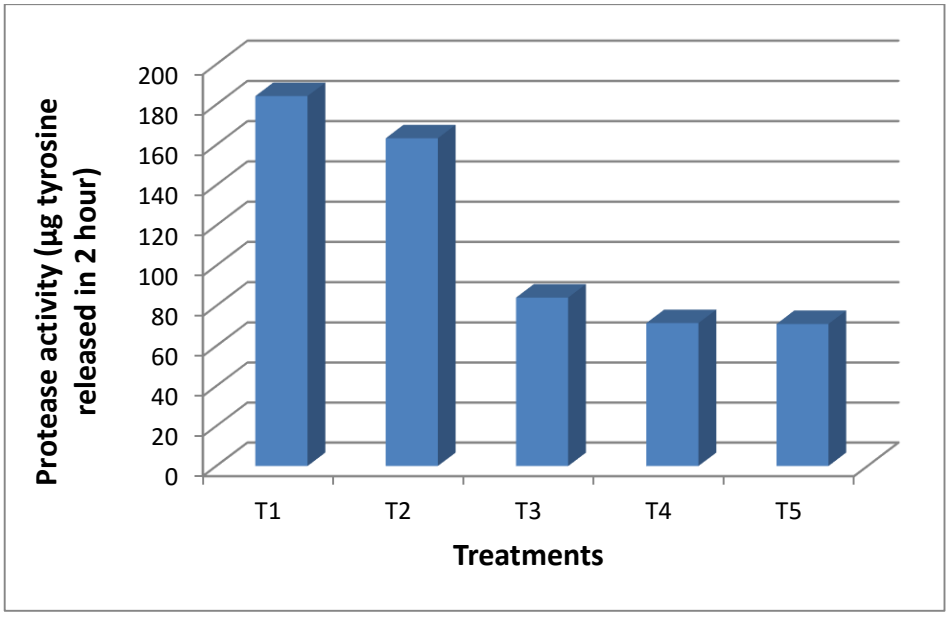

Fig.4. Protease activity of microorganisms isolated

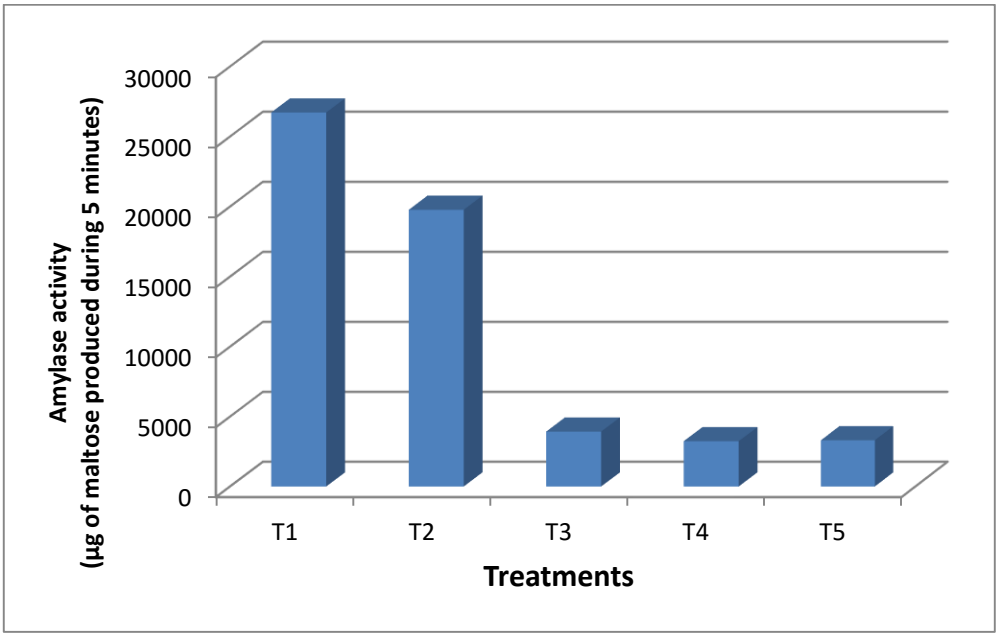

Fig.5. Amylase activity of microorganisms isolated

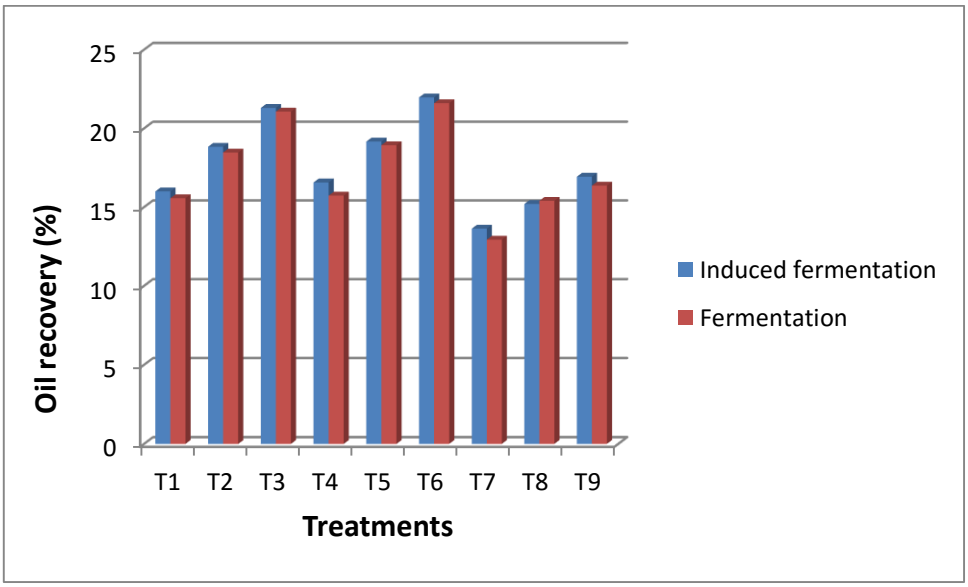

Fig.6. Effect of induced fermentation and fermentation on oil recovery (%)

induced fermentation was brought about by only one per cent of the lactobacillus inoculum which might be the reason for only a slight increase in oil recovery compared to fermentation method. The efficiency of separation of oil is directly proportional to the inoculum concentration. According to Satheesh and Prasad (2012) the inoculum concentration of 1, 2 and 5 per cent of *Lactobacillus plantarum* on yield of virgin coconut oil revealed 5 per cent as more efficient with an efficiency of 82.91 per cent compared to one per cent inoculum concentration which showed an efficiency of only 65.67 per cent.

Carmen *et al.* (1970) proposed that *Lactobacillus plantarum* enhanced the yield of coconut oil in wet process than other *Lactobacillus* species. Che Man *et al*. (1996) discovered a 74 per cent oil yield from one per cent enzyme mixture of cellulase, α-amylase, polygalacturonase, and protease. Christensen (1989) also reported a very significant oil yield of more than 90 per cent by the use of galactomannase in combination with a polysaccharide enzyme. A maximum virgin coconut oil yield of 27.2 per cent was achieved by adding five per cent microbial inoculum of *Lactobacillus bulgaricus* as enzymatic starter (Rini *et al*., 2009).

5.2.2.Induced fermentation

5.2.2.1. Free fatty acid

There was no significant difference between the treatments on free fatty acid content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with one per cent of *Lactobacillus* culture and kept for 18, 24 and 36 hours (Table 20). High free fatty acid in the oil is an indication of change caused by oxidation resulting in the rancidity of the oil. In the present treatments the fatty acids were in the range of 0.200 to 0.233mg KOH/ g of oil. Thus the results were of indication that extraction of virgin coconut oil by any of the methods followed would not alter the free fatty acid content of the virgin coconut oil. Free fatty acid value of virgin coconut oil recovered by adding microbial

inoculum (*L. bulgaricus*) as enzymatic starter was recorded as 0.22 per cent (Rini *et al*., 2009).

5.2.2.2. Total phenolic content

The effect of induced fermentation on total phenolic content of virgin coconut oil is presented in Table 20. The total phenolic content did not differ significantly between virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with one per cent of *Lactobacillus* culture and kept for 18, 24 and 36 hours and dried at 50°C. This might be because of low temperature which was used for drying.

5.2.2.3. Moisture content

The effect of induced fermentation on moisture content of virgin coconut oil is represented in Table 20. There was no significant difference between the treatments on moisture content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with one per cent of *Lactobacillus* culture and kept for 18, 24 and 36 hours. Moisture content of virgin coconut oil recovered by adding microbial inoculum (*L. bulgaricus*) as enzymatic starter was recorded as 0.30 per cent (Rini *et al*., 2009).

5.2.2.4. Oil recovery

The effect of induced fermentation on oil recovery is presented in Table 20. Virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and inoculated with one per cent of *Lactobacillus* culture and kept for 18, 24 and 36 hours showed significant difference between the treatments. The treatment, T_6, coconut milk extracted from grated coconut with water in the ratio 1:1 inoculated with one per cent of *Lactobacillus* culture and kept for 36 hours recorded maximum oil recovery (21.966 per cent). The treatment T_3, in which coconut milk

was extracted from grated coconut with coconut water in the ratio 1:1 inoculated with one per cent of *Lactobacillus* culture and kept for 36 hours showed 21.300 per cent oil recovery. The higher oil recovery in these treatments was an indication that for complete separation of oil from coconut milk 36 hours were required whether coconut milk was extracted from coconut gratings with water in the ratio 1:1 or with coconut water in the ratio 1:1.

According to Rini *et al*. (2009) maximum virgin coconut oil yield of 27.2 per cent was achieved by adding 5.0 per cent microbial inoculum of *Lactobacillus bulgaricus* as enzymatic starter. Satheesh and Prasad (2012) conducted a study and reported that the yield of virgin coconut oil by using *Lactobacillus plantarum* was 28.47±1.070 per cent.

5.2.2.5. Atmospheric temperature

Observations on maximum and minimum temperature when the *Lactobacillus* inoculated samples were kept for fermentation is presented in Table 21. Virgin coconut oil was recovered by induced fermentation method in November 10 and 11 of the year 2014 and March 7 and 8 of the year 2015. On January 5 and 6 of the year 2015, the oil was not separated from the *Lactobacillus* inoculated coconut milk. The maximum temperature when the oil was separated from coconut milk inoculated with lactobacillus was 30.55°C and 32.20°C with corresponding minimum temperature of 23.40°C and 23.95°C. The oil was not separated when the maximum temperature was 30.40°C and the minimum temperature was 22.15°C even though the coconut milk was inoculated with one per cent *Lactobacillus.* This emphasise that maximum and minimum atmospheric temperature is a critical factor which determines the separation of oil from coconut milk.

It was also reported that *Lactobacillus plantarum* was well metabolised at 40-50°C (Marina *et al.*, 2009). In a study by Raghavendra and Raghavarao (2010) at a maximum limit of temperature of 40°C, the virgin coconut oil was high. Satheesh and Prasad (2014) observed that better oil yield was achieved by the

combination of two parameters pH and temperature Kumalaningsih and Masdiana (2012) analysed the fermented coconut milk for number and type of Lactic Acid Bacteria (LAB) at duration of 0, 6, 12, 18 and 24 hours, incubated at 25°C, 30°C and 40°C.The results indicated that the bacteria could grow well at 25°C, 30°C and 40°C and the best temperature of growing was 30°C. According to Satheesh and Prasad (2013) for *Lactobacillus plantarum*, the optimum fermentation temperature needed was 45±1°C.

5.2.2.6. Relative humidity

Observations on maximum and minimum relative humidity (per cent) during the period of induced fermentation is presented in Table 21. During the period of separation of oil from inoculated coconut milk, the maximum relative humidity was 89 and 83 per cent with corresponding minimum relative humidity of 82 and 63 per cent and the average relative humidity being 85.5 and 73 per cent. However the oil was not separated when the maximum relative humidity was 96 per cent. The higher relative humidity of 96 per cent could not separate the oil from the *Lactobacillus* inoculated coconut milk. The higher relative humidity might not be a favourable factor for the multiplication of the *Lactobacillus*. The fermentation process was carried out by the acidic condition created by lactic acid produced by *Lactobacillus* which destabilize the proteins finally resulting in the accumulation of oil.

5.2.2.7. Sensory parameters

Table 22 depicts the sensory parameters of virgin coconut oil produced by induced fermentation method. Virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2, inoculated with one per cent *Lactobacillus* and kept for 18, 24 and 36 hours did not show any significant difference for the sensory parameters like colour, odour and taste. Satheesh and Prasad (2012) reported that virgin coconut oil produced in induced fermentation (semi-controlled) was pure and water white in colour with characteristic coconut smell.

5.3. STANDARDISATION OF CENTRIFUGATION METHOD

5.3.1. Revolutions per minute and time required

Effect of revolutions per minute (rpm) expressed in terms of gravitational force for different time on oil recovery is presented in Table 23. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 showed significant difference between different rpm at different time intervals. Oil recovery (plate 12.) was more for the treatment T_{21}, coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000rpm for 20 minutes (27.806 per cent) and was on par with T_{20}, coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 15 minutes (27.800 per cent).

Higher recovery of oil at higher rpm of 12000 (12096 N) is an indication of the requirement of sufficient gravitational force required to separate the oil. The centrifugation at 15 and 20 minutes did not make any significant change in oil recovery. Hence for further continuation of the study of quality parameters and for comparison of centrifugation method with other methods, the revolutions per minute was taken as 12000 rpm which produced 12096 N and the time required for centrifugation of cooled coconut milk was taken as 15 minutes. The oil recovery at 15 minutes was on par with 20 minutes and hence lower time was taken for further continuation of the project.

According to Nour *et al.* (2009) the trend in increasing centrifugation speed resulted in increase of the rate of sedimentation and the emulsion separation of two immiscible liquids. The close contact among large droplets (higher interaction time) and applied force during centrifugation led to destabilization of emulsion, resulting in the phase separation and formation of oil and aqueous layers (Chiewchan and Tansakul., 2004). Wong and Hartina (2014), studied the production of virgin coconut oil by using various centrifugation speed, temperature and time intervals. The results showed that the yield of virgin coconut

oil was 13.53 per cent at 12000 rpm, at 120 minutes. The highest yield of virgin coconut oil was 13.80 per cent at centrifugation temperature of 40ºC.

5.3.2. Free fatty acid

The free fatty acid content of virgin coconut oil recovered by centrifugation method is depicted in Table 24. The results revealed no significant difference between the treatments on free fatty acid content (mg KOH/g of oil) of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was subjected to centrifugation at 12000 rpm with gravitational force of 12096 N for 15 minutes and was dried at 50°C.

5.3.3. Total phenolic content

Effect of centrifugation on total phenolic content (mg catechin equivalent /kg of oil) of virgin coconut oil recovered from centrifugation method is presented in Table 24. The results showed that there was no significant difference between the treatments on total phenolic content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and dried at 50°C. Attempts have been made by few investigators to determine the phenolic content in virgin coconut oil. Dia *et al*. (2005) determined the total phenolic content in virgin coconut oil produced from different methods. The results revealed that virgin coconut oil contained higher total phenolic content compared to refined coconut oil.

5.3.4. Moisture content

The effect of moisture content on the virgin coconut oil extracted by different methods from coconut milk and centrifuged at 12000 rpm for 15 minutes is presented in Table 24.There was no significant difference between the treatments on moisture content of virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the

ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and dried at 50°C (Table 24). Moisture content was also an important parameter which played important role in the determination of the quality control of the virgin coconut oil samples that had been produced. The study conducted by Wong and Hartina (2014) showed that the moisture content of centrifuged virgin coconut oil at varying centrifugation speed (rpm), centrifugation time (minutes) and centrifugation temperature ranged from 0.88 per cent to 1.03 per cent and did not fall in the range of APCC standards of moisture percentage maximum range between 0.1 to 0.5 per cent.

4.3.5. Oil recovery

The effect of centrifugation on oil recovery (per cent) of virgin coconut oil is presented in Fig.7. The results showed that there was significant difference on virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes. The treatment coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 15 minutes showed maximum oil recovery (28.087 per cent).

Attempts have been made to break the protein stabilized oil-in-water emulsion with heating and centrifugation, freezing and thawing, chilling and thawing the coconut cream obtained after centrifugation (Seow and Gwee, 1997).

The removal of solids present in high percentages in the dispersion of oil seed was important for efficient recovery of oil by centrifugation (Rosenthal e*t al.*, 1996). Wong (2010) reported that the highest yield of virgin coconut oil obtained by centrifugation method at 12000 rpm and 105 minutes was 37.3 per cent. For the combination of microwave and centrifugation method, the highest yield of 46.88 per cent virgin coconut oil was obtained at 720 watt of microwave power at 12000 rpm and 105 minutes. Wong and Hartina (2014) reported that the oil recovery was 13.53 per cent at 12000 rpm, at 120 minutes by centrifugation.

5.3.6. Sensory parameters

The effect of centrifugation on sensory parameters of virgin coconut oil is presented in Table 25. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 which was centrifuged at 12000 rpm for 15 minutes and dried at 50°C did not show any significant difference between the treatments on sensory parameters like colour, odour and taste. This showed that different treatments did not influence the colour, odour and taste of virgin coconut oil extracted.

5.4. COMPARISON OF VIRGIN COCONUT OIL PRODUCED BY FERMENTATION, INDUCED FERMENTATION AND CENTRIFUGATION WITH TRADITIONAL BOILING METHOD

5.4.1. Quality parameters

5.4.1.1. Oil recovery

The effect of different methods of extraction on oil recovery (per cent) is presented in Fig.8. Virgin coconut oil recovered from centrifugation method was found to be significantly superior (28.180 per cent) compared to other methods. Wong (2010) reported that the highest yield (37.3 per cent) of virgin coconut oil was obtained by centrifugation method at 12000rpm and 105 minutes. According to Wong and Hartina (2014) the yield of virgin coconut oil by centrifugation method was 13.53 per cent at 12000 rpm, at 120 minutes. Bawalan and Chapman (2006) reported that by modified natural fermentation method about 19.8 Kg oil recovered from 100 Kg of fresh grated meat. According to Satheesh and Prasad (2012) the oil recovery from natural fermentation method was 25.68± 0.963 per cent.

5.4.1.2. Moisture content

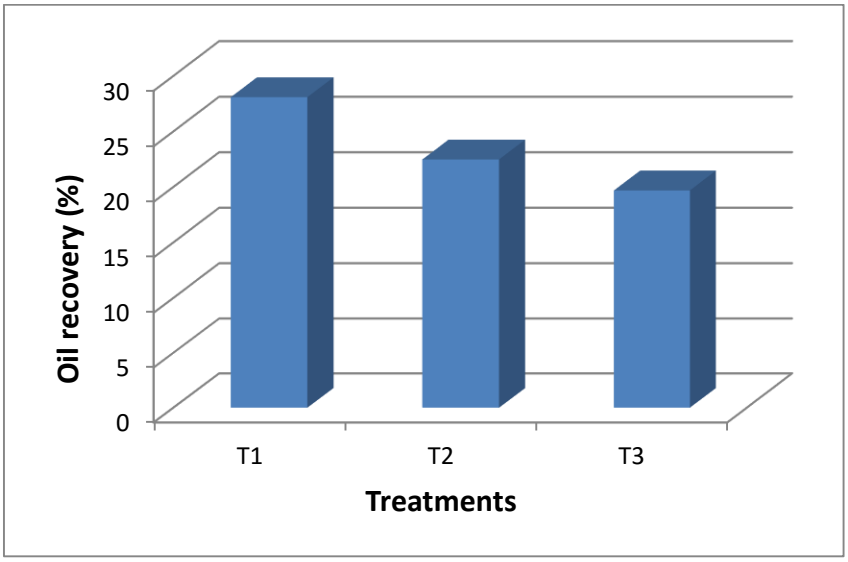

Fig.7. Effect of centrifugation on oil recovery (%)

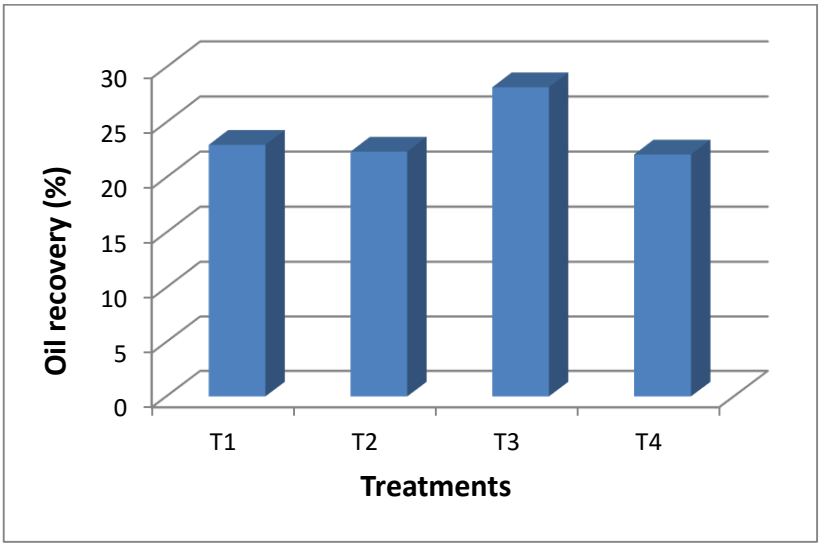

Fig.8. Effect of different methods of extraction on oil recovery (%)

The effect of different methods of extraction of virgin coconut oil on moisture content of virgin coconut oil is depicted in Table 27. The mean value for moisture content of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method did not show any significant difference among the treatments. Vermont *et al*. (2005) observed the moisture percentage of laboratory produced virgin coconut oil as 0.06 to 0.12 per cent. Manaf *et al*. (2007) observed that the moisture content of the oil was one of the parameters which affects the shelf life. The higher the moisture content, it will adversely influence the oxidation process and thus promoting rancidity.

5.4.1.3. Refractive index at 40°C

The effect of different methods of extraction of virgin coconut oil on refractive index of virgin coconut oil is presented in Table 27. There was a significant difference in refractive index of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. The higher mean value was noticed for the treatment traditional boiling method (1.453) and lowest mean value was obtained for the treatment centrifugation (1.421).

Madhavan *et al*. (2005) reported that the refractive index at 40°C of the oil extracted by natural fermentation method was (1.4483-1.4491) which indicated good quality. The refractive index of the oil measures the extent to which a beam of light is refracted on passing from air into oil. The refractive index can also be used for establishing oil purity. It is generalized that the refractive index of oils increases with increase in the number of double bonds. The refractive index can also be influenced by oxidative damage of the oil. A total of ten virgin coconut oil samples from Malaysian market were collected and analysed for their physic chemical and quality characteristics. Two of the samples were produced using MARDI's technologies i.e. dry and wet processes and the remainders of the samples were produced either through natural fermentation or mechanical process. The range of the refractive index was found to be very narrow from minimum

1.4467 to maximum 1.4472 (Kamariah *et al*., 2008). Sashya and Coorey(2012) in a study reported that the refractive index of pale yellow and dark yellow coconut oil varied in the range of 1.498 −1.466 and the refractive index of white coconut oil varied in the range of 1.484 −1.466.

According to Satheesh and Prasad (2012) refractive index at 40°C of the oil extracted by natural fermentation method was 1.4490±0.0036 and the oil from induced fermentation method was 1.4483±0.0016.

5.4.1.4. Specific gravity at 30°C

The effect of different methods of extraction of virgin coconut oil on specific gravity at 30°C of virgin coconut oil is presented in Table 27. No significant difference was found in the specific gravity of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. Specific gravity at 30^0C of the virgin coconut oil extracted by natural fermentation method was 0.918 and from induced fermentation method was 0.920 (Satheesh and Prasad, 2012).

5.4.1.5. Relative density

Relative density of virgin coconut oil produced by different methods of extraction did not show any significant difference among the treatments (Table 28). However a lower value was noticed in the virgin coconut oil produced by traditional boiling method (0.912) compared to other methods, which showed that the higher temperature in the production of virgin coconut oil might have resulted in lower relative density.

According to Kamariah *et al*. (2008) the density of vegetable oils is temperature dependent and decreases in value when temperature increases. A study conducted in ten virgin coconut oil samples collected from Malaysian market and analysed to know the physico - chemical and quality characteristics of virgin coconut oil observed that the relative density of the virgin coconut oil

ranged from 0.9185 to 0.9194. The value obtained was very close to the Asian Pacific Coconut Community (APCC) standard range (0.915–0.920).

5.4.1.6. Colour

The effect of different methods of extraction of virgin coconut oil on colour (yellowing index) is presented in Fig.9. A significant difference in yellowing index of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method was noticed. The yellowing index was highest for the traditional method (7.810) and lowest for treatment T_1, fermentation (1.616) and was on par with T_3, centrifugation (1.620) and T_2, induced fermentation (1.650). The highest yellowness index from the traditional oil method might be because, some of the coconut meat might have been over-heated, causing slight yellowish discolouration.

According to Mansor *et al.* (2012) the colour measurement is important because it reflects the quality, consistency and safety of the virgin coconut oil. Virgin coconut oil should have a high brightness and low yellowness as well as being consistent from batch to batch production and from different techniques of extraction, regardless of the cultivar types and production plants. According to APCC (2003) for the coconut oil to be categorised as virgin coconut oil, its colour should be water clean. Sashya and Coorey (2012) observed that yellow coconut oil could result when virgin coconut oil is heated to excessive temperatures where oil undergoes hydrogenation resulting in trans fatty acids. When heated beyond smoke point of 180°C of coconut oil, it turns to dark yellow and will have a strong flavour. In order to make coconut oil to be more stable and long lasting, manufacturers heat coconut oil excessively and is subjected to partially or fully hydrogenation which creates trans - fatty acids which are unhealthy for human consumption.

5.4.1.7. Iodine value

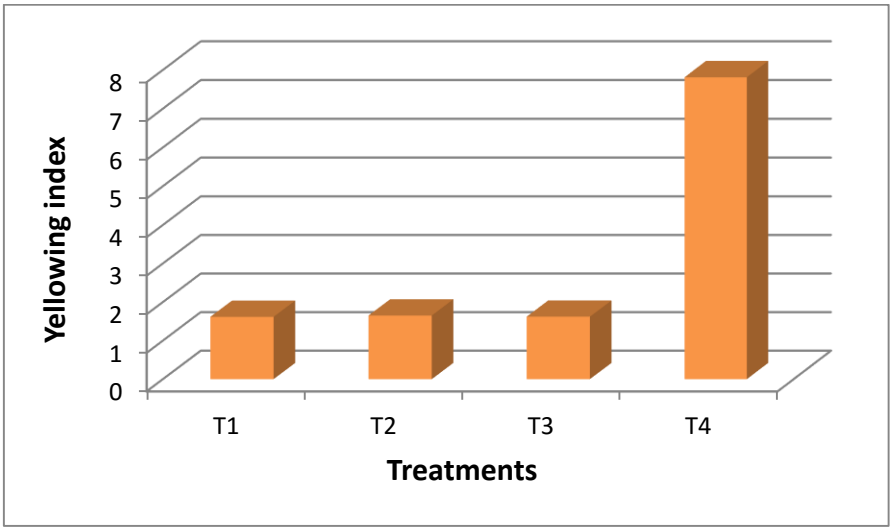

Fig.9. Effect of different methods of extraction on yellowing index

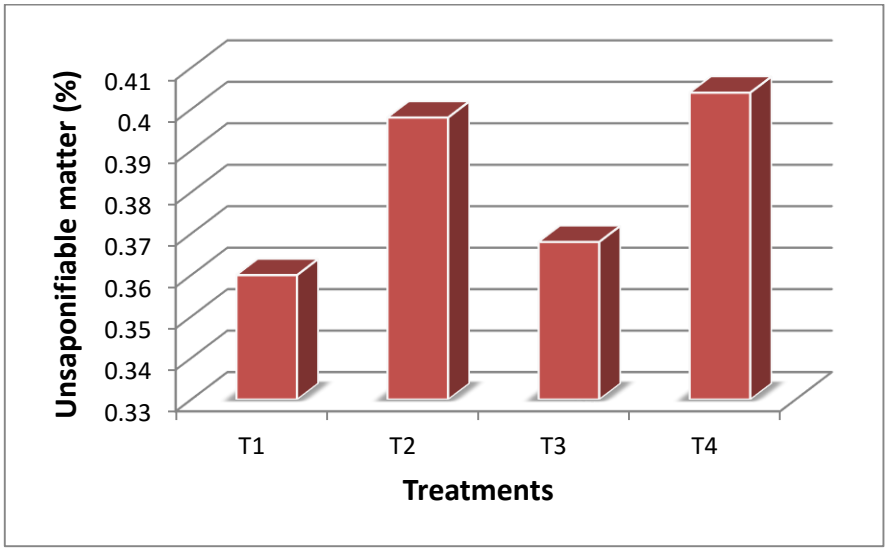

Fig.10. Effect of different methods of extraction on unsaponifiable matter (% by weight)

There was not any significant difference in iodine value of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method. However, higher iodine value was noticed for the treatment, T_4, traditional boiling method (6.140g of iodine/ 100g of oil) and lower values were noticed for the treatment, T_2, induced fermentation method (5.532g of iodine/ 100g of oil).

Dayrit *et al*. (2007) reported that the iodine value of virgin coconut oil samples ranged from 5.64- 10.34g iodine per 100g fats. Kamariah *et al*. (2008) recorded the iodine value of virgin coconut oil samples as 5.5 to 7.3 per cent. Mansor *et al*. (2012) conducted a study to compare the physiochemical properties of virgin coconut oil extracted from different processing methods. The iodine value of all the samples were 4.13 – 4.33g iodine per 100g fats and the lowest iodine value was obtained from the chilling and thawing method while the highest was from fermentation method.

5.4.1.8. Unsaponifiable matter

The effect of different methods of extraction of virgin coconut oil on unsaponifiable matter is presented in Fig.10. The unsaponifiable matter of virgin coconut oil recovered from fermentation method (0.360 per cent) was on par with centrifugation method (0.368). The unsaponifiable matter of oil serves as a check for contamination by foreign materials such as mineral oils and damage to the oil by oxidation. Highly oxidized oils contain polymerized fatty acids which are extracted together with the unsaponifiable matter. All natural fats contain minor quantities of substance other than fatty acid glycerides. The unsaponified constituent is mostly sterols. The unsaponifiable constituent of coconut oil include a small amount of tocopherols and phytosterols (GopalaKrishna *et al*., 2010).The average unsaponifiable matters for 10 samples analysed by Kamariah *et al*. (2008) was found to be 0.116 with a range from minimum of 0.085 per cent to maximum 0.135 per cent and a standard deviation of 0.0184. Unsaponifiable matter of the

virgin coconut oil extracted by natural fermentation method was 0.38 per cent and from induced fermentation was 0.4 per cent (Satheesh and Prasad, 2012).

5.4.1.9. Acid value

There was a significant difference in acid value of virgin coconut oil recovered by fermentation, induced fermentation, centrifugation and traditional boiling method (Fig.11). The virgin coconut oil recovered from centrifugation (0.200mg KOH/g of oil) was on par with induced and fermentation method compared to virgin coconut oil recovered from other methods. A significantly higher acid value obtained for traditional boiling method might be due to the higher temperatures which accelerated the hydrolysis.

The acid value (AV) is a measure of the free fatty acids (FFA) present in the fat or oil, a common parameter in the specification of fats and oils. An increment in the amount of free fatty acid in a sample of oil or fat indicates hydrolysis of triglycerides (due to moisture, temperature or enzymes). According to the Thailand Ministry of Public Health (1981), the acid value for virgin coconut oil should be less than 4.00 mg KOH/g oil. The acid values of the studied oils ranged from 0.06 to 0.63 mg KOH/g oil which was far below the maximum limit. According to Lawson (1985) and CheMan *et al*. (1997), hydrolysis was accelerated by high temperatures and excessive amounts of water. Thus, virgin coconut oil produced through the fermentation method would have high free fatty acid content due to the action of lipolytic enzymes, which was enhanced by the addition of water (Lalas and Tsaknis, 2002).

Vermont *et al*. (2005) reported the free fatty acid value of laboratory produced virgin coconut oil as 0.09 to 0.18 per cent. Dayrit *et al*. (2007) observed that the average free fatty acid value of virgin coconut oil samples as 0.131 per cent with a range of 0.037 to 0.337 per cent. The free fatty acid values for virgin coconut oil samples ranged from 0.15 to 0.25 (Marina *et al*., 2009). A study was carried out by Arlee *et al*. (2013) to compare the differences in chemical components and antioxidant related substances in virgin coconut oil from coconut

hybrids and their parents and the virgin coconut oil was extracted by cold pressing and fermentation methods. In this study, the acid value of the virgin coconut oil after different method of processing ranged from 0.06 to 0.63 mg KOH per gram oil.

5.4.1.10. Polenske value

The effect of different methods of extraction on polenske value of virgin coconut oil produced by different methods is presented in Table 29. Virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method did not showed any significant difference in polenske value. Polenske value of the virgin coconut oil extracted by natural fermentation method was 13.9 ± 0.6 and from induced fermentation method was 13.9 ± 0.3 minimum (Satheesh and Prasad, 2012).

5.4.1.11. Peroxide value

The effect of different methods of extraction on peroxide value of virgin coconut oil produced by different methods is presented in Fig.12. The peroxide value of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method differed significantly. Virgin coconut oil recovered from centrifugation (0.196 meq/ kg of oil) showed less peroxide value compared to virgin coconut oil recovered from other methods. The peroxide value were within the range given by APCC standard of $\leq$ 3 meq/ kg of oil.

Peroxide value gives an indication of the primary oxidation state of oil. The peroxide value of the virgin coconut oil recorded by Dayrit *et al*. (2007) was 1.86 meq peroxide/kg oil. According to (Marina *et al*., 2009) the peroxide values of the virgin coconut oil ranged from 0.21 to 0.63 meq oxygen/kg oil. According to the Codex Standard, the maximum peroxide value for virgin coconut oil was 15 meq oxygen/kg oil.

5.4.1.12. Saponification value

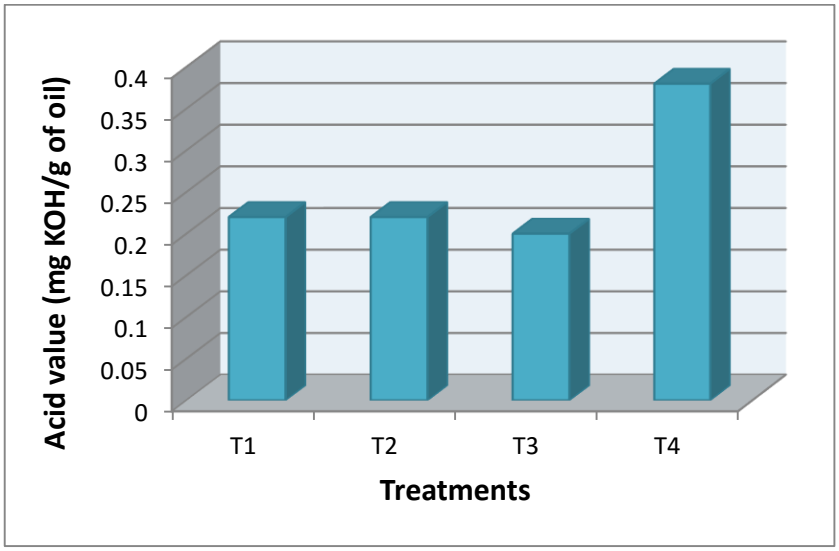

Fig.11. Effect of different methods of extraction on acid value (mg KOH/g of oil)

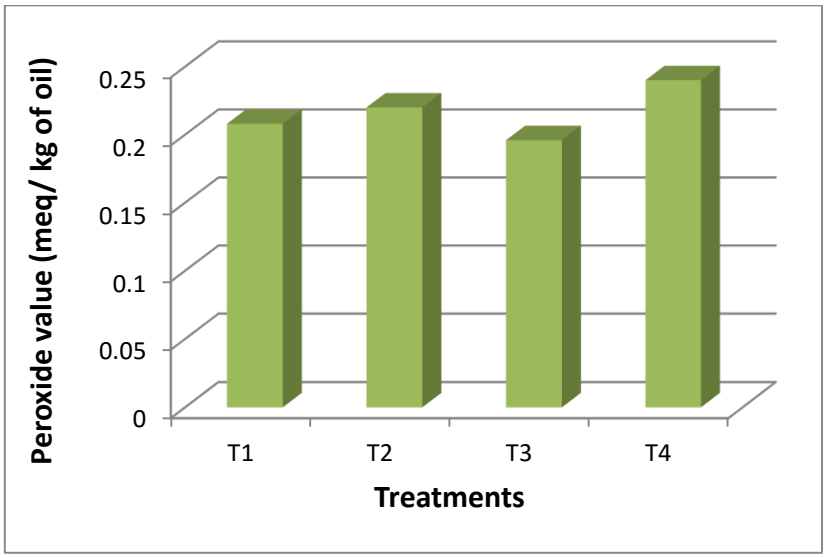

Fig.12. Effect of different methods of extraction on peroxide value (meq/ kg of oil)

Saponification value of virgin coconut oil produced by different methods of extraction did not show any significant difference among the treatments (Table 29). The saponification value of the virgin coconut oil extracted by different methods ranged from 262.428 to 262.652mg KOH/g oil and was within the range specified by APCC (2003). According to the Codex standard, specification for saponification value of edible coconut oil should be between 248 and 265 mg KOH/g oil. The saponification value of virgin coconut oil by fermentation method as reported by Madhavan *et al*. (2005) was 253 - 280 mg KOH/g oil.

Vermont *et al*. (2005) observed the saponification value of laboratory produced virgin coconut oil as 264 to 274 mg KOH /g oil. Kamariah *et al*. (2008) mentioned the saponification value of virgin coconut oil samples and recorded as 258.8 to 263.7 mg KOH/g oil. The saponification value of virgin coconut oil samples analysed by Marina *et al*. (2009) ranged from 250.07 - 258.26 mg KOH/g oil.

5.4.1.13. Sensory parameters

The effect of different methods of extraction on sensory parameters of virgin coconut oil is presented in Fig.13. The sensory parameters like colour, odour and taste showed significant difference for the virgin coconut oil recovered by different methods.

5.4.1.13.1. Colour

The virgin coconut oil recovered from induced fermentation and centrifugation method showed higher score (9.00) and the treatment, T_4, traditional boiling method showed lowest score (6.30) for the sensory parameter colour (Fig.13).

The colour of virgin coconut oil extracted by centrifugation and induced fermentation was significantly superior. The virgin coconut oil recovered by centrifugation, induced fermentation and fermentation was water clear. However the colour of virgin coconut oil recovered by traditional boiling method was

slightly yellowish in colour and scored lower values. Soeka *et al*. (2008) stated that the traditional coconut oils are considered to be low quality products which was indicated by high moisture and free fatty acid content. It is therefore easily to rancid and turn to be brown and exhibit relatively short life time by sensory test.

Descriptive sensory analysis was conducted to describe and differentiate Philippine virgin coconut oil and refined, bleached and deodourized coconut oil samples. Evaluation of the samples revealed that refined, bleached and deodourized oil significantly differed in colour having a distinct yellow colour while all virgin coconut oil samples were almost colourless. Ratings on colour demonstrated that the application of heat may affect the colour of the samples. Refined, bleached and deodorized oil undergo severe mechanical, chemical and heat treatments. Fermented samples received the highest colour scores among the virgin coconut oil samples (Villarino *et al*., 2007).

5.4.1.13.2. Odour

Virgin coconut oil recovered from traditional boiling method showed higher score (9.00) and the treatment induced fermentation method showed the lower score (7.80) (Fig.13). The highest score for traditional method may be due to the peculiar aroma obtained due to cooking of the coconut solid matter.

According to Villarino *et al*. (2007) the refined, bleached and deodourized oil sample have no perceptible aroma. The virgin coconut oil samples were described to have an acid, cocojam, latik, nutty and rancid aromas which differed among samples. The differences in the aroma ratings among virgin coconut oil samples may be attributed to the processes applied. Centrifugation with heat had the lowest acid aroma rating amongst all virgin coconut oil samples while fermentation and fermentation with heat samples were described to have slightly perceptible acid aroma. The acid aroma may be attributed to the acetic acid produced during the fermentation process. Another possible explanation of the rancid aroma in the non - heated virgin coconut oil sample was that the microorganisms present in the sample degrade the oil to methyl ketones. Methyl

ketones provide undesirable odours noted as perfume rancidity. The presence of moisture aggravated the release of free C_8–C_{12} fatty acids and their partial degradation to methyl ketones.

5.4.1.13.3. Taste

Virgin coconut oil recovered from traditional boiling method showed significantly superior mean value score (8.90) for taste. The lowest score was noticed for the virgin coconut oil recovered from fermentation and induced fermentation (6.80) method (Fig.13). According to Gopalakrishna *et al*. (2010) the main disadvantages of fermentation process was fermented odour, which masks the characteristic coconut flavour of the oil. It might be affect the taste of virgin coconut oil produced by fermentation method.

5.4.1.14. Antioxidant properties

5.4.1.14.1. Total phenolic content

The effect of different methods of extraction on total phenolic content of virgin coconut oil is presented in Table 31. The total phenolic content of virgin coconut oil produced by centrifugation, fermentation and induced fermentation were on par and significantly superior than the total phenolic content of virgin coconut oil extracted by traditional boiling method. The less total phenolic content in traditional boiling method may be because of loss of some of the antioxidants at higher temperatures. This is substantiated by the fact that hydroxyl tyrosol derivatives present in virgin olive oil were also destroyed during thermal oxidation (Nissiotis and margari, 2002).

The total phenolic content of the laboratory produced virgin coconut oil ranged from 22.88 to 91.90 mg catechin equivalent per kg oil while that of the commercial virgin coconut oil ranged from 35.26 to 49.07 mg catechin per kg oil (Vermont *et al*., 2005). Arlee *et al*. (2013) studied the differences in the chemical components and antioxidant related substances in virgin coconut oil extracted by

cold pressing and fermentation methods and reported that virgin coconut oil is also a source of phenolic compounds. The results indicated that virgin coconut oil extracted by cold pressing method was richer in phenolic substances compared with the fermentation method. This was because during the fermentation process coconut milk was left to ferment overnight; the oil was released and settled on the top layer, separating from the aqueous phase. Phenolic compounds are polar compounds that are easily dissolved in the aqueous phase of coconut milk and thus are subsequently lost during oil collection. Therefore, the total phenolic content in virgin coconut oil using the fermentation method was less than when the cold pressing method was used.

The total phenolic content in coconut oil produced by traditional and commercial methods (dry processing) were also compared by Seneviratne and Dissanayake (2008). The result indicated that the total phenolic content of traditional coconut oil was nearly seven times higher than that of commercial coconut oil. The authors did not use the term virgin coconut oil for the traditional coconut oil, even though the oil was produced by wet method, probably because of the high temperature used, which was 100-120°C. Marina *et al*. (2009) conducted a study on commercial virgin coconut oil in Malaysian and Indonesian markets, confirmed that virgin coconut oil samples were significantly higher in total phenolic content compared to refined, bleached and deodourised coconut oil. It was suggested that the refined, bleached and deodourised process being applied through dry method had considerably destroyed some of the phenolic compound in the coconut oil.

The phenolic compounds in virgin coconut oil was determined by Marina *et al*. (2008). Some of the phenolic acids identified in virgin coconut oil were protocatechuic, vanillic, caffeic, syringic, ferulic and p-coumaric acids. The study suggested that the contribution of antioxidant activity in virgin coconut oil could be due to phenolic compounds. Seneviratne and Dissanayake (2008) also reported the presence of caffeic, p-coumaric and ferulic acids as well as catechin in the commercial and traditional coconut oil.

5.4.1.14.2. DPPH radical scavenging activity

The effect of different methods of extraction on DPPH radical scavenging activity of virgin coconut oil is presented in Fig.14. Percentage inhibition was more for the virgin coconut oil recovered from fermentation method followed by induced fermentation and centrifugation method. Virgin coconut oil recovered from traditional boiling method showed the lower value of DPPH radical scavenging activity. The radical scavenging activity of the virgin coconut oil extracted by different methods increased with increase in concentration. With 35 µg/ml of the virgin coconut oil by different methods more than 50 per cent DPPH scavenging activity was noticed. From the present results it may be postulated that the virgin coconut oil extracted by different methods reduced the DPPH radical to corresponding hydrazine when it is reacted with hydrogen donors in antioxidant principles. More inhibitory was brought about by the oil recovered from fermentation method which showed the presence of higher antioxidant principles.

Unlike laboratory generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Amarowicz *et al*., 2004). A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. This purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (*i.e.*, by providing hydrogen atoms or by electron donation, conceivably *via* a free radical attack on the DPPH molecule) and convert them to a colourless or bleached product (*i.e*., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale (Ferreira *et al.*, 2007).

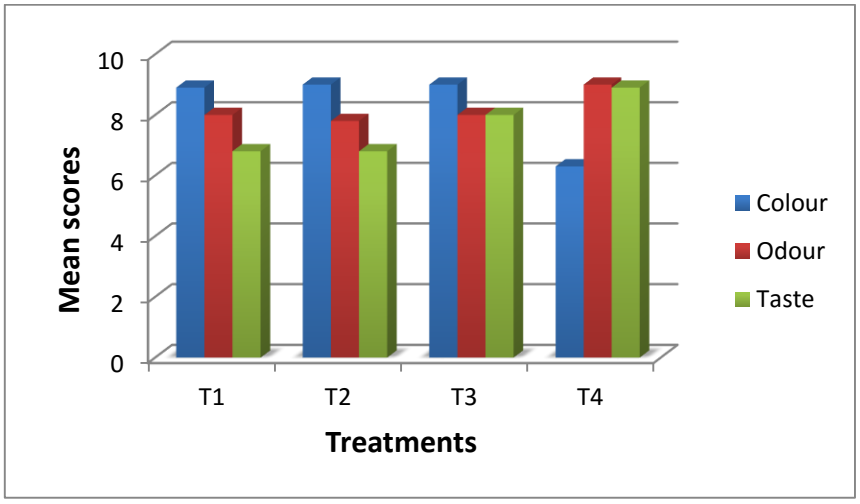

Fig.13. Effect of different methods of extraction on sensory parameters of virgin coconut oil

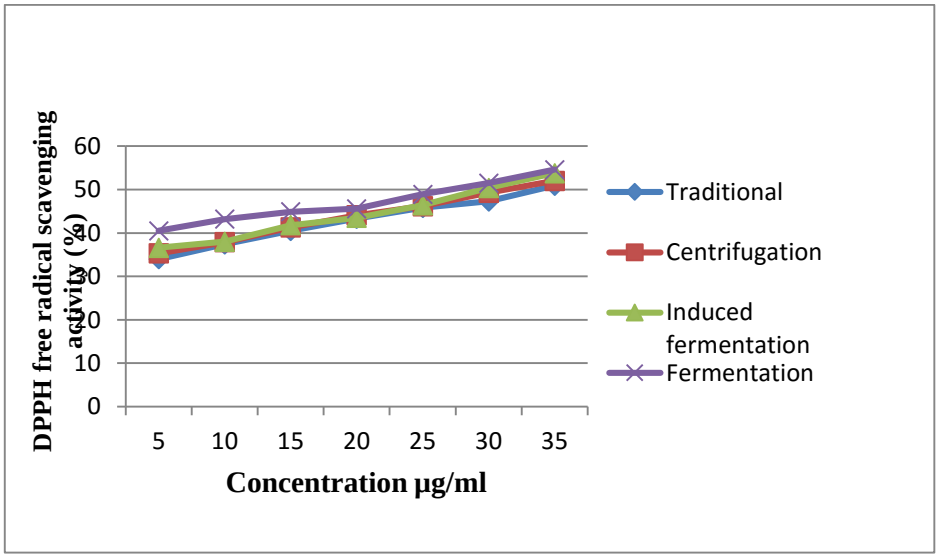

Fig 14. Effect of different methods of extraction on DPPH radical scavenging activity (%) of virgin coconut oil

Marina *et al.* (2009) reported that fermented virgin coconut oil had a strong scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH). The phenolic extract of virgin coconut oil was able to scavenge 39.1 per cent DPPH radicals at greater than 500 μg/ml (Lim *et al*., 2014).

5.4.1.14.3. Total antioxidant activity

The effect of different methods of extraction on total antioxidant activity of virgin coconut oil is presented in Fig.15. Total antioxidant activity was more for the virgin coconut oil recovered from fermentation method followed by induced fermentation, centrifugation and traditional boiling method. The lowest antioxidant properties of virgin coconut oil recovered by traditional method might be due to high temperature at which the oil was exposed during the process.

The antioxidant properties of phenolic substances vary significantly, depending on their functional groups (Evans *et al*., 1996). Therefore, when the antioxidant activity of a mixture of phenolic substances is evaluated it is important to compare the effect of concentration as well as the quality of phenolic substances on the antioxidant activity. For virgin coconut oil, the phenolic substances were protocatechuic, vanillic, caffeic, syringic, ferulic and p-coumaric acids (Marina *et al*., 2008).

Arlee *et al.* (2013) compared the chemical composition and antioxidant activity of the virgin coconut oil obtained from three varieties and three hybrids prepared by cold pressing and fermentation methods. The antioxidant activity of virgin coconut oil samples ranged from the EC_{50} of 0.48 to 1.27 mg GAE/ml. The highest antioxidant activity was observed in WAT and its hybrid Chumphon 60 (cold pressing method) ($p \leq 0.05$), which might be because they had a high content of phenolic compounds. Compared with cold pressed virgin coconut oil, fermented virgin coconut oil had gone through more processing steps during sample preparation, such as heat during fermentation and drying off water from the oil.

The antioxidant activity in virgin coconut oil was reported to be high in virgin coconut oil compared to refined coconut oil (Dia *et al*., 2005). Vermont *et al*. (2005) reported the antioxidant activity of laboratory produced virgin coconut oil as 47.4 to 78 per cent relative peroxidation. According to Marina *et al*. (2008) virgin coconut oil with the highest total phenolic content possessed the highest antioxidant activity. The virgin coconut oil produced through fermentation had the highest antioxidant activity based on beta-carotene-linoleate bleaching method. The antioxidant activity of virgin coconut oil samples ranged from 52 to 80 per cent (Marina *et al*., 2009).

5.4.1.14.4. Reducing power

Reducing power was more for the virgin coconut oil recovered from fermentation method followed by induced fermentation, centrifugation method and traditional method for all the concentrations observed (Fig.16). Virgin coconut oil recovered from traditional boiling method showed the lower absorbance value for reducing power for all the concentrations observed. The higher reducing power of virgin coconut oil under fermentation might be due to their higher hydrogen donating ability (strong reducing power potential) compared to other methods. Accordingly, virgin coconut oil recovered by fermentation method might contain higher amounts of reductone, which could react with free radicals to stabilise and block radical chain reactions.

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2). The presence of reducers (*i.e*., antioxidants) causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Marina *et al*. (2008) reported that virgin coconut oil obtained through chilling method had the highest reducing power.

5.4.1.14.5. IC_{50} values

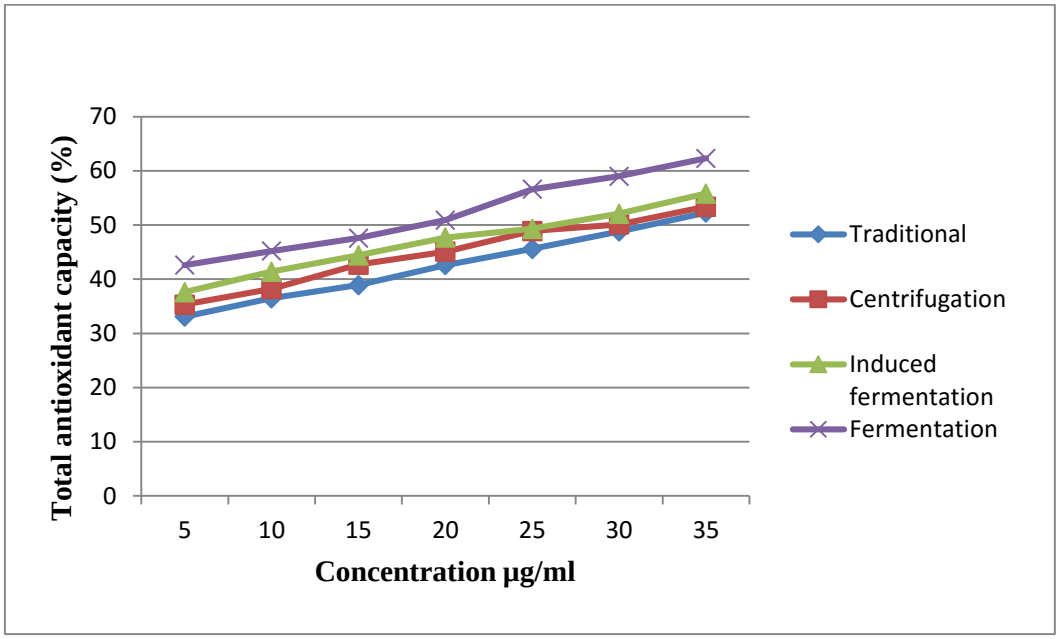

Fig.15. Effect of different methods of extraction on total antioxidant capacity (%) of virgin coconut oil

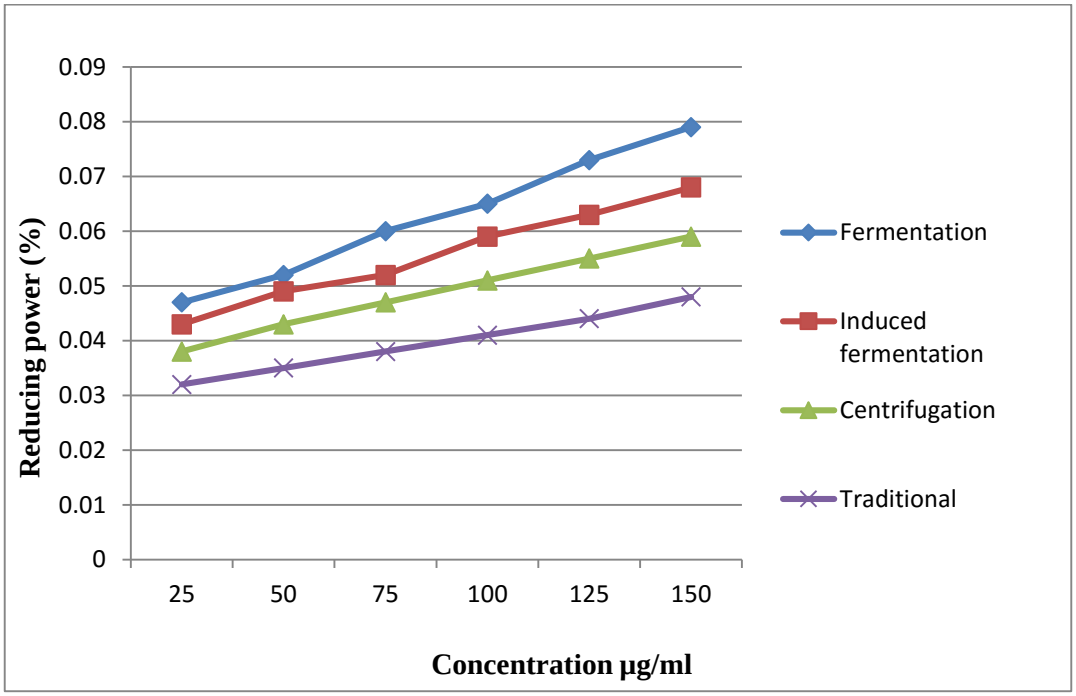

Fig.16. Effect of different methods of extraction on reducing power (%) of virgin coconut oil

IC_{50} values, the effective concentration at which 50 per cent of DPPH radicals were scavenged by the virgin coconut oil extracted by different methods is presented in Table 34. IC_{50} values were lower for fermentation method and highest for the traditional method. Thus overall, fermentation method (lower IC_{50} values) revealed better antioxidant properties than other methods, which is in agreement with the higher content of total phenols found in the fermentation method.

The free radical scavenging activity of virgin coconut oil samples ranged from the EC_{50} of 0.48 to 1.27 mg GAE/ml (Arlee *et al*., 2013).

5.4.2. Total plate count

The microbial load present in the virgin coconut oil produced by different methods of extraction after drying is found to be nil in all the media (Table 36). However, virgin coconut oil recovered from induced fermentation method showed 2×10^2 colony forming units in *Lactobacillus* media. Microbial contamination in virgin coconut oil products is due to the quality of production and not the type of process (Dayrit *et al*., 2007).Total aerobic plate count of 31 ± 3.1 and 49 ± 2.8 cfu per 0.1mg was determined in virgin coconut oil by the natural and induced fermentation method (Satheesh and Prasad, 2012).

5.4.3. Shelf life

5.4.3.1. Moisture content

The effect of different methods of extraction on moisture content of virgin coconut oil under storage is presented in Table 37. No significant difference was noticed in moisture content of virgin coconut oil under storage. However, moisture content increased with the time period. The lower moisture content was noticed in virgin coconut oil recovered from traditional boiling method for the initial period (0.052 per cent) and it was increased after 3 months (0.272 per cent) and 6 months (0.352 per cent). Virgin coconut oil recovered from other methods

also moisture content increased with the time period. The high moisture content will assist in hyrolysis process (Osawa *et al*., 2007). It is desirable to keep the moisture content low as it will increase the shelf life by preventing oxidation and rancidity processes.

5.4.3.2. Total plate count

The effect of different methods of extraction on microbial load of virgin coconut oil under storage is presented in Table 38 and Table 39. Microbial load of virgin coconut oil recovered from different methods under storage showed an increasing rate with the time period.

5.4.3.3. Acid value

The effect of different methods of extraction on acid value of virgin coconut oil under storage is presented in Table 40. Acid value of virgin coconut oil recovered from different methods showed significant difference among the treatments under storage. Acid value increased with the time period. The lower acid value was noticed in virgin coconut oil recovered from centrifugation method for the initial period (0.200mg KOH/g of oil) and it was increased after 3 months (0.320mg KOH/g of oil) and 6 months (0.400mg KOH/g of oil). For other treatments also acid value increased with the time period. The moisture content of the oil was one of the parameters which affected the shelf life (Manaf *et al*., 2007). The higher the moisture content, it will adversely influence the oxidation process and thus promoting rancidity. Free fatty acids were higher in coconut oil having higher moisture content. So, the reason to keep the moisture content as low as possible was in order to increase the shelf life of virgin coconut oil while preventing the oxidation and rancidity process to occur that can affect the quality of virgin coconut oil. Wong and Hartina (2014) observed that rancidity can also occur due to exposure to light which occurred due to the active oxygen, heat, metal, or light. Rancidity is the condition reached by certain food where oxidation reaction occurs by lipid material (fat) due to production of hydroxy acids, keto

acids, aldehydes, short-chain fatty acids, and other compounds which are responsible for the characteristics of off flavours and off odours in stale food.

Srivastava *et al*. (2013) reported that both cold extracted virgin coconut oil and hot extracted virgin coconut oil have low free fatty acid (0.05 and 0.04 per cent lauric acid) content. During storage, free fatty acid content was found to increase in all the packaging system. Free fatty acid value increase was more pronounced in cold extracted virgin coconut oil samples as compared to hot extracted virgin coconut oil samples. The rate of hydrolysis reaction was found to be slightly higher in samples stored at 37˚C than the ones stored at room temperatures.

5.4.3.4. Peroxide value

The effect of different methods of extraction on peroxide value of virgin coconut oil under storage is presented in Table 41. Peroxide value of virgin coconut oil recovered from different methods showed significant difference among the treatments under storage. Peroxide value increased with the time period. The lower peroxide value was noticed in centrifugation method for the initial period (0.196meq/ kg of oil) and it was increased after 3 months (0.560meq/ kg of oil) and 6 months (0.840meq/ kg of oil). For virgin coconut oil recovered from other methods also peroxide value increased with the time period.

Peroxide value is a good guide to judge about the quality of oil. The peroxide value of cold extracted virgin coconut oil and hot extracted virgin coconut oil was observed as 4.95 and 5.65 meq O_2/kg oil, respectively which was increased as a function of storage time in all samples up to 12 months of storage (Srivastava *et al*., 2013).

5.4.3.5. Saponification value

The effect of different methods of extraction on saponification value of virgin coconut oil under storage is presented in Table 42. No significant

difference was noticed in saponification value of virgin coconut oil under storage. The higher saponification value was noticed in traditional boiling method for the initial period (262.652mg/g of oil) and it was decreased after 3 months (242.798mg/g of oil) and 6 months (222.592mg/g of oil). For other treatments also saponification value decreased with the time period.

5.4.3.6. Total phenolic content

The effect of different methods of extraction on total phenolic content of virgin coconut oil under storage is presented in Table 43. Total phenolic content showed significant difference among the treatments. However, total phenolic content decreased with the time period. The maximum total phenolic content was noticed in virgin coconut oil recovered from centrifugation method for the initial period (71.0mg catechin equivalent /kg of oil) and it was decreased after 3 months (68.4mg catechin equivalent /kg of oil) and 6 months (60.0mg catechin equivalent /kg of oil). For other treatments also total phenolic content decreased with the time period.

5.4.3.7. Sensory parameters

The sensory parameters like colour, odour and taste of virgin coconut oil recovered from fermentation, induced fermentation, centrifugation and traditional boiling method is presented in Table 44, 45 and 46. The observation showed significant difference among the treatments.

5.4.3.7.1. Colour

Virgin coconut oil recovered from induced fermentation and centrifugation method showed maximum score (9.00) and the treatment traditional boiling method showed the minimum score (6.30) for the parameter colour (Table 44).

5.4.3.7.2. Odour

Virgin coconut oil recovered from traditional boiling method showed maximum score (9.00) and the treatment induced fermentation method showed the minimum score (7.80) (Table 45).

5.4.3.7.3. Taste

Virgin coconut oil recovered from traditional boiling method showed maximum score (8.90) for taste. The minimum score for taste was obtained for virgin coconut oil recovered from the treatment fermentation and induced fermentation (6.80) method. All the sensory parameters decreased with the time period (Table 46).

5.4.4. Economics of production

Economics of production of virgin coconut oil by different methods is depicted in Table 46. For the processing of 100kg coconut per day for one year, the cost of production was more (22,65,190/-Rs.) for the centrifugation method (Table 47). The income (57,96,900Rs.) and net profit (35,31,710Rs.) was also more from the centrifugation mehod since the recovery was highest for the centrifugation method. The benefit cost ratio was also more for centrifugation method (2.55) followed by fermentation method (2.50). In the fermentation method the initial cost involved was less compared to all other method and turned out to be the cheapest method that can adopted with modest investment for home scale. The centrifugation technology requires a high initial investment and can be recommended for the small scale industry.

Future line of work

1.Standardisation of climatic conditions for fermentation and induced fermentation
2. Standardisation of *Lactobacillus* culture as probiotics.
3. Effect of chilling on centrifugation.
4. Standardisation of methods to improve the shelf life of virgin coconut oil by different methods.

SUMMARY

6.SUMMARY

The present investigation entitled “Cost effective technology for home scale and small scale production of virgin coconut oil” was conducted during the period of 2013-15 in Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The experiment was conducted with the objective of standardising the cost effective technique for home scale and small scale production of virgin coconut oil through fermentation, induced fermentation and centrifugation and to compare the technology developed with the traditional boiling method.

The study was conducted in four experiments. Standardising the fermentation method, induced fermentation method, centrifugation method and the comparison of virgin coconut oil produced by fermentation, induced fermentation and centrifugation with traditional boiling method was carried out.

Standardisation of drying temperatures of 40°C, 45°C and 50°C were carried out for the virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and kept for fermentation at 24, 36 and 48 hours. The drying temperature 50°C was found to be the best compared to 40°C and 45°C since it showed higher oil recovery (%) and maximum scores for sensory parameters like odour and taste. However there was no difference in moisture content, free fatty acid content and total phenolic content of virgin coconut oil dried at 40°C, 45°C and 50°C.

Standardisation of fermentation method was carried out for the virgin coconut oil recovered from coconut milk extracted with coconut gratings and coconut water in the ratio 1:1 and water in the ratio 1:1 and 1:2 and kept for fermentation for 24, 36 and 48 hours at June- July, November- December and March - April. The virgin coconut oil recovered from coconut milk extracted with coconut gratings and water in the ratio 1:1 kept for 36 and 48 hours showed better oil recovery, 20.466 per cent during June- July and 22.900 and 23.066 per cent during March- April respectively.

The maximum scores for taste (7.20) compared to other treatments was noted in the virgin coconut oil recovered from coconut milk extracted with coconut gratings and water in the ratio 1:1 and kept for 36 hours. However there was apparently no significant difference in moisture content, colour and odour of virgin coconut oil recovered.

The investigation on the effect of different seasons on fermentation method revealed that the atmospheric temperature and relative humidity was critical for the separation of virgin coconut oil.

Screening and isolation of microorganism was done from the fermented coconut milk extracted from coconut gratings and water in the ratio 1:1 and kept at 24 and 36 hours. The microorganism *Lactobacillus* showed maximum protease (184.200µg tyrosine released in 2 hour) and amylase (26715.000µg of maltose produced during 5 minutes) activity and was selected for inducing fermentation.

A comparison was made to analyse the effect of induced fermentation and fermentation method on virgin coconut oil recovery. Virgin coconut oil recovered from induced fermentation method was significantly superior (17.733 per cent) compared to fermentation method (17.341). Coconut milk extracted from grated coconut with water in the ratio 1:1 and kept for 36 hours (21.780 per cent) showed significantly superior oil recovery compared to other treatments. The interaction effect also showed significant difference.

Standardisation of induced fermentation method was carried out using the *Lactobacillus* culture (1 per cent). Virgin coconut oil recovered from coconut milk extracted with coconut gratings and water in the ratio 1:1 and kept for 36 hours showed the higher oil recovery (21.966 per cent). The quality parameters like moisture content, free fatty acid content and total phenolic content of virgin coconut oil recovered did not show any significant difference between treatments.

Effect of revolutions per minute and duration on virgin coconut oil recovered from centrifugation method showed that coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 20 minutes (27.806 per cent) was better and was on par with the treatment coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 15 minutes (27.800 per cent). Considering the effectiveness, 12000 rpm and 15 minutes duration was selected for further experiment.

Standardisation of centrifugation method revealed that the treatment coconut milk extracted from grated coconut and coconut water in the ratio 1:1 centrifuged at 12000 rpm for 15 minutes showed maximum virgin coconut oil recovery (28.087 per cent). There was no significant difference in the moisture content, free fatty acid content and total phenolic content of virgin coconut oil recovered from coconut milk extracted with grated coconut and coconut water in the ratio 1:1, grated coconut and water in the ratio 1:1 and grated coconut and water in the ratio 1:2 centrifuged at 12000 rpm for 15 min and dried at 50°C.

Comparison of virgin coconut oil recovered from different methods like fermentation, induced fermentation, centrifugation and traditional boiling method was made and showed significant difference among the treatments for quality parameters. Virgin coconut oil recovered from centrifugation method (28.180 per cent) showed higher oil recovery compared to fermentation, induced fermentation and traditional boiling method. Moisture content, specific gravity, relative density, polenske value, iodine value and saponification value of recovered virgin coconut oil did not show any significant difference among the treatments.

Refractive index of virgin coconut oil recovered from centrifugation method showed significantly superior value (1.421) compared to other methods. The colour (yellowing index) of virgin coconut oil showed significant difference and the highest value was noted for traditional boiling method (7.810).

Virgin coconut oil recovered from fermentation method showed significantly superior unsaponifiable matter (0.360 per cent) compared to virgin coconut oil recovered from other methods. The acid value and peroxide value showed significant difference among different methods and lower acid value (0.200mg KOH/g of oil) and peroxide value (0.196meq/ kg of oil) was noted under centrifugation.

Significant difference in sensory parameters were noted between the virgin coconut oil extracted by different methods. Virgin coconut oil recovered from induced fermentation and centrifugation method showed higher score (9.00) and virgin coconut oil recovered from traditional boiling method showed lower score (6.30) for the sensory parameter colour. Virgin coconut oil recovered from traditional boiling method showed higher score for odour (9.00) and taste (8.90).

Studies on antioxidant properties of virgin coconut oil recovered from different methods revealed that the total phenolic content showed significant difference and virgin coconut oil recovered from centrifugation method was significantly superior (71.0 mg catechin equivalent /kg of oil). Antioxidant properties like DPPH radical scavenging activity, total antioxidant activity and reducing power were higher for the virgin coconut oil recovered from fermentation method.

The microbial load present in the virgin coconut oil produced by different methods of extraction after drying was found to be zero in all the media except *Lactobacillus* (2×10^2 cfu).

Studies on the shelf life of virgin coconut oil revealed no significant difference in moisture content and saponification value of virgin coconut oil recovered from different extraction

techniques. However it increased with the time period. The microbial load in virgin coconut oil increased with the time period and it was above the permissible limit. Acid value, peroxide value, total phenolic content and sensory parameters of virgin coconut oil recovered from

different extraction techniques showed significant difference under storage and the acid value and peroxide value increased on storage. Total phenolic content decreased on storage.

Comparing the economics of production of virgin coconut oil recovered from different extraction techniques, centrifugation technique was found to be more profitable. Fermentation method can be adopted for homescale production while centrifugation and induced fermentation can be used as a better method for small scale production of virgin coconut oil.

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COST EFFECTIVE TECHNOLOGY FOR HOMESCALE AND SMALL SCALE PRODUCTION OF VIRGIN COCONUT OIL

by

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2015

ABSTRACT

The investigation on "Cost effective technology for home scale and small scale production of virgin coconut oil" was carried out during the period 2013-2015 at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala to standardise the cost effective technique for home scale and small scale production of virgin coconut oil through fermentation, induced fermentation and centrifugation and to compare the technology developed with the traditional boiling method.

The study was conducted in four experiments and the coconuts for the experimental purpose were collected from the variety WCT (West Cost Tall). Standardisation of fermentation method was done by extracting coconut milk with coconut water (CM+CW 1:1) and with water in the ratio 1:1 (CM+W 1:1) and 1:2 (CM+W 1:2) and kept for 24, 36 and 48 hours for fermentation. The oil recovered was subjected to drying at 40, 45 and 50°C and the sensory parameters and percentage oil recovery were significantly superior at 50°C. The fermentation method was replicated during June- July, Nov- Dec and March- April. The atmospheric conditions was not congenial for the VCO production during Nov-Dec. The oil recovery was nil below an atmospheric temperature of 31.05°C. The maximum relative humidity when the oil was separated ranged from 90.50 per cent to 97 per cent. The minimum relative humidity during that period ranged from 67 per cent to 83 per cent.

For the standardisation of induced fermentation method, isolation of microorganisms from fermented coconut milk at 24 and 36 h was done and the best isolate identified belonged to the genus *Lactobacillus*. Induced fermentation with one per cent of *Lactobacillus* broth showed significantly higher oil recovery compared to natural fermentation.

Standardisation of virgin coconut oil production by centrifugation was also carried out by extracting coconut milk after 10 hours of chilling at different

revolutions per minute (rpm) and time. The coconut milk extracted with coconut water at 12000 rpm for 15 minutes and dried at 50°C recorded significantly higher oil recovery (28.087 per cent).

The virgin coconut oil produced by fermentation, induced fermentation and centrifugation were compared with traditional boiling method. A higher oil recovery and B:C ratio with minimum refractive index was observed under centrifugation while moisture content, specific gravity, relative density, acid value, peroxide value, iodine value, polenske value and saponification value did not vary significantly between different methods of extraction. The yellowing index and sensory parameters like odour and taste were significantly superior for traditional method while unsaponifiable matter, total phenolic content, free radical scavenging activity, total antioxidant capacity and the reducing power were significantly superior for fermentation method. The shelf life of the VCO produced by different methods was less than 3 months.

The present study implies that virgin coconut oil produced by fermentation, induced fermentation and centrifugation had better quality parameters, antioxidant properties and cost effectiveness compared to traditional boiling method. Fermentation method can be adopted for home scale production while centrifugation and induced fermentation can be suggested as a better method for small scale production of virgin coconut oil.

APPENDICES

APPENDIX I

Media compositions

1. Nutrient Agar

Peptone	-	5g
Sodium chloride	-	5g
Beef extract	-	3g
Agar	-	20g
Distilled water	-	1000ml
pH	-	7

2. Yeast Extract Manitol Agar

Manitol	-	10g
K2HPO4	-	0.5g
MgSO4	-	0.2g
NaCl	-	0.1g
Yeast Extract	-	1.0g
Agar	-	20g
Distilled water	-	1000ml
Congo red (%)	-	2.5ml

3. Lactobacillus Selection Agar

Pancreatic digest of casein	-	10g/L
Yeast extract	-	5g
Dextrose	-	20g
Sodium acetate	-	25g
Monopotassium phosphate	-	6g
Ammonium citrate	-	2g
Polysorbate 80	-	1g
Magnesium sulphate	-	0.575g
Ferrous sulphate	-	0.034g
Manganese sulphate	-	0.12g
Agar	-	15g

APPENDIX II

Kerala Agricultural University, College of Agriculture

Department of Plantation Crops and Spices

SCORE CARD FOR ORGANOLEPTIC EVALUATION OF VIRGIN COCONUT OIL

Name of the student: Thanuja T.T.

Title of the thesis: Cost effective technology for home scale and small scale production of virgin coconut oil.

Sample

Criteria	1	2	3	4	5	6	7	8	9
Appearance									
Colour									
Flavour									
Texture									
Taste									
Overall acceptability									

Like extremely – 9
Like very much – 8
Like moderately – 7
Like slightly – 6
Neither like nor dislike – 5
Dislike slightly – 4
Dislike moderately – 3
Dislike very much – 2
Dislike extremely – 1

Name:

Date: Signature :