QUALITY EVALUATION OF HOT AND COLD PROCESSED VIRGIN COCONUT OIL AND VCO CAPSULE

By

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(2019-24-004)

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

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KERALA, INDIA

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled "**Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other Universities or Society.

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Date:

CERTIFICATE

Certified that the thesis entitled "**Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule**" is a bonafide record of research work done independently by **Ms. Nivya E. M.** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, fellowship, associateship or other similar title of any other University or Society.

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CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

1. INTRODUCTION

Coconut (*Cocos nucifera*) is generally referred to as the "*Kalpavriksha*" or “tree of life” because it provides food security and opportunities to the people for livelihood through farming, processing, marketing, and trade related activities, especially in tropical and subtropical areas (DebMandal and Mandal, 2011). Every part of the coconut tree can be used by humans such as furniture from the trunk, thatch roof from weaved leaves, mats and brushes from coir, ladles and spoons from coconut shells. Coconut kernel (solid endosperm or meat) is the major part of the coconut used to make a variety of food products including coconut milk, desiccated coconut, cream, milk powder, coconut oil, chips, *etc*.

Coconut oil traditionally used as a cooking oil is extracted using the dry method and then undergoes refining, bleaching, and deodorisation (RBD) procedure before being used. The RBD process degrades oil quality in a number of ways, including masking pleasant natural aroma and flavour, producing high levels of free fatty acids, and eliminating beneficial compounds that are good for our health (Nevin and Rajamohan, 2004). High free fatty acid level reduces oil quality and shelf life by increasing oxidative rancidity and degradation (Raghavendra and Raghavarao, 2010). To overcome these limitations, wet extraction methods are currently used to extract oil. The oil extracted through the wet method is called virgin coconut oil (VCO).

VCO is a high value product gaining popularity worldwide as a functional food because of its pharmaceutical, nutritional and cosmetic qualities. VCO is extracted from the fresh and mature coconut kernels using mechanical or natural methods, whether heat is present or not, and without any alteration or transformation of the oil (APCC, 2003). VCO is healthier than commercial copra oil since its natural active components including antioxidants, vitamins, and polyphenols, are retained during extraction because no thermal, chemical, or UV treatments are used (Marina *et al*., 2009).

Several methods for extracting VCO from fresh and mature coconuts. These methods can be generally divided into dry and wet methods. The coconut meat or kernel does not undergo drying in the wet method, but it is heated under particular conditions to eliminate moisture to prevent scorching and microbial invasion in the dry method.

While the main objective is to destabilise the coconut milk emulsion, the wet approach can be further separated into fermentation, chilling, enzymatic, thawing, and pH methods, or any of these methods in combination. In the dry method, the kernel was mechanically pressed to extract the oil after being dried using controlled heating.

The quality of VCO depends on intrinsic factors like coconut varieties and maturity and extrinsic factors like the method of extraction (Seneviartne and Dissanayake, 2008). The maturity and variety of coconuts affect the oil recovery and the amount of micro constituents present in the oil such as phenolic compounds, tocopherol and pigments (Baccouri *et al*., 2008). The VCO shows various fatty acid profiles due to the use of various extraction techniques, which can impact their health benefits and storage stability (Raghavendra and Raghavarao, 2010). Hence, the present study attempts to assess the impact of extraction methods of VCO on oil recovery and its physicochemical properties.

Compared to other vegetable oils, VCO has unique characteristics due to its high lauric acid concentration. Lauric acid improves functional characteristics such as high digestibility, anti-obesity, antibacterial, antiviral, antiplaque, anti-inflammatory, Alzheimer's and dementia (German and Dillard, 2004). VCO can increase antioxidant enzymes and reduce lipid peroxidation (Nevin and Rajamohan, 2004). VCO protects liver function, minimizes liver damage caused by peroxidation and improves the level of liver fatty acids and hepatic antioxidant enzymes's activity (Narayankutty *et al.*, 2018).

VCO has a number of health benefits due to the presence of retained vitamins, polyphenols and antioxidants. The high concentration lauric acid and its monoglyceride form, monolaurin provides immunity to the body and protects infants from various viral and bacterial infections. Lauric acid exhibits antibacterial, antifungal and antiviral properties against human pathogens (Nakatsuji *et al*., 2009). Therefore, VCO could be taken as a daily supplement or an alternative remedy for microbial infections (Nasir *et al*., 2018).

VCO contain phenolic compounds like syringic acid, caffeic acid, p-coumaric acid and vanillic acid (Marina *et al*., 2009). Polyphenols in VCO possess therapeutic

potential such as antioxidant, anticarcinogenic, antiproliferative and antimutagenic properties that benefit the human being. This study also aims to evaluate the antimicrobial, antiproliferative and antioxidant activities of VCO for understanding its medicinal properties.

The capsulized version of the VCO is more effective than its liquid version. Recent studies show that the demand of coconut oil in capsule form increased significantly more than those of their bottled counterparts. In cold conditions, VCO liquid either freezes or solidifies. Thus, the capsulized version, which has no storage issues, is preferred by the public. Growing concerns about oxidisation of VCO when exposed to air are another reason to encapsulate it. Hence, this study also attempts to develop a capsulized version of VCO and to evaluate its shelf life.

The study entitled “Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule” was undertaken with an objectives of assessing the physico-chemical properties, antioxidant activity and medicinal properties of virgin coconut oil. It also tends to develop virgin coconut oil capsule and study its quality attributes.

Review of Literature

2. REVIEW OF LITERATURE

In this chapter, the literature review of the current study entitled ‘Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule’ is discussed under the following headings

2.1. Coconut: The tree of life

2.2. Virgin coconut oil (VCO) and extraction methods

2.3. Nutritional composition of VCO

2.4. Health benefits of VCO

2.5. Value added products of VCO

2.1. Coconut: The tree of life

The coconut tree (*Cocos nucifera*) is a monocot perennial tropical palm that is cultivated in humid and sub-humid coastal regions around the world. Coconut belongs to the palm family (*Palmae* or *Arecaceae*), which includes more than 2800 species and 190 genera. Because of its multifarious uses, it is considered as *'kalpavriksha'* (the tree of heaven) in Indian mythology (DebMandal and Mandal, 2011). The coconut palm is also known as the ‘Tree of heaven,’ the ‘Tree of Life,’ the ‘Tree of Paradise,’ the ‘Tree of Bounty,’ and the ‘Lord of Palms,’ as well as "God's blessing to humankind" (Kalimuthu and Dharani, 2020).

The coconut palm is one of the most valuable crops, and its entire part is highly useful to humans. The root is utilised as medicine, and the stem and leaf offer building materials. The fruit is the most valuable portion, while the husk (mesocarp) is used to make geotextiles, carpets, rope and growing media. The endocarp (hard brown shell) can be used to make high quality activated charcoal. The endosperm (the interior part of nut) is separated into two edible parts: a white kernel and clear liquid: coconut water (Ediriweera, 2003; Bourdeix *et al*., 2005).

Coconut has a variety of uses, including stems for furniture; leaves for handicrafts and thatch roofing; coconut sap for making wine, vinegar, or sugar production; kernel or coconut meat for making oil, milk, or cream; husks for coir fiber,

coconut water for wine and beverages; and coconut shells for utensils and charcoal, *etc*. Because of its importance in supporting the lives of individuals who grow it for various economic goals, coconut is often referred to as a 'tree of life' (Yang *et al*., 2018).

Coconut holds religious and cultural significance in some countries, particularly in India. Coconuts are inseparable from Hindu religious practices and are widely used. Coconuts have been associated with the worship of gods and goddesses in Hindu religion from ancient times. It is thought to be a symbol of luck and prosperity. As part of an initiation or inauguration, coconuts are crushed on the ground to propitiate *Ganesha*, the remover of all obstacles. In Indian tradition, coconut is referred to as *kalpavriksha*, and it is believed that the coconuts are helpful in fulfilling one's desires (Ahuja *et al*., 2014).

There are mainly two varieties of coconut palms: tall and dwarf. The tall types, which flower 6 to 10 years after planting, produce medium to big size nuts, and have a life span of 60 to 70 years, are the ones most usually planted for commercial purposes. The dwarf variants developed as a result of a mutation in tall types, live roughly for 30 years. Despite their difficulty in cultivation, dwarf types are prized for bearing fruit early (Ishiaq and Odeyemi, 2012).

Coconut is mostly grown in tropical countries, with over 90 countries cultivating it globally. It is considered as one of the most important cash crops throughout the world. Coconut is a oil crop that is primarily cultivated by people in tropical places where water is plenty, as the crop requires a huge amount of water to grow. Coconut's origin is still a matter of debate, however many researchers say that it comes from Southeast Asia's coastal regions (Gadhe and Mathur, 2018).

Coconuts are grown on around 12 million hectares around the world, with an annual potential yield of 70 billion nuts. India, Indonesia, and Philippines are currently the top coconut producers in the world, accounting for more than 75 per cent of total production (Hoe, 2018).

Coconut is grown in India's large coastline region, which is favourable for its growth, and it is planted in 13 states with a total area of 2.09 million hectares. India ranks third in the world in terms of production, contributing 31 per cent of total output, whereas Indonesia and the Philippines are first and second, respectively (Paul and Lakshmi, 2017).

Coconut has been grown in India for centuries and plays an important role in the people's social, economic, and cultural activities. In India, coconut is primarily grown in the east and west coast regions of the country. Kerala is India's leading coconut-growing state, followed by Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra, which together account for 90 per cent of area and 91 per cent of production (Gadhe and Mathur, 2018; Palanivelu and Muthukrishnan, 2019).

The coconut palm provides a variety of products, including coconut leaves, coir pith, husk fibre, coconut shell, fresh coconut meat, dried coconut meat (copra), coconut oil, tender coconut water, coconut toddy, coconut cake, and wood based goods (Onifade and Jeff-Agboola, 2003). Coconut kernel can also be included in the cakes, pies, candies, desserts and cookies to provide the desired texture (Kyari, 2008). The demand for canned coconut water, coconut milk, coconut powders and cream is rising day by day (Hoe, 2018). Hard shells, long leaves, and husk can be used to make a variety of furnishing and decoration items (Muralidharan and Jayasree, 2011).

Since ancient times, India has recognised the medicinal potential of coconut. In Ayurveda, coconut is used for the following ailments: chronic fever, acid gastritis, fissures in the feet, worms, wounds, and vomiting (Sharma, 1996). Coconut oil is also good for nourishing and strengthening the body. The oil is valued for its antimicrobial properties. Coconut oil in various forms promotes hair growth while also protecting the skin from bacterial and viral infections (Joshi *et al*., 2020).

Coconut is a nutrient-dense food that is abundant in minerals, vitamins, and fibre. It also contains medium chain fatty acids that shows good digestibility (Che Man and Marina, 2006). Ajenu *et al.* (2021) reported that coconut has good proximate (carbohydrate, fat, protein, ash, moisture and fibre) qualities and also contain

micronutrients (vitamin C, E, sodium, zinc, iron and copper). The availability of coconut is high and consuming it frequently will help prevent micronutrient deficiencies because it is rich in micronutrients like vitamin C, E, *etc*.

Coconut is considered as a functional food since it has numerous health benefits in addition to its nutritional value. Coconut oil is one of the primary natural products derived from the dried coconut kernel (copra), and it has been used as food, food additive, and functional food since the dawn of time, as well as in pharmaceuticals, nutraceuticals, cosmetics, and various industrial applications such as biofuel. Because of its health benefits, coconut oil is also referred to as 'miracle oil' (Rethinam, 2002). Coconut milk contains approximately 27 per cent of coconut oil and zeatin. Zeatin is an adenyl cytokinin that exhibits antiaging property and also minimizing carcinogenic effects (Rattan and Sodagam, 2005). Coconut milk is also considered as a better lactose free alternative to animal milk. It's also been suggested that coconut milk and cow milk be combined to make more appealing probiotic goods (Sanful, 2009). Algar and Mabesa (2015) reported that some proteins in coconut milk had antimicrobial properties against *Debaryomyces hanseni* and *Candida* sp. that cause food spoilage.

Coconut kernel is high in crude fat and carbohydrates. All nine essential amino acids are present in the protein found in coconut meat. In addition, coconut is a good source of essential minerals and vitamins for the body's metabolic activities, such as vitamin A, vitamin C, potassium, magnesium, calcium and so on. Coconut contains vitamins such as A, C, B_2 and B_6 and it can be used to treat various diseases including cancer (Okwu and Okwu, 2004). The presence of tannin content indicates that it has anti-inflammatory property. The high ascorbic acid content of the coconut also suggests that it can be used to prevent or reduce the production of carcinogenic chemicals from food (Ojobor *et al*., 2018).

Coconut water is composed of a variety of carbohydrates, alcohols, amino acids, vitamins, organic acids, inorganic elements, nitrogenous compounds, and growth factors like auxins and cytokines. It also contains significant amount of mineral elements especially sodium and potassium (George, 1993). The coconut water is an excellent source of electrolytes that can be used as rehydrating and refreshing drink

after physical activity (Saat *et al*., 2002). Coconut water has also been used as a rehydration fluid to treat diarrhoea. For individuals with diarrhoea, oral rehydration is recommended to replenish fluid loss from the gastro intestinal tract (Khan *et al*., 2003).

The various products of coconut other than copra and coconut oil offers vast scope for further development, value addition and commercialization. Commercially, coconut products and by-products can be used for a variety of purposes. Coconut sugar, syrup, jaggery, and other products made from the inflorescence; tender coconut water, coconut water concentrate, coconut honey, nata de coco, vinegar and other products made from coconut water; coconut milk, coconut cream, milk powder, desiccated coconut, virgin coconut oil, coconut chips, and other products made from the coconut kernel are some of the products getting impetus all over the world. Because of their high medicinal properties, there is a growing demand for these items on the market (Prades *et al*., 2012).

2.2. Virgin coconut oil (VCO) and extraction methods

Virgin coconut oil (VCO) is defined as the oil extracted from the fresh and mature kernels of the coconut using mechanical or natural methods, with or without the use of heat, which does not lead to any alteration or transformation of the nature of oil (APCC, 2009). VCO is considered as a functional food due to its ability to deliver various biological activities that are beneficial to human health. This is due to the presence of some components such as tocopherol and phenolic compounds (Marina *et al*., 2009).

For thousands of years, man has relied on coconuts and its oil from copra as key meals. Currently, VCO is gaining popularity among consumers because of its nutritional and medicinal properties. VCO is thought to be healthier than commonly available copra oil since the extraction method preserves more beneficial active compounds including polyphenols and vitamin E (Nevin and Rajamohan, 2004). Any kind of chemical treatments like refining, bleaching and deodorizing (RBD) processes are not involved in the extraction methods. VCO is colourless and it has a mildly detectable acid or nutty aroma and flavour, and sweet and salty taste. On the other hand,

copra oil is yellow in colour, mild salty taste and has no detectable aroma and flavour (Villarino *et al*., 2007).

There are numerous ways to extract VCO, which can be broadly divided into two categories: dry and wet processes (Bawalan and Chapman, 2006). In dry method, the kernel is dried using controlled temperature to remove the moisture content, and pressed mechanically to extract the oil (APCC, 2009). In the wet extraction method, VCO is extracted from the coconut milk using fermentation, chilling and thawing, pH method, enzymatic or any combination of these methods to destabilise the coconut milk emulsion without the need of drying (Raghavendra and Raghavarao, 2010).

The yield of VCO is highly influenced by a number of factors, including the the age of the coconut, the time of harvesting, location of the plantation, and copra's age before the extraction (Carandang, 2008). The extraction procedure has an impact on the quality and quantity of oil (Amri, 2011).

2.2.1. Hot extraction method

In Southern India, the heat extraction process was widely utilised, and traditionally, VCO was used in the preparation of ayurvedic medicines to treat skin diseases in children. In the hot extraction method, the coconut milk is heated at 100 to 120°C for 60 minutes or longer, until the oil is totally extracted from the coconut milk. Then, oil is collected after filtration. It has been found that hot extraction method can improve oil recovery and increase the release of bound phenolic compounds (Narayankutty *et al*., 2018).

Seneviratne *et al*. (2009) also found that more phenolic compounds were retained from the oil extracted by hot process. The hot extraction process used a high temperature, which allowed for the incorporation of more thermally stable phenolic antioxidants into the coconut oil. When the water in the coconut milk emulsion evaporated during the hot extraction, the concentration of phenolic compounds increased. Srivastava *et al*. (2016) reported that, compared to copra oil and cold

extracted oil, VCO extracted by hot process had high content of tocopherols, total polyphenols, monoglycerides, diglycerides, phytosterols and more antioxidant activity.

2.2.2. Cold extraction method

In cold extraction method, VCO is extracted from coconut milk without the application of heat. This method also known as aqueous extraction method. In this procedure, coconut milk is kept at lower temperature (2 to 8°C) for chilling. Then milk is centrifuged to separate the oil and filtered it before storing. This is the simplest approach for preparing VCO. This method is also environment friendly (Srivastava *et al*., 2018).

According to Hamid *et al.* (2011) the meat of a fresh coconut is mechanically crushed to obtain coconut milk. The coconut milk is refrigerated at 10°C to separate the water and coconut butter, which helped to break the emulsion. After transferring the coconut butter, it is heated at 45°C and centrifuged to separate the oil easily. Then, solid materials were removed from the VCO using filter paper. VCO is colorless and it has a fresh aroma and sweet taste. The cold extraction process can maximize the yield of oil approximately of 30 to 40 per cent, which is 10 to 20 per cent higher than traditional method with minimum time and energy.

Dumancas *et al*. (2016) reported that VCO extracted from cold process preserves more beneficial components than copra oil since it is produced straight from the coconut milk using controlled temperature. This extraction procedure, in particular, prevents the loss of small components including polyphenols, vitamin A and vitamin E owing to UV radiation from sunshine during copra drying.

2.2.3. Fermentation method

VCO is extracted by the microbial activity through natural or induced fermentation. Natural fermentation is widely used for extracting VCO. The coconut milk is extracted from the freshly shredded coconut kernels, and then left at room temperature (or till 45°C) for 24 hours to allow natural fermentation and separation of the oil layer. After 24 hours, the oil is centrifuged, scooped out from the tubes and

filtered for eliminating the solid particles (Nevin and Rajamohan, 2006). The induced fermentation method is not much popular than natural fermentation. Oil recovery is high in induced fermentation compared to natural method (Oseni *et al*., 2017). In induced fermentation, microbes like *Lactobacillus delbrueckii Lactobacillus plantarum*, and *Saccharomyces cerevisiae* were used for the extraction of VCO from coconut milk (Masyithah, 2017).

According to Bawalan and Chapman (2006) VCO can be easily extracted at home by natural fermentation method using a clean manual coconut grater and common kitchen utensils. The maturity and the freshness of the coconuts are important in this process. To ensure that the oil naturally separates from the coconut milk after 24 hours, fully matured coconuts should be processed within three days after the time of harvest.

According to Bawalan (2011), after 16 hours of fermentation, five separate layers could be seen in the fermenting container when adequate operational conditions and sanitary precautions were properly followed. The bottom layer is a gummy material. The watery portion of fermented skim milk is the next layer up. Above the watery skim milk layer is a solid layer of curd, followed by the separated oil, and finally another layer of fermented curd on top. There is a lot of oil in the fermented curd, especially on the top layer.

Extraction of VCO can also be done by changing the pH of the milk emulsion. By adjusting the emulsion's pH to a range between 3 and 5.6 and introducing bacterial cultures that destabilise coconut cream using acetic acid treatments. (Chen and Diosady, 2003). This technique is possible because of the proteins in coconut milk coagulated and then precipitated easily at pH 4.

2.2.4. Enzymatic method

VCO can also be extracted using an enzymatic method in an aqueous extraction system. Inside plant cells, oil can be found attached to proteins and a variety of carbohydrates, including cellulose, hemicellulose, starch and pectins. Cell wall degrading enzymes can be used to extract oil from the oil seeds by dissolving the

structural cell wall elements of the seed. Che Man *et al*. (1996) obtained a 74 per cent oil yield using one per cent of enzyme mixtures including protease, polygalacturonase, a-amylase, and cellulase.

The oil portion of the coconut milk is extracted using a variety of enzymes in this method. The enzymes included alpha-amylase, which breaks down starch into simple carbohydrates, protease, which breaks down polygalacturonase, cellulase and plant proteins, and which break down cell wall components (Narayankutty *et al*., 2016).

2.3. Nutritional composition of VCO

VCO is known as a multifunctional nutrient supplement due to the high nutritional content and therapeutic benefits of medium chain fatty acids (MCFAs), amino acids, vitamins, antioxidants, antibacterial, and antiviral components (Nevin and Rajamohan, 2006). The major components of VCO are triglycerides (TGs), which contribute the majority of the oil's weight. Free fatty acids (FFAs), Monoglycerides (MGs), sterols, and diglycerides (DGs) are the minor components. The concentration of minor components can vary depending on the extraction methods, age of the oil, and storage conditions (Dayrit *et al*., 2008). Additionally, VCO retains a higher number of bioactive compounds such as vitamin E, polyphenols and sterols (Lima and Block, 2019).

2.3.1. Primary metabolites of VCO

Coconut oil is made up predominantly of medium chain triglycerides. Two-thirds of the fatty acids in coconut oil are saturated medium chain fatty acids; less than one-third are saturated long chain fatty acids, and less than one-tenth are unsaturated fatty acids. Therefore, nearly 90 per cent of coconut oil's fatty acids are saturated. Coconut oil has the largest concentration of medium chain fatty acids when compared to other fats (Dia *et al*., 2005). According to Ahmed *et al*. (2015) the medium chain fatty acids in VCO is ranged from 62.2 to 63.7 per cent with long chain fatty acids of about 1.34 to 29.05 per cent and unsaturated fatty acids of about 0.12 to 6.33 per cent. The linolenic acid cannot be detected in VCO.

According to Nagao and Yanagita (2010) VCO is mainly composed of saturated fats and it contains 60 per cent of medium chain triglycerides. The high content of MCFAs in VCO makes it a potential functional food that may have some health advantages. MCFAs are saturated fatty acids having a 6 to 12 atom carbon chain. Capric, lauric, stearic, palmitic, myristic, oleic, and linoleic acids are among the fatty acids that are readily absorbed by the body (DebMandal and Mandal, 2011). Among these MCFAs, lauric acid is most noticeable with antibacterial and antiviral properties similar to monolaurin (monoglyceride form) in human breast milk (Mansor *et al*., 2012).

According to Asian Pacific Coconut Community (2003) the major triacylglycerol in VCO composed of 43 to 53 per cent lauric acid, 16 to 21 per cent myristic acid, 5 to 10 per cent of caprylic acid and oleic acid, 7.5 to 10 per cent palmitic acid, 4.5 to 8 per cent capric acid, 2 to 4 per cent stearic acid, 1 to 2.5 per cent linoleic acid and 0.4 to 0.6 per cent caproic acid. In fatty acid composition of VCO, lauric acid is dominated with a percentage ranging from 43 to 53%. Espino (2006) reported that due to the abundance of medium chain fatty acids, especially 48 to 53 per cent of lauric acid, VCO has drawn significant attention as the healthy oil in the world. According to Kamariah *et al*. (2008), compared to other vegetable oils, VCO is a unique oil because it is the only oil where 48 per cent of the fatty acid composition is lauric acid. Most of the advantages of VCO are due to lauric acid and its secondary metabolite monolaurin.

According to Kappally *et al.* (2015), the fatty acids composition in VCO is determined by gas liquid chromatography. VCO is mainly composed of saturated fats such as capric acid (4 to 8%), caproic acid (0.5 to 1%), caprylic acid (5 to 10%), lauric acid (45 to 52%), palmitic acid (7 to 10%), myristic acid (16 to 21%), stearic acid (2 to 4%), and unsaturated fats such as oleic acid (5 to 8%), linolenic acid (up to 0.2%) and linoleic acid (1 to 3%). Compared to unrefined coconut oil and refined coconut oil, virgin coconut oil has a significantly higher concentration of medium chain fatty acids (69.65%), lauric acid (55.8%), and medium chain triglycerides (59.27%) mainly trilaurin (21.88%), dilauricmonocaprin (18.89%) and dicapricmonolaurin (14.32%) (Kumar and Krishna, 2015).

Lauric acid (49%) is the most dominant fatty acids present in VCO followed by 18 per cent myristic, 9 per cent palmitic, 7 per cent capric, 2 per cent stearic and small percentages of unsaturated oil such as oleic (6 per cent) and linoleic acids (2 per cent) (DebMandal and Mandal, 2011). The amount of lauric acid (C12) in VCO ranges from 48.40 to 52.84 per cent. Extraction of oil through oven dry process is an effective method to get high lauric acid content (Ghani *et al*., 2018).

Dia *et al*. (2005) reported that lauric acid is the major fatty acid in VCO and it ranges from 49.05 to 52.55% among the commercial VCO samples and from 47.63% (dry processed VCO) to 49.97% (cold processed VCO) among laboratory produced VCO samples. Additionally, wet processed VCO had the largest quantity of oleic acid and stearic acid, while dry processed VCO had the higher amount of palmitic acid. The total fatty acid composition of the VCO samples varied between 60 and 70 per cent short chain to medium chain fatty acids.

Dayrit *et al.* (2008) observed that the level of 1-monoglycerides is higher in VCO (0.027%) than refined coconut oil (0.019 %) and total sterols are more in VCO (0.096 %) compared to refined oil (0.032 %), while diglyceride is lower in VCO (1.55 %) than refined oil (4.10 %). Marina *et al*. (2009) reported that VCO contained 60.5 to 63.6 per cent medium chain fatty acids. With a percentage between 46 and 48, lauric acid (C12:0) was the most predominant fatty acid in the VCO samples. A small per cent of caproic fatty acids (0.52 to 0.69%) and linoleic fatty acids (0.9 to 1.7%) were found in the VCO.

Osani *et al*. (2017) stated that the fatty acid composition of oil is not significantly changed by the extraction process. Additionally, the triglycerides of oil obtained from centrifugation, chilling and thawing, spontaneous fermentation, forced fermentation, and refined coconut oil are relatively similar. Ghani *et al*. (2018) reported that, depending on the procedures used for extraction, VCO's fatty acid composition may vary. The overall MCFA content of extracted VCO using cold centrifugation, fermentation, oven drying and sun drying processes is 70.1%, 69.6%, 71.3%, and 65.7% respectively.

VCO is more advantageous than commonly available copra oil because the extraction process retains more bioactive components like vitamin E (Nevin and Rajamohan, 2006). Compared to copra oil, VCO contained higher amount of vitamin E and A. Nevin and Rajamohan (2010) reported that when VCO is exposed to sunlight for several days, the amount of vitamins it contained gradually decreased, which confirms the loss of vitamins from copra oil when it exposed to UV radiation of sunlight. Mansor *et al*. (2012) pointed that VCO contains around 38 mg/kg vitamin E.

Vitamin E (tocopherol), a naturally occurring lipophilic antioxidant, is present in VCO. Tocopherol is present in various forms: alpha, beta, delta and gamma, Alpha tocopherol is present in large amounts in coconut testa (732 mg). Because the coconut testa is removed during manufacture, alpha-tocopherol is absent from VCO. (Dia *et al*., 2005). Mansur *et al*. (2012) found three kinds of tocopherols in VCO including beta, delta and gamma. The beta tocopherol varied from 0.04 to 0.05 mg/kg, delta tocopherol was detected at a very low concentration levels (1.30×10^{-5} to 1.10×10^{-3} mg/kg) and gamma tocopherol ranged from 0.01-0.05 mg/kg in VCO. Kumar and Krishna (2015) reported that VCO contains high amount of tocopherol (4.9 mg·100 g-1) than copra oil (2.8 mg·100 g-1).

The proximate and nutritional analysis showed that VCO contains 28.45 per cent of carbohydrates, 138.15 mg/kg of potassium, 58.6 mg/kg of sodium and 38.55 mg/kg of calcium. VCO contains almost all the minerals needed for the body in order to enhance proper functioning of the body system. The regular intake of VCO help in the enhancement of these minerals. Other minerals such as zinc, iron, and phosphorous are also present in VCO but in trace amounts (Adaji *et al*., 2020).

2.3.2. Secondary metabolites of VCO

VCO contains high concentrations of polyphenols. They are mainly, ferulic, syringic, caffeic, vanillic, protocatechuric and p-coumaric derivatives, all of which significantly increase the VCO's antioxidant activity (Marina *et al*., 2009). The advantages of phenolic antioxidants and the high concentration of these compounds in VCO make it one of the edible oils with a high concentration of phenolic compounds.

Ghani *et al*. (2018) reported that the extraction or processing techniques have a significant impact on total phenol content (TPC). The highest total phenol content was found in the VCO extracted by fermentation (12.54 ± 0.96 mg GAE/g oil), followed by sun dry process (8.57 ± 0.36 mg GAE/g oil), oven dry process (1.56 ± 0.24 mg GAE/g oil), and cold centrifugation (1.16 ± 0.05 mg GAE/g oil).

The phenolic compounds found in VCO are a good source of potential antioxidants. The higher level of total phenolic content in VCO is responsible for its higher antioxidant properties. Depending on the types of coconuts and the methods used to extract the oil, the total phenolic levels in VCO will change (Mansor *et al*., 2012). The total phenolic content of VCO is between 48.17 and 57.89 mg GAE/100 g oil (Arlee *et al*., 2013). According to Babu *et al.* (2014), the high antioxidant characteristics of VCO (antioxidant activity ranging from 52.54 to 79.87 per cent) are the result of its high total phenolic content (11.8 to 29.18 mg gallic acid equivalents per100 g oil).

Marina *et al*. (2009) reported that VCO samples had greater total phenolic content (7.78 to 29.18 mg GAE per 100 g oil) than refined, bleached and deodorized (RBD) coconut oil (6.14 mg GAE per 100 g oil). Some of the phenolic content in oil is being destroyed by the refining process and high heat. In addition, phenolic content may vary among VCO samples due to the difference in geographical origin of coconuts, coconut varieties, processing methods and duration of storage. Kumar and Krishna (2015) reported that total phenol content is higher in unrefined coconut oil (5.7 mg to 19.1 mg·100 g^{-1}) compared to refined, bleached and deodorised oil (2.1 mg 100 g^{-1}) and VCO (19.1 mg·100 g^{-1}).

Seneviratne and Dissanayake (2006) reported that phenolic content varies with the extraction process. VCO extracted by traditional method has a nearly seven times higher total phenol concentration than commercial coconut oil. In addition, the presence of some phenolic compounds such as, ferulic, caffeic acid, catechin and p-coumaric acid are identified in traditional and commercial oils. The total phenol content of VCO samples extracted by various methods was also examined by Mulyadi *et al.* (2018), who

came to the conclusion that the phenol concentration varies depending on the extraction methods.

Dia *et al*. (2005) reported that wet processed VCO had the highest polyphenol content, which was 91.90 mg catechin per kg oil. Dry processed VCO had the least amount of polyphenols, ranging from 22.88 to 32.37 mg catechin equivalent per kg oil due to the degradation of phenolics during the expulsion step in the dry processing. Some of the phenolics that were initially present in the oil may have been changed by the increase in temperature of 47°C that occurred during oil expulsion. Marina *et al*. (2009a) reported that phenolic acids and its derivatives such as ferulic acid, p-coumaric acid, syringic acid, protocatechuic acid, caffeic acid, and vanillic acid detected in VCO by fermented and chilling technique.

According to Nevin and Rajamohan (2004), polyphenol content is higher in wet processed VCO (80 mg of fraction per 100 g of oil) compared to copra oil (64.4 mg/100 g). The biological activity of the minor components in the oil may have been retained by the wet extraction process, which is carried out in complete darkness and at a controlled temperature. In addition, exposure to sunlight or high temperatures may inactivate the minor components like tocotrienols and polyphenols. Ngampeerapong *et al*. (2018) reported that phenolic compounds are hydrophilic in nature, therefore, they are eliminated during the oil separation processes and are not present in VCO. Compared to centrifuged VCO, fermented VCO has a slightly higher phenolic content.

According to Srivastava *et al*. (2016) polyphenols are higher in hot extracted VCO (HE-VCO) than cold extracted VCO (CE-VCO) and copra oil. During the hot extraction process, the water in the emulsion evaporates, increasing the concentration of the phenolic compounds. The polyphenols like gallic acid, ferulic acid, p-coumaric acid, sinapic, caffeic acid, vanillic acid, rutin, chlorogenic, epicatechin, quercetin, catechin and homovallin are also present in greater proportions in HE-VCO than CE-VCO. Rohman *et al.* (2021) reported that VCO contains high amount of polyphenols (250.6 mg/kg) than copra oil (91 mg/kg). Phenolic compounds such as 2.08 mg/ kg of vanilic acid, 0.45 mg/kg of syringic acid, 0.75 mg/kg of p-coumaric acid, 0.12 mg/kg

of caffeic acid and 5.09 mg/kg of ferulic acid are also present in VCO. Syringic acid and vanillic acid are deficient in the copra oil.

Illam *et al*. (2017) revealed that VCO contains 4 ± 2.3 mg GAE/100 g oil of polyphenols and its derivatives such as ferulic acid, myricetin-3-o-glucoside, rosmarinic acid, quercetin, gallic acid, dihydrokaempferol, *etc*. Mulyadi *et al*. (2018) reported that phenolic content is high in the oil extracted by fermentation method, followed by the chilling method and copra oil. Some of the phenolic compounds identified in VCO include p-coumaric acids, protocatechuic, ferulic, syringic, vanillic and caffeic acids. Some volatile compounds such as 2-pentanone, 2-heptanone, nonanal, acetic acid, octanoic acid, noctane, limonene, ethyl octanoate, dodecanoic acid, ethyl acetate, hexanal, ethyl decanoate, δdecalactone, and δ-octalactone, are also identified in VCO.

Various forms of alpha, beta, delta and gamma tocotrienols also present in VCO. These are biologically active substances, collectively called tocols. Some studies show that tocotrienols are better than tocopherols as antioxidants and are beneficial for the prevention and treatment of many diseases (Theirault *et al*., 1999). In terms of the chemistry of the side chain, tocols and tocopherols are different. The side chain of tocotrienols has three double bonds and is unsaturated, whereas the side chain of tocopherols is saturated with phytyl. The presence tocols and tocotrienols are identified in coconut oil (Carandang, 2008). Kumar and Krishna (2015) reported that tocotrienols content of VCO is significantly higher (4.9 mg·100 g-1) than other oils such as copra oil (2.8 mg·100 g -1) and unrefined oil (4 mg·100 g-1).

Plant oils are a concentrated source of phytosterol. Sabir *et al*. (2003) reported that VCO contains 0.08 mg/g of sterols. Refined coconut oil contains only 0.032 per cent total sterols, while VCO has an average total sterol concentration of around 0.096 (Dayrit *et al*., 2008). Okpuzor *et al*. (2009) showed that VCO contains 1.64 mg/g of phytosterol. A study conducted by Kumar and Krishna (2015) found that unrefined coconut oil contains high amount of phytosterol (74.5 mg·100 g−1 oil) than VCO (54.9 mg·100 g−1 oil).

According to Srivastava (2016), VCO samples contained high phytosterol content compared to copra oil. In addition, hot extracted VCO (0.573%) had a greater sterol content than cold extracted VCO (0.426%) and copra oil (0.162%). Ngampeerapong *et al.* (2018) stated that because phytosterols are lipophilic, they are more abundant in VCO than coconut milk. The sterol content is almost similar in between the VCO from centrifugation and fermentation.

2.4. Health benefits of VCO

VCO is becoming more popular as a functional food oil due to its various health benefits. VCO possess a variety of therapeutic benefits, including antioxidant, antistress, analgesic, antipyretic, anti-inflammatory and antimicrobial characteristics. VCO can be used to treat degenerative disorders like atherosclerosis, diabetes, obesity, stroke, *etc*. (Kabara, 2000). VCO has the ability to treat gastrointestinal problems, injuries, and swellings (Lans, 2007). Additionally, it prevents the growth of human pathogens like *Candida sp*., which is responsible for the fungal infection and also inhibit the action of cancer causing agents (Ogbolu *et al*., 2007). VCO has both antiviral and immunomodulatory properties that can prevent the spread of viruses and boost the immune system's ability to fight against pathogens, respectively (Dacasin *et al*., 2021).

The MCFA in VCO are used to enhance insulin secretion and glucose utilisation, which significantly reduces the symptoms and health concerns associated with diabetes (Enig, 1996). The monolaurin content of VCO has a similar beneficial effect to that of human breast milk, which is known to be healthy for infants and boost their immune system (Kabara, 2000). Lauric acid content in VCO has the ability to protect arteries from atherosclerosis and prevent cardiovascular diseases. Tocotrienols in VCO have strong antioxidant properties that protect the nervous system, prevent cancer and atherosclerosis (Das, 2007). VCO has higher levels of phenolic compounds and superior antioxidant activity compared to copra oil. The phenolic compound in VCO exhibits its antioxidant properties, including antiproliferative, antimutagenic, and anticarcinogenic which are beneficial to humans (Marina *et al*., 2009).

The medium chain fatty acids in VCO are one of the primary factors that make it an effective adjuvant in treating a variety of diseases. Capric acid inhibits the growth of human pathogen, *Candida albicans* (Bergsson *et al*., 2001). Caproic acid can improve the transdermal distribution of proteins like phenylalanyl-glycine that have undergone chemical alteration by caproic acid, improving their stability in the skin (Yamamoto *et al*., 2003). Lauric acid has antimicrobial property that helps in the treatment of inflammatory acne vulgaris (Nakatsuji *et al*., 2009). Caproic acid can be used to improve gene delivery without compromising biocompatibility (Layek and Singh, 2013). Lauric acid prevents cardiovascular diseases because it increases high density lipoprotein (HDL) and lowers the total/HDL cholesterol ratio (Kumar *et al*., 2014). Stearic acid offers protection against oxidative stress and it can be used to promote the growth of hair (Noor *et al*., 2017). Myristic acid helps type 2 diabetes patients by significantly lowering hyperglycemia by decreasing body weight and insulin-responsive glucose levels (Takato *et al*., 2017). Capric acid has plays an imporatnat role on the stimulation of the human osteoblast (MG63) (Venugopal *et al*., (2017). As a result, VCO can be included in the daily diet as a supplement to improve the quality of life.

2.4.1. Antioxidant activity of VCO

Antioxidants are the substances that can slow down or stop the oxidation process by scavenging free radicals. The free radicals cause oxidative stress and have a negative impact on biomolecules such as DNA, lipids, and proteins (Pisoschi and Negulescu, 2012). Antioxidant activity is evaluated by measuring lipid peroxidation and oxidative stress, while antioxidant capacity is assessed by measuring the levels of malondialdehyde (MDA) and other antioxidant enzymes, which frequently increase in response to oxidative stress. Antioxidant enzymes may include, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase (Jordan *et al*., 2012; Kappally *et al*., 2015).

VCO has a strong antioxidant activity because it contains polyphenols and vitamin E. Phenolic compounds are mainly caffeic, ferulic, p-coumaric, vanillic, protocatechuic, syringic and its derivatives, which significantly increase antioxidant

activity of the VCO (Marina *et al*., 2009). Zakaria *et al*. (2011) reported that high polyphenol content of VCO may contribute to increased levels of antioxidant enzymes, which in turn reduce lipid peroxidation and inflammation in mice treated with VCO. Arunima and Rajamohan (2015) compared the effect of VCO with sunflower oil, olive oil and copra oil on endogenous antioxidant status and results showed that VCO improved the antioxidant status in comparison to the other three oil fed groups, as evidenced by increased superoxide dismutase, glutathione reductase, glutathione peroxidase and catalase, activities in tissues.

VCO shows strong antioxidant activity than copra and groundnut oil because it had a greater inhibitory effect on microsomal lipid peroxidation. VCO also decreased the level of LDL cholesterol and increased HDL cholesterol (Nevin and Rajamohan, 2004). In most cases, antioxidants do not lower the total cholesterol levels, rather, they prevent plaque from building up by binding to and eliminating free radical scavengers and other oxidants, which inhibits LDL oxidation. In addition, VCO is high in polyphenols, which help to raise levels of antioxidant enzymes and, in turn, prevent peroxidation of lipid. Restoring antioxidant levels in the brain blocks more neuronal damage thereby preventing subsequent monoamine depletion (Nevin and Rajamohan, 2006). Treatment with VCO has been shown to protect liver function while improving liver fatty acid levels, hepatic antioxidant enzyme activity, and reducing lipid peroxidation induced liver damage (Eid *et al*., 2015).

The antioxidant properties of VCO varies depending on the extraction method used. Villarino *et al.* (2007) found that strong scavenging effect and higher antioxidant activity were higher in VCO extracted from fermentation method than VCO from chilling method. In addition, VCO obtained from chilling method showed a higher reducing power than VCO from fermentation method and refined oil. Seneviratne *et al*. (2009) reported that VCO extracted from hot method contained more phenolic compound than a VCO extracted under cold conditions. They concluded that VCO extracted from hot method showed higher antioxidant activity than coconut oil extracted from cold methods as demonstrated by deoxyribose assay, DPPH assay, and *in vivo* assay of serum antioxidant capacity because phenolic compounds act as free

radical scavengers. Marina *et al*. (2009) pointed that high temperature applied in the hot extraction process of VCO allowed the addition of more thermally stable phenolic antioxidants into VCO. Ghani *et al*. (2018) reported that VCO obtained from fermentation method showed the highest antioxidant activity than cold centrifugation method and dry process.

2.4.2. Antimicrobial activity of VCO

VCO has excellent antimicrobial property that destroy dangerous viruses, bacteria, fungi and parasites. VCO is rich in medium chain fatty acids (MCFA) like caprylic, caproic, and lauric acids that are responsible for the antifungal, antibacterial and antiviral properties of VCO (ChiawMei *et al*., 2010). The monoglyceride forms of lauric acid such as monocaprin monocaprylin, and monolaurin, prevent microbes from disturbing the immune system. Monolaurin is considered to have the strongest antibacterial, antiviral and antifungal properties (Shilling *et al*., 2013).

Daftary *et al.* (2008) reported that monolaurin has high antibacterial property that can be used as a medicine to prevent the growth of *Candida albicans Escherechia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Oyi *et al*. (2010) found that monolaurin exhibits bactericidal activity against *Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris*, and *E. coli*. The medium chain fatty acids and its derivatives, monoglycerides present in VCO are effective against lipid coated bacteria by rupturing their lipid membrane. For instance, they can be effectively used against the bacteria that cause urinary tract infections, food poisoning, sinusitis, stomach ulcers, and sinusitis (DebMandal and Mandal, 2011).

MCFA is very effective against gram positive bacteria, but not against gram negative bacteria (McKellar *et al*., 1992). Widiyarti *et al*. (2009) found that lauric acid inhibits the growth of *S. aureus*. Parfene *et al*. (2013) observed that growth of gram negative bacteria such as *Salmonella enteritidis* and *Escherechia coli* was inhibited by MCFA. Sihombing *et al.* (2014) compared the effectiveness of VCO on gram positive and gram negative bacteria and concluded that VCO was more effective against *B.*

cereus (gram positive bacterium) compared to *Escherechia coli* due to the presence of lauric acid and its monoglyceride form monolaurin.

Verallo-Rowell *et al*. (2008) reported that monolaurin content in VCO shows antibacterial effect on *S. aureus* and can be helpful in the proactive treatment of atopic dermatitis colonisation. VCO also inhibit the growth of antibiotic resistant bacteria *Clostridium difficile* (Shilling *et al*., 2013) and *Pseudomonas aeruginosa* (Silalahi *et al*., 2014). Elysa *et al*. (2014) found that growth of *Salmonella* species was decreased by the enzymatic hydrolysis of VCO in both *in vitro* and *in vivo* investigations.

Wang and Johnson (1992) conducted a study to examine the effectiveness of monolaurin on the growth of *Listeria monocytogenes* and observed the morphological changes in the cells of bacteria via transmission electron scanning (TEM) analysis. The results showed that cytoplasm of treated bacteria cell separated from cell envelop and the cell envelop was ruptured. Parfene *et al*. (2013) reported that extracted fatty acids from coconut fat exhibited strong antibacterial activity against gram positive bacteria including *Bacillus cereus* and *Listeria Monocytogenes* as well as gram negative bacteria like *Escherechia coli* and *S. enteritidis*.

Monolaurin prevents the growth of fungus species such as *Cladosporium sp*., *Penicillium sp*., *Fonsecaea pedrosoi*, *Fusarium sp*., *Alternaria sp*., *Cryptococcus neoformans Aspergillus sp*., and *C. albicans* (Esquenazi *et al*., 2002). Rihakova *et al*. (2002) found that 0.5 mg/mL and >1mg/mL dosage of monolaurin was effective against *Aspergillus niger* by inhibiting the spore germination and radial growth respectively. Ogbolu *et al*. (2007) conducted a study to know the effectiveness of VCO and fluconazole against drug resistant *Candida albicans*. They concluded that VCO was very effective against *Candida albicans* at 100 per cent concentration when compared to fluconazole and therefore VCO can be used in the treatment of *Candida* infections. In addition, Capric acid kills *Candida albicans* most quickly and effectively while lauric acid is more active at lower concentrations.

Currently, VCO and its MCFAs are widely used against fungi species especially *Candida albicans*, the most prevalent and commonly isolated fungus from the human

body Arnfinnsson *et al*. (2001) reported that capric acid and lauric acid exhibited a strong inhibitory effect on *C. albicans* growth. Huang *et al*. (2011) pointed those different kinds of fatty acids showed different patterns of inhibition against oral fungus. They concluded that MCFA had a strong anti *Candida* activity while SCFA and LCFA displayed limited bioactivity against oral fungal species.

Winarsi and Purwanto (2008) reported that zinc enriched VCO successfully used as an immunostimulator for the treatment of vaginal candidiasis patients. Enriched VCO retained neutrophil, improved number of T-cytotoxic and T-helper cells and increased the level of IL-2. Shino *et al*. (2016) conducted a study to compare the antifungal activity of coconut oil, ketoconazole and probiotics on *Candida albicans* isolated in children with early childhood caries. The results showed that coconut oil had comparable antifungal activity with ketoconazole. Additionally, it was discovered that coconut oil had higher antifungal action than probiotics against *C. albicans*.

Lauric acid and its monoglyceride form monolaurin which have antiviral activity against many enveloped human RNA and DNA viruses. Hornung *et al*. (1994) reported that the maturation of vesicular stomatitis was found to be decreasing after the treatment with lauric acid. The action of visna virus and herpes simplex virus were inactivated by free fatty acids and monoglycerides (Thormar *et al*., 1994). Daily inclusion of coconut oil in the diet reduced the viral load of HIV patients (Enig, 1997). Enig (1998) reported that monolaurin has the ability to inactivate viruses including HSV, visna virus, measles virus, cytomegalo virus HIV and VSV.

VCO can be effectively used against lipid coated viruses such as influenza virus, Epstein-Barr virus, visna virus, CMV, leukaemia virus, hepatitis C virus and pneumono virus. These viruses are mostly destroyed by the MCFAs in VCO, which also interferes with virus assembly and maturation (Arora *et al*., 2011). In MCFAs, lauric acid showed greater antiviral activity than capric acid, caprylic acid and myristic acid (DebMandal and Mandal, 2011). Bhatt *et al*. (2021) reported that free fatty acids such as capric acid, uric acid, and monoglycerides has high antimicrobial activity. Capric acid and its monoglyceride, monocaprin exhibits potential antiviral activity against HIV-1 infection.

Lauric acid and its monoglyceride form, monolaurin shows antiviral properties through three crucial mechanisms include disintegration of the virus envelop, inhibition of late maturation stage in the replicative virus cycle and prevent the binding of viral proteins onto the host cell membrane (Dayrit and Newport, 2020). Tan-Lim and Martinez (2020) reported that monolaurin has the ability to destroy the viral cell envelop of both the DNA and RNA viruses by incorporating the fatty acids that can destabilize the lipid bilayer. Lauric acid can help to prevent viral maturation during a phase when viral glycoproteins are declining and incorporation is increasing in the host plasma membrane of triacylglycerol. Bhatt *et al*. (2021) mentioned that VCO has been considered as a potential supplement for COVID-19 patients due to its inhibitory activity against single stranded RNA viruses.

2.4.3. Anti-inflammatory activity of VCO

Inflammation is a natural response to fight off infection and repair tissue damage (Aggarwal *et al*., 2007). However, prolonged chronic inflammation can cause the onset of diseases such as cardiovascular diseases, diabetes, cancer, colitis, arthritis, sepsis, autoimmune diseases, and neurodegenerative diseases (Alzheimer's disease) (Bhatelia *et al*., 2014).

VCO has the potential to reduce inflammation because of its high phenolic content. VCO increases antioxidant enzymes and decrease the expression of inflammatory genes such as IL-6, iNOS and COX-2. In acute inflammatory models, carrageenin and arachidonic acid induced paw oedema in rats as well as ethyl phenylpropiolate-induced ear edema were both moderately reduced by VCO (Intahphuak *et al*., 2010). Anti-inflammatory properties of VCO limit the production of tumor necrosis factor, an inflammatory agent, as well as Interferon (IFN) and Interleukins (IL-5, IL-6 and IL-8), which decrease these inflammatory markers and protect the skin by improving skin barrier function (Varma *et al*., 2019). Chew (2019) stated that the antioxidant property of VCO effective to scavenge free radicals that reduce inflammation.

Zakaria *et al*. (2011) conducted a study to observe the anti-inflammatory effect of VCO extracted drying and fermentation process on rats with acute and chronic inflammation. VCO from fermentation process showed high anti-inflammatory effect on acute inflammation, whereas, VCO was found to be less effective on chronic inflammation. Visakh *et al*. (2014) studied the anti-inflammatory effects of the polyphenolic fraction isolated from VCO on animal models of arthritis. The results showed the potential health benefits of VCO on adjuvant induced arthritis in rats due to its antioxidant and anti-inflammatory effects.

Nakatsuji *et al*. (2009) found that VCO showed therapeutic potential against *Propionibacterium acnes* (*P. acnes*) induced inflammation. Lauric acid successfully reduced the amount of *P. acnes* colonised with mouse ears, reducing ear edoema and granulomatous inflammation brought on by *P. acnes*. Dreno (2010) also investigated the anti-inflammatory property of lauric acid on *acnes vulgaris* and concluded that lauric acid can be used as a substitute for antibiotic therapy for acne vulgaris.

2.4.4. Antidiabetic activity of VCO

VCO is easily digested in the body and it prevents the complications of diabetes mellitus by producing more insulin. The lauric and capric acids in VCO enhance the pancreatic Langerhans cells' ability to secrete insulin inside the body and reduce blood sugar level (Garfinkel *et al.*, 1992). Supriatna *et al*. (2018) found that VCO suppressed blood glucose level and inhibited the damage of pancreatic beta cells in rats with diabetes.

According to Eckel *et al.* (1992), VCO is proven to balance blood sugar levels, which has an antidiabetic effect. A comparison study by Siddalingaswamy *et al*. (2011) on the protective potential in a streptozotocin induced diabetic model using cold pressed VCO, hot extracted VCO (HVCO) and commercial coconut oil found that hot extracted VCO to be a better hypoglycemic and insulin sensitizing agent compared to the other oils used. Iranloye *et al*. (2013) and Madin and Ahamed (2015) found that fermented VCO effectively lowered hyperglycemia in alloxan induced diabetic mice.

Narayanankutty *et al*. (2016) discovered that fermented VCO protected rats fed high-fructose diets from developing insulin resistance and dyslipidemia.

Akinnuga *et al*. (2014) reported that diabetic nephropathy in animals could be prevented by the use of 10 per cent of VCO. According to Lekshmi *et al*. (2016) phenolic acids present in VCO help to prevent dipeptidyl peptidase-4 or insulin sensitivity. Additionally, by blocking the polyol pathway, phenolic chemicals found in VCO are believed to provide protection against diabetic complications such as diabetic retinopathy, nephropathy, *etc*.

2.4.5. Cardioprotective effect of VCO

VCO consist of high amount (65 per cent) of medium chain triglycerides (MCTs). These MCTs are a rapid source of energy because they are directly absorbed from the intestinal tract and transported to the liver without taking part in the biosynthesis and transport of cholesterol (Enig, 1999). As a result, VCO was found to be effective in lowering cholesterol levels. VCO was found to increase HDL and decrease total cholesterol, triglycerides, phospholipids, and LDL in the serum and tissues (Nevin and Rajamohan, 2004). Nevin and Rajamohan (2006) stated that polyphenolic compounds of VCO have the ability to lower lipid levels and LDL significantly.

Liau *et al*. (2011) found that VCO supplementation (30 mL per day) for four weeks significantly decreased waist circumference and improved lipid profiles, and it is considered as safe for use in humans. Feranil *et al*. (2011) conducted a cohort study to know the benefits of coconut oil on lipid profile in 1839 premenopausal women and the results showed that consumption of coconut oil did not increase the serum total cholesterol or triglyceride levels. Nurul-Iman *et al*. (2013) reported that VCO prevented hypertension and improved endothelial function in rats. Kamisah *et al.* (2015) found that VCO supplementation showed a cardioprotective effect via lowering blood pressure in rats fed with repeatedly heated palm oil. The outcome of VCO supplementation (1.43 mL/kg) in rats were fed with repeatedly heated palm oil showed

that VCO had protective effects on the remodeling of the cardiac and vascular tissues (Subermaniam *et al*., 2015).

Compared to normal coconut oil, administration of VCO increased antioxidant activity and lowered lipid and thrombotic factor levels in rats (Nevin and Rajamohan, 2008). Babu *et al*. (2014) reported that VCO fed animals had a better prothrombin time and improved coagulation with reduced fibrin levels compared to copra oil and sunflower oil fed animals. Polyphenols in VCO prevent atherosclerosis and cardiovascular disease due to its anti-inflammatory and antioxidant property (Vysakh *et al*., 2014).

Feranil *et al.* (2011) found a positive association between VCO consumption (9.54 ± 8.92 g/day) and increase the level of serum HDL cholesterol. High HDL level lowers the risk for cardiovascular diseases (Vivas *et al*., 2013). Cardoso *et al*. (2015) found that addition of VCO in the daily diet helps to prevent coronary artery disease via decreasing the waist circumstance and improving lipid profile especially increasing the level of serum HDL cholesterol.

2.4.6. Anticancer property of VCO

Winarsi *et al*. (2009) found that zinc enriched VCO increased the number of cytotoxic Tc cells and helper Tcd4 cells. Tc cells generate cytokines that can activate macrophages and kill cancerous cells as well as virus infected cells. Jordan *et al*. (2012) developed tocopherol rich nanoemulsions, which were demonstrated to have anticancer action. This result indicates that the tocopherols and tocotrienols found in VCO may have some anticancer properties.

VCO has the capacity to impede the action of compounds that cause cancer due to its anticarcinogenic effect (Lim-Sylianco *et al*., 1987). VCO shows anticancer property against various cancer cells. The HT29 malignant human colon cells were more effectively inhibited by VCO rich in lauric and palmitic acids (Salerno and Smith, 1991). The incidence of ulcerative colitis and azoxymethane induced colon cancer was effectively decreased by the inclusion of VCO in the daily diet. The treatment with

coconut oil raises the levels of the intestinal protein (mucin 2), which is essential for the healthy maintenance of intestinal barrier integrity (Enos *et al*., 2016).

Lauric acid, the primary form of fatty acid in VCO, has recently been shown to induce apoptotic alterations in a variety of colorectal cancer cells as well as breast and endometrial cancer cells through reactive oxygen species in an *in vitro* system (Fauser *et al*., 2014; Lappano *et al*., 2017). Sheela *et al*. (2019) found that lauric acid exhibited cytotoxicity *in vitro* and *in silico* against the human cancer cells HepG2 (hepatocellular carcinoma), HCT-15 (colon cancer), and Raw 264.7 (murine macrophages). Additionally, in experimental animal models, VCO has demonstrated an inhibitory effect on mammary carcinogenesis (Deen *et al*., 2020).

Kamalaldin *et al.* (2010) reported that VCO was safe to consume and induced apoptosis in the lung cancer cell lines NCI-H1299 and A549. After being treated to VCO (8.64% and 12.04%), both cell lines had distinct morphological alterations, including the emergence of extensive cytoplasmic vacuolization and blebbing of the cell membrane. Deen *et al*. (2020) revealed that VCO, fractionated coconut oil and processed coconut oil, demonstrated anticancer effectiveness against liver and oral cancer cells.

2.4.7. Other health benefits of VCO

VCO was proven to decrease the effects of paracetamol induced toxicity by restoring hepatic morphology and liver function markers. Zakaria *et al*. (2011) discovered that higher levels of liver damage serum indicators (aspartate aminotransferase, alanine transaminase, and alkaline phophatase) caused by paracetamol induced toxicity were decreased at the highest concentration of VCO used. Otuechere *et al*. (2014) found that consuming cold pressed VCO (600 mg/kg body weight per day) lowers the toxicity caused by the popular antibiotic trimethoprim-sulfamethoxazole (TMP-SMX) by restoring the levels of lactate dehydrogenase, alkaline phosphatase and total bilirubin. Application of VCO led to a natural reversion of the established hepatosteatosis condition by raising HDL-C levels and lowering hepatic and serum triglycerides (Narayanankutty *et al*., 2018).

Nevin and Rajamohan (2010) assessed the healing capacity of VCO by monitoring the time needed for complete epithelisation. In this study, wounds that were treated with VCO healed much more quickly as shown by a shorter period for complete epithelization and higher levels of different skin components. Intahphuak *et al*. (2010) reported that VCO had a moderate analgesic impact on mice's acetic acid induced writhing response as well as an antipyretic effect in yeast induced hyperthermia.

Free radicals and oxidative stress are linked to the pathophysiology of osteoporosis. Antioxidants are therefore probably effective at preventing the disease. Hayatullina *et al*. (2012) reported that polyphenols in VCO improved bone structure and prevented bone loss in rats with osteoporosis. Abujazia *et al.* (2012) found that VCO supplementation (8%) significantly improved the bone's antioxidant status by preventing lipid peroxidation and raising levels of the enzymes glutathione peroxidase and superoxide dismutase in the osteoporotic rat model.

VCO is beneficial for those with immunosuppression because it is also high in medium-chain fatty acids. VCO consumption is a potential therapy for HIV/AIDS patients whose immune systems are severely weakened (Dayrit, 2000). Soerjodibroto (2006) found that immune response of HIV positive patients noticeably improved among those who took VCO as a supplement (10 to 15 mL/day). Law *et al*. (2014) reported that the consumption of VCO (10 mL twice daily) during chemotherapy of patients with breast cancer can help alleviate the symptoms related to the side effects of their chemotherapy.

Coconut oil has been used to treat a variety of skin disorders in Ayurvedic medicine, including microbial infections and wound healing. Agero and Verallo-Rowell (2004) compared the efficacy of mineral oil and VCO as therapeutic moisturiser for mild to moderate xerosis and the results revealed that both oils significantly hydrated skin and raised levels of skin surface lipids. They concluded that VCO is more effective than mineral oils to cure xerosis. Nevin and Rajamohan (2010) have shown the effectiveness of VCO (0.5 to 1.0 mL) for the rat dermal wound healing process. VCO is also used as a remedy for inflammation caused by burn wounds (Intahphuak *et al*., 2010).

2.5. Value added products of VCO

VCO is a value added coconut product with edible quality that has several uses for people. VCO plays a significant role in a variety of industries, including food, medicine, cosmetics, and nanotechnology. Foale (2003) reported that VCO is acting as a main ingredient in high grade cooking lotions, drinks, *etc*. due to its unique flavour and aroma properties. According to Choo *et al*. (2010), milk fat can be replaced with VCO to create healthy ice cream with a delicious coconut flavour and aroma. They concluded that formulations containing VCO were more widely accepted in terms of their general acceptability, appearance, texture, aroma, and flavour.

The edible by products produced during the hot processing of VCO include VCO cake, mature coconut water, and milk residue. Commercial applications for mature coconut water include the production of nata de coco, vinegar, squash, fruit juice blends, and ready to serve (RTS) beverages. Milk residue is being explored for use in a variety of meals, including baked goods, chocolate, and steamed goods (Bawalan, 2000). VCO cake is often discarded as waste and in frequently used as animal feed. Large amounts of whey, or waste water containing little coconut cream and coconut pulp, were produced during the fermentation process used to produce VCO. This could be used efficiently in the manufacturing of bio-extract along with other forms of biowastes including fish waste and fruit peel (Tripetchkul *et al*., 2010).

Manikantan *et al*. (2015) reported that recovery of VCO cake ranged from 6.3 to 8.8 per cent of fresh kernel and it is a rich source of protein. Beegam *et al*. (2017) developed VCO cake based muffins and the product had a potential market as high nutritious high caloric snack food. VCO cake based extrudates were developed by Beegam *et al*. (2019) and it was highly acceptable. Proximate analysis showed that protein content of extrudates significantly increased as the level of VCO cake was raised. They concluded that VCO cake is a perfect substitute for flour mix for the preparation of expanded snacks.

VCO has an oily unpleasant taste when consumed directly. VCO may taste better when it is emulsified into beverages, and customers may benefit from the

convenience of consumption and health-promoting effects. VCO beverages were developed by Yani *et al*. (2018) by combining VCO with water and using soybean lecithin as a natural emulsifier. The findings demonstrated that, in terms of viscosity and stability, the 20% VCO beverage emulsion was the most stable emulsion.

Srivastava *et al*. (2010) prepared a biscuit by incorporating virgin coconut meal (VCM) into refined wheat flour at 5 to 25 per cent. The addition of VCM had a noticeable impact on the colour values of biscuits as the concentration of VCM was increased. According to the sensory analysis, the most acceptable biscuit was 15% VCM. All biscuits had high content of protein and fibre compared to the control (100% refined wheat flour based biscuit). Mondal *et al*. (2022) formulated antioxidant rich cookies by replacing hydrogenated hydrogenated vegetable fat in cookies with gamma irradiation induced deodorized (rancid acid odor removed) VCO. They concluded that VCO formulated cookies had good sensory acceptability and long shelf life. It can be safely consumed up to 150 days when stored at 25 ± 2 °C.

VCO is used in the development of hypoallergenic cosmetics and skin care products (Agero and Verallo-Rowell, 2004). Pandiselvam *et al*. (2019) reported that VCO is added in many beauty products including moisturiser, shampoo, lip balm, mouthwash, sunscreen, hair oil, and massage oil. Satheeshan *et al*. (2020) developed a VCO based body lotion consisting 76% of VCO, bee wax (8 per cent), aloe vera (12 per cent), cocoa butter (2 per cent) and rose oil (2 per cent). They concluded that the body lotion developed has a long shelf life, is stable and safe, and doesn't irritate the skin.

VCO is included in the preparation of natural shampoos in which the extract of soap nut powder and amla fruit are sometimes incorporated to add the value of the product (Rethinam, 2002). Sulphate is not present in the shampoo made with VCO. VCO based shampoo promotes hair growth, removes impurities, rehydrates hair, and keeps it shiny. According to Viste *et al*. (2013), the shampoo is quite successful at preventing fleas, ticks, lice, and mites. The shampoo with 80% VCO is the most effective concentration for getting rid of dog mites and other ectoparasites as early as the sixth week of treatment. Virgin coconut oil, olive oil and vegetable fat are used as

natural ingredients in the formulation of the herbal lipsticks (Azwanida *et al*., 2014). VCO is an excellent raw material for manufacturing soap since it has lathering and active cleansing properties (Pandiselvam *et al*., 2019).

VCO is a healthy and highly acceptable product but people do not like the taste and greasiness of it. Acceptability of oil is increased by microencapsulation by hiding the natural taste, aroma and texture of oil. Oxidative stability, thermostability, shelf life and biological activity of oil also can be improved by microencapsulation (Bakry *et al*., 2016). Hee *et al*. (2017) encapsulated VCO from oil in water emulsion with supercritical carbon dioxide spray drying at 12 to 16 MPa pressure and 40 to 60°C temperature. The microcapsules were spherical and the size ranged from 27 to 72 μm. They concluded that VCO and other food oils can be effectively encapsulated using SC-CO_2 spray drying.

Aini *et al*. (2020) used VCO as a fat substitute and determined the effect of VCO content on the quality of cheddar cheese substitute made from corn milk. The results showed that adding more VCO to the cheese analogue production process increased fat and moisture content while lowering sensory value. The best cheese substitute was made with 25% VCO and the qualities of this product were good. Juliyarsi *et al*. (2021) developed biodegradable packaging from cheese waste by combining lactic acid bacteria found in fermented virgin coconut oil (VCO) with edible whey film. The study aimed to know the effect of VCO on barrier, mechanical and microstructure whey edible films. The results showed that thickness of whey based edible films increased along with the increase of VCO concentration. They concluded that addition of VCO had a significant impact on the physical properties such as solubility time and thickness and no effect on microstructure and mechanical properties such as elongation and tensile strength.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule' was proposed to assess the physico-chemical properties, antioxidant activity and medicinal properties of virgin coconut oil. The study also aims to develop VCO capsule and to study its quality attributes. The investigation was carried out in the Department of Community Science, College of Agriculture, KAU during the year 2019-2023. The materials and methods used for the study are given under the following headings.

3.1. Collection of raw materials

3.2. Quality evaluation of VCO extracted using different methods

3.2.1. Extraction of virgin coconut oil using different methods

3.2.2. Organoleptic evaluation of VCO

3.2.3. Physico-chemical evaluation of VCO

3.3. Storage studies of the extracted VCO

3.3.1. Organoleptic evaluation

3.3.2. Physico-chemical evaluation

3.3.3. Total microbial population

3.4. Selection of the best VCO

3.5. Evaluation of medicinal properties of VCO

3.5.1. Antimicrobial properties

3.5.2. Antiproliferatory activities

3.5.3. Antioxidant properties

3.6. Development of VCO capsule

3.7. Storage studies of VCO capsule

3.7.1. Acceptability studies

3.7.2. Physico-chemical evaluation

3.7.3. Total microbial population

3.8. Cost of production

3.9. Statistical analysis

3.1. Collection of raw materials

Mature coconuts (11 to 12 months old) of west coast tall (WCT) variety and KAU released hybrid, *Kerasree* were used for the extraction of VCO. WCT variety collected from the instructional farm Kerala Agricultural University, Vellanikkara, Thrissur and *Kerasree* from RARS, Pilicode, Kasaragode.

3.2. Quality evaluation of VCO extracted using different methods

3.2.1. Extraction of virgin coconut oil using different methods

VCO was extracted from WCT variety and *Kerasree* hybrid by four different methods: traditional method, fermentation method, cold centrifugation method and enzymatic method. Various treatments used for the extraction of VCO are given in Table 1.

Table 1: Different treatments for the extraction of VCO

Treatment	Process
T_1	Traditional method (WCT)
T_2	Fermentation method (WCT)
T_3	Cold centrifugation method (WCT)
T_4	Enzymatic method (WCT)
T_5	Traditional method (*Kerasree*)
T_6	Fermentation method (*Kerasree*)
T_7	Cold centrifugation method (*Kerasree*)
T_8	Enzymatic method (*Kerasree*)

3.2.1.1. Preparation of coconut milk

Fully matured, 11 to 12 months old coconuts were chosen for milk extraction. After dehusking and breaking the coconut into two halves, the coconut meat was grated using a coconut grater. Coconut milk was extracted from the grated coconut meat using

a white cloth by manual pressing. The coconut milk obtained from the first extraction was collected for the preparation of oil.

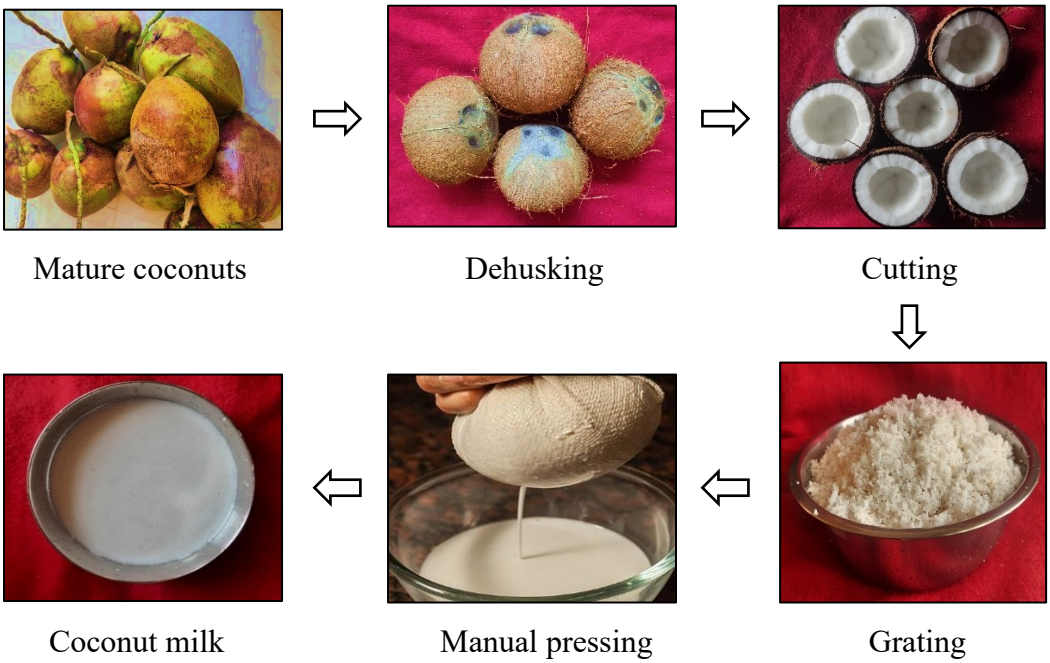


Plate 1: Preparation of coconut milk

3.2.1.2. Extraction of virgin coconut oil using the traditional method

In the hot extraction process, oil was extracted from coconut milk by heating as per the methodology of Manikanatan *et al*. (2016). Coconut milk was heated for 15 minutes at 100 to 120°C to evaporate the water completely. After that, the temperature was lowered to 90°C to allow the protein to coagulate and when the temperature was reduced to 60°C, oil started to separate. The coagulated and sedimented protein was separated from oil by filtering through muslin cloth. To extract more oil, further heat was applied to the residual material.

3.2.1.3. Extraction of virgin coconut oil using fermentation method

The fermentation was carried out according to Manikantan *et al*. (2016) method with modifications. The coconut milk was allowed to ferment naturally at room temperature. After 24 hours, the mixture was centrifuged at 6000 rpm for 15 minutes to separate the different layers of VCO and water. The top layer (VCO) was separated

from the centrifuge tube. Then, VCO was filtered through Whatman filter paper no. 1 to remove solid particles that escaped into the oil while the separation and stored in glass bottles.

3.2.1.4. Extraction of virgin coconut oil using the cold centrifugation method

In cold centrifugation, VCO was extracted using a standard procedure recommended by Raghavendra and Raghavarao (2010) with modifications. Coconut milk was centrifuged at 10000 rpm for 10 minutes at 4ºC and the top layer of cream was separated for chilling. The cream was chilled at 4°C for 24 hours before gradually thawed in a water bath at 50°C. Then oil was stored in glass bottle after filteration.

3.2.1.5. Extraction of virgin coconut oil using the enzymatic method

VCO was extracted by enzymatic method according to Raghavendra and Raghavarao (2010) procedure with modifications. Papain enzyme was added to coconut milk at a rate of 0.1 per cent (w/w). The mixture was stirred into a homogeneous solution and it was incubated for 3 hours at 55°C. After incubation, the mixture was centrifuged for 25 minutes at 4900 rpm to extract the oil. Then, oil was collected from the centrifuge tube and filtered through Whatman filter paper no. 1.

3.2.2. Organoleptic evaluation of VCO

Organoleptic evaluation was conducted by using score cards by a panel of twenty judges.

3.2.2.1. Selection of judges

A series of acceptability trials were carried out using simple triangle test at the laboratory level to select a panel of twenty judges between the age group of eighteen to thirty five years, as Jellinek (1985) suggested.

3.2.2.2. Preparation of score card

The score card contained six sensory quality parameters: appearance, colour, texture, flavour, taste and overall acceptability. A nine point hedonic scale was used for the organoleptic evaluation of VCO by the panel members. The prepared score card is given in Appendix I.

[T_1]
Traditional method
WCT variety

[T_2]
Fermentation method
WCT variety

[T_3]
Cold centrifugation method
WCT variety

[T_4]
Enzymatic method
WCT variety

[T_1]
Traditional method
Kerasree hybrid

[T_6]
Fermentation method
Kerasree hybrid

[T_7]
Cold centrifugation method
Kerasree hybrid

[T_8]
Enzymatic method
Kerasree hybrid

Plate 2: VCO extracted from WCT variety and *Kerasree* hybrid using different methods

3.2.3. Physico-chemical evaluation of VCO

3.2.3.1. Oil recovery

The recovery of oil was calculated according to the initial weight of coconut milk and the weight of oil extracted from different methods (AOAC, 2012).

$$\text{Oil recovery (\%)} = \frac{\text{Weight of oil extracted}}{\text{Weight of coconut milk used}} \times 100$$

3.2.3.2. Iodine value

The iodine value indicates the amount of unsaturated fatty acids in the oil. Iodine value was estimated using the method recommended by Sadasivam and Manickam (2007). Oil (0.25 mL) was taken in an iodine flask and dissolved in

chloroform. Then Hanus iodine solution (25 mL) was poured to the flask and left for 30 minutes in the dark with intermittent shaking. Freshly boiled and cooled 100 ml water and 10mL of 15 per cent potassium iodide were then added. The solution was then titrated until the yellow colour disappeared against 0.1N sodium thiosulphate. Later, a few drops of starch were added as an indicator, and the amount was measured until the blue colour disappeared completely. The readings were recorded, and the iodine value of oil was expressed in I_2 per 100 mg.

3.2.3.3. Peroxide value

Peroxide value of VCO was estimated to find out the rate of rancidity during storage. It was determined by the procedure given by Sadasivam and Manickam (2007). Solvent mixture was prepared using glacial acetic acid and choloform. A boiling tube was filled with one mL of the sample, 20 mL of solvent mixture, and one gram of potassium iodide. After 30 seconds of boiling in a water bath, the mixture was transferred to a standard conical flask containing 20mL of 5 per cent potassium iodide solution. The tubes were rinsed twice using 25mL of water and collected in a conical flask. This was quantified against N/500 sodium thiosulphate solution until the disappearance of yellow colour. Later starch solution (0.5mL) was added and titrated till the disappearance of blue colour. A blank solution was also prepared and the peroxide value was computed and expressed in milli equivalent per kg of the sample.

3.2.3.4. Saponification value

The saponification value of VCO samples was estimated by the method of Sadasivam and Manickam (2007). About 1.5 to 2.0 mL of the test sample was taken in a conical flask. The potassium hydroxide alcoholic solution (25 mL) was added and connected the reflux condenser to the flask. Then, the flask was heated on an electric hot plate or water bath for about one hour. It was slowly and gradually boiled until the sample was totally saponified, which was demonstrated by the complete absence of oil matter and the appearance of a clear solution. Then, the condenser and flask were allowed to cool. Inside of the condenser was washed using hot neutral ethyl alcohol (10 mL). One mL of phenolphthalein was added and titrated with standard hydrochloric acid. A blank sample was also carried at the same time.

3.2.3.5. Fatty acids

The AOAC (2012) method was followed to extract the fatty acid methyl ester (FAME). Approximately 50 mL of VCO was dissolved in 4 mL of methanolic HCl (0.5 mol/L). The mixture was well mixed before being incubated at 50°C for 4 hours and then cooled to room temperature. 10 mL of hexane was used to purify the FAME, and the clear upper layer containing the FAME was then passed through anhydrous Na_2SO_4. The extracted fatty acid methyl ester was identified using gas chromatography following the protocols outlined by Moigradean *et al*. (2013). A split/split less injector-equipped gas chromatography-mass spectrometer (GC-MS) QP 2010 (Shimadzu, Japan) was used to analyse the composition of FAME. On a DB-5 ms column with dimensions of 30 m, 0.25 mm in diameter, and 0.25 m film thickness, the compounds were separated. A flow rate of one mL per minute and a split ratio of 100 were employed with helium. The temperature of the injector was 250°C. For 10 minutes, the oven's temperature was maintained at 60 °C. After that, it was raised to 140 °C at a rate of 10 °C/min., and then maintained there for 10 minutes. The temperature was subsequently raised to a final level of 250°C at a rate of 7°C/min, and it was maintained there for 10 min. The conditions for the mass-selective detector were set to full-scan mode with a mass range of 40-850 amu, a capillary direct interface temperature of 230°C, and an ionisation energy of 70 eV. By comparing the retention indices and mass spectra of the unknown chemicals with those of standard compounds, the FAME in the VCO was identified. Based on the ratio of the area of the associated peak to the total area of all peaks, the weight fractions of the FAMEs were computed.

3.2.3.6. Moisture content

Moisture content was analysed by the method of AOAC (2012). Oil (25 mL) was poured in a Petri dish, heated in a hot air oven at 105°C, cooled and then weighed. To get a constant weight, this process was repeated. The moisture content was expressed in percentage and calculated from the loss of weight while drying.

$$\text{Moisture} = \frac{(\text{Initial weight of petridish} + \text{Sample}) - (\text{Final weight of petridish} + \text{Dry sample})}{\text{weight of the sample}} \text{ X } 100$$

3.2.3.7. Viscosity and Colour

The viscosity of the VCO samples was measured using a Brookfield viscometer. Approximately 60 mL of the oil was taken in the conical flask and tested for viscosity using spindle 61. The rotation speed was adjusted to 50 rpm for 30 seconds to measure viscosity (cP) (Wiyani *et al*., 2018).

The colour of the oil was analysed by using a Hunter Lab spectrophotometer. About 25 to 50 mL of oil was taken in a plastic Petri dish and placed on the spectrophotometer. The colour coordinates 'L', 'a' and 'b' (brightness, redness and yellowness, respectively) values were recorded from the device (Tijskens *et al*., 2001).

3.2.3.8. Tocopherol

Tocopherol analysis was done according to the procedure described by Sadasivam and Manickam (2007). For centrifugation, sample (1.5 mL), standard (1.5 mL) and water (1.5 mL) were pipetted out into three tubes (test, standard and blank) and tubes were capped. Ethanol (1.5 mL) was added to the test and blank tubes and 1.5 mL of water was added to standard tubes and centrifuged. Xylene (1.5 mL) was added to each tube and mixed thoroughly before centrifugation. 1.0 mL of the xylene was transferred into another stopper tube. The reagent 2, 2-dipyridyl (1.0 mL) was added to each tube and mixed well. This mixture (1.5 mL) was pipetted into the cuvette and measure the extinction of standard and test against the blank at 460 nm. Ferric chloride (0.33 mL) solution was then added and mixed well. After 15 minutes, the standard and test were read against the blank at 520 nm.

3.2.3.9. Total fat

The fat content was estimated by using modified Batch Solvent Extraction (Min and Steenson, 1998). The sample (1 mL) and 20 mL of the solvent (Hexane) were added to a separatory funnel. The organic solvent and aqueous phase were allowed to separate by gravity after the container was vigorously shaken. The aqueous phase was then separated, and the amount of fat in the solvent was calculated by measuring the mass of fat after the solvent had evaporated.

$$\text{Per cent of weight of fat} = \frac{\text{Weight of fat}}{\text{Initial weight of sample taken}} \times 100$$

3.2.3.10. Total phenol content

The total phenolic content in VCO was assessed using the Folin-Ciocalteu reagent (Mallick and Singh, 1980). Different concentration of samples was pipetted out and mixed with distilled water to make up the volume to 3.0 mL. After adding 0.5 mL Folin-Ciocalteau reagent and 20% Na_2CO_3 (2 mL) to the test tube, it was placed in a water bath for one minute. The tubes were allowed to cool and the absorbance was measured at 750nm using spectrophotometer against a reagent blank. Gallic acid solutions (2.5-100 µg/mL) were taken as standard and also treated as above.

3.2.3.11. Total antioxidant activity

According to the procedure suggested by Prieto *et al*. (1999), the total antioxidant activity of the VCO was assessed using the phosphor molybdenum method. The reagent solution was made up of 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid. One mL of VCO was mixed with 3 mL of reagent solution. The standard used was ascorbic acid, which was also mixed with 3 mL of the reagent solution in 1 mL of different standard concentrations. The reaction solution-filled tubes were incubated for 90 minutes at 95°C. After cooling to room temperature, the solution's absorbance at 695 nm was measured using a spectrophotometer against the blank solution. The number of micrograms equivalent to ascorbic acid is used to express the total antioxidant activity. The calibration curve was created by combining methanol with ascorbic acid.

3.2.3.12. Volatile and nonvolatile compounds

3.2.3.12.1. Sample preparation

Oil (0.1 mL) was taken into a 10 mL standard flask using a micropipette and prepared a 1000 ppm solution by adding HPLC grade hexane. Then, 0.1 mL of solution was pipetted out from 1000 ppm solution into another standard flask and prepared 100 ppm solution.

3.2.3.12.2. GC-MS analysis

The phytochemicals in VCO were characterised using the GC-MS QP2010 Plus (Shimadzu, Japan) system. Using a QP2010 gas chromatography with Thermal Desorption System, TD 20 paired with Mass Spectroscopy (Shimadzu), the phytochemicals in the samples were detected. 70 eV was the ionisation voltage. A Restek column (0.25 mm, 60 m, XTI-5) was used for gas chromatography in the temperature programming mode. The initial column temperature was 80°C for one minute, followed by a linear increase of 70°C per minute to 220°C, held for three minutes, and then a linear increase of 10°C per minute to 290°C for ten minutes. The GC-MS interface was kept at 290°C, and the injection port's temperature was 290°C. An all-glass injector operating in split mode and using a helium carrier gas flow rate of 1.2 mL min^{-1} was used to insert the sample. The compounds were identified by comparing fragmentation pattern, mass spectra and retention time, from the GC-MS.

3.3. Storage studies of the extracted VCO

The extracted VCO samples were stored in glass bottles for six months. Quality aspects of the VCO samples were studied throughout the storage period. The organoleptic evaluation was done at weekly intervals. All the other quality parameters were evaluated at 2 months intervals. The parameters studied and the methods followed are mentioned below.

3.3.1. Organoleptic evaluation

Organoleptic qualities of VCO were conducted initially and at weekly interval for six months. The organoleptic qualities of the VCO were conducted as per the procedure mentioned in 3.2.2.

3.3.2. Moisture content

The moisture content of VCO was determined as per the procedure mentioned in 3.2.3.6.

3.3.3. Free fatty acids

The free fatty acid content of products was estimated by the method given by Sadasivam and Manickam (2007). The sample (10 mL) was dissolved in 50 mL of neutral solvent. The neutral solvent is a mixture of 25mL of 95 per cent alcohols, 25mL of ether and 1mL of phenolphthalein solution, neutralized with N/10 alkali in a 250mL of conical flask. Then two drops of phenolphthalein indicator were added, and an aliquot of the sample was quantified against 0.1N potassium hydroxide with constant stirring until a pink colour was formed. The free fatty acid value was calculated and expressed as milligram KOH per gram.

3.3.4. Peroxide value

The peroxide value of VCO was determined as per the procedure mentioned in 3.2.3.3.

3.3.5. Total microbial population

The microbial population in the VCO samples were estimated using serial dilution plate count method as Agarwal and Hasija (1986) suggested. The microbial analysis was carried out initially and at two monthly storage intervals.

3.3.5.1. Preparation of samples and media

The sample was prepared by mixing 10g of sample with 90 mL of distilled water and shaken well using a shaker to obtain a homogeneous suspension. The serial dilution was carried out in the prepared sterile water blank. Transfer one mL of the prepared suspension to nine mL of water blank with a dilution of 10^{-2}. This is then diluted to 10^{-3} using serial dilution techniques. Bacteria, fungi and yeast count were assessed using Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Sabouraud's Dextrose Agar (SDA) media respectively and results were given as cfu/mL.

3.3.5.2. Total bacterial population

The total number of bacterial colonies was computed in 10^{-2} dilution in a nutrient agar medium. In a sterile Petri plate, one mL of 10^{-2} dilution was poured using a micropipette. About 20 mL of melted and cooled nutrient agar media was poured in

a Petri plate and mixed by rotating it clockwise and anti clockwise direction for 3 to 4 times. Petri plates were incubated for 48 hours at 28℃ ± 2 for bacterial colony count. The total number of bacterial population was counted and expressed as cfu/mL.

3.3.5.3. Total fungal population

The total number of fungal colonies was enumerated in 10^{-3} dilution in potato dextrose agar medium. One mL of 10^{-3} dilution was poured into a Petri plate using a micropipette. About 20 mL of the potato dextrose agar medium was poured into the Petri plate and uniformly spread. For fungal count, the Petri plates were incubated for 4 to 5 days at 28℃ ± 2. The total number of fungal population were counted and expressed as cfu/mL.

3.3.5.4. Total yeast population

The total number of yeast colonies was enumerated in 10^{-3} dilution using Sabouraud's dextrose agar medium. In a sterile Petri plate, one mL of 10^{-3} dilution was poured using a micropipette. About 20 mL of Sabouraud's dextrose agar medium was poured into a Petri plate and uniformly spread by rotating. The Petri plates were incubated for 4 to 5 days at 28℃ ± 2. The total number of yeast population was counted and expressed as cfu/mL.

3.4. Selection of the best VCO

Based on the organoleptic scores, physico-chemical properties and shelf life studies, the best treatment was selected for further studies. A rank score method was used for the selection of the best treatment. According to the rank scores, the lowest sum of the summary scores is considered as the best treatment.

3.5. Evaluation of medicinal properties of VCO

3.5.1. Antimicrobial properties

Culture Media: Mueller-Hinton Agar (Himedia M173)
Potato Dextrose Agar (Himedia MH096)

Solvent: Methanol

Bacterial strains: *Staphylococcus aureus* MTCC87
Bacillus subtilis MTCC2413

Streptococcus pyrogenes MTCC442

Candida albicans MTCC227

Media preparation and antibacterial analysis:

The antibacterial activity of VCO analysed using agar well diffusion method following the method of Valgas *et al*. (2007). Muller Hinton Agar medium (HIMEDIA-M173) was used for evaluation of the susceptibility of bacterial strains to antibacterial agents. The Mueller-Hinton Agar (3.8 g) was dissolved in 100 mL of distilled water and autoclaved for 15 minutes at 121°C under 15 lbs of pressure. After sterilisation, the medium was chilled in a water bath to 45°C. The same-sized glass Petri plates were then filled with 25 mL of medium and given time to set. Using a sterile cotton swab, a standardised inoculum of the test organism was evenly dispersed across the surface of the plates. A sterile cork borer was used to aseptically punch four 8 mm-diameter wells into each plate, 20 mm apart from one another. The sample (100 and 500 µL) was poured into the wells T_1 and T_2 directly from the test sample. Gentamycin (160 µg) was used as positive control and the solvent for sample dilution was used as negative control. Under aerobic conditions, the plates were incubated at 36ºC ± 1ºC. After 24 hours of incubation, the plates were examined and the zone of inhibition around the wells was measured in milli metre.

Media preparation and antifungal analysis:

The antifungal activity of VCO evaluated as per the procedure of Magaldi *et al.* (2004). Potato Dextrose Agar and Mueller-Hinton Agar (1:1) were used for the evaluation of susceptibility of fungal strains to antifungal agents. PDA and MHA were dissolved in distilled water (100 mL) and autoclaved for 15 minutes at 121°C under 15 lbs pressure. After sterilisation, media was kept in a water bath (45 to 50°C) for cooling. PDA and MHA (1:1) were poured into the Petri plates of the same size and allowed to solidify at room temperature. Using a sterile cotton swab, a standardised inoculum of the test organism was evenly distributed across the surface of the plates. Each plate was punctured aseptically using a sterile cork borer to create four wells that were each 7 mm in diameter and 20 mm apart from one another. The test samples (100 and 500 µL) were added into the wells T_1 and T_2 directly from the sample. Clotrimazole (400

μg) was added as the positive control and the solvent for sample dilution was used negative control. Then, plates were incubated at 27°C ± 1°C for 24 hours, under aerobic conditions. After 24 hours of incubation, the plates were examined and the zone of inhibition around the wells was measured in milli metre.

3.4.2. Antiproliferatory studies

Antiproliferative assay in human hepatic cell lines using MTT [3-(4, 5 dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] assay was done in VCO according to the method of Mosmann (1983).

3.4.2.1. Cell line and maintenance

Human hepatic cell line (MCF-7) was purchased from National centre for cell sciences, Pune. The cells were grown in Dulbecco's Modified Eagle Media (DMEM-Himedia), which was supplemented with 10 per cent heat inactivated Fetal Bovine Serum (FBS) and one per cent antibiotic cocktail made up of amphotericin B (2.5 μg/mL), streptomycin (100 U/mL), and penicillin (100 μg/mL), The cell containing flasks (25 cm^2) were incubated at 37 ^{O}C at 5 per cent CO_2 environment with humidity in a cell culture incubator.

3.4.2.2. Cytotoxicity analysis

On 96-well plates, human hepatic cell lines were seeded and given 24 hours to adjust to the culture conditions of 37°C and 5 per cent CO_2 in the incubator. The test samples were made in DMEM medium (100 mg/mL), and a 0.2 m Millipore syringe filter was used for filter sterilisation. The samples were further diluted in DMEM media and introduced to the wells containing grown cells at final concentrations of 6.25, 12.5, 25, 50, and 100 μg/mL, respectively. The untreated wells are considered as control. After the test samples were applied, the plates underwent another round of incubation for another 24 hours. After the incubation time, the wells' media were aspirated and discarded. The MTT solution in PBS (100 μL of 0.5 mg/mL) was added to the wells. The plates were further kept for two hours incubation. Afterthat, the developed formazan crystals during incubation was observed using inverted phase contrast microscope. Hundred per cent of DMSO (100 μL) was added per well after removing

the supernatant. A micro plate reader (Multiskan Sky High, Thermo Scientific) was used to measure the absorbance at 570 nm. Two wells per plate without cells served as blank. Triplicates of each experiment were performed to minimise errors. The absorbance of the control well was then compared with in order to calculate cell viability.

3.5.3. Antioxidant properties

The free radical scavenging properties of VCO were assessed. The antioxidant activities such as DPPH (1,1- diphenyl 1-2-picrylhydrazyl) radical scavenging activities, reducing power (RP) assay, nitric oxide (NO) scavenging, superoxide and hydroxyl scavenging activity and total antioxidant activity were assessed in VCO.

3.5.3.1. DPPH radical scavenging assay

Radical scavenging activity of the test sample against stable 2, 2- diphenyl 2-picrylhydrazylhydrate (DPPH) was determined by the standard procedure of Brand-William *et al*. (1995) with some modification. Ascorbic acid was taken as standard for the DPPH assay. The ascorbic acid stock solution was prepared using distilled water (1 mg/ mL; w/v). A 200 µL of freshly made 60 µM solution of DPPH in methanol solution was mixed with 50 µL of the test sample at different concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 µg/mL). The plates were left at room temperature in the dark for 15 minutes. The decrease in absorbance was detected at 515 nm. The control was prepared using only DPPH solution. As a blank, methanol (95%) was used. The percentage inhibition was computed and the IC_{50} value was estimated.

3.5.3.2. Reducing power assay (FRAP)

The reducing power was analysed as per the procedure of Rashad *et al.* (2011). Different concentrations of (10, 100, 200, 500, 1000 µg) sample and ascorbic acid (standard) were combined with sodium phosphate buffer (1.2 mL - pH 6.6) and one per cent of potassium ferric-cyanide (2.5 mL). After incubating this mixture (20 min at 50°C), 1.25 mL of 10 per cent trichloroacetic acid (w/v) was added. Then, mixture was centrifuged at room temperature for 10 min. The upper layer of mixture (1 mL) was separated from the centrifuge tube and mixed with deionised water (1 mL) and 0.1 per

cent of ferric chloride (125 µL). The absorbance was recorded at 700 nm. The reducing power is higher when the absorbance is higher. The effective concentration was calculated by comparing the absorbance value of the experimental tubes and control.

3.5.3.3. Nitric oxide scavenging activity

Nitric oxide radical scavenging activity of sample was estimated using Griess Ilosvay reaction as per the method of Patel *et al*. (2010). Griess Ilosvay reagent was modified by using 0.1 per cent of napthyl ethylene diaminedihydrochloride. Three mL reaction mixture was incubated for 150 minutes at 25°C. Reaction mixture contained 2 mL sodium nitroprusside (10 mM), 0.5 mL saline phosphate buffer and standard solution (0.5 mL) or samples (6.25-100 µg/mL). After incubation, one mL of mixture was combined with one mL sulfanilic acid reagent (0.33 per cent in 20 per cent glacial acetic acid) and the mixture was left to stand for 5 minutes for the completion of the diazotization reaction. Then, one mL napthyl ethylene diaminedihydrochloride was added, mixed well, and let to stand at 25°C for 30 minutes. The nitrite concentration was measured at 546 nm. It was computed with the control absorbance of the standard solution. Ascorbic acid was used as the standard solution and buffer was taken as the blank solution.

3.5.3.4. Superoxide radical scavenging assay

The test sample's ability to scavenge superoxide radical was assessed using the nitro blue tetrazolium (NBT) reduction technique (Cord and Fridovich, 1969). It depends on the production of superoxide by riboflavin in response to light and the concomitant reduction of nitro blue tetrazolium. The reaction mixture (0.1 M EDTA) was prepared by mixing 0.067 M phosphate, 0.12 Mm riboflavin, 0.3 mM NaCN, and 1.5 mM NBT. Different concentrations of the sample were added to the reaction mixture and made up to 3 mL total volume. The optical density at 560 nm was measured before and after the tubes received uniform illumination from an incandescent bulb for 15 minutes. The absorbance readings of the control and experimental tubes were compared to determine the percentage inhibition of superoxide production.

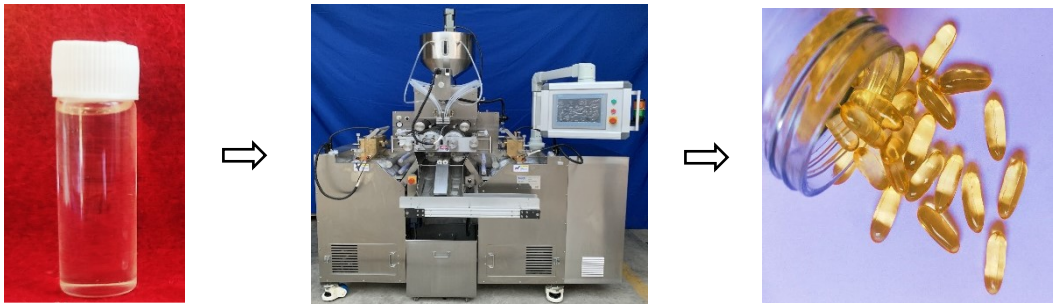
3.5.3.5. Hydroxyl radical scavenging activity

The hydroxyl radical generated in the Fe^{3+}-ascorbate - EDTA-H_2O_2 system (Fenton reaction) degrade 2-deoxyribose the product of which condense with Thiobarbituric acid Reacting Substances (TBAR's) forming a pink coloured complex (Elizebeth *et al*., 1990). The efficacy of test compounds to interfere with the reaction was assessed. One ml reaction mixture was prepared by adding KH_2PO_4-KOH buffer (20 mM, pH 7.4), 2-deoxy-2-ribose (2.8 mM), EDTA (0.1 mM), H_2O_2 (0.1 mM), $FeCl_3$ (0.1 mM), ascorbic acid (0.25-4.0 µg/mL) of sample. After incubation for 1 hour at 37°C, transferred 0.4 mL of the reaction mixture into test tubes, and added with 1.5 mL TBA (0.8%), 1.5 mL acetic acid (20% pH 3.5), 200 µL SDS (8.1%) and made up the volume to 4.0 mL with distilled water. The incubation was carried out at 100°C for one hour. After cooling the solution, the absorbance was measured at 532 nm against an appropriate blank solution. The positive control was Vitamin C.

3.6. Development of VCO capsule

Development of VCO capsule was done according to Bhawna and Aggrawal (2007) and Javed *et al*. (2010). 20 to 30 per cent plasticizers (glycerol), 30 to 40 per cent water, preservatives (0.2% of methyl paraben) and flavouring agents were used to formulate soft gel capsule. The gel mass was prepared under vaccum condition, by dissolving the gelatin in water at 80°C temperature, followed by the addition of glycerol. Flavours and preservatives were added after the complete dissolution of gelatin. The temperature of the melting tank was maintained at 57 - 60°C. A casting technique that creates two distinct gelatin ribbons was used to convey the hot gel mass from the transfer pipes to the encapsulation machine. The gel mass was fed by gravity to a metering device, which regulates the flow of mass onto rotating drums that are heated to between 13 and 14°C. Gelatin ribbons were produced and each ribbon's thickness was kept within a range of 0.1 mm as the gelatin passed through the soft gel transformer throughout the casting process. The thickness was checked regularly during the process. The two gel ribbons were then passed through rollers and onwards to the rotatory die encapsulation. Each gelatin ribbon provided one-half of the soft gel. VCO was contained in the liquid fill matrix. The rotating drum pulled together the continuous ribbon of liquid gelatin that was flowing from an overhead tank and brought

it together between two rotating dies. The liquid was injected between the gel ribbons, forcing the gel to spread out and into the dies packets, which in turan controlled the shape and size of the capsule. The capsules were sealed by applying mechanical pressure to the die rolls and heating the ribbons with the wedge. After being manufactured, it was dried using infrared radiation before being separated on trays and placed in funnel dryers that supplied air with a twenty per cent relative humidity.



VCO extracted by cold centrifugation method

Soft gel encapsulation machine

VCO capsule

Plate 3: Development of VCO capsule

3.7. Storage studies of VCO capsule

The developed VCO capsules were filled in glass bottles and stored for a period of three months. Acceptability studies were done at weekly intervals by evaluationg organoleptic parameters. Physico-chemical properties of VCO capsule including moisture, free fatty acid and peroxide value, and total microbial population were studied at weekly intervals throughout the storage period.

3.7.1. Acceptability studies

Acceptabiity studies were done by evaluating the organoleptic parameters like appearance, colour, texture, flavour, taste, and overall acceptability of VCO capsule. The nine hedonic scale was used for the evaluation by a panel of twenty selected judges (Jellinek, 1985).

3.7.2. Moisture

The method suggested by AOAC (2012) for analysing the moisture content of capsule was followed as mentioned in 3.2.3.6.

3.7.3. Free fatty acids

Free fatty acids in the VCO capsules were estimated as per the procedure suggested by Sadasivam and Manickam (2007). The methods are detailed in 3.3.3.

3.7.4. Peroxide value

The peroxide value was estimated using the method described in 3.2.3.3.

3.7.5. Total microbial population

The count of total microbes were done using the methods described in 3.3.5.

3.8. Cost of production

The cost of production of VCO and VCO capsule was computed using the market price of raw materials incurred for the oil extraction and VCO capsule development along with electricity charge, fuel charge, labour charge, and packaging cost.

3.9. Statistical analysis of the data

The results obtained were statistically analysed using methods like the Duncan's multiple range test (DMRT) and Kendall's coefficient of concordance.

Results

4. RESULTS

The results of the present study entitled "Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule" are presented in this chapter under the following headings.

4.1. Organoleptic evaluation of VCO extracted using different methods

4.2. Physico-chemical evaluation of VCO extracted using different methods

4.3. Storage studies of the extracted VCO

4.3.1. Organoleptic evaluation

4.3.2. Physico-chemical evaluation

4.3.3. Total microbial population

4.4. Evaluation of medicinal properties of VCO

4.4.1. Antimicrobial properties

4.4.2. Antiproliferatory activity

4.4.3. Antioxidant properties

4.5. Development of VCO capsule and its quality evaluation

4.5.1. Storage studies of VCO capsule

4.5.1.1. Acceptability studies

4.5.1.2. Physico-chemical evaluation

4.5.1.3. Total microbial population

4.6. Cost of production

4.1. Organoleptic evaluation of VCO extracted using different methods

VCO was extracted from the mature coconuts of the west coast tall variety and *Kerasree* hybrid using four different methods: traditional method, fermentation method, cold centrifugation method and enzymatic method using standard procedures.

A panel of twenty selected judges organoleptically evaluated the extracted VCO samples. A nine-point hedonic scale was used to evaluate the organoleptic qualities of VCO, including appearance, colour, texture, flavour, taste and overall acceptability. The mean scores obtained for the organoleptic parameters of each treatment were statistically analysed using Kendall's coefficient of concordance and the results are given in Table 2.

Based on the organoleptic scores of VCO, the mean score for appearance ranged from 8.95 to 9.00 with a mean rank score from 3.85 to 4.65. All treatments had highest mean score for appearance (9.00) except T_1 and T_5 (8.95).

In the case of colour, the mean score and mean rank score varied from 8.95 to 9.00 and 3.55 to 4.75 respectively. The lowest mean score for colour was obtained in T_1 and T_5 (8.95). All other treatments had a higher mean score and mean rank score for colour (9.00 and 4.75 respectively).

The mean score for flavour of VCO ranged from 8.20 to 9.00 with the mean rank scores from 1.10 to 6.40. In VCO, the highest mean score for flavour was obtained in T_1 and T_5 (9.00) and the lowest in T_8 (8.20).

The mean score and mean rank score for taste of VCO ranged from 8.33 to 9.00 and 1.35 to 6.25 respectively. T_1 and T_5 had the highest mean score for taste (9.00) and T_6 showed the lowest score (8.33). All treatments had high mean scores and mean rank scores for texture (9.00 and 4.50 respectively).

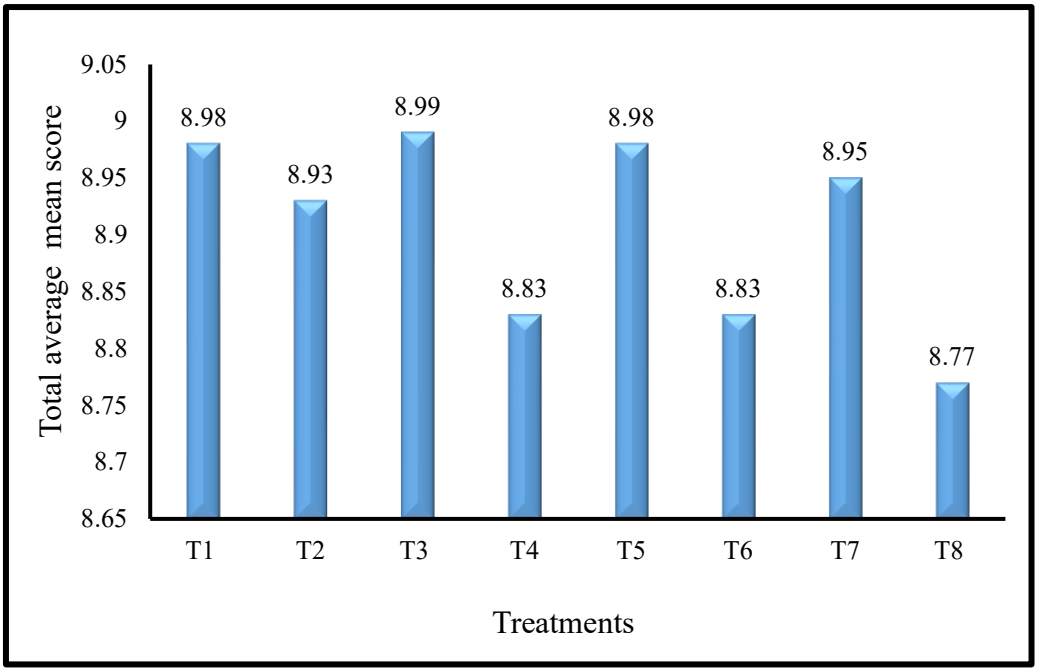
From the different treatments, T_3 obtained the highest mean rank score (8.99) for overall acceptability followed by T_1 and T_5 (8.98). The mean score and mean rank score for overall acceptability ranged from 8.77 to 8.99 and 1.30 to 6.55 respectively. The lowest mean score was obtained for T_8 (8.77).

Table 2. Mean scores obtained for the organoleptic evaluation of VCO

Parameters	WCT variety				*Kerasree* hybrid				Kendall's [W]
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Appearance	8.95 (4.25)	9.00 (4.65)	9.00 (4.65)	9.00 (4.65)	8.95 (3.85)	9.00 (4.65)	9.00 (4.65)	9.00 (4.65)	0.148**
Colour	8.95 (3.55)	9.00 (4.75)	9.00 (4.75)	9.00 (4.75)	8.95 (3.95)	9.00 (4.75)	9.00 (4.75)	9.00 (4.75)	0.226**
Flavour	9.00 (6.40)	8.83 (4.30)	8.98 (6.15)	8.30 (1.90)	9.00 (6.40)	8.82 (4.15)	8.95 (5.60)	8.20 (1.10)	0.842**
Taste	9.00 (6.25)	8.80 (3.70)	8.97 (5.75)	8.87 (5.00)	9.00 (6.25)	8.33 (1.35)	8.85 (4.65)	8.62 (3.05)	0.642**
Texture	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	9.00 (4.50)	-
Overall acceptability	8.98 (6.55)	8.93 (4.85)	8.99 (7.00)	8.83 (2.35)	8.98 (6.50)	8.83 (2.55)	8.95 (4.90)	8.77 (1.30)	0.837**

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

** Significant at 1% level; Values in parentheses indicate mean rank scores



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig. 1. Total average mean score for the organoleptic qualities of VCO

Fig. 1 shows the total average mean score for organoleptic qualities for VCO extracted using different methods in WCT and *Kerasree* hybrid. From the graph, it is evident that treatment T_3 (VCO extracted from WCT variety using cold centrifugation method) had the highest total mean score (8.99) for organoleptic qualities, followed by the VCO samples from WCT variety and *Kerasree* hybrid by traditional method (T_1 and T_5 - 8.98) and the lowest score was recorded in the VCO extracted from *Kerasree* hybrid by enzymatic method (T_8 - 8.77).

4.2. Physico-chemical evaluation of VCO extracted using different methods

The physico-chemical properties of VCO such as oil recovery, iodine value, peroxide value, saponification value, fatty acids, moisture content, viscosity, colour, tocopherol, total fat, total phenol content and total antioxidant activity were evaluated. Bioactive compounds present in VCO were identified using GC-MS analysis. The results were statistically compared by Duncan's multiple range test (DMRT) and given in Table 3 to 7.

4.2.1. Oil recovery

Oil recovery of VCO varied from 38.82 per cent to 54.34 per cent. Oil recovery was higher in T_2 (54.34%) followed by T_6 (52.33%). The fermentation method was found to have the highest oil recovery in WCT variety and *Kerasree* hybrid. But a significant difference was observed between the varieties. Oil recovery of T_1 (39.53%) was found to be on par with that of T_5 (39.37%) as per the DMRT analysis. Cold centrifugation method showed the lowest oil recovery in WCT variety and *Kerasree* hybrid (T_3 - 38.97% and T_7 - 38.82%).

4.2.2. Iodine value

Iodine value denotes the level of unsaturation of oil. The iodine value of VCO varied from 4.03 to 5.95 I_2/100 mg. The highest iodine value was found in the VCO extracted from WCT variety by traditional method (T_1 - 5.95 I_2/100 mg) whereas the lowest was found in the VCO extracted from WCT variety by fermentation method (T_2 - 4.03 I_2/100 mg). The iodine values of all treatments were within the range of APCC (2009) and FSSAI standards (2011) of 4.0 to 11 I_2/100 mg. The results were statistically analysed and found that the iodine value was significantly different in all treatments. DMRT result revealed that the iodine value of T_5 (5.24 I_2/100 mg) was on par with the value of T_7 (5.21 I_2/100 mg).

4.2.3. Peroxide value

Peroxide value indicates the oxidative rancidity or level of oxidation of oil. The peroxide value was low in all treatments, ranging from 0.16 to 0.34 MEq/kg. The lowest peroxide value was detected in T_3 (0.16 MEq/kg) whereas highest value was detected in T_4 (0.34 MEq/kg) and T_8 (0.32 MEq/kg). The peroxide values of all the treatments were within the limits of CODEX standard (2006) of <15 MEq/kg, APCC standard (2009) of <3 MEq/kg and FSSAI standards (2011) of <15 MEq/kg. DMRT analysis showed that peroxide value of T_1, T_2, T_5, T_6 and T_7 were on par. The VCO extracted from WCT variety and *Kerasree* hybrid using cold centrifugation method (T_3 and T_7) had the lowest peroxide value and the highest value was detected in the VCO extracted by enzymatic method (T_4 and T_8).

Table 3. Physico-chemical properties of VCO extracted using different methods

Parameters	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Oil recovery (%)	39.53^{e}	54.34^{a}	38.97^{f}	49.60^{c}	39.37^{e}	52.33^{b}	38.82^{f}	48.80^{d}	0.175*
Iodine value (I_2/100 mg)	5.95^{a}	4.03^{g}	5.49^{b}	4.84^{d}	5.24^{c}	4.14^{f}	5.21^{c}	4.63^{e}	0.046*
Peroxide value (MEq/kg)	0.23^{b}	0.22^{b}	0.16^{c}	0.34^{a}	0.24^{b}	0.24^{b}	0.20^{b}	0.32^{a}	0.040*
Saponification value (mg KOH/g)	258.07^{b}	259.86^{a}	256.58^{c}	254.52^{d}	259.37^{a}	259.48^{a}	257.01^{c}	256.15^{c}	0.982*
Moisture content (%)	0.09^{c}	0.11^{b}	0.11^{b}	0.12^{ab}	0.10^{bc}	0.12^{ab}	0.11^{b}	0.13^{a}	0.020*
Tocopherol (µg/g)	14.82^{g}	27.64^{a}	27.68^{a}	18.53^{e}	17.81^{f}	26.25^{c}	27.25^{b}	20.82^{d}	0.214*
Total fat (%)	95.02^{a}	94.00^{c}	92.89^{d}	94.71^{ab}	94.91^{a}	94.50^{abc}	93.01^{d}	94.12^{bc}	0.676*
Total phenol content (GAE µg/mg)	8.82^{d}	10.87^{a}	10.63^{b}	9.82^{c}	7.78^{e}	10.54^{b}	8.87^{d}	5.28^{f}	0.110*
Total antioxidant activity (µg/mg)	17.23^{h}	27.28^{b}	27.45^{a}	25.28^{e}	17.52^{g}	25.95^{d}	26.44^{c}	23.45^{f}	0.142*

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

4.2.4. Saponification value

The saponification value determines the average molecular weight of all the fatty acids present in the oil. All treatments had high saponification value. It ranged from 254.52 to 259.86 mg KOH/g and the values were significantly different as per the statistical analysis. The highest saponification value was from T_2 (259.86 mg KOH/g), T_6 (259.48 mg KOH/g) and T_5 (259.37 mg KOH/g) and the lowest was from T_4 (254.52 mg KOH/g). The saponification value of T_3 (256.58 mg KOH/g), T_7 (257.01 mg KOH/g) and T_8 (256.15 mg KOH/g) were found to be on par based on the result of DMRT. The saponification value of all treatments were within the range of the CODEX standard (2006) of 248 to 265 mg KOH/g, APCC standard (2009) of 250 to 260 mg KOH/g and FSSAI standard (2011) of >250 mg KOH/g.

4.2.5. Moisture content

The moisture content of extracted VCO samples varied from 0.09 to 0.13 per cent and were within the limits of APCC (2009) and FSSAI (2011) standards of <0.5 per cent. The minimum moisture content was found in T_1 (0.09%) whereas the maximum was in T_8 (0.13%). As per the results of statistical analysis, there was a significant variation in moisture content of all treatments with a CD value of 0.020. In WCT variety and *Kerasree* hybrid, the lowest moisture content was detected in the VCO samples extracted by traditional method (T_1 and T_5) and the highest in the VCO samples extracted by enzymatic method (T_4 and T_8). Moisture content of all treatments except T_1 was found to be on par based on the results of DMRT analysis.

4.2.6. Tocopherol

Tocopherol content was assessed on each treatment and the results are presented in Table 3. Tocopherol was detected in all treatments with a range of 14.82 to 27.68 µg/g. The highest tocopherol content was found in T_3 (27.68 µg/g) and T_2 (27.64 µg/g) and the lowest was found in T_1 (14.82 µg/g). A statistically significant difference in tocopherol content was observed in all treatments. As per the results of DMRT analysis, the tocopherol content of T_2 (27.64 µg/g) was on par with that of T_3 (27.68 µg/g). In WCT variety and *Kerasree* hybrid, tocopherol content was higher in the VCO extracted

by cold centrifugation method (T_3 and T_7) and the traditional method showed the lowest amount of tocopherol content (T_1 and T_5).

4.2.7. Total fat

All treatments contained high per cent of total fat, ranging from 92.89 to 95.02 per cent. The highest total fat was observed in T_1 (95.02%) and T_5 (94.91%) followed by T_4 (94.71%) and lowest in T_3 (92.89%). Statistical analysis showed a significant difference in total fat of all treatments. The total fat content of T_1 (95.02%), T_4 (94.71%), T_5 (94.91%) and T_6 (94.50%) were found to be on par based on the result of DMRT.

4.2.8. Total phenol content

The total phenol content of VCO varied from 5.28 to 10.87 GAE µg/mg. The highest total phenol content was noted in T_2 (10.87 GAE µg/mg) followed by T_3 (10.63 GAE µg/mg) and the lowest content was noted in T_8 (5.28 GAE µg/mg). Based on DMRT analysis, a significant variation was observed in the total phenol content of the treatments. The VCO extracted by fermentation method had high total phenol content (T_2 and T_6) in both WCT variety and *Kerasree* hybrid followed by the cold centrifugation method (T_3 and T_7).

4.2.9. Total antioxidant activity

The total concentration of antioxidants presents in oil ranged between 17.23 and 27.45 µg/mg. The total antioxidant activity was higher in T_3 (27.45 µg/mg) followed by T_2 (27.28 µg/mg), T_7 (26.44 µg/mg) and T_6 (25.95 µg/mg). T_1 had the lowest total antioxidant activity (17.23 µg/mg). Statistically, the values differed significantly with a CD value of 0.142. Among the WCT variety and *Kerasree* hybrid, theVCO extracted by cold centrifugation method (T_3 and T_7) showed high total antioxidant activity followed by the fermentation method (T_2 and T_6**).**

4.2.10. Fatty acid profile

The fatty acid profile of VCO extracted by different methods is presented in Table 4. It indicated that the VCO mainly composed of a high per cent of saturated fatty acids (caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid and stearic acid) and a small per cent of unsaturated fatty acids (oleic acid and linoleic acid). Lauric

acid (medium chain fatty acid) was the predominant fatty acid present in VCO which ranged from 45.03 to 47.06 per cent and was within the range of CODEX standard (2006) of 45.1 to 53.2 per cent and APCC standard (2009) of 43.00 to 53.00 per cent. T_3 had the highest per cent of lauric acid (47.06%), followed by T_2, T_1 and T_7 (46.94%, 46.86% and 46.15%). Statistically, the lauric acid content of all treatments was significantly different. VCO extracted by cold centrifugation method had high lauric acid content in WCT variety and *Kerasree* hybrid. In WCT variety, VCO extracted by enzymatic method (T_4 - 45.03%) had the lowest lauric acid content whereas, in the *Kerasree* hybrid, VCO extracted by traditional method (T_5 - 45.14%) had the lowest lauric acid content.

Caprylic acid (short chain fatty acid) content of VCO samples varied from 5.54 to 7.60 per cent and was within the prescribed limit of 4.60 to 10.00 per cent (CODEX, 2006; APCC, 2009). The highest caprylic acid content was observed in T_7 (7.60%) followed by T_2 (7.39%) and T_1 (7.36%) and the lowest content was in T_3 (5.54%). Statistical analysis showed that caprylic acid content was significantly different in all treatments. In WCT variety, VCO extracted by traditional (T_1 - 7.36%) and fermentation method (T_2 - 7.39%) had the highest caprylic acid whereas in *Kerasree* hybrid, VCO extracted by cold centrifugation (T_7 - 7.60%) was having the highest caprylic acid content.

Capric acid (medium chain fatty acid) content of VCO samples ranged from 5.01 to 6.60 per cent and was within the range of CODEX standard (2006) of 5.0 to 8.00 per cent and APCC standard (2009) of 4.50 to 8.00 per cent. Statistical analysis showed that the capric acid content of all treatments was significantly different. But, the capric acid content of T_1 (6.58%) was on par with T_2 (6.60%). Likewise, the capric acid content of T_7 (5.83%) and T_8 (5.79%) was on par. The treatments T_2 and T_1 contained the highest per cent of capric acid (6.60% and 6.58%) whereas, the lowest capric acid was observed in T_3 (5.01%). VCO extracted by traditional method (T_1 - 6.58% and T_5 - 5.95%) was found to have the highest capric acid content in both WCT variety and *Kerasree* hybrid, but, a significant difference was observed between the variety and hybrid. The lowest capric acid content was found in the VCO extracted

from WCT variety by cold centrifugation method (T_3 - 5.01%) and the VCO extracted from *Kerasree* hybrid by fermentation method (T_6 - 5.56%).

The highest myristic acid (medium chain fatty acid) content was present in T_3 (21.69%) and T_4 (21.67%) and the lowest content in T_7 (19.73%). Myristic acid content of all treatments varied from 19.73 to 21.69 per cent and was within the prescribed limit of 16.00 to 21.00 per cent (CODEX, 2006; APCC, 2009). A statistically significant difference in myristic acid content were found in all treatments. As per DMRT analysis, the myristic acid content of T_3 and T_4 were on par. In the WCT variety, the myristic acid content was higher in the VCO extrcated by cold centrifugation method (T_3 - 21.69%) and enzymatic method (T_4 - 21.67%) and lower in the traditional method (T_1 - 20.13%). In *Kerasree* hybrid, highest myristic acid content was found in the VCO extracted by traditional method (T_5 - 21.04%) and lowest in the cold centrifugation method (T_7 - 19.73%).

Palmitic acid (long chain fatty acid) content of VCO samples varied from 8.66 to 9.58 per cent and was within the range of CODEX standard (2006) of 7.50 to 10.20 per cent and APCC standard (2009) of 7.50 to 10.00 per cent. There was a significant difference in the palmitic acid content of all the treatments. Highest palmitic acid was observed in the VCO extracted from the WCT variety using cold centrifugation method (T_3 - 9.58%) followed by VCO from the *Kerasree* hybrid by fermentation method (T_6 - 9.50%). Lowest palmitic acid content was obtained for the traditional method in WCT variety (T_1 - 8.66%).

Table 4. Fatty acid profile of VCO extracted using different methods

Fatty acids (%)	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Caprylic acid (C8:0)	7.36^{b}	7.39^{b}	5.54^{f}	6.97^{d}	7.00^{d}	6.60^{e}	7.60^{a}	7.24^{c}	0.052*
Capric acid (C10:0)	6.58^{a}	6.60^{a}	5.01^{f}	5.61^{d}	5.95^{b}	5.56^{e}	5.83^{c}	5.79^{c}	0.046*
Lauric acid (C12:0)	46.86^{c}	46.94^{b}	47.06^{a}	45.03^{h}	45.14^{g}	45.38^{f}	46.15^{d}	45.57^{e}	0.048*
Myristic acid (C14:0)	20.13^{f}	20.17^{e}	21.69^{a}	21.67^{a}	21.04^{b}	20.72^{c}	19.73^{g}	20.43^{d}	0.039*
Palmitic acid (C16:0)	8.66^{h}	8.77^{f}	9.58^{a}	8.97^{e}	9.20^{c}	9.50^{b}	8.71^{g}	9.13^{d}	0.031*
Stearic acid (C18:0)	1.84^{a}	1.12^{f}	1.86^{a}	1.47^{c}	1.23^{d}	1.59^{b}	1.19^{e}	1.17^{e}	0.042*
Oleic acid (C18:1)	7.39^{g}	7.92^{f}	8.03^{e}	9.07^{d}	9.46^{c}	9.58^{b}	9.69^{a}	9.46^{c}	0.049*
Linoleic acid (C18:2)	1.18^{a}	1.09^{b}	1.23^{a}	1.21^{a}	0.98^{c}	1.07^{b}	1.10^{b}	1.21^{a}	0.057*

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

The maximum stearic acid (long chain fatty acid) content was observed in T_1 (1.84%) and T_3 (1.86%) and the minimum was observed in T_2 (1.12%). The stearic acid content of VCO samples ranged from 1.12 to 1.86 per cent and was lower than the prescribed limit of 2.00 to 4.00 per cent (CODEX, 2006; APCC, 2009). A statistically significant variation in stearic acid content was observed in all treatments. The stearic acid content of T_1 and T_3 was found to be on par. In the WCT variety, VCO extracted by cold centrifugation and the traditional method had the maximum per cent of stearic acid (T_3 - 1.86% and T_1 - 1.84%), whereas in *Kerasree* hybrid, the VCO extracted by fermentation method attained the maximum stearic acid content T_6 - 1.59%).

The per cent of unsaturated fatty acids including oleic acid and linoleic acid present in VCO samples varied from7.39 to 9.69 per cent and 0.98 to 1.23 per cent respectively. The values were statistically analysed and found that there was a significant difference in oleic acid content and linoleic acid content of all the treatments. The oleic acid content of VCO samples was within the prescribed limit of 5.00 - 10.00 per cent (CODEX, 2006; APCC, 2009), but linoleic acid content of treatments was lower than the recommended value (1.00 - 2.50%). The VCO extracted from *Kerasree* hybrid by cold centrifugation method (T_7 - 9.69%) had the highest oleic acid content and the lowest was observed in the VCO extracted from the WCT variety by traditional method (T_1 - 7.39%). The maximum linoleic acid content was found in T_3 (1.23%), T_4 (1.21%), T_8 (1.21%) and T_1 (1.18%) and the minimum was observed in T_5 (0.98%). As per the DMRT analysis, the linoleic acid content of T_3, T_4, T_8, T_1 were found to be on par.

4.2.11. Viscosity and colour

The viscosity of VCO samples ranged from 47.60 to 51.72 cP. The VCO extracted from *Kerasree* hybrid by cold centrifugation method (T_7 -51.72 cP) had the highest viscosity followed by the VCO samples extracted from the WCT variety by cold centrifugation method (T_3 - 50.90 cP) and enzymatic method (T_4 - 50.40 cP). As per the results of statistical analysis, a significant difference was observed in the viscosity of all treatments.

Table 5. Viscosity and colour of VCO

Parameters	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Viscosity (cP)	47.88^{g}	48.16^{e}	50.90^{b}	50.40^{c}	47.60^{h}	48.00^{f}	51.72^{a}	49.80^{d}	0.059*
Colour L* value	97.01^{e}	98.35^{c}	98.75^{a}	98.31^{cd}	97.06^{e}	98.28^{d}	98.32^{cd}	98.44^{b}	0.061*
a* value	0.02^{a}	-0.70^{e}	-0.41^{d}	0.03^{a}	-1.59^{f}	-0.25^{c}	0.02^{a}	-0.07^{b}	0.029*
b* value	0.72^{b}	0.07^{f}	0.16^{e}	0.34^{d}	2.72^{a}	0.15^{e}	0.18^{e}	0.45^{c}	0.037*

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

L*- Brightness; a*- redness; b*- yellowness

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

In WCT variety and *Kerasree* hybrid, the highest viscosity was found in the VCO extracted by cold centrifugation method (T_3 – 50.60 cP and T_7 – 51.72 cP) and the lowest viscosity was recorded in the VCO samples extracted by traditional method (T_5 - 47.60 cP and T_1 - 47.88 cP).

Colour analysis was done using a Hunter Lab spectrophotometer, mainly to measure the brightness (L) and yellowness (b) of the VCO. The highest brightness (L) was detected in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 98.75) followed by the VCO from *Kerasree* hybrid by enzymatic method (T_8 - 98.44) and the VCO extracted from WCT variety by fermentation method (T_2 -98.35). The lowest brightness was found in the VCO extracted by traditional method from WCT variety and *Kerasree* hybrid (T_1 - 97.01 and T_5 - 97.06). Statistical analysis proved a significant variation in the brightness and yellowness of all treatments. The minimum yellowness was observed in the VCO extracted from WCT variety by fermentation method (T_2 - 0.07) followed by the VCO from *Kerasree* hybrid by fermentation method (T_6 - 0.15). The yellowness was higher in the VCO extracted from the *Kerasree* hybrid by traditional method (T_5 - 2.72), followed by the VCO from WCT variety by traditional method (T_1 - 0.72). The redness (a) value of VCO samples ranged from -1.59 to 0.02.

4.2.12. Volatile and nonvolatile compounds

Bioactive compounds such as hexadecane, heneicosane, octadecane, 1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters, dibutyl phthalate, eicosane, pentacosane, tetracosane, nonacosane, 1-2-Benzenedicarboxylic acid, bis[2-methyl propyl] ester and heptadecane were identified in VCO using GC-MS analysis.

Compounds like hexdecane, heneicosane, octadecane and pentacosane were identified in all the treatments. 1-2-benzenedicarboxylic acid, butyl 8-methyl nonyl esters were present in T_1, T_2, T_7, and T_8. A compound dibutyl phthalate was identified only in T_1 (28.91%) and heptadecane was only present in T_4 (3.62%). Eicosane was detected in almost all the treatments except T_4 and T_6.

Table 6. Volatile and non-volatile compounds identified in VCO

Sl. No.	Compounds	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1.	Hexadecane	✓	✓	✓	✓	✓	✓	✓	✓
2.	Heneicosane	✓	✓	✓	✓	✓	✓	✓	✓
3.	Octadecane	✓	✓	✓	✓	✓	✓	✓	✓
4.	1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters	✓	✓	×	×	×	×	✓	✓
5.	Dibutyl phthalate	✓	×	×	×	×	×	×	×
6.	Eicosane	✓	✓	✓	×	✓	×	✓	✓
7.	Pentacosane	✓	✓	✓	✓	✓	✓	✓	✓
8.	Tetracosane	✓	×	✓	✓	✓	✓	×	✓
9.	Nonacosane	✓	×	✓	×	✓	×	✓	×
10.	1-2-Benzenedicarboxylic acid, bis[2-methyl propyl] ester	×	✓	✓	✓	✓	✓	✓	✓
11.	Heptadecane	×	×	×	✓	×	×	×	×

✓ Present; × Absent

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method

Table 7. Relative abundance (%) of volatile and non-volatile compounds present in VCO

Sl. No.	Compounds	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1.	Hexadecane	4.21	7.38	5.98	6.43	5.89	5.94	5.67	6.41
2.	Heneicosane	13.54	24.83	15.102	15.57	17.42	20.48	18.67	17.86
3.	Octadecane	4.44	7.91	6.23	6.61	7.513	6.74	6.52	7.26
4.	1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters	5.80	7.68	×	×	×	×	3.42	3.63
5.	Dibutyl phthalate	28.91	×	×	×	×	×	×	×
6.	Eicosane	3.20	5.39	7.46	×	7.83	×	7.64	8.88
7.	Pentacosane	31.54	3.51	16.09	18.01	9.32	18.21	16.87	14.06
8.	Tetracosane	4.79	×	7.9	7.49	7.48	7.68	×	8.92
9.	Nonacosane	3.58	×	8.92	×	8.62	×	6.68	×
10.	1-2-Benzenedicarboxylic acid, bis[2-methyl propyl] ester	×	43.29	32.26	42.27	35.93	40.95	34.53	32.92
11.	Heptadecane	×	×	×	3.62	×	×	×	×

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Tetracosane was identified in all treatments except T_2 and T_7. Nonacosane was present in the VCO extracted by traditional method (T_1 and T_5) and cold centrifugation method (T_3 and T_7) from WCT variety and *Kerasree* hybrid. 1-2-Benzenedicarboxylic acid, bis [2-methyl propyl] ester was identified in all the treatments except T_1.

Table 7 showed that VCO extracted from WCT variety by fermentation method (T_2) contained high per cent of bioactive compounds like hexadecane (7.38%), heneicosane (24.83%), octadecane (7.91%), 1-2-benzenedicarboxylic acid, butyl 8-methyl nonyl esters (7.68%) and 1-2-benzenedicarboxylic acid, bis[2-methyl propyl] ester (43.29%). The VCO extracted from *Kerasree* hybrid by enzymatic method (T_8) had a high per cent of eicosane (8.88%). The VCO from the WCT variety by traditional method (T_1) contained the highest per cent of pentacosane (31.54%) and lowest per cent of hexadecane (4.21%), heneicosane (13.54%), octadecane (4.44%), eicosane (3.20%), tetracosane (4.79%) and nonacosane (3.58%).

The lowest per cent of 1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters, pentacosane and 1-2-Benzenedicarboxylic acid, bis[2-methyl propyl] ester were found in the VCO extracted from the *Kerasree* hybrid by cold centrifugation method (T_7 - 3.42%), VCO from the WCT variety by fermentation method (T_2 - 3.51%) and cold centrifugation method (T_3 - 32.26%).

4.3. Storage studies of the extracted VCO

VCO samples were stored in glass bottles for six months. The organoleptic evaluation was done in weekly intervals. Physico-chemical properties such as moisture, free fatty acids, and peroxide value of VCO samples were evaluated in two months intervals. The count of total microbial population was also done in two months interval.

4.3.1. Organoleptic evaluation of VCO during storage

Organoleptic evaluation of VCO was done throughout the storage period at weekly intervals using standard procedures. A panel of twenty judges used nine point hedonic scale to evaluate the different organoleptic quality parameters including appearance, colour, texture, flavour, taste and overall acceptability. The results of organoleptic evaluation done at weekly intervals are presented in Table 8a to Table 8f.

The results for initial and final organoleptic evaluation is given in Table 9. The initial mean score for appearance of VCO samples varied from 8.95 to 9.00. All treatments except T_1 and T_5 had the highest mean score of 9.00 for appearance from the start to the end of the storage period. The initial mean score for appearance of T_1 and T_5 (VCO extracted from the WCT variety and *Kerasree* hybrid using traditional method) was 8.95 and it was decreased to 8.48 and 8.50, respectively, at the end of the storage period.

During the initial stage of storage, the mean score for colour ranged from 8.95 to 9.00. The highest score of 9.00 was observed in the VCO extracted by fermentation method (T_2 and T_6), cold centrifugation method (T_3 and T_7) and enzymatic method (T_4 and T_8) from the both WCT variety and *Kerasree* hybrid throughout the storage period. The lowest mean score was recorded in the VCO extracted using traditional method from WCT variety and *Kerasree* hybrid (T_1 and T_5 - 8.95). At the end of the storage period, the mean score for colour of T_1 and T_5 decreased to 8.77 and 8.83.

Table 9 shows that, the mean score for flavour initially varied from 8.20 to 9.00. The highest mean score was obtained for the VCO extracted by traditional method from WCT variety and *Kerasree* hybrid (T_1 and T_5 -9.00) and the lowest for the VCO from the *Kerasree* hybrid by enzymatic method (T_8 - 8.20). During the storage period, a gradual decrease was observed in the mean score for flavour in all the treatments. The VCO extracted by traditional method from the WCT variety and *Kerasree* hybrid (T_1 and T_5) had the highest mean score (8.00) for flavour at the end of the storage period and the lowest score for the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 5.00).

Table 8a. Mean scores for the weekly organoleptic evaluation of VCO on 1st month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Appearance	8.95	8.95	8.95	8.95	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.95	8.95	8.95	8.95	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	9.00	9.00	8.82	8.82	8.80	8.80	8.98	8.97	8.97	8.97	8.30	8.28	8.25	8.25
Taste	9.00	9.00	9.00	9.00	8.80	8.78	8.77	8.77	8.97	8.95	8.95	8.93	8.87	8.85	8.83	8.83
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.98	8.98	8.98	8.98	8.93	8.92	8.91	8.91	8.99	8.98	8.98	8.97	8.83	8.83	8.82	8.82

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8a. Mean scores for weekly organoleptic evaluation of VCO on 1st month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Appearance	8.95	8.95	8.95	8.95	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.95	8.95	8.95	8.95	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	9.00	9.00	8.82	8.80	8.78	8.78	8.93	8.93	8.93	8.88	8.18	8.18	8.16	8.16
Taste	9.00	9.00	9.00	9.00	8.32	8.32	8.30	8.30	8.83	8.83	8.83	8.80	8.65	8.65	8.62	8.62
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.98	8.98	8.98	8.98	8.83	8.83	8.81	8.81	8.95	8.95	8.95	8.93	8.77	8.77	8.76	8.76

[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 8b: Mean scores for the weekly organoleptic evaluation of VCO on 2nd month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week
Appearance	8.93	8.92	8.92	8.90	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.93	8.92	8.92	8.88	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	9.00	9.00	8.78	8.77	8.77	8.73	8.93	8.92	8.92	8.85	8.23	8.22	8.15	8.15
Taste	9.00	9.00	9.00	8.98	8.78	8.77	8.77	8.72	8.95	8.93	8.93	8.86	8.85	8.83	8.78	8.78
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.97	8.97	8.97	8.95	8.91	8.91	8.91	8.89	8.98	8.97	8.97	8.94	8.82	8.81	8.78	8.78

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8b: Mean scores for the weekly organoleptic evaluation of VCO on 2nd month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week	5th week	6th week	7th week	8th week
Appearance	8.93	8.92	8.92	8.92	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.93	8.92	8.92	8.92	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	9.00	9.00	8.75	8.73	8.73	8.70	8.90	8.90	8.90	8.85	8.12	8.10	8.10	8.03
Taste	9.00	9.00	9.00	8.97	8.28	8.27	8.25	8.22	8.82	8.80	8.80	8.75	8.55	8.53	8.53	8.45
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.97	8.97	8.97	8.96	8.80	8.78	8.77	8.75	8.94	8.90	8.90	8.88	8.73	8.73	8.73	8.70

[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 8c. Mean scores for the weekly organoleptic evaluation of VCO on 3rd month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week
Appearance	8.90	8.88	8.83	8.83	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.88	8.85	8.82	8.78	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	8.95	8.95	8.73	8.70	8.60	8.49	8.85	8.78	8.70	8.60	8.15	8.10	8.03	7.97
Taste	8.98	8.98	8.93	8.85	8.72	8.62	8.53	8.45	8.86	8.73	8.67	8.56	8.78	8.67	8.57	8.40
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.95	8.94	8.91	8.88	8.89	8.86	8.83	8.79	8.94	8.90	8.87	8.83	8.78	8.75	8.72	8.67

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8c. Mean scores for the weekly organoleptic evaluation of VCO on 3rd month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week	9th week	10th week	11th week	12th week
Appearance	8.92	8.88	8.83	8.80	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.92	8.90	8.87	8.83	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	9.00	8.95	8.92	8.70	8.53	8.43	8.28	8.85	8.73	8.63	8.35	8.03	8.03	7.98	7.87
Taste	8.97	8.97	8.93	8.90	8.22	8.17	8.13	8.03	8.75	8.57	8.50	8.33	8.45	8.45	8.30	8.10
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.96	8.95	8.92	8.89	8.75	8.74	8.71	8.66	8.88	8.86	8.83	8.73	8.70	8.70	8.65	8.59

[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 8d. Mean scores for the weekly organoleptic evaluation of VCO on 4th month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week
Appearance	8.80	8.75	8.75	8.58	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.80	8.80	8.80	8.80	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.90	8.50	8.50	8.23	8.00	7.89	7.85	7.63	8.36	8.30	8.12	8.11	7.76	7.70	7.52	7.40
Taste	8.82	8.80	8.55	8.54	8.18	8.16	8.03	8.00	8.26	8.17	8.03	7.94	8.00	7.98	7.55	7.28
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.86	8.77	8.72	8.63	8.64	8.61	8.58	8.53	8.72	8.70	8.63	8.61	8.55	8.54	8.41	8.34

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8d. Mean scores for the weekly organoleptic evaluation of VCO on 4th month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week	13th week	14th week	15th week	16th week
Appearance	8.80	8.76	8.69	8.63	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.85	8.85	8.85	8.85	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.88	8.80	8.80	8.65	7.98	7.82	7.80	7.25	8.01	7.97	7.90	7.43	7.66	7.45	7.21	7.04
Taste	8.82	8.80	8.69	8.55	7.93	7.80	7.79	7.65	8.03	7.90	7.53	7.21	7.96	7.55	7.09	7.08
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.87	8.84	8.81	8.74	8.58	8.54	8.52	8.38	8.61	8.57	8.49	8.33	8.52	8.40	8.26	8.22

[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 8e: Mean scores for the weekly organoleptic evaluation of VCO on 5th month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
Appearance	8.53	8.53	8.52	8.50	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.80	8.80	8.78	8.78	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.00	8.00	8.00	8.00	7.55	7.15	7.12	6.99	7.95	7.25	7.21	7.18	7.25	7.05	6.93	6.70
Taste	8.52	8.30	8.25	8.25	7.98	7.90	7.57	7.55	7.79	7.53	7.50	7.09	7.07	6.93	6.92	6.50
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.57	8.53	8.51	8.51	8.51	8.41	8.34	8.31	8.55	8.36	8.34	8.25	8.26	8.20	8.17	8.04

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8e. Mean scores for the weekly organoleptic evaluation of VCO on 5th month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	17th week	18th week	19th week	20th week	17th week	18th week	19th week	20th week	17th week	18th week	19th week	20th week	17th week	18th week	19th week	20th week
Appearance	8.53	8.53	8.52	8.52	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.85	8.85	8.85	8.85	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.60	8.59	8.32	8.28	7.13	7.00	6.98	6.70	7.01	6.87	6.29	6.00	6.97	6.59	6.36	6.05
Taste	8.40	8.35	8.32	8.19	7.50	7.42	7.10	7.00	7.05	6.98	6.56	6.15	6.97	6.77	6.75	6.23
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.68	8.66	8.60	8.57	8.33	8.28	8.22	8.14	8.17	7.97	7.83	7.50	8.19	8.07	8.02	7.86

[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 8f. Mean scores for the weekly organoleptic evaluation of VCO on 6th month storage (T_1 to T_4)

Parameters	WCT variety															
	T_1				T_2				T_3				T_4			
	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week
Appearance	8.50	8.48	8.48	8.48	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.78	8.77	8.77	8.77	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.00	8.00	8.00	8.00	6.57	6.52	6.20	6.00	6.98	6.65	6.17	6.08	6.68	6.25	6.10	5.98
Taste	8.25	8.13	8.02	8.02	6.95	6.52	6.11	5.98	6.58	6.43	6.19	6.00	6.35	6.33	5.99	5.93
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.51	8.48	8.45	8.45	8.10	8.01	7.87	7.80	8.11	8.02	7.86	7.82	8.01	7.92	7.82	7.78

[T_1 - Traditional method; T_2 - Fermentation method; T_3 - Cold centrifugation method; T_4 - Enzymatic method]

Table 8f. Mean scores for the weekly organoleptic evaluation of VCO on 6th month storage (T_5 to T_8)

Parameters	*Kerasree* hybrid															
	T_5				T_6				T_7				T_8			
	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week	21st week	22nd week	23rd week	24th week
Appearance	8.52	8.50	8.50	8.50	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.85	8.83	8.83	8.83	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	8.25	8.10	8.00	8.00	6.00	5.93	5.81	5.70	5.99	5.83	5.60	5.65	5.62	5.57	5.03	5.00
Taste	8.13	8.01	7.97	7.97	6.87	6.53	6.00	6.00	6.01	5.89	5.70	5.68	6.03	5.90	5.67	5.66
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.55	8.49	8.46	8.46	7.97	7.89	7.76	7.74	7.75	7.74	7.66	7.67	7.73	7.69	7.54	7.53

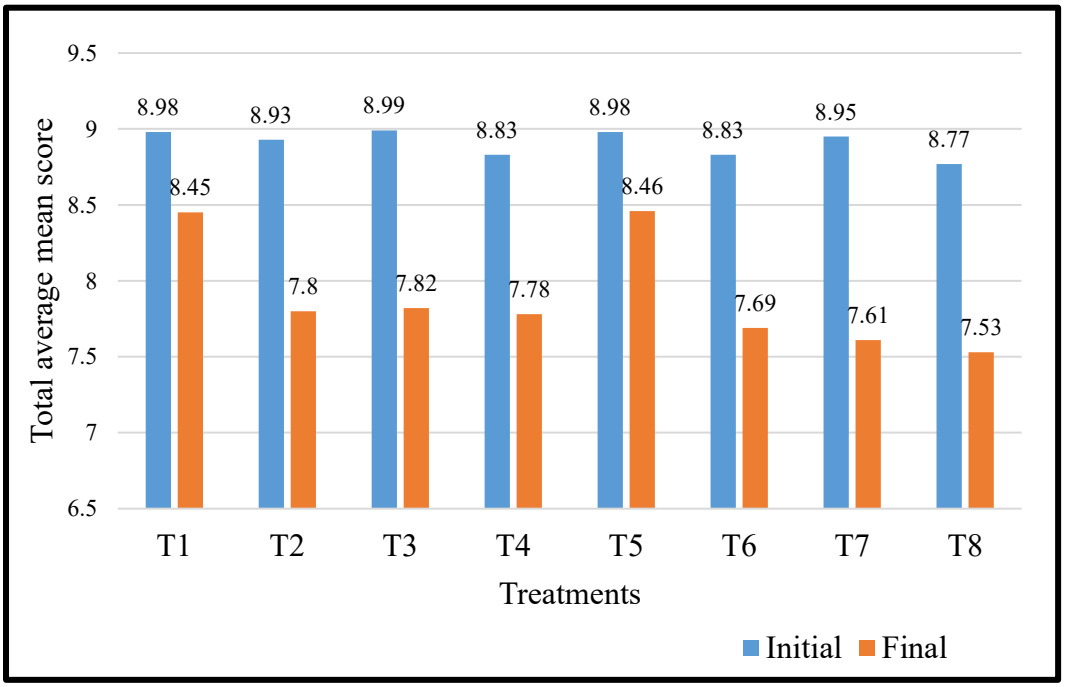
[T_5 - Traditional method; T_6 - Fermentation method; T_7 - Cold centrifugation method; T_8 - Enzymatic method]

Table 9. Mean scores for initial and final organoleptic evaluation of VCO on storage

Parameters	WCT variety								*Kerasree* hybrid							
	T_1		T_2		T_3		T_4		T_5		T_6		T_7		T_8	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Appearance	8.95	8.48	9.00	9.00	9.00	9.00	9.00	9.00	8.95	8.50	9.00	9.00	9.00	9.00	9.00	9.00
Colour	8.95	8.77	9.00	9.00	9.00	9.00	9.00	9.00	8.95	8.83	9.00	9.00	9.00	9.00	9.00	9.00
Flavour	9.00	8.00	8.83	6.00	8.98	6.08	8.30	5.98	9.00	8.00	8.82	5.70	8.95	5.65	8.20	5.00
Taste	9.00	8.02	8.80	5.98	8.97	6.00	8.87	5.93	9.00	7.97	8.33	6.00	8.85	5.68	8.62	5.66
Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Over all acceptability	8.98	8.45	8.93	7.80	8.99	7.82	8.83	7.78	8.98	8.46	8.83	7.69	8.95	7.61	8.77	7.53

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

The mean score for the taste of VCO ranged from 8.33 to 9.00 initially. The VCO extracted by traditional method from the WCT variety and *Kerasree* hybrid (T_1 and T_5) showed the highest score (9.00) for taste and the lowest score was observed in the VCO from the *Kerasree* hybrid by fermentation method (T_6 - 8.33). After six month of storage period, the mean score for taste was reduced in all the treatments. At the end of the sixth month, the highest score for taste was recorded in the VCO extracted from the WCT variety by traditional method (T_1 - 8.02) followed by the VCO extracted from the *Kerasree* hybrid by traditional method (T_5 - 7.97) and the lowest in the VCO extracted using enzymatic method from the *Kerasree* hybrid (T_8 - 5.66). In the case of flavour, all treatments (T_1 to T_8) had a maximum mean score of 9.00 for texture initially and till the end of the storage period.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig. 2. Total average mean scores for organoleptic qualities of VCO on storage

At the beginning of the storage period, the treatment T_3 (VCO extracted from the WCT variety by cold centrifugation method - 8.99) had the highest mean score for overall acceptability followed by T_1 and T_5 (VCO extracted from the WCT variety and

Kerasree hybrid using traditional method - 8.98) and the lowest score was obtained for T_8 (VCO extracted from the *Kerasree* hybrid by enzymatic method - 8.77). During the storage period, a gradual reduction was observed in the mean score for overall acceptability among all the treatments. At the end of the storage period, the mean score obtained for T_3 (8.99) decreased to 7.82. The highest mean score was recorded in the VCO extracted from the *Kerasree* hybrid and WCT variety using traditional method (T_5 - 8.46 and T_1 - 8.45) and lowest in the VCO extracted from the *Kerasree* hybrid using enzymatic method (T_7 - 7.53).

Fig 2. showed that initially, the VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest total average mean score (8.99) for organoleptic qualities followed by the VCO extracted from both WCT variety and *Kerasree* hybrid using traditional method (T_1 and T_5 - 8.98) and the lowest score was observed in the VCO extracted from the *Kerasree* hybrid using enzymatic method (T_8 - 8.76). A gradual decrease was noted in the total average mean score in all treatments till the end of the storage period. The total average mean score of T_3 declined to 7.82 after six months. The VCO extracted from the *Kerasree* hybrid by traditional method (T_5) showed the highest total average mean score (8.46) followed by the VCO extrcated from the WCT variety by traditional method (T_1 - 8.45). The VCO extracted from the *Kerasree* hybrid using enzymatic method (T_8) had the lowest total average mean score (7.53) for organoleptic qualities at the end of storage.

4.3.2. Physico-chemical evaluation of VCO during storage

4.3.2.1. Moisture content of VCO during storage

The moisture content of stored VCO was analysed and statistically compared using DMRT analysis and is presented in table 10. Initially moisture content varied from 0.09 to 0.13 per cent and was within the limits of CODEX (2006) and APCC (2009) standards of <0.5 per cent. A statistically significant difference was observed in the moisture content of all treatments. The lowest moisture content was found in the VCO extracted from the WCT variety by traditional method (T_1 - 0.09%) followed by the VCO from the *Kerasree* hybrid by traditional method (T_5 - 0.10%) and highest in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.13%).

Table 10. Moisture content (%) of VCO on storage

Storage period	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Initial	0.09^{c}	0.11^{b}	0.11^{b}	0.12^{ab}	0.10^{bc}	0.12^{ab}	0.11^{b}	0.13^{a}	0.020*
2 MAS	0.11^{a}	0.18^{c}	0.16^{c}	0.23^{a}	0.13^{d}	0.20^{b}	0.20^{b}	0.25^{a}	0.022*
4 MAS	0.16^{f}	0.26^{d}	0.25^{d}	0.32^{bc}	0.19^{e}	0.33^{b}	0.31^{c}	0.39^{a}	0.019*
6 MAS	0.24^{h}	0.42^{e}	0.40^{f}	0.54^{b}	0.29^{g}	0.48^{c}	0.46^{d}	0.58^{a}	0.022*

MAS - Month after storage

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

The moisture content of stored VCO gradually increased during the storage period. At the end of the fourth month, moisture content ranged from 0.16 to 0.39 per cent. The moisture content of all treatments was within the prescribed limit of <0.5 per cent (APCC, 2009). The VCO extracted from the WCT variety and *Kerasree* hybrid using traditional method (T_1 and T_5) showed the lowest moisture content (0.16% and 0.19% respectively) and the highest level in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.39%).

At the end of the sixth month, the moisture content of all the treatments steadily increased from 0.09 - 0.13 per cent to 0.24 - 0.58 per cent. The maximum increase in moisture was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.58%) and the minimum in the VCO extracted using traditional method from WCT variety and *Kerasree* hybrid (T_1 - 0.24% and T_5 - 0.29%). The moisture content of all the treatments was within the prescribed limit of <0.5 per cent (APCC, 2009) except for the VCO extracted enzymatic method from both WCT variety and *Kerasree* hybrid (T_4 and T_8).

4.3.2.2. Free fatty acid content of VCO during storage

Free fatty acid is an important factor in determining the quality of oil during storage. Initially free fatty acid content of VCO samples ranged from 0.22 to 0.47 mg KOH/g. The results were statistically analysed and free fatty acid content was significantly different in all the treatments

Table 11. Free fatty acid content (mg KOH/g) of VCO on storage

Storage period	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Initial	0.22^{f}	0.38^{c}	0.28^{e}	0.43^{b}	0.26^{e}	0.39^{c}	0.31^{d}	0.47^{a}	0.023*
2 MAS	0.28^{h}	0.62^{d}	0.49^{f}	0.85^{b}	0.36^{g}	0.65^{c}	0.54^{e}	0.94^{a}	0.020*
4 MAS	0.34^{h}	0.86^{d}	0.63^{f}	1.09^{b}	0.42^{g}	0.98^{c}	0.72^{e}	1.13^{a}	0.019*
6 MAS	0.41^{h}	1.04^{d}	0.88^{f}	2.03^{b}	0.50^{g}	1.11^{c}	0.99^{e}	2.12^{a}	0.022*

MAS - Month after storage

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

. The highest free fatty acid content was recorded in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.47 mg KOH/g) and lowest in the VCO extracted using traditional method from both WCT variety and *Kerasree* hybrid (T_1 and T_5 - 0.22 mg KOH/g). DMRT analysis showed that the free fatty acid content of the VCO extracted from the WCT variety by cold centrifugation method (T_3) and VCO from the *Kerasree* hybrid by traditional method (T_5) was on par.

During the storage period, the free fatty acid content of all the treatments steadily increased but was still within the limits of CODEX (2006) and FSSAI (2011) standards. At the end of the fourth month of storage, the free fatty acid content of all

the treatments varied from 0.34 to 1.13 mg KOH/g. Maximum free fatty acid content was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 1.13 mg KOH/g) and minimum in the VCO extracted from the WCT variety by traditional method (T_1 - 0.34 mg KOH/g).

Free fatty acid content ranged from 0.41 to 2.12 mg KOH/g at the end of sixth month of storage. A statistically significant difference was found in the free fatty acid content of all treatments. The lowest free fatty acid content was recorded in the VCO extracted from the WCT variety by traditional method (T_1 - 0.41 mg KOH/g) followed by the VCO from the *Kerasree* hybrid by traditional method (T_5 - 0.50 mg KOH/g) and highest in the extracted using enzymatic method from both WCT variety and *Kerasree* hybrid (T_8 - 2.12 mg KOH/g and T_4 - 2.03 mg KOH/g). However, free fatty acid values of all the treatments were within the limits of CODEX (2006) and FSSAI (2011) standards of 4.0 mg KOH/g.

4.3.2.3. Peroxide value of VCO during storage

Initially, the peroxide value ranged from 0.16 to 0.34 MEq/kg and was within the limits of CODEX (2006) and FSSAI (2011) standards of <15 MEq/kg. Statistical analysis showed that the peroxide value of all treatments was significantly different. As per the results of DMRT analysis, the peroxide value of T_1, T_2, T_5, T_6, and T_7 were on par. The VCO extracted from the WCT variety by cold centrifugation method (T_3) had the lowest peroxide value (0.16 MEq/kg). The maximum peroxide value observed in the VCO extracted using enzymatic method from both WCT variety and *Kerasree* hybrid (T_4 - 0.34 MEq/kg and T_8 - 0.32 MEq/kg).

The peroxide value of VCO significantly increased during the storage period. It ranged from 0.16 - 0.34 MEq/kg to 0.68 - 0.79 MEq/kg. However, the values were within the range of CODEX standard (2006) and FSSAI standard (2011) till the end of the sixth month. The lowest peroxide value was found in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 0.68 MEq/kg) followed by T_2 (0.70 MEq/kg), T_6 and T_7 (0.72 MEq/kg). The maximum increase in peroxide value was found in the VCO extracted from the *Kerasree* hybrid by traditional method (T_5 - 0.79 MEq/kg) followed by the VCO from the *Kerasree* hybrid by enzymatic method (T_8 -

0.77 MEq/kg) and VCO from the WCT variety by traditional method (T_1 - 0.75 MEq/kg).

Table 12. Peroxide value (MEq/kg) of VCO on storage

Storage period	WCT variety				*Kerasree* hybrid				CD value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Initial	0.23^{b}	0.22^{b}	0.16^{c}	0.34^{a}	0.24^{b}	0.24^{b}	0.20^{b}	0.32^{a}	0.040*
2 MAS	0.37^{b}	0.35^{bc}	0.31^{e}	0.42^{a}	0.37^{b}	0.34^{cd}	0.32^{de}	0.43^{a}	0.028*
4 MAS	0.52^{bc}	0.50^{c}	0.49^{c}	0.58^{a}	0.55^{b}	0.52^{bc}	0.50^{c}	0.61^{a}	0.033*
6 MAS	0.75^{b}	0.70^{cd}	0.68^{d}	0.71^{cd}	0.79^{a}	0.72^{c}	0.72^{c}	0.77^{ab}	0.033*

MAS - Month after storage

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogenous group.

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

4.3.3. Total microbial population during storage

The presence of bacteria, fungi and yeast was evaluated in all the treatments at monthly intervals using standard procedures. Initially bacterial population was not detected in all treatments. At the end of the second month, the presence of bacteria was found in the VCO extracted using fermentation method from both WCT variety and *Kerasree* hybrid (T_2 and T_6 - 0.67 x 10^2 cfu/mL). An increase was observed in the total bacterial count of VCO samples during storage. At the end of the fourth month, 1.67 x 10^2 cfu/mL of bacterial count was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8).

Table 13. Total bacterial population on storage (x 10^2 cfu/mL)

Storage period	WCT variety				*Kerasree* hybrid			
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Initial	ND	ND	ND	ND	ND	ND	ND	ND
2 MAS	ND	0.67 (1.83)	ND	ND	ND	0.67 (1.83)	ND	ND
4 MAS	ND	1.33 (2.12)	ND	ND	ND	1.67 (2.22)	ND	1.67 (2.22)
6 MAS	ND	2.00 (2.30)	1.33 (2.12)	2.00 (2.30)	ND	2.67 (2.43)	1.33 (2.12)	2.33 (2.37)

MAS - Month after storage; ND - Not Detected

All values are mean of three independent enumerations

Figures in parenthesis indicates log cfu/mL

[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

At the end of the storage period, bacterial count in the VCO extracted using fermentation method from WCT variety and *Kerasree* hybrid (T_2 and T_6) increased to 2.00 x 10^2 cfu/mL and 2.67 x 10^2 cfu/mL respectively. The highest bacterial count was found in T_6 (2.67 x 10^2 cfu/mL) followed by T_8 (2.33 x 10^2 cfu/mL), T_2 (2.00 x 10^2 cfu/mL), T_4 (2.00 x 10^2 cfu/mL), T_3 (1.33 x 10^2 cfu/mL) and T_7 (1.33 x 10^2 cfu/mL). Bacterial count was not detected in the VCO samples extracted using traditional method from both WCT variety and *Kerasree* hybrid (T_1 and T_5) during the storage period. The bacterial population of all the treatments was within the range of APCC standard (2009) of <10 x 10^2 cfu/mL.

In the case of fungi and yeast, there were no fungal and yeast colonies detected in the VCO samples till the end of storage period.

The best treatment was selected for further studies based on the high organoleptic scores, physico-chemical properties and storage stability.

WCT variety was superior to the *Kerasree* hybrid in all the quality parameters. In organoleptic evaluation, the initial rank scores were summarised in higher order of rankings for all the parameters for which concordance was done. The overall grading

was done as the sum of the summary scores. According to the rank scores, the lowest sum of the summary scores is considered the best. Considering the final and initial rank scores, the lowest sum of the summary scores was also attained for VCO extracted by cold centrifugation method (T_3).

In physico-chemical evaluation, T_3 had high tocopherol content, total phenol content and antioxidant activity compared to other treatments. Iodine value, saponification value, moisture content and peroxide value of T_3 were within the permissible limits. The moisture content, free fatty acid value, peroxide value and total microbial count of T_3 increased during storage but still within the permissible limit. Therefore, the VCO extracted from the WCT variety using cold centrifugation method (T_3) was selected for further studies.

4.4. Evaluation of medicinal properties of VCO

Medicinal properties such as antimicrobial, antiproliferatory and antioxidant properties of VCO were evaluated. The results are presented under the following headings.

4.4.1. Antimicrobial properties

4.4.1.1. Antibacterial activity of VCO

Antibacterial activity of VCO against human pathogens namely *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes* were evaluated using agar well diffusion method. Gentamycin at 160 µg concentration was used as a standard drug and 100 µl (T_1) and 500 µl (T_2) of VCO were added to the well directly. The results showed that the zone of bacterial growth inhibition was not found in the T_1 (100 µl concentration of VCO). 500 µl concentration of VCO (T_2) was effective against *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition was higher in the plate containing *Bacillus subtilis* (15 mm) followed by *Staphylococcus aureus* (10 mm). The zone of inhibition was not observed in the plate containing *Streptococcus pyogenes* at both the concentrations.

Table 14: Antimicrobial properties of VCO

Sl. No.	Parameters	Zone of inhibition (mm)			
		T_1 (100 µl)	T_2 (500 µl)	Standard drug	Solvent control
1	Bacteria *Staphylococcus aureus*	A	10 mm	18 mm (Gentamycin, 160 µg)	A
2	*Bacillus subtilis*	A	15 mm	29 mm (Gentamycin, 160 µg)	A
3	*Streptococcus pyogenes*	A	A	36 mm (Gentamycin, 160 µg)	A
4	Fungus *Candidia albicans*	A	10 mm	21 mm (Clotrimazole, 400 µg)	A

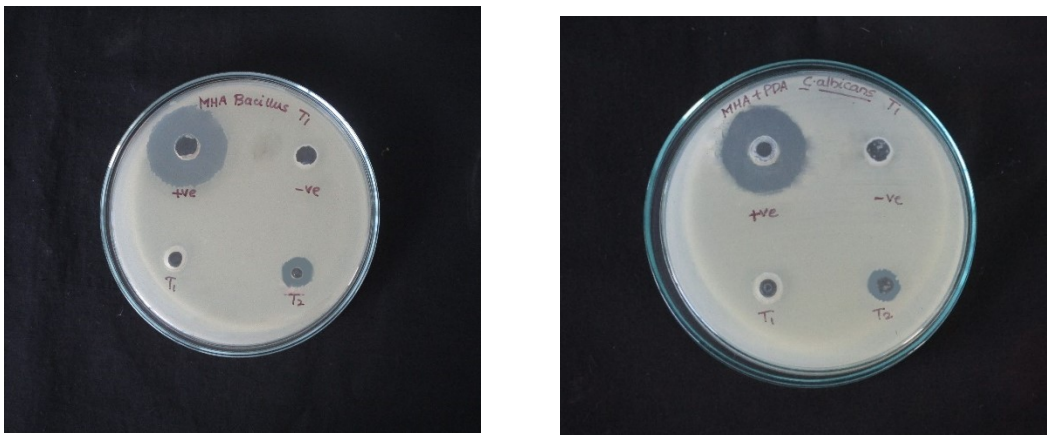
A: absent

4.4.1.2. Antifungal activity of VCO

The antifungal activity of VCO against *Candidia albicans* was evaluated using agar well diffusion method. Clotrimazole at 400 mg was used as a standard drug and 100 µl (T_1) and 500 µl (T_2) of VCO were added to the well directly. VCO was effective against *Candidia albicans* at 500 µl concentration. Zone of fungal growth inhibition was not found in T_1 (100 µl) whereas T_2 (500 µl) showed 10 mm zone of inhibition. The antimicrobial activity of VCO against human pathogens is shown in plate 4.

Staphylococcus aureus

Streptococcus pyogenes



Bacillus subtilis *Candidia albicans*

Plate 4: Antimicrobial activity evaluated against certain human pathogens

4.4.2. Antiproliferatory activity of VCO

Antiproliferative activity of VCO was evaluated in hepatic cancer cell lines using MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay. Different concentrations of VCO (6.25, 12.5, 25, 50 and 100 µg/mL) was added and the result are shown in figure 3.

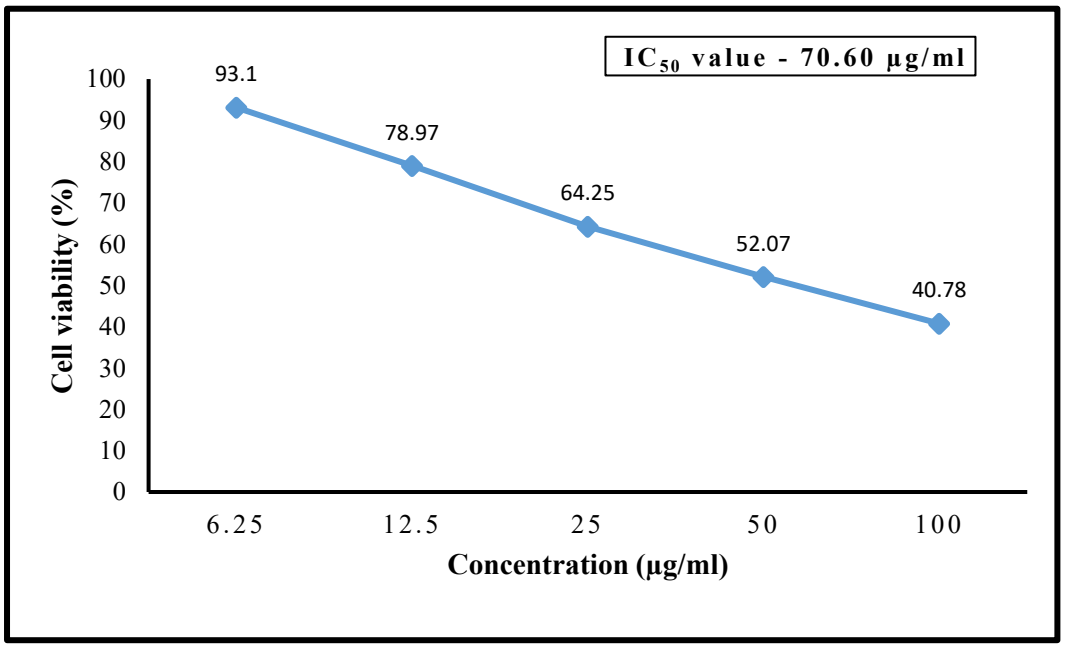


Fig 3. Antiproliferatory activity of VCO

The figure 3 showed that VCO effectively decreased the viability of cancer cell lines in a dose-dependent way. The cancer cell viability was reduced from 93.1 per cent to 40.78 per cent by the action of VCO at different concentrations (6.25 to 100 µg/mL respectively). The measured IC_{50} value was 70.60 µg/ml, which is considered to be a moderately active concentration. The normal cell lines and the VCO induced cell death at different concentrations are shown in plate 5.

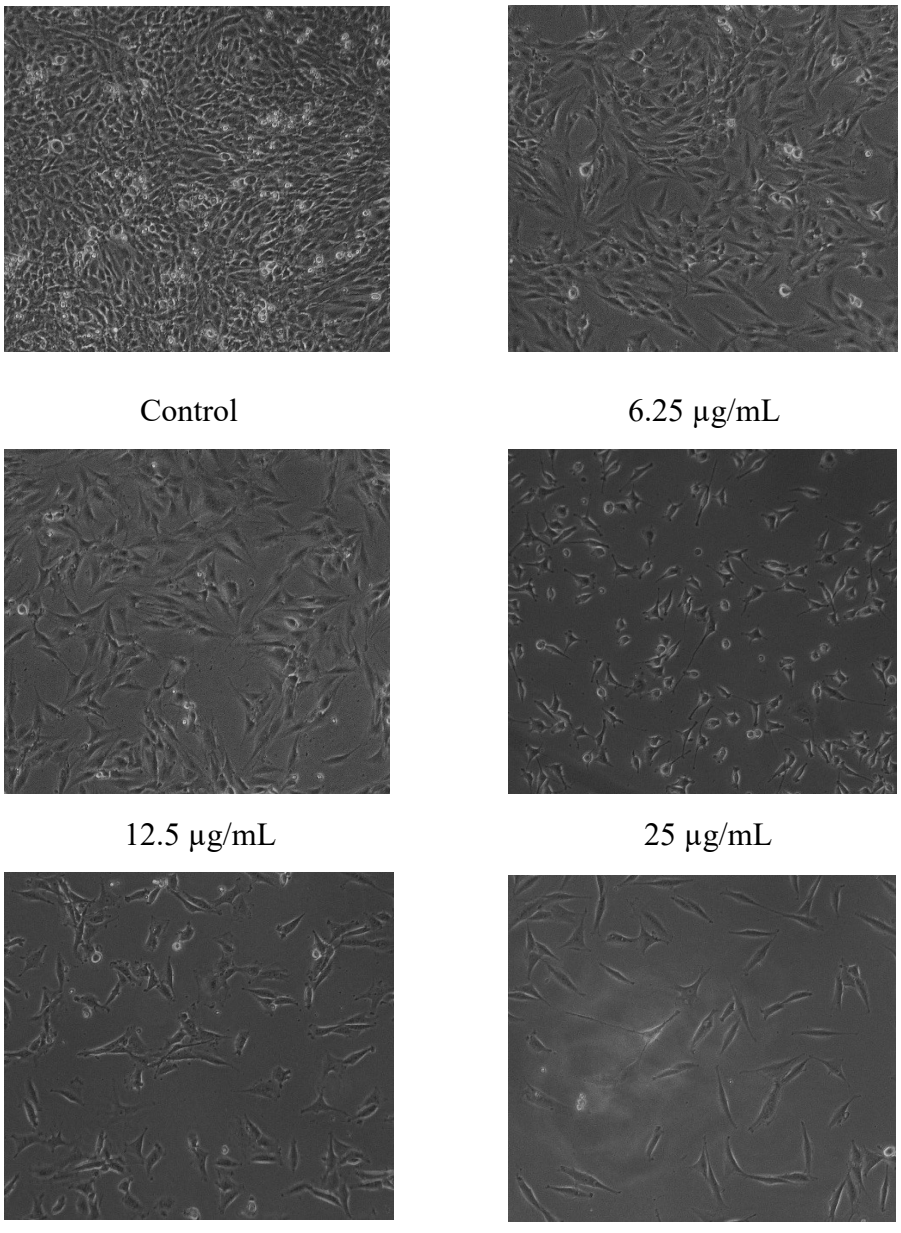


Plate 5. Cytotoxicity activity of VCO on hepatic cancer cell line

4.4.3. Antioxidant properties of VCO

Antioxidant activities such as DPPH radical scavenging activity, reducing power assay, nitric oxide scavenging activity, superoxide and hydroxyl scavenging activity were evaluated in VCO. The results are given 0below.

4.4.3.1. DPPH radical scavenging activity of VCO

A stable free radical called DPPH (1, 1-diphenyl-1,2-picrylhydrazyl) is commonly used to assess the antioxidant capacity of natural products like virgin coconut oil. The DPPH assay assesses an antioxidant's capacity to scavenge free radicals by analysing the reduction of DPPH absorbance at 515 nm. In this study, the different concentrations of VCO (1.56 to 1000 µg/mL) were used to evaluate the DPPH radical scavenging activity and the results are expressed in table 15 and figure 4.

Table 15: DPPH radical scavenging activity of VCO

Concentration (µg/mL)	Inhibition (%)
1.56	2.44
3.12	9.16
6.25	12.18
12.50	17.93
25	19.83
50	23.04
100	27.47
200	29.65
400	32.55
800	36.42
1000	40.22

The results showed that VCO exhibited antioxidant activity by scavenging the DPPH radicals with an IC_{50} value of 1236.29 µg/mL. The IC_{50} value denotes the concentration of VCO needed to scavenge fifty per cent of DPPH free radicals and the higher IC_{50} value indicates lower antioxidant activity.

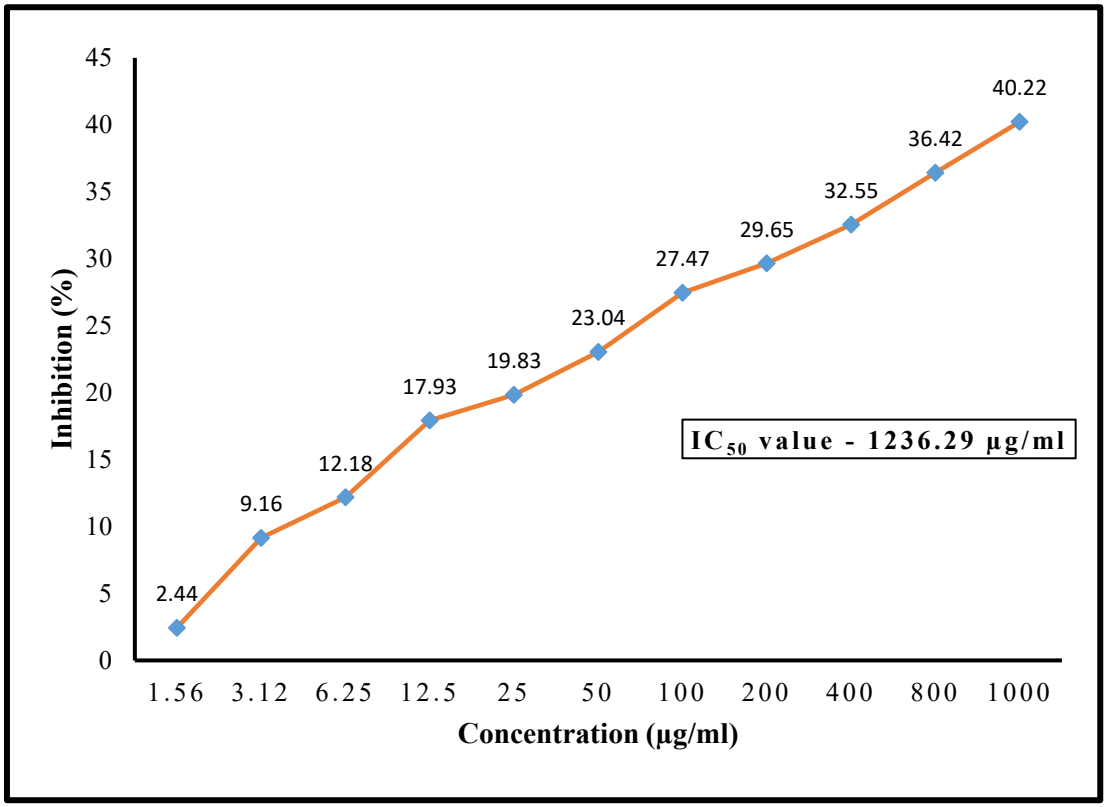


Fig 4. DPPH radical scavenging activity of VCO

4.4.3.2. Reducing power assay of VCO

The reducing power assay assesses the capacity of a sample to scavenge free radicals and reduce oxidative stress by measuring its ability to donate electrons and reduce oxidised molecules. In this study, a reducing power assay was used to evaluate the antioxidant activity of VCO by measuring the absorbance of the reaction mixture at 700 nm. A greater absorbance value indicates a higher reducing power and hence a stronger antioxidant activity. The findings are presented in figure 5 and table 16.

Table 16. Reducing power assay of VCO

Concentration (µg/mL)	Absorbance (700 nm)
20	0.011
40	0.018
60	0.026
80	0.032
100	0.046

The results showed that the absorbance value ranged from 0.011 to 0.046 at 20 to 100 µg/mL concentration of VCO. It indicates that VCO has a higher reducing power and thus a higher antioxidant activity.

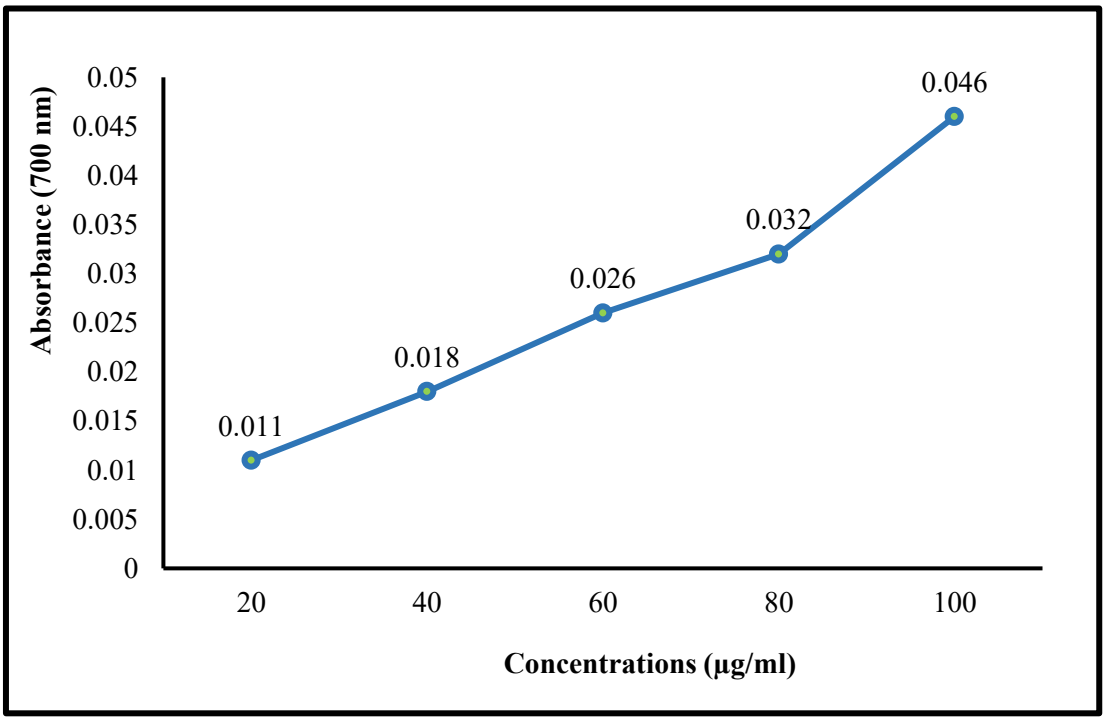


Fig 5. Reducing power assay of VCO

4.4.3.3. Nitric oxide scavenging activity of VCO

Nitric oxide scavenging activity assess the ability of samples to neutralise the excess nitric oxide in the body. Nitric oxide scavenging activity of VCO ranged from 16.87 to 38.14 per cent at 6.25 to 200 per cent of concentration of VCO respectively. The measured IC_{50} value (concentration required to scavenge 50% of the nitric oxide) was 295.59 µg/mL. This denotes that VCO has a strong nitric oxide scavenging activity, as a lower IC_{50} indicates higher activity.

Table 17. Nitric oxide scavenging activity of VCO

Concentration (µg/mL)	Inhibition (%)
6.25	16.87
12.5	19.56
25	23.47
50	29.10
100	32.52
200	38.14

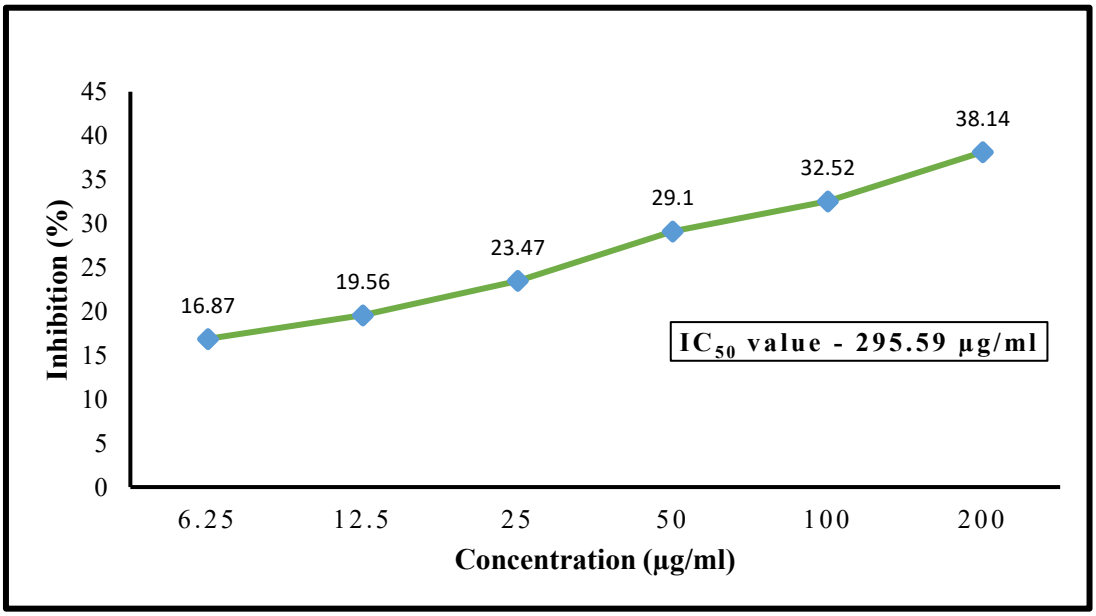


Fig 6. Nitric oxide scavenging activity of VCO

4.4.3.4. Superoxide scavenging activity of VCO

Superoxide radicals were produced in a non-enzymatic PMS-NADH (Phenazinemethosulfate - nicotinamide adenine dinucleotide) system through the interaction of oxygen, NADH, and PMS. The reduction of nitro blue tetrazolium (NBT) was used to measure the superoxide radicals produced. The results showed that superoxide scavenging activity of VCO varied from 8.65 to 45.28 percent of inhibition at various doses of 6.25 to 100 µg/ml, respectively. VCO had higher superoxide scavenging property with an IC_{50} value of 108.71 µg/mL.

Table 18. Superoxide scavenging activity of VCO

Concentration (µg/mL)	Inhibition (%)
6.25	8.65
12.5	12.75
25	21.03
50	29.71
100	45.28

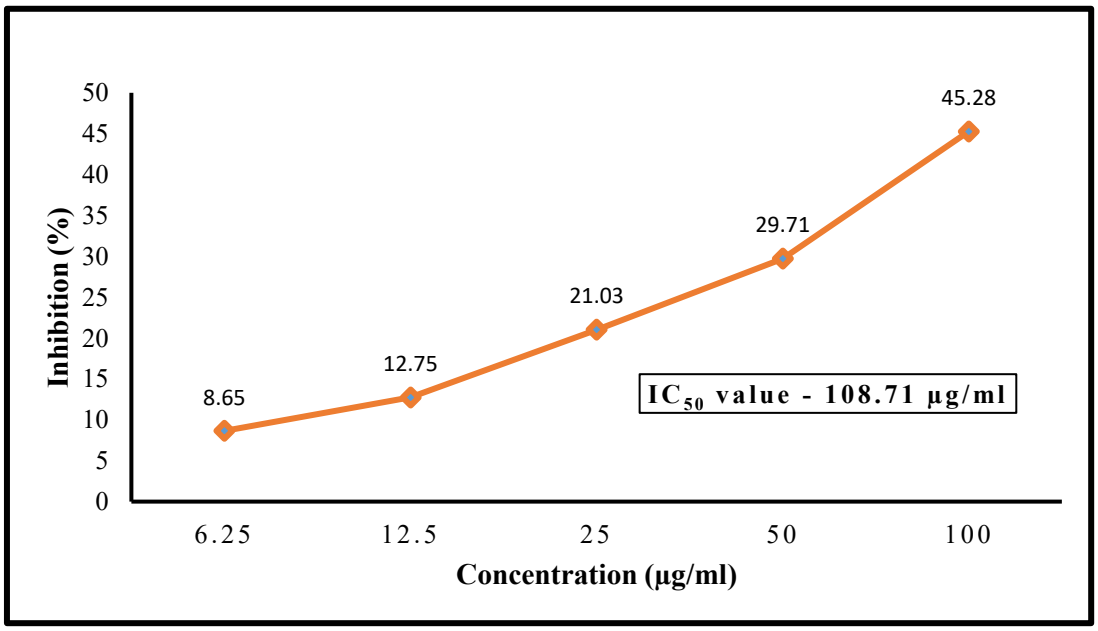


Fig 7. Superoxide scavenging activity of VCO

4.4.3.5. Hydroxyl scavenging activity of VCO

The hydroxyl scavenging activity of VCO was analysed to evaluate the ability of VCO to scavenge hydroxyl radicals which are extremely reactive and capable of harming biomolecules including DNA, lipids and proteins. The results showed that the hydroxyl scavenging activity of VCO was higher with the projected IC_{50} value of 120.65 µg/mL. The hydroxyl scavenging activity ranged from 5.78 to 38.01 at the concentration of 6.25 to 100 µg/mL respectively.

Table 19. Hydroxyl scavenging activity of VCO

Concentration (µg/mL)	Inhibition (%)
6.25	5.78
12.5	9.68
25	21.97
50	35.12
100	38.01

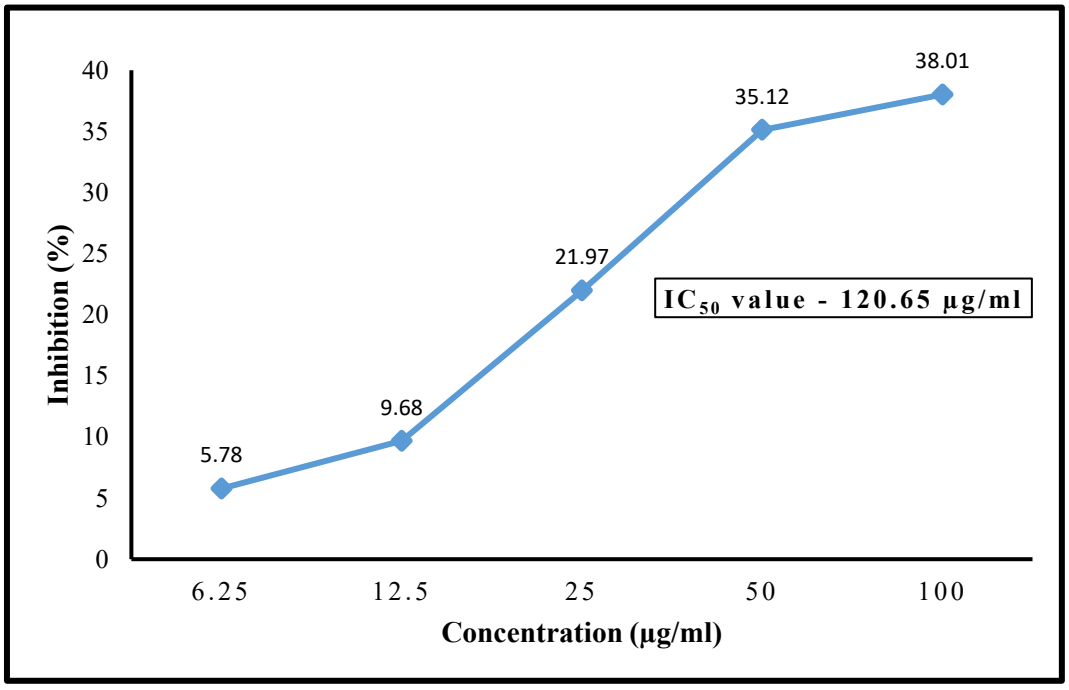


Fig 8. Hydroxyl scavenging activity of VCO

4.5. Development of VCO capsule and its quality evaluation

VCO capsule was developed using standard procedure. The capsule coating was formulated with gelatin and the dosage of VCO was one mL for encapsulation. The organoleptic evaluation was done to assess the acceptability of the capsule. Physico-chemical properties such as moisture, free fatty acids and peroxide value were evaluated.

The results of the organoleptic evaluation showed that VCO capsules were highly acceptable with a high score for sensory parameters including appearance (8.40), colour (8.87), flavour (8.43), taste (8.27), texture (8.63) and overall acceptability (8.52). The moisture content, free fatty acid value and peroxide value of VCO capsule were 0.10 per cent, 0.24 mg KOH/g and 0.17 MEq/kg, respectively. These values were within the range of CODEX (2006), APCC (2009) and FSSAI (2011) standards. The results of microbial population showed that there was no bacterial, fungal or yeast growth detected in the VCO capsules.

4.5.1. Storage studies of VCO capsule

VCO capsules were stored in glass bottles for a period of three months. Organoleptic evaluation, physico-chemical properties and total microbial population were done at weekly intervals. The results are given below.

4.5.1.1. Organoleptic evaluation of VCO capsule during storage

Using standard procedure, the organoleptic evaluation of VCO capsule was done throughout the storage period at weekly intervals. A panel of twenty judges used a nine point hedonic scale for the organoleptic evaluation. Sensory attributes such as appearance, colour, flavour, taste, texture and overall acceptability were analysed and the total average mean score was calculated. The results of organoleptic scores initially and the end of the first, second and third months of storage are depicted in Table 20. Appendix VII to IX. Lists the organoleptic scores at weekly intervals during storage.

Table 20. Organoleptic evaluation of VCO capsule on storage

Parameters	VCO Capsule			
	Initial	1 MAS	2 MAS	3 MAS
Appearance	8.40	8.38	8.35	8.33
Colour	8.87	8.87	8.86	8.85
Flavour	8.43	8.37	8.33	8.30
Taste	8.27	8.20	8.18	8.17
Texture	8.63	8.63	8.62	8.62
Overall acceptability and Total average mean score	8.52	8.49	8.47	8.45

MAS - Month after storage

The results of the organoleptic evaluation showed that VCO capsules were highly acceptable among the judges till the end of the storage period. VCO capsules had high organoleptic scores for all sensory attributes and also a slight variation was observed among the scores during the storage period. The mean score obtained for appearance was 8.40 initially and it slightly decreased to 8.33 at the end of storage. The mean score for colour and texture ranged from 8.87 to 8.85 and 8.63 to 8.62, respectively. For flavour and taste, the mean scores obtained were 8.43 and 8.27 at the initial stage and decreased to 8.30 and 8.17, respectively, at the end of the third month. The mean scores obtained for overall acceptability ranged from 8.52 to 8.45 during storage. At the initial stage of storage period, the total average mean score was 8.52 and decreased to 8.45 at the end.

4.5.1.2. Physico-chemical properties of VCO capsule during storage

Moisture content, free fatty acids, peroxide value and total microbial population were done in weekly intervals. The results of physico-chemical evaluation at the initial stage and the end of the first, second and third months of storage are presented in Table 20. The physico-chemical evaluation at weekly intervals during storage is furnished in appendix X.

4.5.1.2.1. Moisture content

The moisture content of stored VCO capsules was observed and statistically analysed. The results are revealed in Table 21. The moisture content of VCO capsules was found increasing slightly on storage. Initially, moisture content was 0.10, increased to 0.12 per cent at the end of storage with a significant difference. The values were within the limit of APCC (2009) and FSSAI (2011) standards of <0.5 per cent throughout the storage period.

Table 21. Physico-chemical evaluation on storage

Parameters	VCO Capsule				CD value
	Initial	1 MAS	2 MAS	3 MAS	
Moisture (%)	0.10^{b}	0.10^{b}	0.11^{ab}	0.12^{a}	0.011*
Free fatty acids (mg KOH/g)	0.24^{c}	0.24^{bc}	0.25^{ab}	0.26^{a}	0.013*
Peroxide value (MEq/kg)	0.17^{c}	0.18^{b}	0.18^{b}	0.20^{a}	0.013*

MAS - Month after storage

DMRT row wise comparison (significant at 5% level)

Values with same alphabet for all treatments represented in each row form a homogeneous group.

4.5.1.2.2. Free fatty acids

The free fatty acid content of VCO capsule increased during storage but, the values were within the prescribed limit of CODEX (2006), APCC (2009) and FSSAI (2011) standards. Initially, free fatty acid content of the VCO capsule was 0.24 mg KOH/g and increased to 0.26 mg KOH/g at the end of the storage period with significant difference.

4.5.1.2.3. Peroxide value

During the storage period, peroxide value ranged from 0.17 to 0.20 MEq/kg and was within the range of CODEX (2006), APCC (2009) and FSSAI (2011) standards of <15 MEq/kg. Statistically significant difference was observed in the peroxide value of the capsule throughout the storage period. The peroxide value of the capsule at the end of first and second month of storage was found to be on par based on the DMRT analysis. A slight increase in peroxide value was observed during storage. The initial peroxide value (0.17 MEq/kg) increased to 0.20 MEq/kg at the end of the third month with a significant difference (Table 21).

4.5.1.3. Total microbial population during storage

The count of bacteria, fungi and yeast was done in the VCO capsule at weekly intervals using the appropriate media and standard procedures. The colonies of bacteria, fungi and yeast were not detected throughout the storage period.

4.6. Cost of production

The cost of production for the VCO extracted by different methods and VCO capsule were calculated by considering the cost of raw materials, packaging cost, labour charges, fuel and electricity costs. The cost was calculated per 100 mL and presented in the Table 22.

The production cost for VCO varied from Rs. 93 to 115 per 100 mL. VCO extracted by cold centrifugation method had the higher cost (Rs. 115 per 100 mL) followed by fermentation (Rs. 100 per 100 mL) and traditional method (Rs. 93 per 100 mL). The lowest cost of production was found in the VCO extracted by enzymatic method (Rs. 90 per 100 mL). The cost of production for one mL capsule was estimated to be Rs. 7.00 per one mL.

Table 22. Cost of production for VCO and VCO capsule

Products	Cost (Rs.)
VCO extracted by traditional method	93.00/100 ml
VCO extracted by fermentation method	100.00/100 ml
VCO extracted by cold centrifugation method	115.00/100 ml
VCO extracted by enzymatic method	90.00/100 ml
VCO capsule	7.00/1 ml

Discussion

5. DISCUSSION

The results of the present study entitled 'Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule' are discussed in this chapter under the following headings.

5.1. Organoleptic evaluation of VCO extracted using different methods

5.2. Physico-chemical evaluation of VCO extracted using different methods

5.3. Storage studies of the extracted VCO

5.3.1. Organoleptic evaluation

5.3.2. Physico-chemical evaluation

5.3.3. Total microbial population

5.4. Evaluation of medicinal properties of VCO

5.4.1. Antimicrobial properties

5.4.2. Antiproliferatory activity

5.4.3. Antioxidant properties

5.5. Development of VCO capsule and its quality evaluation

5.5.1. Storage studies of VCO capsule

5.5.1.1. Acceptability studies

5.5.1.2. Physico-chemical evaluation

5.5.1.3. Total microbial population

5.6. Cost of production

5.1. Organoleptic evaluation of VCO extracted using different methods

Organoleptic evaluation of VCO extracted using different methods was done using a standard procedure. A panel of twenty judges was selected and a nine point hedonic scale was used for the the organoleptic evaluation. The mean scores obtained

for the sensory parameters including appearance, colour, flavour, taste and texture were statistically evaluated using Kendal's coefficient of concordance. The results of the organoleptic evaluation showed that VCO samples extracted by different methods were highly acceptable.

All the treatments had the maximum mean score for appearance (9.00) and colour (9.00) except the VCO extracted using traditional method from WCT variety (T_1 - 8.95) and *Kerasree* hybrid (T_5 - 8.95). Villarino *et al*. (2007) noted that VCO was almost colourless and the hot process may alter the colour of the oil. Lisna and Purnama (2010) reported that VCO extracted using hot process had the lowest score for appearance and colour in organoleptic evaluation. They also pointed out that the change in colour of VCO was the result of prolonged heating and continuous stirring. Indarto and Li (2019) also found that VCO extracted using the boiling method had the lowest score for colour (7.40) compared to other treatments such as centrifugation (8.90), the addition of protease enzyme (9.20) and acetic acid (9.30).

The highest mean score for flavour was recorded in the VCO extracted using traditional method (T_1 and T_5 - 9.00) followed by the cold centrifugation method from both WCT variety and *Kerasree* hybrid (T_3 and T_7 - 8.98 and 8.95). The lowest mean score for flavour was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 8.20). The treatments T_1 and T_5 had the highest mean score for taste (9.00), followed by T_3 (8.97), and the VCO extracted from the *Kerasree* hybrid by fermentation method (T_6) showed lowest score for taste. Thanuja (2015) also found that VCO extracted from the traditional boiling method had the maximum mean score for odour (9.00) and taste (8.90) and VCO from fermentation method had the minimum score for odour (7.80) and taste (6.80). Indarto and Li (2019) reported that VCO extracted from the boiling method had the highest organoleptic score for aroma (8.70) and taste (8.50).

According to Villarino *et al.* (2007) VCO has different aromas such as acid, nutty, cocojam, latik and rancid aroma based on the method of extraction. Hot extracted VCO has cocojam and latik flavour. VCO from the fermentation method has a rancid flavour and VCO from the centrifugation method has a sweet and nutty flavour. VCO extracted from the fermentation method had a noticeable acid aroma due to acetic acid

production during fermentation. This acid aroma affected the flavour and taste of the VCO (Gopalakrishna *et al*., 2010).

All the treatments had high mean scores for texture (9.00). The VCO extracted from the WCT variety by cold centrifugation method (T_3) showed the highest mean score for overall acceptability (8.99) followed by the VCO extracted using traditional method from both WCT variety and *Kerasree* hybrid (T_1 and T_5). Hamid *et al.* (2011) found that VCO extracted using centrifugation method was acceptable with a sensory analysis score of 7.58 out of 9. VCO from the centrifugation method had a fresh and coconut aroma due to the short time of extraction method. Ramesh *et al.* (2020) also pointed out that VCO extracted from the hot process and centrifugation process had desirable aroma and taste among the VCO samples.

The lowest mean score for organoleptic qualities was recorded in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8). Prayitno (2019) found that VCO extracted from an enzymatic method using bromelain enzyme showed different aroma and colour. They concluded that the flavour, taste and colour of oil depended on the enzyme used for the extraction. Indarto and Li (2019) also extracted VCO using paddy oats protease enzyme and received a lower score for aroma (7.6) and taste (7.3) in organoleptic evaluation compared to boiling method and centrifugation method.

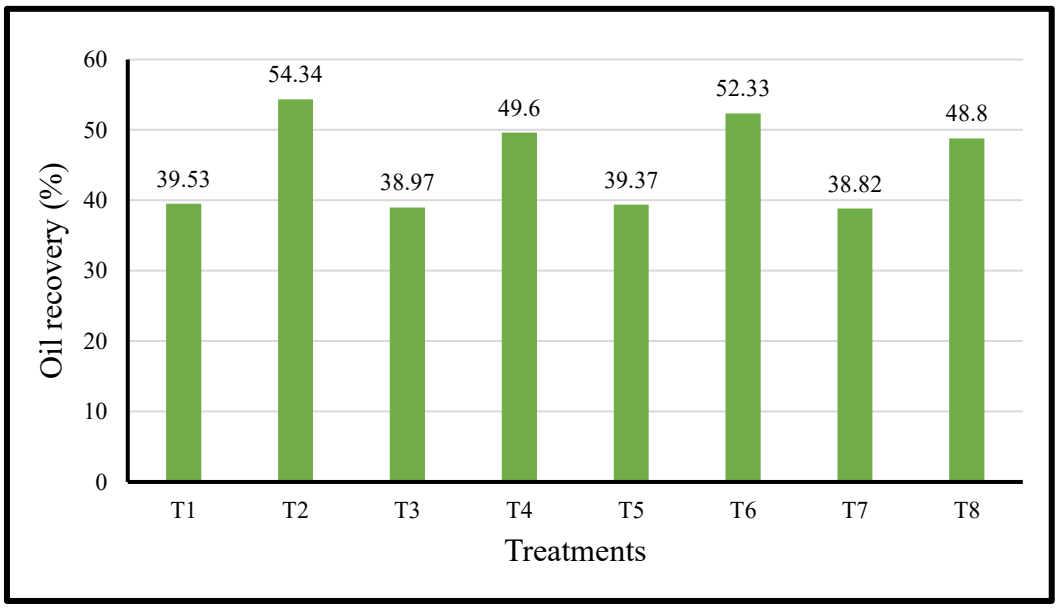
5.2. Physico-chemical evaluation of VCO extracted using different methods

The physico-chemical properties of VCO such as oil recovery, iodine value, peroxide value, saponification value, moisture content, tocopherol, total fat, total phenol content, total antioxidant activity, fatty acids, viscosity and colour were evaluated. Bioactive compounds present in VCO were identified using GC-MS analysis.

5.2.1. Oil recovery

The amount of oil recovered is dependent on a number of variables, such as the extraction technique, the age of the coconuts, the time of coconut harvesting, the location of the plantation and copra's age (Ghani *et al.*, 2018). Oil recovery provides a

quantitative evaluation of the impact of different extraction methods on the amount of oil produced. In this study, oil recovery varied from 38.82 to 54.34 per cent (Fig 9).



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 9. Oil recovery of VCO extracted from different methods

The fermentation method (T_2 and T_6) gave the highest oil recovery in both WCT variety and *Kerasree* hybrid followed by the enzymatic method (T_4 and T_8). According to Masyithah (2017), oil recovery in the fermentation method was affected by various factors such as stirring time, concentration of inoculation and the time of fermentation. They concluded that 16 to 24 hours of fermentation gave the highest yield for oil.

Bacterial cultures destabilise coconut milk emulsion by altering the pH between 3.0 and 5.6 (Chen and Diosady, 2003). In natural fermentation, air borne lactic acid bacteria destabilise the coconut milk emulsion by altering the acidity to around pH 4.0 at 35 to 40°C temperatures (Tangsuphoom and Coupland, 2008; Manikandan *et al*., 2016). Patil and Benjakul (2018) pointed out that the action of microbial proteases in the fermentation method enhances the breakdown of coconut milk emulsion and separate oil on the top portion. Mohammed *et al.* (2021) reported that the highest VCO recovery was obtained for the fermentation method among all the extraction methods.

Mansor *et al*. (2012) found 65.55 per cent oil yield from the fermentation method and 60.09 per cent from the enzymatic method using 0.1 per cent of papain. Coconut milk can be destabilised by the enzymatic hydrolysis process. Enzymatic method was time consuming and very effective in getting maximum yield of VCO (Senphan and Benjakul. 2015). Yulirohyamii *et al*. (2022) successfully extracted VCO using papain enzyme at 70℃ temperature and found that 0.6 g/L of papain was the optimum concentration for extracting VCO. Laga *et al.* (2023) applied 0.5 per cent of papain enzyme for the extraction of VCO and concluded that the quality and yield of VCO were affected by the incubation time and temperature. In addition, 50℃ to 60℃ and 18 hours were found to be the optimal temperature and incubation time respectively for the higher yield of VCO.

Enzymes enhances the liberation of oil from the plant cellular matrix. Carbohydrases (cellulases and pectinases) could breakdown the cell wall and proteases enhance the separation of oil by disrupting the covering of protein around the oil droplets (Rosenthal *et al*., 1996). Che Man *et al*. (1996) enhanced the oil recovery by adding the enzymes cellulase, polygalacturonase, protease and alpha-amylase in combination. The combination of cellulase, hemicellulase and protease can effectively extract oil (Debrah and Ohta, 1997). The oil recovery increased up to 76 per cent by using the combination of protease and cellulase enzymes (Sharma *et al*., 2001). In the enzymatic method, the oil recovery was influenced by the type of enzyme, extraction conditions such as concentration of enzyme, pH, and temperature (Jiang *et al.,* 2010).

This study found that the lowest oil recovery from the cold centrifugation method (T_3 and T_7). Nour *et al*. (2009) extracted VCO using the centrifugation method at different speed (6000 to 12000 rpm) and time (30 to 105 minutes). They concluded that the centrifugation speed and time affected the oil recovery and the highest VCO yield was obtained from 12000 rpm and 105 minutes. Raghavendra and Raghavarao (2010) reported that the chilling temperature is an important parameter in the extraction. The recovery of oil was higher (92%) at lower chilling temperature (5℃). The yield of VCO from the centrifugation method depends on centrifugation speed, time and temperature. The maximum yield was reported when the centrifugation speed, time and temperature increased to 12,000 rpm, 120 minutes and 40℃, respectively (Wong and

Hartina, 2014). Compared to other extraction methods, centrifugation of coconut milk at 10000 rpm for one hour produced lower oil recovery (Indarto and Li, 2019).

In this study, VCO samples extracted from the WCT variety showed the highest oil recovery than *Kerasree* hybrid. Muthukkannan and Balasravanan (2021) also found that maximum oil yield was obtained from the WCT variety compared to other Arasampatti Tall, Deejay Vishwas and Tiptur Tall varieties. Pathirana *et al*. (2021) reported no variation in the oil recovery among different varieties. Mudiyanselage and Wickramasinghe (2023) extracted VCO from different traditional and hybrid varieties and found that all varieties except green dwarf variety were good source of oil.

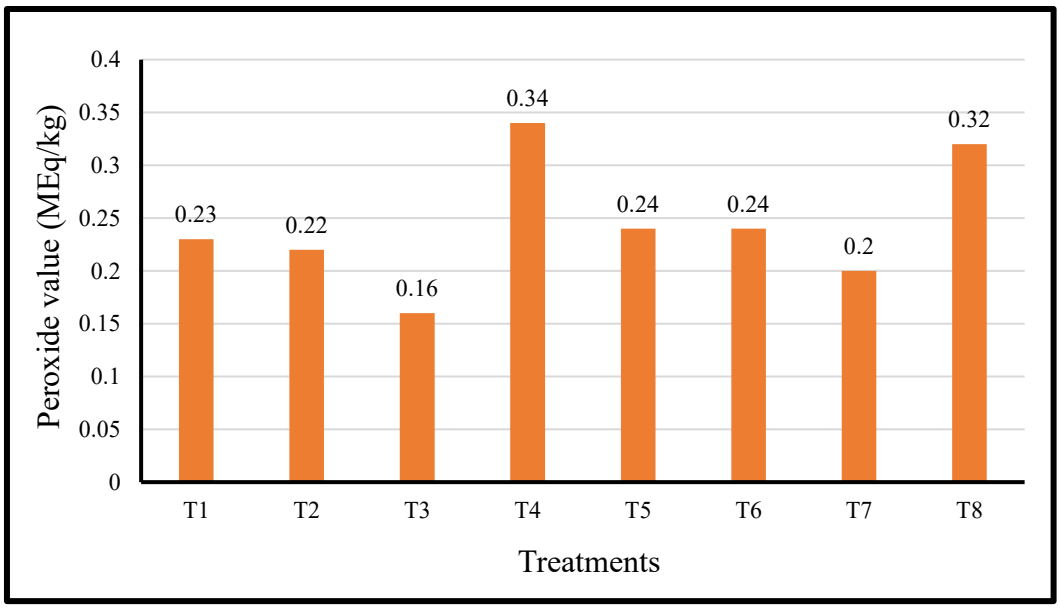
5.2.2. Iodine value

The iodine value determines the degree of unsaturation of the oil or the presence of double bonds in fatty acids of the oil. The iodine value of VCO samples ranged between 4.03 and 5.95 I_2/100 mg and was within the range of APCC (2009) and FSSAI standards (2011) of 4.0 to 11 I_2/100 mg. Low iodine value in VCO indicated that they contained high levels of saturated fatty acids and were oxidation resistant. Compared to other treatments, the highest iodine value was found in the VCO extracted from WCT variety by traditional method (T_1) and the lowest in the VCO extracted by fermentation method (T_2). Thanuja (2015) also reported that the highest iodine value was observed in the VCO extracted by traditional method (6.14 I_2/100 mg).

According to Dayrit *et al.* (2007), the iodine value of VCO samples ranged from 5.64 to 10.34 I_2/100 mg. Kamariah *et al.* (2008) reported that the iodine value of all the VCO samples varied from 5.5 to 7.3 I_2/100 mg. Mansor *et al.* (2012) found that the highest iodine value was recorded in the VCO extracted using fermentation method (4.30 I_2/100 mg) and the lowest value in the VCO extracted by chilling method (4.13 I_2/100 mg). As per the results of Srivastava *et al*. (2016), iodine value was higher in cold extracted VCO (8.58 I_2/100 mg) followed by hot extracted VCO (7.86 I_2/100 mg) and copra oil (7.48 I_2/100 mg).

5.2.3. Peroxide value

Peroxide value indicates the quality and freshness of fats and oils. A higher peroxide value signifies more oxidation along with possible rancidity. According to Choe and Min (2006), the peroxide value depended on several factors including light, content of oxygen and fatty acids composition. Unsaturated fatty acids exhibit a higher oxidation rate than saturated fatty acids (Cunha and Oliveira (2006).



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 10. Peroxide value of VCO extracted from different methods

In this study, the peroxide value were low in all the treatments and ranged from 0.16 to 0.34 MEq/kg (Fig 10). This may be because of the high concentration of saturated fatty acids in VCO. The lowest peroxide value was recorded in the VCO extracted from the WCT variety by cold centrifugation method (T_3) whereas the highest peroxide value was in the VCO extracted from the WCT variety by enzymatic method (T_4). The recorded values were within the limits of CODEX standard (2009) of <15 MEq/kg, APCC standard (2009) of <3 MEq/kg and FSSAI standard (2011) of <15 MEq/kg. This indicates that all the VCO samples were fresh and highly resistant to oxidation.

The lowest peroxide content in VCO extracted using cold centrifugation may be because of the absence of heat in the extraction method (Dia *et al.,* 2005). The highest peroxide value in the VCO extracted by enzymatic method may be due to the higher water content. Compared to other treatments, the moisture content was higher in the enzymatic method, leading to the formation of peroxides. Akinoso *et al*. (2010) also found that peroxide value increased with increasing moisture content.

According to Seneviratne and Jayathilake (2016), peroxide value changes with different extraction methods but not with different varieties. This finding is in agreement with the present study, as the peroxide value significantly varied with the extraction methods. The lowest and highest peroxide values of both WCT variety and *Kerasree* hybrid were in the VCO extracted by cold centrifugation and enzymatic methods, respectively. Ghani *et al*. (2018) also found that there was a significant difference in the peroxide value of VCO extracted from different wet and dry methods. VCO extracted from chilling and centrifugation showed the lowest peroxide value than the fermentation method and other dry methods. Pathirana *et al.* (2021) stated that there was no significant difference in the peroxide value of VCO extracted from different coconut varieties.

Marina *et al*. (2009) found that the peroxide value of VCO ranged between 0.21 and 0.57 MEq/kg which was below the standard limits. Compared to cooking coconut oil, VCO showed the lowest peroxide value. The high content of peroxides in copra oil may be due to the use of poor quality copra and high temperature applied for the extraction (Kumar and Krishna, 2015; Natalia *et al*., 2019). Satheeshan *et al*. (2019) also reported that copra oil (1.35 MEq/kg) had a significantly higher peroxides compared to cold extracted VCO (0.40 MEq/kg) and hot extracted VCO (0.86 MEq/kg).

5.2.4. Saponification value

Saponification value (SV) indicates the average molecular weight of a fatty acid or a combination of fatty acids in oils. Saponification value is directly proportional to the level of short chain fatty acids present in the oils. In this study, all the treatments had high saponification value and it ranged between 254.52 and 259.86 mg KOH/g. These values were within the range of CODEX standard (2009) of 248 to 265 mg

KOH/g, APCC standard (2009) of 250 to 260 mg KOH/g and FSSAI standard (2011) of >250 mg KOH/g. A high saponification value indicated a low impurity level (Marina *et al*., 2009). Coconut oils had higher saponification value than other edible oils due to more chain fatty acids (Satheeshan *et al*., 2019).

Saponification value is depended on the molecular weight of oils. Oil with lower molecular weight had a higher saponification value (Nainggolan *et al.,* 2016). Extraction methods also affected the saponification value (Ghani *et al*., 2018). The current study's findings also revealed a significant variation in the saponification value of VCO samples extracted from different methods. The highest saponification value was observed in the VCO extracted using fermentation method from the WCT variety and *Kerasree* hybrid (T_2 and T_6) and the VCO from the *Kerasree* hybrid by traditional method (T_5). Satheeshan *et al*. (2019) also found that VCO extracted by fermentation method (263.29 mg KOH/g) had the highest saponification value than hot extracted VCO (262.70 mg KOH/g) and copra oil 256.87 mg KOH/g). Ramesh *et al.* (2020) also noted that saponification value slightly varied with various extraction methods. Compared to other wet extraction methods, the fermentation method showed maximum saponification value (Mohammed *et al*., 2021).

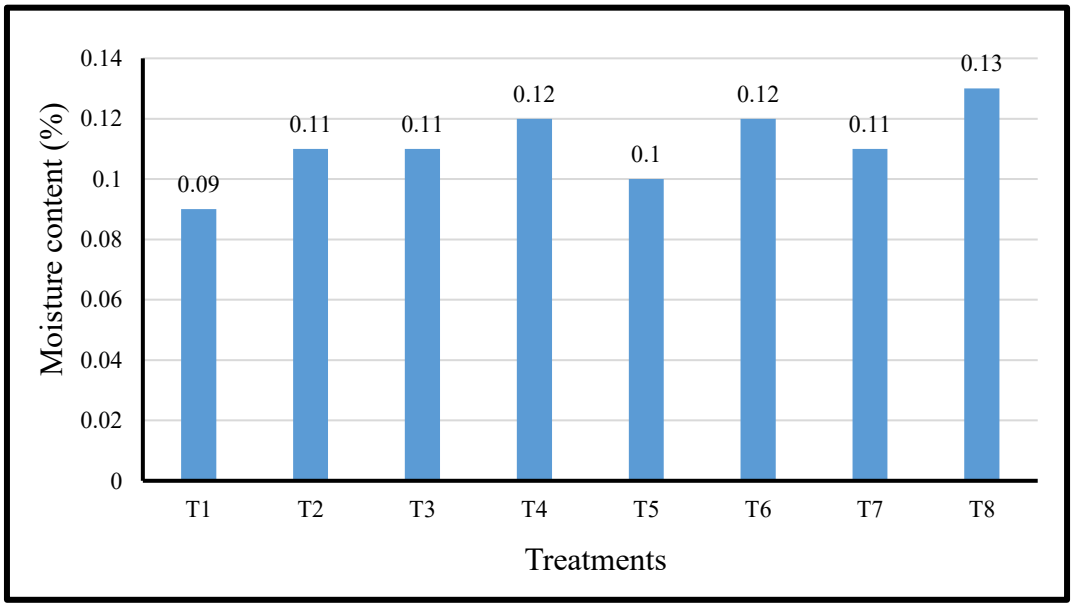
In this study, the lowest saponification value was noted in the VCO extracted from the WCT variety by enzymatic method (T_4). But, Mansor *et al*. (2012) found that VCO from the enzymatic method had high a saponification value of 262.72 mg KOH/g. Senphan and Benjakul (2016) reported that the saponification value of VCO extracted by the enzymatic method varied from 254.21 to 255.83 mg KOH/g. These values were almost similar to the findings of the present study. According to Seneviratne and Jayathilake (2016), saponification values may change because of the different coconut varieties. Pathirana *et al*. (2021) reported no difference in the saponification value of VCO among varieties. The present study observed a slight variation in the saponification value among the WCT variety and *Kerasree* hybrid.

5.2.5. Moisture content

Moisture content is an important parameter for determining the quality of VCO (Choe and Min, 2006). High moisture content in the oil leads to rancidity via hydrolysis,

which contributes to the formation of free fatty acids. High water content in oil also causes bacterial contamination (Raharja and Dwiyuni, 2008). Therefore, it is ideal to keep the moisture content low since it will lengthen the shelf life of the products by reducing the oxidation and rancidity processes (Satheeshan *et al*., 2019).

The moisture content of VCO varied with the various extraction methods. In this study, the moisture content of VCO extracted by different methods varied from 0.09 to 0.13 per cent. These values were within the range of APCC (2009) and FSSAI (2011) standards of <0.5 per cent. Similarly, Mansor *et al.* (2012) found that the moisture content of VCO extracted by different processes varied from 0.04 to 0.11 per cent. Ghani *et al.* (2018) also found that the moisture content of VCO samples varied between 0.10 and 0.17 per cent due to the different extraction techniques. Pathirana *et al.* (2021) observed a significant variation in the moisture content of VCO from different varieties. Similarly, a slight difference in the moisture content of VCO was noted in the current study among WCT variety and *Kerasree* hybrid.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 11. Moisture content of VCO extracted from different methods

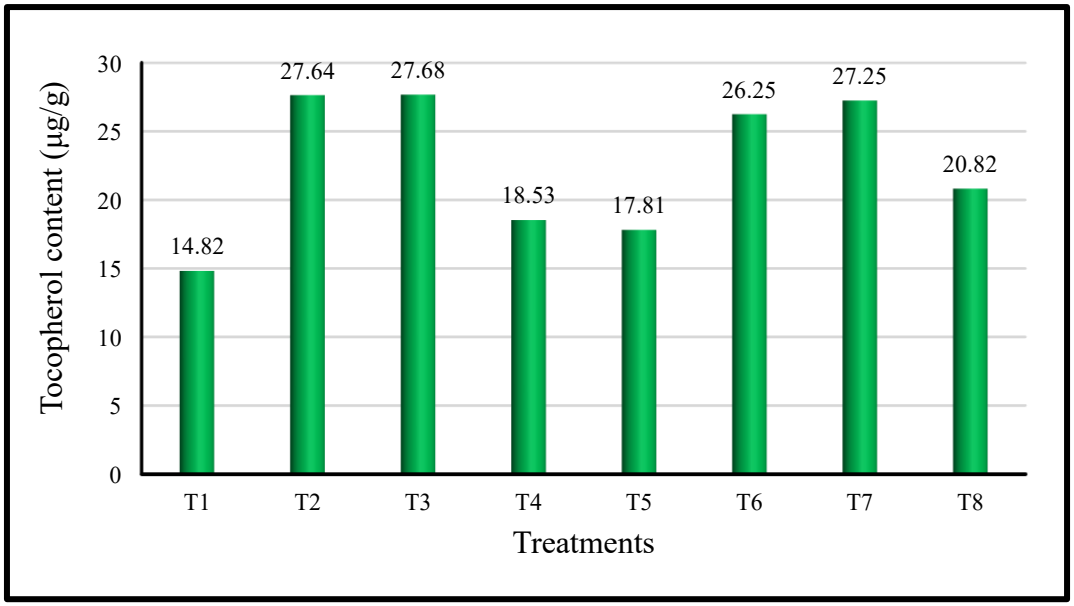
The lowest moisture content was found in the VCO extracted from the WCT variety by traditional method (T_1), whereas the maximum was found in the VCO

extracted from the *Kerasree* hybrid by enzymatic method (T_8). In the traditional method, high temperature was used to extract VCO which may be the reason for the lower amount of moisture in it. Senphan and Benjakul (2016) pointed out that low moisture content in oil is because of the low capacity of triglycerides to hold or bind the water molecules during the extraction process. Cheetangdee (2017) found that VCO extracted by enzymatic method had 0.15 per cent moisture. VCO extracted by combining fermentation and enzymatic method showed 0.14 to 0.36 per cent moisture (Prayitno, 2019). Ramesh *et al.* (2019) opined that high moisture content in the VCO extracted by cold processes may be due to the lower temperature or heat and low mechanical pressure.

Marina *et al.* (2009) reported that high moisture content was recorded from the enzymatic method (0.35%) compared to the chilling and fermentation method. Mansor *et al*. (2012) reported that the lowest was from the fresh dry method and the highest was from the enzymatic and chilling method. Mohammed *et al.* (2021) also observed the moisture content of VCO extracted by different methods and found that fermentation and enzymatic content had 0.15 per cent moisture content. The lowest moisture content was observed in the VCO obtained from dry method (0.12%). These findings were in accordance with the results of the present study.

5.2.6. Tocopherol

Tocoherol or vitamin E is a fat soluble natural antioxidant and also a free radical scavenger. The tocopherol is a general term that includes eight subtypes (alpha, beta, gamma, delta, - tocopherols, and alpha, beta, gamma, delta - tocotrienols). VCO has more antioxidant capacity than refined, bleached and deodorised oil due to tocopherols (Nevin and Rajamohan, 2006; Che Man and Marina, 2009). Marina *et al.* (2009) reported that VCO contained only a trace amount of tocopherol because of the removal of coconut testa before the extraction of coconut milk. The thin brown layer of coconut is called coconut testa which is rich in tocopherol content.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 12. Tocopherol content of VCO extracted from different methods

In this study, the tocopherol content of VCO samples varied from 14.82 to 27.68 µg/g. The highest tocopherol content was recorded in the VCO extracted using cold centrifugation method (T_3) and fermentation method from the WCT variety (T_2). The lowest tocopherol content was observed in the VCO extracted by traditional method. That may be because of the application of high heat during extraction that led to the degradation of tocopherol (Casal *et al*., 2010). Mansor *et al*. (2012) also reported that tocopherol content varied with extraction methods. They detected three forms of tocopherols in the VCO samples: delta, beta, and gamma. The findings of the present study are in accordance with the results of Srivastava *et al*. (2016). They reported that VCO extracted by fermentation method had a higher amount of tocopherol (27.65 µg/g) than hot extracted VCO (17.87 µg/g) and copra oil (3.72 µg/g). Similarly, Prapun and Cheetangdee (2016) found that VCO extracted by fermentation had higher tocopherol content than the enzymatic method. Satheeshan *et al*. (2019) also reported that cold extracted VCO had a higher amount of vitamin E (1.76 ppm) than hot extracted VCO (1.23 ppm) and copra oil (1.095 ppm).

VCO contained only a minimum amount of tocopherol than other vegetable oils like olive oil and palm oil (Dia *et al*., 2005). The lowest tocopherol content in VCO

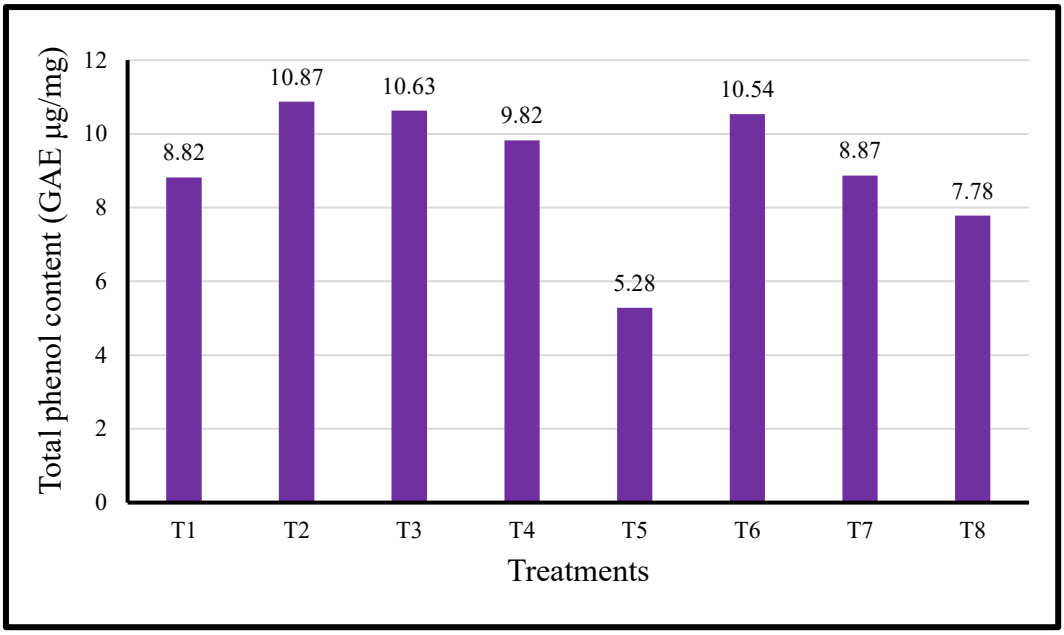
might be because of three effects such as polar or nonpolar compatible, dilution effect and oxidation effect. The tocopherol content in coconut kernel was diluted in the water and might oxidise during extraction (Arlee *et al*., 2013). According to Kumar and Krishna (2015), the cause of lower tocopherol content in VCO is unknown, but it could be because of changes in the quality of copra, storage temperature, degradation of tocopherol during storage, higher initial moisture content, drying techniques like sun or oven drying, or processing conditions used during the oil extraction process.

5.2.7. Total fat

Oil is mainly composed of more than ninety per cent triacylglycerols followed by diacylglycerols and free fatty acids. Oil also contains phospholipids, sterol esters, free esters, tocopherol, tocotrienols, alcohols, hydrocarbons and fat soluble vitamins (Gunstone, 2009). In this study, all the treatments contained a high per cent of total fat, with a range of 92.89 to 95.02 per cent. This variation may be due to the changes in extraction methods and coconut varieties (Prapun *et al*., 2016).

5.2.8. Total phenol content

VCO contains phenolic compounds, which are natural antioxidants (Parr and Bolwell, 2000). In this study, the total phenol content of VCO ranged from 5.28 to 10.87 GAE μg/mg. The variation in phenolic content may be because of different coconut varieties and extraction methods (Arlee *et al*., 2013). The genotype or variety of the coconuts, the crop's age and the coconut's maturity stage at the time of extraction all had an impact on the variation in phenolic content in VCO (Ramesh *et al.,* 2019).



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 13. Total phenol content of VCO extracted from different methods

Marina *et al*. (2009) reported that the extraction process had an impact on the phenolic content in the oil. They found that the VCO extracted by fermentation method had the highest phenolic content followed by the chilling method and the lowest phenolic content in RBD oil. These findings were in accordance with the results of the present study. The highest total phenol content was noted in the VCO samples extracted by fermentation method followed by the cold centrifugation method and the lowest content was noted in the VCO samples extracted by traditional method (Fig 13). Ngampeerapong *et al*. (2018) also found that VCO extracted by fermentation method had high phenolic content (59.44 mg GAE/100 mL oil).

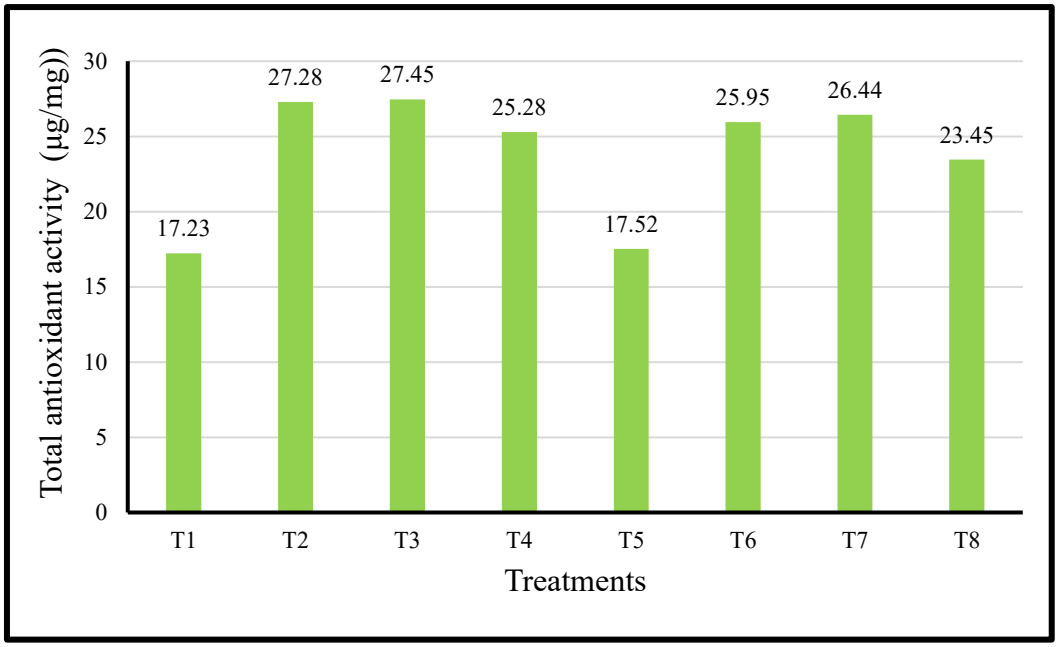
During the fermentation process, the pH of the coconut milk was brought into an acidic range that can impair the ability to hydrolyse bound phenolics, increasing the phenolic concentration of the extracted oil (Baublis *et al*., 2000). More phenolic components were incorporated into the VCO due to the increased contact period of oil and phenolic solution during the fermentation extraction. Compared to the fermentation method, VCO extracted by chilling method showed a low content of phenolics. This might be a result of the centrifugation process used to separate the aqueous phase from

the coconut milk, which contained some water soluble phenolic compounds (Marina *et al*., 2009). Ghani *et al.* (2018) also found that total phenol content was higher in the VCO extracted by the fermentation method. Mohammed *et al.* (2021) reported that phenols present in VCO varied from 37.42 to 68.12 mg GAE/100 mL oil. VCO extracted by chilling and fermentation method showed the highest phenols, whereas RBD oil showed the lowest phenol content. The high phenol content from chilling and fermentation may be due to low heat application during the extraction.

Nevin and Rajamohan (2004) reported that VCO extracted by wet methods showed high phenolic content. The lowest phenolic content in the RBD oil may be because of the refining process during the extraction. The refining process resulted in the loss or degradation of several phenolic components. Dia *et al*. (2005) opined that the lowest number of phenolic compounds from dry methods may be due to the expulsion step in the dry processing of VCO. In addition, some of the phenolics that were initially present in the oil may have been altered by a rise in temperature during oil expulsion. Seneviratne and Dissanayake (2008) reported that phenolic compounds were thermally unstable. The lowest phenolics content in VCO may be due to the exposure of oil to high temperature during the extraction (Marina *et al*., 2009). Srivastava and Semwal (2015) reported that the polyphenol content of VCO was significantly decreased due to the oxidation of polyphenolic compounds during continuous heating.

5.2.9. Total antioxidant activity

Tocopherols and phenolic compounds are the natural antioxidants. The oils rich in tocopherol and phenolics were shown to have better antioxidant activity (Mateos *et al*., 2003). VCO is a rich source of unsaponified compounds including tocopherol, tocotrienol, and polyphenols since it is extracted under mild conditions and has powerful antioxidant activity (Dia *et al*., 2005). VCO had high phenolic content and better antioxidant activity (Marina *et al*., 2009). Arunima and Rajamohan (2013) reported that VCO had higher antioxidant activity than coconut oil, olive oil and sunflower oil.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 14. Total antioxidant activity of VCO extracted from different methods

In this study, the antioxidant activity of all treatments ranged from 17.23 to 27.45 µg/mg. Marina *et al*. (2009) reported that the antioxidant activity of VCO samples ranged from 52 to 80 per cent. This variation in the antioxidant activity among VCO may be due to the different extraction methods, geographical origin of coconuts and the duration of storage. In addition, the assessed antioxidant activity was significantly correlated with the total phenolic content of the samples. Therefore, the strong antioxidant activity of VCO may be related to its high phenolic content. Pathirana *et al*. (2021) also pointed out that the variation in antioxidant activity may be due to the difference in total phenol content among the treatments.

The total antioxidant activity was higher in the VCO extracted using cold centrifugation method (T_3 - 27.45 µg/mg) followed by the fermentation method from the WCT variety (T_2 - 27.28 µg/mg). This may be because of the high content of phenolic and tocopherol. VCO extracted using cold centrifugation method (T_3 and T_7) showed high total antioxidant activity in both WCT variety and *Kerasree* hybrid (Fig. 14). Ghani *et al.* (2018) also found that the VCO extracted by chilling and centrifugation method showed the highest antioxidant activity than other extraction methods. They

concluded that chilling and centrifugation methods could preserve thermally unstable antioxidants in VCO.

Renee (2018) opined that the antioxidant activity of VCO extracted by the chilling method was higher due to the absence of heat during the extraction method. Mohammed *et al*. (2021) reported that the antioxidant activity of VCO varied with extraction method. They found that the fermentation method had higher antioxidant activity followed by chilling and the lowest activity was recorded in the dry method. Pathirana *et al*. (2021) observed a significant variation in the antioxidant activity of VCO among different coconut varieties.

Thermal treatment may also have an impact on antioxidant activity. Therefore, applying heat to extract VCO could reduce its antioxidant activity (Gazzani *et al*., 1998). These findings were in accordance with the results of the present study. In both WCT variety and *Kerasree* hybrid, the traditional method (T_1 and T_5) showed the lowest total antioxidant activity (17.23 µg/mg and 17.52 µg/mg respectively). Similarly, Dia *et al*. (2005) reported that VCO extracted from fresh dry process had the lowest antioxidant activity due to the degradation of phenolic compounds by high temperature. Nevin and Rajamohan (2006) also found that coconut oil obtained by the dry process had lower antioxidant properties than VCO obtained from the cold process, which contained a high concentration of active components.

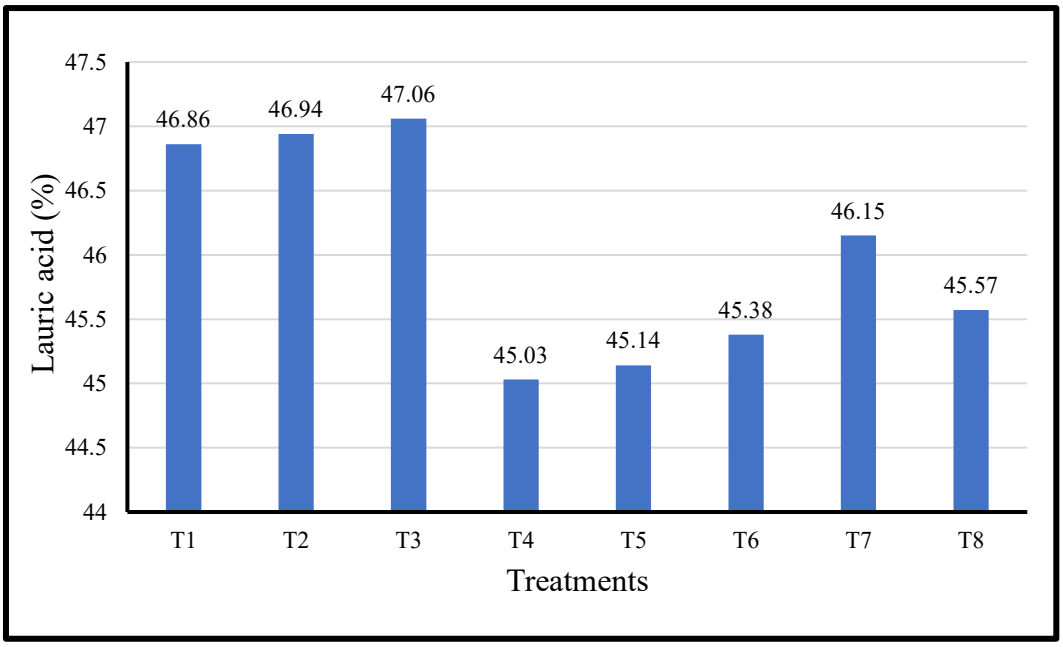
Seneviratne *et al*. (2009) observed that the phenolic components in coconut oil significantly improved the antioxidant activity of oil. The polyphenol content of VCO significantly decreased due to the oxidation of polyphenol components during continuous heating, which reduced the antioxidant activity of oil (Srivastava and Semwal, 2015). Oseni *et al*. (2017) reported that VCO extracted by fermentation method had the highest antioxidant activity than other methods due to the higher content of phenolic compounds. Ramesh *et al*. (2020) also found that the antioxidant activity of VCO was directly correlated with the total phenolic content.

5.2.10. Fatty acid profile

VCO mainly consists of a high per cent of saturated and a low per cent of unsaturated fatty acids. Lauric acid was the predominant fatty acid present in the VCO

samples (Chowdhury *et al*., 2007). Marina *et al.* (2009) found that lauric acid content varied from 46 to 48 per cent. In this study, the lauric acid content of VCO samples ranged from 45.03 to 47.06 per cent (Fig. 15). The highest lauric acid content was found in the VCO extracted by cold centrifugation method (T_3 - 47.06%) followed by the fermentation method (T_2 - 46.94%) from WCT variety. Oil extracted from WCT variety showed the highest lauric acid content than *Kerasree* hybrid.

Dia *et al.* (2005) reported that the lauric acid content of VCO varied from 48 to 50 per cent and this variation may be a result of ecological conditions and geographical origin. Mansor *et al.* (2012) reported that fatty acid profile slightly varied with different coconuts and extraction methods. Lauric acid content ranged from 46.36 to 48.42 per cent and the highest lauric acid was obtained from the fermentation method (48.42%) followed by the fresh dry method (48.07%), chilling method (48.05%) and enzymatic method (46.36%). Mohammed *et al*. (2021) found that the lauric acid content of VCO extracted by different processes ranged from 47.95 to 48.83 per cent and this variation indicated that the different extraction methods affected the fatty acid composition of oils.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 15. Lauric acid content of VCO extracted from different methods

In this study, saturated fatty acids like caprylic acid (5.54% - 7.60%), capric acid (5.01% - 6.60%), myristic acid (19.73% - 21.69%), palmitic acid (8.66% - 9.58%) and stearic acid (1.12% - 1.86%) and unsaturated fatty acids like oleic acid (7.39% - 9.69%) and linoleic acid (1.09% - 1.23%) were present in the VCO samples. These values were within the range of CODEX (2009) and APCC (2009) standards. The total saturated and unsaturated fatty acids content of VCO samples ranged from 89.21 to 91.43 per cent and 8.57 to 10.79 per cent respectively. These variations may be due to the use of different coconut varieties and extraction methods. These results are in agreement with the findings of Ajogun *et al.* (2020). They reported that VCO extracted by hot and cold method contained 90.95 to 91.60 per cent of saturated fatty acids and 8.40 to 9.07 per cent of unsaturated fatty acids.

The fatty acid concentration of the VCO samples differed significantly when compared to copra oil, especially for the fatty acids lauric, myristic, capric, caprylic, linoleic and oleic. The variations in coconut varieties used to make the oil and the breakdown of particular fatty acids during manufacture may be responsible for these variations (Dia *et al*., 2005). Ghani *et al.* (2018) reported that VCO mainly contained lauric acid (48.40 - 52.84%) followed by myristic (12.93 - 15.37%), caprylic (9.67 - 10.11%), capric (6.86 - 7.50%), palmitic (3.95 - 5.30%) and small per cent of unsaturated fatty acids (2.1 - 2.9%).

Prapun *et al*. (2016) opined that the extraction method and maturity of coconuts impacted the fatty acid profile of the extracted VCO samples. They found higher content of unsaturated fatty acids for the VCO extracted by enzymatic method. Similarly, the present study also found that the enzymatic method contained a higher amount of unsaturated fatty acids (T_4 - 10.28% and T_8 - 10.67%) than other methods. Rupasinghe *et al.* (2016) found that VCO extracted from different coconut varieties like dwarf yellow, green and brown showed significant differences in the fatty acid profile. Pathirana *et al*. (2021) reported no significant difference in the fatty acid profile of VCO extracted from different coconut varieties.

5.2.11. Viscosity and colour

The viscosity of a fluid determines its capacity to flow against resistance, with slow flow occurring at high viscosity and high flow occurring at low viscosity (Apriani, 2013). The viscosity of a fluid depends on various factors including molecular weight, size, molecular shape, and temperature. Azizah *et al.* (2015) pointed out that viscosity value depends on the presence of fatty acids in the oil. The viscosity value will be high when there are many fatty acids with double bonds in the oil sample, while the viscosity value will be low if saturated fatty acid is high. In this study, the viscosity of VCO samples ranged from 47.60 to 51.72 cP. VCO had low viscosity due to the presence of 90 per cent saturated fatty acids (Asiah *et al*., 2019).

According to Mansor *et al*. (2012), the viscosity of VCO varied from 48.73 to 50.93 Pa.s. The VCO extracted by fresh dry method showed the highest viscosity followed by chilling and thawing (48.93), enzymatic (48.93) and fermentation method (48.3). Ghani *et al*. (2018) opined that the viscosity of oil varied with the number of fatty acids present in the oil. They found that the viscosity of VCO extracted by different methods varied from 48.4 to 52.5 cP. The VCO extracted by fermentation method showed the highest viscosity and the dry method had the lowest viscosity. Mohammed *et al*. (2021) also reported that the viscosity of VCO samples ranged from 48 to 51 cP. The highest viscosity was measured for the VCO extracted by the fermentation method, while the lowest value was for the dry method.

Colour is an important parameter that affects the acceptability of oil by the consumers. In this study, the various factors of colour like brightness (L) yellowness (b), and redness (a) of the VCO samples were compared. The highest brightness (L) was detected in the VCO extracted by cold centrifugation method from the WCT variety (T_3 - 98.75), followed by the enzymatic method (T_8 - 98.44) and fermentation method (T_2 - 98.35). The lowest brightness was found in the VCO extracted from the WCT variety by traditional method (T_1 - 97.01 and T_5 - 97.06). The minimum yellowness was observed in the VCO extracted from the WCT variety and *Kerasree* hybrid by fermentation method (T_2 -0.07 and T_6 - 0.15) and the maximum yellowness was in the VCO extracted using traditional method from both WCT variety and *Kerasree* hybrid (T_5 - 2.72 and T_1 - 0.72). The redness value of VCO samples ranged from -1.59 to 0.02.

Yellow colour in the VCO samples extracted by traditional method may be due to the application of high temperature during extraction (Fife, 2006). Pathirana *et al.* (2021) reported that the colour of VCO samples depended on the extraction method.

According to Mansor *et al*. (2012), colour assessment is important because it represents the quality, safety and consistency of the VCO. They found that the VCO extracted by enzymatic method showed the highest brightness (92.25), followed by the fresh dry method (92.17), fermentation (92.06), and chilling and thawing method (91.91). The VCO extracted by fresh dry method (3.88) had the highest yellowness compared to other methods, possibly because of over-heating during the extraction process. The light yellow colour in VCO may be due to the extraction of coconut milk without removing the brown outer layer of coconut (Satheeshan *et al*., 2019).

Kumar and Krishna (2015) reported that VCO was a clear liquid with a colour of zero lovibond units because the coconut testa was removed before oil extraction. According to Mohammed *et al.* (2021), the colour of oil depends on various factors like the variety of coconut, its ripeness and extraction methods. They reported that VCO extracted by the enzymatic method (99.73) had the highest brightness followed by the chilling and thawing method (99.34), fermentation (99.34) and dry method (98.85). The highest yellowness was noted for the dry method (2.75), followed by the fermentation method (2.11), chilling and thawing (1.78). The VCO obtained from the enzymatic method (0.74) had the lowest yellowness compared to other methods.

5.2.12. Volatile and nonvolatile compounds present in VCO

Coconut kernel is the major source of volatile compounds responsible for VCO's aroma. The extraction method of oil, the degradation processes that occur before, during, and after oil separation, and the coconut meat itself can all contribute to the formation of volatile compounds in oil (Dimzon *et al*., 2011). Various physico-chemical and microbiological procedures used in the manufacturing and storing oil can also produce certain volatile compounds (Dayrit *et al*., 2011).

In this study, bioactive compounds such as hexadecane, heneicosane, octadecane, 1-2-benzenedicarboxylic acid, butyl 8-methyl nonyl esters, dibutyl phthalate, eicosane, pentacosane, tetracosane, nonacosane, 1-2-benzenedicarboxylic

acid, bis [2-methyl propyl] ester, heptadecane were identified in VCO using GC-MS analysis. Studies showed these compounds have different medicinal properties (Table 22).

Fresh coconut oil contained volatile compounds like 1-alkanes, n-alkene volatiles, methyl alkanones, n-alkanals, alkenals, methyl and ethyl esters, free fatty acids, delta - and gamma- lactones. Depending on the method of heat treatment, the relative levels of these compounds changed (Pai *et al*., 1979). Santos *et al.* (2011) identified fourteen organic volatile compounds in VCO via SPME-GC-MS technique that were acetic acid, 2-heptanone, 2-pentanone, n-octane, limonene, hexanal, nonanal, dodecanoic acid, ethyl octanoate, octanoic acid, ethyl acetate, ethyl decanoate, δ-decalactone, and δ-octalacton. These compounds were responsible for the aroma and flavour of VCO.

Mulyadi *et al*. (2018) reported that coconut oil contained different types of alcohols, aldehydes, hydrocarbons, methyl ketones and δ-octalactone responsible for its distinctive aroma. The identified volatile compounds in VCO were limonene, nonanal, acetic acid, n-octane, 2-heptanone, 2-pentanone, octanoic acid, ethyl octanoate, ethyl decanoate, ethyl acetate, δ-decalactone, δ-octalactone, and dodecanoic acid. Chang *et al.* (2020) found some volatile compounds in VCO including ethanol, 1-propanol, dimethyl ketone, 2-heptanone, hexanal *etc*.

The presence of alkane and alkene group compounds was detected in the home made virgin coconut oil using Fourier-transform infrared (FTIR) spectroscopy technique (Suaniti *et al*., 2019). Dimzon *et al*. (2020) identified fourteen major volatile organic compounds in VCO extracted by fermentation, centrifugation and expeller methods that were acetaldehyde, butanoic acid, acetoin, hexanal, benzaldehyde, 2-heptanone, ethyl acetate, benzoic acid, pentadecanoic acid, methyl tetradecanoate, formic acid, ethanol, n-hexane and toluene.

Table 23. Medicinal properties of bioactive compounds present in VCO

Sl. No.	Compounds	Medicinal properties	Reference
1.	Hexadecane	Antimicrobial and antioxidant activity	Yogeswari *et al.* (2012)
2.	Heneicosane	Antimicrobial activity	Bhutia et al. (2010)
3.	Octadecane	Antimicrobial activity	Abubacker and Devi (2015)
4.	1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters	Antimicrobial activity	Ogunlesi *et al*. (2009)
5.	Dibutyl phthalate	Antibacterial activity	Maruthupandian and Mohan (2011)
6.	Eicosane	Anti-inflammatory, analgesic and antipyretic activity	Pizon *et al*. (2018)
7.	Pentacosane	Antibacterial activity	Mihailovi *et al*. (2011)
8.	Tetracosane	Antioxidant activity	Dendekar *et al.* (2015)
9.	Nonacosane	Antibacterial activity	Mihailovi *et al*. (2011)
10.	1-2-Benzenedicarboxylic acid, bis[2-methyl propyl] ester	Antiproliferatory activity	Kumar *et al*. (2021)
11.	Heptadecane	Anti-inflammatory activity and antioxidant activity	Kim *et al*. (2013)

5.3. Storage studies of the extracted VCO

5.3.1. Organoleptic evaluation of VCO during storage

The temperature variations during storage affected the sensory qualities of oil especially aroma due to the instability of volatile compounds present in the VCO (Santos *et al*., 2011). Srivastava *et al*. (2013) reported that VCO stored in metallised polyester (70 µ) and HDPE (500 µ) had high storage stability and sensory acceptable scores. They concluded that the stability of VCO extracted by hot and cold methods depended on the packaging system, storage temperature and time. Echeverria *et al*. (2021) opined that the sensory properties of VCO depend on external factors like temperature and storage conditions.

In this study, VCO samples were stored in glass bottles six months. Organoleptic evaluation of VCO was done throughout the storage period at weekly intervals using standard procedures. A panel of twenty judges used nine point hedonic scale to evaluate the organoleptic quality attributes including appearance, colour, texture, flavour, taste and overall acceptability. Initially, all the treatments had high organoleptic scores (8.76 to 8.99). The VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest total average mean score (8.99) for organoleptic qualities followed by the VCO extracted by traditional method from both WCT variety and *Kerasree* hybrid (T_1 and T_5 - 8.98) and the lowest score was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 8.76).

A gradual decrease was noted in the total average mean score in all the treatments till the end of the storage period. The total average mean score of T_3 declined to 7.80 after six months. The VCO extracted from the *Kerasree* hybrid by traditional method (T_5) showed the highest score for taste, flavour and total average mean score (8.46) compared to other treatments and this may be due to the low level of free fatty acid content in the oil. Ramesh *et al.* (2020) also found that VCO extracted by hot and centrifugation method had a pleasant aroma and taste due to the low level of free fatty acids in the oil.

The VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8) had the lowest total average mean score (7.53) for organoleptic qualities at the end of

storage. The lowest organoleptic scores may be due to the increased free fatty acid and moisture content of the oil which are responsible for the off flavour and taste in oils (Osawa et al., 2007). Pathirana *et al*. (2021) also opined that high free fatty acid level gives a rancid taste and smell to oil that affects the organoleptic qualites of oil.

Thanuja (2015) also reported that organoleptic scores obtained for the sensory parameters such as colour, odour and taste decreased with time period. At the end of six months storage period, the VCO extracted by traditional method showed the highest score for odour and taste compared to fermentation and centrifugation methods. The minimum score for odour and taste was obtained for the VCO extracted by fermentation method.

5.3.2. Physico-chemical evaluation of VCO during storage

5.3.2.1. Moisture content of VCO during storage

The amount of moisture in oil is one factor affecting its shelf life and quality. An increase in moisture content will have an adverse effect on the oxidation process and promote hydrolytic rancidity (Manaf *et al*., 2007). In this study, the moisture content of VCO samples increased during the storage period (Fig. 16). Initially, moisture content varied from 0.09 to 0.13 per cent. At the end of the storage period, it increased from 0.21 to 0.58 per cent. The moisture content of all the treatments was within the prescribed limits of CODEX (2009) and APCC (2009) standards of <0.5 per cent except for the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8). The VCO extracted by traditional method had the lowest moisture content throughout the storage period, while the VCO obtained from the enzymatic method showed the highest level. The increased level of moisture during storage was due to the prolonged exposure of oil to room temperature (Wong and Hartina, 2014).

Similarly, Thanuja (2015) observed the variation of moisture content during storage in VCO samples extracted by fermentation, centrifugation and traditional boiling method and found that moisture content increased from 0.05 - 0.07 per cent to 0.35 - 0.39 per cent at the end of the sixth month. The lowest moisture content was recorded in the VCO extracted by traditional method and the highest in the fermentation method. The lowest moisture content in the VCO obtained from the traditional method

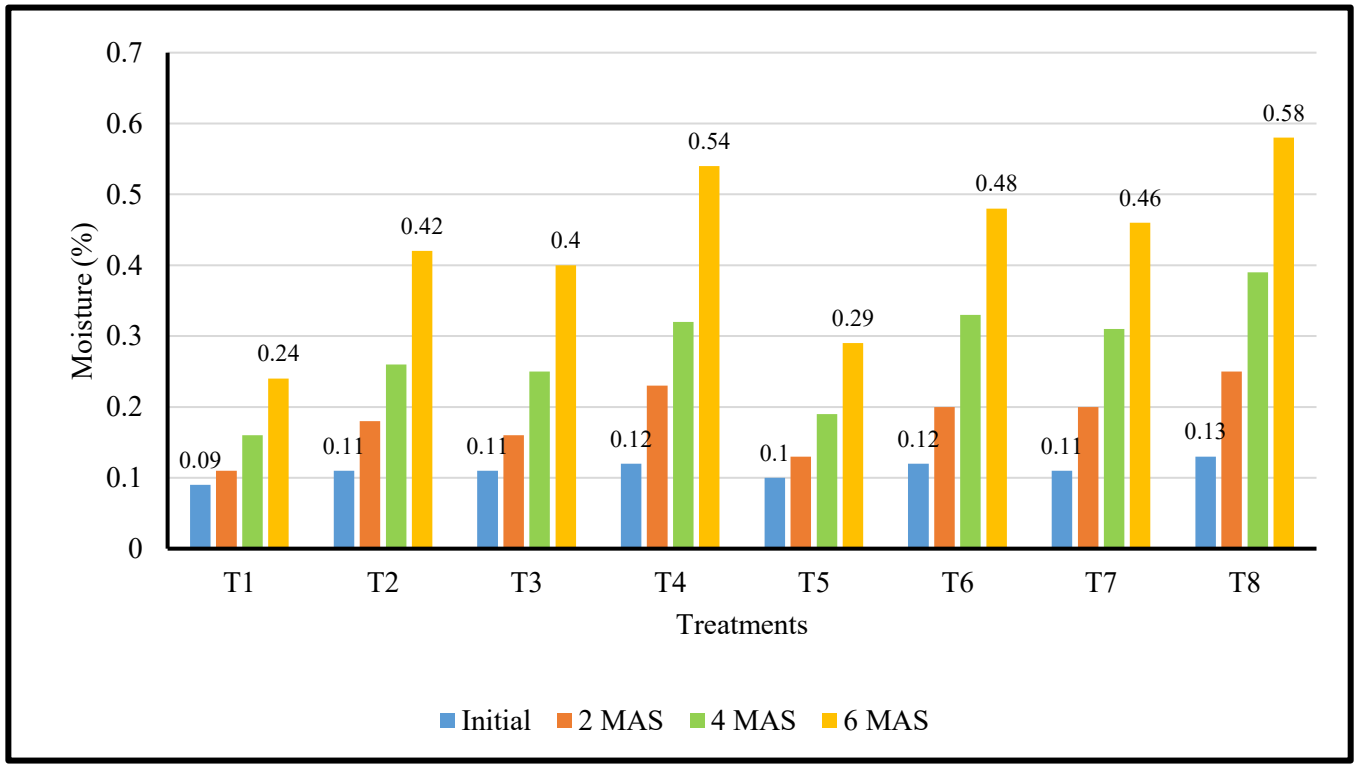
may be due to the heat treatment during extraction that significantly remove excess moisture from the oil (Mohammed *et al*., 2021).

5.3.2.2. Free fatty acid content of VCO during storage

The quality of oil during storage is determined by the free fatty acid content. A higher acid number indicates a lower quality of the oil and it affects the shelf life (Che Man *et al*., 1997). These free fatty acids, responsible for the off flavour and odour of fats, are formed when an ester is hydrolyzed by moisture or lipase (Osawa *et al*., 2007). Free fatty acids are more reactive to the oxidative factors such as temperature and light, leading to oil quality destruction of oil (Wildan *et al*., 2013).

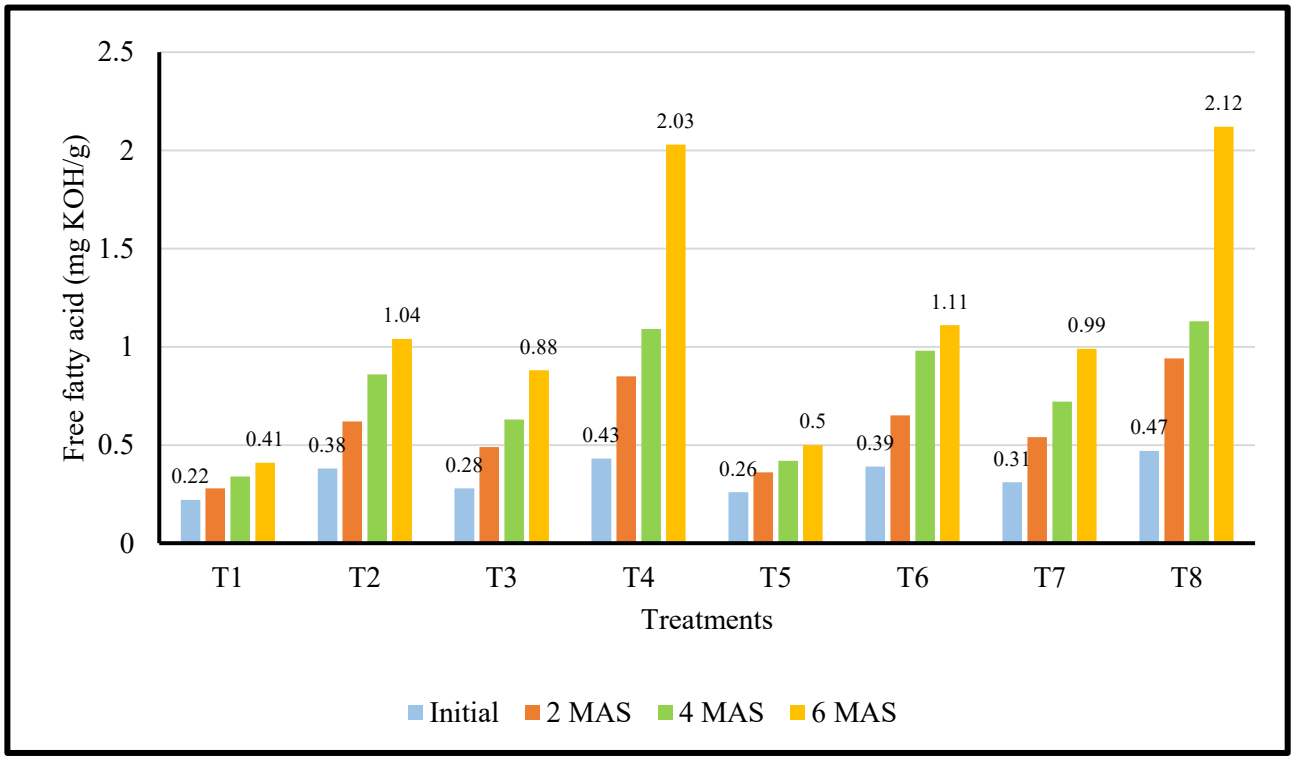
In this study, the initial free fatty acid value of VCO ranged from 0.22 to 0.47 mg KOH/g (Fig. 17). These values were within the limits of CODEX (2009) and FSSAI (2011) standards. The variation in free fatty value may be due to the difference in extraction methods. Marina *et al*. (2009) also reported that free fatty acid values varied with different extraction techniques and found that values ranged from 0.15 to 0.25. Similarly, Mansor *et al.* (2012) observed that free fatty acids contents ranged from 0.29 to 0.46 mg KOH/g in VCO extracted from different methods.

In this study, the highest free fatty acid value was noted in the VCO extracted by enzymatic method followed by the fermentation method, which may be due to its higher moisture content. These results agree with the findings of Lawson *et al.* (1985) and Che Man *et al*. (1997). They reported that coconut oil with a high moisture has high levels of free fatty acids.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 16. Moisture content of VCO extracted from different methods on storage



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 17. Free fatty acid content of VCO extracted from different methods on storage

The high content of free fatty acid in the VCO extracted by fermentation method may be due to the action of lipolytic enzymes (Lalas and Tsaknis, 2002). Senphen and Benjakul (2016) also reported that the VCO extracted by fermentation method showed high free fatty acid content (0.69%) due to the increased extraction time. They concluded that hydrolysis process may be increased with increased time and temperature of extraction. However, the free fatty acid value of VCO samples in this study was comparatively low, indicating that the oils were of high quality.

Satheeshan *et al.* (2019) also reported that the free fatty acid content obtained for different VCO samples ranged from 0.23% to 0.57%, which were relatively low and showed that the oils were of high quality. They also found that the acid value was lower for hot extracted VCO (0.64%) followed by cold extracted VCO (0.66%) and higher for copra oil (1.60%). The lower acid value indicates that the oil contains relatively low water or moisture content. Similarly, the VCO extracted by traditional method showed low acid value in this study, possibly due to its lowest moisture content.

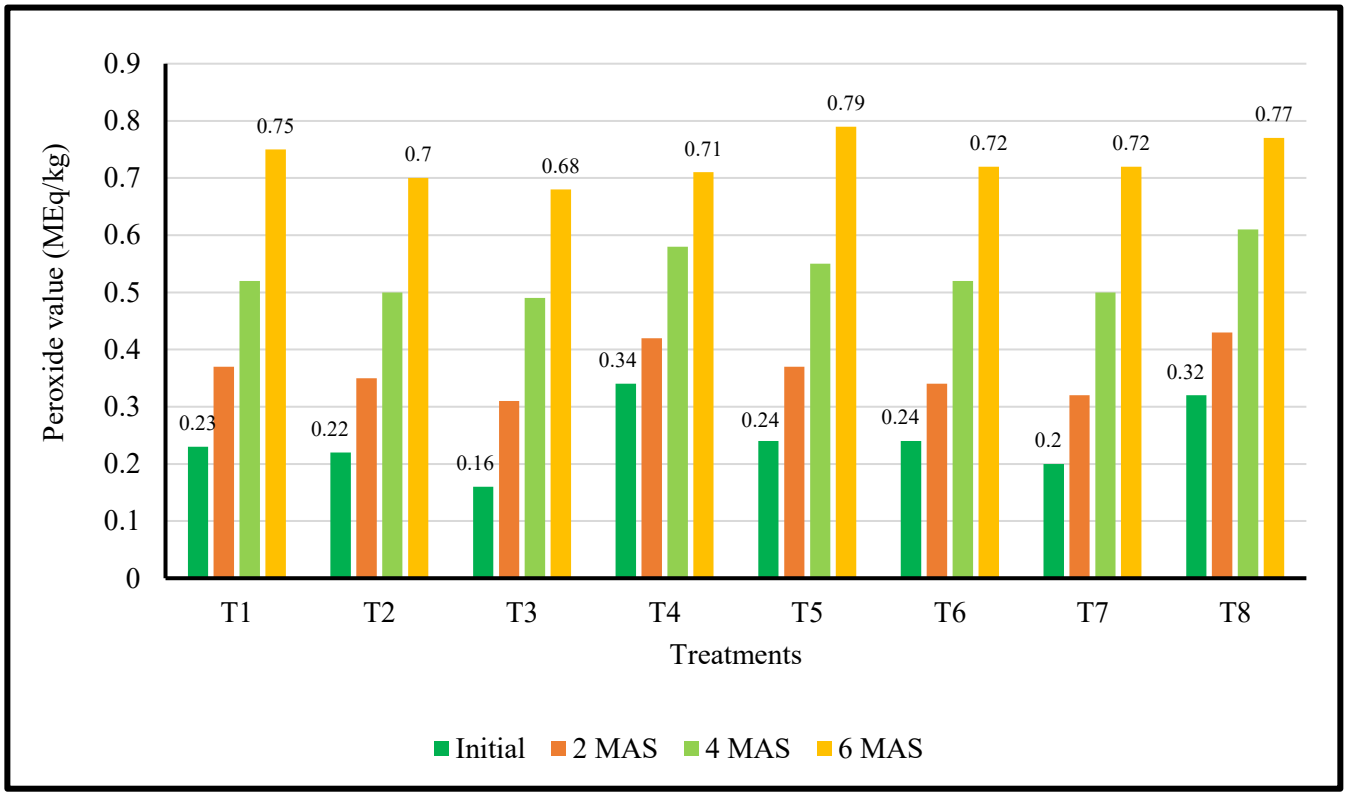
In this study, the VCO extracted by cold centrifugation method showed a low level of free fatty acids compared to the fermentation and enzymatic methods. Raghavendra and Raghavarao (2011) also found that VCO extracted by fermentation method showed higher free fatty acid value than the centrifugation method due to the longer duration taken to extract the oil by fermentation, which may lead to rancidity. Similarly, Ngampeerapong *et al*. (2018) reported that VCO extracted using centrifugation had low free fatty acid value than fermentation. At the end of the storage period, the free fatty acid value of all the treatments increased from 0.22 - 0.47 mg KOH/g to 0.41 - 2.12 mg KOH/g. This may be due to the increased amount of moisture and temperature variations. Lawson *et al.* (1985) opined that higher temperature and excess water increases the free fatty acid content in oil. However, these values were within the recommended levels of CODEX (2009) and FSSAI (2011) standards of 4.0 mg KOH/g. The results of the present study showed that the minimum acid value was obtained for the VCO extracted by traditional method and the maximum for the enzymatic method followed by the fermentation method throughout the storage period. Ramesh *et al.* (2020) also found that hot extracted VCO had a low level of free fatty acid that gives a pleasant odour and taste to the oil.

Natalia *et al.* (2019) also pointed out that the free fatty acid content of VCO increased with the storage period. Ajogun *et al*. (2020) found that the free fatty acid level of cold pressed and hot pressed oil increased from 0.054 to 0.742% and 0.700%, respectively, after three months of storage period at room temperature. Cold extracted VCO had a higher acid value than hot extracted VCO at the end of the storage. However, acid values of VCO were within the range of CODEX standard (2009).

5.3.2.3. Peroxide value of VCO during storage

Peroxide value is an important parameter for determining oil quality during storage. High peroxide value leads to rancidity and decreases the quality of oil. In this study, the peroxide value of all the treatments increased from 0.16 - 0.32 MEq/kg to 0.68 - 0.79 MEq/kg during the storage period (Fig. 18). These values were within the prescribed limits of CODEX (2009), APCC (2009) and FSSAI (2011) standards. The low level of peroxide value in VCO may be due to the presence of high saturated fatty acids. Peroxides are produced easily by the reaction of oxygen with unsaturated fatty acids (Marina *et al*., 2009). Low peroxide value during room-temperature storage indicates high storage stability for coconut oil (Ajogun *et al*., 2020).

Similarly, Srivastava *et al*. (2013) reported that peroxide value increased with storage period. They found that the peroxide value of cold and hot extracted VCO increased to 4.95 and 5.65 MEq/kg, respectively at the end of 12 months of storage. However, these values were within the limits of CODEX (2009) and FSSAI (2011) standards of <15 MEq/kg and indicated that oil had good storage stability till the end of the storage period.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

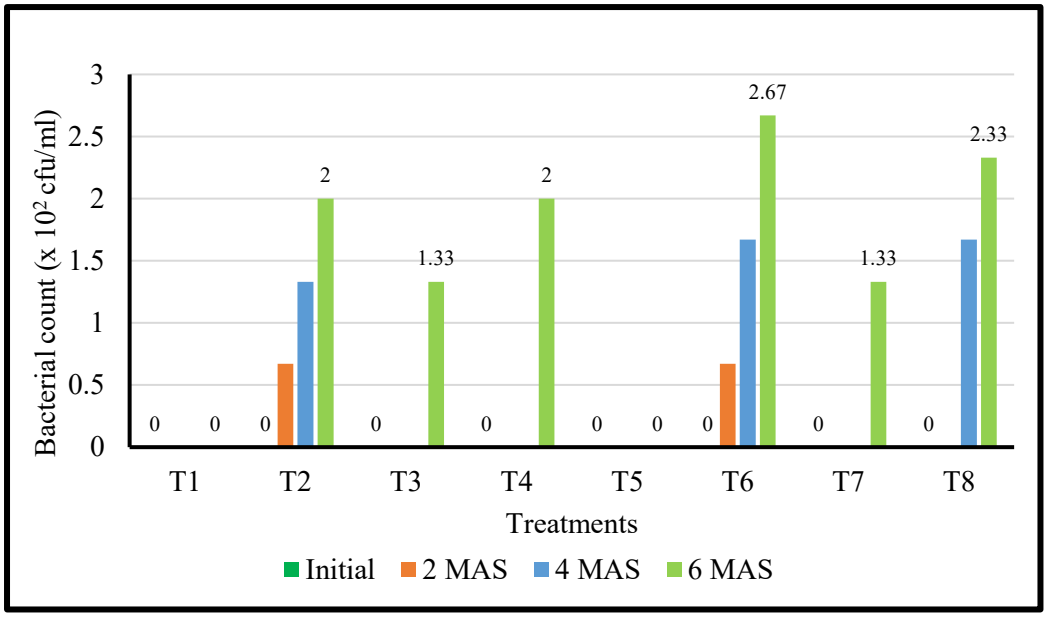
Fig 18. Peroxide value of VCO extracted from different methods on storage

Thanuja (2015) observed that peroxide value increased with the storage period. They found that the peroxide value of VCO extracted from different methods including centrifugation, fermentation and traditional boiling method, increased from 0.19 - 0.24 MEq/kg to 0.84 - 0.89 MEq/kg during the six month storage period. The VCO extracted by centrifugation method initially showed a lower peroxide value (0.19 MEq/kg), which increased after three (0.56 MEq/kg) and six months (0.84 MEq/kg). Compared to other treatments, the VCO extracted by traditional method showed a high level of peroxides, whereas the centrifugation method showed a low level of peroxides throughout the storage period. These findings were in accordance with the findings of the present study. During the storage period, the VCO extracted by traditional method showed the highest peroxide value and lowest was in the cold centrifugation method.

Ajogun *et al*. (2020) also found that the peroxide value of cold pressed VCO increased significantly from 1.17 MEq/kg to 2.27 MEq/kg during the three months of storage at room temperature. The peroxide value of hot extracted VCO also increased from 1.29 to 2.20 MEq/kg. These values were not beyond the standard limit and they concluded that VCO had good storage stability throughout the storage period.

5.3.3. Total microbial count during storage

The microbial count in a product determines the shelf life of food products. A product with a high microbiological count has a short shelf life and cannot be advised for safe consumption. In this storage study, initially, microbes were not detected in all the treatments. An increase was observed in the total bacterial count of VCO samples during storage (Fig. 19). At the end of the second month, the presence of bacteria was found in the VCO samples extracted by fermentation method from the WCT variety and *Kerasree* hybrid (T_2 and T_6 - 0.67 x 10^2 cfu/mL). At the end of the storage, bacteria were detected in all the treatments except in the VCO samples extracted by traditional method from the WCT variety and *Kerasree* hybrid.



[T_1, T_5 - Traditional method; T_2, T_6 - Fermentation method; T_3, T_7 - Cold centrifugation method; T_4, T_8 - Enzymatic method]

Fig 19. Total bacterial population during storage

The highest bacterial count was found in T_6 (2.67 x 10^2 cfu/mL) followed by T_8 (2.33 x 10^2 cfu/mL), T_2 (2.00 x 10^2 cfu/mL), T_4 (2.00 x 10^2 cfu/mL), T_3 (1.33 x 10^2 cfu/mL) and T_7 (1.33 x 10^2 cfu/mL). However, these counts were within the APCC standard (2009) limits of <10 x 10^2 cfu/mL. In the case of fungi and yeast, no fungal or yeast colonies were found in the VCO samples until the end of the storage period, which may be related to the antimicrobial activity of the VCO. As a result, VCO can be consumed safely till the end of the sixth month.

Arumugam *et al.* (2014) reported that microbes were not detected in the cold pressed VCO during the 10 months of storage period. Thanuja (2015) found that microbial load increased with the storage period. Initially, microbes were not present in the VCO samples. After three months of storage, bacteria, fungi, actinomycetes, lactobacillus and yeast were detected in the VCO samples. VCO extracted by traditional and centrifugation methods showed a low level of microbial count. Natural fermentation and induced fermentation methods had high levels of microbial load during storage.

Ndife *et al.* (2019) found that the total microbial count in the VCO samples extracted using different methods varied between 5.05 and 12.93 x 10^2 cfu/mL. The VCO extracted by fermentation method showed the highest microbial load (12.93 x 10^2 cfu/mL) followed by centrifugation (9.54 x 10^2 cfu/mL), freezing (6.17 x 10^2 cfu/mL) and solvent methods (5.05 x 10^2 cfu/mL). These counts were within the permissible limits suggested by APCC standard (2009) of <10 x 10^2 cfu/mL except for VCO extracted by fermentation method. Hanjaya *et al*. (2020) reported that the total plate count of VCO extracted using the fermentation method was high (11.67 cfu/mL), which indicated poor oil quality because it exceeded the permissible limit for VCO. The contamination of the coconut meat, the equipment used to shred the coconut, and unhygienic handling practices during coconut milk wet extraction may be the reason for these issues.

5.4. Evaluation of medicinal properties of VCO

5.4.1. Antimicrobial properties

VCO contains various strong bioactive components, most of which might have antibacterial, antifungal and antiviral effects. The findings from the current study reveals that VCO had antimicrobial activity against human pathogens including *Staphylococcus aureus, Bacillus subtilis* and *Candidia albicans.* However, VCO was not effective against *Streptococcus pyogenes* at 100 and 500 µl concentrations. The effectiveness of VCO against pathogenic organisms may be due to the high concentration of medium chain fatty acids, particularly lauric acid and its monoglyceride form, monolaurin (Nasir *et al.*, 2017).

Abraham and Verallo-Rowell (2001) also found that VCO inhibited the growth of *Staphylococcus aureus* by the action of the monoglyceride form of lauric acid, monolaurin. The lipase enzyme produced by *Staphylococcus aureus* converts triglycerides in VCO to monoglycerides form (Verallo-Rowell *et al*., 2008). The small molecules of monoglycerides might more easily cross the membrane barrier, disrupt the bacterial cell membrane, stop the action of the core enzymes, and ultimately cause bacterial cell death (Shilling *et al*., 2013).

According to Bergsson *et al*. (2001) and Skrivanova *et al*. (2005), VCO and some of its fatty acids, including lauric, capric, and caprylic acid, can prevent the growth of pathogens such as enveloped viruses, yeast (*Candida albicans*), and bacteria (*Clostridium perfringens*). Shilling *et al*. (2013) reported that ingested VCO was digested by lipase enzyme in the digestive tract to release fatty acids and ultimately prevent the growth of microorganisms. They concluded that, compared to capric and caprylic acids, lauric acid was the most effective fatty acid against pathogens, inhibiting hundred per cent microbial growth.

Loung *et al.* (2014) reported that free fatty acids and monoglycerides have antibacterial properties, whereas triglycerides and diglycerides do not. They found hydrolysed VCO had more antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* than nonhydrolysed VCO. In addition, increased concentrations of hydrolysed VCO increased antibacterial activity. Lauric acid and monolaurin effectively inhibited the growth of bacteria *Staphylococcus aureus* and fungi *Candida albicans* (Nitbani *et al*., 2016). Widianingrum *et al*. (2019) found that VCO had inhibiting effect on the growth of *S. aureus* in an *in vitro* test at the concentration of 200 µL.

Free fatty acids from hydrolyzed VCO were found to inhibit the growth of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella enteritidis*, at minimum inhibitory concentrations of 60%, 50%, 40%, and 20%, respectively (Nguyen *et al*., 2017). Margata *et al.* (2019) reported that fifty per cent concentration of hydrolysed VCO showed the best antimicrobial activity with an inhibition zone of 10.88 mm for *Propionibacterium acne*, 10.58 mm for *Staphylococcus epidermidis*, and 10.53 mm for *Bacillus subtilis*. They also observed that the inhibition zone widened with increasing doses of VCO. The same ratio of VCO and *Swietenia mahagoni* seed extract was found to be effective against *Bacillus subtilis*, *Staphylococcus aureus* and *C. albicans* with an inhibition zone of 8.46 mm, 8.49 mm and 6.00 mm, respectively (Hartati *et al*., 2019).

According to Bergsson *et al.* (2001), capric acid and lauric acid are highly effective at preventing the growth of *C. albicans*. Ogbolu *et al.* (2007) found that coconut oil had the maximum susceptibility to *C. albicans* (100%), with a minimum

inhibitory concentration of 25 per cent (1:4 dilution), while fluconazole had 100 per cent susceptibility at a minimum inhibitory concentration of 64 g/mL (1:2 dilution). Kannan and Mohammed (2014) observed the antifungal effect of 500 µL concentration of VCO and clotrimazole (1%) against *C. albicans*. They found that VCO inhibited the growth of the microbe with an inhibition zone of 19.6 mm and 23.5 mm for clotrimazole. Tjin *et al*. (2016) reported that VCO at 25 per cent concentration was effective against the *Candida albicans*. The antifungal effects of coconut oil were comparable to those of ketoconazole. Additionally, coconut oil was reported to have stronger antifungal action than probiotics against *C. albicans* (Shino *et al*., 2016).

5.4.2. Antiproliferatory activity of VCO

According to Carandang (2008), VCO had anticancer properties due to natural antioxidants lie tocopherols, tocotrienols and phenolic compounds. Phenolic compounds could decrease cell proliferation, induce apoptosis and prevent the progression of cell cycle in different types of human cancer cell lines, including skin, lung, breast, colon, prostate, pancreatic and liver cancer cell lines (Vanamala *et al*., 2010). Tocopherols and tocotrienols also had antiproliferative and apoptotic effects on cancerous human cells. This effect could result from the induction of apoptosis through a mitochondria-mediated pathway or the suppression of cyclin D, which would cause the cell cycle to be arrested (Rizvi *et al*., 2014). The medium chain fatty acids in VCO, such as lauric acid, myristic acid, and caprylic acid also exhibited anticancer property (Sandhya *et al*., 2016; Pruseth et al., 2020).

Coconut oil was noticeably more protective than other vegetable oils for colon and breast cancers (Cohen et al., 1987). Calderon *et al.* (2009) reported that VCO inhibited the proliferation of breast cancer cell lines. VCO is rich in saturated fatty acids, especially lauric acid. Exposure to lauric acid causes apoptic changes in a large number of colorectal cancer cells (Fauser *et al*., 2013). Yahaya *et al.* (2015) found that VCO induced apoptosis and showed anticancer effect on lung cancer cell lines. Kamalaldin *et al.* (2015) reported that VCO reduced the proliferation of lung cancer cells and triggered cell death via the apoptosis pathway at doses as low as 8.64% and 12.04%

In this study, VCO effectively decreased the viability of hepatic cancer cells (HepG2) in a dose dependent way. The cancer cell viability was reduced from 93.1% to 40.78% by the action of VCO at 6.25 to 100 µg/mL concentrations respectively. The measured IC_{50} value was 70.60 µg/mL, considered a moderately active concentration. The presence of high lauric acid, natural antioxidants like tocopherol and phenolic compounds in VCO may be the cause of its antiproliferative properties. Lim *et al.* (2014) pointed out that the phenolic extract of VCO is a free radical scavenger that may inhibit the proliferation of cancer cell lines. In addition, VCO showed cytotoxic effects on human hepatocarcinoma cells by increasing hydroxyl radicals that kills cancer cells.

Famurewa *et al*. (2017) reported that VCO had hepatoprotective effect by preventing oxidative stress; and lipid peroxidation and boosting the activities of antioxidant enzymes. Verma *et al.* (2019) found that VCO (20%) significantly inhibited cancer growth in hepatic cell lines. They concluded that VCO had anticancer properties and could be used to treat cancer, particularly liver and oral cancer. Pruseth *et al.* (2020) observed the anticancer efficacy of VCO using *in silico* and *in vitro* methods. They found that VCO was effective against HepG2 liver cancer cells. Tasdan *et al.* (2023) studied the cytotoxic effect of coconut on hepatocellular carcinoma cells and found that coconut extract, coconut water and coconut milk had a cytotoxic effect on HepG-2 with an IC_{50} value of 93.13 µg/ml, 96.37 µg/mL and 71.96 µg/mL, respectively.

5.4.3. Antioxidant properties of VCO

Compared to refined copra oil, VCO showed higher antioxidant activity (Dia *et al*., 2005). The higher phenolic acid concentration in VCO may be the reason for its increased antioxidant properties (Marina *et al.,* 2009). In this study, VCO possessed antioxidant activity by scavenging DPPH radicals, nitric oxides, superoxides and hydroxyl groups. Librado and Von Luigi (2013) also found that phenolic compounds in VCO inhibited the general free radicals such as DPPH radical (IC_{50} -14.9 mg/mL), nitric oxide (IC_{50} - 2.31 x 10^{-2} mg/mL), hydroxyl and hydrogen peroxides (IC_{50} - 1.29 mg/mL).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, a stable organic compound, was commonly used to determine the free radical scavenging activity, which was

expressed as IC_{50}. During the DPPH scavenging process, the DPPH radical in methanolic solution receives an electron or an active hydrogen atom from VCO, formating the yellow molecule (diphenylpicryl hydrazine). Ahmad *et al*. (2015) found that fifty per cent of DPPH radicals were inhibited by 5.07 mg/L concentration of VCO extract.

In this study, VCO showed DPPH scavenging activity with an IC_{50} value of 1236.29 µg/mL and reducing power with an absorbance of 0.046 at 100 µg/mL. Similarly, Marina *et al.* (2009) found that VCO extracted by fermentation and chilling method showed better antioxidant activity by scavenging DPPH radicals with an EC_{50} value of 1.24 mg/mL and 1.66 mg/mL, respectively. With an absorbance of 1.02, the chilling method had the strongest reducing power, followed by fermentation (0.64) and commercial copra oil (0.37) at 10 mg/mL. They concluded that VCO had better reducing power and antioxidant activity than commercial copra oil but showed lower antioxidant activity than tocopherol.

Librado and Von Luigi (2013) reported that the free radical scavenging and antioxidant activity of the VCO samples were affected by the processing conditions. They found that VCO showed lower free radical scavenging activity with an IC_{50} value of 313.46 mg/mL. Ghani *et al*. (2018) also reported that IC_{50} values of VCO extracted by different methods ranged between 7.49 and 104.52 mg/mL and indicated lower VCO antioxidant capacity.

Adaji *et al.* (2020) found that VCO had strong antioxidant activity against DPPH free radicals. The results showed that VCO inhibited the free radicals in the range of 37.0 to 61.0 per cent at 25 to 400 µg/mL concentration. Pathirana *et al*. (2021) reported that the DPPH scavenging activity of VCO varied from 857.19 to 1282.5 µg/mL. This variation was due to the use of four different coconut varieties and their total phenolic content. Mohammed *et al*. (2021) found that the IC_{50} values of VCO varied with extraction methods, ranging between 205.15 and 248.16 mg/mL.

In this study, the measured IC_{50} values for the nitric oxide, superoxide and hydroxyl scavenging activity of VCO were 295.59 µg/ml, 108.71 µg/mL and 120.65 µg/mL, respectively. The antioxidant activity of VCO in the present study was low

compared to the findings of Janu *et al*. (2014). They reported that coconut oil showed higher DPPH scavenging activity (IC_{50} - 229.76 µg/mL), ABTS radical scavenging activity (IC_{50} - 51.75 µg/mL), superoxide radical scavenging activity (IC_{50} - 20 µg/mL) and nitric oxide scavenging activity (IC_{50} - 11.14 µg/mL). VCO inhibited the free radicals such as ABTS, DPPH, and superoxide with IC_{50} values of 1.39 ± 0.01 mg/mL, 78.16 ± 0.60 mg/mL, and 27.43 ± 0.37 mg/mL respectively (Jamjai *et al*., 2020). VCO extracted by the hot method and fermentation method showed nitric oxide scavenging efficacy with IC_{50} values of 14.84 ± 0.81 µg/mL and 29.41 ± 1.7 µg/mL, respectively (Illam *et al*., 2021).

5.5. Development of VCO capsule and its quality evaluation

Capsules are solid dosage forms in which one or more active ingredients are combined with inert ingredients and enclosed in small tubes, typically made of gelatin. The outer gelatin covering of the capsule is made up of gelatin, plasticiser and water. Soft gels are a practical and often used dosage form due to its appealing attributes and swallowability, as well as its tamper resistance, preservation of the active ingredients from light and oxidation and masking of unpleasant odour and taste (Lachman *et al*., 1991). There are different sizes of capsules available to allow for flexible dosing. Soft gels can be made in a variety of shapes including round, oval and oblong.

Soft capsules are most suitable for oils. In this study, one ml, oval shaped soft gel VCO capsules were developed using standard procedure. The organoleptic evaluation was done to evaluate its acceptability. The size and shape of capsules are important parameters that affects the acceptability of capsule. In addition, the size and shape of capsules, which affect how the product travels through the pharynx and oesophagus, may directly impact a consumer's ability to swallow a particular product (Jackson *et al*., 2008). Kelly *et al*. (2010) reported that people showed difficulties swallowing capsules larger than 8 mm in diameter. Yamamoto *et al*. (2014) also opined that oval shaped capsules can more easily pass through the oesophagus than round capsules, especially when the capsule is large.

According to the results of the organoleptic evaluation, VCO capsules were highly acceptable with a high score for sensory attributes including appearance (8.40),

colour (8.87), flavour (8.43), taste (8.27), texture (8.63) and overall acceptability (8.52). The high score for taste and flavour may be due to the gelatin coating. The gelatin shells can mask the unpleasant taste and odour of the active ingredients (Aulton, 2007). The moisture content, free fatty acid content and peroxide value of the VCO capsule were 0.10 per cent, 0.24 mg KOH/g and 0.17 MEq/kg respectively. These parameters were within the limits of CODEX (2009), APCC (2009) and FSSAI (2011) standards.

5.5.1. Storage studies of VCO capsule

VCO capsules were stored in glass bottles for three months. Organoleptic evaluation, physico-chemical evaluation of oil including moisture, free fatty acid value and peroxide value, and total microbial population were done at weekly intervals.

5.5.1.1. Organoleptic evaluation of VCO capsule during storage

The results of organoleptic evaluation during the storage period showed that VCO capsule had a high score for all the sensory parameters until the end of the third month. Compared to the initial organoleptic mean scores, a slight decrease was observed at the end of the storage period, but capsules were highly acceptable (Fig. 20). Nedovic *et al.* (2011) opined that encapsulation technology not only masks the unpleasant taste and odour, but also protect the sensory qualities. Similarly, Yenipazar and Sahin-Yesilcubuk (2023) reported that capsulated oil samples showed high sensory scores until the end of the storage. Since, no rancid odour or flavour was found in the samples, demonstrating that the encapsulating material provided strong protection against oxidation, the sensory evaluation scores indicated that samples that had been capsuled were better protected.

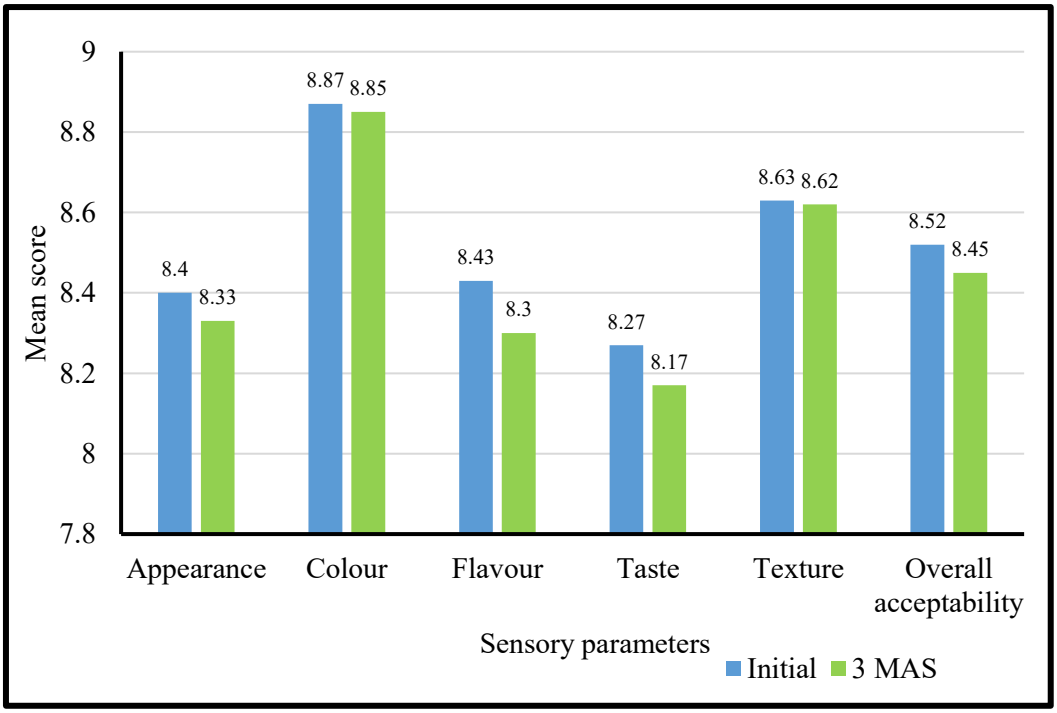


Fig 20. Organoleptic evaluation of VCO capsule on storage

5.5.1.2. Physico-chemical properties of VCO capsule during storage

The physico-chemical properties of the VCO capsule, including moisture, free fatty acid value and peroxide value slightly increased during the three months of storage period. The moisture content, free fatty acid value and peroxide value of the capsule varied from 0.10 to 0.12 per cent, 0.24 to 0.26 mg KOH/g and 0.17 to 0.20 MEq/kg respectively during storage. These values were within the standard limits of CODEX (2009), APCC (2009) and FSSAI (2011) standards. This result indicates that VCO capsule had good storage stability until the end of three months, possibly due to the gelatin coating. Marques (2010) reported that gelatin gives protection from heat and light. Gelatin also reduces peroxide by preventing oxidation (Liu *et al*., 2015).

Liquid-filled soft gel preparations have been useful for drugs that degrade through oxidation or hydrolysis. A protective nitrogen environment prepares and encloses the liquid, and the resulting dried shell has very low oxygen permeability. The drug is protected from moisture by being formulated in a lipophilic vehicle and is packaged in blister packs that are carefully developed and made of materials with low

moisture transmission (Benza and Munyendo, 2011). Film forming properties of gelatin as an outer covering that protect the food products during the storage period from dryness, light and exposure to oxygen (Ramos *et al*., 2016). These are the reasons for the storage stability of soft gel capsules. The outer covering acts as a protective barrier that prevent the oil from interacting with environmental factors, thus extending shelf life (Hosseini and Jafari, 2020). Pattnaik and Mishra (2022) reported that the physicochemical and oxidative stability of oils may be improved by adopting encapsulating methods (such as gels, emulsions, colloidal forms, and powders).

Tabibi *et al*. (2008) reported that soft gelatin capsules are sensitive to heat and humidity. Soft gel capsules may stick together or even crack open in hot or humid conditions, which decreases their shelf life. VCO capsules had stability problems when it stored for longer than six months (Benza and Munyendo, 2011). This limitation of gelatin can be improved by incorporating it with other biopolymers like chitosan, pectin, *etc*. (Hosseini *et al*., 2013). To enhance the the shelf-life and functional properties of gelatin, including antimicrobial and antioxidant properties, various substances such as additives or plasticizers, strengthening agents, and crosslinkerswere added (Mellinas *et al*., 2016).

5.5.1.3. Total microbial population during storage

The colonies of bacteria, fungi and yeast were not detected in the VCO capsules during storage. It may be because of the low moisture content of the capsule (Hamad, 2012). In soft gel capsules, gelatin acts as a barrier that protects the active ingredients from exposure to external factors such as microbes (Ramos *et al*., 2016). Nasir *et al*. (2017) opined that VCO had an antimicrobial activity that prevent the growth of bacteria, fungi, yeast, *etc*.

5.6. Cost of production

The cost of VCO extracted by different methods varied from Rs. 90 to 115 per 100 mL. The lowest production cost was found in the VCO extracted by the enzymatic method and highest in the cold centrifugation method. The cost of VCO extracted by traditional and fermentation methods was Rs. 93 and 100 per 100 mL, respectively. The result showed that the cost of VCO varied with the extraction methods.

The cost of commercially available hot processed VCO varies from Rs. 100 to 180 per 100 mL. Cold processed VCO has a higher price than hot processed VCO. The cost of commercially available cold processed VCO ranges from Rs. 150 to 280 per 100 mL. KAU released hot and cold processed VCO had Rs. 110 and 120 per 100 mL respectively.

The cost of production for one mL capsule was Rs. 7.00 in this study. The commercially available VCO capsules rate ranges between Rs. 10 and 24 per 1000 mg. It indicates that the cost of the VCO capsules prepared in this study was lower than the commercially available VCO capsules.

Summary

6. SUMMARY

The study entitled "Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule" was carried out with the objectives of assessing the physico-chemical properties, antioxidant activity and medicinal properties of virgin coconut oil (VCO). The study also intends to develop a VCO capsule and to study its quality attributes.

VCO was extracted from the mature coconuts of west coast tall (WCT) variety and *Kerasree* hybrid using four different methods: traditional method, fermentation method, cold centrifugation method and enzymatic method using standard procedures. The extracted VCO samples were organoleptically evaluated by a panel of twenty selected judges. A nine-point hedonic scale was used to evaluate the organoleptic qualities of VCO, including appearance, texture, colour, flavour, taste and overall acceptability. The result of the organoleptic evaluation showed that VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest total mean score (8.99) for organoleptic qualities, followed by the VCO extracted using traditional method from the WCT variety and *Kerasree* hybrid (T_1 and T_5 - 8.98) and the lowest score was recorded in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 8.77).

The physico-chemical properties of VCO such as oil recovery, iodine value, peroxide value, saponification value, fatty acids, moisture content, viscosity, colour, tocopherol, total fat, total phenol content and total antioxidant activity were evaluated by following standard procedures. The result showed that the fermentation method was found to have the highest oil recovery from WCT variety (54.34%) and *Kerasree* hybrid (52.33%). The cold centrifugation method showed the lowest oil recovery from both the WCT variety and *Kerasree* hybrid (T_3 - 38.97% and T_7 - 38.82%). The iodine value of VCO samples ranged from 4.03 to 5.95 I_2/100 mg. The peroxide value was low in all the treatments and it varied from 0.16 to 0.34 MEq/kg. The lowest peroxide value was detected in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 0.16 MEq/kg) whereas highest value was detected in the VCO extracted by enzymatic method from the WCT variety and *Kerasree* hybrid (T_4 - 0.34 MEq/kg and T_8 - 0.32 MEq/kg).

All the treatments had high saponification value and it ranged from 254.52 to 259.86 mg KOH/g. The moisture content of extracted VCO samples ranged from 0.09 to 0.13 per cent and the minimum moisture content was found in the VCO extracted by traditional method from the WCT variety (T_1 - 0.09%) whereas the maximum was found in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.13%). Tocopherol content was detected in all the treatments with a range of 14.82 to 27.68 µg/g. The highest tocopherol content was found in the VCO extracted by cold centrifugation method (T_3 - 27.68 µg/g) and fermentation method from the WCT variety (T_2 - 27.64 µg/g) and the lowest was found in the VCO extracted from the WCT variety by traditional method (T_1 - 14.82 µg/g).

All the treatments contained high per cent of total fat with a range of 92.89 to 95.02 per cent. Total phenol content of VCO ranged from 5.28 to 10.87 GAE µg/mg. The highest total phenol content was noted in the VCO extracted from the WCT variety by fermentation method (T_2 - 10.87 GAE µg/mg) followed by the VCO sample from the WCT variety by cold centrifugation method (T_3 - 10.63 GAE µg/mg) and the lowest content was noted in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 5.28 GAE µg/mg). The total concentration of antioxidants presents in oil varied from 17.23 to 27.45 µg/mg. The total antioxidant activity was higher in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 27.45 µg/mg) followed by fermentation method (T_2 - 27.28 µg/mg). VCO extracted from the WCT variety by traditional method (T_1) had the lowest total antioxidant activity (17.23 µg/mg).

The fatty acid profile showed that VCO was mainly composed of high per cent of saturated fatty acids (capric, caprylic, myristic, lauric, stearic and palmitic acids) and small per cent of unsaturated fatty acids (oleic and linoleic acid). Caprylic acid (short chain fatty acid) content of VCO samples varied from 5.54 to 7.60%. The highest caprylic acid content was observed in the VCO extracted from the *Kerasree* hybrid by cold centrifugation method (T_7 - 7.60%) followed by fermentation method (T_2 - 7.39%) and the lowest content was in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 5.54%). The VCO extracted from the WCT variety by fermentation method (T_2) and traditional method (T_1) contained the highest per cent of

capric acid (6.60% and 6.58%) whereas, lowest capric acid was observed in the VCO from WCT variety by cold centrifugation method (T_3 - 5.01%).

Lauric acid (medium chain fatty acid) was the predominant fatty acid present in VCO which was ranged from 45.03 to 47.06 per cent. VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest per cent of lauric acid (47.06%) followed by fermentation method (T_2 - 46.94%). The lowest lauric acid content was recorded in the VCO extracted by enzymatic method (T_4 - 45.03%). The highest myristic acid (medium chain fatty acid) content was present in the VCO extracted from the WCT variety by cold centrifugation method (T_3 -21.69%) and enzymatic method (T_4 - 21.67%) and lowest content in the VCO extracted from the *Kerasree* hybrid by cold centrifugation method (T_7 - 19.73%).

Palmitic acid (Long chain fatty acid) content of VCO samples varied from 8.66 to 9.58%. The highest palmitic acid was observed in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 9.58%) followed by VCO from the *Kerasree* hybrid by fermentation method (T_6 - 9.50%). Lowest palmitic acid content was obtained for the VCO from the WCT variety by traditional method (T_1 - 8.66%). The maximum stearic acid (long chain fatty acid) content was observed in the VCO extracted by traditional method (T_1 - 1.84%) and cold centrifugation method from the WCT variety (T_3 - 1.86%) and minimum was observed in the VCO extracted from the WCT variety by fermentation method (T_2 - 1.12%).

The per cent of unsaturated fatty acids such as oleic acid and linoleic acid present in VCO samples varied from 7.39 to 9.69% and 0.98 to 1.23% respectively. The VCO extracted from the *Kerasree* hybrid by cold centrifugation method (T_7 - 9.69%) had the highest oleic acid content and the lowest was observed in the VCO extracted from the WCT variety by traditional method (T_1 - 7.39%). The maximum linoleic acid content was found in the VCO extracted by cold centrifugation method from the WCT variety (T_3 - 1.23) and minimum was observed in the VCO extracted by traditional method from the *Kerasree* hybrid (T_5 - 0.98%).

Viscosity of VCO samples ranged from 47.60 to 51.72 cP. The VCO extracted from the *Kerasree* hybrid by cold centrifugation method (T_7 -51.72 cP) had the highest

viscosity followed by the VCO samples extracted from the WCT variety by cold centrifugation method (T_3 - 50.90 cP) and enzymatic method method o (T_4 - 50.40 cP). The results of colour analysis showed that the minimum yellowness was observed in the VCO extracted from the WCT variety by fermentation method (T_2 -0.07) followed by the VCO from the *Kerasree* hybrid by fermentation method (T_6 - 0.15) and VCO extracted from both WCT variety and *Kerasree* hybrid by cold centrifugation method (T_3 - 0.16 and T_7 - 0.18). The yellowness was higher in the VCO extracted from the *Kerasree* hybrid by traditional method (T_5 - 2.72) followed by the VCO obtained from the WCT variety by traditional method (T_1 - 0.72). The brightness (L) and redness (a) values of VCO samples varied from 97.06 to 98.31 and -1.59 to 0.02 respectively.

Bioactive compounds such as hexadecane, heneicosane, octadecane, 1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters, dibutyl phthalate, eicosane, pentacosane, tetracosane, nonacosane, 1-2-benzenedicarboxylic acid, bis[2-methyl propyl] ester, heptadecane were identified in VCO using GC-MS analysis. Compounds like hexdecane, heneicosane, octadecane and pentacosane were identified in all the treatments. Eicosane was detected in almost all the treatments except VCO extracted from the WCT variety by enzymatic method (T_4) and VCO extracted from the *Kerasree* hybrid by fermentation method (T_6).

VCO extracted from the WCT variety by fermentation method (T_2) contained high per cent of bioactive compounds like hexadecane (7.38%), heneicosane (24.83%), octadecane (7.91%), 1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters (7.68%) and 1-2-benzenedicarboxylic acid, bis[2-methyl propyl] ester (43.29%). VCO extracted by enzymatic method from the *Kerasree* hybrid (T_8) had high per cent of eicosane (8.88%). The VCO extracted by traditional method from the WCT variety (T_1) contained the highest per cent of pentacosane (31.54%) and lowest per cent of hexadecane (4.21%), heneicosane (13.54%), octadecane (4.44%), eicosane (3.20%), tetracosane (4.79%) and nonacosane (3.58%). These compounds have various medicinal properties such as antimicrobial, antiproliferatory, antioxidant, anti-inflammatory antipyretic, and analgesic properties.

For storage studies, VCO samples were stored in glass bottles for a period of six months. The organoleptic evaluation of VCO was done throughout the storage

period at weekly intervals using standard procedures. Initially, VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest total average mean score (8.99) for organoleptic qualities followed by VCO obtained from the WCT variety and from the *Kerasree* hybrid by traditional method (T_1 and T_5 - 8.98) and the lowest score was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 8.77). A gradual decrease was noted in the total average mean score in all treatments till the end of storage period. The total average mean score of T_3 declined to 7.82 after six months. VCO extracted by traditional method from the *Kerasree* hybrid (T_5) showed the highest total average mean score (8.46) followed by the VCO extracted by traditional method from the WCT variety (T_1 - 8.45). The VCO obtained from the *Kerasree* hybrid by enzymatic method (T_8) had the lowest total average mean score (7.53) for organoleptic qualities at the end of storage.

Physico-chemical properties such as moisture, free fatty acids, and peroxide value of VCO samples were evaluated in two months interval. Moisture content of stored VCO gradually increased during the storage period. Initially, moisture content varied from 0.09 to 0.13%. At the end of sixth month, moisture content of all the treatments steadily increased from 0.09 - 0.13 per cent to 0.24 - 0.58 per cent. The maximum increase in moisture was observed in the VCO extracted from the *Kerasree* hybrid by enzymatic method (T_8 - 0.58%) and minimum in the VCO extrcated from both WCT variety and *Kerasree* hybrid by traditional method (T_1 - 0.24% and T_5 - 0.29%).

During the storage, free fatty acid value of all the treatments steadily increased. Initially, free fatty acid content of VCO samples varied from 0.22 to 0.47 mg KOH/g and it increased to 0.41 to 2.12 mg KOH/g at the end of sixth month. The lowest free fatty acid content was recorded in the VCO extracted by traditional method from the WCT variety (T_1 - 0.41 mg KOH/g) followed by VCO from the *Kerasree* hybrid by traditional method (T_5 - 0.50 mg KOH/g) and highest in the VCO extracted by enzymatic method from WCT variety and *Kerasree* hybrid (T_8 - 2.12 mg KOH/g and T_4 - 2.03 mg KOH/g). The peroxide value of VCO significantly increased during the storage period. Initially it was ranged from 0.16 to 0.34 MEq/kg and at the end of storage period, peroxide value was increased to 0.68 - 0.79 MEq/kg. Lowest peroxide

value was found in the VCO extracted by cold centrifugation method from the WCT variety (T_3 - 0.68 MEq/kg) and the maximum increase was noted in the VCO extracted from the *Kerasree* hybrid by traditional method (T_5 - 0.79 MEq/kg).

The presence of bacteria, fungi, and yeast was observed in all the treatments at monthly intervals using standard procedures. Initially, bacterial population was not detected in all the VCO sapmles. At the end of storage period, bacterial count was detected in all the treatments except the VCO extracted by traditional method from the WCT variety and *Kerasree* hybrid (T_1 and T_5). The highest bacterial count was found in the VCO extracted by fermentation method from the *Kerasree* hybrid (T_6 - 2.67 x 10^2 cfu/mL) and lowest in the VCO extracted from both WCT variety and *Kerasree* hybrid using cold centrifugation method (T_3 - 1.33 x 10^2 cfu/mL and T_7 - 1.33 x 10^2 cfu/mL). In the case of fungi and yeast, there were no fungal and yeast colonies detected in the VCO samples till the end of storage period. Based on the high organoleptic scores, physico-chemical properties and storage stability, VCO extracted from the WCT variety using cold centrifugation method (T_3) was selected for further studies.

Medicinal properties such as antimicrobial, antiproliferatory and antioxidant properties of selected VCO were evaluated using standard procedure. Antibacterial activity of VCO against human pathogens namely *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes* were evaluated by agar well diffusion method. The results showed that zone of bacterial growth inhibition was not found in the T_1 (100 µl concentration of VCO). 500 µl concentration of VCO was effective against *Staphylococcus aureus* and *Bacillus subtilis*. The zone of inhibition was higher in the plate containing *Bacillus subtilis* (15 mm) followed by *Staphylococcus aureus* (10 mm). The zone of inhibition was not observed in the plate containing *Streptococcus pyogenes* at both the concentrations. Antifungal activity of VCO against *Candidia albicans* was evaluated using agar well diffusion method. VCO was effective against *Candidia albicans* at 500 µl concentration. Zone of fungal growth inhibition was not found in the T_1 (100 µl) whereas T_2 (500 µl) showed 10 mm zone of inhibition.

Antiproliferative activity of VCO was evaluated in hepatic cancer cell lines using MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay. The results showed that VCO effectively decreased the viability of cancer cells in a dose

dependent way. The cancer cell viability decreased from 93.1 per cent to 40.78 per cent by the action of VCO at different concentration (6.25 to 100 µg/mL respectively) with a measured IC_{50} value of 70.60 µg/mL.

Antioxidant activities of VCO such as DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radical scavenging activity, reducing power assay, nitric oxide scavenging activity, superoxide and hydroxyl scavenging activity were evaluated. VCO exhibited the antioxidant property by scavenging the DPPH free radicals with an IC_{50} value of 1236.29 µg/mL. The results of reducing power assay showed that the absorbance value ranged from 0.011 to 0.046 at the concentration of 20 to 100 µg/mL of VCO. It indicates that VCO has a higher reducing power and thus a higher antioxidant activity.

Nitric oxide scavenging activity of VCO ranged from 16.87 to 38.14 per cent at 6.25 to 200 per cent of concentration of VCO respectively. The measured IC_{50} value was 295.59 µg/mL. Superoxide scavenging activity of VCO varied from 8.65 to 45.28 percent of inhibition at various doses of 6.25 to 100 µg/ml, respectively. The hydroxyl scavenging activity ranged from 5.78 to 38.01 at the concentration of 6.25 to 100 µg/mL, respectively. VCO had higher superoxide scavenging property with an IC_{50} value of 108.71 µg/mL, and higher hydroxyl scavenging activity with the projected IC_{50} value of 120.65 µg/mL.

VCO capsule was developed using standard procedure. VCO capsule was golden in colour, one mL in size, oval in shape and coated by gelatin. Organoleptic evaluation was done for assessing the acceptability of capsule. The results of organoleptic evaluation showed that VCO capsules were highly acceptable with high score for sensory parameters including appearance (8.40), colour (8.87), flavour (8.43), taste (8.27), texture (8.63) and overall acceptability (8.52). Physico-chemical properties including moisture, free fatty acids, peroxide value was evaluated. The moisture content, peroxide value and free fatty acid value of VCO capsule were 0.10 per cent, 0.17 MEq/kg and 0.24 mg KOH/g respectively. The results of microbial population showed that there was no bacterial, fungal and yeast growth detected in the VCO capsules.

VCO capsules were stored in glass bottles for a period of three months. Organoleptic evaluation of VCO capsule was done throughout the storage period at weekly intervals using standard procedure. The results of organoleptic evaluation showed that VCO capsules were highly acceptable among the judges until the end of storage period. Initially, the total average mean score was 8.52 and it decreased to 8.45 at the end of storage.

Moisture content, free fatty acid value, peroxide value and total microbial population were done in weekly intervals. The moisture content of VCO capsules were found slightly increasing on storage. Initially moisture content was 0.10% and it was slightly increased to 0.12% at the end of storage with significant difference. The free fatty acid content of VCO capsule was increased during storage. Initially free fatty acid content of capsule was 0.24 mg KOH/g and increased to 0.26 mg KOH/g at the end of storage period with significant difference. During the storage period, a slight increase in peroxide value was observed and the values ranged from 0.17 to 0.20 MEq/kg. A during storage. The population of bacteria, yeast and fungi was observed in the VCO capsule at weekly intervals using the appropriate media and standard procedures. Throughout the storage period, no colonies of bacteria, fungus, or yeast were found.

The production cost for VCO varied from Rs. 93 to 115 per 100 mL. VCO extracted by cold centrifugation method had the higher cost (Rs. 115 per 100 mL) followed by fermentation (Rs. 100 per 100 mL) and traditional method (Rs. 93 per 100 mL). The lowest cost of production was found in the VCO extracted by enzymatic method (Rs. 90 per 100 mL). The cost of production for one mL capsule was estimated to be Rs. 7.00 per one mL.

The study revealed that VCO can be considered as a functional oil because of the presence of various beneficial compounds and its therapeutic properties. The quality of VCO varies with the coconut variety, hybrid (WCT and *Kerasree*) and extraction methods (traditional, fermentation, cold centrifugation, and enzymatic methods). VCO extracted by cold centrifugation and traditional methods showed high organoleptic scores than other methods but, all the samples were acceptable. The oil recovery was found to be higher in the fermentation and enzymatic methods. The physico-chemical properties of oil varied with extraction methods and coconut variety. The iodine value,

peroxide value, saponification value, moisture content, free fatty acid value and microbial population of the VCO samples were within the permissible limits. VCO samples were rich in saturated fatty acids, especially medium chain fatty acids. VCO also contained tocopherols, total phenols and various bioactive compounds. VCO was highly acceptable product and showed shelf life stability till the end of six months. Based on the organoleptic evaluation, physico-chemical properties and shelf life studies, WCT variety and cold centrifugation method were found to be better for the VCO extraction. VCO possessed antimicrobial, antiproliferatory and antioxidant activities. VCO effectively inhibited the growth of human pathogens like *Staphylococcus aureus, Bacillus subtilis* and *Candidia albicans.* The cell viability of hepatic cancer cells was inhibited by VCO with an IC_{50} value of 70.60 µg/mL. VCO also exhibited the antioxidant activity by high reducing power and scavenging the DPPH radicals, nitric oxides, superoxides and hydroxyl groups. VCO capsules were successfully developed with high acceptability scores and storage stability.

Future line of work

- Antiproliferatory activity of VCO against various cancer cell lines
- *In silico* molecular docking studies
- *In vivo* studies
- Value added products from VCO

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Appendices

APPENDIX – I

Score card for the organoleptic evaluation of virgin coconut oil

Name:

Date:

Signature:

Sl. No.	Parameters	Treatments							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	Appearance								
2	Colour								
3	Flavor								
4	Texture								
5	Taste								
6	Overall acceptability								

Nine point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

APPENDIX – II

Score card for the organoleptic evaluation of VCO capsule

Name:

Date:

Signature:

Sl. No.	Parameters	T_1
1	**Appearance**	
2	**Colour**	
3	**Flavour**	
4	**Texture**	
5	**Taste**	
6	**Overall acceptability**	

Nine point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

APPENDIX – III

Organoleptic evaluation of VCO capsule on storage

Parameters	1st month				2nd month				3rd month			
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week
Appearance	8.40	8.40	8.40	8.38	8.38	8.38	8.35	8.35	8.35	8.35	8.33	8.33
Colour	8.87	8.87	8.87	8.87	8.87	8.87	8.86	8.86	8.86	8.86	8.85	8.85
Flavour	8.43	8.43	8.43	8.37	8.37	8.37	8.33	8.33	8.33	8.33	8.30	8.30
Taste	8.27	8.27	8.27	8.20	8.20	8.20	8.18	8.18	8.18	8.18	8.17	8.17
Texture	8.63	8.63	8.63	8.63	8.63	8.63	8.62	8.62	8.62	8.62	8.62	8.62
Overall acceptability	8.52	8.52	8.52	8.49	8.49	8.49	8.47	8.47	8.47	8.47	8.45	8.45

APPENDIX – IV

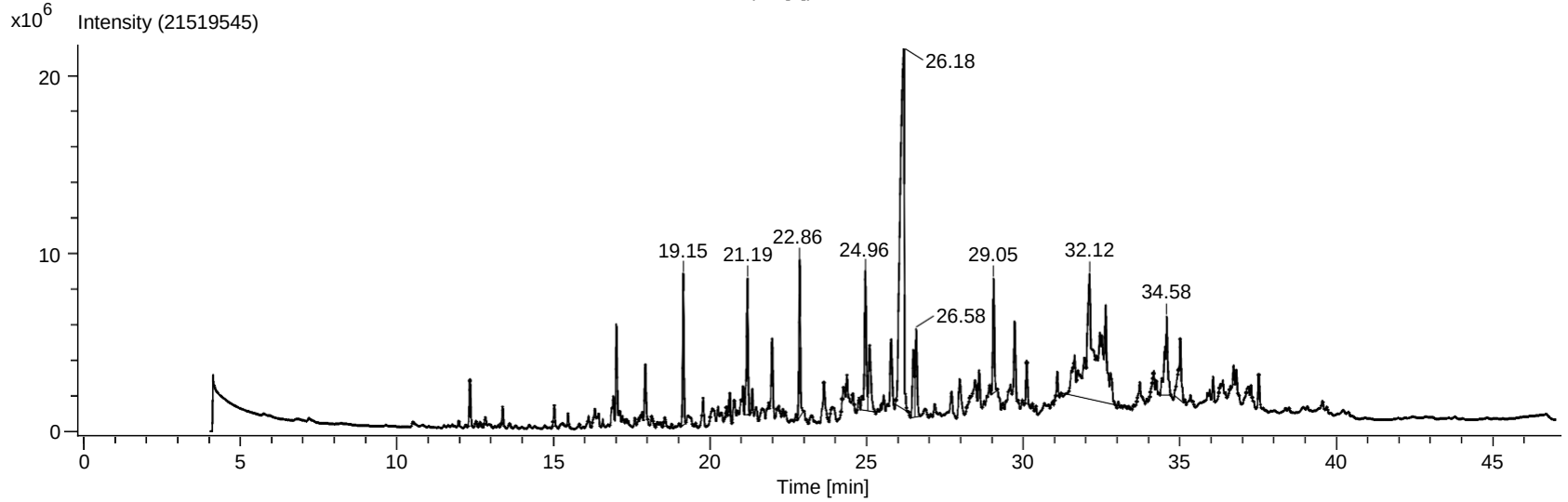
Physico-chemical evaluation of VCO capsule on storage

Parameters	1st month				2nd month				3rd month			
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week
Moisture (%)	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.12	0.12
Free fatty acids (mg KOH/g)	0.24	0.24	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.26
Peroxide value (MEq/kg)	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.20

APPENDIX - V

The results of GC-MS analysis

External Sample Id: SAMPLE H1 Acq. Data Name: SAMPLE H1 Ionization Mode: 1:EI+
Experiment Date/Time: 7/6/2022 9:56:07 AM Experiment Title: -
Comment: SPLITLESS:60-1M-6-200-2M-8-275-10M-5-28... Creation Parameters: TIC(MS[1])

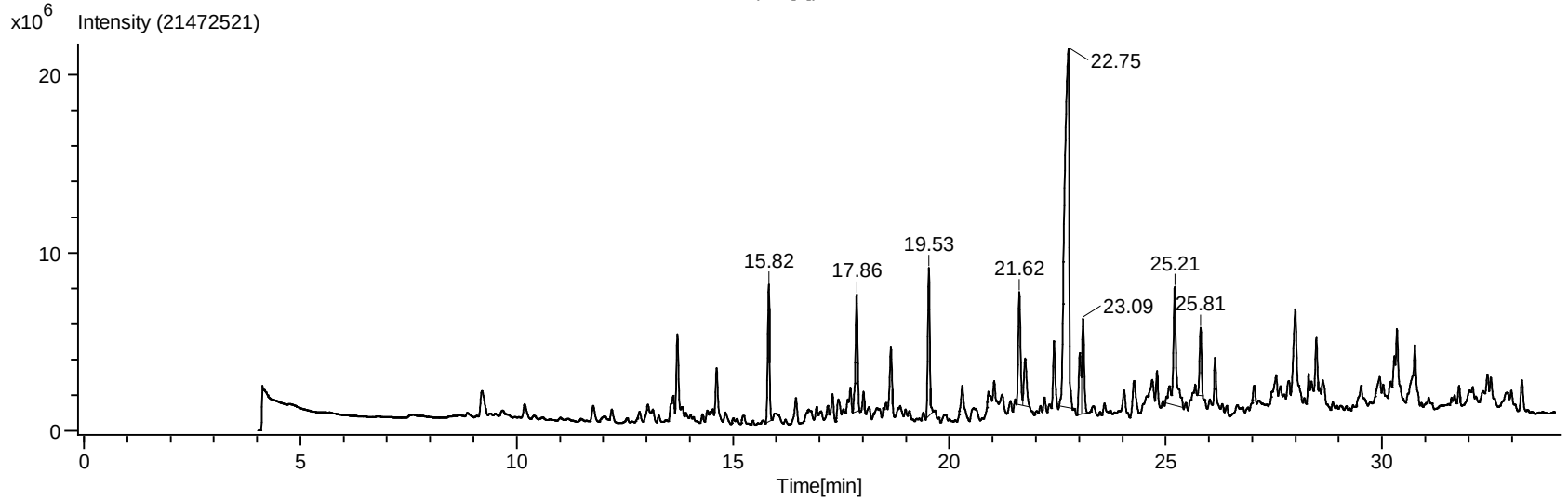


Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	19.15	BB	0.0481	26382387.47	8509271.57		19.07	269784	19.22	461167
2	21.19	BB	0.0539	28506056.82	7682682.52		21.09	962838	21.27	919748
3	22.86	BB	0.0488	27836909.46	8773853.70		22.79	623760	22.96	1110698
4	24.96	BV	0.0591	33067765.45	7789606.39		24.80	1227172	25.03	1170438
5	25.10	VB	0.0793	20338574.08	3759249.52		25.03	1170438	25.26	1113693
6	26.18	BB	0.1523	181048321.60	20270885.24		25.92	1539162	26.29	1132459
7	26.49	BV	0.0776	15967857.52	3804150.52		26.40	770672	26.54	815695
8	26.58	VB	0.0667	20019910.91	4953542.21		26.54	815695	26.73	880964
9	29.05	BB	0.0554	23234113.50	6437562.75		28.97	2057784	29.15	2314781
10	32.12	BB	0.1129	197567314.58	7010918.20		31.23	2176384	32.94	1513032
11	34.58	BB	0.0557	29975190.15	4439808.15		34.38	2056620	34.72	2026173
12	35.02	BB	0.0830	22405377.73	3495136.76		34.82	2034391	35.13	1617655

External Sample Id: H2 | Acq. Data Name: H2 | Ionization Mode: 1:EI+

Experiment Date/Time: 7/6/2022 2:25:33 PM | Experiment Title: -

Comment: SPLITLESS:80-1M-6-200-1M-8-275-2M-5-280-... | Creation Parameters: TIC(MS[1])



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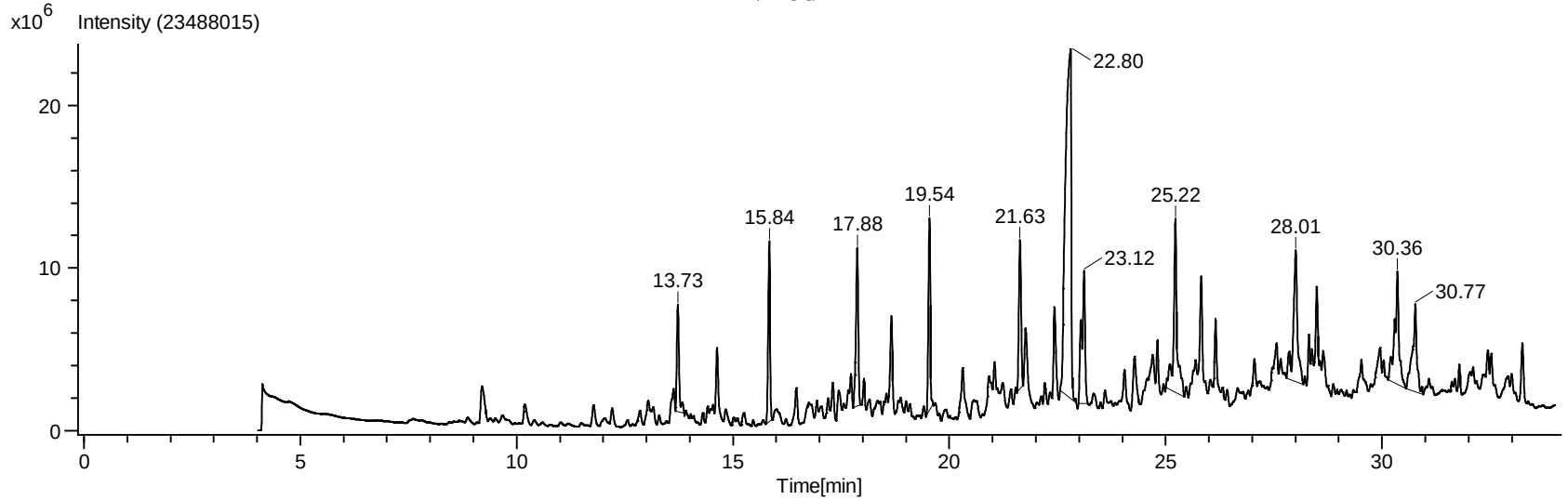
1	15.82	BB	0.0495	24414859.40	7717492.13		15.76	445603	15.90	584459
2	17.86	BB	0.0531	24102787.25	6601553.70		17.76	987262	17.92	1115523
3	19.53	BB	0.0481	26164763.53	8330507.87		19.45	616277	19.63	1059030
4	21.62	BV	0.0550	22266712.93	6336726.46		21.55	1496269	21.69	1423113
5	21.75	VB	0.0753	12516371.48	2658083.29		21.69	1423113	21.86	1340018
6	22.75	BB	0.1202	143226891.19	20173665.22		22.54	1404889	22.90	1225962
7	23.02	BV	0.0708	12893180.67	3450231.80		22.94	861436	23.06	935372
8	23.09	VB	0.0557	17820714.48	5330378.05		23.06	935372	23.18	1013393
9	25.21	BB	0.0548	35788575.57	6669595.33		24.99	1549970	25.40	1296698
10	25.81	BB	0.0484	11626117.45	3825238.90		25.74	2004705	25.86	1969594

External Sample Id: H3 | Acq. Data Name: H3 | Ionization Mode: 1:EI+

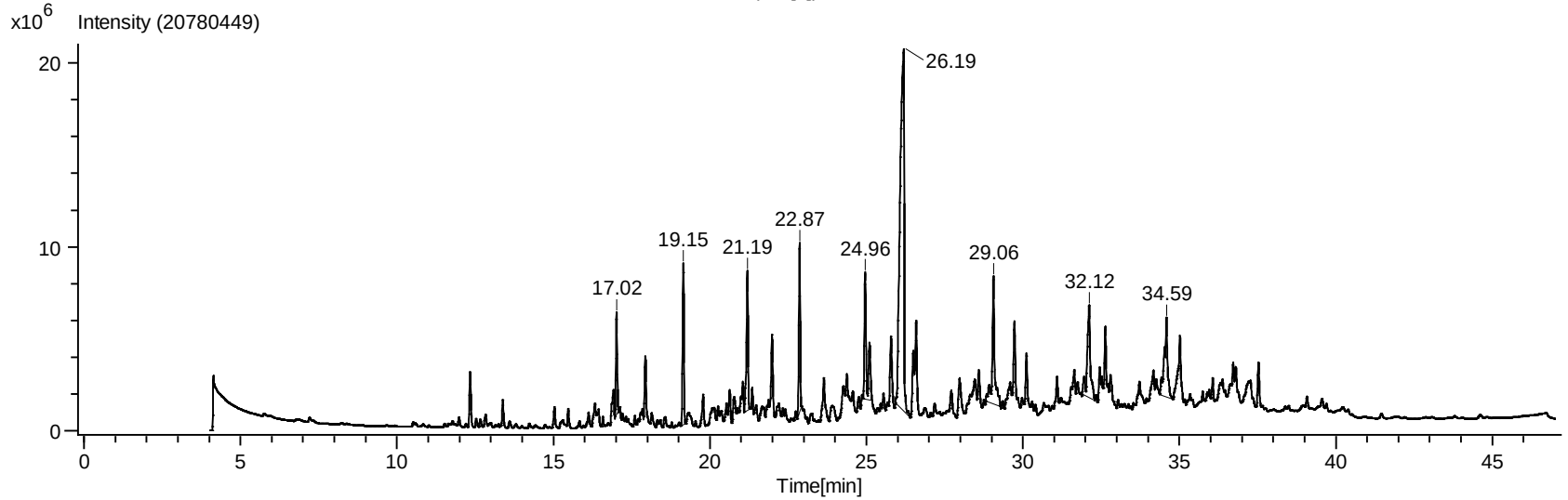
Experiment Date/Time: 7/6/2022 3:12:24 PM | Experiment Title: -

Comment: SPLITLESS:80-1M-6-200-1M-8-275-2M-5-280-... | Creation Parameters: TIC(MS[1])



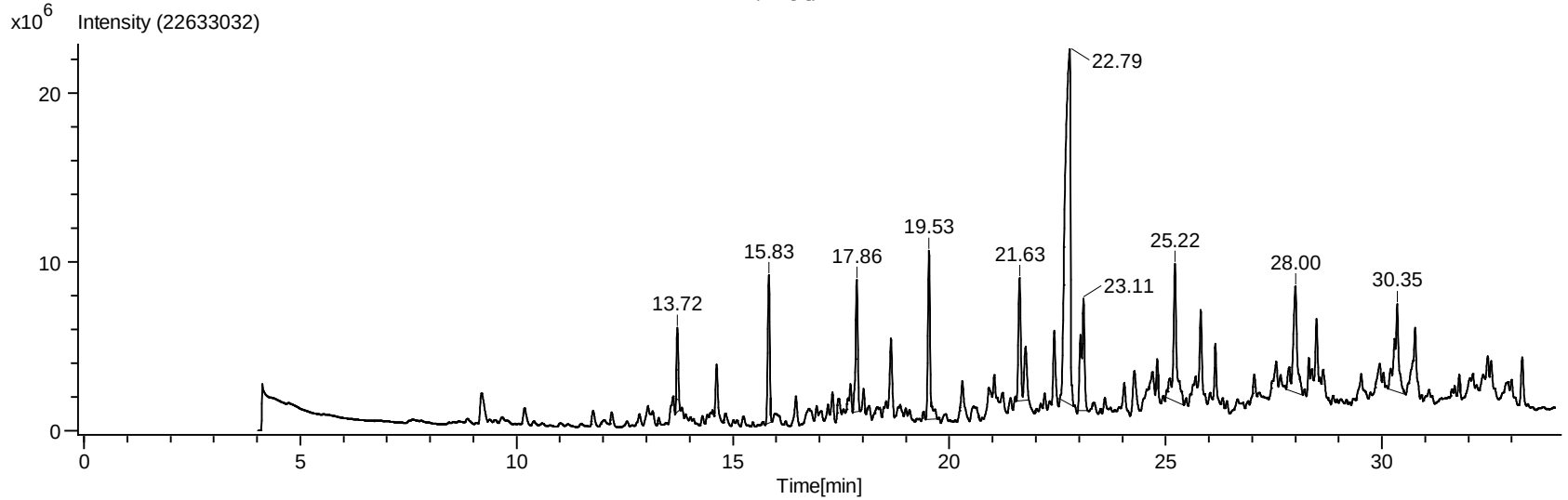
Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	13.73	BB	0.0510	23255718.83	6597555.27		13.67	1203561	13.87	1100986
2	15.84	BB	0.0517	36223403.13	11078120.73		15.76	405902	15.90	696657
3	17.88	BB	0.0544	36451323.77	9733436.75		17.77	1420854	17.96	1602780
4	19.54	BB	0.0502	37728581.58	11812847.50		19.45	889654	19.63	1643239
5	21.63	BB	0.0556	31704562.14	9154551.70		21.57	2392679	21.70	2759076
6	22.80	BB	0.1564	195277403.42	21517693.07		22.56	2465776	22.94	1702534
7	23.12	BB	0.0550	45156138.13	8153984.34		22.98	1693290	23.20	1688524
8	25.22	BB	0.0551	59065879.38	10681075.64		25.00	2669732	25.43	2079650
9	28.01	BB	0.0796	53963361.67	8080637.75		27.79	3256419	28.18	2837541
10	30.36	BB	0.0570	48136173.13	6952704.54		30.15	3114575	30.56	2586759
11	30.77	BB	0.0869	38328727.70	5388984.67		30.56	2586759	30.96	2238295

External Sample Id: SAMPLE H4
Acq. Data Name: SAMPLE H4
Ionization Mode: 1:EI+
Experiment Date/Time: 7/6/2022 8:13:14 AM
Experiment Title: -
Comment: SPLITLESS:60-1M-6-200-2M-8-275-10M-5-28...
Creation Parameters: TIC(MS[1])



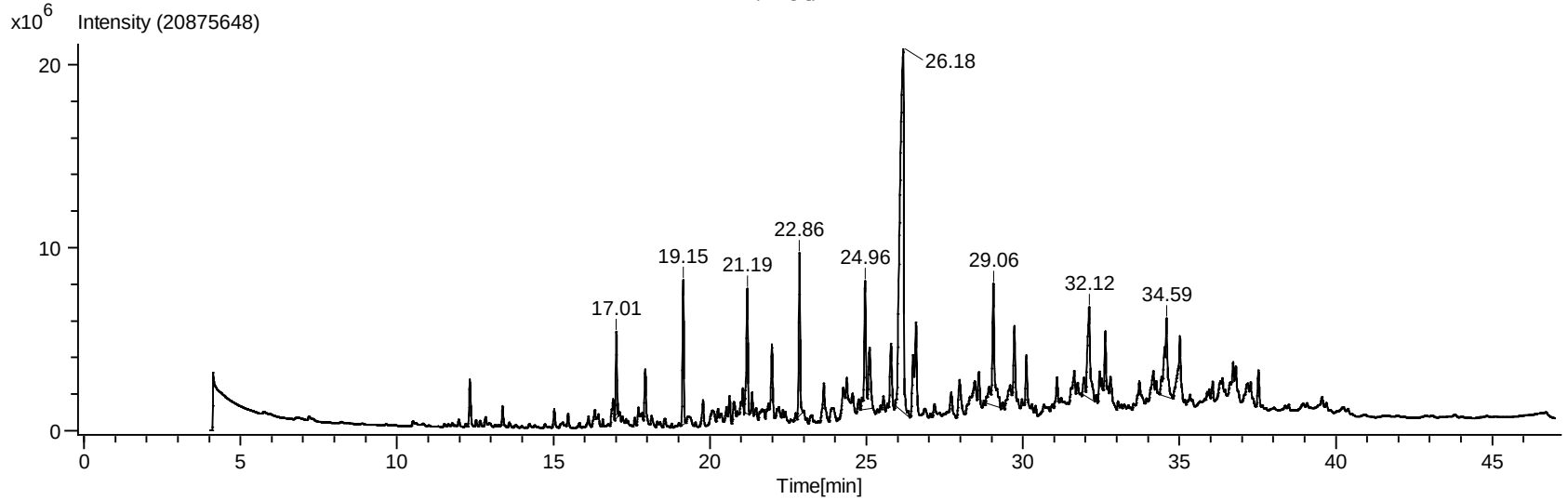
Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	17.02	BB	0.0443	15266777.35	5552494.36		16.96	702297	17.13	1272108
2	19.15	BB	0.0486	27159283.73	8777470.83		19.08	269670	19.22	383675
3	21.19	BB	0.0521	27579257.55	7594372.03		21.09	1008880	21.29	1185655
4	22.87	BB	0.0483	27884802.69	9187362.25		22.80	692402	22.93	1304847
5	24.96	BB	0.0568	38151150.39	6915501.37		24.89	1739575	25.18	1642463
6	26.19	BB	0.1537	178432935.92	19706783.22		25.92	1528393	26.40	709884
7	29.06	BB	0.0590	39540601.46	6940930.38		28.81	1606946	29.28	1316523
8	32.12	BB	0.0934	36458100.22	5035476.31		31.90	1981740	32.30	1636036
9	34.59	BB	0.1102	31635768.80	4334144.46		34.37	1979343	34.80	1698338

External Sample Id: H5
Acq. Data Name: SAMPLE H5
Ionization Mode: 1:EI+
Experiment Date/Time: 7/6/2022 11:33:48 AM
Experiment Title: -
Comment: SPLITLESS:80-1M-6-200-1M-8-275-2M-5-280-...
Creation Parameters: TIC(MS[1])



Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	13.72	BB	0.0492	15084065.24	4966084.92		13.67	1022762	13.81	1323509
2	15.83	BB	0.0515	28623244.65	8766733.27		15.76	384139	15.91	526505
3	17.86	BB	0.0539	28840524.45	7826999.61		17.77	1097565	17.94	1155716
4	19.53	BB	0.0511	36529948.75	9980595.13		19.45	678401	19.73	692641
5	21.63	BB	0.0579	40763947.95	7257020.95		21.55	1771531	21.84	1837930
6	22.79	BB	0.1428	174682460.10	21070644.65		22.55	1881191	22.92	1380254
7	23.11	BB	0.0585	38074684.47	6621716.83		22.97	1207474	23.19	1209731
8	25.22	BB	0.0561	45321175.19	8136068.91		25.00	1998608	25.42	1511049
9	28.00	BB	0.0792	41926003.87	6297896.33		27.78	2468019	28.18	2120582
10	30.35	BB	0.0587	36363244.57	5206856.36		30.14	2474670	30.55	2146179

External Sample Id: SAMPLE H6
Acq. Data Name: SAMPLE H6
Ionization Mode: 1:EI+
Experiment Date/Time: 7/6/2022 9:04:30 AM
Experiment Title: -
Comment: SPLITLESS:60-1M-6-200-2M-8-275-10M-5-28...
Creation Parameters: TIC(MS[1])

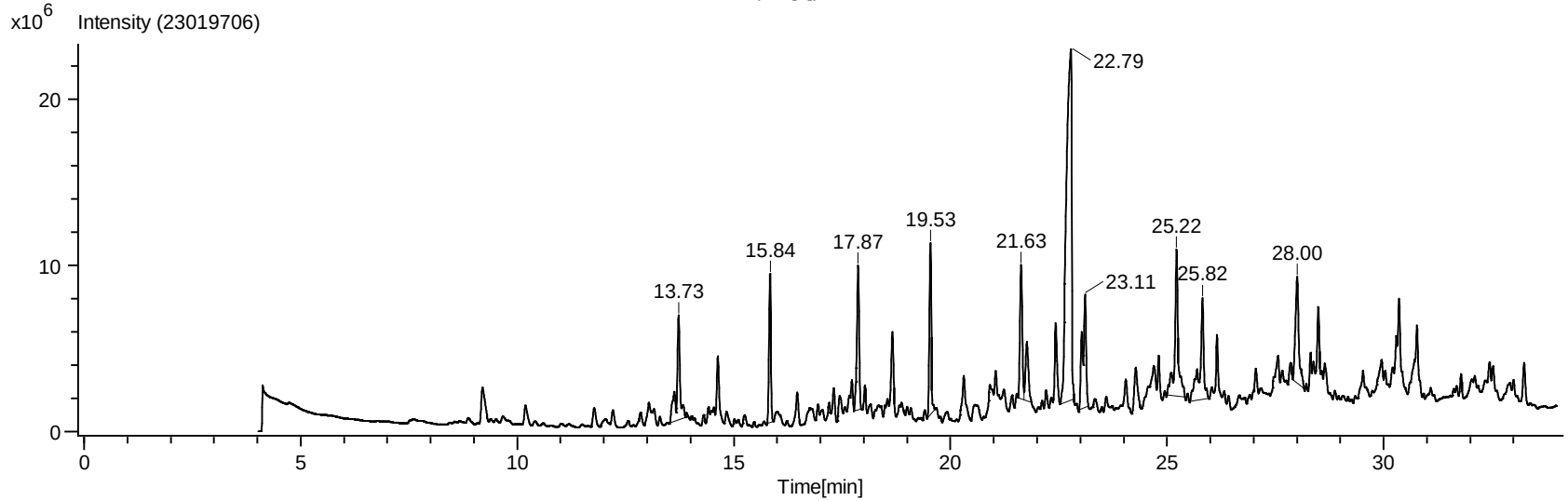


Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	17.01	BB	0.0451	13381910.25	4714178.50		16.96	537281	17.07	830519
2	19.15	BB	0.0486	24716240.11	7912085.89		19.08	269962	19.22	418419
3	21.19	BB	0.0533	25114486.92	6857531.05		21.10	959795	21.26	894851
4	22.86	BB	0.0485	28028285.47	8891676.00		22.80	708470	22.98	1033517
5	24.96	BB	0.0593	46665982.30	7017622.39		24.80	1129090	25.22	1237321
6	26.18	BB	0.1454	170283886.87	19840600.68		25.92	1403575	26.40	723202
7	29.06	BB	0.0613	39933559.83	6655916.25		28.81	1511235	29.28	1261031
8	32.12	BB	0.0946	35804227.71	4990818.52		31.90	1997213	32.30	1585718
9	34.59	BB	0.1117	31950927.48	4302455.46		34.37	1980474	34.79	1746456

External Sample Id: H7 | Acq. Data Name: H7 | Ionization Mode: 1:EI+

Experiment Date/Time: 7/6/2022 3:55:52 PM | Experiment Title: -

Comment: SPLITLESS:80-1M-6-200-1M-8-275-2M-5-280-... | Creation Parameters: TIC(MS[1])

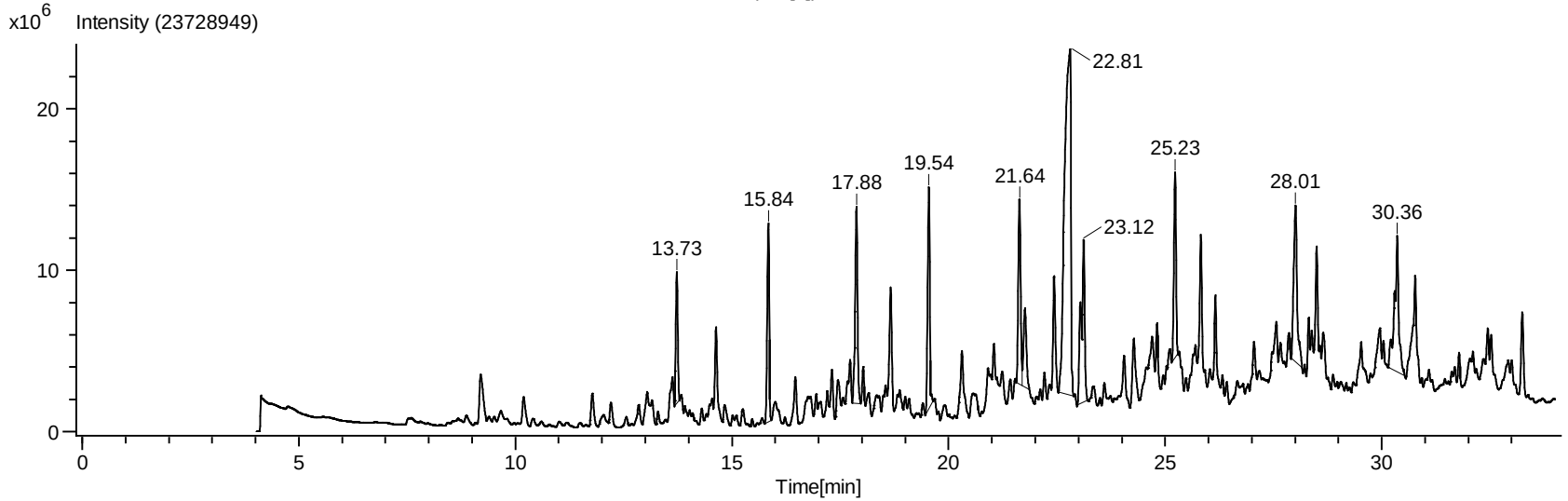


Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	13.73	BB	0.0540	33921306.53	6295699.94		13.49	485025	13.89	870337
2	15.84	BB	0.0509	28833456.94	8960703.68		15.73	463290	15.91	597324
3	17.87	BB	0.0529	31892521.96	8676755.70		17.78	1237454	17.94	1351159
4	19.53	BB	0.0494	33122786.15	10340840.74		19.45	780562	19.64	1327015
5	21.63	BV	0.0574	29047989.25	8058912.67		21.56	2018715	21.69	1907390
6	21.77	VB	0.0773	17372698.10	3543835.74		21.69	1907390	21.87	1757065
7	22.79	BB	0.1395	175485733.98	21118846.18		22.53	1628963	22.85	1972425
8	23.11	BB	0.0554	38834737.12	6796203.61		22.95	1279890	23.19	1535322
9	25.22	BB	0.0553	47174376.92	8825002.16		24.97	2216934	25.45	2061879
10	25.82	BB	0.0546	38536974.53	6097661.40		25.54	1836963	25.96	1982291
11	28.00	BB	0.0752	33957461.85	6366886.63		27.90	3144737	28.16	2613993

External Sample Id: H8 | Acq. Data Name: H8 | Ionization Mode: 1:EI+

Experiment Date/Time: 7/7/2022 10:36:53 AM | Experiment Title: -

Comment: SPLITLESS:80-1M-6-200-1M-8-275-2M-5-280-... | Creation Parameters: TIC(MS[1])



Peak Number	Time [min]	Type	Peak Width(FWH [min]	Area [Intens. * sec]	Height	Description	Start Point		End Point	
							Time[min]	Height	Time[min]	Height
1	13.73	BB	0.0497	25075253.10	8132146.04		13.67	1569296	13.80	1974743
2	15.84	BB	0.0535	41408194.85	12307210.68		15.75	485201	15.91	702338
3	17.88	BB	0.0561	47716725.80	12212683.75		17.78	1749015	17.97	1742684
4	19.54	BB	0.0533	46900094.79	13771919.79		19.46	1010752	19.64	1867771
5	21.64	BV	0.0591	42641397.67	11467785.25		21.56	3045287	21.70	2851140
6	21.76	VB	0.0762	23477818.39	4878609.78		21.70	2851140	21.87	2619981
7	22.81	BB	0.1699	213187699.07	21479830.80		22.53	2448516	22.89	2192126
8	23.12	BB	0.0561	57414937.42	10023570.62		22.98	1669969	23.21	1963770
9	25.23	BB	0.0524	38600979.40	11471858.89		25.15	4269584	25.30	4865220
10	28.01	BB	0.0783	52290091.43	9689780.79		27.92	4567913	28.16	3962562
11	30.36	BB	0.1023	57661469.26	8432888.91		30.13	4015864	30.53	3465692

QUALITY EVALUATION OF HOT AND COLD PROCESSED VIRGIN COCONUT OIL AND VCO CAPSULE

By

NIVYA E. M.

(2019-24-004)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2023

ABSTRACT

Virgin coconut oil (VCO) is a high value product extracted from fresh and mature coconut kernels using mechanical or natural methods, with or without the application of heat. The study entitled "Quality evaluation of hot and cold processed virgin coconut oil and VCO capsule" was carried out with the objectives to assess the physico-chemical properties, antioxidant activity and medicinal properties of virgin coconut oil. The study also envisaged the development of VCO capsule and evaluation of its quality attributes. VCO was extracted from the mature coconuts of West Coast Tall variety (WCT) and *Kerasree* hybrid using four different methods such as traditional, fermentation, cold centrifugation and enzymatic method using standard procedures.

The VCO extracted by the above mentioned methods were subjected to organoleptic evaluation and all the treatments were found to have high sensory qualities. VCO extracted from the WCT variety by cold centrifugation method (T_3) had the highest total mean score (8.99) for organoleptic qualities followed by VCO extracted from both WCT variety and *Kerasree* hybrid by traditional method (T_1 and T_5 - 8.98). The maximum oil recovery was from the fermentation method (T_2 - 54.34 % and T_6 - 52.33%) followed by enzymatic method (T_4 - 49.60% and T_8 - 48.80%) and lowest from the cold centrifugation method (T_3 - 38.97% and T_7 - 38.82%) in both WCT variety and *Kerasree* hybrid.

The iodine value of VCO ranged from 4.03 to 5.95 I_2/100 mg. Peroxide value was low in all the treatments and it ranged from 0.16 to 0.34 MEq/kg. VCO had high saponification value and it ranged from 254.52 to 259.86 mg KOH/g. Moisture content in extracted VCO samples varied from 0.09 to 0.13 per cent with significant difference and the minimum moisture content was noticed in the VCO extracted from the WCT variety by traditional method (T_1 - 0.09%) whereas the maximum was found in the VCO extracted from the *Kerasree* variety by enzymatic method (T_8 - 0.13%). These values were within the range specified by CODEX (2009), APCC (2009) and FSSAI (2011) standards.

Tocopherol was present in all the treatments within a range of 14.82 to 27.68 µg/g. The highest tocopherol content was found in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 27.68 µg/g) and oil from the WCT variety by fermentation method (T_2 - 27.64 µg/g). VCO prepared by different treatments had total fat in the range of 92.89 to 95.02 per cent.

Total phenol content of VCO ranged from 5.28 to 10.87 GAE µg/mg. The highest total phenol content was noted in the VCO extracted from the WCT variety by fermentation method (T_2 - 10.87 GAE µg/mg) followed by cold centrifugation method (T_3 - 10.63 GAE µg/mg). The concentration of total antioxidants present in the oil ranged from 17.23 to 27.45 µg/mg. The total antioxidant activity was higher in the VCO extracted from the WCT variety by cold centrifugation method (T_3 - 27.45 µg/mg) followed by fermentation method (T_2 - 27.28 µg/mg).

Fatty acid profile showed that VCO was mainly composed of saturated fatty acids (caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid and stearic acid) and 8.57 to 10.79 per cent of unsaturated fatty acids (oleic acid and linoleic acid). Lauric acid (medium chain fatty acid) was the predominant fatty acid present in VCO which ranged from 45.03 to 47.06 per cent. VCO from the WCT variety by cold centrifugation method (T_3) had the highest per cent of lauric acid (47.06%) followed by fermentation method (T_2 - 46.94%).

Viscosity of VCOs ranged from 47.60 to 51.72 cP. The results of colour analysis showed that compared to other treatments, the maximum yellowness was observed in the VCO extracted from the *Kerasree* variety by traditional method (T_5 - 2.72) followed by oil from the WCT variety by traditional method (T_1 - 0.72).

Bioactive compounds such as hexadecane, heneicosane, octadecane, 1-2-Benzenedicarboxylic acid, butyl 8-methyl nonyl esters, dibutyl phthalate, eicosane, pentacosane, tetracosane, nonacosane, 1-2-benzenedicarboxylic acid, bis[2-methyl propyl] ester, heptadecane were identified in VCO using GCMS analysis. These compounds have various medicinal properties such as antioxidant, antimicrobial, antiproliferatory, antipyretic, analgesic and anti-inflammatory properties.

Organoleptic qualities of VCO samples from all the treatments steadily decreased during the storage period, but were acceptable till the end of six months. The physico-chemical properties such as moisture, free fatty acid value and peroxide values were increased with significant difference and was within the permissible limits till the end of sixth month of storage. The total bacterial population increased during the storage period. Fungi and yeast were not detected in the VCOs till the end of the storage period.

Based on the organoleptic evaluation, physico-chemical properties and shelf life studies, VCO extracted from the WCT variety extracted by cold centrifugation method (T_3) was selected for further studies.

VCO effectively inhibited the growth of human pathogens like *Staphylococcus aureus, Bacillus subtilis* and *Candida albicans.* The cell viability of hepatic cancer cells was inhibited by VCO with an IC_{50} value of 70.60 µg/mL. VCO exhibited the antioxidant activity by its high reducing power and scavenging the DPPH radicals, nitric oxides, superoxides and hydroxyl groups. The projected IC_{50} value for DPPH, nitric oxides, superoxides and hydroxyl groups were 1236.29 µg/mL, 295.59 µg/mL, 108.71 µg/mL and 120.65 µg/mL respectively.

The developed soft gel VCO capsules containing one mL of oil were highly acceptable with a high score for sensory parameters. During the three months of storage period, the organoleptic mean scores slightly decreased and the physico-chemical properties including moisture content, free fatty acid value and peroxide value of capsules slightly increased with significant difference and was within the specified standards. The colonies of bacteria, fungi and yeast were not detected throughout the storage period. VCO capsules showed good storage stability till the end of three months.

The cost of production of VCO varied with the extraction methods. The oil extracted by cold centrifugation method had the highest cost (Rs.115/100 mL) followed fermentation (Rs.100/100 mL), traditional (Rs.93/100 mL) and enzymatic method (Rs.90/100 mL). The estimated cost of production for one mL capsule was Rs. 7.00.

Quality of VCO varied with the coconut variety, hybrid and different extraction methods. VCO extracted by cold centrifugation and traditional methods showed high organoleptic scores than fermentation and enzymatic methods. The presence of lauric acid content, tocopherols, phenols, various bioactive compounds and antioxidant activity contributed to the medicinal properties of VCO. Cold centrifuged oil proved to have antimicrobial activity against human pathogens like *Staphylococcus aureus, Bacillus subtilis* and *Candidia albicans*. Antiproliferatory activity was exhibited against hepatic cancer cell lines. VCO capsules were successfully developed with high acceptability scores and storage stability for three months.

The beneficial potential of VCO therapy needs to be evaluated clinically through *in silico* molecular docking and *in vivo* studies.

സംക്ഷിപ്തം

തേങ്ങാപ്പാലിൽ നിന്ന് പരമ്പരാഗതമായ രീതിയിലോ അല്ലെങ്കിൽ മെക്കാനിക്കൽ രീതിയിലോ താപം ഉപയോഗിച്ചോ അല്ലാതെയോ വേർത്തിരിച്ചെടുക്കുന്ന ഉയർന്ന മൂല്യമുള്ള ഒരു ഉല്പന്നമാണ് വെർജിൻ കോക്കനട്ട് ഓയിൽ അഥവാ വി. സി. ഒ. വിവിധ രീതികളിൽ വേർത്തിരിച്ചെടുത്ത വി.സി.ഓയുടെ ഭൗതിക രാസ ഗുണങ്ങളും ആന്റി ഓക്സിഡന്റ് പ്രവർത്തനവും ഔഷധഗുണങ്ങളെയും വിലയിരുത്തുന്നതായിരുന്ന ഈ പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യം. കൂടാതെ വി.സി.ഒ. കാപ്സ്യൂളിന്റെ വികസനവും അതിന്റെ ഗുണനിലവാരം വിലയിരുത്തലും ഈ പഠനത്തിൽ ഉൾപ്പെടുത്തിയിരിക്കുന്നു. പ്രസ്തുത പഠനത്തിനായി വെസ്റ്റ് കോസ്റ്റ് ടോൾ (ഡബ്ല്യൂ. സി. ടി.) ഇനത്തിൽ നിന്നും കേരശ്രീ ഹൈബ്രിഡിൽ നിന്നും ശേഖരിച്ച മൂപ്പെത്തിയ തേങ്ങകൾ ഉപയോഗിച്ച് പരമ്പരാഗതം, പുളിപ്പിക്കൽ, കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ, എൻസൈമാറ്റിക്ക് എന്നീ നാല് വ്യത്യസ്ത രീതികളിലൂടെ വികസിപ്പിച്ചെടുത്ത വി.സി.ഒ ആണ് പ്രസ്തുത പഠനത്തിന് വിധേയമാക്കിയത്.

ഇത്തരത്തിൽ വേർത്തിരിച്ചെടുത്ത എട്ട് വി. സി. ഒ സാമ്പിളുകൾക്കും രുചിഗുണങ്ങളുടെ മൂല്യനിർണ്ണയത്തിൽ ഉയർന്ന സ്കോറുകൾ ലഭിച്ചു. ഈ സാമ്പിളുകളിൽ കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ (ടി. 3) വഴി ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും വേർത്തിരിച്ചെടുത്ത വി.സി.ഒയ്ക്ക് ഉയർന്ന സ്കോറും (8.99) പരമ്പരാഗത രീതിയിൽ ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും കേരശ്രീ ഹൈബ്രിഡിൽ നിന്നും വികിസിപ്പിച്ചെടുത്ത വി.സി.ഒയ്ക്ക് രണ്ടാം സ്ഥാനവുമാണ് (ടി. 1, ടി. 5 – 8.98) ലഭിച്ചത്. പുളിപ്പിക്കൽ (ടി 2 – 54.34%, ടി. 6. –52.36%), എൻസൈമാറ്റിക്ക് (ടി. 4 – 49.60%, ടി.8 – 48.80%) രീതികളിലൂടെ കൂടുതൽ എണ്ണയും കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ (ടി. 3 –38.97%, ടി. 7 – 38.82%) രീതിയിൽ കുറഞ്ഞ എണ്ണയുമാണ് വേർത്തിരിച്ചെടുക്കാൻ സാധിച്ചത്.

വി. സി. ഒ സാമ്പിളുകളിൽ 4.30–5.92 ഐ2/100 മില്ലി ഗ്രാം വരെ അയോഡിൻ മൂല്യവും 0.16–0.34 മില്ലി ഇക്വിവാലെന്റ്/കിലോ ഗ്രാം വരെ കുറഞ്ഞ പെറോക്സൈഡ് മൂല്യവും 254.52 – 259.86 കെ.ഒ.എച്ച്/ഗ്രാം കൂടിയ സാപ്പോണിഫിക്കേഷൻ മൂല്യവും കണ്ടെത്തി. വിവിധ വി. സി. ഒ സാമ്പിളുകളിൽ 0.092 – 0.13 ശതമാനം ഈർപ്പം നിർണ്ണയിക്കപ്പെടുകയും ഇവയിൽ പരമ്പരാഗത രീതിയിൽ ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയ്ക്ക് കുറഞ്ഞ ഈർപ്പവും (0.09%) എൻസൈമാറ്റിക്ക് രീതി വഴി കേരശ്രീ ഹൈബ്രിഡിൽ നിന്നും വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയ്ക്ക് ഉയർന്ന ഈർപ്പവുമാണ് കണ്ടെത്തിയത്. ഈ മൂല്യങ്ങൾ കോഡെക്സ് (2009), എ.പി.സി.സി. (2009) എഫ്. എസ്. എസ്. ഐ (2011) എന്നീ മാനദണ്ഡങ്ങൾക്കനുസൃതമാണ്.

എല്ലാ സാമ്പിളുകളിലും ടോക്കോഫിറോളിന്റെ (14.82 – 27.68 മൈക്രോ ഗ്രാം/ ഗ്രാം) സാന്നിധ്യം കണ്ടെത്തിയിരുന്നു. ടോക്കോഫിറോളിന്റെ ഉയർന്ന അളവ് ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ (ടി.3. – 27.68 മൈക്രോ ഗ്രാം/ ഗ്രാം) വഴിയും പുളിപ്പിക്കൽ (ടി.2. – 27.64 മൈക്രോ ഗ്രാം/ ഗ്രാം) വഴിയും വേർത്തിരിച്ചെടുത്ത വി.സി.ഒയിലായിരുന്നു. വി. സി. ഒ സാമ്പിളുകളിൽ 92.89– 95.02 ശതമാനം കൊഴുപ്പ് അടങ്ങിയിരിക്കുന്നു.

സാമ്പിളുകളിൽ 5.28 – 10.87 മൈക്രോ ഗ്രാം/മില്ലി ഗ്രാം ഫീനോളുകൾ അടങ്ങിയിട്ടുള്ളതായി കണ്ടെത്തി. ഫീനോളിന്റെ ഉയർന്ന അളവ് ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും പുളിപ്പിക്കൽ (ടി.2. –

10.87 മൈക്രോ ഗ്രാം/മില്ലി ഗ്രാം) വഴിയും കോൾഡ് സെന്റ്രിഫ്യൂഗേഷൻ (ടി.3. – 10.63 മൈക്രോ ഗ്രാം/ മില്ലി ഗ്രാം) വഴിയും വേർത്തിരിച്ചെടുത്ത വി.സി.ഒയിലായിരുന്നു. ആന്റിഓക്സിഡന്റുകളുടെ ഉയർന്ന അളവ് ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും കോൾഡ് സെന്റ്രിഫ്യൂഗേഷൻ (ടി.3. – 27.45 മൈക്രോ ഗ്രാം/മില്ലി ഗ്രാം) വഴിയും പുളിപ്പിക്കൽ (ടി.2. – 27.28 മൈക്രോ ഗ്രാം/മില്ലി ഗ്രാം) വഴിയും വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയിലായിരുന്നു.

വി. സി. ഒ സാമ്പിളുകൾ പ്രധാനമായും പൂരിത ഫാറ്റി ആസിഡുകളാൽ (കാപ്രിലിക്ക് ആസിഡ്, കാപ്രിക്ക് ആസിഡ്, ലോറിക്ക് ആസിഡ്, മിറിസ്റ്റിക്ക് ആസിഡ്, പാല്മിറ്റിക്ക് ആസിഡ്, സ്റ്റീറിക്ക് ആസിഡ്) നിർമ്മിതമാണ്. കൂടാതെ 8.57 – 10.79 ശതമാനം അപൂരിത ഫാറ്റി ആസിഡുകളും (ഒലീക്ക് ആസിഡ്, ലിനോലിക്ക് ആസിഡ്) കാണപ്പെടുന്നു. ലോറിക്ക് ആസിഡാണ് വി. സി. ഒ സാമ്പിളുകളിൽ കൂടുതലായി കാണപ്പെടുന്ന ഫാറ്റി ആസിഡ്. പ്രസ്തുത പഠനത്തിൽ അത് 45.03 – 47.06% എന്ന തോതിൽ കാണപ്പെട്ടു. ഡബ്ല്യൂ. സി. ടി. തേങ്ങയിൽ നിന്നും കോൾഡ് സെന്റ്രിഫ്യൂഗേഷൻ വഴി വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയിൽ ആണ് കൂടുതൽ ലോറിക്ക് ആസിഡ് (47.06%) കണ്ടെത്തിയത്. കൂടാതെ പുളിപ്പിക്കൽ രീതിയിൽ വേർതിരിച്ചെടുത്ത എണ്ണയിലും കൂടുതൽ അളവ് ലോറിക്ക് ആസിഡ് (46.94%) കണ്ടെത്തിയിരുന്നു.

വി. സി. ഒ സാമ്പിളുകളുടെ വിസ്കോസിറ്റി 47.60 – 57.72 സെന്റിപോയിസ് ആയിരുന്നു. സാമ്പിളുകൾ തമ്മിൽ താരതമ്യം ചെയ്യുത്തപ്പോൾ ഡബ്ല്യൂ. സി. ടി. ഇനത്തിൽ നിന്നും കേരശ്രീ ഹൈബ്രിഡിൽ നിന്നും പരമ്പരാഗത രീതിയിൽ വേർത്തിരിച്ചെടുത്ത എണ്ണയ്ക്കാണ് കൂടുതൽ മഞ്ഞ നിറം ഉള്ളതായി കണ്ടെത്തിയത്.

ഹെക്സാഡെക്കേൻ, ഹെനിക്കോസൈൻ, ഒക്റ്റാഡെക്കേൻ, 1–2, ബെൻസീൻ ടൈകാർബോക്സിലിക്കാസിഡ്, ബ്യൂട്ടൈൽ 8 മീതൈൽ നൊണൈൽ എസ്റ്റെർസ്, ഡൈ ബ്യൂട്ടൈൽ ഫത്തല്ലേറ്റ്, ഇക്കോസൈൻ പെന്റാകൊസൈൻ ടെട്രാകൊസൈൻ നൊണാകൊസൈൻ 1,2 ബെൻസീൻ ടൈകാർബോക്സിലിക്കാസിഡ് ബിസ് 2 മീതൈൽ പ്രൊപൈൽ എസ്റ്റെർസ്, ഹെക്റ്റഡെക്കേൻ എന്നീ സംയുക്തങ്ങൾ വി. സി. ഒ സാമ്പിളുകളിൽ ഉള്ളതായി കണ്ടെത്തി. ഈ സംയുക്തങ്ങൾ ആന്റി ഒക്സിഡന്റ്, ആന്റി മൈക്രോബിയൽ, ആന്റി പ്രോലിഫറേറ്റിവ്, ആന്റി പൈറെറ്റിക്ക്, ആന്റി ഇൻഫ്ളാമേറ്ററി, വേദനസംഹാര പ്രവർത്തനങ്ങൾ ഉള്ളവയാണ്.

വി. സി. ഒ സാമ്പിളുകൾ ചില്ലു കുപ്പികളിൽ ആറു മാസകലയളവിൽ സൂക്ഷിക്കുകയും ഒരോ ആഴ്ച്ചയുടെ ഇടവേളയിൽ ഇവയുടെ സ്വീകാര്യതയും ഭൗതികരാസഗുണങ്ങളും നിരീക്ഷിക്കുകയും ചെയ്തിരുന്നു. എല്ലാ സാമ്പിളുകളും ആറ് മാസകാലയളവിലും രുചിഗുണ മൂല്യനിർണ്ണയത്തിൽ സ്വീകാര്യത ഉള്ളതായിരുന്നു. സൂക്ഷിപ്പുകാലം അധികരിക്കുന്നതനുസരിച്ച് ഭൗതികരാസഗുണങ്ങളായ ഈർപ്പം ഫ്രീ ഫാറ്റി ആസിഡ് തുടങ്ങിയവയിൽ ഗണ്യമായ വ്യത്യാസം കണ്ടെത്തിയെങ്കിലും ഇവയെല്ലാം അനിവദനീയമായ പരിതിക്കുള്ളിലായിരുന്നു.

രുചി ഗുണ മൂല്യനിർണ്ണയം ഭൗതിക രാസഗുണനിലവാരം, സൂക്ഷിപ്പുകാല പഠനം എന്നിവയുടെ അടിസ്ഥാനത്തിൽ ഡബ്ല്യൂ. സി. ടി. ഇനത്തിൽ നിന്നും കോൾഡ് സെന്റ്രിഫ്യൂഗേഷൻ വഴി വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയാണ് തുടർ പഠനത്തിനായി തിരഞ്ഞെടുത്തത്.

സ്റ്റഫൈലോകോക്കസ് ഓറിയസ്, ബാസില്ലസ് സബ്റ്റിലിസ്, കാൻഡിട ആല്ബികൻസ്, തുടങ്ങിയ മാനുഷിക സംക്രമ രോഗകാരികൾക്കെതിരെ വി. സി. ഒയുടെ ആന്റി മൈക്രോബിയൽ പ്രവർത്തനം നടക്കുന്നതായി പഠനത്തിൽ തെളിഞ്ഞു. 70.60 മൈകോഗ്രാം/ മില്ലി എന്ന ഐ. സി. 50 മൂല്യമുള്ള വി. സി. ഒ. കരൾ അർബുദ കോശങ്ങളുടെ പ്രവർത്തന ക്ഷമതയെ തടയുകയും ഡി. പി. പി. എച്. നൈട്രിക്ക് ഒക്സൈഡ്, സുപ്പരോക്സൈഡ്, ഹൈഡ്രോക്സിൽ എന്നിവയ്ക്കായുള്ള ഐ. സി. 50 മൂല്യം യഥാക്രമം 1236.29, 295.59,108.71,120.65 മൈക്രോഗ്രാം / മില്ലി എന്നിങ്ങനെയാണ്.

ഒരു മില്ലി ഗ്രാം വി. സി. ഒ ഉൾക്കൊള്ളുന്ന ജെലാറ്റിൻ കാപ്സ്യൂളുകൾ വികസിപ്പിച്ചെടുത്തു. മൂന്നു മാസത്തെ സൂക്ഷിപ്പുകാലയളവിൽ രുചി ഗുണങ്ങളുടെ ശരാശരി സ്കോറുകൾ ചെറിയ അളവിൽ കുറയുകയും ഭൗതിക രാസ ഗുണങ്ങളായ ഈർപ്പം ഫ്രീ ഫാറ്റി ആസിഡ് പെറോക്സൈഡ് എന്നിവയുടെ മൂല്യം ചെറുതായി വർദ്ധിക്കുകയും ചെയ്തെങ്കിലും അനുവദനീയമായ പരിതിക്കുള്ളിൽ തന്നെയായിരുന്നു. മൂന്നു മാസ കാലയളവിലും സൂക്ഷ്മ ജീവികളായ ബാക്ടീരിയ,യീസ്റ്റ്, ഫംഗൈ എന്നിവയൊന്നിന്റെയും സാന്നിധ്യം കണ്ടെത്തിയില്ല. ഈ കാരണങ്ങളാൽ തന്നെ കാപ്സ്യൂളുകൾ മൂന്നു മാസം വരെ ഭക്ഷ്യയോഗ്യമായിരുന്നു.

വി. സി. ഒയുടെ ഉല്പ്പാദന ചിലവ് അവ വേർതിരിച്ചെടുക്കുന്ന രീതികൾക്കനുസരിച്ച് വ്യത്യാസപ്പെട്ടിരിക്കുന്നു. ഉല്പ്പാദന ചിലവ് യഥാക്രമം കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ, പുളിപ്പിക്കൽ, പരമ്പരാഗതം, എൻസൈമാറ്റിക്ക് രീതികളിൽ 115, 100, 93, 90 രൂപ എന്നതായിരുന്നു. ഒരു മില്ലി ഗ്രാം കാപ്സ്യൂളിന് 7 രൂപയാണ് ചിലവ് വരുന്നത്.

വി. സി. ഒയുടെ ഗുണ നിലവാരം വ്യത്യസ്ത ഘടകങ്ങളെ ആശ്രയിച്ചിരിക്കുന്നു എന്നാണ് ഈ പഠനം വ്യക്തമാക്കുന്നത്. നാളികേരത്തിന്റെ ഇനം ഹൈബ്രിഡ് വി. സി. ഒ വേർത്തിരിച്ചെടുക്കുന്ന രീതികൾ എന്നിവയനുസരിച്ച് എണ്ണയുടെ ഗുണനിലവാരം വ്യത്യാസപ്പെട്ടിരിക്കുന്നു. രുചിഗുണ മൂല്യനിർണ്ണയത്തിൽ കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ വഴിയും പരമ്പരാഗത രീതി ഉപയോഗിച്ചും വേർത്തിരിച്ചെടുത്ത വി. സി. ഒയ്ക്കാണ് ഉയർന്ന സ്വീകാര്യത ലഭിച്ചത്. ലോറിക്ക് ആസിഡ്, ടൊക്കോഫിറോൾ, ഫീനോളുകൾ, ബയോ ആക്റ്റിവ് സംയുക്തങ്ങൾ, ആന്റി ഓക്സിഡന്റ് പ്രവർത്തനം എന്നിവ വി. സി. ഒയുടെ ഔഷധ ഗുണങ്ങൾക്ക് കാരണമാകുന്നു. കോൾഡ് സെന്ട്രിഫ്യൂഗേഷൻ വഴി വേർത്തിരിച്ചെടുത്ത വി. സി. ഒ സ്റ്റഫൈലോകോക്കസ് ഓറിയസ്, ബാസില്ലസ് സബ്റ്റിലിസ്, കാൻഡിട ആല്ബികൻസ്, തുടങ്ങിയ മാനുഷിക സംക്രമ രോഗകാരികൾക്കെതിരെ ആന്റി മൈക്രോബിയൽ പ്രവർത്തനം തെളിയിക്കുന്നു. കരൾ അർബുദ കോശങ്ങൾക്കെതിരെ ആന്റി പ്രൊലിഫറേറ്റിവ് പ്രവർത്തനം ഉള്ളതായും കണ്ടെത്തി. ഉയർന്ന സ്വീകാര്യതയും മൂന്നു മാസം വരെ സൂക്ഷിപ്പു കാലമുള്ളതുമായ കാപ്സ്യൂളുകൾ വിജയകരമായി വികസിപ്പിച്ചെടുത്തു.

മൊളിക്കുലാർ ഡോക്കിങ്ങിലൂടെയും ഇൻ വൈവോ പഠനങ്ങളിലൂടെയും വി. സി. ഒ തെറാപ്പിയുടെ പ്രയോജനകരമായ സാധ്യതകൾ കൂടുതലായി വിലയിരുത്തേണ്ടതുണ്ട്.