

Physicochemical Properties of Virgin Coconut Oil Extracted from Different Coconut (*Cocos nucifera* L) Varieties

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ABSTRACT

Virgin coconut oil (VCO) is a superior edible oil extracted from fresh coconut (*Cocos nucifera* L) kernel using mixed coconut varieties without considering the varietal effect. Therefore, this research focuses on the quality evaluation of VCO extracted from four types of coconut varieties, namely Sri Lanka Tall×Tall (TT), a tall variety of Gon Thambili (GT), a tall variety of Ran Thambili (RT) and Philippines tall variety of San Ramon (SR). Mature coconuts from each variety were collected from the Bandirippuwa Estate of the Coconut Research Institute, Sri Lanka to extract VCO by cold press oil extraction method. The extractability of VCO from different varieties was investigated. Moisture, free fatty acid (FFA), fatty acid profile (gas chromatography), peroxide value (PV), color (Lovibond scale), total phenolic substances (Gallic acid equivalent), antioxidant capacity (α,α -diphenyl- β -picrylhydrazyl, 0.1mM – DPPH method) and sun protection factor (SPF) of VCO extracted from each variety were analyzed. The experiment was conducted as a completely randomized design with three replicates. Data were analyzed using ANOVA using Tukey's test by MINITAB 17. Oil extractability (58%-59%), FFA (0.04%-0.12%), color (0.43–0.93) and fatty acid profile of VCO did not show variation among varieties. A higher concentration of *total* phenolic substances was observed in GT (0.24±0.03mg GAE/100g) while antioxidant capacity (857.19±14.99mg/ml) and SPF (8.99±1.26) was rich in RT.

Key words: *Coconut variety, dry processing, physicochemical properties, virgin coconut oil*

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a versatile plantation crop that is widely grown in tropical regions of the world. It has a variety of benefits to the human being such as food, drink, fuel, animal feed and shelter. Coconut kernel is the main part of coconut fruit for diverse coconut-based products such as coconut oil, desiccated coconut (DC), coconut milk, coconut cream, coconut milk powder and coconut chips. White coconut oil, virgin coconut oil (VCO), refined, bleached, deodorized coconut oil, coconut pairing oil and industrial, coconut oil are produced from coconut kernel by changing processing conditions and status of raw materials. VCO is defined as, an oil that is obtained from the fresh, mature kernel of the coconut by mechanical or natural means, with or without the use of heat, without undergoing chemical refining, bleaching or deodorizing, and which does not lead to an alteration of the nature of the oil (Philippine National Standards, 2004). Sri Lankan standard institute (2017) defined the virgin coconut oil as "Product obtained from the fresh, mature kernel without testa of the coconut by mechanical processes,

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with or without the use of heat not exceeding 60°C, without undergoing chemical refining, bleaching or deodorizing and which does not lead to the alteration of the nature of the oil. The dry processing method is used to extract the VCO from dehydrated coconut kernel while the wet method utilizes coconut milk from the fresh kernel. The combination of water with oil in wet processing reduces the shelf life of the VCO (Senphan and Benjakul, 2016). However, dry processing (Cold press method) is a prominent VCO extraction method in Sri Lanka.

Pure color, natural aroma free of sediments, rancid odor or taste and hygienic production conditions increase the therapeutic benefit of VCO (Che Man and Marina, 2006). Medium-chain fatty acid (MCFA—capric, caproic, caprylic, Lauric and myristic) accelerates functional properties such as high digestibility, anti-obesity, anti-bacterial, anti-viral, anti-plaque, anti-inflammatory, Alzheimer's and dementia (German and Dillard, 2004). Therefore, it can be used in functional foods, health foods, pharmaceuticals, infant foods and cosmetic formulations.

Based on the morphological characteristics and growing habitat, external features of the coconut palm are changed and it was grouped into three distinct groups of *typica* (Tall palm), *nana* (Dwarf palm) and *aurantica* (Intermediate – king coconut) (Liyanage, 1958). The yield of VCO depends on factors such as the age of coconut, location, time of harvesting (Carandang, 2008). A nutritional composition such as protein and fat content of defatted coconut testa flour has been changed with the varietal effect (Marasinghe *et al.*, 2019).

Coconut oil extraction is done through mixed several varieties and the most common variety is Sri Lanka Tall×Tall in Sri Lanka. However, the variety of coconut can affect the nutritional and physiochemical properties of

VCO extracted such as fatty acid profile and antioxidant capacity. Gon thambili and Ran thambili and Sri Lankan Tall varieties are indigenous tall coconut varieties whereas San Raman is an exotic tall coconut variety introduced from the Philippines to Sri Lankan plantations due to its yield performance similar to the Sri Lankan Tall variety (Fernando, 1999).

Therefore, this research focuses on the evaluation of the quality of virgin coconut oil extracted from four types of coconut varieties found in Sri Lanka, namely Tall×Tall (TT), Gon Thambili (GT), Ran Thambili (RT) and San Ramon (SR).

MATERIAL AND METHODOLOGY

Sample Collection

Mature coconuts aged at 12 months of Sri Lanka Tall×Tall (TT), a tall variety of Gon Thambili (GT), a tall variety of Ran Thambili (RT) and Philippines tall variety of San Ramon (SR), were collected from a germplasm collection block of the Bandirippuwa Estate of the Coconut Research Institute, Sri Lanka. Fifty coconuts were collected from each variety to extract the oil and samples were collected from three picking to representing triplicates. The coconuts were separately kept for three weeks under shade for seasoning before processing to increase the easiness of deshelling and reduce the moisture content of the coconut kernel. The seasoned coconut was used for VCO extraction using the cold press method as described below.

Extraction of Virgin Coconut Oil

The seasoned, mature coconuts of each variety were de-husked and then de-shelled. The testa of fresh kernels was removed (de-paired) manually using a peeler. The white kernels were cut into halves and opened to remove water. Then, white kernels were washed with clean

water and drained to remove excess water. The white kernels were disintegrated using a disintegrator (Unitex Engineers, Sri Lanka) and were dehydrated at 60°C until its moisture content reached 3% using a cabinet type dehydrator (Unitex Engineer, Sri Lanka). Finally, the dehydrated coconut kernels were expelled for oil extraction using cold press oil expeller (Udaya industries, Sri Lanka) at 60°C. VCO of each coconut variety was filtered manually using cotton wool and volume (ml) of oil extracted from each variety was measured. Then the oil was bottled in sterilized glass containers and kept at ambient temperature for further analysis.

Data Collection

Moisture content in dehydrated coconut and oil extractability: The moisture content of desiccated coconut was determined using the standard AOAC method (1999) by calculating moisture reduction in 5g of sample in a fan force oven at 103±2°C. Oil extractability of dehydrated coconut was measured as a percentage of oil weight in dehydrated coconut (w/w).

The moisture content of virgin coconut oil: The moisture content of oil was determined according to the standard method given by SLSI (2012) by measuring moisture reduction in 5g of coconut oil in a fan force oven at 103±2°C.

Free Fatty Acid (FFA) content: Five grams of oil sample were mixed with 50ml of fresh neutralized 95% of ethyl alcohol. Then, the mixture was heated to boiling and titrated against, standard 0.1N NaOH solution until the pink color persists for 15 seconds. The free fatty acid content of the samples was calculated as Lauric acid (SLSI, 2012).

Peroxide Value: The peroxide value (PV) of the sample was determined according to the standard method of SLSI (2012). Five grams of oil were mixed with 30ml of glacial acetic acid and

chloroform solution (3:2) followed by adding about 0.5ml of saturated KI. Then, the solution was swirled for one minute, 30 of distilled water and 1 of freshly prepared starch solutions were added and mixed vigorously. Then the contents were titrated immediately with 0.01N of Na₂SO₃ until the contents turned colorless endpoint.

Colour: The color of the oil sample was measured using LovibondTintometer (PFX-I UK) and the result was expressed in terms of the number of red (R) and yellow (Y) units (Y+5R) as given in SLSI (2012).

Fatty acid profile: Fatty acid methyl ester (FAME) of the oil sample was prepared according to the AOCS Official Method Ce 1-62, (1998). VCO sample (0.4g) was dissolved in 0.1 of 1M methanolic KOH and 4ml of methanol. The solution was mixed thoroughly and was incubated in a boiling water bath for 10 min. Then the solution was allowed to cool (28°C) followed by the addition of 2ml of n-hexane and 4ml distilled water. Then the sample was mixed gently and allowed to settle for 2 hours. The separated upper layer of methyl ester (1ml) in hexane was taken for analysis. Extracted FAME was analyzed by gas chromatography (GC) (Shimadzu-2010 plus) equipped with an FID detector and auto-injector. Separation of each fatty acid was performed by a capillary column Restec (length 30m, diameter 0.25mm and thickness 0.2µm). AOCS, 1998-Method (Ce 2-66) was followed for separation of fatty acids by adjusting N₂ (1ml/min) and H₂ (1ml/min) flow rate and injector temperature at 220 °C. The temperature gradient of oven was 35°C for 0.5 min, 35°C -195°C at 25°C/min, 195°C -205°C at 3°C/min, 205°C-230°C at 8°C/min and 230°C for 1min. Percentages of each fatty acid were determined relative to the total area of fatty acids.

Determination of total phenolic content of oil: Phenolic compounds were extracted into

80% aqueous methanolic solution by dissolving 5g of oil in 1ml of methanolic solution. Then the mixture was vortexed for 2min and centrifuged (2500 rpm for 10min at room temperature). The methanolic layer was separated and extraction was repeated four times and volume was adjusted to 4 ml with 80% methanol as described by Seneviratne and Dissanayake (2008).

The total phenolic content of the sample was determined by the Folin-Ciocalteu reagent method as described by Lister *et al* (2001). One milliliter of the extract was mixed with 5ml of 10% Folin-Ciocalteu and followed by the addition of 4ml of 7.5% Na₂CO₃ solution. The contents were kept in the dark for 30min. The absorbance of the sample was measured at 765nm using a spectrophotometer (Shimadzu UV-1800) concerning a blank sample of methanol with other solution. The total phenolic content was expressed as mg Gallic acid equivalent (GAE) per 100g of oil using a calibration curve of Gallic acid.

Antioxidant activity by DPPH method: DPPH (α , α -diphenyl- β -picrylhydrazyl) scavenging activity of oil sample was measured in terms of hydrogen-donating or radical scavenging ability (Marina *et al.*, 2008) with modifications. Three milliliters of oil extract in methanol were mixed with 1ml of 0.1mM methanolic DPPH and mixed gently for 1min and kept in the dark for 60min. Methanol (1ml) was used as a control sample. The absorbance of each sample was measured at 517nm using a UV spectrophotometer (UV 1800 SHIMADZU) with the blank sample of methanol and scavenging activity of each sample calculated by the following equation.

$$\text{Scavenging Activity (\%)} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$$

Where,

Abs (Control) = Absorbance of solvent methanol

Abs (Sample) = Absorbance of the sample

IC₅₀ values of the graph were obtained by plotting the percentage of scavenging activity (y) against the sample concentration (ppm) in (x). The regression line was fitted to $y=mx+C$.

Sun Protection Factor (SPF): The ultraviolet absorption capacity of each type of oil was measured using a UV spectrophotometer (UV 1800 SHIMADZU) using a 0.1% oil sample in ethanolic solution (ethanol:water 4:6). Absorption of the sample was measured from 290 to 320nm at 5nm intervals through a 1cm cell with path length. Correction factor (10) was used to take the summation to calculate the sun protection factor as the following equation.

$$\text{Sun protection factor (SPF)} = CF \times \sum_{290}^{320} (EE(\lambda) \times I(\lambda) \text{Absorbance}(\lambda))$$

Whereas CF=correction factor (10), EE(λ)=Erythrogenic effect of radiation with wavelength λ , I=solar intensity spectrum, Absorbance(λ)=spectrophotometric absorbance values at wavelength (λ). The constant values of EE×I are determined by Sayre *et al.* (1979).

Statistical Analysis

The experiment was arranged as a complete randomized design (CRD) with three replicates. Data were analyzed by MINITAB 17 software using one-way ANOVA. Mean separation was done for moisture, total phenol, antioxidant activity and sun protection factor through Tukey's test due to significant differences among the treatments.

RESULTS AND DISCUSSION

The moisture content of dehydrated coconut and oil recovery: The moisture content of dehydrated coconut is directly related to the moisture content and free fatty acid concentration of the oil to be extracted.

Significant moisture variation was observed in the dehydrated coconut sample while the highest moisture was persisted at dehydrated kernels of GT ($2.79\pm 0.20\%$) while the lowest of RT ($1.74\pm 0.20\%$). Results showed that (Table 1) extractability of oil from the dehydrated kernel is similar among the different varieties ranging from 58.47% to 59.83%. Ghani *et al.*, (2018) reported lower oil extractability (47.92%) using dehydrated kernels with expeller press than oil extractability from kernels of different coconut varieties. Therefore, the varieties are good for VCO production on a commercial scale if they show good physicochemical and nutritional characteristics due to their having better oil extractability.

The moisture content of virgin coconut oil: The moisture content of virgin coconut oil

obtained from different varieties of coconut varied significantly ($p < 0.05$). Higher moisture content was observed from an SR variety ($0.12\pm 0.05\%$) whereas the lowest moisture content was observed in RT ($0.04\pm 0.01\%$) variety. However, moisture contents of virgin coconut oil from all varieties were within the moisture contents recommended by the Asian Pacific Coconut Community in 2009 ($\leq 0.3\%$) and Sri Lankan Standard Institute (SLSI) in 2017 ($\leq 0.2\%$). These values are similar to the moisture content of VCO extracted from the dry processing method reported by Mansor *et al.*, (2012). Moreover, the moisture content of the VCO extracted from hot extraction was $0.237\pm 0.083\%$, whereas the mechanical extraction method increase the moisture content up to $0.286\pm 0.070\%$ (Ramesh *et al.*, 2020). The positive direct relationship between moisture

Variety	DC Moisture	Oil extraction %	Characteristic of virgin coconut oil						
			Moisture %	FFA %	Color	Peroxide (meq O ₂ /kg)	Total Phenol (mg/100g GAE)	Anti-oxidant Activity*	Sun Protection Factor
GT	2.79 ± 0.20^a	58.78 ± 2.66^a	0.06 ± 0.02^{ab}	0.08 ± 0.05^a	0.43 ± 0.32^a	ND	0.24 ± 0.03^a	936.1 ± 32.2^b	4.92 ± 0.59^c
RT	1.74 ± 0.49^b	59.83 ± 6.36^a	0.04 ± 0.01^b	0.08 ± 0.04^a	0.93 ± 0.51^a	ND	0.20 ± 0.01^{ab}	857.19 ± 14.99^c	8.99 ± 1.26^a
SR	1.99 ± 0.25^{ab}	58.66 ± 9.41^a	0.12 ± 0.05^a	0.08 ± 0.05^a	0.47 ± 0.25^a	ND	0.12 ± 0.00^c	863.24 ± 8.67^c	6.74 ± 0.18^{bc}
TT	1.97 ± 0.22^b	58.47 ± 8.09^a	0.05 ± 0.04^{ab}	0.06 ± 0.05^a	0.80 ± 0.50^a	ND	0.17 ± 0.01^b	1282.5 ± 18.3^a	6.81 ± 0.36^b
APCC Standard	≤ 3	NM	≤ 0.3	≤ 0.2	NM	≤ 3	NM	NM	NM

Each value represents the mean of three replicates. Means with different superscripts are significant ($p < 0.05$) different from each other's along each column * DPPH Assay as 0.1mM.

DC – Dehydrated coconut; FFA – Free Fatty Acid; GT – Gonthambili; RT- Ran thambili; SR- San Ramon; TT - Tall×Tall; NM – Not mentioned ND – Not detected; APCC – Standard of Asian and Pacific Coconut Community

Table 1. Physicochemical properties of dehydrated kernel and oil in different coconut varieties

content and the free fatty acid content of oil was identified by Che Man *et al.*, (1997). Therefore, low moisture contents in four types of VCO in this study will have an extended shelf life due to low free fatty acids.

Free Fatty Acid (FFA) content: Free fatty acid concentrations of the oil of different varieties have ranged from 0.06% to 0.08% and the values are not significant ($p > 0.05$) different among varieties (Table 1). However, these values are within the recommended level of FFA ($\leq 0.2\%$) by APCC (2009) and SLSI (2017). Results proved that the dry processing method has resulted in a low concentration of FFA compared with high FFA contents (0.69%) reported for the fermentation method (Senphan and Benjakul, 2016). FFA can be used as an indicator for evaluating the organoleptic quality (taste and aroma) of coconut oil. If the oil has high FFA is produced a rancid taste and aroma to reject the organoleptic quality.

Peroxide Value (PV): There was no detectable peroxide formation in VCO prepared from different varieties (Table 1). Peroxide

formation is not changed with different varieties but changed to the method of preparation (Seneviratne and Jayathilake, 2016). According to Rupasinghe *et al.*, (2013), wet-processed coconut oil produced from different varieties did not show peroxide formation. However, higher oxidation has been identified from natural fermentation (7.75 meq O_2 /kg) by Senphan and Benjakul (2016). Several factors such as light, oxygen, metal and fatty acid composition of coconut oil affect the formation of hydroperoxides (Choe and Min, 2006). Results proved that the stability of VCO of all varieties was at the highest level without the tendency of formation rancid and the values are within the APCC standard of ≤ 3 meq O_2 /kg.

Colour: The color of VCO did not change with the variety of coconut significantly and it was changed from 0.43 to 0.93 when analyzed from the Lovibond color scale. Based on the SLSI standards, the color of the VCO should be less than 1. The processing method and type of material affect the color of the oil. During VCO processing in this method brown testa is removed from the fresh coconut kernel. As

Treat-ments	Caprylic (C8)	Capric (C10)	Lauric (C12)	Myristic (C14)	Palmitic (C16)	Stearic (C18)	Oleic (C18:1)	Linoleic (C18:2)
GT	9.20a	6.10a	51.92a	19.46a	5.87a	1.73a	4.57a	1.15a
RT	9.58a	6.35a	52.55a	17.98a	6.68a	1.34a	4.41a	1.10a
SR	8.95a	6.08a	51.14a	18.90a	7.16a	1.89a	4.75a	1.11a
TT	9.76 a	6.27a	51.43a	18.30a	6.70a	2.47a	4.10a	0.98a
APCC Stan- dards	4-10	4-8	45-56	16-21	7.5-10.2	2-4	4.5-10.0	0.7-2.5

Each value represents the mean of three replicates. Means with different superscripts are significant ($p < 0.05$) different from each other along each column. GT – Gonthambili; RT- Ran thambili; SR- San Ramon; TT - Tall×Tall; APCC – Asian and Pacific Coconut Community

Table 2. Fatty acid profile of VCO extracted from different varieties

brown testa is responsible for the color intensity, the color of VCO produced by this method is low. As observed in dehydration, no caramelization was seen. Therefore, the color of virgin coconut oil obtained from different varieties did not change significantly.

Fatty acid profile: The results in Table 2 show that the fatty acid composition of virgin coconut oil obtained from different varieties shows characteristics of coconut oil without significant effect among varieties of coconut. Lauric acid, unsaturated fatty acids (oleic and linoleic acid) concentrations of virgin coconut oil from different varieties are within the conformity of APCC (2009) and show insignificant variation among the varieties. Although Seneviratne and Jayathilake (2016) reported that iodine value and saponification value of coconut may change due to different cultivars, the present study did not show such variation. In addition, Rupasinghe *et al.* (2013) reported that wet-processed coconut oil extracted from a different cultivar of coconuts such as dwarf green, dwarf yellow and dwarf brown showed significantly different fatty acid composition as explained from iodine value. However, the varieties used in this study have not shown significant variation when the dry process is applied to virgin coconut oil production.

Total phenolic substances content: The total phenolic content of VCO significantly ($p < 0.05$) changed with the variety of coconut. A higher concentration of phenolic compounds was observed in VCO from the variety of GT (0.24 ± 0.03 mg GAE/100g) and RT (0.20 ± 0.01 mg GAE/100g) while lowest from the SR (0.12 ± 0.00 mg GAE/100g). The hot VCO extraction method has higher total phenolics (2.867 ± 0.152 mg GAE/100g) than the fermentation (0.566 ± 0.020 mg GAE/100g) method and mechanical extraction method (0.63 ± 0.121 mg GAE/100g) (Ramesh *et al.*, 2020).

The total phenolic content of oil was changed with the processing methods (Marina *et al.*, 2009). Senevirathne and Dissanayake (2008) reported that the dry processing method destroyed phenolic compounds in VCO compared to the wet processing method. The previous finding also stated that the total phenolic content of VCO was 0.65 mg GAE/100g (Henna and Tan 2009) and 0.2 ± 0.04 mg GAE/100g (Appaiah *et al.*, 2014). The phenolic substances of whole copra oil (1.4 ± 0.19 mg GAE/100g) are higher than the VCO extracted without brown testa which is the phenolic substances-rich portion of coconut kernel. Vanillic acid ($63.8 \mu\text{g}/100\text{g}$) and Gallic acid ($24.7 \mu\text{g}/100\text{g}$) are richer acids in whole copra oil and it was devoted to the Syringic ($37.3 \mu\text{g}/100\text{g}$) acid and hydroxybenzoic acid ($34.7 \mu\text{g}/100\text{g}$) in wet coconut white kernel oil (Appaiah *et al.*, 2014). Seneviratne *et al.*, (2009) also reported that the concentration of polyphenols in coconut kernel is not evenly distributed and also is a poor source of polyphenolic substances. Fresh white kernel without brown testa contains only 61 mg/kg phenolic substances while brown testa contains 3946 mg/kg. Although dehydrated whole coconut kernel (copra) contains 405 mg/kg of polyphenols, the amount concentrated to 2156 mg/kg in copra meal which is the residue obtained after copra is expelled for oil. Therefore, the type of raw material, processing practices, extraction method and variety have a significant effect on the concentration of phenolic in coconut oil.

Antioxidant activity by DPPH method: IC_{50} values of VCO prepared from different varieties of coconut are shown in Table 1. During the scavenging action, an electron or an active hydrogen atom of VCO is donated to the DPPH radical in methanolic solution and converts into the yellow color compound (diphenylpicrylhydrazine). If IC_{50} values are lower, it has a high power of neutralizing active hydrogen even it has a low concentration

(Shimamura *et al.*,2014). There is a significant ($p<0.05$) variation of antioxidant activity of four types of VCO due to the difference of total phenol compounds among the coconut variety.

The RT and SR showed a higher antioxidant capacity with $857.19\pm 14.99\text{mg/ml}$ and $863.24\pm 8.67\text{mg/ml}$, respectively. The VCO extracted from the TT variety had the lowest radical scavenging ability ($1282.5\pm 18.3\text{mg/ml}$).

Sun Protection Factor (SPF): Coconut oil is a popular emulsion in the cosmetic industry. *In-vitro* measurement of sun protection factor provides clues for sunscreen formulation. To be an effective sun screening ability it should have better absorbance between 290 to 400nm, which is the most biologically damaging radiation (Chanchal and Swarnalatha, 2010). Our findings show that the virgin coconut oil produced from variety RT (8.99 ± 1.26) has a significance ($p<0.05$) higher value for SPF than the other varieties do (Table 1). Chanchal and Swarnalatha, (2010) have reported that olive oil and coconut oil had good sun protection factors of 7.55 and 7.12 respectively. The UV absorption spectrum of each variety of VCO is shown in Figure 1. Significantly higher UV absorbance is shown by the RT variety except for the absorbance at 290nm. Therefore, the results can be concluded that RT has the best therapeutic

action for the sunscreen formulations than the oil extracted from TxT, GT and SR.

CONCLUSIONS

Physicochemical properties of virgin coconut oil extracted from coconut varieties TxT, GT, RT and SR did not differ significantly. However, antioxidant activity and sun protection factor of RT has a significant effect on therapeutic action.

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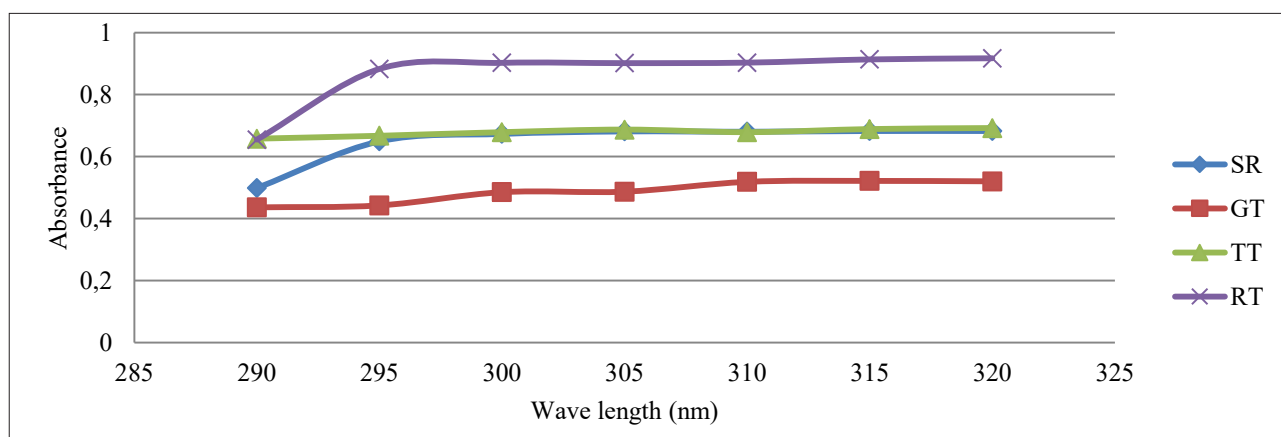


Figure 1. UV absorption spectrum VCO from SR, GT, TT and RT

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