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Cord

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

H. P. D. T. Hewa Pathirana[1*], L. L. W. C. Yalegama[1], J. A. D. Madusanka[1], L. M. I. Senarathne[1]

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 (Galic 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 (Marasingnhe *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 Na_2SO_3 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 N_2 (1ml/min) and H_2 (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-Ciocalteau reagent method as described by Lister *et al* (2001). One milliliter of the extract was mixed with 5ml of 10% Folin-Ciocalteau and followed by the addition of 4ml of 7.5% Na_2CO_3 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

IC_{50} 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.

$$\text{Sun protection factor (SPF)} = CF \times \sum_{0290}^{320} (EE\ (\lambda) \times I(\lambda) Absorbance(\lambda)$$

Whereas CF=correction factor (10), EE (λ)=Erythmogenic 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±0.20%) while the lowest of RT (1.74±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±0.05%) whereas the lowest moisture content was observed in RT (0.04±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 (≤0.3%) and Sri Lankan Standard Institute (SLSI) in 2017 (≤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±0.083%, whereas the mechanical extraction method increase the moisture content up to 0.286±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± 0.18b^{c}
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. Physichochemical 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 (≤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

Treatments	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 Standards	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±0.03mg GAE/100g) and RT (0.20±0.01mg GAE/100g) while lowest from the SR (0.12±0.00mg GAE/100g). The hot VCO extraction method has higher total phenolics (2.867±0.152mg GAE/100g) than the fermentation (0.566±0.020mg GAE/100g) method and mechanical extraction method (0.63±0.121mg 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 0.65mg GAE/100g (Henna and Tan 2009) and 0.2±0.04mg GAE/100g (Appaiah *et al*., 2014). The phenolic substances of whole copra oil (1.4±0.19mg GAE/100g) are higher than the VCO extracted without brown testa which is the phenolic substances-rich portion of coconut kernel. Vanillic acid (63.8µg/100g) and Gallic acid (24.7µg/100g) are richer acids in whole copra oil and it was devoted to the Syringic (37.3µg/100g) acid and hydroxybenzoic acid (34.7µg/100g) 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 61mg/kg phenolic substances while brown testa contains 3946mg/kg. Although dehydrated whole coconut kernel (copra) contains 405mg/kg of polyphenols, the amount concentrated to 2156mg/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 VCOis 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±14.99mg/ml and 863.24±8.67mg/ml, respectively. The VCO extracted from the TT variety had the lowest radical scavenging ability (1282.5±18.3mg/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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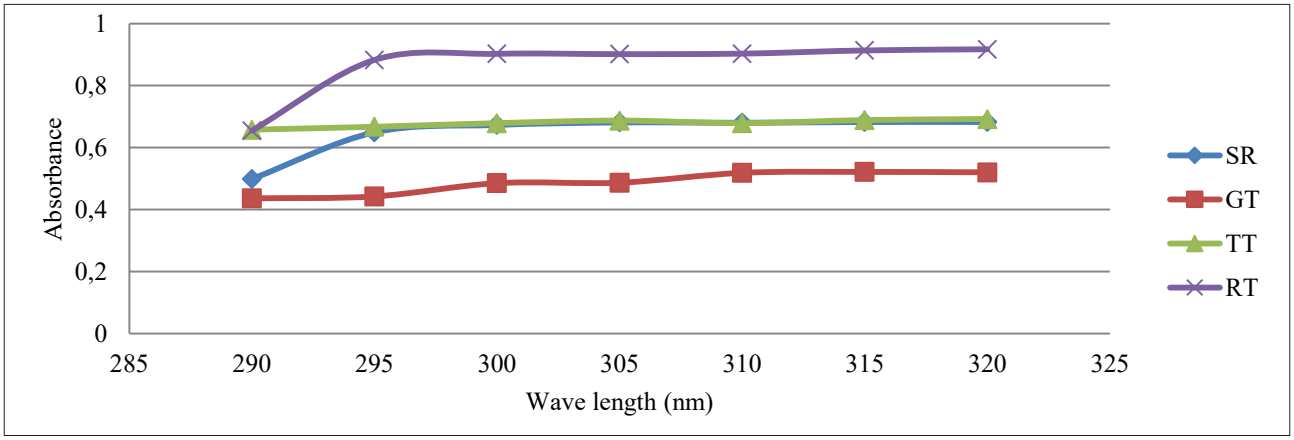

Figure 1. UV absorption spectrum VCO from SR, GT, TT and RT

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The Physical and Functional Properties of Partially Defatted Coconut Testa Flour

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ABSTRACT

Coconut testa is an important byproduct of the coconut industry. In this study, particle size distribution, physical and functional properties of flour produced from partially coconut testa of four local cultivars namely san raman, gon thembili, ran thembili, TallxTall were compared with those of commercial hybrid (COM) using relevant procedures. Results showed that particle size distribution, physical and functional properties of flours of different coconut cultivars were varied significantly ($p<0.05$). The highest bulk density value was observed for SR (0.67 g/ml) while the lowest for TxT (0.54 g/ml) ($p<0.05$). Maximum swelling capacity (35.00 ml) and oil absorption capacity (142.67%) were recorded for COM while the least swelling capacity (20.67 ml) and oil absorption capacity (85.67%) were recorded for RT ($p<0.05$). The highest emulsion activity was found for COM (50.00%) while the least value recorded for SR (42.95) ($p<0.05$). The maximum emulsion stability was displayed by COM (54.86%) while the least emulsion stability was recorded for GT (27.51%) ($p<0.05$). The observed physical properties suggested that coconut testa flour of COM variety has certain advantages over others. It could be used for partial replacement with wheat flour for value addition leading to non-cereal based products.

Key words: *Agro-waste utilization, coconut byproducts, coconut testa, coconut testa flour, flour properties*

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a tropical monocotyledon perennial crop belonging to the family arecaceae. The fruit of coconut has a number of sub-components, each of which finds beneficial uses to humankind. Among the different sub-component parts of the coconut, the white kernel is the most valuable part contributing to the highest economic value. The thin brown outer layer present adjacent to the hard shell covering the white kernel is called testa (Lima *et al*., 2015). According to previous studies, testa constitutes approximately 18% (w/w, wet basis) of the fresh coconut kernel weight (Marikkar and Madurapperuma, 2012). Often, testa is removed during the production of virgin coconut oil, desiccated coconut, coconut milk, and coconut milk powder due to the unappealing brown colour imparted on the finished products. According to current practice, removal of testa takes place after deshelling the coconut, done manually using paring knives (Marikkar and Madurapperuma, 2012). As the total annual production of coconut in Sri Lankan is in the range of 2500 – 3000 million coconuts (CDA, 2016), a considerable amount of testa is left under-utilized in the above-mentioned industries. The current utilization of testa is merely

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limited to the extraction of low-grade oil and using the residue for animal feed formulation. A number of past investigations have given focus to study the importance of testa as a source of bioactive compounds and associated health benefits (Marasinghe *et al*., 2019; Arivalagan *et al*., 2018; Appaiah *et al*., 2016; Geetha *et al*., 2016; Zhang *et al*., 2015). In a recent study, Adekola *et al*. (2017) compared the antioxidative and anti-diabetic activities of whole coconut testa with those of seed coats of selected beans. However, previous authors did not investigate the physical and functional properties of coconut testa flour produced out of residue coming from the cold-press mechanical oil extraction process, which involves low-temperature heating to protect severe burning or thermal degradation effects. This type of investigation would be beneficial because different food processing methods including oil extraction are known to bring changes to the bioactivities and functional properties of original raw materials. According to our literature search, an investigation into the physical and functional properties of the flour produced from residues of coconut testa has not been considered in most of the previous studies. Hence, the study aimed to compare the physical and functional properties of partially defatted coconut testa flour of four local coconut cultivars namely, san raman (SR), gon thembili (GT), ran thembili (RT), TallxTall (TxT) and commercial hybrid (COM) grown in Sri Lanka.

MATERIALS AND METHODS

Materials and Sampling

Fifty mature coconuts (12 months) from five different local cultivars (SR, RT, GT, TxT and COM) were collected from the varietal blocks of Coconut Research Institute of Sri Lanka, Lunuwila from August to October 2018. In the varietal blocks, fresh emerging bunches of each cultivar were earmarked for sampling after 12 months of maturity. Samples were subjected to 3 weeks of seasoning to make de-shelling easy. Coconuts seasoned for 3 weeks were de-husked manually and their hard shell was removed carefully. Coconut testa of the nuts peeled off manually were pulverized into medium size particles using a disintegrator (Unitex Engineers, Sri Lanka). Inter-particulate bonds of coconut testa can be broken down in this way. The disintegrated testa (moisture content of 42 to 45%) were oven-dried at 70°C using a cabinet-type dryer (Wessberg, Martin, Germany) for 8 h. Low-temperature drying was adopted to minimize severe burning or thermal degradation. Two kilograms of dried testa (medium size particles) of individual cultivar were then subjected to cold press oil extraction using a micro oil expeller (Komet DD85 machine, Germany). The residues left after oil extraction were ground into fine coconut testa flour (CTF) using a commercial grinder (Panasonic, Model: MK-MG 1000). The grounded flour samples were packed in low-density polyethylene (LDPE) bags and then stored at refrigerated (4°C) condition until further analyses. All chemicals used in this study were of analytical grade unless otherwise specified.

Methods

Particle size analysis: Analysis of flour particle size distribution was carried out according to Nishita and Bean (1982). CTF (50g) was sifted through 500, 420, 297, 150 and 63 µm sifters (500 µm sifter on the top and 63 µm sifter at the end), on a ro–tap type sieve shaker (Heiko Seisakusho, Japan) operated at 278 rpm for 15 min. After shaking, the weight of flour remained on each sifter was recorded. The weight of flour remained in each sifter was divided by the total weight of flour (50 g) to determine the percentage of flour retained on each sifter.

Determination of protein content: The protein contents of samples were determined

according to a method described in the AOAC (2005) manual with a conversion factor of 6.25.

Bulk density: The bulk density (BD) was measured according to the method described by Okaka and Potter (1977) with some modifications. CTF (20g) was loaded in to a measuring cylinder (50 ml). The cylinder was constantly knocked until a consistent volume was attained. The bulk density was calculated as shown below:

$$Bulk\ density = Weight\ of\ flour\ (g) \div Volume\ of\ flour\ (ml)$$

Swelling capacity: The swelling capacity (SC) was performed according to the procedure described by Okaka and Potter (1977). Each sample of CTF was added into 100 ml measuring cylinder to fill up to 10 ml mark. A portion of distilled water was added up to 50 ml mark. The topmost of the measuring cylinder was firmly enclosed by the cap. The contents were mixed thoroughly by inverting the cylinder. After 2 min, the contents were inverted again and kept on a table without any motion for further 8 min. The volume of the flour was recorded after 8 min and reported as SC.

Water absorption capacity: The water absorption capacity (WAC) was measured according to the method described by Sosulski *et al*. (1976). A sample of CTF (1.0 g) was mixed with distilled water (10 ml) in a centrifuge tube and kept at 30°C ± 2°C for 30 min. The mixture was centrifuged (Beckman, USA) at 6000 rpm for 1 h. The weight of the paste was recorded after removing the supernatant. The WAC was expressed as the percentage of water absorbed per g of flour.

$$WAC = \frac{Final\ weight\ of\ paste\ (g) - Initial\ weight\ of\ flour (g)}{Initial\ weight\ of\ flour\ (g)} \times 100$$

Oil absorption capacity: The oil absorption capacity (OAC) was measured according to the method described by Sosulski el al. (1976). A sample of CTF (1.0 g) was mixed with soybean oil (specific gravity 0.9092) (10 ml) in a centrifuge tube and kept at 30°C ± 2°C for 30 min. The mixture was centrifuged (Beckman, USA) at 6000 rpm for 1 h. The weight of the paste was recorded after removing the supernatant. The OAC was expressed as the percentage of water absorbed per g of flour.

$$OAC = \frac{Final\ weight\ of\ paste\ (g) - Initial\ weight\ of\ flour\ (g)}{Initial\ weight\ of\ flour\ (g)} \times 100$$

Emulsion activity: The emulsion activity (EA) was estimated according to the method of Yasumatsu *et al*. (1972). A sample of CTF (1.0 g), distilled water (10 ml), soybean oil (10 ml) were blended together in a calibrated centrifuge tube to prepare the emulsion. The prepared emulsion was centrifuged at 3000 rpm for 5 min. The EA was calculated as shown below:

$$EA = \frac{Height\ of\ the\ emulsified\ layer(cm)}{Total\ height\ of\ the\ mixture(cm)} \times 100$$

Emulsion stability: The emulsion stability (ES) was estimated according to the method of Yasumatsu *et al*. (1972). A sample of CTF (1.0 g), distilled water (10 ml), soybean oil (10 ml) were blended together in a centrifuge tube to prepare the emulsion. The prepared emulsion was heated at 80°C for 30 min in a water bath. The mixture was cooled under running tap water for 15 min and centrifuged at 3000 rpm for 15 min. The ES was calculated as shown below:

$$ES = \frac{Height\ of\ the\ emulsion\ layer(cm)}{Total\ height\ of\ the\ mixture(cm)} \times 100$$

Foam capacity: The foam capacity (FC) was determined according to the method described by Narayana and Narasinga (1982) with slight modification. A sample of CTF (1.0 g) was mixed with distilled water (50 ml) in a measuring cylinder. The foam was formed

by shaking the mixture for 5 min. The volume of foam was recorded after 3 min while the foam was stabilized (volume of foam before whipping). The mixture was shaken again and the volume of foam was recorded after 30 sec (volume of foam after whipping). The FC was expressed as shown below:

$$FC = \frac{\text{Volume of foam after whipping (ml)} - \text{Volume of foam before whipping(ml)}}{\text{Volume of foam beore whipping (ml)}} \times 100$$

Foam stability: The foam stability (FS) was determined according to the method described by Narayana and Narasinga (1982) with slight modification. A sample of CTF (1.0 g) was mixed with distilled water (50 ml) in a measuring cylinder. The foam was formed by shaking the mixture for 5 min. The volume of foam was recorded after 3 min when the foam was stabilized (initial volume of foam). The contents were kept steady and volume of foam was recorded after 1 hr (final volume of foam). The FS was expressed as shown below:

$$FS = \frac{Initial\ volume\ of\ foam\ (ml)}{Final\ volume\ of\ foam(ml)} \times 100$$

Least gelation concentration: The least gelation concentration (LGC) was assessed using the procedure described by Coffman and Garcia (1977) with modification. The flour suspensions of 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30% (w/v) were prepared in distilled water (5 ml). The contents were heated in a water bath at 90°C for 1 h. The mixtures were cooled down under running tap water. The contents were further cooled at 10 ± 2°C for 2 h. The concentration at which the contents remained in the tube without moving when the tube was inverted was taken as the LGC.

Statistical Analysis

All results from analyses were expressed as the mean value ± standard deviation. Data were statistically analyzed by one-way analysis of variance (ANOVA) using Tukey's test of MINITAB (version 14) statistical package at 0.05 probability level.

RESULTS AND DISCUSSIONS

Particle size distribution

Particle size is one of the important characteristics of any flour that determines its functional attributes in product formulations. The particle size distribution of CTF of different coconut cultivars is depicted in Fig. 1. According to statistical analysis, significant relationships were existed [F (16, 50) = 6288.81, p = 0.0001] between cultivars and particle size distribution with few exceptions. For all cultivar types, no significant differences were noticed for particle size ranges of 63-150 µm and 150–297 µm, but a significant difference was noticed for the particle size range of 420–500 µm. SR and the rest of the cultivars displayed significant differences for the particle size range of 297–420 µm, meanwhile COM and TxT displayed significant differences for particle size range >500 µm.

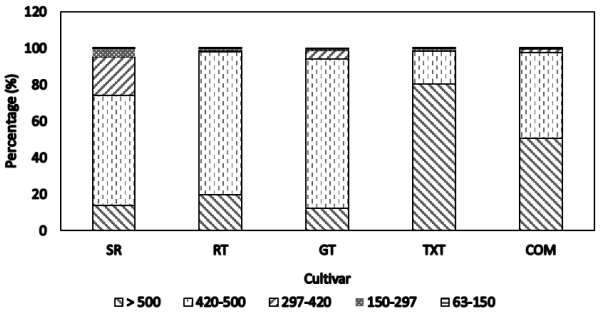

Figure 1. Particle size distribution of coconut testa flour of different coconut cultivars

Generally, the particle size distribution of flours would be influenced by the nature of grinding used in flour making process (Suntharalingam and Ravindran, 1993). This could be probably because the sieving process is affected by flour constraints (intrinsic) as well as sieve constraints such as aperture opening, rate, direction of movement of sieve,

sieve cloth etc. According to Wang and Flores (2000), the observed particle size distribution of wheat flour would have some influence on its physicochemical properties. In a subsequent study, De la Hera *et al*. (2013) found that the particle size heterogeneity of rice flour affected its hydration properties. For instance, rice flour hydration properties were increased with the reduction of particle size. In another study involving lentil flour, Ahmed *et al*. (2016) noticed that the decrease in the magnitude of particles influenced considerably the constitution, but it did not affect the water retention ability. In coconut kernel flour, the increase in the magnitude of flour particles tended to decrease the oil absorption capacity but enhanced the swelling and water absorption capacities (Dat *et al*., 2017). Enhanced WAC would favor more water to be absorbed by the flour matrices, which in turn would result in increased SC. According to Guillon and Champ (2000), modification of microstructure of particles caused by grinding would lead to altered OAC and hydration attributes of flour. For instance, the reduction of particle size by grinding leads to alteration of the structure of carbohydrate matrices in flour. This causes a reduction in water absorption by particles, which ultimately leads to modification of the hydration properties of flour.

Functional properties

The data presented in Table 1 compares various properties of coconut testa flour. Generally, assessment of functional characteristics of CTF would be useful to predict the effects of the addition of a new source of protein, carbohydrate, fat, and fiber to a specific food system (Chandra *et al*., 2013). This could also be valuable information for investigators engaged in coconut breeding programs.

The bulk density of flours would depend on factors such as particle size distribution, density and initial moisture content of flour. According to Table 1, bulk density of CTF of

Functional Property	Cultivar				
	SR	RT	GT	TxT	COM
Bulk density (g/ml)	0.67 ± 0.00^{c}	0.59 ± 0.00^{b}	0.55 ± 0.00^{a}	0.54 ± 0.00^{a}	0.65 ± 0.01^{c}
Swelling capacity (ml)	29.67 ± 1.53^{b}	20.67 ± 3.06^{a}	$31.33 \pm 1.15^{b,c}$	29.00 ± 1.73^{b}	35.00 ± 1.73^{c}
Water absorption capacity (%)	320.00 ± 6.08^{d}	194.33 ± 10.69^{a}	238.67 ± 14.29^{b}	$215.00 \pm 7.21^{a,b}$	275.00 ± 11.53^{c}
Oil absorption capacity (%)	124 ± 3^{c}	85.67 ± 7.02^{a}	127.33 ± 3.21^{c}	97.33 ± 3.21^{b}	142.67 ± 2.52^{d}
Emulsion activity (%)	42.95 ± 1.11^{a}	$48.72 \pm 1.11^{b,c}$	$45.51 \pm 1.11^{a,b}$	$48.72 \pm 1.11^{b,c}$	50.00 ± 1.92^{c}
Emulsion stability (%)	43.24 ± 2.15^{c}	$28.86 \pm 1.03^{a,b}$	27.51 ± 0.89^{a}	34.23 ± 3.28^{b}	54.86 ± 2.85^{d}
Foam capacity (%)	23.33 ± 2.89^{a}	28.67 ± 7.51^{b}	50.00 ± 0.00^{b}	41.11 ± 8.39^{b}	31.67 ± 16.07^{b}
Foam stability (%)	93.33 ± 11.55^{b}	0.00 ± 0.00^{a}	15.00 ± 8.66^{a}	17.78 ± 13.47^{a}	83.33 ± 14.43^{b}
Least gelation concentration (% , w/v)	12.00 ± 0.00^{a}	26.00 ± 0.00^{e}	22.00 ± 0.00^{d}	18.00 ± 0.00^{c}	16.00 ± 0.00^{b}

Each value in the table represents the mean ± standard deviations (SD) from three replicates. Means within each row bearing different superscripts are significantly ($p < 0.05$) different.

Table 1. Functional properties of coconut testa flour of different coconut cultivars

different cultivars varied from 0.54 to 0.67 g/ml; the highest value being observed for SR (0.67 ± 0.00 g/ml), followed by COM (0.65 ± 0.01 g/ml) and RT (0.59 ± 0.00 g/ml). The bulk density of SR was significantly higher than that of COM ($p<0.05$). The bulk density of GT and TxT were similar ($p>0.05$) but significantly ($p<0.05$) lower than those of SR, RT, and COM. In fact, bulk density values of CTF were slightly higher than that of coconut kernel flour (0.51 ± 0.02 g/ml) as reported previously by Igbabul *et al*. (2014). In another study, Chandra and Samsher (2013) found that the bulk density of rice flours (0.914±0.01 g/cc) was comparatively higher than that of wheat flour (0.762 ± 0.00 g/cc). Packing requirements and handling of materials during wet processing are usually affected by the bulk density of flour (Abioye *et al*., 2011). Hence, during packaging and transportation, higher bulk density is desirable as it can reduce the cost significantly. From a product development point of view, flours with high bulk density are preferable as food thickeners (Chandra *et al*., 2015) while those with low bulk density such as CTF of TxT (0.54 g/ml) would be suitable for preparing infant foods.

The swelling capacity is the maximum volume a flour sample can occupy as a result of water absorption. This water absorption continues until a colloidal suspension is formed. The volume expansion would be stopped as a result of prevention of water absorption which is caused by intermolecular forces present among swelled molecules (Adetuyi *et al*., 2009). According to a previous report, the SC of flour is generally affected by particle size, varietal differences and processing methods (Chandra and Samsher, 2013). The data presented in Table 1 compared the swelling capacity of CTF of local coconut cultivars, which was varied from 20.67 to 35 ml. The maximum value was recorded for COM (35.00 ± 1.73 ml), followed by GT (31.33 ± 1.15 ml) and SR (29.67 ± 1.53 ml). The least value was recorded for RT (20.67 ± 3.06 ml) which was significantly ($p<0.05$) lower than those of other cultivars. The swelling capacity values of SR (29.67 ± 1.53 ml), GT (31.33 ± 1.15 ml) and TXT (29.00 ± 1.73 ml) were similar but significantly ($p < 0.05$) lower than that of COM (35.00 ± 1.73 ml). According to a previous study by Dat *et al*. (2017), the swelling capacity of coconut kernel flour was found to range from 10.31 to 13.45 ml, which was lower than those of CTF found in the present study. Likewise, swelling capacity values of CTF of the present study were higher than those of wheat (17.60 ± 1.85 ml) and rice flours (15.20 ± 0.84 ml) as reported by Chandra and Samsher (2013).

The water absorption capacity (WAC) is a parameter, which determines the amount of water to be added during food processing. The flour with high water absorption may have more hydrophilic constituents such as polysaccharides (Chandra *et al*., 2015). According to Table 1, WAC values of different cultivars were ranged from 194.33 to 320%; maximum being recorded for SR (320.00 ± 6.08%), followed by COM (275.00 ± 11.53%) and GT (238.67 ± 14.29%). The WAC of TXT and RT were more or less similar ($p>0.05$), but significantly ($p<0.05$) lower than those of SR, COM, and GT. There was no statistically significant ($p>0.05$) difference between WAC of GT and TXT ($p<0.05$), but the values were significantly ($p<0.05$) lower than those of SR and COM. WAC of SR was significantly higher than that of COM ($p<0.05$). In an attempt to assess the effect of flour particle size on functional properties of coconut kernel flour, Dat *et al*. (2017) observed a higher WAC value (7.91 to 11.88 g/ g of flour) for coconut kernel flour compared to those of CTF observed in this study. However, WAC of CTF of different cultivars were higher than those of flours such as wheat (140 ± 12.25%) and rice (192 ± 10.95%) as reported by Chandra and Samsher (2013). In fact, higher WAC values for CTF suggests its suitability for the preparation of foods such as sausages, soups and baked products (Aremu *et al*., 2007).

Generally, the water absorption of flour is influenced by its protein content and hemicellulose level. As stated by Narayana and Narasinga (1982), polar amino acid residues of proteins attracts more water molecules. Hence, flours with high protein content

might absorb more water than those with low protein contents. As shown in Fig. 2, crude protein contents of

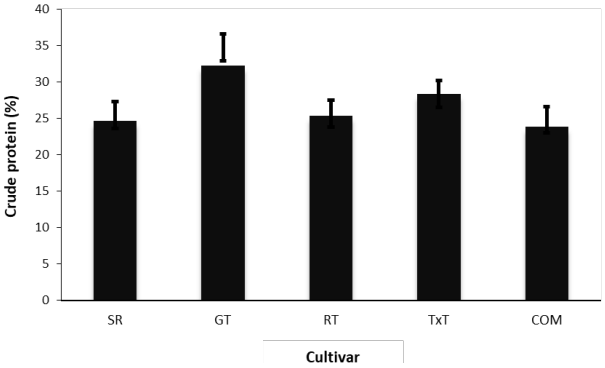

Figure 2. Crude protein content of coconut testa flour of different coconut cultivars

CTF of this study were comparably higher than those of wheat and rice flours as reported by other researchers. However, a statistically significant correlation was not found ($p>0.05$) between crude protein contents and WAC of CTF among different coconut cultivars. Perhaps, the differences in the amount of polar amino acids and conformational characteristics of proteins may be attributed to varied values of WAC among different cultivars (Narayana and Narasinga, 1982). Swelling of crude fiber might occur after absorbing water and expands the volume of flour further (Narayana and Narasinga, 1982). These might also contribute to the increased WAC of CTF when compared to other flour types such as wheat and rice.

Oil absorption capacity (OAC) contributes to improving palatability as well as the preservation of flavor. According to Table 1, OAC values of CTF were ranged from 85.67 to 142.67%; the maximum being recorded for COM (142.67 ± 2.52%), followed by GT (127.33 ± 3.21%) and SR (124 ± 3%). The least OAC was recorded for RT (85.67 ± 7.02%), which was significantly ($p<0.05$) lower than those of all other cultivars. The OAC value of GT (127.33 ± 3.21%) and SR (124 ± 3%) were not statistically different ($p>0.05$), but significantly ($p<0.05$) higher than those of TxT (97.33 ± 3.21%) and RT (85.67 ± 7.02%). The OAC of COM (142.67 ± 2.52%) was significantly higher than that of either GT (127.33 ± 3.21%) or SR (124 ± 3%) ($p<0.05$). In a previous study, Dat *et al*. (2017) observed a higher OAC value (3.28 to 3.93 g/g of flour) for coconut kernel flour compared to those of CTF of this study. Chandra and Samsher (2013) reported the OAC of wheat flour (146 ± 8.94%) which was higher than those of CTF used in the present study. In contrast, the OAC of rice flour (124) was found to be higher than those of cultivars namely RT and TxT. The variation in chemical composition among these flours was attributed to the differences in OAC values. For instance, hydrophobic interactions would be formed between non-polar side chains of amino acids and hydrocarbon chains of lipids, leading to the enhancement of OAC (Jitngarmkusol *et al*., 2008). Hence, the protein content and nature of amino acids present are important determinants of OAC of flours. High OAC of CTF of local cultivars would make them suitable for the preparation of food such as whipped toppings, sausages and bakery products where fat absorption is necessary (Aremu *et al*., 2007).

The emulsion activity (EA) of CTF of different coconut cultivars are given in Table 1. The EA of different coconut cultivars were ranged from 42.95 to 50.00%. The highest EA was reported for COM (50.00 ± 1.92%), followed by TxT (48.72 ± 1.11%) and RT (48.72 ± 1.11%). The emulsion activity of RT (48.720 ± 1.109%), TXT (48.720 ± 1.109%) and COM (50.00 ± 1.92%) were not statistically different ($p> 0.05$), but significantly ($p< 0.05$) higher than that of SR (42.95 ± 1.11%). The emulsion activity of GT (45.510 ± 1.109%) and SR (42.950 ± 1.109%) were more or less similar ($p>0.05$). The emulsion capacities reported for coconut kernel flour (50.00 ± 0.20%) (Igbabul *et al*., 2014), wheat (43.88 ± 4.119%) and rice flours (41.48 ± 1.842%) (Chandra and Samsher, 2013) were somewhat similar to those of CTF samples

of local cultivars. Emulsifying properties are usually influenced by the hydrophobicity of proteins in the flour. Owing to their surface-active properties, proteins are able to form electrostatic repulsions on the surface of oil droplets and stabilize the emulsion (Kushal *et al*., 2012). Solubility and conformational stability of proteins are generally attributed to variations in the emulsifying activity of proteins (Kushal *et al*., 2012). Flours with the least protein solubility might display the least emulsification activity but the highest emulsifying stability (Kushal *et al*., 2012). According to Peyrano *et al*. (2016), the state of proteins (native/denatured) also affects emulsification properties; native proteins isolated from yellow cowpea showed higher emulsification activity compared to denatured ones. Researchers suggested that compositional changes in proteins and non-protein components such as carbohydrates might contribute collectively to emulsifying characteristics of flours (Kushal *et al*., 2012).

The emulsion stability (ES) of CTF of local coconut cultivars are compared as shown in Table 1. The ES of CTF were ranged from 27.51 to 54.86%; the maximum ES value being recorded for COM (54.86 ± 2.85%), followed by SR (43.24 ± 2.15%) and TXT (34.23 ± 3.28%). The mean ES values of GT (27.51 ± 0.85%) and RT (28.86 ± 1.03%) were not statistically different ($p>0.05$), but significantly lower than those of the rest. ES could be enhanced by the existence of soluble proteins in food systems (Kushal *et al*., 2012). The ES was significantly enhanced when firm globular protein molecules, which were impervious to physical distortion, involved in the formation of an adhesive layer in emulsions (Kaushal *et al*., 2012). High EA and ES are generally desired in food preparations such as comminuted meat products, salad dressings, frozen desserts and mayonnaise. The high ES of CTF from COM indicates its suitability for preparation of food such as cakes, soups and sausages where stability of emulsion is required. ES of CTF from local coconut cultivars were comparably similar to those of wheat (38.38 ± 4.79%) and rice flours (37.31 ± 5.41%) (Chandra and Samsher, 2013).

Foam is a colloid of small gas bubbles surrounded by a thin liquid layer trapped in a liquid or solid medium. The extent of the area of an air-water interface that can be formed by proteins is referred to as foam capacity (FC) (Chandra *et al*., 2015). The FC of CTF of local coconut cultivars are compared with that of commercial hybrid as depicted in Table 1. The FC (%) of GT (50.00 ± 0.00), TxT (41.11 ± 8.39) COM (31.67 ± 16.07) and RT (28.67 ± 7.51) were more or less similar ($p>0.05$), but significantly ($p<0.05$) higher than that of SR (23.33 ± 2.89). Previously, Chandra and Samsher (2013) reported FC values for wheat (12.92 ± 5.03%) and rice flours (3.52 ± 0.89%), which were comparably lower than those of CTF of this study. This is attributed to low protein content in wheat flour (16%) and rice flour (9.1%) when compared to the protein content of CTF (23.82-32.22%) (Fig. 2). Previously, Yasumatsu *et al*. (1972) observed the association between FC and water-soluble nitrogen content in soybean products. FC of a food material also depends on its pH, type of proteins, processing method, surface tension, viscosity etc. According to Oladele and Aina (2007), foam capacity could be influenced by solubilized protein content and polar and non-polar lipid content of flour. Soluble proteins might reduce the surface tension at the gas-water interface and form a continual sticky layer throughout the gas bubbles (Kaushal *et al*., 2012). It is believed that the arrangement of protein molecules also affects the FC of flours. Stretchy proteins would give well foaming ability while greatly organized globular proteins would result in low FC (Baljeet *et al*., 2010).

The foam stability (FS) is defined as the capability of proteins to maintain the foam against gravitational and physical forces

(Chandra *et al*., 2015). The FS of CTF of local coconut cultivars are compared with that of commercial hybrid as shown in Table 1. The FS (%) of SR (93.33 ± 11.55) and COM (83.33 ± 14.43) were more or less similar ($p > 0.05$), but significantly ($p < 0.05$) higher than that of RT (0.00 ± 0.00), GT (15.00 ± 8.66) and TxT (17.78 ± 13.47). Previously, Chandra and Samsher (2013) reported the FS values for wheat (1.94±0.048 %) and rice flours (0.98 ± 0.00%), which were considerably lower than those of CTF of all cultivars except RT. In CTF of RT, there was hardly any foam that could be seen after one hour. Air from small bubbles disperse into larger bubbles due to high pressure inside small bubbles compared to larger ones or the atmospheric pressure from the surrounding. Hence, this phenomenon would decrease the stability of the foam. FS can be reduced by draining liquid or through the foam layer due to gravity. Instability of liquid layer between bubbles would cause a combination of bubbles leading to reduction of foam. According to Jitngarmkusol *et al*. (2008), an inverse correlation existed between FC and FS. Bulky gas bubbles bordered by skinny, less stretchy protein layers can be formed due to high FC of flours. These gas bubbles can be easily broken due to the instability of the liquid layer. This results in low FS in flours with high FC. Food ingredients with good FC and FS are used in bakery products and whipping toppings (Aremu *et al*., 2007).

The least gelation concentration (LGC) which is used as a measurement of gelation capacity is the lowest protein concentration upon which the gel is retained in the tube without slipping when a tube is inverted (Aremu *et al*., 2007). The LGC of CTF of local coconut cultivars are compared with that of commercial hybrid as shown in Table 1. The LGC of CTF were ranged from 12 to 26% (w/v); maximum being recorded for RT (26 ± 0.00%), followed by GT (22 ± 0.00%) and TxT (18 ± 0.00%). The LGC of each variety was significantly ($p<0.05$) different from each other and decreased in the order of RT>GT>TxT>COM>SR. A lower LGC implies a better ability to forming gels by the protein component of the flour. The LGC values of CTF found in this study were remarkably higher than those of wheat (8%) and rice (6%) as reported previously (Chandra and Samsher, 2013). Differences in gelation properties among different legume flours were attributed to differences in the distribution of proteins, lipids, and carbohydrates (Adebowale and Maliki, 2011). According to Kaushal *et al*. (2012), the gelation ability of flours is affected by the competition between proteins and starch to water absorption. Gelation properties are attributed to protein type present in the system but not to protein quality (Adebowale and Maliki, 2011). Gelation properties could be affected by interactions among chemical constituents of the flour. When protein concentration is increased by adding more flour, thermodynamic attractions between protein and water molecules are reduced leading to increased synergistic effects between proteins that leads to enhanced gelation (Aremu *et al*., 2007). The gel forming ability of a food ingredient is important in food applications as well as new product developments. The gel provides a physical medium to retain water, sugars, flavors, and other nutrients in a food system (Aremu *et al*., 2007). Owing to its binding properties, flours with low LGC such as CTF, particularly from SR variety may provide consistency in food preparations such as semi-solid beverages (Ogunlakin *et al*., 2012). Flours with low LGC are useful for the preparation of foods such as sauce, puddings etc where thickening and gelling are desired (Chandra *et al*., 2015).

CONCLUSIONS

This study investigated the physical and functional properties and particle size distribution of CTF of five local coconut cultivars. Significant relationships were existed between

cultivars and particle size distribution with few exceptions. Among all cultivars, COM hybrid was found to display the highest values for EA, ES, SC and OAC. The observed differences in particle size and functional properties would make them suitable for different applications. Information of this kind would be beneficial not only for product development activities but also for those who are engaged in plant breeding.

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Preliminary Investigation of The Potential of Coconut Sugar Production Using Dwarf Varieties

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ABSTRACT

Coconut sugar is one of the high economic value products. The production of coconut sugar In Indonesia is about 300,000 tonnes/year. During the last few years, the supply of raw materials for the coconut sugar product has diminished due to stagnant production caused by the lack of coconut climbers collecting coconut sap. The scarcity of labor is caused by several factors, especially young tappers who are less interested in climbing tall coconut palms. To solve this problem, it is necessary to evaluate the potency of dwarf coconuts, with short trunk and fast fruiting. The objective of this study was the evaluation and selection of Dwarf coconut varieties that have high yield potential as a producer of sap and coconut sugar. The research was conducted in North Sulawesi Province by evaluating 9 Dwarf coconut varieties. The results of research on the production of coconut sap and sugar from 9 Dwarf coconut varieties with different plant ages, gave mixed results among coconut varieties. The length of tapping sap varied between 31.42 - 43.21 days/bunch, the volume of sap varied between 1.1 - 3.3 liters/tree/day, the pH of sap ranged between 6.49 - 7.86 and the Brix value varied between 14.01 - 17.64. The most important traits such as the sap yield and sugar production also varied from 14.54 to 18.95% among varieties, and between 0.16 to 0.42 kg/tree/day respectively. The Dwarf coconut varieties with the highest potential yield of sap and sugar are the Waingapu Red Dwarf (WRD), Salak Green Dwarf (SGD), and Nias Yellow Dwarf (NYD), with the potential sugar yield of 2.09 tons, 1.64 tons, and 1.56 tons/month/ha respectively. It is hoped that these Dwarf coconut varieties would be attractive for young tapers and could be introduced to farmers in several Provinces for tapping purposes for the production of coconut sugar.

Key words: *Dwarf coconut variety, short trunk, sap, brix, coconut sugar*

INTRODUCTION

Coconut palm, in addition to producing fruit that can be processed into various high economic value products, and used to produce sap (also called neera) by tapping inflorescences. Coconut sap contains a higher concentration of fructose and glucose with a lower concentration of sucrose compared with those of sugar palm and sugarcane juices (Asghar *et al.*, 2020). Coconut sap also contains higher amounts of vitamins (C, B1, B3, B4, and B10) as compared to sugar palm and sugarcane juices (Asghar *et*

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al., 2020). Hence, coconut sap could be a better potential source for the production of healthier sugar (Asghar *et al*., 2020).

According to Supomo (2007), each inflorescence can produce 2-4 liters of sap per palm per day. The results of an economic analysis showed that the coconut sap yields from coconut palms and made coconut sugar from them turned out to be more profitable for coconut farmers compared to coconuts being processed into copra or sold as fresh coconut.. Some evaluation reports (personal information) published says that income from palm sugar is 5-10 times higher than income from copra products. The main advantage of coconut sugar is that its glycemic index value is relatively low at 35-42, and it is a safe sweetener for diabetes (Trinidad *et al*., 2010). Coconut sugar also has sufficient nutrition compared to granulated sugar (cane sugar). The literature survey on coconut sap showed that there were about 30 developed products from coconut sap such as under suitable category; white crystalline sugar, fresh drinks, and jelly drinks and under a quite suitable category; syrup, candy, soy sauce, nectar, yakult, and yogurt and under not suitable category; kefir (Adiluhung *et al*., 2019) based on the priority recommendations for MSME (Medium and Small Micro Enterprise) in Banyumas District.

Coconut sugar which is known in the trade as Javanese or brown sugar is the result of processing coconut palm sap with a distinctive taste so that its use cannot be replaced by other types of sugar. Besides functioning as a sweetener, Javanese sugar also functions as a chocolate dye. Javanese sugar is produced from coconut sap which is then cooked by a family of farmers in a very simple way, then printed with bamboo molds, and then sold to small traders (baskets). From this basket Java sugar products are sold to collectors, then from, the collectors it is resold to dealers/suppliers who supply and sell directly to soy sauce factories in very large numbers (http://www.pidra-indonesia.org). The result of the effect of soaking tube with an anti inverse solution and concentration addition of sodium metabisulphite for naturally fermented coconut sap during 8 hours showed that the best parameter based on physicochemical properties were obtained by concentration addition of sodium metabisulphite 500 ppm and anti inverse concentration 3000 ppm (Pratama *et al*., 2015). Coconut sap sugar can be used as an alternative source for sugar because of its low glycemic index and since it possesses α – amylase inhibitory activity but is also used as a therapeutic agent in treating type II diabetes mellitus (Devi *et al*., 2015).

Nationally the production of coconut sugar is around 300,000 tons/year. The need for coconut sugar in Indonesia, especially for soy sauce raw materials, continues to increase every year by around 10%. In recent years, the supply of raw materials for coconut sugar for soy sauce products has decreased, due to stagnant production. This problem is due to the decreasing number of coconut sap that is caused by several factors, most significantly the less interest in climbing tall coconut trees by the youth. Climbing tall coconut trees is at high risk as falling from trees is quite evident. For this reason, it is necessary to find a solution so that coconut sugar production can be increased, and youth in rural and gender areas are interested in working in the area of tapping coconut sap. The use of coconut varieties that have short stem morphology is one solution to this problem. Coconut palms in the farmer's plantations are generally tall type coconut that has a high stem morphology.

The type of coconut that has a short stem morphology is the Dwarf coconut. The Indonesia Palma Crops Research Institute, IAARD has several dwarf coconut varieties collected as germplasm from several regions in

Indonesia, such as; Nias Yellow Dwarf (NYD), Raja Brown Dwarf (RBD), Salak Green Dwarf (SGD), Sagerat Orange Dwarf (SOD), Jombang Green Dwarf (JGD), Tebing Tinggi Dwarf (TTD) and Bali Yellow Dwarf (BYD). The difference in sap production is due to the differences in the variety and age of palms. According to Aristya *et al*. (2013) younger coconut palms have higher yields than older plants, in addition to the skills of tappers.

The objective of this study was to select and recommend Dwarf coconut varieties suitable for taping for coconut sugar production.

MATERIALS AND METHODS

The research was carried out in the Mapanget and Paniki Experimental Garden, IPCRI, North Sulawesi for six months starting in January-June 2016. The materials used in this study are productive trees from dwarf coconut varieties.

The research was carried out in the Mapanget and Paniki Experimental Garden, IPCRI, North Sulawesi for six months from January to June 2016. The materials used in this study were productive trees from dwarf coconut varieties.

The study used Single Block Design with 9 treatments and 20 sample palms for each coconut variety as a replication so that a total of 180 palms were used for the study along with KHINA-1 Hybrid coconut as the control (2 sample palms). The treatments tested were 9 dwarf coconut varieties consisting of:

1. Tebing Tinggi Dwarf (TTD)
2. Bali Yellow Dwarf (BYD)
3. Nias Yellow Dwarf (NYD)
4. Jombang Green Dwarf (JGD)
5. Nias Green Dwarf (NGD)
6. Waingapu Red Dwarf (WRD)
7. Raja Brown Dwarf (RBD)
8. Sagerat Orange Dwarf (SOD)
9. Salak Green Dwarf (SGD)
10. KHINA-1 hybrid as the control.

The observed variables consisted of:

1. Coconut stem height (m), measured from the bottom of the trunk to the old petiole of the crown of the leaf,
2. Inflorescence length (cm), measured from the base to the end of the bunch before starting the first tapping process,
3. Inflorescence of the circle (cm), measured in the middle of the inflorescence,
4. The production of sap per inflorescence/day, measured by the volume of sap per inflorescence per day (twice tapping),
5. Sap production per palm, measured by the volume of sap produced by each palm (for 6 months).
6. Sap sugar levels, measured using a refractometer.
7. The level of acidity (pH) of the sap.
8. Duration of tapping per bunch.
9. Number of bunches harvested for 6 months per tree (bunches).
10. Yield of coconut sugar produced (%).

MATERIALS AND METHODS

Palm ages, Stem length, and morphology of Bunch

The nine varieties of dwarf coconut with different ages were evaluated (Figure 1). The average duration of tapping of nine dwarf coconut varieties and one hybrid coconut as along with morphological characteristics are presented in Table 1.

The results in Table 1 shows that hybrid coconut of KHINA-1 (NYD x Tenga tall) has the highest duration of tapping (50 days). Of

Figure 1. Nine of dwarf coconut varieties in Mapanget Experimental Garden

No.	Varieties*)	Palms ages (years)	Average stem length (m)	Average Inflorescence length (cm)	Average Inflorescence circumference (cm)	Average days of tapping/ Inflorescence
1	WRD	17	5.43	63.26	26.32	43.21
2	SGD	15	4.93	50.32	21.66	31.42
3	NGD	38	10.33	54.25	21.08	42.00
4	NYD	39	9.61	56.00	22.27	37.70
5	JGD	38	9.43	48.92	22.79	36.33
6	SOD	30	7.20	57.44	17.19	36.13
7	RBD	36	8.66	61.22	19.64	34.90
8	TTD	37	8.24	50.20	23.53	33.90
9	BYD	10	4,06	51.09	21.53	32.21
10	KHINA-1 (Control)	38	11.90	64.13	26.75	50.00

Note: *) WRD (Waingapu Reda Dwarf), SGD (Salak Green Dwarf), NGD (Nias Green Dwarf), NYD (Nias Yellow Dwarf), JGD (Jombang Green Dwarf), SOD (Sagerat Orange Dwarf), RBD (Raja Brown Dwarf), TTD (Tebing Tinggi Dwarf), BYD (Bali Yellow Dwarf).

Table 1. The average length of tapping and the morphological characteristics of the inflorescence of nine dwarf coconut varieties and one hybrid

the nine dwarf coconut varieties, MRD has the highest duration of tapping (43.21 days) and the lowest was SGD with 31.42 days. The younger coconut palms have higher yields than older palms. It was observed that the circumference of the inflorescence, influences the duration of the tapping, as was seen in WRD and Khina 1 which have a larger inflorescence circumference than other varieties and have the highest duration of tapping. It was interested in observing that however, the length of the inflorescence does not affect the duration of the tapping.

In general, the production of one inflorescence of sap in the first week of tapping is below 500 ml/inflorescence/day which is then increasing in the second week, and become stable until the third week, and thereafter begins to fall in the fourth week onwards. But in the next / inflorescence, sap production in the first week on average was above 1,000 ml/inflorescence/ day and in the second week, it has reached above 2,000 ml/ Inflorescence/ day. The process of tapping sap in dwarf coconut variety can be seen in Figure 2.

The Sap and Coco sugar Production of Dwarf Varieties

The average volume of sap/tree/day and the production of sugar/palm/day are presented in Table 2. The number of palms of the nine dwarf coconut varieties is not uniform, because the palms conditions in the field are already quite high and some palms are considered to be quite risky to be tasted and there are limitations for tappers. From Table 2, it can be seen that the highest production of sap/palm/day and sugar/palm/day production are found in hybrid coconut (KHINA-1), While it was WRD among 9 dwarf coconut varieties showed the highest average production of sap /palm/day and the highest production of sugar /palm/day. The results of this observation are quite stable for four months of observation. Tulalo and Mawardi (2018) found that the coconut variety of WRD is

Figure 2. Tapping sap on dwarf coconut in Mapanget Experimental Garden

No.	Varieties	Number of palm sample (palms)	Average volume sap/ palm/day (ml)	Average production coco sugar/palm/day (kg)
1	BYD	20	1.56	0.29
2	SOD	7	1.36	0.23
3	RBD	5	1.33	0.22
4	WRD	10	2.60	0.45
5	TTD	7	1.48	0.27
6	JGD	6	1.28	0.23
7	SGD	11	2.07	0.35
8	NYD	4	1.91	0.33
9	NGD	2	1.48	0.30
10	KHINA-1	2	3.58	0.67

Table 2. Number of palms sample, sap volume/palm/day, sugar yield/palm/day, and average coco sugar/palm/day of nine Dwarf coconut varieties and hybrid KHINA-1 in Mapanget Experimental Garden

produced more sap compare to SGD, and RBD and the yield of sap are 1,007 ml, 741 ml, and 628 ml/ palm/day respectively. In India, on an average, a spadix can produce 1.5–3 liters of sap per day or 60–80 liters in 40–45 days (Hebbar *et al*., 2015). Although it was planned to use 20 sample palms for each coconut variety, but due to practical reasons between 2 to 20 palms were evaluated.

Compared to Hybrid coconut, the production of sap and coco sugar from nine dwarf coconut varieties is lower but seen from the condition of dwarf coconut palms with slower stem growth and faster first flowering of fruit bunches (2-3 years) compared to hybrid coconut (3-4 years) and tall coconut (5-7 years), shows the great potential of dwarf

coconut to be developed in producing coconut sap and sugar. In addition, the number of dwarf coconut palms in one hectare of land is more than that of tall coconut and hybrid coconut because the spacing of Genjah coconut. The seed nuts needed for rejuvenation and development are easier to obtain because they do not go through an artificial pollination process such as hybrid coconut.

pH, Brix and Yield of Coco sugar

The damage to the sap is characterized by a decrease in pH due to a breakdown of sugar into organic acids by microbes, such as yeast (*Saccharomyces sp.*) and the bacterium *Acetobacter sp.* Sucrose is converted into glucose and fructose, then the fermentation process of glucose and fructose into ethanol and CO2 ends with the process of formation of acetic acid, the process of changing ethanol into acetic acid (Naufalin *et al.*, 2013).

The values of pH, Brix, and sugar yield during the period March-June 2016 are presented in Table 3. The pH of the sap is observed when the sap is lowered from the coconut palm in the morning and evening. The results of the observation of the pH of the sap from the nine varieties of Dwarf coconut and hybrid coconut which were evaluated were neutral, an average of 6.80 - 7.44. In general, it appears that the variation of sap in terms of acidity in the nine dwarf varieties is very small, illustrating that the pH of the sap is not affected by the variety. From the results of observations in the field, the pH of the sap is more likely to be influenced by the preservatives used as well as the weather during erosion, for example during very hot weather the sap of the pH tends to be lower than the weather that is not too hot. Based on PT. Unilever Palm Sugar Manufacturing SOP for coconut sap as raw material for making soy sauce, the expected pH of sap is 6-8. Karseno *et al.*, 2018 reported that the browning intensity and antioxidant activity of sugars were increased with increasing pH of coconut neera and temperature. It was found that the effect of pH at 8 and temperature at 115°C shows the highest total phenolics (0.48%) and browning intensity (0.35) of sugar. The treatment also exhibited good antioxidant activity (DPPH scavenging activity) as high as 40%. This result also indicates that there is a significant correlation between browning intensity and the antioxidant activity of coconut sugar.

No.	Varieties	pH	Brix	Rendement
1	BYD	6.88	15.20	16.60
2	SOD	7.44	14.78	16.89
3	RBD	7.02	14.80	15.57
4	WRD	6.82	14.34	16.21
5	TTD	6.99	14.74	17.08
6	JGD	6.88	15.49	17.29
7	SGD	7.00	14.91	16.94
8	NYD	6.80	14.20	15.90
9	NGD	6.80	14.65	16.65
10	KHINA-1	7.30	15.45	17.64

Table 3. The average of pH, Brix and rendement coco sugar of nine Dwarf coconut varieties and hybrid KHINA in Mapanget Experimental Garden on period March - June 2016

The range of evaluated coconut brix value is 14.20-15.49 and the variety that has the highest brix value is GHJ which is 15.49. The sugar content of the nine sap from seven Dwarf coconut varieties in this study was almost the same as that was obtained by Xia *et al.* (2011) in his study, which was 14% in freshly tapped sap.

The extraction process is carried out twice a day, ie morning and evening, and from each palm two bunches could be tapped. The process of tapping and storing affects the freshness of the sap because the sugar in the sap is very easily fermented (Indahyanti *et al*., 2014). The yield range of coco sugar is among 15.57 - 17.29%, respectively and the average yield of JGD is 17.29%, TTD 17.08%, SGD 16.95%, SOD 16.89%, BYD 16.60 %, NGD 16.65%, WRD 16.21%, NYD 15.90% and RBD 15.57%. This data shows that to get as much as 1 kg of coconut sugar, it requires ±6 liters of palm sap.

The results of evaluations carried out on nine Dwarf coconut varieties showed all Dwarf coconut varieties evaluated could produce good quality sap and coconut sugar with varying production potential. All observations, obtained from the potential production of sugar /palm/ month and the potential sugar/month/hectare from Dwarf coconut planted with a spacing of 8 m x 8 m square planting system and hybrid coconut 9 m X 9 m is presented in Table 4. This evaluation shows that the nine Dwarf coconut varieties have the potential to be developed for the production of sap and coconut sugar, but of the nine varieties evaluated there are three high potential varieties with the highest sugar/hectare/month production potential, namely WRD coconut of 2,106 kg, evidenced by SGD 1,638 kg and 1,544 kg NYD. This is not only based on the production of sap and sugar but also based on palm morphology such as the number of flower bunches being tasted and visually more palms performance in the field.

Based on the experience delivered by the tappers, BYD and RBD varieties are less preferred by them due to the risk of tappers falling from the tree as the base of the fronds they support are loosely attached to the base of the stem.

No.	Varieties	Coco sugar/palm/day (kg)	Coco sugar/palm/ month (kg)	Potential coco sugar/ month/ha (kg)
1	BYD	0.46	13.37	2.106
2	SOD	0.3	8.85	1.404
3	RBD	0.33	10.01	1.544
4	WRD	0.23	6.94	1.076
5	TTD	0.23	7.02	1.076
6	JGD	0.23	7.02	1.029
7	SGD	0.27	8.17	1.263
8	NYD	0.29	8.6	1.357
9	NGD	0.35	10.54	1.638
10	KHINA-1	0.67	20.13	2.436

Table 4. Potential and sap production and coco sugar of nine dwarf varieties in Mapanget Experimental Garden

Figure 3. Processing Dwarf coconut sap to produce coco sugar

The process of cooking sap to produce coco sugar is shown in Figure 3. Coconut sap that has been spoiled or fermented when processed will produce coconut sugar with a texture that is difficult to converted to crystal sugar, resulting in loss for coconut sugar craftsmen (Febryanti *et al.,* 2014). To overcome this problem, it is necessary to provide preservatives in the sap container during tapping (Naufalin, *et al*., 2012). Usually, the male member of the family does the tapping twice in day and the sap cooks to become coco sugar is done by the female member of the family.

CONCLUSIONS

1. The thickness of the inflorescence, influences the duration of the tapping, but not for the length of bunches.
2. The production of palm sap and coco sugar from nine dwarf coconut varieties with different old palms, shown mixed results among coconut varieties. The length of tapping varies between 31.42 - 43.21 days / bunch, the volume of sap is between 1.1 - 3.3 liters / tree / day, the sap of pH is 6.49 - 7.86, Brix 14.01 - 17.64, sugar yield of 14.54 - 18.95%, and coco sugar production between 0.16 - 0.42 kg / palm / day.
3. Waingapu Red Dwarf (WRD), Salak Green Dwarf (SGD), and Nias Yellow Dwarf (NYD) were identified as the high potential varieties with the highest sugar/hectare/month with the potential yield of 2.09 tons, 1.64 tons, and 1.56 tons/month/ha respectively.

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Effect of Selected Leguminous Cover Crop Species on the Productivity of Coconut Cultivated in Reddish Brown Latosolic Soils in Sri Lanka

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ABSTRACT

This study was conducted for six years to assess the effects of two widely grown cover crops *Gliricidia sepium* (T_1) and *Puereria phasioloides* (T_2) on coconut yields and soil characteristics. Experiments were carried out on coconuts planted in Reddish Brown Latosolic soils located in the low country intermediate zone-IL1 of Sri Lanka. Results showed that treatments 1 and 2 were significantly ($p \leq 0.05$) effective over the control treatment (no cover crop) in suppressing weed biomass. Soil samples were collected and analyzed for physical, chemical and biological properties. Results showed that soils where *G. sepium* and *P. phasioloides* were grown were superior in most of the properties compared to the control. Soils under *P. phasioloides* showed significantly higher moisture contents compared to *G. sepium* and control treatments. Significant increases on soil properties were likewise observed in *G. sepium* and *P. phasioloides* plots: nitrogen (by 77% and 76 %), organic carbon content (by 86% and 148%), soil microbial activity (by 52% and 73%), respectively. On the other hand, soil bulk densities were reduced significantly by 20% and 27% under *G. sepium* and *P. phasioloides*, respectively. There was no significant increase observed on available P but P content improved with the establishment of cover crops. Significant increases in mean annual nut yields were observed during the 4th, 5th, and 6th years after cover crops establishment. Nut yields were found to increase with *G. sepium* and *P. phasioloides* by 46% and 58%, respectively when compared to that of control treatment plots.

Key words: *Gliricidia, Pueraria, coconut, cover crops, soil amendment*

INTRODUCTION

The coconut (*Cocos nucifera*) contributes to the livelihoods of millions of people in the developing world, not only through its production but also through employment generated by the many associated industries. It is the most widespread, economically useful palm of the wet tropics. Coconut is ideally grown under tropical climatic conditions. The degradation and loss of organic matter from soils under tropical climatic conditions is high due to optimal temperature, porous, light textured soil types and high precipitation. The growth habit of the coconut palm and the canopy structure requires a wide spacing between palms which permits the penetration of abundant sunlight to the ground vegetation. Thus, a wide range of perennial and annual weed species often invade the unutilized space beneath coconut palms (Senarathne *et al*., 2003). However, this space can be utilized to grow beneficial plant species depending on a particular purpose the farmer wants.

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Establishment of different leguminous cover crops in unutilized space between coconut palms helps to minimize surface run-off during heavy rains thereby controlling soil erosion. Cover crops also provide large quantities of mulch that help improve organic matter content in the soil. Penetration of leguminous cover crop roots into the soil improves the soil structure, improves infiltration of water, reduces leaching of nutrients, reduces ground surface temperature, controls the growth of weeds and provides nitrogen to the soil (Liyanage and Dasanayake, 1993). Leguminous cover crops are considered green manure, hence their cultivation *in situ* and their incorporation were considered to be a viable alternative to inorganic fertilization. This was practiced by the farmers as it is the most convenient and economically viable method for enhancing the organic matter status in the soil. Growing of green manuring crops improves the soil structure and nutrient status in the soil. In addition, they aid in releasing plant nutrients, reducing leaching, regulating soil temperatures, and enhances the activities of soil microbes. Several plant species have been tested as a cover crop in coconut plantations in the past. However, the suitability of a cover crop in coconut plantation depends on the climatic and soil characteristics in a particular growing area. Major cover crops recommended for coconut lands in Sri Lanka are, *Pueraria phaseoloides*, *Calopogonium mucunoides*, *Centrosema pubescens* and bush cover crops such as *Gliricidia sepium* (Coconut Research Institute, 2012). These cover crops produce large amount of green biomass and litter. These green biomass can be used as a green manure for coconut palms.

The application of green manure materials such as tree or shrub pruning with relatively high nutrient composition and fast decomposition properties have been recommended either as sole soil amendment or in combination with mineral fertilizers (Gachengo *et al*., 1999; Nziguheba *et al*., 2000 and Quinkenstein *et al*., 2009). The addition of these tree or shrub prunings through alley cropping or biomass transfer systems had made substantial contribution to the development of sustainable land use systems in the tropics by providing a cost effective mechanism for optimizing crop yields for efficient and stable crop production (Kang 1997 and Young 1997). The periodic pruning and return of biomass from hedgerow trees or shrubs through alley cropping or biomass transfer, contribute to recycling of plant nutrients, improvements in soil temperature, enhancement of soil structure, erosion control, and maintenance of microbial activity and high soil nutrient status (Isaac *et al*., 2003; Lin *et al*., 2009 and Wang *et al*., 2010). Moreover, long term productivity of alley cropping or biomass transfer systems requires shrubs or tree species that can coppice vigorously after each cutting (Latt *et al*., 2000). With many of the soil fertility and nutrient cycling benefits of agroforestry systems derived from the production and decomposition of tree biomass (Nair *et al*., 1999), optimal biomass production would be expected at each cutting to provide sufficient amounts of nutrients to crop nutrient demands (Latt *et al*., 2000). Some areas of the intermediate zone of Sri Lanka are characterized by the presence of Reddish Brown Latasolic soils. Since soil fertility of this soil group is comparatively much lower to its counterpart the Reddish Brown Earth soil. However, the impact of different leguminous cover crops as mulch or incorporated on soil development and the subsequent productivity of coconut palms in Reddish Brown Latasolic soils has not been clearly identified especially in field studies on coconut farming systems. The objectives of this study were to monitor the long term effects of leguminous cover crop establishment in coconut plantations for its land productivity in Reddish Brown Latasolic soils in the region.

MATERIALS AND METHODS

This experiment was carried out at the Ridigama Estate, Dodangaslanda, in the Low

country Intermediate Zone of North Western Province of Sri Lanka for six years, from January 2007 to December 2012. The area is characterized by bi-modal pattern of rainfall with an annual mean precipitation of 1500mm. Approximately 65% of the annual rainfall is received from September to February (Maha). There is a smaller peak of rainfall from March to May (Yala), but the rainfall is erratic. Higher ambient air and soil temperatures (about 28°C-32°C) and bright sunshine hours (about 6 – 8 hours per day) are more common especially during the dry periods from May to September. The soil at the site is a Reddish Brown Latasolic (RBL) (USDA soil taxonomy - Rhodudalfs fine loamy, non-calcareous, isohyperthermic), (FAO/ UNESCO soil taxonomy - RhodicCutanicLuvisols). Soils are very deep and well drained. Surface soil is dark brown in colour with a sandy clay loam texture. The sub surface soil is dark reddish brown to dark red in colour with a texture that ranges from clay loam to clay. The structure of the sub surface soil is moderately developed coarse sub angular blocky with patchy cutans on ped faces. However, during the dry season; the ground water table remains at 10 – 12 m below the ground surface. Reaction of the soil is slightly acidic (pH 6.0 – 6.5) throughout the soil profile (Mapa *et al*., 2005).

Treatments

T_1 - Establishment of *Gliricidia sepium* under coconut
T_2 - Establishment of *Pueraria phaseoloides* under coconut
T_3 - Control

Establishment of the experiment

One bush type (*Gliricidia sepium*) and one creeping (*Pueraria phaseoloides*) leguminous cover crops were selected for this experiment. Age of the coconut plantation was 50 years and trees were planted at a spacing of 8m × 8m (giving a density of 137 trees/ha). The cuttings of *Gliricidia* and *Pueraria* seeds were established during the 2007 rainy season. Three feet long well matured *Gliricidia* sticks were used for planting the trials. Double rows of *Gliricidia* cuttings were planted in between two rows of coconut palms, at a spacing of 1m x 2m (2250 cuttings ha^{-1}). The distance between coconut palms and *Gliricidia* was 3 meters. Each experimental plot was $384m^2$ (consisting of six coconut squares) in size and plots were separated by a single row of coconut. The *Pueraria phaseoloides* cover crop was established in the harrowed plots at a seeding rate of 5 kg ha-1. Overgrown conditions of the cover crop were managed to overcome competition by tractor harrowing twice a year and excess biomass was thatched around the coconut palms.

Experimental treatments were arranged in a Complete Randomized Block Design with three replicates. Gliricida plants were left to grow during the first 18 months without pruning. The first pruning was done on May 2009. Threafter, all the coppiced sprouts were cut back to within 4cm of the stump. This was done two times per year at the onset of monsoon rains. Pruned green biomass was thatched as a mulch around the coconut plams. This kind of management allowed the establishment of a vigorous stand of *Gliricidia*.

Before starting the experiment, all the coconut palms were fertilized each with 3 kg of fertilizer mixture (800g Urea, 600g Rock Phosphate and 1600g Muriate of Potash) with 1000g of Dolomite/palm/year. Fertilizer applications were carried out as per the recommendations given by the Coconut Research Institute of Sri Lanka.

Collection of ground cover weed biomass

The ground cover weed biomass was collected once in every month using 1m x 1m

quadrates from four random points per plot. Plant biomass samples were dried at 80°C for five days until it reached to a constant weight and dry weight was recorded.

Soil sampling and analysis

From the commencement of the experiment, soil samples were randomly collected 2.5m away from the effective root zone of coconut palms and at 0-30cm depth for determination of available and exchangeable plant nutrients in the soil. Simultaneously, an undisturbed soil sample was also collected using a core-sampler at depths of 0-15cm and 15-30cm for the determination of bulk density. Samples were processed under laboratory conditions by air drying separately at room temperature for 48-72 hours without any contaminations. Air dried soil samples were crushed and sieved through 2mm sieve. In addition, undisturbed soil samples were collected from same locations to determine microbial activity. For physio-chemical characterization, the following soil parameters were determined: organic carbon of the samples were measured by Walkey-Black method (Walkley and Black, 1934); N was estimated by the Kjeldahl method (Jackson, 1973) and the P and K contents of the samples were analyzed by calorimetric method (Anderson and Ingram, 1993) and flame photometric method (Simard, 1993), respectively. For the soil biological property, microbial activity was determined by trapping CO_2 with alkali solutions, followed by the precipitation of carbonates with barium chloride, and the titration of any remaining hydroxide with standardised acid (Stotzky, 1965).

Determination of soil moisture content

Soil samples were collected from four random points that were 2.5m away from the effective coconut palms and at a 30cm depth to determine the treatment effect on soil moisture content during drier months (January and July). These soil samples were oven dried at 105°C until constant weight is attained and gravimetric soil moisture content was determined afterwards.

Data analysis

Experimental data were analysed following the Analysis of Variance (ANOVA) procedure using the statistical software SAS and the significance of the differences between means was tested using the Least Significant Differences (LSD) at a P0.05 Value (SAS Institute 1999).

RESULTS AND DISCUSSION

Effect of treatments in controlling ground cover weed biomass

The lowest weed biomass was recorded in *Pueraria phaseoloides* established treatment plots (Figure 1). Initially *P. phaseoloides* took several months to establish a good cover. The weed biomass was very high at initial stages in cover cropped treatments plots but declined gradually later. When *Pueraria* was established, the weed biomass was reduced but weed seeds in the soil seed bank initiated germination with the onset of rainy season. Most of the new emerging weeds were annual dicotyledonous species (*Allmania nodiflora*, *Mitracarpus villosus*, *Tephrosia purpurea*, *Vernonia cinerea*). The emergence of monocotyledonous (*Panicum maximum*, *Pennisetum polystachion*, *Imperata cylindrica*) weed species was comparatively low. *P. phaseoloides* became self-seeded, and formed a good ground cover a few months after sowing, thereby suppressing weed populations by 90% (Figure 1). However, management of cover crops was essential to avoid possible competition between coconut palms and cover crops. Planting *Gliricidia sepium* between coconut rows was found to be a less effective method of controlling weeds in coconut plantations (Figure 1). However, *G. sepium* grows rapidly producing

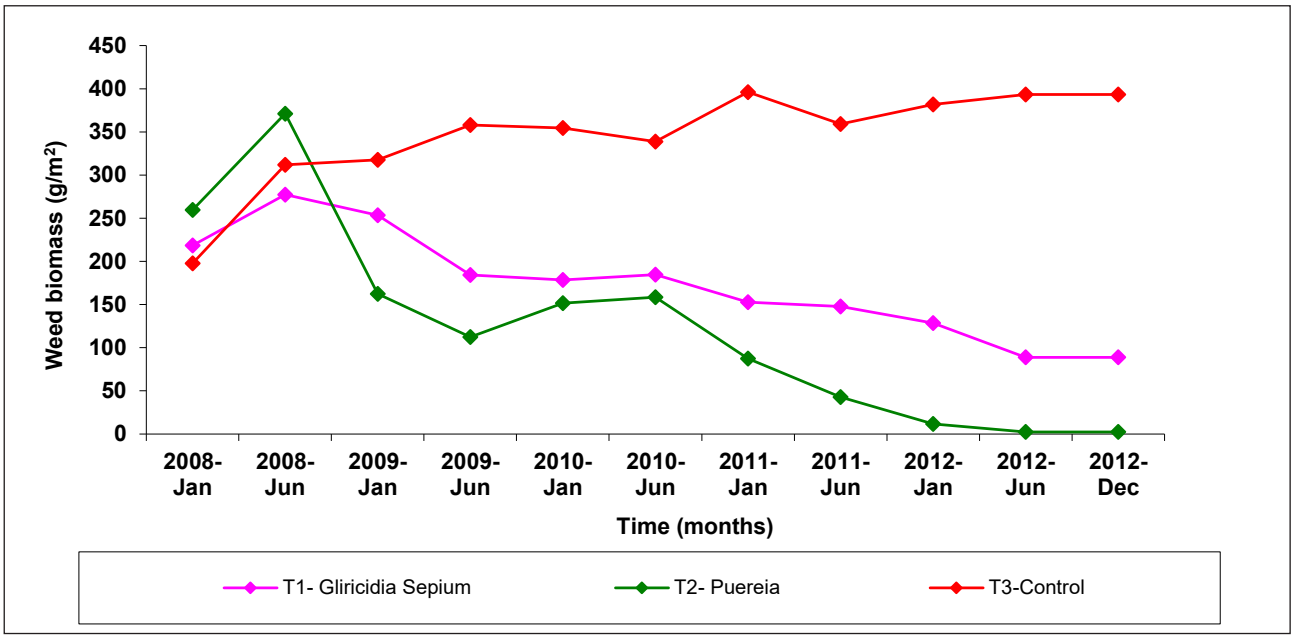

Figure 1. Effect of treatments on total weed biomass from January 2008 to December 2012

large quantities of foliage which can be used as mulch around the coconut palms thereby aiding in the control of weed population (Senarathne and Sangakkara, 2009).

Effect of treatments on soil physical properties (soil moisture and bulk density)

During the experimental period, results indicated that the moisture content significantly differ during the last three years (2010-2012) of experimental period (Table 1). However, cover crops established treatments have shown a quantitatively higher soil moisture compared to the control treatment. Areas planted to *P. Phaseeoloide*s cover crop had a comparatively higher soil moisture content during the whole experimental period.

Treatments	Soil moistures content (%)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	2.76	3.62	1.44	4.56	2.98
T_2- *Pueraria phaseoloides*	3.14	4.12	3.27	6.78	5.68
T_3- Control	3.15	3.13	1.27	2.98	1.58
Significance	ns	ns	*	*	*
LSD (P<0.05)	-	-	1.21	2.82	2.15

* Significantly different at P=0.05; ns - not significant

Table 1. Effect of treatments on soil moisture content (%)

However, although *P. phaseoloides* covered soils were found to be quantitatively higher in soil moisture content, their differences were found to be significant (P<0.05) only during the last three years (2010-2012) of the experimental period (Table 1). The control showed the lowest soil moisture content during the whole experimental period, except in 2008.

These increases in soil moisture contents might be due to the contribution of cover crop plant biomass litter to the soil surface which might have improved the soil's physical properties. Ji and Under (2001) reported that higher organic carbon contents of the soil increases its water holding capacity. Soil organic matter is responsible to a great extent, directly or indirectly for a good physical soil environment making it suitable for the plant root growth (Jeyamala and Soman, 1999). There is also a body of evidence that supports the ability of cover crops to increase soil carbon or soil organic

matter (McDaniel *et al*., 2014, Moore *et al*., 2014 and Poeplau and Don, 2015) and to improve the soil physical properties which enhance soil water dynamics (Daigh *et al*., 2014a, Steele *et al*., 2012 and Villamil *et al*., 2006). Furthermore, there is a complex interaction of soil physical and chemical properties that contribute to soil water storage capacity, including soil organic matter concentration, aggregation and porosity (Emerson, 1995; Hudson, 1994 and Kay, 1998).

The establishment of cover crops was found to reduce the bulk density of soil (Table 2) compared to the control treatment. Bulk density is a vital soil characteristic for successful root development (Kuchenbuch and Ingram, 2004). There was no significant difference in the bulk density in T_1 and T_2 during the first two years. However, significant effects were observed in soil bulk densities three years after treatment. Soils in *G. sepium* and *P. phaseoloides* were found to be lesser in bulk densities compared to control. The lowest bulk density was observed in T_2 during the last three years of the experimental period. Accumulated organic matter from litters of covercrops during the first three years and the succeeding years increases the organic matter of the soil. A high level of organic matter in the soil indicates reduced bulk density, improved soil structure, better aeration and highwater holding capacity all of which are attributes of a productive soil (Hseih and Hseih, 1990).

Treatments	Soil bulk density (g/cm^3)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	1.63	1.58	1.49	1.42	1.36
T_2- *Pueraria phaseoloides*	1.72	1.48	1.36	1.32	1.24
T_3- Control	1.71	1.64	1.68	1.73	1.69
Significance	ns	ns	*	*	*
LSD (P<0.05)	-	-	0.15	0.21	0.24

* Significantly different at P=0.05; ns - not significant

Table 2. Effect of treatments on soil bulk density (g/cm^3)

Effect of treatments on soil biological properties (microbial activity)

Significant effect of treatments on soil microbial activity were observed during the last four years of the experimental period (Table 3). The highest soil microbial activities were recorded in coconut areas with covercrops (T_1 and T_2). The lowest soil microbial activity was observed in control treatment (T_3) during the whole period of the experiment (Table 3).

Treatments	Microbial activity (mg/day)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	77.5	91.9	109.8	116.2	116.4
T_2- *Pueraria phaseoloides*	76.2	98.1	123.9	118.6	132.4
T_3- Control	71.5	74.7	68.3	84.5	76.4
Significance	ns	*	*	*	*
LSD (P<0.05)	-	14.8	23.2	12.8	22.3

* Significantly different at P=0.05; ns - not significant

Table 3. Effect of treatments on soil microbial activity (mg/day)

The establishment of cover crops is considered as a good management practice as it stimulates soil microbial growth and activity. Subsequent mineralization of plant nutrients (Eriksen, 2005) increases soil fertility and quality (Doran *et al*., 1988). Likewise, cover crops provide active living roots that form symbiotic relationships with fungi. These relationships are crucial in building a healthy soil. Different types of mycorrhizal fungi can be found on almost 90% of all plants in the world (Steenwerth and Belina, 2008). Other factors that may had contributed to the increased microbial activities were the abundance of organic matter due to litters, reduced ground surface temperature, and increased soil moisture creating a microclimate suitable for microbial growth.

Effects of treatments on soil chemical properties (organic C, available P, total N and exchangeable K)

The establishment of selected leguminous cover crops had no significant impact on available phosphorus content in the soil during the whole experimental period (Table 4). The available P, however, was observed to gradually increases throughout the experimental period, with *P. phaseoloides* treatment exhibiting the highest gain.

Treatments	Soil P (ppm)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	11.32	12.41	13.42	14.94	15.34
T_2- *Pueraria phaseoloides*	10.11	16.32	15.88	16.28	16.95
T_3- Control	11.45	10.98	11.48	12.85	12.01
Significance	ns	ns	ns	ns	ns
LSD (P<0.05)	-	-	-	-	-

* Significantly different at P=0.05; ns - not significant

Table 4. Effect of treatments on soil available P (ppm) content

Available N and K in the soil were shown to be significantly higher compared to control (Table 5 and 6). From 2010 to 2012, N contents in T_1 and T_2 are comparatively higher than T_3 (Table 5). Leguminous cover crops are known to improve the nutrient availability in the soil (Seiter and Horwath, 2004). The mean increments in soil N due to the establishment of leguminous cover crops (*G. sepium* and *P. phaseoloides*) were 76.5% and 75.6% respectively compared to the control. Both leguminous cover crops increased the total N content of the soil (Table 5). Leguminous plants improve soil N by returning N-rich organic litter to the soil, which may help maintain soil N pools in tropical soils (Hedin *et al.*, 2009). Soil nitrogen plays an important role in improving the productivity and sustainability of farms. Similar effect can also be observed with the use of high quality organic manures (i.e. organic manures with low C: N ratio) (Six *et al*., 2002). In tropical regions, however, the combination of low availability of organic carbon and nitrogen leads to poor-quality soil and low sustainability (Egodawatta *et al*., 2012).

Treatments	Soil N (ppm)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	287.2	324.4	332.4	495.0	581.7
T_2- *Pueraria phaseoloides*	292.5	328.4	324.8	505.2	578.9
T_3- Control	311.8	315.7	265.2	322.1	329.1
Significance	ns	ns	*	*	*
LSD (P<0.05)	-	-	22.7	39.7	29.4

* Significantly different at P=0.05; ns - not significant

Table 5. Effect of treatments on soil total N (ppm) content

A similar trend was observed with the effects of the treatments to the availability of exchangeable K content in the soil. Mean value of soil K were enhanced by 190% and 150% in T_1 and T_2, respectively (Table 6). Higher exchangeable K contents were recorded in T_1 and T_2 compared to the control (T_3). The lowest exchangeable K content was observed in the control treatment during the whole experiment. Results clearly show the importance of establishing cover crops for improving soil quality and fertility.

Treatments	Soil K (meq/100g)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	0.068	0.082	0.108	0.116	0.296
T_2- *Pueraria phaseoloides*	0.065	0.091	0.115	0.118	0.256
T_3- Control	0.061	0.077	0.091	0.087	0.102
Significance	ns	ns	*	*	*
LSD (P<0.05)	-	-	0.009	0.022	0.124

* Significantly different at P=0.05; ns - not significant

Table 6. Effect of treatments on soil exchangeable K (meq/100g)

Effect of selected leguminous cover crops on soil organic C content (%)

Results in Table 7 indicate increased soil organic carbon contents in T_1 and T_2. There was a significant effect of treatments on soil organic C content during the last three years of the experimental period (Table 7). The highest soil organic C content was recorded in T_2 while the lowest was recorded in T_3. Similar results were observed by Follett *et al*., (2007). Past studies showed that the addition of organic residues increases the soil organic carbon level initially. However, it gradually decreases in the soil up to a certain period (Gulser *et al*., 2010 and Manivannan *et al*., 2009). Incorporation of legume residues is really useful to the soil for growing soil natural carbon awareness which is not only vital to agricultural productiveness but also to sequestration of C from atmospheric CO_2 (Ayarza *et al*., 2007). When leguminous cover crops are used as green manure and incorporated into the soil, their residues enhance the availability of N, P, K, and trace elements to the succeeding plants due to the lowering of the soil pH brought about by the CO_2 produced in the process of decomposition (Benchaar *et al*., 2001).

Treatments	Soil organic C (%)				
	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	1.08	1.18	1.38	1.78	1.98
T_2- *Pueraria phaseoloides*	1.14	1.56	2.11	2.46	2.63
T_3- Control	1.04	1.12	1.09	1.11	1.06
Significance	ns	ns	*	*	*
LSD (P<0.05)	-	-	0.94	1.02	1.14

* Significantly different at P=0.05; ns - not significant

Table 7. Effect of selected leguminous cover crops on soil organic C content (%)

Effect of the treatments on coconut yield (nuts/palm/year)

Results show that the establishment of leguminous cover crops increases nut yields. Significant effects on nut yields were observed during the last three years of the experimental period in coconuts palms with *P. phaseoloides* cover crop (Table 8). Although there was an increase in yield of palms with *G. sepium* covercrop, the difference was insignificant compared with the control. Cover crops should be viewed as a long-term investment that gradually improves farm management in multiple areas, for altering soil physical, chemical or biological properties, to minimize erosion, or to improve soil fertility. While it has been long known that cover crops can influence soil properties (Masiunas, 1998). The yield benefits were likely due to soil nutrient credits provided from the mineralization of cover crop biomass prior to or during the crop production (Belfry *et al*., 2017). Further, the tomato yield benefits with vs. without cover crops in the crop rotation after 6- years were linked to 8.4–9.3% greater surface soil organic carbon concentrations (Chahal and Van Eerd, 2018). Decreased weed abundance might also contribute to yield benefits, as cover crop mulches may provide a physical barrier to weed growth and thereby improve vegetable crop production (Altieri *et al*., 2011). Thus, cover crops may benefit multiple soil health functions such as carbon sequestration, mitigating nutrient losses and supporting crop production (Chahal and Van Eerd, 2018).

Treatments	Nuts/palm/year					
	2007	2008	2009	2010	2011	2012
T_1- *Gliricidia sepium*	45	53	53	33	68	63
T_2- *Pueraria phaseoloides*	36	42	51	42	71	68
T_3- Control (APM)	45	50	43	32	42	43
Significance	ns	ns	ns	*	*	*
LSD (P<0.05)	-	-	-	8	14	11

* Significantly different at P=0.05; ns - not significant

Table 8. Effect of treatments on coconut yield (nuts/palm/year)

CONCLUSION

This study highlighted the importance of cover crop establishment for coconut farming systems. It demonstrated the possibility of *in situ* cultivation of two different leguminous cover crops (*G. sepium* and *P. phaseoloides*) in coconut plantations and their effect on increasing organic matter content of the soil which ultimately adds nutrients to the soil besides improving soil physical and chemical properties. *P. phaseoloides* is the most effective cover crop for managing the ground surface between coconut palms. Cover crops also stimulated soil biological activities which is necessary for soil health and fertility. The results also showed a positive effect on coconut yield and are environmentally friendly and economical. Based on the results of the study, it can be concluded that the use of *in situ* cover cropping system based green manures along with chemical fertilizers, is important for increasing soil productivity in coconut plantations established in Reddish Brown Latasolic soils in Sri Lanka.

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Perceptions and Efficacy of Oral Rinsing with Two Types of Coconut Oil: A Comparative Study

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ABSTRACT

This study focuses on the efficacy of virgin and regular coconut oil on plaque-related gingivitis and the perceptions of the subjects regarding its taste and odor. A study was carried out on 80 subjects, divided into 4 groups, 20 participants each. Group A: virgin coconut oil (VCO) gargling, Group B: regular cooking coconut oil (RCCO) gargling, Group C: chlorhexidine mouthwash gargling, and Group D: routine toothbrushing. The Modified gingival Index (MGI) was assessed to check the gingival inflammation on the 15th and 30th days. Perceptions of the subjects on the taste and odor were measured with the Hedonic Scale, and texture of VCO and RCCO in comparison with chlorhexidine. The baseline means MGI values are: 1.62±0.47, 1.74±0.22, 1.78±0.22, 1.68±0.66 for Group A, B, C, and D respectively. There is a significant difference in gingival index scores across all the study groups on the 15th day and 30th day (intra-group comparison). There is a significant difference in mean scores when group VCO, RCCO, and Chlorhexidine are compared with the control (inter-group comparison). Hedonic rating scale shows: chlorhexidine has a better odor (3.2) than VCO (3.1), RCCO (2.9). Chlorhexidine scored (3.4) in taste compared with VCO (3.1) and RCCO (2.8). Texture and mouthfeel scores for Chlorhexidine and VCO (3.6) and RCCO (3.4). VCO and RCCO are as efficient in reducing gingivitis. VCO has better taste, odor, and texture in the mouth than RCCO.

Key words: *Efficacy, oral rinsing, virgin coconut oil, regular cooking coconut oil, mouthwash gargling*

INTRODUCTION

Oil rinsing is a type of traditional procedure in the Indian system of medicine that involves swishing edible oil in the mouth and then spitting it out. This procedure is also called 'Oil Pulling' because the oil used for swishing is pulled and swirled to all parts of the oral cavity by movements of the tongue and oral musculature (Ripari *et al*., 2020). Ancient Ayurveda textbooks like Charaka Samhita and Arthashastra have mentioned these procedures as Kavala Gandoosha and Kavala Graha. Kavala Gandoosha is a procedure in which the mouth is completely filled with a large amount of oil and is spitted after a few minutes, whereas Kavala Graha is a procedure in which the oil is retained in the mouth and swished. Some Ayurveda textbooks say that such practices cure about 30 systemic diseases and have an effect on the overall well-being of the individuals practicing it (Pedikayil *et al*., 2015; Singla *et al*., 2015).

Oil rinsing or oil pulling is advised to be done in the morning on empty stomach, the oil is taken in the mouth before or after tooth brushing and is moved between the teeth for a few minutes till the

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oil turns thin and milky white and is spitted out (Peedikayil, 2019).

A variety of common edible oils are used for oil pulling therapy such as sesame oil, coconut oil, sunflower oil, groundnut oil, olive oil, mustard oil, and leaf extracts of gooseberries and mango. The advantage of these natural oils is that they neither cause any staining as seen in the use of mouthwashes nor there is any after taste or allergic reactions and are readily available (Shanbhag, 2016).

Various types of coconut oils are available in the market depending upon the method of extraction of oil from the coconut (*Cocos nucifera* L). In the present study, two types of coconut oil are considered for oil gargling. Regular Cooking Coconut Oil (RCCO) is made from dried coconut kernel called 'copra'. The copra is pressed; extracted oil is refined, decolorized and bleached. This process makes it suitable for consumption and has a high content of medium-chain fatty acids. Virgin coconut oil (VCO) is obtained from the fresh and mature kernel of the coconut by mechanical or natural means with or without the application of heat, which does not lead to alteration of the nature of the oil. VCO doesn't undergo any chemical refining, bleaching or deodorizing (Wallace, 2019; Deen *et al*., 2021; Dayrit *et al*., 2011).

Therefore, a study was conducted to compare the efficiency of regular coconut oil and virgin coconut oil in comparison with chlorhexidine mouth wash. The study also accesses the individual perceptions such as taste, odor and mouthfeel on its use.

MATERIALS AND METHODS

A prospective interventional comparative study was carried out in 80 male subjects in the age group of 14-18 years. The number of participants was calculated using the online software (https://clincalc.com) Minimum number needed in each group was 18, which was rounded to 20 in each group. Before the start of the study, ethical approval was obtained from the Institutional Review Board Committee (KDC/ETH/18/PED11/4A).

The inclusion criteria for the study were subjects with plaque-related gingivitis. Individuals with systemic disease, individuals on antibiotic or steroid medications and history of any dental treatment in the past 6 months, were excluded from the study. The study was explained to the participants and parents and informed written consent was obtained from the parents before proceeding with the study.

The selected study population was divided into four groups which included:

- Group A with a total of 20 participants for virgin coconut oil (VCO) gargling
- Group B with a total of 20 refined for regular cooking coconut oil (RCCO) gargling
- Group C with a total of 20 for chlorhexidine mouthwash gargling
- Group D (Control) with a total of 20 participants for routine toothbrushing only

The subjects designated for group A and group B were advised to routinely perform swishing for 3-4 minutes in the morning with 5ml of oil provided to them and group C participants were advised to routinely perform 5ml mouthwash gargling for 3-4 minutes in the morning. In addition, the subjects were directed to perform their routine tooth brushing 30 minutes after the oil/mouth wash gargling. While swishing, the subjects were advised to swish the fluids in all parts of the oral cavity. The participants in group D were advised to carry only routine tooth brushing only. All subjects were provided with a new toothbrush and toothpaste to use during the period of study and standard toothbrushing was demonstrated to them to ensure standard oral hygiene

practices. The Modified gingival Index (MGI) was assessed at baseline 15th day and 30th day for all participants. Following criteria are adopted 0 = absence of inflammation; 1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary gingiva; 2 = mild inflammation, in all portions of gingival marginal or papillary; 3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of gingival marginal or papillary; 4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration.

After 30 days of a 5-point questionnaire was given to the participants of group 1 and group 2 and group 3 to know their perceptions of using coconut oil swishing in comparison with coconut oil mouth wash use. A hedonic scale was used to find the acceptance of taste, odor and mouth feel of coconut oil.

The data obtained in the study were tabulated and analyzed using SPSS software (version 21, IBM, USA). One-way ANOVA was done to find the significance (P<0.5). Bonferroni *post hoc* test was done to compare mean scores within groups.

RESULTS

The baseline means MGI values obtained for each group are 1.62±0.47, 1.74±0.22, 1.78±0.22, 1.68±0.66 for group A, group B, group C, Group D respectively. Figure 1 shows the modified gingival index values obtained for rach groups at baseline, 15th day and 30th day. Table 1 shows an inter-group Comparison of mean modified gingival index scores at baseline, on the 15th day and 30th day between the groups. The results show that there is a significant difference across the study groups

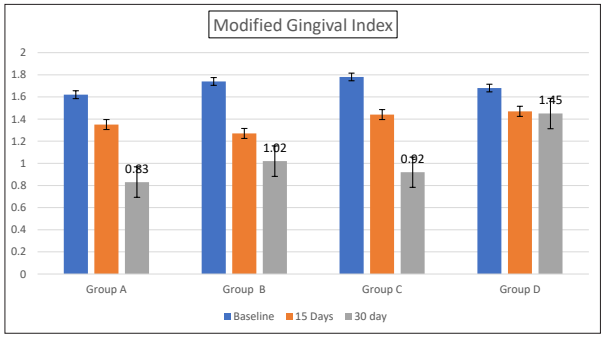

Figure 1. Modified Gingival Index at baseline, 15 and 30 days

Time Period	Group A (mean with SD)	Group B (mean with SD)	Group C (mean with SD)	Group D (mean with SD)	F Value	Significance
Baseline	1.62±0.47	1.74±0.22	1.78±0.22	1.68 ±0.66	2.232	0.254
15th day	1.35±0.28	1.27±0.26	1.44±0.86	1.47±0.30	16.17	0.000 #
30th day	0.83±0.22	1.02±0.28	0.92±0.28	1.55±0.32	39.26	0.000#

One way ANOVA, # $P < 0.05$ = statistically significant

Table 1. Inter Comparison of mean modified gingival index scores

Time Period	Baseline	15th Day	30th Day	F Value	Significance
Group A	1.62±0.47	1.35±0.28	0.83±0.22	51.5	0.02#
Group B	1.74±0.22	1.27±0.26	1.02±0.28	33.56	0.02#
Group C	1.78±0.22	1.44±0.86	0.92±0.28	44.76	0.02#
Group D	1.68 ±0.66	1.47±0.30	1.55±0.32	29.98	0.452 NS

$P < 0.05$ = statistically significant, # denotes significance

Table 2. Intra-group comparison of gingivitis scores at different time periods

Time Period	Group A vs Group B	Group A vs Group C	Group A vs Group D	Group B vs Group C	Group B vs Group D	Group C vs Group D
Baseline	0.543	0.343	0. 725	0.823	0.634	0.732
15th day	0.876	0.363	0.034 #	0.652	0.012#	0.043#
30th day	0.654	0.876	0.022#	0.735	0.047#	0.036#

Bonferroni post hoc test ($P < 0.05$ = significant). # denotes significance

Table 3. Comparison of mean gingivitis scores between study groups using Bonferroni post hoc test

	Ratings by participants																				Total Score	Average Score
Virgin Coconut Oil	Aroma	4	3	3	3	3	4	3	3	3	2	3	3	3	3	3	2	3	4	4	3	62	3.1
	Taste	4	3	3	4	4	3	3	3	3	2	3	2	3	3	3	3	3	3	3	3	62	3.1
	Texture Mouthfeel	3	4	4	4	3	4	4	3	4	4	3	3	3	3	4	4	4	4	3	4	72	3.6
Regular Cooking Coconut Oil	Aroma	3	3	2	3	3	4	3	3	3	2	3	4	3	3	3	2	4	2	2	3	58	2.9
	Taste	2	3	3	2	4	3	3	3	3	2	3	2	3	3	3	3	3	2	2	3	56	2.8
	Texture Mouthfeel	3	2	3	4	5	3	4	3	4	4	4	3	4	3	3	4	4	3	3	2	68	3.4
Chlorhexidine	Aroma	4	3	4	4	3	2	3	3	3	3	3	3	3	3	3	3	3	4	4	3	64	3.2
	Taste	4	3	4	3	3	4	4	3	3	2	3	2	3	4	5	3	4	3	4	4	68	3.4
	Texture Mouthfeel	4	4	4	4	3	4	4	3	4	4	3	3	4	3	4	4	2	4	4	3	72	3.6

Like a lot =5, like a little= 4, neither like or dislike= 3 dislike a little =2 dislike a lot=1

Table 4. Perceptions of subjects using Hedonic Rating scale

on the 15th day and 30th day. there is no statistical difference in mean gingival index scores in the control group (one-way ANOVA, $P<0.05$).

Table 2 shows the intragroup comparisons. statistically significant changes from baseline to 30 days in all groups whereas no statistical significance is seen in the control group.

Table 3 shows a comparison of mean gingivitis scores between study groups using the Bonferroni *post hoc* test. The results show as there is a highly statistical difference in mean scores when groups A, B and C are compared with control Group D, ie Group A vs Group D (p=0.034 on 15th day and p=0.022 on 30th day), Group B vs Group D (P=0.012 on 15th day and p=0.047 on 30th day), group C vs group D (p=0.043 on 15th day p=0.36 on 30th day).

Table 4 shows the perceptions of the subjects using different mouthwashes/oils as a part of this study. Hedonic rating scale shows that chlorhexidine has a better odor (3.2) than VCO (3.1) followed by RCCO (2.9). Chlorhexidine scored higher (3.4) in taste when compared with VCO (3.1) and RCCO (2.8). Texture and mouthfeel scores were the same for Chlorhexidine and virgin coconut oil (3.6) followed by RCO (3.4).

DISCUSSION

Chemo mechanical procedures is a part of oral hygiene maintenance as it reduces the incidence of plaque-related diseases such as gingivitis by decreasing plaque accumulation (Peedikayil, 2015). Modified Gingival Index is used for clinical assessment as it is the most widely used indices in trials for therapeutic

agents. The Modified Gingival Index (MGI) uses a visual scale to assess gingival health. The MGI relies on a visual assessment of gingival changes to measure the severity of inflammation (Asokan *et al*., 2009). The results of our study show that there was a significant decrease in the gingival index at the end of 15 days and 30 days on coconut oil rinsing especially with virgin coconut oil and is comparable to chlorhexidine which is considered as a gold standard among antiplaque and gingivitis agents.

Oil rinsing helps in decreasing plaque accumulation thereby and gingival inflammation. The mechanical shear forces exerted on the oil during swishing leads to an increase in the surface area of the oil film. The oil film thus formed on the surface of the teeth can reduce plaque adhesion and bacterial co-aggregation. It was also proposed that the alkalis in the saliva can react with the oil leading to saponification and formation of a soap-like substance which can reduce the adhesion of plaque. Coconut oil has a high saponification value and is one of the most commonly used oils in making soaps. Coconut oil-based soaps can lather well and have an increased cleansing action. The lauric acid in the coconut oil can easily react with sodium hydroxide in saliva during oil pulling to form sodium laureate, the main constituent of soap which might be responsible for the cleansing action and decreased plaque accumulation (Peedikayil *et al*., 2015; Singla *et al*., 2014; Peedikayil *et al*., 2016). Another reason for the action of the coconut oil in the oral cavity may be that the lipase enzyme present in the saliva is responsible for the breakdown of Medium Chain fatty acids and therefore lauric acid can enhance the anti-inflammatory effect in the oral cavity (Lai, 2019).

Virgin coconut oil (VCO) consists mainly of medium-chain triglycerides, which are resistant to peroxidation. The fatty acids in virgin coconut oil are distinct from animal fats which contain mainly long-chain saturated fatty acids. Virgin coconut oil is colorless, free of sediment with a natural fresh coconut scent. It is free from rancid odor or taste whereas refined coconut oil is refined by neutralization with alkali, bleached with bleaching earth or activated carbon or both and deodorized with steam; no other chemical agents being used. One of the most immediate differences between Virgin and regular cooking coconut oil is the taste and aroma. While Virgin Coconut Oil boasts a delicious, tropical coconut scent and flavor, Regular cooking Coconut Oil has a mild coconut scent and flavor (Deen *et al*., 2021; Dayrit *et al*., 2011).

Polyphenols are abundant dietary micronutrients protecting cells from damage due to oxidative stress. Several phenols have been identified in coconut oil such as protocatechuic acid, vanillic, caffeic, ferulic, and p-coumaric acids (Dimzon *et al*., 2011). Marina *et al.* in Williamson (2017) found phenolic content was 7% higher in Virgin coconut oil than in refined coconut oil. Polyphenol amount was highest in virgin coconut oil produced by fermentation and lowest in refined coconut oil. This may also be the reason for better action of virgin coconut oil than regular cooking coconut oil in our study.

The present study also shows that coconut oil pulling and chlorhexidine gargling when used as an adjuvant has a statistically significant reduction in modified gingival index scores when compared to routine oral hygiene maintained with brushing alone. Coconut oil contains a high amount of lauric acid and has been shown to reduce markers of inflammation in animal studies (Wallace, 2019). Peedikayil *et al.* (2015) in a study found that the coconut oil pulling practice reduces plaque formation and plaque-induced gingivitis significantly from day 7 of oil pulling, and the scores showed a continued decrease during the study period of 30 days. A study by Kaliamoorthy *et al.* (2018)showed coconut oil gargling showed a significant reduction in the severity of gingivitis in the coconut oil group than the sesame oil

group at all postintervention stages of their study. In the latest study by Ripari *et al.* (2020), coconut oil pulling showed a significant decrease in reducing plaque formation and gingivitis. The results of these studies are in agreement with our study results. In another study by Sezgin (2019) to find the plaque-inhibiting effects of oil pulling using 4-days plaque regrowth study model compared to 0.2% chlorhexidine gluconate (CHX) containing mouth rinse concluded that coconut therapy presented similar inhibitory activity on plaque regrowth compared with chlorhexidine.

Coconut oil is a readily accessible and cheap material for most when compared to chlorhexidine. Chlorhexidine mouthwashes are the most effective chemotherapeutic agent and are considered a gold standard against plaque-related gingivitis. But some studies have shown that Chlorhexidine on long-term use may alter taste sensation and also induce staining on the teeth surfaces. The mucous membranes and the tongue can also be affected and may be related to the precipitation of chromogenic bacteria (James *et al*., 2017).

The study also took into account the perceptions of the subjects regarding the Characteristic such as aroma, taste, texture/ mouthfeel of the oils/ mouth wash by using the Hedonic Rating Scale. Hedonic Rating scale is a widely used scale for measuring the acceptability of foods and beverages (Pimentel *et al*., 2016). Results show that chlorhexidine has a better taste and aroma. Among the oils tested virgin coconut oil has a better aroma, taste and texture taste of the oil. The perceptions of taste vary from person to person. Virgin coconut oils have a natural coconut flavor whereas regular cooking oil has a delicate, nutty flavor without a strong coconut taste. Future studies can be directed towards improving the aroma, taste and texture/ mouthfeel by the addition of certain herbs or natural substances without compromising on the efficacy of the coconut oil used as a mouth wash.

The limitations of this study are that the study is of short duration. The perceptions reported by the patients are based on a limited number of participants and can vary from person to person. Therefore, future studies have to be based on a greater number of participants and longer time period to check for long term efficacy and side effects.

CONCLUSION

This study focuses on the effectiveness of two different types of coconut oil in the rinsing procedure. The study is of clinical merit and proves that coconut oil is efficient, safe natural adjuvant to routine oral hygiene procedures. Virgin coconut oil and Regular cooking coconut oil safely used for oral swishing and can be an adjuvant to routine oral hygiene procedures. Taste perceptions need to be improved for better compliance with the oil rinsing procedure. More research has to be carried out to find a suitable natural flavoring addictive for better aroma, taste and oral texture of coconut oil to be used as a mouth wash.

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