Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

COLD PRESSED VIRGIN COCONUT OIL FROM FULL FAT COCONUT FLAKES A FUNCTIONAL OIL

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Received: 02 Apr 2014 Revised and Accepted: 28 Apr 2014

ABSTRACT

Objective: The general objective of the study is to process cold pressed virgin coconut oil from full fat coconut flakes, as functional oil, and the specific objectives are as follows: (a) to determine the proximate composition, microbial load and storage stability of Cold Pressed Virgin Coconut Oil (CPVCO) (b) to explore anti diabetic and cholesterol lowering effect of CPVCO.

Methods: The CPVCO was prepared out of fresh full fat coconut flakes by cold pressed method. The CPVCO was administrated to two experimental groups of rats at a dosage of 8 ml and 10 ml/kg/day for a period of three weeks. The negative and positive control groups were also reared for comparison. Similarly, another set of four groups of male Wister rats were used for experimenting cholesterol lowering effect of CPVCO. Apart from control group (I), rest of the groups were fed with cholesterol powder (1g/kg/day) as prescribed by Reeves et al 1993 [15]. The II group was maintained as negative control group and IIIrd and IVth groups were force fed with 8ml/kg/day and 10 ml/kg/day of CPVCO respectively. Baseline and the post treated lipid profile were assessed by using the blood sample drawn by retro orbital plexus.

Results: The present study showed that more than 70% of its saturated fatty acid was medium chain fatty acids (C_6-C_{12}). Out of this, a majority of 49% was lauric acid. In accordance with APCC (Asian and Pacific Coconut Committee), the microbial load and peroxide value of CPVCO were within safe limits (up to 10 months). When, 10ml/kg/day of virgin coconut oil was administered to diabetic experimental group, appreciably showed a decrease of 33%, 44% and 53% ($p < 0.01$) decrease of blood glucose at the end of 1st 2nd and 3rd week respectively. Serum total cholesterol, LDL, TGL except HDL of the experimental cholesterol group was significantly lower after the administration of 8ml/kg/day and 10ml/kg/day of virgin coconut oil for thirty days ($P<0.01$); The results indicated 8 ml/kg/day and 10ml/kg/day of CPVCO reduced blood glucose and lipids viz total cholesterol, low density lipoprotein and triglycerides to a significant level.

Conclusion: Promising results from the above study can be the basis in the development of cold pressed virgin coconut oil as functional oil.

Keywords: Coconut flakes, CPVCO, Functional oil.

INTRODUCTION

Virgin coconut oil (VCO) is defined as the oil resulting from the fresh and mature kernel of the coconut (*Cocos nucifera* L.) through mechanical and natural means, either with the use of heat or not provided that it does not lead to alteration or transformation of the oil [1]. Virgin coconut oil (VCO) is gaining popularity as functional oil and the public awareness of it is increasing. It is expected that VCO will experience a dramatic growth in the market. The introduction of VCO has opened up new research avenue that basically expects new benefits besides what has already been known with commercial coconut oil [2].

VCO can be extracted from the fresh and mature kernel of the coconut meat from several methods [3]. There are no specific processing prerequisites that were established according to [4], however, several methods to produce VCO were found to measure up with the definition of the VCO have been reported [3], [5], [6], [7] . These methods can be largely divided into wet and dry methods. In wet method, the coconut meat/kernel do not go through drying process while in dry method, the kernals were heated under specific conditions to remove the moisture in it preventing scorching and microbial invasion. Wet method can be further divided into chilling and thawing, fermentation, enzymatic and pH method or any of these in combination as the main aim is to destabilize the coconut milk emulsion [7].

Virgin coconut oil (VCO) a preparation of coconut oil without harsh processing such as refining, hydrogenation, deodorization, bleaching etc may retain native bioactive compounds present in it Okava et al., 1979 [4]. VCO extracted in cold and hot conditions shown to be rich in poly phenols. Lauric acid, present in coconut oil has been shown to possess insulino tropic properties in isolated per fused mouse islet model but not proven in diabetic animals [8].

VCO has many advantages, which include the health benefits from the retained vitamins and antioxidants, the antimicrobial and antiviral activity from the lauric acid components and through its easy digestibility due to Medium Chain Fatty Acids (MCFA) [3]. In this context, any dietary oil that lowers LDL cholesterol and elevates HDL cholesterol is considered to have health benefits. Coconut oil is believed to elevate blood cholesterol since it contains mostly saturated fatty acids. Hence, in the present study, an effort was taken to explore the lipoglycemic benefits of Cold Pressed Virgin Coconut Oil (CPVCO) in animal models, along with assessing the storage stability.

The general objective of this study is to analyze proximate composition and shelf life of virgin coconut oil and to determine the anti-diabetic activity and the cholesterol lowering effect of virgin coconut oil.

MATERIALS AND METHODS

Virgin coconut oil processing

Fresh meat of the coconut was shredded and dried in a fluid bed dryer at 40^{0}C for approximately 30- 35 minutes. The time taken for drying was 30 – 35 minutes one tonne of scraped coconut meal. The dehydrated full fat coconut flakes were then subjected to cold pressed at 10 – 15^{0}C temperature without applying any heat. The virgin coconut oil extracted on cold press was filtered using poly propylene filter sheet at duration of 45 to 50 minutes. The Cold Pressed Virgin Coconut Oil (CPVCO) obtained was packed in High Density Poly Ethylene (HDPE) bottles and stored at room temperatures. The shelf life study was carried out for a period of 10 months.

Proximate composition of CPVCO

CPVCO was analyzed for caprylic acid, capric acid lauric acid, myristic acid, palmitic acid, steric acid, unsaturated fat and poly unsaturated fat using AOAC 18 th Edition method. Energy content

was assessed by calculation using Nutritive Value of Indian Foods, ICMR [9].Arsenic, Cadmium, Mercury and lead were determined by using SO-CHML-CTS-C-01-QU-063 by ICPMS.

Physico chemical Properties

Total ash, peroxide value and acid insoluble ash percentage by weight were analyzed by the method suggested by AOAC (2005) [10]. Iodine value, refractive index, saponification value and free fatty acid were determined by using AOCS Cd 1-25, AOCS Cc 7-25, AOCS 3-25, AOCS Ca 5a -40 methods [11] [12] [13] [14]. Specific gravity was determined using IS 548 (part I):1964.

Assessment of Microbial Quality

Assessment of Aflatoxin (B_1, B_2, G_1 and G_2) was performed in accordance with the procedure of **AOAC (2005)** [10]. Whereas total plate count, coli form count, detection of Salmonella and yeast mold plate count were determined by ISO methods.

Anti diabetic activity

Animal experiment was carried out as per guidelines of Institutional Animal Ethical Committee and approval (Bearing number: 1012/c/06/CPCSEA). 32 male Wister albino rats reared at animal house of RVS College of Pharmaceutical Science, Coimbatore, Tamil Nadu, INDIA, Body weight of 150 – 200 g were selected based on uniform food intake and weight gain were maintained with standard laboratory conditions. The animals were divided into 4 groups of 8 rats, the first group being non-diabetic control. Animals of groups II, III and IV were rendered diabetic by a single intraperitoneal (i.p.) injection of 60 mg/kg of Streptozotocin (STZ) freshly prepared in 0.1 M of citrate buffer (pH 4.5). Second, the diabetic control group was administered ½ unit of Insulin per day for avoiding morbidity and mortality as it was positive control group. Third and fourth groups were force fed with 8 ml and 10 ml of Virgin coconut oil /kg/day. The experimental duration was for three weeks. Food intake and body weight gain were monitored weekly. The blood glucose levels were monitored 5 days once, by obtaining blood samples from tail vein; with standard kit. At the end, animals were sacrificed, after overnight fasting, by cervical dislocation and the blood sample was collected by cardiac puncture for final analysis of blood glucose levels.

Cholesterol activity

The rats were divided into four groups, each of six animals were maintained under standard laboratory conditions (12 h light/dark cycles). The first group (gp I) was kept as the control (receiving Vehicle). The second group (gp II) third and fourth groups (gp III and gp IV) were fed with 1% cholesterol powder (1g/kg/day) of hypercholesterolemic gp by oral administration for 10 days as prescribed by Reeves et al (1993)[15]. Then, the hypercholesterolemic condition was confirmed by using respective diagnostic kits on the 11th day of experiment using blood drawn by retro orbital plexus. After the confirmation of hypercholesterolemic conditions of rats, the day on which rats were treated with virgin coconut oil at a low dose of 8 ml/kg /day (gp III) and high dose of 10 ml/kg/day (gp IV) body weight was considered as 1st day of experiment. However, the II group was maintained as negative control group. Blood samples from the retro orbital plexus were drawn after an overnight fasting (more than 12 hr) before treatment (0 week), then 30 days of treatment. Serum total lipids were estimated by using kits (Bio-Diagnostic). Total cholesterol (Tc), triglycerides TGs) and high density lipoprotein (HDL)-cholesterol were measured using kits. Low density lipoprotein (LDL)-cholesterol was calculated by Friedwalds formula.

Table 1: Proximate composition (g/100g) and energetic value (Kcal/100g) of cold pressed Virgin Coconut Oil (mean ± SD)

S. No.	Testing Parameters	CPVCO	Test Methods	APCC standard for Virgin coconut oil
1	Total fat %	99.79 ± 0.01	AOAC 18th Edition	-
2	Moisture %	0.1 ± 0.01	AOAC 18th Edition	0.1
3	Caprylic acid C_8 g/100g	8 ± 0.2	AOAC 18th Edition	4-10
4	Capric acid C_{10} g/100g	10 ± 0.01	AOAC 18th Edition	4-8
5	Lauric acid C_{12} g/100g	49 ± 0.06	AOAC 18th Edition	45-56
6	Myristic acid C_{14} g/100g	17 ± 0.08	AOAC 18th Edition	16-21
7	Steric acid C_{18} g/100g	2 ± 0.5	AOAC 18th Edition	2-4
8	Palmitic acid C_{16} g/100g	7.8 ± 0.04	AOAC 18th Edition	7.5-10.2
9	Unsaturated fatty acid g/100g	6 ± 0.1	AOAC 18th Edition	-
10	Poly unsaturated fatty acid g/100g	2 ± 0.07	AOAC 18th Edition	-
11	Energy kcal	898.1 ± 0.02	Nutritive value of Indian foods, ICMR (Gopalan et al., 1996).	-
12	Free fatty acid (as lauric acid) g/100 g	0.07 ± 0.03	AOCS Ca5a-40	Max 0.2
13	Arsenic (as AS)	BLQ (LOQ: 0.05)	SO-CHML-CTS-C-01-QU-063 by ICPMS	Max 0.1
14	Cadmium (as Cd)	BLQ (LOQ: 0.01)	SO-CHML-CTS-C-01-QU-063 by ICPMS	-
15	Mercury (as Hg)	BLQ (LOQ: 0.01)	SO-CHML-CTS-C-01-QU-063 by ICPMS	-
16	Lead (as Pb)	0.1 ± 0.03	SO-CHML-CTS-C-01-QU-063 by ICPMS	Max 0.1

RESULTS AND DISCUSSION

Fatty acid Composition of cold pressed Virgin coconut oil

Virgin coconut oil (VCO) characterized by its high level of lauric acid (C_{12}), which ranged from 47-50% APCC, (2003) [1]. In the present study, the lauric acid (C_{12}) content was 49% (Table 1). Other fatty acid composition determined include caprylic acid C_8 (8%), capric acid C_{10} (10%), myristic acid C_{14} (17%), stearic acid C_{18} (2%) and palmitic acid C_{16} (7.8%) (Table 1).Out of 92% of saturated fatty acids, medium chain fatty acids possessing a carbon chain from C_6 to C_{12}, constitutes as 49% lauric acid and other MCTs. However, unsaturated and poly unsaturated fatty acid were constituting only 6% and 2% respectively.

In a study conducted by kamariah et al (2008) [16], the that fatty acid composition of virgin coconut oil possessed medium chain fatty acids more than 64%, within which lauric acid was ranging from 47-50% and total saturated fatty acids were 93%. Always water is very slightly soluble in oils and fats, and its presence is confined only to very small amount. In this study the moisture content of CPVCO was only 0.1%.

Table 2: Physico chemical properties of CPVCO (g/100g) (mean ± SD)

S. No.	Testing parameters	CPVCO	Test methods
1	Total ash %	0.02 ± 0.1	AOAC 18th Edition, 2005
2	Moisture %	0.28 ± 0.01	AOAC 18th Edition, 2005
3	Iodine value	7.61 ± 0.03	AOCS Cd 1-25
4	Refractive index at 40 ^{0} C	1.4488 ± 0.05	AOCS Cc 7-25
5	Saponification value	260.8 ± 0.02	AOCS 3-25
6	Specific gravity	0.9225 ± 0.01	IS 548(part I): 1964
7	Peroxide value	Nil	AOAC 18th Edition, 2005

Iodine Value (IV) is a measure of the degree of unsaturation in oil. The IV of CPVCO in fresh sample was 7.6 ± 0.03(Table.2). The values are similar to that was reported in literature Marina et al 2009 [17] and Henna et al 2009 [18]. The Refractive index (RI) of the oil measures the extent to which a beam of light is refracted on passing from air in to the oil. For CPVCO extracted in the present study, the RI was measured at 40 ^{0}C and was at 1.44±0.05. Whereas, kamariah et al 2008 [16], documented RI of VCO as 1.44± 0.0001. The Saponification Value (SV) is a measure of the free and esterified acids present in fats and oil. The SV measured in the recorded present study 260.8± 0.02. Free Fatty Acid (FFA) is the most important characteristic of VCO quality that are considered as criteria for sales and contracts. The FFA is an indication of the care taken during VCO production Lide et al 1996 [19].

The mean FFA value of CPVCO obtained from the present study was only 0.07± 0.03 as against the standard of max 0.2 (AOCS ca5a-40). Peroxide value (PV) value gives an indication of the primary oxidation state of oil. However, VCO was found to have nil value of peroxide. Up to four month.

Table 3: Assessment of Microbial Quality (cfu/g) for Fresh cold pressed Virgin coconut oil

S. No.	Testing parameters	CPVCO	Test methods
1	Escherichia coli per g	Absent	ISO 7251:2005
2	Salmonella Spp per 25 g	Absent	ISO 6579 :2002
3	Total plate count	0	ISO 4833:2003
4	Yeast and mould count	Absent	ISO 21527(part 2) : 2008
5	Aflatoxin B_1 µg/kg	BLQ *(LOQ: 1.0)	AOAC 999.07
6	Aflatoxin B_2 µg/kg	BLQ *(LOQ: 0.5)	AOAC 999.07
7	Aflatoxin G_1 µg/kg	BLQ *(LOQ: 1.0)	AOAC 999.07
8	Aflatoxin G_2 µg/kg	BLQ *(LOQ: 0.5)	AOAC 999.07

Table 4: Changes in Peroxide value (mεq O_2 / kg fat) of CPVCO at room temperature over 10 months of period

Test food	Month interval / Peroxide value (mεq O2 / kg fat)									
	1	2	3	4	5	6	7	8	9	10
VCO	Nil	Nil	Nil	Nil	0.2	0.2	0.4	0.4	0.9	1.5
Specification	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3

From the Table no 4, it was clearly evident that peroxide value of CPVCO was within safe limits up to 10 months which indeed was falling within the APCC (Asian and Pacific Coconut Committee) prescribed level of <3 maximum. In this line, a study conducted by Dayrit (2008) [20] stated that the Standards for Essential Composition and Quality Factors of Commercial Virgin Coconut Oil and its Differentiation from Refined Bleach Deodorant (RBD) Coconut Oil and Copra Oil, standards for peroxide value should be <3.

Table no 5 shows the total plate count of the experimental substances. Total plate count of CPVCO was absent over a period of 10 months. This in fact was in accordance with APCC (Asian and Pacific Coconut Committee) standard, which prescribes of within the limit of (<10 cfu) VCO without treating with any preservatives over a period of 10 months. Conversely, our storage of CPVCO was also carried out without adding any preservatives and antibiotics.

Table 5: Changes in Microbial load (cfu/g) of CPVCO at room temperature over 10 months of period

Test food	Month interval / Microbial Load colonies / g									
	1	2	3	4	5	6	7	8	9	10
VCO	0	0	0	0	0	0	0	0	0	0
Specification	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10	≤10

Table 6: Effect of different concentration level of CPVCO for anti diabetic activity

Groups	Food Intake g/kg/day	Weight gain/loss (g/21 days)	Blood glucose mg/dl			
			5th day	10th day	15th day	21th day
Control Non diabetic	12.8 ± 1.7^a	25.2 ± 1.6	108.31±1.03	110.10±2.3	115.2±3.1	106.4±1.04
Control diabetic (Insulin)	21.2 ± 1.5^b	-28 ± 3.2	264.20±3.6	252.31±4.2 (4%)	235.18±4.16* (10%)	221.24±4.2* (16%)
CPVCO (8ml)	20 ± 1.1^b	-20 ± 4.2	343.71±6.72	266.21±4.78 (22%)	236.52±5.27** (31%)	207.41±7.12** (39%)
CPVCO (10ml)	20 ± 1.3^b	-14.5 ± 0.6	287.23±3.79	190.21±10.6 (33%)	159.16±10.1** (44%)	133.50±11.3** (53%)

Values bearing different stars in the same row are significantly different at (p <0.05) and (p<0.01) respectively

Effect of CPVCO on Blood Glucose level Values are mean of ± SD of 8 rats

Diabetes, a dreadful disease with many metabolic manifestations is recently developing alarmingly. The diabetics are treated with many modalities and of course their diet if not prudent would lead to aberrations in their metabolic outcome. In this, context natural functional foods with least ill effects and low cost are desirable for favorable management diabetes.

Increased food intake with body weight loss was observed in all the diabetic groups, lowest weight loss was observed in 10ml /kg/day concentration (Table 6) of CPVCO (-14.5 ± 0.6).It is interesting to note that medium chain fatty acids and triglyceride with their fatty acids can metabolize fast and assist in preventing obesity and in turn stimulate weight loss in diabetic obese individuals Nevin and RajaMohan 2006 [21]. With this context, the present weight loss in the study even after feeding CPVCO may also be corroborated. With Gradual decrease in blood glucose was observed in all the 3 groups of animals. Insulin (control diabetic) was able to decrease the blood glucose level from 264.20 mg to 221.24 mg during 3 weeks (4%, 10% and 16% at the end of 1st, 2nd and 3rd week respectively). Significant decrease in Insulin fed was observed at the end of 2nd week and 3rd week ($p<0.05$) respectively. In animals fed with CPVCO 8 ml/kg /day, a significant decrease was observed from 1st and 2nd week till 3rd week (The percentage decrease was 22%, 31% and 39% ($p<0.01$) at the end of 1st, 2nd and 3rd week respectively). The group fed with 10 ml/kg/day of CPVCO was noted to have a decrease in blood glucose at 33% 44% and 53% ($p < 0.01$) at the end of 1st 2nd and 3rd week respectively. The present results of our study appreciably matched the results of the animal research carried out for four week with VCO by Bolanle et al 2013 [22]. In yet, another animal study, the blood glucose and cholesterol levels of treated groups of rats showed significant reduction after 7 weeks of treatment with Virgin coconut oil Siddhalingasamy 2011 [23].

The plausible reduction of blood glucose may be attributed to the rich content of lauric acid of CPVCO. It has been reported that lauric acid in coconut oil has insulin tropic properties but reported in isolated islet of mouse Girotti 1984 [8].

Table 7: Cholesterol lowering effect of CPVCO Values are mean of ± SD of 8 rats

Test food	Total cholesterol		LDL		HDL		Triglycerides	
	Day 1	Day 42	Day 1	Day 42	Day 1	Day 42	Day 1	Day 42
Control	293±7.84	292.5±3.9	144±3.7	145.4±4.9	49.2±5.4	47.68±3.1	223±14.14	223.2±18.0
1% Cholesterol powder	285±7.11	280±3.96	134.3±6.9	132±0.11	57.4±4.20	45.4±7.53	267±16.02	245±14.5*
CPVCO (8ml)	295±22.0	266±15.5**	162.7±7.8	149.7±9.1**	52.4±4.54	49.28±4.9	318±21.3	261±20.14**
CPVCO (10ml)	289±10.1	241±7.52**	152.8±4.7	136±3.10**	51.9±3.19	50.68±7.6	232±19.10	196±16.48**

*Significantly different at $P<0.05$ and ** significantly different at $p<0.01$

Cholesterol lowering effect

Hyperlipidemia, dyslipidemia and elevated oxidative stress may lead to cardiac complications despite in control diabetes status. It has been strongly reported that VCO reduces blood lipid even under normal conditions Cohen et al 1970 [24]. Further, it is known according to Nevin and RajaMohan 2004 [25] Evans 2007 [26], that VCO is rich in medium chain fatty acid and can metabolize quickly and thus, accumulation of fat is inhibited. Table 7 shows the serum total, LDL, HDL cholesterol, and triglycerides of rats before and after feeding of the CPVCO. Serum total cholesterol were significantly lower after consumption of 8 ml CPVCO and 10 ml CPVCO for 30 days ($P<0.05$; Table 7). Similar results were also observed for LDL cholesterol. For both the concentration of CPVCO, there was a significant reduction in serum triglycerides ($P<0.05$; Table 7). However, there was no significant increase or decrease in HDL cholesterol that was observed in the study after treatment. The findings of Arunima and Raajamohan 2012 [27] Bolanle 2013 [22] supported the phenomenon of cholesterol reduction with diets containing VCO; they reported decrease in serum cholesterol of rats by providing normal diet with VCO. They concluded that VCO can alter lipid metabolism. In another study are also in corroboration with recent findings of decreasing plasma lipid profile especially cholesterol with VCO Siddhalingasamy 2011 [23]. The same kind of reduction in cholesterol and level other lipid except HDL was also observed in the present study after feeding with CPVCO. Such dramatic reduction in glucose and lipids in the present research could be attributed to CPVCO. It may also be possible that other vital antioxidant vitamins present in this oil due to saponification may have role in reducing lipids Nevin and RajaMohan 2006 [21]. Yet another study, which has used hot extracted VCO rich in antioxidants supplementations was stated to have a role in regulating cholesterol synthesis by regulating HMG –COA reductase activity Hetono et al 1988 [28]. Though the basic source is coconut, yet it has not impaired the lipid parameters in vivo. Thus, the results make us to get interested to further explore the mechanism of attenuation of lipo-glycemic entities.

CONCLUSION

In conclusion: (a) Virgin coconut oil is a rich source of medium chain fatty acids especially lauric acid (49%) (b) CPVCO stored up to 10 months at ambient conditions, did not produce any rancid odour and the microbial load was also within the safe limits (c) CPVCO administration substantially brought down the blood glucose level and reduced the lipid parameters of the rats. Hence, CPVCO prepared from full fat coconut flakes rendered as safe, odourless therapeutic functional oil. As CPVCO potentially ameliorates glucose and lipid levels, this may be used as functional oil for human beings too. Further, a systematic human study may be carried out using CPVCO in order to explore its real impact in humans.

ACKNOWLEDGEMENTS

The authors are thankful to Mr.R.S.Ganesh, Director of Vama oil Private Limited, Coimbatore, INDIA for his constant support, encouragement and financial assistance.

CONFLICT OF INTEREST: None

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